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HBM4EU science and policy for a healthy future

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List of effect biomarkers for the first set of prioritized substances

Deliverable Report

D14.2

WP14 Biomarkers of effect

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2 Abstract/Summary

Background: The ultimate objective of HBM is to link biomarkers of exposure to biomarkers of effect and susceptibility to understand the public-health implications. Biomarkers of effect are defined as measurable biochemical, physiologic, behavioral, or other alteration in an organism that, depending on the magnitude, can be recognized as associated with an established or possible health impairment or disease.

Objective: Deliverable 14.2 aimed to carry out a wide literature survey in order to create an **inventory of available biomarkers of effects** for the first set of prioritized chemical families: Bisphenols, Phthalates, Polycyclic Aromatic Hydrocarbons (PAHs), Perfluorinated compounds (PFAS, PFOAs), Organophosphate (OPFR) and Brominated (BFR) Flame retardants, and Cadmium (Cd).

Methods: Different research teams participating in WP14 covered each of the prioritized chemical family based on their previous expertise. Comprehensive literature searches with defined search terms for both the exposure of interest and the selected health endpoints were conducted in the PubMed/MEDLINE database. Given the enormous amount of information collected, effect biomarkers used in epidemiological settings were prioritized following the criteria previously established in D14.1.

Results: An inventory of effect biomarkers for the first set of prioritized substances has been created based on the information gathered from the literature searches. They have been classified in four levels of biological complexity (biochemical/physiological, *in vitro*/*ex vivo*, omics, and anthropometrics biomarkers) and in two groups according to their use (long-established “traditional biomarkers” *versus* less studied “novel biomarkers”). As an example, commonly used biochemical parameters such as steroid hormones and serum lipids would fit the category of “traditional” biomarkers, while other biomarkers such as epigenetic marks or understudied biomarkers such as the brain-derived neurotrophic factor (BDNF) would fit the category of “novel biomarkers”.

Conclusions: Although not all the biomarkers found show good applicability to the easily accessible bio-speciments normally collected in HBM studies, a preliminary proposal of effect biomarkers that could be of interest has been included in this deliverable. However, it should be noted that it only provides an initial orientation. A thorough process is needed to go from this initial list or inventory of effect biomarkers, to the final selection of the effect biomarkers that will be implemented in the HBM4EU aligned studies. D14.3, “Report on available biomarkers of effect of utility in human epidemiological studies for the first set of prioritized substances”, will continue the selection process, narrowing down the number of possible effect biomarkers and also taking into account the critical period of development in which exposure is assessed in the HBM4EU aligned studies (children, adolescents and/or adults), among other variables.

3 List of abbreviations

2-MeO-E1: 2-metoxi-estrone

2-MeO-E2: 2-metoxi-estradiol

4-MeO-E1: 4-metoxi-estrone

4-MeO-E2: 4-metoxi-estradiol

8-OHdG: 8-oxo-7,8-dihydro-2'-deoxyguanosine

11β-HSD2: 11β-hydroxysteroid dehydrogenase

ACTH: adrenocorticotropic hormone

AGD: anogenital distance

AhR: aryl hydrocarbon receptor

ALP: alkaline phosphatase

ALT: alanine transaminase

AOPs: adverse outcome pathways

AR: androgen receptor

AST: aspartate transaminase

B2MG: beta-2 microglobulin

BASC-2: Behavior Assessment System for Children

BC: breast cancer

BF: body fat

BFRs: brominated flame retardants

BDNF: brain-derived neurotrophic factor

BL: birth length

BMI: body max index

BPA: bisphenol A

BPF: bisphenol F

BPS: bisphenol S

BRIEF-P: Behavior Rating Inventory of Executive Function-Preschool

BW: birth weight

Cd: cadmium

CAS: Chemical Abstracts Service

CBCL: Child Behavior Check-List

CBMN: cytokinesis-block micronucleus

CC16: club cell secretory protein 16

CD4: cluster of differentiation 4

CHD: coronary heart disease

CpG: cytosine-phosphate-guanine nucleotides

CRC: colorectal cancer

CRH: corticotropin releasing hormone

CRP: c-reactive protein

Cys: cystatin c

CYP19: aromatase

DHEA-S: dehydroepiandrosterone-sulfate

E2: estradiol

ECHA: European Chemicals Agency

EDCs: Endocrine Disrupting Chemicals

ELISA: enzyme-linked immunosorbent assay

EPA: Environmental Protection Agency

ER: estrogen receptor

FBG: fasting blood glucose

FSH: follicle-stimulating hormone

GC-MS: gas chromatography-mass spectrometry

GGT: gamma-glutamyl transferase

GSTM1: glutathione S-transferase M1 enzyme

HbAC1: glycated hemoglobin

HBM: human biomonitoring

HC: head circumference

HDL: high-density lipoprotein

HOMA-IR: homeostatic model assessment for insulin resistance

HPA: hypothalamic-pituitary-adrenocortical

HPLC-EC: high-performance liquid chromatography electrochemical detection

hsCRP: high sensitivity c-reactive protein

HSP: heat shock proteins

IARC: International Agency for Research in Cancer

IGF: insulin growth factor

IL-6: interleukin 6

IL-10: interleukin 10

Ig-E: immunoglobulin E

INSL3: insulin-like factor 3

kDa: kilo Daltons

KE: Key Event

Kim-1: kidney injury molecule-1

LBW: low birth weight

LDL: low density lipoprotein

LH: luteinizing hormone

LINE: long interspersed nuclear elements

MCF-7: Michigan Cancer Foundation-7

MDA: malondialdehyde

MIE: Molecular Iniciating Event

MN: micro nuclei

MOA: Mechanism of Action

mRNA: messenger ribonucleic acid

MT: metallothionein

NAG: N-acetyl-β-D glucosaminidase

NHANES: National Health and Nutrition Examination Survey

OPFRs: organophosphate flame retardants

PAHs: polycyclic aromatic hydrocarbons

PBA: polyclonal B cell activation

PBMCs: peripheral blood mononuclear cells

PFAS: perfluorinated alkylated substances

PFOA: perfluorooctanoic acid

PPARγ: peroxisome proliferator-activated receptor gamma

PSA: prostate-specific antigen

PT: prothrombin time

RBCs: red blood cells

RBP: retinol binding protein

SFLT1: soluble fms-like tyrosine kinase-1

SDQ: Strengths and Difficulties Questionnaire

Sp4: specific factor 4

SHBG: sex hormone binding-globulin

T3: triiodothyronine

T4: thyroxine

TC: total cholesterol

TG: triglycerides

TPO: thyroperoxidase

TSH: thyrosine stimulating hormone

TSLP: thymic stromal lymphopoietin

TT: total testosterone

TTR: transthyretin

WISC: Wechsler Intelligence Scale for Children

4 Introduction

Biological markers, or biomarkers, reflect molecular and cellular alterations that occur along the temporal and mechanistic pathways connecting exposure to toxic chemicals or physical agents and the presence or risk of clinical disease. Biomarkers of effect are defined as measurable biochemical, physiologic, behavioral, or other alteration in an organism that, depending on the magnitude, can be recognized as associated with an established or possible health impairment or disease (Human Biomonitoring for Environmental Chemicals, 2006). Although effect biomarkers are increasingly used for risk assessment purposes, to our knowledge no previous comprehensive reviews of effect biomarkers in the field of environmental research have been previously conducted.

The specificity of effect biomarkers in relation to chemical exposures has been the topic of several previous efforts, such as the US National Academy of Sciences (Human Biomonitoring for Environmental Chemicals, 2006) or the German Research Foundation (Biological Biomonitoring: Prospects and Environmental Medicine, 2002). Those programs pointed out several challenges: a) the difficulties in systematizing the literature search on effect biomarkers because of their generally non-specific nature; b) the considerable work load due to the numerous references to be covered.

One of the objetives of WP14 (“Effect biomarkers”) was to carry out a wide literature survey on existing biomarkers of effects for the first set of prioritized substances covered in the first two years of the HBM4EU Project [AWP17]: Bisphenols, Phthalates, Polycyclic Aromatic Hydrocarbons (PAHs), Perfluorinated compounds (PFAS, PFOAs), Organophosphate (OPFRs) and Brominated (BFRs) Flame retardants, and Cadmium (Cd). Therefore, based on the previously defined **criteria** for the prioritization of biomarkers of effect (D14.1), we aimed to carry out a wide literature survey in order to create an **inventory of available biomarkers of effects** for the first set of prioritized substances. To the best of our knowledge, this is the first comprehensive review on effect biomarkers of interest for human biomonitoring related to environmental chemical exposures.

5 Methodology

The first criteria for the prioritization of biomarkers of effect, defined in D14.1, include two categories: a qualitative criteria (yes/no) and quantitative criteria (a numerical score assigned depending on the characteristics of the specific effect biomarker under study). In accordance with the qualitative criteria, the scientific articles retrieved from databases have been included or discarded. Moreover, the quantitative criteria have been considered to rank them. In order to reduce the number of articles to be reviewed, the scope of the search for biomarkers included selected health endpoints: Neurodevelopment, Reproductive diseases, Endocrine diseases, Obesity and metabolic disorders, Cardio-vascular diseases, Allergies and immunological diseases, and Cancer.

Following D14.1, priority has been given to; i) Biomarkers implemented in epidemiologic studies over those that had not been validated in human populations, ii) Biomarkers of effect related to mechanisms of action (MOA) and/or Key Events (KE) in Adverse Outcome Pathways (AOPs; iii) The cost-benefit ratio in relation to the efficacy/innovative potential of the biomarker under study;

iv) Biomarkers of effect applied in both clinical and epidemiological settings, as well as those applied in human matrices not invasively, and v) Specificity, sensitivity, and reliability of the biological changes in relation to the chemical exposure and interpretation of the biological change.

Finally, the selected effect biomarkers have been categorized according to their biological level of complexity: i) Omics, ii) Biochemical / physiological biomarkers, iii) In vitro/ex vivo cell-based biomarkers, and iv) Anthropometric biomarkers.

5.1 Search Terms, Filters and Division of work among WP14 partners

5.1.1 Terms used for the exposure.

Several search terms for each chemical family were selected in order to retrieve as much information as possible. To gain specificity in the search, some synonyms or MeSH terms were used, depending on the chemical family. As an example, in the literature search for bisphenols, we first introduced the term ‘’bisphenol’’, resulting in 11000 references, many more than obtained using the MeSH terms related to ‘’bisphenol’, such as ‘bisphenol A [Supplementary Concept]’, ‘bisphenol A disulfate [Supplementary Concept]’, ‘bisphenol A glucuronide [Supplementary Concept]’, ‘bisphenol A 3,4-quinone [Supplementary Concept]’, ‘2,4'-bisphenol F [Supplementary Concept]’, or ‘2,2'-bisphenol F [Supplementary Concept]’. In PubMed, "bisphenol" alone produced 11446 references, bisphenol A produced 11207, bisphenol S 174, bisphenol F 216, and bisphenol AF 84. When bisphenol A + bisphenol S + bisphenol F + bisphenol AF were entered together, they produced 11274 references. No relevant differences were found between the references obtained using ‘Bisphenol’ or the MeSH terms related to ‘’bisphenol’. For this reason, "bisphenol" alone was finally used.

5.1.2 Terms used for the effect: Selected health-endpoints.

We use a selection of the health endpoints of highest concern for exposure to environmental chemicals. The final number of references was larger with this health-endpoint model than with the previous biomarker-oriented-search, avoiding the possibility of missing information.

Six groups of health outcomes/endpoints were selected:

- Behavioral/Neurobehavioral disorders;
- Cancer,
- Endocrine system disorders,
- Immune system disorders and Allergies;
- Obesity, Metabolic and Cardiovascular diseases; and
- Reproductive disorders.

MeSH terms were also used in order to gain precision and reduce the scope of the search, especially for those endpoints with too many references (e.g., endocrine system or reproductive system). The search sequence was as follows: First, a MeSH terms was selected from the MeSH specific option provided by PubMed data-base; second, all MeSH terms were joined together (through the selection of "OR" instead of "AND" in order not to lose any MeSH term), and finally, "chemical family" AND "all selected MeSH terms" were included in the search entry. Because some important references were not retrieved using the MeSh search strategy alone, it was proposed to use further search terms for each health endpoint. For instance, besides the specific MeSH term for Reproduction, we used other synonyms/related terms (e.g., puberty, pregnancy, etc.).

5.1.3 Literature Filters

In order to reduce the number of references and to focus on the most relevant ones, PubMed filters were used. The selected filters were: i) full text articles, ii) within the last decade (no more than 10 years). All of the articles retrieved were divided into two groups: human studies (e.g., epidemiologic studies such as NHANES, CHAMACOS, MIREC, etc.), and animal studies (experimental).

5.1.4 Division of references among partners based on their expertise/field of knowledge

It was decided, as the best approach, to divide the chemical families among WP14 partners according to their area of expertise (Table 1.0). For example, within the bisphenol group, INSERM focused on effect biomarkers related to Omics, while UGR on those related to ex-vivo/in vitro studies and biochemical, physiological, and anthropometrical biomarkers.

Table 1. Division of chemical families among WP14 partners

Chemicals Family	Partner	Responsible
Phthalates; DINCH	VITO*	Nathalie Lambrechts*
	UCY	Christiana Neophytou Andreas Constantinou
Poly/Perfluorinated compounds	AU*	Eva Ceciline Bonefeld-Jorgensen*; Christian B.Olesen
	DTU	Anne Marie Vinggaard; Hanna Johansson
	RIVM	Aldert Piersma Stella Fragki
Brominated and Organophosphate Flame Retardants	MU*	Lola Bajard* Ludek Blaha
	EASP*	Marina Lacasaña* Antonio Hernández
Bisphenols A, F, S	CNRS	Jean-Baptiste Fini Stephan Couderq
	INSERM	Arthur David; Cynthia Shereen
	NIPH	Hubert Dirven Tim Hofer Inger-lise Karin Steffensen
	UGR*	Vicente Mustieles* Andrea Rodríguez Mariana F. Fernández Nicolás Olea
Cadmium and Chromium (VI)	MUW	Claudia Gundacker
	BfR*	Axel Oberem* Alfonso Lampen
	UH	Tim Nawrot
8 Carcinogenic PAHs in REACH 16USEPA priority PAHs	UCY	Christiana Neophytou Andreas Constantinou
	NRCWE*	Anne Thoustrup Saber*
	BfR	Axel Oberem Alfonso Lampen

* Coordinator for each chemical family

AU: Aarhus University (Denmark); BfR: German Federal Institute for risk assessment; CNRS: National Center for Scientific Research; DTU: Denmark Technical University; EASP: Andalusian School of Public Health; INSERM: French National Institute of Health and Medical Research; MU: Masaryk University; MUW: Medical University of Vienna; NIPH: Norwegian Institute of Public Health; NRCWE: National Research Centre for the Working Environment; RIVM: UCY: University of Cyprus; UGR: University of Granada; UH: University of Hasselt; VITO: Flemish institute for technological research

6 Results and Discussion

6.1 Classification and selection of potential effect biomarkers by chemical families

Once the information from the literature search was collected, all the chemical groups taking part in Task 14.1 discussed and revised the approach for the selection of the effect biomarkers with higher scores at the Granada Workshop (see AD14.2).

6.2 Bisphenols (BPA, BPF, BPS) (Coordinated by UGR)

6.2.1 Search terms used for Bisphenol chemical family.

Table 2. List of MeSH and No-MeSH terms for the health endpoints used in the literature search.

Health Endpoint	Bisphenol AND (MeSH Terms OR synonym)
Behavior/ Neurobehavior	(Bisphenol) AND ((((((((((("Behavior"[Mesh]) OR ("Behavior and Behavior Mechanisms"[Mesh] OR "Reproductive Behavior"[Mesh])) OR "Social Behavior Disorders"[Mesh]) OR ("Child Behavior Disorders"[Mesh] OR "Adolescent Behavior"[Mesh])) OR "Antisocial Personality Disorder"[Mesh]) OR ("Infant Behavior"[Mesh] OR "Spatial Behavior"[Mesh])) OR "Sucking Behavior"[Mesh]) OR ("Sexual Behavior, Animal"[Mesh] OR "Sexual Behavior"[Mesh])) OR ("Paternal Behavior"[Mesh] OR "Maternal Behavior"[Mesh] OR "Impulsive Behavior"[Mesh] OR "Feeding Behavior"[Mesh] OR "Exploratory Behavior"[Mesh])) OR ("Compulsive Behavior"[Mesh] OR "Child Behavior"[Mesh] OR "Behavior, Animal"[Mesh])) OR "Mental Disorders"[Mesh])) OR (Behavior OR Neurobehavior OR Neurodevelopment OR Neurology OR Parkinson OR Alzheimer OR Autism OR Hyperactivity OR ASD OR ADHD OR mental retardation OR IQ loss OR internalizing OR externalizing))
Cancer	(Bisphenol) AND (((((("Neoplasms"[Mesh] OR "Uterine Cervical Neoplasms"[Mesh] OR "Urologic Neoplasms"[Mesh] OR "Liver Neoplasms"[Mesh] OR "Hereditary Breast and Ovarian Cancer Syndrome"[Mesh] OR "Early Detection of Cancer"[Mesh]) OR ("Urogenital Neoplasms"[Mesh] OR "Testicular Neoplasms"[Mesh] OR "Endometrial Neoplasms"[Mesh] OR "Vaginal Neoplasms"[Mesh] OR "Uterine Neoplasms"[Mesh])) OR ("Prostatic Neoplasms"[Mesh] OR "Ovarian Neoplasms"[Mesh] OR "Endocrine Gland Neoplasms"[Mesh])) OR ("Breast Neoplasms"[Mesh] OR "Neoplasms, Germ Cell and Embryonal"[Mesh] OR "Tumor Microenvironment"[Mesh])) OR ("Thyroid Neoplasms"[Mesh] OR "Pituitary Neoplasms"[Mesh] OR "Brain Neoplasms"[Mesh]))) OR (Cancer OR hormone-dependent cancer OR neoplasm OR malignant tumor OR tumor OR tumour) OR (Colon neoplasms)))

Endocrine	(Bisphenol) AND ("Endocrine System"[Mesh] OR "Endocrine Glands"[Mesh] OR "Endocrine System Diseases"[Mesh] OR "Hormones"[Mesh] OR "Gonadal Hormones"[Mesh] OR "Placental Hormones"[Mesh] OR "Pituitary Hormones"[Mesh] OR "Growth Hormone"[Mesh] OR "Thyroid Hormones"[Mesh] OR "Gastrointestinal Hormones"[Mesh] OR "Sex Hormone-Binding Globulin"[Mesh] OR "Adrenocorticotropic Hormone"[Mesh] OR "Adrenal Cortex Hormones"[Mesh] OR Endocrine system OR hypothyroidism OR hyperthyroidism OR adrenal)
Immune System AND Allergy	(bisphenol) AND ("Allergy and Immunology"[Mesh] OR "Hypersensitivity"[Mesh] OR "Rhinitis, Allergic, Seasonal"[Mesh] OR "Food Hypersensitivity"[Mesh] OR "Drug Hypersensitivity"[Mesh] OR "Shellfish Hypersensitivity"[Mesh] OR Allergy OR Hypersensitive OR respiratory allergy OR gastrointestinal allergy OR multiple chemical sensitivity OR allergic hypersensitivity disease OR contact allergy OR "Immune System"[Mesh] OR "Immune System Diseases"[Mesh] OR Immune system OR autoimmune disease OR cytokines OR white cells OR innate immune system OR adaptive immune system)
Obesity, Metabolic AND Cardiovascular	(Bisphenol) AND ("Metabolic Syndrome"[Mesh] OR "Nutritional and Metabolic Diseases"[Mesh] OR "Metabolic Diseases"[Mesh] OR "Metabolism"[Mesh] OR "Glucose Metabolism Disorders"[Mesh] OR "Acidosis"[Mesh] OR "Metabolome"[Mesh] OR "Metabolomics"[Mesh] OR "Receptor, Insulin"[Mesh] OR "Lipolysis"[Mesh] OR "Gout"[Mesh] OR "Diabetes Mellitus, Type 2"[Mesh] OR "Acidosis, Renal Tubular"[Mesh] OR "Homocysteinemia" [Supplementary Concept] OR "Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha"[Mesh] OR "Obesity"[Mesh] OR "Pediatric Obesity"[Mesh] OR "Obesity, Abdominal"[Mesh] OR "Abdominal obesity metabolic syndrome" [Supplementary Concept] OR "Cardiovascular System"[Mesh] OR "Cardiovascular Abnormalities"[Mesh] OR "Pregnancy Complications, Cardiovascular"[Mesh] OR "Cardiovascular Diseases"[Mesh] OR "Myocardial Infarction"[Mesh] OR obesity OR abdominal obesity OR waist hip ratio OR adipose tissue OR adipokine OR visceral fat OR body fat OR overweight OR Metabolic OR Metabolic disorder OR Metabolic syndrome OR glucose homeostasis OR Hyperlipidemia OR Dyslipidemia OR hypertriglyceridemia OR HOMA-IR OR insulin resistance OR pancreas OR liver OR kidney)
Reproductive	(Bisphenol) AND (reproductive OR puberty OR pregnancy OR infertility OR semen quality OR placenta OR anogenital distance OR hypospadias OR cryptorchidism OR "Reproductive Health"[Mesh] OR "Reproductive Medicine"[Mesh] OR "Reproduction"[Mesh] OR "Reproductive Techniques, Assisted"[Mesh] OR "Infertility"[Mesh])

6.2.2 Exploratory Search

The final exploratory search used in the selection of potential references is summarized below, including the final number of articles reviewed after applying the eligibility criteria (full text and published in the last 10 years).

Figure 1. Number of articles gathered in the exploratory search for bisphenol chemical family group

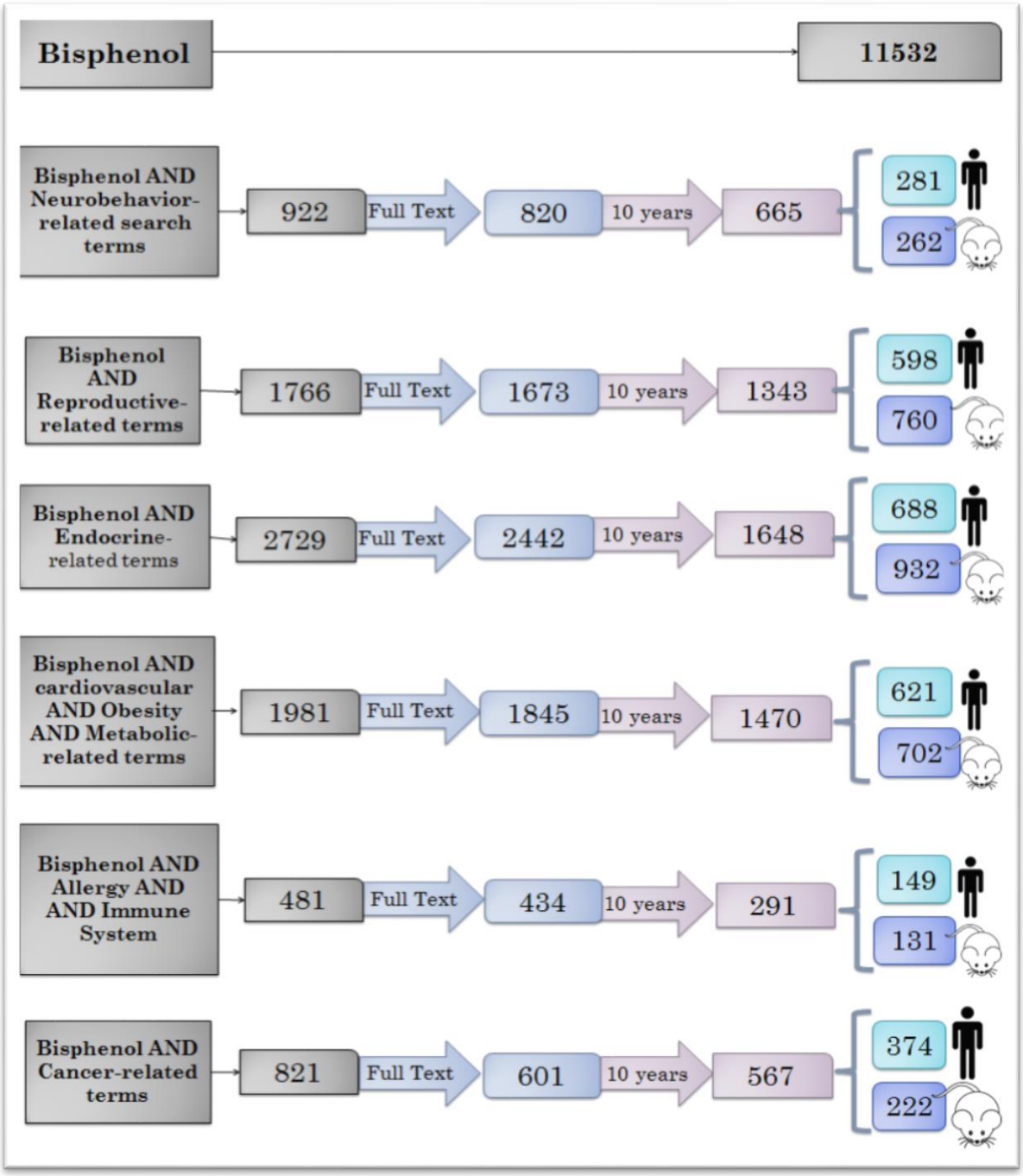


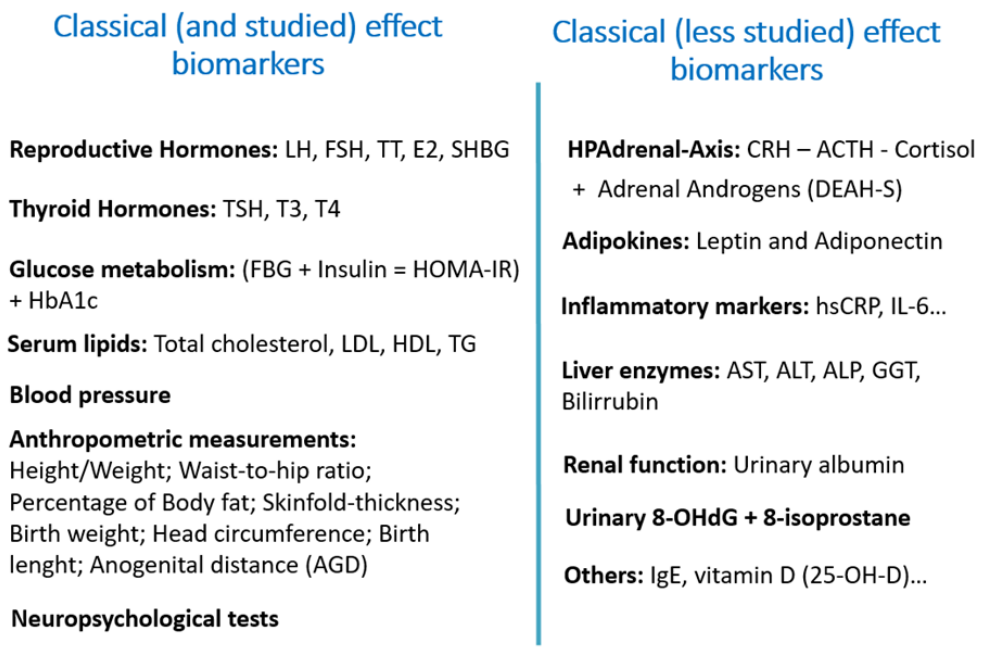
Table 3: Distribution of selected references among WP14 partners in the bisphenols group

	HBM4EU. WP14-Bisphenols Final number of references per partner									
	GENERAL		UGR		INSERM		CNRS		NIPH	
Health Outcome	With Duplicates	**Without Duplicates**	With Duplicates	**Without Duplicates**	With Duplicates	**Without Duplicates**	With Duplicates	**Without Duplicates**	With Duplicates	**Without Duplicates**
Neurodevelopment	29	29	27	**24**	1	**1**	0	**0**	1	**1**
Reproductive Diseases	114	41	99	**71**	9	**3**	4	**2**	2	**0**
Endocrine Diseases	75	75	60	**6**	3	**3**	8	**8**	4	**4**
Obesity and Cardio-Metabolic Diseases	119	79	92	**86**	11	**8**	3	**1**	13	**8**
Allergy-Immunological Diseases	16	14	11	**8**	2	**2**	0	**0**	3	**3**
Cancer	22	11	14	**1**	3	**3**	1	**0**	4	**2**
Total	375	**249**	303	**196**	29	**20**	16	**11**	27	**18**

6.2.3 List of traditional effect biomarkers found for Bisphenols

As shown in Table 3, UGR analyzed 196 references regarding biochemical/physiological effect biomarkers that have been studied in relation to Bisphenols exposure. Most of these biomarkers were traditional or classic effect biomarkers. Therefore, since it was not possible to provide and comment all the references found, we have decided to provide a summary of the traditional biomarkers of effect most commonly associated with Bisphenols exposure, citing some of the most relevant references and/or previous reviews that have been conducted on the topic.

Figure 2. Traditional effect biomarkers studied in relation to bisphenols chemical family.



6.2.4 Traditional (and studied) effect biomarkers:

All the following biomarkers have been found in the literature search in relation to Bisphenols. For space reasons, only some of the most relevant articles are cited below.

6.2.4.1 Biochemical/Physiological effect biomarkers (UGR)

Reproductive Hormones [Luteinizing hormone (LH), follicle stimulating hormone (FSH), Total testosterone (TT), estradiol (E2), sex hormone binding-globulin (SHBG)]. Reproductive hormones coordinate a myriad of physiological functions, being responsible for the organization of reproductive, neurological and metabolic organs during development and the maintenance of their functions during adulthood among other processes. Each of these markers has been associated with BPA exposure, but not always in the same direction or in a dose-dependent manner (for a review, see Mínguez-Alarcón et al., 2016).

Thyroid Hormones [thyroid stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4), anti-thyroperoxidase (TPO) antibodies]. Thyroid hormones (TH) play a critical role in differentiation, growth, and metabolism and are required for the normal function of all tissues (Yen PM, 2001). Mild or transient TH insufficiency affects offspring cognitive outcome (Bernal et al., 2005). In epidemiological studies, BPA exposure has been associated with increased TSH levels in serum of adults (Wang et al., 2013; Meeker et al., 2011) and in cord blood of newborns (Romano et al., 2015; Chevrier et al., 2013), altering levels of circulating T3 (Wang et al., 2013), total T4 (Meeker et al., 2011; Chevrier et al., 2013), free T4 (Sriphrapradang et al., 2013; Aker et al., 2016), and anti-TPO antibodies (Chailurkit et al., 2016). However, further investigations are warranted to link these BPA-mediated disruptions with behavior and other health endpoints.

Glucose metabolism [Fasting blood glucose (FBG), fasting insulin levels and glycated hemoglobin (HbA1c)] and the homeostatic model assessment (HOMA), a method used to quantify insulin resistance and beta-cell function: HOMA-IR= (Fasting blood glucose (FBG) x Insulin) / 450) (Matthews et al., 1985)]. Dysregulation of glucose metabolism is a well-known risk factor for obesity and cardio-metabolic disorders, including atherosclerosis, type 2 diabetes, and high blood pressure. BPA exposure has been associated with alterations in glucose metabolism in several epidemiologic studies (Bi et al., 2016; Chiu et al., 2017).

Serum lipids [Low density lipoprotein (LDL), high density lipoprotein (HDL), total cholesterol (TC) and triglycerides (TG)], related to obesity and cardio-metabolic diseases]. Serum lipids have been tested in some human epidemiological studies designed to study the relationship between BPA exposure and cardiovascular-metabolic diseases. However, no associations have been previously found with serum lipids (Eng et al., 2013; Vafeaidi et al., 2016; Milošević et al., 2017; Perng et al., 2017).

6.2.4.2 Other physiological measures: Blood pressure

There is a close link between hypertension and atherosclerosis. The risk of mortality associated with hypertension increases with blood pressure values below the cutoff point of normality (140/90 mm Hg) and even below 130/85 mm Hg (Román et al., 2010). Although blood pressure has been

commonly studied in adults, it has been less studied in children. It is increasingly highlighted by epidemiologic studies as a sensitive marker of early developmental cardio-metabolic health that can be assessed non-invasively (Balat et al., 2014). BPA has been positively associated with children's blood pressure (Bae et al., 2017; Khalil et al., 2014)

6.2.4.3 Anthropometric markers

Birth Weight, Birth Length and Head circumference (Huo et al., 2015; Troisi et al., 2014).

Body Mass Index (BMI), BMI z-scores (for children), waist circumference, hip circumference, waist-to-hip ratio. Skinfold thickness (bicipital, tricipital, subscapular, etc.), for a review see Ranciére et al. (2015).

Body fat mass (Hoepner et al., 2016).

Anogenital Distance (AGD): In rodents, the anogenital distance (AGD), measured from the anus to the base of genital tubercle, is a sensitive biomarker of androgen exposure during a critical embryonic window of testis development. In humans, several epidemiological studies have shown alterations in AGD associated with prenatal exposure to several chemicals with potential endocrine disrupting properties (Thankamony et al., 2016). Assessment of AGD in newborns has been suggested to determine male feminization and thereby predict neonatal and adult reproductive disorders in a non-invasive way. Bisphenol A has been associated with AGD in some (Barrett et al., 2017; Miao et al., 2011) but not all (Liu et al., 2016) epidemiological studies.

6.2.4.4 Tests of neuropsychological function as neurologic "effect biomarkers"

Neurobehavioral tests are objective, reliable, and valid measures of nervous system function, used in epidemiologic studies to reflect physiologic changes or structural alterations in the nervous system produced by environmental chemical exposure. While neurobehavioral tests have not usually been considered classic biomarkers, they assess the function of neuropsychological abilities that cannot otherwise be measured with non-invasive, non-destructive tests of the human nervous system (Travis, 1992). Therefore, they could be regarded as neurologic effect biomarkers or surrogates of neurologic function. The most widely used neuropsychological tests in epidemiologic studies associated with BPA have been:

CBCL: Child Behavior Check-List (6-18 years).

SDQ: Strengths and Difficulties Questionnaire

BASC-2: Behavior Assessment System for Children

BRIEF-P: Behavior Rating Inventory of Executive Function-Preschool

WISC: Wechsler Intelligence Scale for Children

For an extended review, please see Mustieles et al. (2015) and (2018).

6.2.5 Traditional (but less studied) effect biomarkers

All the following biomarkers have been found in the literature search in relation to Bisphenols. For space reasons, only some of the relevant articles are cited below.

6.2.5.1 Hypothalamic-Pituitary-Adrenal (HPA) axis [Corticotropin releasing hormone (CRH) – Adrenocorticotropin hormone (ACTH) – Cortisol]

Exposure to toxic chemicals found in our environment, including pesticides, metals, and industrial compounds, have been shown to have significant impact on neurological health and disease. Indeed, studies have begun to identify the Hypothalamic-Pituitary-Adrenal (HPA) axis and the limbic system as potential targets of many of these environmental chemicals, suggesting a possible environmental risk of damage to the stress circuit and the response to stressful stimuli (Caudle, 2016). Moreover, there is increasing experimental (Medwid et al., 2018; Lan et al., 2015; Martinez-Arguelles et al., 2015) and epidemiologic evidence (Mustieles et al., 2018; Kim et al., 2018; Watkins et al., 2017) about the potential of non-persistent endocrine disrupting chemicals (EDCs) such as BPA and phthalates to impact the function of the adrenal glands.

6.2.5.2 Adipokines [Leptin and Adiponectin]

Adipose tissue is known to express and secrete a variety of products known as 'adipokines', including leptin, adiponectin, resistin and visfatin, as well as cytokines and chemokines such as tumor necrosis factor-alpha, interleukin-6 and monocyte chemoattractant protein-1. The release of adipokines by either adipocytes or adipose tissue-infiltrated macrophages leads to a chronic subinflammatory state that could play a central role in the development of insulin resistance and type 2 diabetes and in the increased risk of cardiovascular disease associated with obesity, as has been shown by Antuna-Puente et al., 2008. Inverse associations between BPA exposure and adiponectin, resistin, leptin and ghrelin levels have been reported in epidemiologic studies (Menale et al., 2017; Ashley-Martin et al., 2014; Rönn et al., 2014).

6.2.5.3 Liver enzymes

The liver has a significant role in metabolism, regulation of red blood cells (RBCs) and glucose synthesis and storage. Typical markers of liver function include alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), serum bilirubin, prothrombin time (PT), the international normalized ratio (INR), and albumin. These biomarkers can help to determine an area of the liver with possible damage and can assist the differential diagnosis based on the findings. For instance, a disproportionate increase in ALT and AST in relation to alkaline phosphatase and bilirubin denotes hepatocellular disease, whereas a disproportionate increase in alkaline phosphatase and bilirubin in relation to ALT and AST denotes cholestasis. Liver function can be graded according to its ability to produce albumin and vitamin K-dependent clotting factors according to Lala et al., 2018. BPA exposure has been associated in epidemiologic studies with levels of AST, ALT, GGT, and ALP (Lee et al., 2014; Melzer et al., 2010).

6.2.5.4 Renal Function

The kidney is also an important site of injury from chemical exposure, although substantial gaps remain in knowledge of the effects of environmental chemicals on specific aspects of kidney function (Kataria et al., 2015). Recently, emerging evidence from epidemiologic studies has shown an association between higher urinary BPA concentrations and low-grade albuminuria in both adults and children (Li et al., 2012; Trasande et al., 2013; Kataria et al., 2017). The relationship of

high serum BPA levels with increased oxidative stress and inflammatory markers has been reported in patients undergoing hemodialysis with BPA-containing polysulfone dialyzers. These findings underscore the possibility that exposure to BPA during everyday life could have adverse effects on the kidney and might contribute to progressive cumulative renal injury over a lifetime. However, it remains to be determined whether these effects are indeed caused by BPA (Kobroob et al., 2018). BPA exposure has also been associated with higher levels of albuminuria, glomerular filtration rate and blood pressure, all biomarkers of cardiorenal disease (Kataria et al., 2015).

6.2.5.5 Urinary oxidative stress biomarkers of DNA and lipid damage

Oxidatively induced damage affects lipids of cellular membranes, proteins, and DNA. 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo or 8-OHdG) is one of the predominant forms of free radical-induced lesion. Its excretion has been widely used as a biomarker for oxidative stress, and studies have shown that urinary 8-OHdG is increased by certain exposures (e.g., smoking and irradiation) and conditions/diseases (e.g., inflammation). The most widely used methods for 8-OHdG quantification are high-performance liquid chromatography with electrochemical detection (HPLC-EC), gas chromatography-mass spectrometry (GC-MS), HPLC tandem mass spectrometry (LC-MS/MS), and enzyme-linked immunosorbent assay (ELISA). Urinary 8-OHdG has been used to estimate DNA damage in humans after exposure to cancer-causing agents, including as tobacco smoke, asbestos fibers, heavy metals, and polycyclic aromatic hydrocarbons (Valavanidis et al., 2009). Oxidants can also initiate lipid peroxidation, an oxygen (O2)-driven process generating oxidized lipids and various reactive aldehydes (e.g., malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE)) that can react with cellular molecules, including proteins, and form adducts. As urinary biomarkers of lipid peroxidation, 8-iso-prostaglandin F2α (8-isoPF2α or 8-isoprostane [often analyzed using GC-MS; derived from arachidonic acid (Ω-6]) and MDA are commonly measured, however, analysis of MDA generally relies on reaction with 2-thiobarbituric acid (TBA) under heating (30-60 min; 70-100°C), in which artefactual MDA may form. Therefore, the use of MDA as a biomarker has been questioned. TBA may also react with other aldehydes, so that TBA-MDA adducts should be analyzed using a separation method such as HPLC-UV. Urinary oxidative stress biomarkers should preferably be, normalized to the urinary creatinine concentration, because the concentrations are affected by water intake. Although these markers have been used for decades, however, its use in relation to non-persistent chemicals is much more recent and novel. Interestingly, several epidemiologic studies have found positive associations between BPA concentrations and urinary levels of 8-OHdG and 8-isoprostane (a biomarker of lipid peroxidation), among other oxidative stress markers (Asimakopoulos et al., 2016; Ferguson et al., 2016). Although they are not specific, these biomarkers have been widely used in recent years, not only for the measurement of endogenous oxidative DNA damage but also as a risk factor for many diseases, including cancer, and for mediation analyses in environmental health research (Ferguson et al., 2017; Wang et al., 2017).

6.2.5.6 Immune system parameters and inflammatory cytokines

Emerging data suggest that BPA can alter the normal functioning of the immune system, increasing the risk of diseases such as allergy and asthma. Additionally, experiments have suggested a role for BPA in relation to pro-inflammatory cytokines. Scant epidemiologic data are available on this topic, but BPA exposure has been positively associated with IgE levels and the

risk of allergic diseases (Wang et al., 2016) and also with systemic pro-inflammatory markers (CRP, IL-6, IL-10...) [Song et al., 2017].

6.2.5.7 Nutritional biomarkers (Vitamin D)

Vitamin D is a prohormone that plays an integral role in the regulation of bone metabolism and of calcium and phosphorous absorption. BPA exposure has been associated with decreased vitamin D levels in both adults and pregnant women (Johns et al., 2016 and 2017). Although mechanistic studies are lacking, it is plausible that BPA and other non-persistent EDCs may directly and/or indirectly influence the vitamin D endocrine system at multiple points along its axis. Future research is required to determine the public health impact of subclinical changes in circulating 25(OH) D in response to environmental chemicals across diverse populations (Johns et al., 2017). The modulating role of nutritional status on the effect of environmental exposures constitutes a new research area that could improve our current understanding of exposure-health relationships.

6.2.6 List of novel effect biomarkers

Figure 3. Novel effect biomarkers obtained through the bisphenols literature search

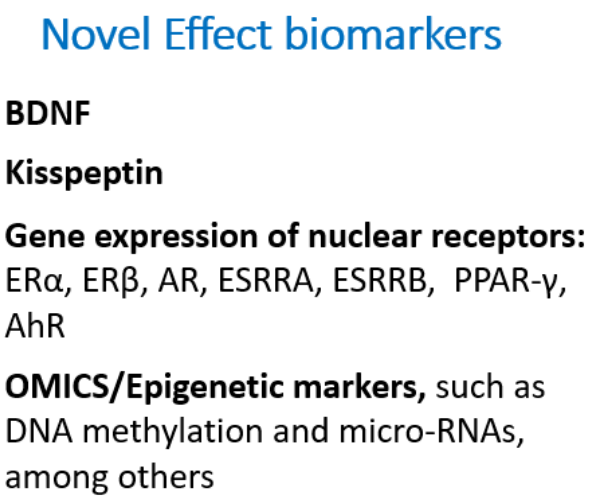


Table 4. List of novel effect biomarkers found in the literature survey on bisphenol chemical substances

Novel effect biomarker	Brief description of the effect biomarker	Scoring table procedure (p: points)	Score
Kisspeptin	Kisspeptin, a hypothalamic peptide encoded by the KiSS1 gene, is a novel neuromodulator that acts upstream of GnRH and is sensitive to sex steroid feedback and metabolic cues. Kisspeptin is recognized as a crucial regulator of the onset of puberty, the regulation of sex hormone-mediated secretion of gonadotropins, and the control of fertility and pregnancy outcomes (Skorupskaite et al., 2014). In a case-control study of 28 girls diagnosed with precocious central puberty compared to 22 control girls, serum kisspeptin levels were significantly different. However, no associations between urinary BPA concentrations and kisspeptin levels were detected. Nevertheless, given the low sample size, more studies are needed to confirm these findings (Özgen et al., 2016).	This effect biomarker has been assessed in **serum (3p)**. There is a plausible mechanism of action between BPA and kisspeptin systems **(2p).** We could not find an AOP for kisspeptin **(0p)**. It has been implemented in one case-control study **(5p)**. The feasibility for HBM was considered high **(5p)**.	15 points
Urinary Hydroxy estrogens: **2-MeO-E1; 2-MeO-E2; 4-MeO-E1; 4-MeO-E2.**	BPA has been shown to alter estrogen metabolism and hormonal homeostasis in both experimental and epidemiologic studies. These 4 urinary metabolites are produced during the metabolism of endogenous estrogens (mainly differentiated by the number of the carbons in which the oxidative reaction is produced), which are: 2-methoxyestrogen, 4-methoxyestrogen, 2-methoxyestrone and 4-methoxyestrone. An increased level of some hydroxyestrogens, especially 4-hydroxyestrogens, in relation to 2-hydroxyestrogens may be a risk factor for breast cancer and other diseases. Associations were found between BPA and altered estrogen metabolism in both men and women (Kim et al., 2014).	These effect biomarkers of estrogen metabolism were assessed in **urine (5p).** There are plausible mechanisms of action by which BPA can interfere with estrogen metabolism **(2p)**. There are several AOPs described for BPA in relation to altered hormonal homeostasis but not directly to these metabolites **(0p).** It has been implemented in at least one epidemiologic study **(5p)**. The feasibility was considered “medium to high” **(3p)**.	15 points
Gene expression of nuclear receptors in peripheral blood leukocytes	BPA has been shown to consistently activate nuclear receptors, especially those implicated in estrogenic pathways, in experimental studies. Associations were found between higher urinary BPA concentrations measured in adult men and higher gene expression of ERβ and ESRRα in peripheral blood leukocytes (Melzer et al., 2011). Mean expression of ESRβ and ESRRα increased by 65% and 38%, respectively, in the highest versus lowest BPA exposure tertile (Melzer et al., 2011).	Gene expression of nuclear receptors was assessed in blood (3p). The plausibility of the findings is high (2p). There are AOPs described for BPA mediated through estrogenic pathways (3p), indeed activation of estrogen nuclear receptors can be interpreted as a molecular initiating event (MIE). Gene expression of nuclear receptors in blood peripheral cell populations has been implemented in several epidemiologic studies (5p). The feasibility was considered high (5p).	18 points

The following epidemiological studies have reported an association between alterations in DNA methylation and exposure to BPA. DNA methylation is a heritable epigenetic mark that involves the covalent transfer of methyl group to the C-5 position of the cytosine to form 5-methylcytosine. More than 98% of DNA methylation occurs in the CpG (cytosine-phosphate-guanine) islands that have promoters associated with them, and this can consequentially affect gene transcription.			
Blood BDNF DNA methylation	Brain-derived neurotropic factor (BDNF) plays an essential role in neurodevelopment (neural differentiation, survival of nerve cells, neurite outgrowth, synaptic plasticity) and has been linked to early-life adversity and psychiatric risk. The Columbia Center for Children's Environmental Health (CCCEH) prospective cohort study showed an association between maternal BPA levels and altered blood BDNF CpG1B methylation in children. Behavioral analyses of the children in this cohort showed that high maternal BPA levels were associated with sex-specific alterations in behavioral profile, with boys exhibiting disturbed emotional regulation and aggressive behavior (Kundakovic et al., 2015). Hence, DNA methylation of BDNF in the blood may be useful to predict epigenetic changes in the brain and behavioral vulnerability induced by early-life adversity.	The biomarker was assessed in blood (3p). There is a plausible AOP between the exposure and the biomarker (3p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "high" (5p).	16 points
Strengths: a) BDNF, a key regulator or synaptic plasticity, is involved in the pathogenesis of various psychiatric disorders, such as major depression, anxiety, schizophrenia, bipolar disorder. b) A correlation between BDNF levels in brain and blood has been established. c) DNA methylation of BDNF could be a stable and reliable biomarker of psychiatric disorders. d) DNA methylation can be measured in a small amount of biological sample, regardless of the storage conditions **Weaknesses:** a) Because BDNF is associated with several psychological disorders, it may not be specific for a given disease. b) Contradictory information has been published on the precise correlation between brain and blood BDNF levels c) Harmonization of techniques for methylation analysis is needed d) Given that changes in DNA methylation caused by psychiatric disorders are subtle, high-resolution techniques are required to reliably detect differences.			
Sperm LINE-1 hypomethylation	The human genome consists of interspersed short (SINEs) and long (LINEs) nuclear elements, of which LINE-1 repeats make up roughly 17% of the genome. For these reasons, LINE-1 promoter methylation is widely used as surrogate marker to identify global levels of DNA methylation. Hypomethylation of LINE-1 elements increase their activity as retrotransposable sequences, and they may induce genomic alterations by insertions and deregulate gene transcription. An epidemiological study of 72 factory workers in China showed that LINE-1 hypomethylation in sperm correlated with increased exposure to BPA (Miao et al., 2014). Analyses of LINE-1 will help to identify global methylation levels and could serve as an important effect biomarker.	The biomarker was assessed in blood (3p) and sperm (1p). There is a plausible mechanism of action between exposure and biomarker (2p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "medium" (2p).	12 points

<table>
<tr><td colspan="4">Strengths: a) Because LINE-1 repeats represent approximately 17-18% of the human genome, the methylation status of LINE-1 and other repetitive elements could serve as a surrogate measure of global DNA methylation. b) Aberrant LINE-1 methylation can be used to detect the onset of various diseases, including cancer.

Weaknesses: Because LINEs are repeat elements, it would be difficult to map them on the genome for identification of their location.</td></tr>
<tr><td>Differential salivary DNA methylation in CpG islands of HOXA10, BRCA1, BEX2, and Xq13.</td><td>Differential salivary DNA methylation in the CpG islands of HOXA10, BRCA1, BEX2, and Xq13.</td><td>Differential salivary DNA methylation in the CpG islands of HOXA10, BRCA1, BEX2, and Xq13.</td><td>Differential salivary DNA methylation in CpG islands of HOXA10, BRCA1, BEX2, Xq13.</td></tr>
<tr><td colspan="4">Strengths: Given the influence of the environment on epigenetic regulation, hypo-or hypermethylation of the DNA would help to obtain a complete picture of the epigenetic regulation of the genes.

Weaknesses: Given the complexity and diversity of the different methods used for DNA methylation analysis, straightforward comparisons may not be possible. The choice of technique depends on the number of samples, the quality and quantity of DNA, and the desired coverage and resolution.</td></tr>
<tr><td>Blood TSP50 gene DNA methylation</td><td>Blood TSP50 gene DNA methylation</td><td>Blood TSP50 gene DNA methylation</td><td>Blood TSP50 gene DNA methylation</td></tr>
<tr><td colspan="4">Strengths/ Weaknesses: As given for the biomarker above</td></tr>
<tr><td>Placental microRNA expression (miR-146a upregulation)</td><td>MicroRNAs (miRNAs) are approximately 22 nucleotides long non-coding RNAs capable of regulating gene expression. They post-transcriptionally regulate gene expression by binding to the 3’-untranslated region of their target mRNA, attenuating protein translation. An Italian epidemiological study has shown an association between placental BPA levels and increased placental microRNA expression (miR-146a upregulation) in therapeutically aborted malformed fetuses (De Felice et al., 2015)</td><td>The biomarker was assessed in placenta (2p). There is a plausible mechanism of action between exposure and biomarker (2p). There is a plausible AOP between the exposure and the biomarker (3p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered “medium” (2p).</td><td>14 points</td></tr>
<tr><td colspan="4">Strengths: a) Circulating miRNA (in serum or plasma) can be used as non-invasive biomarkers for the diagnosis of diseases. B) miRNAs show a tissue-specific expression pattern and can be used as tissue-based biomarkers.

Weaknesses: a) RNA quality and quantity is an important factor in miRNA profiling, which can be influenced by storage conditions b) The accurate measurement of miRNAs is challenging because of their short length and the high sequence similarities between miRNA families. c) Additional challenges depend on the method used for the analysis.</td></tr>
<tr><td>Insulin-like factor 3 - INSL3</td><td>INSL3 is proving useful as a biomarker for testis status as its secretion into the testicular interstitial space and therefore the bloodstream largely reflects INSL3 gene expression and the differentiation status and absolute number of Leydig cells.

INSL3 was negatively correlated with BPA exposure in cord blood of male newborns, (Chevalier et al., 2014), supporting INSL3 as a possible target of endocrine disruption during fetal testis development.</td><td>The biomarker was assessed in blood (3p). There is no plausible mechanism of action between exposure and biomarker (0p). An AOP has been described (5p). The biomarker has been implemented in epidemiology studies in relation to BPA (5p). The feasibility was considered “high” (5p).</td><td>18 points</td></tr>
</table>

3-nitrotyrosine	3-NT is a marker of nitrosative stress. 3-NT levels have been observed in the pathogenesis of several human diseases and are regarded as a marker for Alzheimer's syndrome and Parkinson's disease. 3-NT formation in the placenta has been demonstrated in high-risk pregnancies. Nitrosative stress (3-NT) in both the mother and fetus at time of birth was associated with prenatal BPA (Veiga-López et al., 2015).	*The biomarker was assessed in **plasma (3p).** There is a plausible mechanism of action of BPA induction of ROS through the enzymatic and non-enzymatic formation of phenoxyl radicals **(2p).** No related AOP was found **(0p).** The biomarker has been implemented in epidemiology studies in relation to BPA **(5p).** The feasibility was considered "**high" (5p).***	15 points
Glial cell line-derived neurotrophic factor (GDNF**)**	GDNF has been shown to promote development, differentiation of dopaminergic & serotonergic neurons, and to protect CNS neural & glial cells against oxidative stress, and it is thought to play an important role in various neuropsychiatric disorders.	*The biomarker was assessed in **plasma or serum (3p).** There is a plausible mechanism of action via estrogenic regulation or indirectly via neurotransmitter modulation **(2p).** No related AOP was found **(0p).** The biomarker has been implemented in epidemiology studies unrelated to BPA exposure **(5p).** The feasibility was considered "**high" (5p).***	15 points
Urinary 8-OHdG	Oxidative stress biomarker resulting from nucleoside oxidation	This effect biomarker has been assessed in **urine (5p)**. There is a plausible mechanism of action between BPA and 8-OHdG **(2p).** 8-OHdG is mentioned in two AOPs **(3p)**. It has been implemented in six cross-sectional studies, one interventional study, and one prospective cohort study **(5p)**. The feasibility was considered medium **(2p)**.	17 points
Urinary 8-isoprostane	Oxidative stress biomarker resulting from lipid peroxidation of arachidonic acid (Ω-6)	This effect biomarker has been assessed in **urine (5p)**. There is a plausible mechanism of action between BPA and 8-isoprostane **(2p).** 8-isoprostane is mentioned in one AOP **(3p)**. It has been implemented in one cross-sectional study **(5p)**. The feasibility was considered medium **(2p)**.	17 points

6.2.7 Preliminary proposal of effect biomarkers that could be implemented in HBM

Selection of the effect biomarker need to be based on the requisites extensively discussed in D14.1 “Criteria for the prioritization of effect biomarkers”, including: the availability of the biological matrix, the clinical relevance, potential to predict future diseases, and its reliability and feasibility for HBM. The following proposal has only an orientative aim, and future steps will be conducted in order to reach a final list of effect biomarkers to be implemented in the HBM4EU aligned studies.

6.2.7.1 Gene expression of nuclear receptors in peripheral blood cell populations

BPA has been shown to consistently activate nuclear receptors, especially those implicated in estrogenic pathways, in experimental studies. Associations were found between higher urinary BPA concentrations measured in adult men and higher gene expression of ERβ and ESRRα in peripheral blood leukocytes, but not with ERα, ESRRβ or AR (Melzer et al., 2011). Mean expression of ESRβ and ESRRα increased by 65% and 38%, respectively, in the highest versus lowest BPA exposure tertile (Melzer et al., 2011).

Activation of estrogen nuclear receptors can be interpreted as a molecular initiating event (MIE), and thus be of relevance for AOP development and risk assessment purposes. Gene expression of nuclear receptors in blood peripheral cell populations has been successfully implemented in several epidemiologic studies, and represents a very interesting marker to test the biological plausibility of epidemiologic associations. The great strength of these biomarkers is that they can provide valuable data on possible mechanisms of action and specific pathways (estrogenic, androgenic, thyroid, etc.). Among the limitations are that the implications of altered gene expression in blood leukocytes are unknown in relation to other organs and tissues. However, ther is a lot of interest to validate these gene expression measures as surro-gate markers of effects on hormone-responsive gene expression. Undoubtely, the results from Melzer et al. (2011) suggest that BPA is bioactive in humans, and that the implementation of nuclear gene expression in peripheral blood leukocytes, together with measurements of reproductive hormones could provide a very interesting picture for the interpretation of BPA-related effects in HBM settings.

6.2.7.2 Brain-derived neurotrophic factor (BDNF) and neurotrophins.

Bisphenol A, apart from its acknowledged reproductive toxicity (Peretz et al. 2014), it is widely known by its ability to affect the brain and behavior of experimental animals (Nesan et al. 2018). Moreover, an increasing number of epidemiologic studies supports the effect of BPA on neurobehavior, as assessed by neuropsychological tests (Mustieles et al., 2015 and 2018). However, to date no biochemical effect biomarkers for neurodevelopment has been widely studied, and there is a marked need.

BDNF belongs to the neurotrophins family, and regulates neural circuit structure and synaptic plasticity in both the children and the adult brain. BDNF expression and DNA methylation are altered in several psychiatric disorders that are associated with early-life adversity, including depression, schizophrenia, bipolar disorder, and autism (Cattaneo et al., 2016). Interestingly, several studies have shown that TH can regulate BDNF expression in the brain (Koibuchi et al., 1999; Koibuchi & Chin, 2000; Sui & Li,2010), with the consequent neurodevelopmental consequences. Additionally, it has also been suggested that BDNF is associated with attention, behavior and cognitive skills in children (Yeom et al., 2016). In vivo, BPA has been associated with inhibition of BDNF in the brain (Wang et al., 2016; Patisaul et al., 2012; Castro et al., 2015). In mice, BDNF DNA methylation in the blood may be used as a predictor of BDNF DNA methylation

and gene expression in the hippocampus and of behavioral deficits (Kundakovic et al., 2015). Importantly, in human cord blood, prenatal exposure to high BPA levels was associated with methylation of the CpG1B site of the promoter region IV and may represent a biomarker in humans that predicts BDNF gene expression in the brain and consequent alterations in brain function and behavior (Kundakovic et al., 2015). Therefore, it seems there is sufficient experimental support, and suggestive preliminary epidemiological data to propose further research and implementation of BDNF measurements in serum and/or urine (Koven and Collins, 2014), as well as BDNF related epigenetics as novel biomarkers for neurodevelopment.

Other related neurotrophins such as glial-derived neurotrophic factor could also be of interest (GDNF). Moreover, GDNF expression is controlled by dopamine, serotonin, glutamate or adenosine, and other neurotransmitters, (Saavedra et al., 2008) and many have been shown to be modulated by BPA in vivo or in vitro. Male rats exposed to a low dose of BPA showed permanently decreased transcript levels of Gdnf in the prefrontal cortex along with altered dopamine and serotonin systems (Castro et al., 2015).

6.2.7.3 Insulin-like factor 3 (INSL3)

INSL3 is a major secreted peptide hormone of interstitial Leydig cells of the mature testes. Unlike testosterone, its expression and secretion appear to be constitutive, show minimal diurnal or within-individual fluctuation, and are less acutely regulated by hormones of the hypothalamic-pituitary-gonadal axis. Increasingly, INSL3 is proving useful as a biomarker for testis status because its secretion into the testicular interstitial space and hence into the bloodstream largely reflects INSL3 gene expression and the differentiation status and absolute number of Leydig cells (Ivell et al., 2013). Importantly, INSL3 is required for the maturation of gubernacular cords, which leads to transabdominal descent of the testes (Howdeshell et al., 2017). INSL3 is a target of endocrine disruption, because exposure to several Environmental Endocrine Disruptors with estrogenic or anti-androgenic effects can lead to its inhibition. Indeed, a decrease in INSL3 fetal production has been observed in vitro in human explanted fetal testes cultured with low doses of BPA (TN'Tumba-Byn et al., 2012). Furthermore, INSL3 was negatively correlated with BPA exposure in cord blood of male newborns (Chevalier et al., 2014), supporting INSL3 as a possible target of endocrine disruption during fetal testis development. Regarding adults, while no studies have been published for BPA, an epidemiological study found negative associations between some urinary phthalate metabolites and serum testosterone and INSL3 levels (Chang et al., 2013). Therefore, serum INSL3 levels, together with serum reproductive hormones (TT, E2, LH, FSH and SHBG), and perhaps serum inihibin B levels (a specific marker of Sertolli cells) [Erdos et al., 2013] could provide a valuable picture in order to study developmental testicular function during development.

6.2.7.4 Kisspeptin

It is a novel and relevant effect biomarker involved in reproductive functions and diseases. It is directly implicated in the hypothalamic release of gonadotropin-releasing hormone (GnRH), and therefore able to modulate the Hypothalamic-Pituitary-Gonadal (HPG) Axis. Kisspeptin can feasibly be assessed in serum, and its importance for pregnancy outcomes and during puberty is increasingly acknowledged (Skorupskaite et al., 2014). Additionally, it has been implemented in one small case-control study, with sufficiently good reliability for its implementation in HBM programs. We also found an important study in female rhesus monkeys showing the ability of BPA

to impact kisspeptin and the hypothalamic release of GnRH (Kurian et al., 2015). Furthermore, a study conducted in 262 mother-child pairs from China demonstrated that exposure to environmental contaminants such as BPA, cadmium, and polychlorinated biphenyls could increase placental Kiss1 gene expression (Xu et al., 2015). Animal studies have shown that lack of Kiss1 receptor leads to failure to attain puberty and results in sterility (Funes et al., 2003; Seminara et al., 2003). The significance of kisspeptin in the onset of puberty was highlighted by a case report that gain-of-function mutation of Kiss1 signaling resulted in precocious puberty in an 8-year old girl (Teles at al., 2008). Kisspeptin also plays an important role in the regulation of pregnancy. Decreased kisspeptin levels in pregnant mothers have been linked to an increased likelihood of miscarriage (Sullivan-Pyke et al., 2018) and pre-eclampsia development (Ziyaraa et al., 2016). Therefore, this novel effect biomarker may be relevant for BPA and may complement information on other reproductive hormones, especially during pregnancy and puberty. Moreover, kisspeptin levels can be measured in non-invasive matrices, e.g. plasma/ blood, using relatively simple and cost-effective techniques such as ELISA, which makes it a good candidate for HBM studies.

6.2.7.5 DNA methylation of CpG islands and long nuclear interspersed elements (LINE) methylation

DNA methylation, or addition of methyl groups to the 5'-cytosine residues of DNA, is an important epigenetic marker. DNA methylation mainly occurs in regions that are rich in cytosine-phosphate-guanine nucleotides (also called CpG islands). These regions often have associated promoters that can activate or repress transcriptional activity of genes. Epigenetic markers can provide a wealth of information on the physiological or pathological status of an individual (Ho et al., 2012). An epidemiological study has shown an association between the levels of BPA in urine and genome-wide alterations in the methylation patterns of DNA isolated from saliva of pre-pubescent girls in Egypt (Kim et al., 2013). A functional validation of differentially methylated targets showed alterations in several candidate genes, most of them associated with immune function, transport activity, metabolism, and caspase activity. Specifically, HOXA 10, BRCA1, BEX2 and Xq13 were found to be hypo-methylated. Other studies have shown that global DNA methylation could also be determined by measuring methylation patterns at highly repeated DNA sequences (e.g., LINEs) and that this method could be less labor intensive (Yang et al., 2004). LINE-1 comprises approximately 17% of the human genome and is normally heavily methylated. Hypomethylation of LINE-1 increases its activity as retrotransposable sequences and can cause genome alterations. An epidemiological study conducted in Chinese factory workers showed an association between increased BPA levels and LINE-1 hypomethylation in their sperm (Miao et al., 2014). Given the role of environment in influencing the epigenetic landscape, increased or decreased DNA methylation could be used as a predictive biomarker to identify the genes in a biological pathway that are influenced by toxicant exposure. Because alterations in methylation patterns can be measured in non-invasive matrices such as sperm or saliva, this could serve as an important biomarker for HBM studies. Moreover, it could also serve as a biomarker to predict the pre-disposition of an individual to develop certain diseases. However, one of the difficulties that might be encountered is the genome mapping of differentially methylated LINEs to identify their precise location.

6.2.7.6 MicroRNA (miRNA) expression

MicroRNAs are approximately 22 nucleotide-long non-coding RNAs capable of regulating gene expression. They regulate gene expression by post-transcriptionally binding to the 3'-untranslated region of their target mRNA and consequently reducing translation. Changes in miRNA expression patterns have been observed in several diseases (Tufekci et al., 2014) and after exposure to environmental contaminants (Sollome et al., 2016). An Italian epidemiological study observed a strong association between BPA levels in placenta and upregulation of 34 miRNAs, thereby altering their target genes (De Felice et al., 2015). Notably, the microRNA mir-146a was found to be strongly upregulated in the placental tissues and to be correlated with BPA levels. Given that miRNAs are released into the biofluids and are relatively stable, they can serve as important non-invasive epigenetic effect biomarkers. However, RNA quality and quantity are very important factors in miRNA profiling, and this can be influenced by sample storage conditions. In addition, the accurate measurement of miRNAs is challenging due to their short length and high sequence similarities between miRNA families. Additional challenges and costs depend on the methods used for analysis. For example, qRT-PCR is a less expensive method in comparison to high throughput techniques such as RNA-seq.

6.2.7.7 Urinary 8-oxodG and 8-isoprostane

These species appear to be suitable (traditional but less well-studied) effect biomarkers of oxidative stress. Concentration of urinary biomarkers should be normalized to urinary creatinine concentration. Overall, studies indicate that BPA exposure is positively associated with increased levels of 8-OHdG and 8-isoprostane (and also MDA, although its use as a biomarker is questioned) (Yang et al., 2009, Kim & Hong 2017, Yi et al., 2011, Yang et al., 2014). However, the main limitation is that these biomarkers are not specific for BPA or a given tissue/condition.

6.2.8 Gaps in knowledge

Additionally we include a briefly description about some Gaps in Knowledge identified during the literature search:

- **Neurodevelopment and neurologic effect biomarkers:** An important gap in knowledge was found when reviewing references for biochemical/physiological effect biomarkers associated with BPA exposure and neurobehavioral function. While dozens of epidemiologic studies have found significant associations between BPA exposure and behavioral problems in children (Mustieles et al., 2015; 2018), no validated physiologic or biochemical effect biomarkers for neurobehavior were found during the literature search. The development and/or implementation of novel effect biomarkers for neurological function would be of uttermost importance for improving the ability to make causal inferences in epidemiologic settings and risk assessments. Based on the information obtained through the literature search on bisphenols, as well as other complementary searches, BDNF seems to be an appropriate candidate. Future efforts should investigate the validity of BDNF, including its gene expression in blood and its serum and urinary levels, as an effect biomarker for neurodevelopment and neurological function. Other neurotrophins such as GDNF and transcription factors such as Sp4 and synaptic markers such as Syn1 could also be studied in relation to neurodevelopment and degeneration.

- **Reproduction/ Metabolic disorders:** An important number of epidemiological and animal studies have identified associations between BPA and reproductive/ metabolic disorders, possibly due to the endocrine disrupting properties of the chemical. For both health endpoints, traditional biomarkers such as serum hormones, sperm parameters (for fertility) or anthropometric parameters such as the BMI or waist-hip index (for obesity) are routinely used. Although these are good indicators, there is a need to identify/develop new sensitive biomarkers that would assist biomonitoring. In this context, OMIC biomarkers have emerged as important tools that could help to reveal changes at molecular level. The literature search has revealed that genome-wide methylation/ LINE methylation/ miRNA expression patterns followed by identification of the target genes that have been altered are relevant to identify new effect epigenetic biomarkers. Epigenetic changes can be heritable, and animal studies have shown that these altered epigenetic markers could be transmitted up to three generations with a single ancestral exposure, especially when the exposure occurs during the critical developmental period of gestation. However, many mechanistic aspects in regard to the relationship of epigenetic changes with reproductive and metabolic disorders are still not fully understood. Therefore, revisiting the animal studies may help to provide valuable information and contribute to an overall view.

6.2.9 Conclusions

Not all of the biomarkers found have good applicability to the easily accessible bio-speciments collected in the HBM4EU aligned studies (urine and serum). Moreover, the validity and specificity to adverse outcomes needs to be further addressed. However, it was found that:

- **Gene expression of nuclear receptors** assessed in peripheral blood leukocytes may provide very important information about BPA mechanisms of action and support the biological plausibility of epidemiologic associations. Additionally, it could contribute to delineate molecular initiating events (MIEs).

- While dozens of epidemiologic studies have found significant associations between bisphenols (mainly BPA) exposure and behavioral problems in children (Mustieles et al., 2015; 2018), there are no current available biochemical effect biomarkers for neurodevelopment. Based on the information obtained through the literature search on bisphenols, it seems there is sufficient experimental support, and suggestive preliminary epidemiological data to propose further research and implementation of **BDNF** measurements in serum and/or urine, as well as BDNF gene expression and DNA methylation as novel biomarkers for neurodevelopment.

- **Serum insuline-like factor 3 (INSL3)**, a marker of Leydig cell function, could be used, together with other markers such as **inhibin B,** a marker of Sertolli function, as well as serum reproductive hormones (**TT, E2, FSH, LH, SHBG**) in order to provide a complete picture of testicular function during development (Bay and Andersson et al., 2011; Erdos et al., 2013).

- **DNA methylation** of specific CpG islands and long nuclear interspersed elements (LINE) methylation, as well as other epigenetic markers, could also be proposed to complete the picture regarding a specific adverse outcome.

6.2.10 References

Aker AM, Watkins DJ, Johns LE, Ferguson KK, Soldin OP, Anzalota Del Toro LV, Alshawabkeh AN, Cordero JF, Meeker JD. Phenols and parabens in relation to reproductive and thyroid hormones in pregnant women. Environ Res. 2016; 151:30-37.

Antuna-Puente B, Feve B, Fellahi S, Bastard JP. Adipokines: the missing link between insulin resistance and obesity. Diabetes Metab. 2008; 34(1):2-11.

Ashley-Martin J, Dodds L, Arbuckle TE, Ettinger AS, Shapiro GD, Fisher M, Morisset AS, Taback S, Bouchard MF, Monnier P, Dallaire R, Fraser WD. A birth cohort study to investigate the association between prenatal phthalate and bisphenol A exposures and fetal markers of metabolic dysfunction. Environ Health. 2014; 13:84.

Asimakopoulos AG, Xue J, De Carvalho BP, Iyer A, Abualnaja KO, Yaghmoor SS, Kumosani TA, Kannan K.. Urinary biomarkers of exposure to 57 xenobiotics and its association with oxidative stress in a population in Jeddah, Saudi Arabia. Environ Res 2016; 150: 573-81.

Autry AE, Monteggia LM. Brain-derived neurotrophic factor and neuropsychiatric disorders. Pharmacol Rev 2012; 64(2): 238-258.

Bae S, Lim YH, Lee YA, Shin CH, Oh SY, Hong YC. Maternal Urinary Bisphenol A Concentration During Midterm Pregnancy and Children's Blood Pressure at Age 4. Hypertension. 2017; 69(2):367-374.

Balat A. Children with chronic kidney disease and hypertension: could hypertension footprints be earlybiomarkers? Curr Hypertens Rev. 2014;10(2):86-98.

Barrett ES, Sathyanarayana S, Mbowe O, Thurston SW, Redmon JB, Nguyen RHN, Swan SH. First-Trimester Urinary Bisphenol A Concentration in Relation to Anogenital Distance, an Androgen-Sensitive Measure of Reproductive Development, in Infant Girls. Environ Health Perspect. 2017; 125(7):077008.

Bernal J. Thyroid hormones and brain development. Vitam Horm. 2005; 71:95-122. Review.

Bi Y, Wang W, Xu M, Wang T, Lu J, Xu Y, Dai M, Chen Y, Zhang D, Sun W, Ding L, Chen Y, Huang X, Lin L, Qi L, Lai S, Ning G. Diabetes Genetic Risk Score Modifies Effect of Bisphenol A Exposure on Deterioration in Glucose Metabolism. J Clin Endocrinol Metab. 2016; 101(1):143-50.

Boutillier S, Lannes B, Buée L, Delacourte A, Rouaux C, Mohr M, Bellocq JP, Sellal F, Larmet Y, Boutillier AL, Loeffler JP. Sp3 and sp4 transcription factor levels are increased in brains of patients with Alzheimer's disease. Neurodegener Dis. 2007; 4(6):413-23.

Bromer JG, Zhou Y, Taylor MB, Doherty L, Taylor HS. Bisphenol-A exposure in utero leads to epigenetic alterations in the developmental programming of uterine estrogen response. FASEB J. 2010; 24(7):2273-80.

Castegna A, Thongboonkerd V, Klein JB, Lynn B, Markesbery WR, Butterfield DA. Proteomic identification of nitrated proteins in Alzheimer's disease brain. J Neurochem. 2003; 85(6):1394-401.

Castro B, Sánchez P, Torres JM, Ortega E. Bisphenol A, bisphenol F and bisphenol S affect differently 5α-reductase expression and dopamine-serotonin systems inthe prefrontal cortex of juvenile female rats. Environ Res. 2015; 142:281-7.

Castro B, Sánchez P, Miranda MT, Torres JM, Ortega E. Identification of dopamine- and serotonin-related genes modulated by bisphenol A in the prefrontal cortex of male rats. Chemosphere. 2015; 139:235-9.

Cattaneo A, Cattane N, Begni V, Pariante CM, Riva MA. The human BDNF gene: peripheral gene expression and protein levels as biomarkers for psychiatric disorders. Transl Psychiatry. 2016; 6(11):e958.

Cavalieri EL, Rogan EG. Is bisphenol A a weak carcinogen like the natural estrogens and diethylstilbestrol? IUBMB Life. 2010; 62(10):746-51.

Centonze D, Battistini L, Maccarrone M. The endocannabinoid system in peripheral lymphocytes as a mirror of neuroinflammatory diseases. Curr Pharm Des. 2008; 14(23):2370-42. Review.

Chailurkit LO, Aekplakorn W, Ongphiphadhanakul B. The Association of Serum Bisphenol A with Thyroid Autoimmunity. Int J Environ Res Public Health. 2016; 13(11).

Chang WH, Li SS, Wu MH, Pan HA, Lee CC. Phthalates might interfere with testicular function by reducing testosterone and insulin-like factor 3 levels. Hum Reprod. 2015; 30(11):2658-70.

Chao MR, Hsu YW, Liu HH, Lin JH, Hu CW. Simultaneous Detection of 3-Nitrotyrosine and 3-Nitro-4-hydroxyphenylacetic Acid in Human Urine by Online SPE LC-MS/MS and Their Association with Oxidative and Methylated DNA Lesions. Chem Res Toxicol. 2015; 28(5):997-1006.

Chevalier N, Brucker-Davis F, Lahlou N, Coquillard P, Pugeat M, Pacini P, Panaïa-Ferrari P, Wagner-Mahler K, Fénichel P. A negative correlation between insulin-like peptide 3 and bisphenol A in human cord blood suggests an effect of endocrine disruptors on testicular descent during fetal development. Hum Reprod. 2015; 30(2):447-53.

Chevrier J, Gunier RB, Bradman A, Holland NT, Calafat AM, Eskenazi B, Harley KG. Maternal urinary bisphenol a during pregnancy and maternal and neonatal thyroid function in the CHAMACOS study. Environ Health Perspect. 2013; 121(1):138-44.

Chiu YH, Mínguez-Alarcón L, Ford JB, Keller M, Seely EW, Messerlian C, Petrozza J, Williams PL, Ye X, Calafat AM, Hauser R, James-Todd T; for EARTH Study Team. Trimester-Specific Urinary Bisphenol A Concentrations and Blood Glucose Levels Among Pregnant Women From a Fertility Clinic. J Clin Endocrinol Metab. 2017; 102(4):1350-1357.

Cifre M, Palou A, Oliver P. Cognitive impairment in metabolically-obese, normal-weight rats: identification of early biomarkers in peripheral blood mononuclear cells. Mol Neurodegener. 2018; 13(1):14.

Curtis C Travis. Use of biomarkers in assessing health and environmental impacts of chemical pollutants. 1992. Series A: Life Sciences Vol.250 p.162-167

D'Addario C, Di Francesco A, Arosio B, Gussago C, Dell'Osso B, Bari M, Galimberti D, Scarpini E, Altamura AC, Mari D, Maccarrone M. Epigenetic regulation of fatty acid amide hydrolase in Alzheimer disease. PLoS One. 2012; 7(6):e39186.

De Felice B, Manfellotto F, Palumbo A, Troisi J, Zullo F, Di Carlo C, Di Spiezio Sardo A, De Stefano N, Ferbo U, Guida M, Guida M. Genome-wide microRNA expression profiling in placentas from pregnant women exposed to BPA. BMC Med Genomics. 2015; 8:56

Ejaredar M, Lee Y, Roberts DJ, Sauve R, Dewey D. Bisphenol A exposure and children's behavior: A systematic review. J Expo Sci Environ Epidemiol. 2017; 27(2):175-183.

Eng DS, Lee JM, Gebremariam A, Meeker JD, Peterson K, Padmanabhan V. Bisphenol A and chronic disease risk factors in US children. Pediatrics. 2013; 132(3):e637-45.

Erdos Z, Pearson K, Goedken M, Menzel K, Sistare FD, Glaab WE, Saldutti LP. Inhibin B response to testicular toxicants hexachlorophene, ethane dimethane sulfonate, di-(n-butyl)-phthalate, nitrofurazone, DL-ethionine, 17-alpha ethinylestradiol, 2,5-hexanedione, or carbendazim following short-term dosing in male rats. Birth Defects Res B Dev Reprod Toxicol. 2013; 98(1):41-53.

Ferguson KK, Cantonwine DE, McElrath TF, Mukherjee B, Meeker JD. Repeated measures analysis of associations between urinary bisphenol-A concentrations and biomarkers of inflammation and oxidative stress in pregnancy. Reprod Toxicol. 2016; 66:93-98.

Ferguson KK, Chen YH, VanderWeele TJ, McElrath TF, Meeker JD, Mukherjee B. Mediation of the Relationship between Maternal Phthalate Exposure and Preterm Birth by Oxidative Stress with Repeated Measurements across Pregnancy. Environ Health Perspect. 2017; 125(3):488-494.

Funes S, Hedrick JA, Vassileva G, Markowitz L, Abbondanzo S, Golovko A, Yang S, Monsma FJ, Gustafson EL. The KiSS-1 receptor GPR54 is essential for the development of the murine reproductive system. Biochem Biophys Res Commun 2003; 312(4): 1357-1363.

Gaikwad NW, Yang L, Pruthi S, Ingle JN., Sandhu N, Rogan EG, Cavalieri EL. Urine biomarkers of risk in the molecular etiology of breast cancer. Breast Cancer: Basic and Clinical Research, 2009; 3(1): 1–8.

Gardner LA, Levin MC. Importance of Apolipoprotein A-I in Multiple Sclerosis. Front Pharmacol. 2015; 6:278.

Good PF, Hsu A, Werner P, Perl DP, Olanow CW. Protein nitration in Parkinson's disease. J Neuropathol Exp Neurol. 1998; 57(4):338-42.

Greco R, Gasperi V, Sandrini G, Bagetta G, Nappi G, Maccarrone M, Tassorelli C. Alterations of the endocannabinoid system in an animal model of migraine: evaluation in cerebral areas of rat. Cephalalgia. 2010; 30(3):296-302.

Hanna CW, Bloom MS, Robinson WP, Kim D, Parsons PJ, vom Saal FS, Taylor JA, Steuerwald AJ, Fujimoto VY. DNA methylation changes in whole blood is associated with exposure to the environmental contaminants, mercury, lead, cadmium and bisphenol A, in women undergoing ovarian stimulation for IVF. Hum Reprod. 2012; 27(5):1401-10.

Hiyama M, Choi EK, Wakitani S, Tachibana T, Khan H, Kusakabe KT, Kiso Y. Bisphenol-A (BPA) affects reproductive formation across generations in mice. JVet Med Sci. 2011; 73(9):1211-5.

Ho SM, Johnson A, Tarapore P, Janakiram V, Zhang X, Leung YK. Environmental epigenetics and its implication on disease risk and health outcomes. Ilar J 2012; 53(3-4): 289-305.

Howdeshell KL, Hotchkiss AK, Gray LE Jr. Cumulative effects of antiandrogenic chemical mixtures and their relevance to human health risk assessment. International Journal of Hygiene and Environmental Health 2016; 220(2PtA): 179-188

Huang JT, Wang L, Prabakaran S, Wengenroth M, Lockstone HE, Koethe D, Gerth CW, Gross S, Schreiber D, Lilley K, Wayland M, Oxley D, Leweke FM, Bahn S. Independent protein-profiling studies show a decrease in apolipoprotein A1 levels in schizophrenia CSF, brain and peripheral tissues. Mol Psychiatry. 2008; 13(12):1118-28.

Huang YF, Wang PW, Huang LW, Lai CH, Yang W, Wu KY, Lu CA, Chen HC, Chen ML. Prenatal Nonylphenol and Bisphenol A Exposures and Inflammation Are Determinants of Oxidative/Nitrative Stress: A Taiwanese Cohort Study. Environ Sci Technol. 2017; 51(11):6422-6429.

Huo W, Xia W, Wan Y, Zhang B, Zhou A, Zhang Y, Huang K, Zhu Y, Wu C, Peng Y, Jiang M, Hu J, Chang H, Xu B, Li Y, Xu S. Maternal urinary bisphenol A levels and infant low birth weight: A nested case-control study of the Health Baby Cohort in China. Environ Int. 2015; 85:96-103.

Ivell R, Wade JD, Anand-Ivell R. INSL3 as a biomarker of Leydig cell functionality. Biol Reprod. 2013; 88(6):147.

Johar K, Priya A, Dhar S, Liu Q, Wong-Riley MT. Neuron-specific specificity protein 4 bigenomically regulates the transcription of all mitochondria- and nucleus-encoded cytochrome c oxidase subunit genes in neurons. J Neurochem. 2013; 127(4):496-508.

Johns LE, Ferguson KK, Cantonwine DE, McElrath TF, Mukherjee B, Meeker JD. Urinary BPA and Phthalate Metabolite Concentrations and Plasma Vitamin D Levels in Pregnant Women: A Repeated Measures Analysis. Environ Health Perspect. 2017; 125(8):087026.

Johns LE, Ferguson KK, Meeker JD. Relationships Between Urinary Phthalate Metabolite and Bisphenol A Concentrations and Vitamin D Levels in U.S. Adults: National Health and Nutrition Examination Survey (NHANES), 2005-2010. J Clin Endocrinol Metab. 2016; 101(11):4062-4069.

Kataria A, Levine D, Wertenteil S, Vento S, Xue J, Rajendiran K, Kannan K, Thurman JM, Morrison D, Brody R, Urbina E, Attina T, Trasande L, Trachtman H. Exposure to bisphenols and phthalates and association with oxidant stress, insulin resistance, and endothelial dysfunction in children. Pediatr Res. 2017; 81(6):857-864.

Kataria A, Trasande L, Trachtman H. The effects of environmental chemicals on renal function. Nat Rev Nephrol. 2015; 11(10):610-25.

Khalil N, Ebert JR, Wang L, Belcher S, Lee M, Czerwinski SA, Kannan K. Bisphenol A and cardiometabolic risk factors in obese children. Sci Total Environ. 2014; 470-471:726-32.

Kim JH, Lee J, Moon HB, Park J, Choi K, Kim SK, Kim S. Association of phthalate exposures with urinary free cortisol and 8-hydroxy-2'-deoxyguanosine in early childhood. Sci Total Environ. 2018; 627:506-513.

Kim JH, Lee M-R, Hong Y-C. Modification of the association of bisphenol A with abnormal liver function by polymorphisms of oxidative stress-related genes. Environ Res. 2016; 147:324-330

Kim JH, Hong Y-C. Increase of urinary malondialdehyde level by bisphenol A exposure: a longitudinal panel study. Environ Health. 2017; 16:8.

Kim JH, Rozek LS, Soliman AS, Sartor MA, Hablas A. Bisphenol A-associated epigenomic changes in prepubescent girls: a cross-sectional study in Gharbiah, Egypt. Environ Health 2013; 12: 33.

Klein AB, Williamson R, Santini MA, Clemmensen C, Ettrup A, Rios M, Knudsen GM, Aznar S. Blood BDNF concentrations reflect brain-tissue BDNF levels across species. Int J Neuropsychopharmacol. 2011; 14(3):347-53.

Kobroob A, Peerapanyasut, W, Chattipakorn N, Wongmekiat O. Damaging Effects of Bisphenol A on the Kidney and the Protection by Melatonin: Emerging Evidences from In Vivo and In Vitro Studies. Oxid Med Cell Longev. 2018; 2018:3082438.

Koibuchi N, Fukuda H, Chin WW. Promoter-specific regulation of the brain-derived neurotropic factor gene by thyroid hormone in the developing rat cerebellum. Endocrinology. 1999; 140(9):3955-61.

Koibuchi N, Chin WW. Thyroid hormone action and brain development. Trends Endocrinol Metab. 2000; 11(4):123-8.

Koven NS, Collins LR. Urinary brain-derived neurotrophic factor as a biomarker of executive functioning. Neuropsychobiology. 2014; 69(4):227-34.

Kumar D, Thakur MK. Anxiety like behavior due to perinatal exposure to Bisphenol-A is associated with decrease in excitatory to inhibitory synaptic density of male mouse brain. Toxicology. 2017; 378:107-113.

Kundakovic M, Gudsnuk K, Herbstman JB, Tang D, Perera FP, Champagne FA. DNA methylation of BDNF as a biomarker of early-life adversity. Proc Natl Acad Sci USA. 2015; 112(22):6807-13.

Kurian JR, Keen KL, Kenealy BP, Garcia JP, Hedman CJ, Terasawa E. Acute Influences of Bisphenol A Exposure on Hypothalamic Release of Gonadotropin-Releasing Hormone and Kisspeptin in Female Rhesus Monkeys. Endocrinology. 2015; 156(7):2563-70.

Lala V, Minter DA. Liver Function Tests. SourceStatPearls. Treasure Island (FL): StatPearls. 2018

Lam SH, Hlaing MM, Zhang X, Yan C, Duan Z, Zhu L, Ung CY, Mathavan S, Ong CN, Gong Z. Toxicogenomic and phenotypic analyses of bisphenol-A early-life exposure toxicity in zebrafish. PLoS One. 2011; 6(12):e28273.

Lan HC1, Lin IW2, Yang ZJ2, Lin JH2. Low-dose Bisphenol A Activates Cyp11a1 Gene Expression and Corticosterone Secretion in Adrenal Gland via the JNK Signaling Pathway. Toxicol Sci. 2015; 148(1):26-34.

Lee MR, Park H, Bae S, Lim YH, Kim JH, Cho SH, Hong YC. Urinary bisphenol A concentrations are associated with abnormal liver function in the elderly: a repeated panel study. J Epidemiol Community Health. 2014; 68(4):312-7.

Lee SH, Kim KM, Jung BH, Chung WY, Park CS, Chung BC. Estrogens in female thyroid cancer: alteration of urinary profiles in pre- and post-operative cases. Cancer Lett. 2003; 189(1):27-32.

Li M, Bi Y, Qi L, Wang T, Xu M, Huang Y, Xu Y, Chen Y, Lu J, Wang W, Ning G. Exposure to bisphenol A is associated with low-grade albuminuria in Chinese adults. Kidney Int. 2012; 81(11):1131-9.

Lin PY, Tseng PT. Decreased glial cell line-derived neurotrophic factor levels in patients with depression: a meta-analytic study. J Psychiatr Res. 2015; 63:20-7.

Liu C, Xu X, Zhang Y, Li W, Huo X. Associations between maternal phenolic exposure and cord sex hormones in male newborns. Hum Reprod. 2016; 31(3):648-56.

Lv Y, Rui C, Dai Y, Pang Q, Li Y, Fan R, Lu S. Exposure of children to BPA through dust and the association of urinary BPA and triclosan with oxidative stress in Guangzhou, China. Environ Sci Process Impacts. 2016; 18(12):1492-1499.

Maheu M, Lopez JP, Crapper L, Davoli MA, Turecki G, Mechawar N. MicroRNA regulation of central glial cell line-derived neurotrophic factor (GDNF) signalling in depression. Transl Psychiatry. 2015; 5:e511.

Martinez-Arguelles DB1, Papadopoulos V2. Mechanisms mediating environmental chemical-induced endocrine disruption in the adrenal gland. Front Endocrinol (Lausanne). 2015; 6:29.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28(7):412-9.

Medwid S, Guan H, Yang K. Bisphenol A stimulates adrenal cortical cell proliferation via ERβ-mediated activation of the sonic hedgehog signalling pathway. J Steroid Biochem Mol Biol. 2018; 178:254-262.

Meeker JD, Ferguson KK. Relationship between urinary phthalate and bisphenol A concentrations and serum thyroid measures in U.S. adults and adolescents from the National Health and Nutrition Examination Survey (NHANES) 2007-2008. Environ Health Perspect. 2011; 119(10):1396-402.

Melzer D, Rice NE, Lewis C, Henley WE, Galloway TS. Association of urinary bisphenol a concentration with heart disease: evidence from NHANES 2003/06. PLoS One. 2010; 5(1):e8673.

Melzer D, Harries L, Cipelli R, Henley W, Money C, McCormack P, Young A, Guralnik J, Ferrucci L, Bandinelli S, Corsi AM, Galloway T. Bisphenol A exposure is associated with in vivo estrogenic gene expression in adults. Environ Health Perspect. 2011; 119(12):1788-93.

Menale C, Grandone A, Nicolucci C, Cirillo G, Crispi S, Di Sessa A, Marzuillo P, Rossi S, Mita DG, Perrone L, Diano N, Miraglia Del Giudice E. Bisphenol A is associated with insulin resistance and modulates adiponectin and resistin gene expression in obese children. Pediatr Obes. 2017;12(5):380-387.

Miao M, Zhou X, Li Y, Zhang O, Zhou Z, Li T, Yuan W, Li R, Li DK. LINE-1 hypomethylation in spermatozoa is associated with Bisphenol A exposure. Andrology. 2014;2(1):138-44.

Miao M, Yuan W, He Y, Zhou Z, Wang J, Gao E, Li G, Li DK. In utero exposure to bisphenol-A and anogenital distance of male offspring. Birth Defects Res A Clin Mol Teratol. 2011;91(10):867-72.

Milošević N, Jakšić V, Sudji J, Vuković B, Ičin T, Milić N, Medić Stojanoska M. Possible influence of the environmental pollutant bisphenol A on the cardiometabolic risk factors. Int J Environ Health Res. 2017;27(1):11-26.

Mínguez-Alarcón L, Hauser R, Gaskins AJ. Effects of bisphenol A on male and couple reproductive health: a review. Fertil Steril. 2016;106(4):864-70.

Mitchelmore C, Gede L. Brain Derived Neurotrophic Factor: epigenetic regulation in psychiatric disorders. Brain Res. 2014;1586:162-72.

Mustieles V, Messerlian C, Reina I, Rodríguez-Carrillo A, Olea N, Fernández MF. Is Bisphenol A (BPA) a threat to children's behavior? J Mental Health & Clin Psychology 2018; 2(1): 6-9.

Mustieles V, Pérez-Lobato R, Olea N, Fernández MF. Bisphenol A: Human exposure and neurobehavior. Neurotoxicology. 2015;49:174-84.

Mustieles V, Ocón-Hernandez O, Mínguez-Alarcón L, Dávila-Arias C, Pérez-Lobato R, Calvente I, Arrebola JP, Vela-Soria F, Rubio S, Hauser R, Olea N, Fernández MF. Bisphenol A and reproductive hormones and cortisol in peripubertal boys: The INMA-Granada cohort. Sci Total Environ. 2018;618:1046-1053.

Nesan D, Sewell LC, Kurrasch DM. Opening the black box of endocrine disruption of brain development: Lessons from the characterization of Bisphenol A. Horm Behav. 2018;101:50-58.

N'Tumba-Byn T, Moison D, Lacroix M, Lecureuil C, Lesage L, Prud'homme SM, Pozzi-Gaudin S, Frydman R, Benachi A, Livera G, Rouiller-Fabre V, Habert R. Differential effects of bisphenol A and diethylstilbestrol on human, rat and mouse fetal leydig cell function. PLoS One. 2012;7(12):e51579.

Novakovic B, Fournier T, Harris LK, James J, Roberts CT, Yong HEJ, Kalionis B, Evain-Brion D, Ebeling PR, Wallace EM, Saffery R, Murthi P. Increased methylation and decreased expression of homeobox genes TLX1, HOXA10 and DLX5 in human placenta are associated with trophoblast differentiation. Sci Rep. 2017;7(1):4523.

Özgen İT, Torun E, Bayraktar-Tanyeri B, Durmaz E, Kılıç E, Cesur Y. The relation of urinary bisphenol A with kisspeptin in girls diagnosed with central precocious puberty and premature thelarche. J Pediatr Endocrinol Metab. 2016;29(3):337-41.

Patisaul HB, Sullivan AW, Radford ME, Walker DM, Adewale HB, Winnik B, Coughlin JL, Buckley B, Gore AC. Anxiogenic effects of developmental bisphenol A exposure are associated with gene expression changes in the juvenile rat amygdala and mitigated by soy. PLoS One. 2012;7(9):e43890.

Patsenker E, Sachse P, Chicca A, Gachet MS, Schneider V, Mattsson J, Lanz C, Worni M, de Gottardi A, Semmo M, Hampe J, Schafmayer C, Brenneisen R, Gertsch J, Stickel F, Semmo N. Elevated levels of endocannabinoids in chronic hepatitis C may modulate cellular immune response and hepatic stellate cell activation. Int J Mol Sci. 2015;16(4):7057-76.

Perng W, Watkins DJ, Cantoral A, Mercado-García A, Meeker JD, Téllez-Rojo MM, Peterson KE. Exposure to phthalates is associated with lipid profile in peripubertal Mexican youth. Environ Res. 2017;154:311-317.

Pinacho R, Saia G, Fusté M, Meléndez-Pérez I, Villalta-Gil V, Haro JM, Gill G, Ramos B. Phosphorylation of transcription factor specificity protein 4 is increased in peripheral blood mononuclear cells of first-episode psychosis. PLoS One. 2015;10(4):e0125115.

Rancière F, Lyons JG, Loh VH, Botton J, Galloway T, Wang T, Shaw JE, Magliano DJ. Bisphenol A and the risk of cardiometabolic disorders: a systematic review with meta-analysis of the epidemiological evidence. Environ Health. 2015; 31;14:46.

Romano ME, Webster GM, Vuong AM, Thomas Zoeller R, Chen A, Hoofnagle AN, Calafat AM, Karagas MR, Yolton K, Lanphear BP, Braun JM. Gestational urinary bisphenol A and maternal and newborn thyroid hormone concentrations: the HOME Study. Environ Res. 2015;138:453-60.

Rönn M, Kullberg J, Karlsson H, Berglund J, Malmberg F, Orberg J, Lind L, Ahlström H, Lind PM. Bisphenol A exposure increases liver fat in juvenile fructose-fed Fischer 344 rats. Toxicology. 2013;303:125-32.

Rönn M, Lind L, Örberg J, Kullberg J, Söderberg S, Larsson A, Johansson L, Ahlström H, Lind PM. Bisphenol A is related to circulating levels of adiponectin, leptin and ghrelin, but not to fat mass or fat distribution in humans. Chemosphere. 2014;112:42-8.

Saavedra A, Baltazar G, Duarte EP. Driving GDNF expression: the green and the red traffic lights. Prog Neurobiol. 2008;86(3):186-215.

Saczynski JS, White L, Peila RL, Rodriguez BL, Launer LJ. The relation between apolipoprotein A-I and dementia: the Honolulu-Asia aging study. Am J Epidemiol. 2007;165(9):985-92.

Saenen ND, Vrijens K, Janssen BG, Madhloum N, Peusens M, Gyselaers W, Vanpoucke C, Lefebvre W, Roels HA, Nawrot TS. Placental Nitrosative Stress and Exposure to Ambient Air Pollution During Gestation: A Population Study. Am J Epidemiol. 2016;184(6):442-9.

Samavat H, Kurzer MS. Estrogen metabolism and breast cancer. Cancer Lett. 2015;356(2 Pt A):231-43.

Semenkovich Clay F. et al. Williams Textbook of Endocrinology (Thirteenth Edition). 2016.

Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA, Gusella JF, O'Rahilly S, Carlton MB, Crowley WF Jr, Aparicio SA, Colledge WH. The GPR54 gene as a regulator of puberty. N Engl J Med. 200323;349(17):1614-27.

Shim SH, Hwangbo Y, Yoon HJ, Kwon YJ, Lee HY, Hwang JA, Kim YK. Increased levels of plasma glial-derived neurotrophic factor in children with attention deficit hyperactivity disorder.Nord J Psychiatry. 2015;69(7):546-51.

Skorupskaite K, George JT, Anderson RA. The kisspeptin-GnRH pathway in human reproductive health and disease. Hum Reprod Update. 2014;20(4):485-500

Slot RE, Van Harten AC, Kester MI, Jongbloed W, Bouwman FH, Teunissen CE, Scheltens P, Veerhuis R, van der Flier WM. Apolipoprotein A1 in Cerebrospinal Fluid and Plasma and

Progression to Alzheimer's Disease in Non-Demented Elderly. J Alzheimers Dis. 2017;56(2):687-697.

Sollome J, Martin E, Sethupathy P, Fry RC. Environmental contaminants and microRNA regulation: Transcription factors as regulators of toxicant-altered microRNA expression. Toxicol Appl Pharmacol. 2016;312:61-66.

Sullivan-Pyke C, Haisenleder DJ, Senapati S, Nicolais O, Eisenberg E, Sammel MD, Barnhart KT. Kisspeptin as a new serum biomarker to discriminate miscarriage from viable intrauterine pregnancy. Fertil Steril. 2018;109(1):137-141.

Sriphrapradang C, Chailurkit LO, Aekplakorn W, Ongphiphadhanakul B. Association between bisphenol A and abnormal free thyroxine level in men. Endocrine. 2013;44(2):441-7.

Straten G, Eschweiler GW, Maetzler W, Laske C, Leyhe T. Glial cell-line derived neurotrophic factor (GDNF) concentrations in cerebrospinal fluid and serum of patients with early Alzheimer's disease and normal controls. J Alzheimers Dis. 2009;18(2):331-7.

Song H, Park J, Bui PTC, Choi K, Gye MC, Hong YC, Kim JH, Lee YJ. Bisphenol A induces COX-2 through the mitogen-activated protein kinase pathway and is associated with levels of inflammation-related markers in elderly populations. Environ Res. 2017;158:490-498.

Sui L, Li BM. Effects of perinatal hypothyroidism on regulation of reelin and brain-derived neurotrophic factor gene expression in rat hippocampus: Role of DNA methylation and histone acetylation. Steroids. 2010;75(12):988-97.

Swanson CR, Li K, Unger TL, Gallagher MD, Van Deerlin VM, Agarwal P, Leverenz J, Roberts J, Samii A, Gross RG, Hurtig H, Rick J, Weintraub D, Trojanowski JQ, Zabetian C, Chen-Plotkin AS. Lower plasma apolipoprotein A1 levels are found in Parkinson's disease and associate with apolipoprotein A1 genotype. Mov Disord. 2015;30(6):805-12.

Teles MG, Bianco SD, Brito VN, Trarbach EB, Kuohung W, Xu S, Seminara SB, Mendonca BB, Kaiser UB, Latronico AC. A GPR54-activating mutation in a patient with central precocious puberty. N Engl J Med 2008;358(7): 709-715.

Thankamony A, Pasterski V, Ong KK, Acerini CL, Hughes IA. Anogenital distance as a marker of androgen exposure in humans. Andrology. 2016;4(4):616-25.

Trasande L, Attina TM, Trachtman H. Bisphenol A exposure is associated with low-grade urinary albumin excretion in children of the United States. Kidney Int. 2013;83(4):741-8.

Troisi J, Mikelson C, Richards S, Symes S, Adair D, Zullo F, Guida M. Placental concentrations of bisphenol A and birth weight from births in the Southeastern U.S. Placenta. 2014;35(11):947-52.

Tufekci KU, Oner MG, Meuwissen RL, Genc S. The role of microRNAs in human diseases. Methods Mol Biol 2014;1107: 33-50.

Ursin G, Wu AH, Hoover RN, West DW, Nomura AM, Kolonel LN, Pike MC, Ziegler RG. Breast cancer and oral contraceptive use in Asian-American women. Am J Epidemiol. 1999;150(6):561-7.

Use of biomarkers in assessing health and environmental impacts of chemical pollutants. Edited by Curtis C. Travis. 1992. Series A: Life Sciences Vol.250 p.162-167

Vafeiadi M, Roumeliotaki T, Myridakis A, Chalkiadaki G, Fthenou E, Dermitzaki E, Karachaliou M, Sarri K, Vassilaki M, Stephanou EG, Kogevinas M, Chatzi L. Association of early life exposure to bisphenol A with obesity and cardiometabolic traits in childhood. Environ Res. 2016;146:379-87.

Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2' –deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2009;27(2):120-39.

Varayoud J, Ramos JG, Bosquiazzo VL, Lower M, Muñoz-de-Toro M, Luque EH. Neonatal exposure to bisphenol A alters rat uterine implantation-associated gene expression and reduces the number of implantation sites. Endocrinology 2011;152(3):1101-11.

Veiga-Lopez A, Pennathur S, Kannan K, Patisaul HB, Dolinoy DC, Zeng L, Padmanabhan V. Impact of gestational bisphenol A on oxidative stress and free fatty acids: Human association and interspecies animal testing studies. Endocrinology 2015;156(3):911-22.

Villa C, Ghezzi L, Fenoglio C, Clerici F, Marcone A, Benussi L, Ghidoni R, Gallone S, Serpente M, Cantoni C, Ridolfi E, Bonsi R, Cerami C, Cappa S, Binetti G, Franceschi M, Rainero I, Mariani C, Bresolin N, Scarpini E, Galimberti D. Genetics and expression analysis of the specificity protein 4 gene (SP4) in patients with Alzheimer's disease and frontotemporal lobar degeneration. J Alzheimers Dis. 2012;31(3):537-42.

Wang IJ, Chen CY, Bornehag CG. Bisphenol A exposure may increase the risk of development of atopic disorders in children. Int J Hyg Environ Health. 2016;219(3):311-6.

Wang C, Li Z, Han H, Luo G, Zhou B, Wang S, Wang J. Impairment of object recognition memory by maternal bisphenol A exposure is associated with inhibition of Akt and ERK/CREB/BDNF pathway in the male offspring hippocampus. Toxicology 2016;341-343:56-64.

Wang C, Niu R, Zhu Y, Han H, Luo G, Zhou B, Wang J. Changes in memory and synaptic plasticity induced in male rats after maternal exposure to bisphenol A. Toxicology 2014;322:51-60.

Wang IJ, Karmaus WJ, Yang CC. Polycyclic aromatic hydrocarbons exposure, oxidative stress, and asthma in children. Int Arch Occup Environ Health. 2017;90(3):297-303.

Wang T, Lu J, Xu M, Xu Y, Li M, Liu Y, Tian X, Chen Y, Dai M, Wang W, Lai S, Bi Y, Ning G. Urinary bisphenol a concentration and thyroid function in Chinese adults. Epidemiology 2013;24(2):295-302.

Watkins DJ, Ferguson KK, Anzalota Del Toro LV, Alshawabkeh AN, Cordero JF, Meeker JD. Associations between urinary phenol and paraben concentrations and markers of oxidative stress and inflammation among pregnant women in Puerto Rico. Int J Hyg Environ Health. 2015;218(2):212-9.

Watkins DJ, Sánchez BN, Téllez-Rojo MM, Lee JM, Mercado-García A, Blank-Goldenberg C, Peterson KE, Meeker JD. Phthalate and bisphenol A exposure during in utero windows of susceptibility in relation to reproductive hormones and pubertal development in girls. Environ Res. 2017;159:143-151.

Wei J, Sun X, Chen Y, Li Y, Song L, Zhou Z, Xu B, Lin Y, Xu S. Perinatal exposure to bisphenol A exacerbates nonalcoholic steatohepatitis-like phenotype in male rat offspring fed on a high-fat diet. J Endocrinol. 2014;222(3):313-25.

Xu X, Liu X, Zhang Q, Zhang G, Lu Y, Ruan Q, Dong F, Yang Y. Sex-specific effects of bisphenol-A on memory and synaptic structural modification in hippocampus of adult mice. Horm Behav. 2013;63(5):766-75.

Xu X, Gu T, Shen Q. Different effects of bisphenol-A on memory behavior and synaptic modification in intact and estrogen-deprived female mice. J Neurochem.2015;132(5):572-82.

Xu X, Chiung YM, Lu F, Qiu S, Ji M, Huo X. Associations of cadmium, bisphenol A and polychlorinated biphenyl co-exposure in utero with placental gene expression and neonatal outcomes. Reprod Toxicol. 2015;52:62-70.

Yang AS, Estécio MR, Doshi K, Kondo Y, Tajara EH, Issa JP. A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements. Nucleic Acids Res. 2004;32(3):e38.

Yang M, Lee HS, Pyo MY. Proteomic biomarkers for prenatal bisphenol A-exposure in mouse immune organs. Environ Mol Mutagen. 2008;49(5):368-73.

Yang YJ, Hong YC, Oh SY, Park MS, Kim H, Leem JH, Ha EH. Bisphenol A exposure is associated with oxidative stress and inflammation in postmenopausal women. Environ Res 2009;109(6): 797-801.

Yang M, Lee HS, Hwang MW, Jin M. Effects of Korean red ginseng (Panax Ginseng Meyer) on bisphenol A exposure and gynecologic complaints: single blind, randomized clinical trial of efficacy and safety. BMC Complementary & Alternative Medicine. 2014. 14:265.

Yen PM. Physiological and molecular basis of thyroid hormone action. Physiol Rev. 2001;81(3):1097-142.

Yeom CW, Park YJ, Choi SW, Bhang SY. Association of peripheral BDNF level with cognition, attention and behavior in preschool children. Child Adolesc Psychiatry Ment Health. 2016;10:10.

Yi B, Kasai H, Lee HS, Kang Y, Park JY, Yang M. Inhibition by wheat sprout (Triticum aestivum) juice of bisphenol A-induced oxidative stress in young women. Mutat Res. 2011;724(1-2):64-8.

Zbucka-Kretowska M, Zbucki R, Parfieniuk E, Maslyk M, Lazarek U, Miltyk W, Czerniecki J, Wolczynski S, Kretowski A, Ciborowski M. Evaluation of Bisphenol A influence on endocannabinoid system in pregnant women. Chemosphere. 2018;203:387-392.

Zhang T, Xue J, Gao CZ, Qiu RL, Li YX, Li X, Huang MZ, Kannan K. Urinary Concentrations of Bisphenols and Their Association with Biomarkers of Oxidative Stress in People Living Near E-Waste Recycling Facilities in China. Environ Sci Technol. 2016;50(7):4045-53.

Ziyaraa MA, Hamdan FB, Mousa LR. Correlation of Kisspeptin-10 level and fetal well-being in preeclamptic patients. Taiwan J Obstet Gynecol 2016; 55(6): 840-846.

6.3 Phthalates (coordinated by VITO)

6.3.1 Terms used for the search:

- **List of the search terms (MeSH and Non-MeSH) used for exposure.**

Phthalate OR Phthalates OR Diethylhexyl phthalate OR Bis(2-ethylhexyl)phthalate OR Dioctyl Phthalate OR Di-2-Ethylhexylphthalate OR Di(2-ethylhexyl)phthalate OR Butyl benzyl phthalate OR BBPHT OR Dibutyl phthalate OR Di-n-Butyl Phthalate OR Butyl Phthalate OR Diisobutyl phthalate OR Diisononyl phthalate OR ENJ 2065 OR Diethyl phthalate OR Phthalic acid diethyl ester OR Diisodecyl phthalate OR di-isodecyl phthalate OR Di-n-octyl phthalate OR Dimethyl phthalate OR Dimethylphthalate OR Di-n-pentyl phthalate OR Diamyl Phthalate OR Dicyclohexyl phthalate OR Di-n-hexyl phthalate OR Dihexyl Phthalate OR Phthalic acid dihexyl ester OR Di(methoxyethyl) phthalate OR Diisononyl cyclohexane-1,2-dicarboxylate OR Mono-benzyl phthalate OR Monocyclohexyl phthalate OR Mono-(2-ethylhexyl) phthalate OR Monoethyl phthalate OR Mono-isobutyl phthalate OR Mono-isodecyl phthalate OR Mono(carboxy-isooctyl)

phthalate OR Monobutyl phthalate OR Mono-n-octyl phthalate OR Monomethyl phthalate OR Monoisononyl-cyclohexane-1,2-dicarboxylate OR (Diethylhexyl phthalate OR Bis(2-ethylhexyl)phthalate OR Dioctyl Phthalate OR Di-2-Ethylhexylphthalate OR Di(2-ethylhexyl)phthalate [Supplementary Concept]) OR (Butyl benzyl phthalate OR BBPHT [Supplementary Concept]) OR (Dibutyl phthalate OR Di-n-Butyl Phthalate OR Butyl Phthalate [Supplementary Concept]) OR (Diisobutyl phthalate [Supplementary Concept]) OR (Diisononyl phthalate OR ENJ 2065 [Supplementary Concept]) OR (Diethyl phthalate OR Phthalic acid diethyl ester [Supplementary Concept]) OR (Diisodecyl phthalate OR di-isodecyl phthalate [Supplementary Concept]) OR (Di-n-octyl phthalate [Supplementary Concept]) OR (Dimethyl phthalate OR Dimethylphthalate [Supplementary Concept]) OR (Di-n-pentyl phthalate OR Diamyl Phthalate [Supplementary Concept]) OR (Dicyclohexyl phthalate [Supplementary Concept]) OR (Di-n-hexyl phthalate OR Dihexyl Phthalate OR Phthalic acid dihexyl ester [Supplementary Concept]) OR (Di(methoxyethyl) phthalate [Supplementary Concept]) OR (Diisononyl cyclohexane-1,2-dicarboxylate [Supplementary Concept])

- **List of search terms (MeSH and Non-MeSH) used for the health endpoints.**

In this list, we refer to the search terms provided by the WP coordinators (UGR): Exploratory searches on bisphenols and effects biomarkers: Methodology based on D14.1- Criteria for prioritization of effect biomarkers - fully described in Table 2.

6.3.2 Exploratory search

The final exploratory search used to select potential references is summarized in the table below, which exhibits the final number of references on phthalates exposure related to health outcomes and the partner responsible for analysing these references.

Table 5. Final set of references found in Phthalates literature search.

Search terms	Total Hits #	Full text #	<10 years #	 #	 #	Work division
Phthalate AND Neurobehavior-related search terms	219	187	144	69	26	UCY
Phthalate AND Reproductive-related terms	71	651	456	229	202	VITO
Phthalate AND Endocrine-related terms	581	516	320	123	203	
Phthalate AND cardiovascular OR Obesity OR Metabolic-related terms	759	632	393	160	177	
Phthalate AND Allergy OR Immune System	67	152	102	42	59	
Phthalate AND Cancer-related terms	238	202	131	75	55	UCY

6.3.3 List of traditional effect biomarkers

6.3.3.1 Cancer

The abstract screening under this category led to the identification of classic effect biomarkers, including histopathological analysis of tissue sections to confirm breast cancer or uterine lesions. The presence of uterine leiomyoma after exposure to DEHP metabolites was assessed by direct intraoperative visualization and subsequent pathological analysis. Phthalates were detected in the urine of 61 women and a positive association was found between phthalate exposure and uterine leiomyomata. The association of exposure to diethyl phthalate (DEP), butyl benzyl phthalate (BBzP), and dioctyl phthalate (DOP) with the development of breast cancer (BC) was evaluated by histopathology. The chemicals were detected in the urine of 233 women with histologically confirmed BC, and a negative association was found between the development of BC and the consumption of certain flavonoids. The most common mechanism of action for inducing cancer is considered to be the estrogenic activity of phthalates and their metabolites.

6.3.3.2 Behavior/Neurobehavioral

The abstract screening under this category revealed mostly classic and established biomarkers of effect. To evaluate the effect of exposure to phthalates on behavior or neurobehavior in humans, common neurobehavioral tests are applied such as the Child Behavior Checklist (CBCL) and the Strengths and Difficulties Questionnaire (SDQ). Regarding their mechanism of action, it is suggested that these phthalates impact on testosterone levels, which affect brain development and sexual differentiation.

6.3.3.3 Endocrine health effects

Following the screening of abstracts in this relatively large category associated with phthalate exposure, the following classic effect biomarkers were identified. They include the detection of hormones in serum (such as adiponectin or insulin for diabetes, reproductive and thyroid hormones), weight changes to evaluate endocrine disruptors, hip and femur bone density (VitD), and HOMA-IR. Phthalates act as endocrine disruptors by interacting with nuclear receptors such as peroxisome-proliferator-activated receptors (PPARs). According to this mechanism, phthalate exposure could be linked to obesity through PPARγ, which has an important role in adipogenesis and lipid storage.

Given that obesity is a major risk factor for diabetes, PPARs could be a link by which phthalates are involved in diabetes risk.

6.3.3.4 Cardiovascular- Obesity- and Metabolic-related health effects

The majority of abstracts for epidemiological studies in which phthalate exposure was studied concerned metabolic endpoints. Traditional biomarkers described include: hormones (e.g., leptin, IFG-1), fasting c-peptide in serum, oxidative stress markers such as malondialdehyde, HOMA-IR,

anthropometric parameters (weight, BMI, waist circumference…), and self-reported outcomes such as diabetes. Reported mechanisms of action include insulin resistance and oxidative stress.

6.3.3.5 Reproductive health effects

This category was also relatively small, although there was strong overlap with the health effects studied in the endocrine category. Classic biomarkers of effect identified by abstract screening for this category include the measurement of hormone levels, such as testosterone or estradiol, and of sflt1 and PlDGF in serum, and anthropometric measurements such as body size and the cycle of female partners. Semen quality (gene expression) and urinary biomarkers for oxidative stress (e.g. 15-F2t isoprostane and 8-hydroxy-2′-deoxyguanosine (8-OHdG) were also found. Furthermore, questionnaire data and anthropometric parameters were also used, including time to pregnancy (TTP), miscarriages, sexual interest, and anogenital distance (AGD), among others. The two most common mechanisms of action suggested under this category are: **1**. Action of phthalates to disrup estrogen levels, thereby affecting sexual development. For example, urinary concentrations of high-MWP, including di(2-ethylhexyl) phthalate (SDEHP) metabolites, were associated with later pubic hair development during a 7-year multi-ethnic study of 1239 girls in the US11; and **2**. Action of phthalates as oxidative agents affecting DNA. One study reports that phthalate compounds may induce oxidative stress in the male reproductive organs, mainly testes and epididymis. They impair spermatogenic process by inducing oxidative stress and apoptosis in germ cells or target sertoli cells, thereby hampering spermatogenesis. They also impair Leydig cell function by inducing reactive oxidative species (ROS), thereby decreasing levels of steroidogenic enzymes.

6.3.3.6 Allergy & immunology

Screening of abstracts on allergy and immunology endpoints related to phthalate exposure identified relatively few epidemiological studies in comparison to endocrine health effects.

The following ‘traditional’ effect biomarkers are likely to be most studied: IgE, (self-reported), presence of asthma, wheezing, rhinitis through questionnaires, oxidative stress biomarkers (8-OHdG). Inflammation biomarkers: chemokines, C-reactive protein (CRP), neutrophil count. Regarding their mechanism of action, oxidative stress is suggested as a possible trigger of allergic and immune effects.

6.3.4 List of novel effect biomarkers found during the literature search

Using the scoring table and other information, such as the strengths and limitations for HBM purposes, selection is made of the novel effect biomarkers whose characteristics better fit European human biomonitoring needs (e.g., sample availability, the requirement for invasive/non-invasive techniques, such as urine/blood/placenta, and costs of analyses, etc.).

Table 6. List of novel effect biomarkers for Phthalates chemical family

Health Outcome: Cancer			
Novel effect biomarker	Brief description of the effect biomarker	Scoring table procedure	Final Score (max. 20 points)
Detection of Polymorphisms of ESR and CYP17A1 genes	Polymorphisms were detected by DNA sequencing in blood samples. CYP17A1 is an estrogen biosynthesis enzyme, ESR1, and ESR2 are genes encoding estrogen receptors.	*The biomarker was assessed in serum (3p). There is a plausible mechanism of action between exposure and biomarker (2p). A related AOP was found (3p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "unsure" (0p).*	13
Detection of the glutathione S-transferase M1 enzyme	GSTM1 is a major detoxification enzyme. The GSTM1 genotype was determined by polymerase chain reaction (PCR) in blood samples	*The biomarker was assessed in serum (3p). There is no plausible mechanism of action between exposure and biomarker (0p). A related AOP was found (3p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "low" (0p)*	11
Behavior/Neurobehavioral			
Novel effect biomarker	Brief description of the effect biomarker	Scoring table procedure *(Please, specify the points assigned during the scoring procedure)*	Final Score (max. 20 points)
Changes in sex-typical play behaviors	Assessment of parental attitudes towards sex-atypical play. Questionnaires were completed by children and parents	*The biomarker was assessed in 'other' non-invasive matrices (1p). There is a plausible mechanism of action between exposure and biomarker (2p). A related AOP was found (3p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "medium" (2p).*	13
Child visual-spatial abilities evaluated using the Virtual Morris Water Maze (VMWM).	The VMWM is a computerized version of the MWM, a rodent test of learning and visual-spatial reference memory.	*The biomarker was assessed in 'other' non-invasive matrices (1p). There is a plausible mechanism of action between exposure and biomarker (2p). A related AOP was found (3p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "unsure" (0p).*	11

Symptom severity and cortical thickness in ADHD children.	Whole-brain structural MRI was acquired with a T1-weighted magnetization-prepared rapid acquisition gradient echo (MPRAGE) scan	*The biomarker was assessed in other non-invasive matrices (1p). There is no plausible mechanism of action between exposure and biomarker (0p). A related AOP was found (3p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "low" (0p).*	9
Reproductive health effects			
11β-HSD2 activity	*THF+allo-THF)/THE with tetrahydrocortisol (THF), allo-tetrahydrocortisol (allo-THF), tetrahydrocortisone (THE) in urine*	*Paper in Chinese but included because it indicates activation of the HPA axis by phthalates, which is an important possible mode of action*	14
Genotype of CYP17A1, estrogen receptor 1 (ESR1), and 2 (ESR2) in serum	Polymorphisms were detected by DNA sequencing in blood samples. CYP17A1 is an estrogen biosynthesis enzyme, and ESR1 and ESR2 are genes encoding estrogen receptors.	*The biomarker was assessed in serum (3p). There is a plausible mechanism of action between exposure and biomarker (2p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "medium" if consent is available (2p).*	12
Metallothioneins, fatty acid transport protein 1 and heart fatty acid binding protein in umbilical cord blood	Expression of metallothioneins (**MTs**), fatty acid transport protein 1 (**FATP1**) and heart fatty acid binding protein (**HFABP**) in umbilical cord blood by PCR	*The biomarker was assessed in non-invasive matrices (2p). There is a plausible mechanism of action between exposure and biomarker (related to fetal growth and development (2p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "medium" (2p)* .	11
DNA methylation of genes involved in inflammatory response, cancer, endocrine function, and male fertility	**DNA methylation** (Genes with DMRs involved in inflammatory response (IRAK4 and ESM1), cancer (BRCA1 and LASP1), endocrine function (CNPY1), and male fertility (IFT140, TESC, and PRDM8)	*The biomarker was assessed in non-invasive matrices (2p). There is a plausible mechanism of action between exposure and biomarker (2p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "medium" (2p).*	11

Nuclear receptors (NRs) gene expression levels (ERa, ERb, AR, AhR, PPARg, PXR)	Nuclear receptor (NR) gene expression levels (ERa, ERb, AR, AhR, PPARg, PXR) in peripheral blood mononuclear cells from serum	*The biomarker was assessed in invasive matrices (3p). There is a plausible mechanism of action between exposure and biomarker (2p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "medium" (2p).*	12
Metabolic health effects			
DNA methylation of genes involved in inflammatory response, cancer, endocrine function, and male fertility	**DNA methylation** (Genes with DMRs involved in inflammatory response (IRAK4 and ESM1), cancer (BRCA1 and LASP1), endocrine function (CNPY1), and male fertility (IFT140, TESC, and PRDM8))	*The biomarker was assessed in non-invasive matrices (2p). There is a plausible mechanism of action between exposure and biomarker (2p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "medium" (2p)*	11
Endocrine health effects			
Metabolomics in urine	Metabolomics: amino acids and enzymes in urine: increased oxidative stress and fatty acid oxidation, decreased prostaglandin metabolism	The biomarker was assessed in non-invasive matrices (5p). There is a plausible mechanism of action between exposure and biomarker (2p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "medium" (2p)	14
11β-HSD2 activity	*THF+allo-THF)/THE with tetrahydrocortisol (THF), allo-tetrahydrocortisol (allo-THF), tetrahydrocortisone (THE) in urine*	*paper in Chinese but included because it indicates activation of the HPA axis by phthalates, which is an important possible mode of action*	14
Kisspeptin in serum	levels of LH, FSH estradiol and kisspeptin-54 in blood samples were evaluated using radioimmunoassay	*The biomarker was assessed in invasive matrices (3p). There is a plausible mechanism of action between exposure and biomarker (2p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "medium" (2p).*	12

DNA methylation of genes involved in inflammatory response, cancer, endocrine function, and male fertility	DNA methylation (Genes with DMRs involved in inflammatory response (IRAK4 and ESM1), cancer (BRCA1 and LASP1), endocrine function (CNPY1), and male fertility (IFT140, TESC, and PRDM8))	*The biomarker was assessed in non-invasive matrices (2p). There is a plausible mechanism of action between exposure and biomarker (2p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "medium" (2p).*	11
Allergy and immunology			
Genetic polymorphisms of oxidative stress-related genes	Genetic polymorphisms of the oxidative stress-related genes glutathione S-transferase Mu 1 (GSTM1), glutathione S-transferase pi 1 (GSTP1), superoxide dismutase 2 (SOD2), catalase (CAT), myeloperoxidase (MPO) and EPHX1 in buccal samples by SNP genotyping assays	The biomarker was assessed in other non-invasive matrices (1p). There is a plausible mechanism of action between exposure and biomarker (2p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "medium" (2p).	10

6.3.5 Preliminary proposal of effect biomarkers that could be implemented in HBM

The following biomarkers have been selected:

6.3.5.1 The biomarker for oxidative stress, 8-hydroxy-2′-deoxyguanosine (8-OHdG)

These compounds are excreted in urine and provide an assessment of general oxidative stress throughout the body, are considered a classic biomarker of effect for certain chemicals that cause oxidative damage. Studies show that urinary 8-OHdG is a good biomarker for risk assessment of various cancers and degenerative diseases. The advantages of its applicability, such as its ready non-invasive detection in urine, suggest that it can also be applied for different chemical groups and several diseases.

6.3.5.2 Nuclear receptor (NR) gene expression levels (ERa, ERb, AR, AhR, PPARg, PXR) in peripheral blood mononuclear cells (PBMCs)

Based on the literature findings for phthalates, we suggest PPAR-activation might be a central underlying mechanism for phthalate exposure and associated health effects. PPARγ is a ligand-activated transcription factor that belongs to the nuclear receptor family, which also includes the steroid and thyroid hormone receptors. Interest in PPARγ action as a mechanistic basis for effects on the reproductive system arises from the known relationships between activation of this receptor and impairment of steroidogenesis, leading to reproductive toxicity in rodents. PPARs play important roles in the metabolic regulation of lipids, with cholesterol in particular, being a precursor of steroid hormones, linking lipid metabolism to effects on reproduction. However, in research on NR biomarkers, expression of mRNA for NR PPAR-γ did not significantly differ between fertile and

infertile women, unlike mRNA expression for the other NR. However, we believe that as a molecular initiating event (MIE), this is also not necessary. Although detection of an MIE in human biomonitoring studies may be difficult, detection of PPAR-activation in human biomonitoring data could add weight to the evidence of causal associations reported in experimental studies.

An important objective of HBM4EU is to validate plausible toxicity pathways and link them to adverse outcome pathways (AOPs) (WP13). Another target is the creation of an inventory of existing biomarkers of human health effects, based on the scientific literature, and their classification according to their relevance, level of validation and applicability to human biomonitoring studies and AOPs (WP14). Therefore, we propose a strategy by which the inventory of effect biomarkers, associated in this example with phthalates, can be used to establish mechanistic and systematic links between adverse health outcomes in the human population and exposure to priority chemical stressors (Lambrechts et al., in preparation).

6.3.6 Gaps in knowledge

6.3.6.1 Gaps in Knowledge on exposure to Phthalates and Cancer

Novel biomarkers in the Cancer category for exposure to phthalates include the detection of polymorphisms in the genes encoding estrogen receptors, estrogen biosynthesis enzymes and the presence of a detoxification enzyme in the blood of subjects. However, it is questionable whether these biomarkers are specific for the development of cancer or for exposure to the specific chemical group. In addition, the methodology used is costly (DNA sequencing and PCR, respectively) and therefore of doubtful utility for HBM studies.

6.3.6.2 Gaps in Knowledge on exposure to Phthalates and Behavior/Neurobehavior

The methods used to evaluate the effects of Phthalates on Behavior include the Virtual Morris Water Maze (VMWM) and whole-brain structural MRI. The cost and applicability of these methods in wide HBM studies is doubtful. In addition, cortical thickness, used as a biomarker of effect to measure exposure to Phthalates21, may not be specific for the effects of this chemical group. Questionnaires used in several studies to evaluate the effects of phthalates on Behavior may also not be a valid biomarker of effect due to the variability.

6.3.6.3 Gaps in Knowledge on exposure to Phthalates and Endocrine health effects

We found a large number of abstracts on phthalate-associated endocrine health effects. However, there was considerable overlap with other endocrine-related health endpoints, as expected, and we therefore did not define a gap of knowledge for this health endpoint.

6.3.6.4 Gaps in Knowledge on exposure to Phthalates and Metabolic health effects

Compared to the other endpoints, numerous papers were published on phthalates and associated metabolic effects. However, classic effect biomarkers such as glucose or insulin levels were mainly measured, without addressing the underlying mechanisms. It is proposed to include more molecular markers for initiating events, PPAR activation or oxidative stress.

6.3.6.5 Gaps in Knowledge on exposure to Phthalates and Reproductive health effects

A reference on Phthalate exposure and pubertal development concluded that Phthalates are hormonally active pollutants that may alter pubertal timing and their effects on pubertal development, depending on the age at exposure and other factors such as obesity and exposures earlier in life11. One gap in knowledge identified was whether exposures act independently or as part of real-life mixtures. In general, the mechanism of action of Phthalates, such as their ability to affect anogenital distance or levels of oxidative stress marker 8-hydroxy-2′-deoxyguanosine (8-OHdG) in urine is not elucidated and may lead to a variety of disorders. In addition, the AOPs described for this chemical group under this health effect are too wide and non-specific.

Given that **PPAR activation** is often postulated as a central mechanism in phthalate-induced adverse outcomes, biomarkers on this specific pathway are limited. We therefore propose to further elucidate this pathway through either specific biomarkers or at a more holistic scale by -omics profiling.

6.3.6.6 Gaps in Knowledge regarding exposure to Phthalates and immunologic health effects

For allergy and immune or inflammatory end points associated to phthalates, we found relatively little evidence. Therefore, this entire domain could be considered as a gap in knowledge. One of the possible hypotheses is that phthalates induce changes in the epigenome directly or indirectly through alterations in the hypothalamic-pituitary-adrenocortical (HPA) axis or through oxidative stress and thereby causing long term ‘programming’ of organ physiology .

6.3.7 Conclusions

- Traditional (and studied) biomarkers of effect have been identified for Phthalates related to cancer, behavior/neurobehavioral, endocrine, and reproductive health effects.
- Although some classic biomarkers are well established, the mechanisms of action and specific AOPs still need to be deciphered, with oxidative stress, PPAR-activation or HPA-activation being of interest.
- Oxidative stress marker 8-hydroxy-2′-deoxyguanosine (8-OHdG) or PPAR could be re-applied as an effect biomarker for many diseases after exposure to several chemicals.
- The association of allergy and immune effects with phthalate exposure is relatively understudied.

6.3.8 References

Adibi JJ, Lee MK, Naimi AI, Barrett E, Nguyen RH, Sathyanarayana S, Zhao Y, Thiet MP, Redmon JB, Swan SH: Human Chorionic Gonadotropin Partially Mediates Phthalate Association With Male and Female Anogenital Distance. J Clin Endocrinol Metab 2015;100(9):E1216-1224.

AOP: Aromatase (Cyp19a1) reduction leading to impaired fertility in adult female' (Id 7); https://aopwiki.org/aops/7 (dd 04/04/2018)

Braun JM, Bellinger DC, Hauser R, Wright RO, Chen A, Calafat AM, Yolton K, Lanphear BP: Prenatal phthalate, triclosan, and bisphenol A exposures and child visual-spatial abilities. Neurotoxicology 2017;58:75-83.

La Rocca C, Tait S, Guerranti C, Busani L, Ciardo F, Bergamasco B, Stecca L, Perra G, Mancini FR, Marci R, Bordi G, Caserta D, Focardi S, Moscarini M, Mantovani A. Exposure to endocrine disrupters and nuclear receptor gene expression in infertile and fertile women from different Italian areas. Int J Environ Res Public Health. 2014;11(10): 10146–10164.

Dodge LE, Williams PL, Williams MA, Missmer SA, Souter I, Calafat AM, Hauser R, Team ES: Associations between paternal urinary phthalate metabolite concentrations and reproductive outcomes among couples seeking fertility treatment. Reprod Toxicol 2015;58:184-193.

Duan Y, Wang L, Han L, Wang B, Sun H, Chen L, Zhu L, Luo Y: Exposure to phthalates in patients with diabetes and its association with oxidative stress, adiponectin, and inflammatory cytokines. Environ Int 2017;109:53-63.

Franken C, Lambrechts N, Govarts E, Koppen G, Den Hond E, Ooms D, Voorspoels S, Bruckers L, Loots I, Nelen V, Sioen I, Nawrot TS, Baeyens W, Van Larebeke N, Schoeters G. Phthalate-induced oxidative stress and association with asthma-related airway inflammation in adolescents. Int J Hyg Environ Health. 2017;220(2 Pt B):468-477.

Guo Y, Weck J, Sundaram R, Goldstone AE, Louis GB, Kannan K: Urinary concentrations of phthalates in couples planning pregnancy and its association with 8-hydroxy-2'-deoxyguanosine, a biomarker of oxidative stress: longitudinal investigation of fertility and the environment study. Environ Sci Technol 2014; 48(16):9804-9811.

Hu D, Wang YX, Chen WJ, Zhang Y, Li HH, Xiong L, Zhu HP, Chen HY, Peng SX, Wan ZH et al: Associations of phthalates exposure with attention deficits hyperactivity disorder: A case-control study among Chinese children. Environ Pollut 2017; 229:375-385.

Huang PC, Li WF, Liao PC, Sun CW, Tsai EM, Wang SL: Risk for estrogen-dependent diseases in relation to phthalate exposure and polymorphisms of CYP17A1 and estrogen receptor genes. Environ Sci Pollut Res Int 2014; 21(24):13964-13973.

Huang PC, Tsai EM, Li WF, Liao PC, Chung MC, Wang YH, Wang SL: Association between phthalate exposure and glutathione S-transferase M1 polymorphism in adenomyosis, leiomyoma and endometriosis. Hum Reprod 2010; 25(4):986-994.

Kim JH, Park HY, Bae S, Lim YH, Hong YC: Diethylhexyl phthalates is associated with insulin resistance via oxidative stress in the elderly: a panel study. PLoS One 2013; 8(8):e71392.

Kim YA, Kho Y, Chun KC, Koh JW, Park JW, Bunderson-Schelvan M, Cho YH. Increased Urinary Phthalate Levels in Women with Uterine Leiomyoma: A Case-Control Study. Int J Environ Res Public Health 2016; 13(12).

Merida-Ortega A, Hernandez-Alcaraz C, Hernandez-Ramirez RU, Garcia-Martinez A, Trejo-Valdivia B, Salinas-Rodriguez A, Svensson K, Cebrian ME, Franco-Marina F, Lopez-Carrillo L: Phthalate exposure, flavonoid consumption and breast cancer risk among Mexican women. Environ Int 2016; 96:167-172.

Moisiadis VG, Matthews SG: Glucocorticoids and fetal programming part 2: Mechanisms. Nat Rev Endocrinol 2014; 10:403-411.

Moisiadis VG, Matthews SG: Glucocorticoids and fetal programming part 1: Outcomes. Nat Rev Endocrinol 2014; 10:391-402.

National Research Council. 2008. Phthalates and Cumulative Risk Assessment: The Tasks Ahead. Washington, DC: The National Academies Press. https://doi.org/10.17226/12528.

Park S, Lee JM, Kim JW, Cheong JH, Yun HJ, Hong YC, Kim Y, Han DH, Yoo HJ, Shin MS et al: Association between phthalates and externalizing behaviors and cortical thickness in children with attention deficit hyperactivity disorder. Psychol Med 2015; 45(8):1601-1612.

Philippat C, Nakiwala D, Calafat AM, Botton J, De Agostini M, Heude B, Slama R, Group EM-CS: Prenatal Exposure to Nonpersistent Endocrine Disruptors and Behavior in Boys at 3 and 5 Years. Environ Health Perspect 2017;125(9):097014.

Sedha S, Kumar S, Shukla S: Role of Oxidative Stress in Male Reproductive Dysfunctions with Reference to Phthalate Compounds. Urol J 2015;12(5):2304-2316.

Song Y, Hauser R, Hu FB, Franke AA, Liu S, Sun Q: Urinary concentrations of bisphenol A and phthalate metabolites and weight change: a prospective investigation in US women. Int J Obes (Lond) 2014;38(12):1532-1537.

Sun J, Zhang MR, Zhang LQ, Zhao D, Li SG, Chen B: Phthalate monoesters in association with uterine leiomyomata in Shanghai. Int J Environ Health Res 2016;26(3):306-316.

Svensson K, Hernandez-Ramirez RU, Burguete-Garcia A, Cebrian ME, Calafat AM, Needham LL, Claudio L, Lopez-Carrillo L: Phthalate exposure associated with self-reported diabetes among Mexican women. Environ Res 2011;111(6):792-796.

Swan SH, Liu F, Hines M, Kruse RL, Wang C, Redmon JB, Sparks A, Weiss B: Prenatal phthalate exposure and reduced masculine play in boys. Int J Androl 2010; 33(2):259-269.

Valavanidis A, Vlachogianni T, Fiotakis C: 8-hydroxy-2' -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2009; 27(2):120-139.

Watkins DJ, Sanchez BN, Tellez-Rojo MM, Lee JM, Mercado-Garcia A, Blank-Goldenberg C, Peterson KE, Meeker JD: Phthalate and bisphenol A exposure during in utero windows of susceptibility in relation to reproductive hormones and pubertal development in girls. Environ Res 2017; 159:143-151.

Wolff MS, Teitelbaum SL, McGovern K, Windham GC, Pinney SM, Galvez M, Calafat AM, Kushi LH, Biro FM, Breast C et al: Phthalate exposure and pubertal development in a longitudinal study of US girls. Hum Reprod 2014; 29(7):1558-1566.

Zhao B, Chu Y, Huang Y, Hardy DO, Lin S, Ge RS: Structure-dependent inhibition of human and rat 11beta-hydroxysteroid dehydrogenase 2 activities by phthalates. Chem Biol Interact 2010; 183:79-84.

6.4 Polycyclic Aromatic Hydrocarbons (PAHs) (Coordinated by NRCWE)

6.4.1 Terms used for the search:

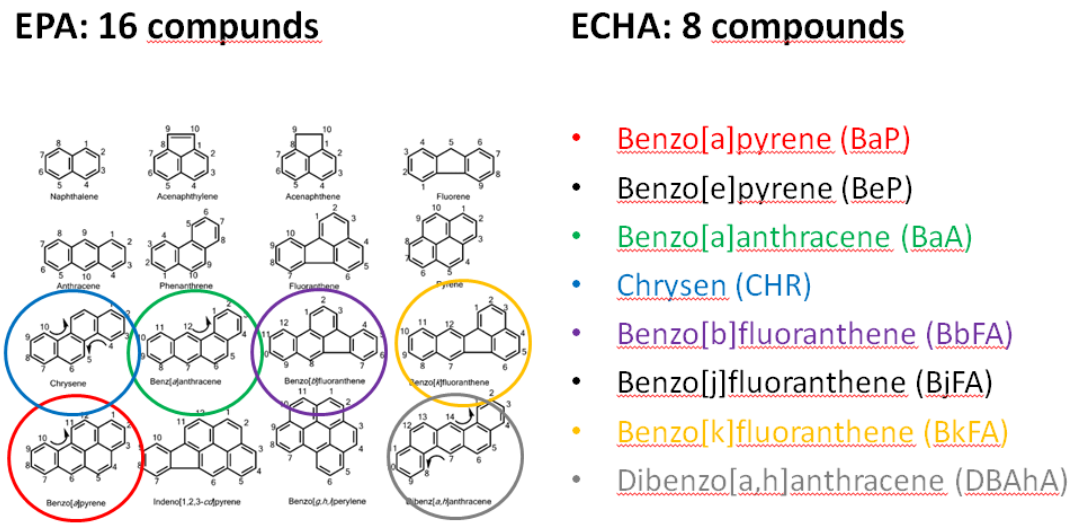
6.4.1.1 List of the search terms (MeSH and No-MeSH) used for the exposure.

An initial PAH search was performed by the UGR team: The term “Polycyclic Aromatic Hydrocarbons” [Mesh] retrieved more than 400,000 references.

To reduce the number of references, it was decided to perform the literature search on the PAHs listed by the Environmental Protection Agency (EPA) and the European Chemicals Agency (ECHA). The EPA list and the ECHA list consist of 16 (Andersson and Achten 2015) and 8 (ECHA 2013) PAHs, respectively. Six compounds are duplicates, yielding a final list of 18 PAHs. The search was performed using both Chemical Abstracts Service (CAS) numbers and chemical names. Please find the complete search profile for the 18 PAHs in appendix A. This search was performed in PubMed and is the final “exposure” search. The search retrieved 181,659 references.

Figure 4. PAHs listed by EPA and ECHA. The cultured PAHs are duplicates contained in both lists.

*EPA= Environmental Protection Agency; ECHA= European Chemicals Agency.



6.4.1.2 List of the search terms (MeSH and Non-MeSH) used for the health endpoints.

An initial search combining the “exposure” search with the search profile for health endpoints as specified by the UGR team, using the filters “full text” and “10 years” retrieved more than 40,000 references in total.

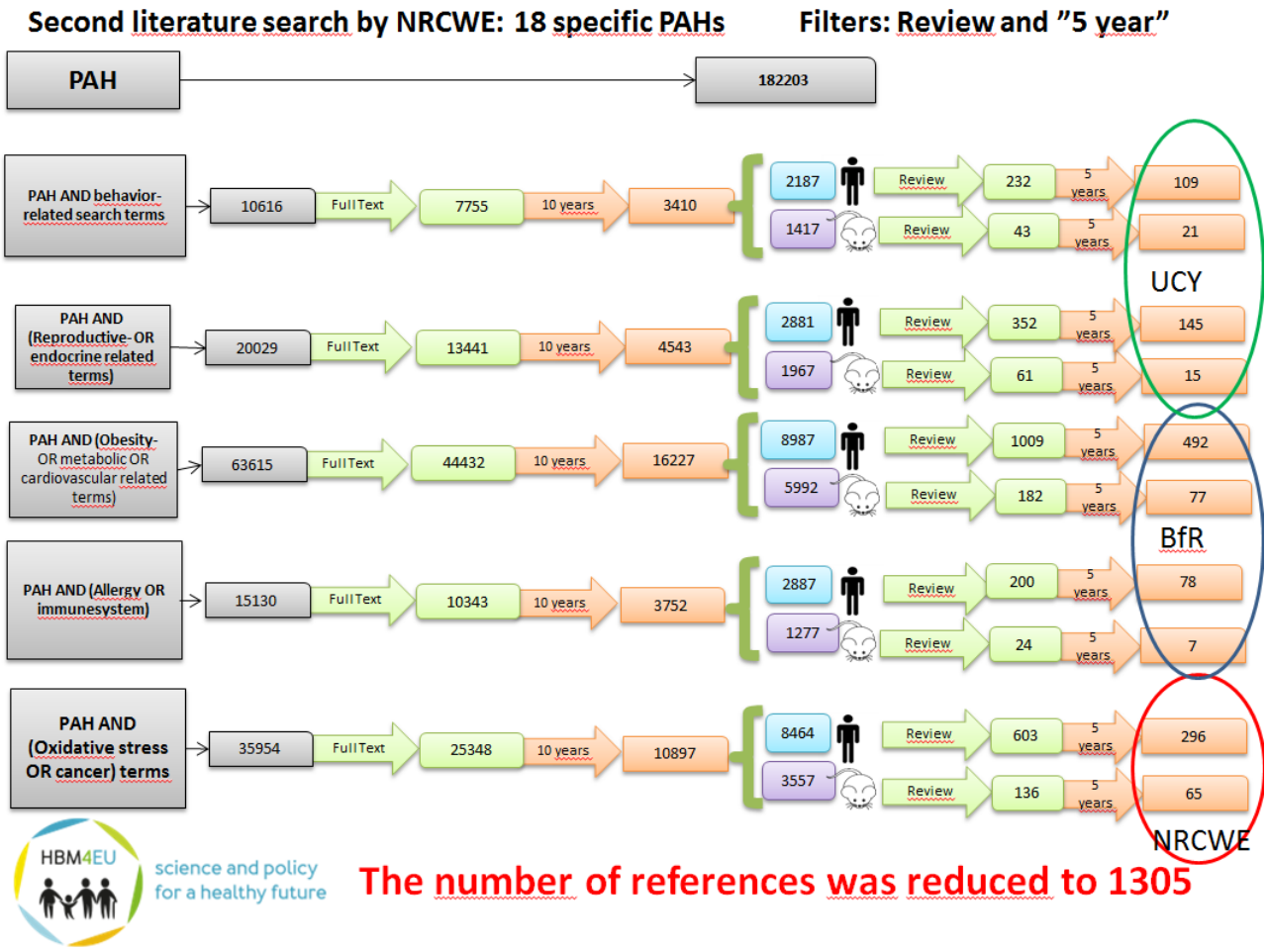
With this background, it was agreed within the “PAH” group and with the UGR team to reduce the number of references by adding the filters “review” and “5 years”.

This final search strategy for PAH biomarkers resulted in much lower number of references: 1,305. Please find the complete search profile in appendix B.

6.4.2 Exploratory Search

NRCWE performed the literature search in PubMed as specified in chapter 1 and appendix A and B in this report. The results of the search are depicted in the figure below.

Figure 5. Second literature search by NRCWE: 18 specific PAHs.



NRCWE prepared word files containing abstracts for each health endpoint divided between human and animal studies, respectively (10 word documents in total) (Appendix C). These were distributed to partners in the PAH group as displayed in table X.

Table 7. Distribution of the 1305 references among BfR, UCY, and NRCWE.

NRCWE		
Oxidative stress and cancer terms	Human	296
	Animal	65
Total		361
BfR		
Obesity, metabolic, cardiovascular	Human	492

	Animal	77
Allergy, immune system	Human	78
	Animal	7
Total		654

UCY

Behavior	Human	109
	Animal	21
Reproductive, endocrinal	Human	145
	Animal	15
Total		290

6.4.3 List of Traditional effect biomarkers

Using the scoring table and other information such as the strengths and limitations for HBM purposes, a selection is made of novel effect biomarkers whose characteristics better fulfil European human biomonitoring needs (e.g. sample availability, need for invasive/non-invasive techniques such as urine/blood/placenta, and costs of analyses, etc.).

6.4.3.1 Cancer and oxidative stress (NRCWE)

The literature search in PubMED showed that the PAH benzo[a]pyrene is by far the most widely studied of the 18 PAHs. Benzo[a]pyrene has been classified as carcinogenic to humans by IARC (Group 1)(IARC 2012) based on its carcinogenicity in many animal species and mechanistic evidence from experimental and human studies. The IARC monograph was found by hand search and is considered the best of the identified reviews. In the table below, a summary of the traditional effect biomarkers are listed based on the IARC monograph.

IARC lists the following effect biomarkers of effect both in humans (in relation to exposure to benzo[a]pyrene containing mixtures) and in rodents (exposure to benzo[a]pyrene or anti-benzo[a]pyrene-7,8-diol-9,10-oxide):

- Chromosomal aberrations
- Sister chromatid exchange
- DNA damage
- 8-oxo-deoxyguanosine formation.

IARC only mentions micronuclei as an effect marker in experimental studies.

However, our literature search identified a recent review on the induction by exposure to polycyclic aromatic hydrocarbons (PAHs) of micronuclei (MN) measured using the lymphocytes cytokinesis-block micronucleus (CBMN) assay in humans in 34 studies: 20 studies in coke oven workers, 7 in aluminum industry workers, rubber factory workers, road construction workers, airport workers and/or diesel exposed workers, 6 in environmentally exposed groups such as police, volunteers, and children (Sram et al., 2016). The authors of the review concluded that the CBMN assay may be a sensitive biomarker of PAH exposure in polluted air.

Table 8. List of the traditional cancer biomarkers for benzo[a]pyrene (IARC monograph, 2012).

Biomarkers of exposure		**Comments**
	anti-benzo[a]pyrene-7,8-diol-9,10-oxide-DNA adduct	The most commonly studied PAH-DNA adduct
		IARC: "In rats exposed to benzo[a]pyrene via oral, intratracheal and dermal routes, anti-benzo[a]pyrene-7,8-diol-9,10-oxide-DNA adducts were formed in white blood cells independently of the exposure route and their numbers correlated with those found in lung DNA, suggesting that anti-benzo[a]pyrene-7,8-diol-9,10-oxide-DNAadduct levels in white blood cells may be used as a surrogate for pulmonary anti-benzo[a]pyrene-7,8-diol-9,10-oxide-DNA adducts"
Biomarkers of effect observed in humans in relation to exposure to benzo[a]pyrene containing mixtures		
	chromosomal aberrations	
	sister chromatid exchange	IARC: "The genotoxic mechanism of action of benzo[a]pyrene involves metabolism to highly reactive species that form covalent adducts to DNA. These anti-benzo[a]pyrene-7,8-diol9,10-oxide-DNA adducts induce mutations in the K-RAS oncogene and the TP53 tumoursuppressor gene in human lung tumours, and in corresponding genes in mouse-lung tumours. Exposure to benzo[a]pyrene and benzo[a]pyrenecontaining complex mixtures also induce other genotoxic effects, including sister chromatid exchange, micronuclei, DNA damage and 8-oxodeoxyguanosine, all of which can contribute to the carcinogenic effects of benzo[a]pyrene and benzo[a]pyrene-containing complex mixtures in exposed humans.
	DNA damage (measured by the comet assay)	
	8-oxo-deoxyguanosine formation	
Experimental studies (exposure to benzo[a]pyrene or anti-benzo[a]pyrene-7,8-diol-9,10-oxide)		
	chromosomal aberrations	
	sister chromatid exchange	
	DNA damage	
	8-oxo-deoxyguanosine formation	
	micronuclei	
Relationship of biomarkers to human cancer		
	Mutations in TP53 (G→T transversions) with hotspots in codons 157, 248 and 273 are common in lung cancers from smokers and less common in nonsmokers. They are associated with anti-benzo[a]pyrene-7,8-diol-9,10-oxide-DNA adduct.	

6.4.3.2 Reproductive or Endocrine Health Effects (UCY)

The screening of abstracts under this category led to the identification of a traditional effect biomarker called the “Gail model score”. The Gail Model score is used to identify women at high risk for breast cancer. It is calculated based on age, age at menarche, age at first birth, prior breast biopsies and atypical hyperplasia, and number of first-degree relatives (mother, sisters, and daughters) diagnosed with breast cancer. PAH metabolites that create DNA adducts were studied in urine (Gaikwad et al., 2008; Gaikwad et al., 2009) and serum (Pruthi et al., 2012) from healthy, high-risk and breast cancer-diagnosed women. The mechanism of action involves formation of depurinating estrogen-DNA adducts that cause mutations and lead to carcinogenesis. Obesity and Metabolic Disorders; Cardiovascular Diseases; Allergies and immunological Diseases (BfR)

For these topics, only 14 relevant references were obtained after the initial screening of 654 abstracts. Most of these references focused on mechanistic considerations or model systems. In

consequence, only one relevant reference was identified. This paper, already mentioned in the section on “Cancer and oxidative stress”, proposes the cytokinesis-block micronucleus (CBMN) assay as biomarker for PAH in polluted air (Sram et al., 2016).

A targeted literature search on PAH-related biomarkers revealed three scientific articles not detected by the scheduled searches. Fan et al. detected 8-OHdG in human urine by HPLC-MS/MS, as already mentioned in the IARC monograph (Fan et al., 2012). Sun et al. described a dose-dependent relationship between urinary PAHs/metabolites and urinary 8-OHdG (Sun et al., 2017).

Plasma lipid damage was described as a potentially useful surrogate marker for DNA adduct formation in target tissues following exposure to BaP (Kwack et al. 2014). This marker could also be used as a biomarker of effect.

6.4.4 List of novel effect biomarkers found in the literature search

6.4.4.1 Cancer (NRCWE):

A recent review focused on the molecular fingerprints of environmental carcinogens in human cancer (Ceccaroli et al., 2015). Based on this review, the table below lists the candidates for novel effect biomarkers of cancer related to PAH exposure. However, further knowledge is needed to establish the relevance of these effect biomarkers.

Table 9. List of novel effect biomarkers found for PAHs chemical family.

Novel effect biomarker	**Brief description of the effect biomarker**	**Scoring table procedure** *(Please, specify the points assigned during the scoring procedure)*	**Final Score (max. 20 points)**
Specific microRNA (miR10, miR-122, miR-320) (Ceccaroli et al. 2015)	Benzo[a]pyrene may induce alterations of specific microRNA (miR10, miR-122 and miR-320). Circulating miRNAs are small non-coding RNAs of 22 nucleotides that can be found in most body fluids, including blood. Circulating miRNAs can act as either oncogenes or tumor suppressors and thereby affect the development and spread of a tumor. Their expression in the blood is altered in various diseases such as cancer (Khoury and Tran 2015).	*The biomarkers were assessed in cell cultures (0p). miRNAs can modulate the development and spread of a tumor by playing multiple roles as either oncogenes or tumor suppressors. However, no specific MoA or AOP between exposure and biomarkers miR10, miR-122 and miR-320 was found (0p). To our best knowledge, these biomarkers have not been implemented in epidemiologic studies (0p). More data are required to evaluate their feasibility (0p).*	0
Heat shock proteins (HSP) 70 and 27	MCF-7 cells exposed to benzo[a]pyrene, and coal tar extract showed an increased expression of the heat shock	*The biomarkers were assessed in cell cultures (0p). No specific MoA or AOP between exposure and biomarker was found (0p). To*	0

(Ceccaroli et al. 2015)	proteins (HSPs) 70 and 27. HSPs are generally involved in the cellular stress response.	*our best knowledge, these biomarkers have not been implemented in epidemiologic studies (0p). More data are needed to evaluate their feasibility (0p).*	

6.4.5 Preliminary proposal of effect biomarkers that could be implemented in HBM

Presentation and brief explanation of the selection of one, two, or more biomarkers for implementation in HBM

6.4.5.1 Cancer and oxidative stress (NRCWE):

The Comet assay is a highly sensitive method to detect DNA strand breaks. The use of frozen cells and tissues has been validated in a high-throughput protocol with fully automated scoring of DNA strand breaks using the comet assay (Jackson et al., 2013). It is an advantage that peripheral blood mononuclear cells can be stored in the freezer after sampling for analysis at a later time point. We recently showed that the level of DNA strand breaks was positively associated with dermal exposure to pyrene and ΣPAHs, and urinary excretion of 1-hydroxypyrene. In summary, we observed that PAH exposure during firefighting activity was associated with genotoxicity in peripheral blood mononuclear cells (Andersen et al., 2018). With this background, we suggest that DNA strand breaks in peripheral lymphocytes analyzed by the Comet assay could be a suitable effect biomarker for PAH exposure in HBM4EU.

6.4.6 Gaps in Knowledge

A brief description of the Gaps in Knowledge identified during the literature search and based on the previous knowledge of the PAH group partners.

6.4.6.1 Cancer and oxidative stress (NRCWE)

Biomarkers of effect are not unique to a specific type of PAH exposure. However, the identified biomarkers of effect may provide knowledge on the mechanism of PAH induced carcinogenesis. In chapter 4, we suggest that microRNAs and heat shock proteins might be candidates as novel effect biomarkers of cancer related to PAH exposure. However, further knowledge on their MoA and AOP is needed before their final evaluation as candidate novel biomarkers.

6.4.6.2 Reproductive or Endocrinal Health Effects (UCY)

The literature search under this category revealed two areas with gaps in knowledge:
Although AOPs have been described for exposure to PAHs and cancer in several studies (Menzie et al., 1992), it is not clear whether these pathways are specific for this disease.

A paper assessing the effects of petroleum streams on thyroid toxicity (Fowles et al., 2016) mainly discussed animal studies and concluded that "human epidemiology evidence found weak and inconsistent effects on the thyroid but without identification of specific chemicals involved". Two studies in petroleum workers reported a statistically significant increase in thyroid cancer, but the small number of cases could not exclude confounding variables as possible explanations. It would

therefore be interesting to investigate whether PAHs can cause thyroid disease (including cancer) and to explore the AOPs, mechanisms of action, and possible effect biomarkers.

6.4.6.3 Obesity and Metabolic Disorders; Cardiovascular Diseases; Allergies and immunological Diseases (BfR)

Due to the scant data found for these topics, no gaps in knowledge are reported.

6.4.7 Conclusions

This part of the wide literature search performed in WP14 revealed an enormous amount of scientific literature on PAHs, making it difficult to analyse the vast number of references related to PAH exposure and its adverse health effects.

6.4.7.1 Cancer (NRCWE):

NRCWE evaluated the IARC monograph on benzo[a]pyrene as the best of reviews on PAH effect biomarkers. This literature review shows that chromosomal aberrations, sister chromatid exchange, DNA damage measured by the comet assay, 8-oxo-deoxyguanosine formation, and micronuclei are established effect biomarkers in humans, related to exposure to benzo[a]pyrene containing mixtures, and in experimental studies, in which rodents were exposed to benzo[a]pyrene or anti-benzo[a]pyrene-7,8-diol-9,10-oxide.

To data, we have been unable to identify promising novel effect biomarkers.

6.4.8 References

Andersen MHG, Saber AT, Clausen PA, Pedersen JE, Lohr M, Kermanizadeh A, Loft S, Ebbehoj N, Hansen AM, Pedersen PB, Koponen IK, Norskov EC, Moller P, Vogel U Association between polycyclic aromatic hydrocarbon exposure and peripheral blood mononuclear cell DNA damage in human volunteers during fire extinction exercises. Mutagenesis. 2018; 33:105-115

Andersson JT, Achten C. Time to Say Goodbye to the 16 EPA PAHs? Toward an Up-to-Date Use of PACs for Environmental Purposes. Polycycl Aromat Compd. 2015; 35:330-354

Ceccaroli C, Pulliero A, Geretto M, Izzotti. A Molecular fingerprints of environmental carcinogens in human cancer. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2015; 33:188-228

ECHA. Substances restricted under REACH. European Chemicals Agency. 2013. ECHA. 19-4-2018

Fan R, Wang D, Ramage R, She J. Fast and simultaneous determination of urinary 8-hydroxy-2'-deoxyguanosine and ten monohydroxylated polycyclic aromatic hydrocarbons by liquid chromatography/tandem mass spectrometry. Chem Res Toxicol. 2012; 25:491-499

Fowles JR, Banton MI, Boogaard PJ, Ketelslegers HB, Rohde AM. Assessment of petroleum streams for thyroid toxicity. Toxicol Lett 2016; 254:52-62

Gaikwad NW, Yang L, Muti P, Meza JL, Pruthi S, Ingle JN, Rogan EG, Cavalieri EL. The molecular etiology of breast cancer: evidence from biomarkers of risk. Int J Cancer 2008; 122:1949-1957

Gaikwad NW, Yang L, Pruthi S, Ingle JN, Sandhu N, Rogan EG, Cavalieri EL. Urine biomarkers of risk in the molecular etiology of breast cancer. Breast Cancer (Auckl). 2009; 3:1-8

IARC. Benzo[a]pyrene. In: IARC Working Group on the Evaluation of carcinogenic risks to humans (ed) Chemical agents and related occupations. vol 100F. IARC monographs on the evaluation of carcinogenic risks to humans. International Agency for Research on Cancer; WHO, Lyon, pp. 2012; 111-144

Jackson P, Pedersen LM, Kyjovska ZO, Jacobsen NR, Saber AT, Hougaard KS, Vogel U, Wallin H. Validation of freezing tissues and cells for analysis of DNA strand break levels by comet assay. Mutagenesis 2013;28:699-707

Khoury S. Tran N Circulating microRNAs: potential biomarkers for common malignancies. Biomark Med. 2015; 9:131-151

Kwack SJ, Kim DY, Kim YJ, Roh TH, Choi SM, Lim DS, Shin HS, Kim HS, Lee BM. Potential application of benzo(a)pyrene-associated adducts (globin or lipid) as blood biomarkers for target organ exposure and human risk assessment. J Toxicol Environ Health A. 2014; 77:1491-1501

Menzie C, Potocki B, Santodonato J. Exposure to carcinogenic PAHs in the environment. Environmental Science and Technology. 1992; 26:1278-1284

Pruthi S, Yang L, Sandhu NP, Ingle JN, Beseler CL, Suman VJ, Cavalieri EL, Rogan EG. Evaluation of serum estrogen-DNA adducts as potential biomarkers for breast cancer risk. J Steroid Biochem Mol Biol. 2012; 132:73-79

Sram RJ, Svecova V, Rossnerova A Systematic review of the use of the lymphocyte cytokinesis-block micronucleus assay to measure DNA damage induced by exposure to polycyclic aromatic hydrocarbons. Mutat Res. 2016; 770:162-169

Sun H, Hou J, Zhou Y, Yang Y, Cheng J, Xu T, Xiao L, Chen W, Yuan J. Dose-response relationship between urinary polycyclic aromatic hydrocarbons metabolites and urinary 8-hydroxy-2'-deoxyguanosine in a Chinese general population. Chemosphere 2017; 174:506-514

6.5 Perfluorinated Compounds PFOS, PFOA (Coordinated by AU)

6.5.1 Terms used for the search:

List of the search terms (MeSH and Non-MeSH) used for the exposure and list of the search terms (MeSH and Non-MeSH) used for the health endpoints.

Table 10. List of search term of the literature search on PFOS, PFOA chemical family.

HBM4EU. WP14-Final Search Terms divided by Health Outcome	
Health Endpoint	PubMed search
Behavior/ Neurobehavior (n ~ 453) Abstract read-through to be conducted by **VITO**	((((((((((((("Behavior"[Mesh]) OR ("Behavior and Behavior Mechanisms"[Mesh] OR "Reproductive Behavior"[Mesh])) OR "Social Behavior Disorders"[Mesh]) OR ("Child Behavior Disorders"[Mesh] OR "Adolescent Behavior"[Mesh])) OR "Antisocial Personality Disorder"[Mesh]) OR ("Infant Behavior"[Mesh] OR "Spatial Behavior"[Mesh])) OR "Sucking Behavior"[Mesh]) OR ("Sexual Behavior, Animal"[Mesh] OR "Sexual Behavior"[Mesh])) OR ("Paternal Behavior"[Mesh] OR "Maternal Behavior"[Mesh] OR "Impulsive Behavior"[Mesh] OR "Feeding Behavior"[Mesh] OR "Exploratory Behavior"[Mesh])) OR ("Compulsive

	Behavior"[Mesh] OR "Child Behavior"[Mesh] OR "Behavior, Animal"[Mesh])) OR "Mental Disorders"[Mesh])) OR (Behavior OR Neurobehavior OR Neurodevelopment OR Neurology OR Parkinson OR Alzheimer OR Autism OR Hyperactivity OR ASD OR ADHD OR mental retardation OR IQ loss OR internalizing OR externalizing)))) AND («(PFAS NOT (patellofemoral OR "pollen-food allergy syndrome" OR "formylglycineamide" OR "phosphoribosylformylglycinamidine synthase" OR "Patient/Family Advisors" OR "posterior fossa abnormalities" OR "purine biosynthesis enzymes" OR "fetal alcohol syndrome")) OR (PFAA NOT ("plasma free amino acid" OR "plasma-free amino acids" OR "precursor-free L-amino acid supplements" OR "Profunda femoris artery aneurysms" OR "pfa cluster members")) OR Perfluorinated OR Perfluoroalkyl OR perfluorosulfonic OR perfluorocarboxylic OR Polyfluorinated OR polyfluoroalkyl OR polyfluorocarboxylic OR fluorotelomer OR (PFBS NOT ("PFB" OR "peripheral fingerstick fasting blood sugar" OR "protofilament bundles" OR "Perceived Family Burden Scale" OR "proximal forward- backward splitting" OR "pulmonary fibroblasts")) OR PFPeS OR PFHxS OR PFHpS OR (PFOS NOT ("PFO" OR "foramen ovale" OR "ferredoxin oxidoreductase“)) OR PFNS OR (PFDS NOT ("PFD" OR "personal flotation devices" OR "personal flotation device" OR "platelet function disorders" OR "platelet function disorder" OR "pelvic floor diseases" OR "pelvic floor disease" OR "pelvic floor disorders" OR "pelvic floor disorder" OR "Prion Forming Domains" OR "Prion Forming Domain" OR "pore-forming domain" OR "photon fluence density" OR "prefoldins" OR "fluence" OR "density" OR "Pelvic floor dysfunctions" OR "pulsatile flow devices" OR "plasma focus devices" OR "partial functional differential equations" OR "pre-coded food diary" OR "penicillin fermentation dregs" OR "positive fluid discrepancy" OR "pubo-femoral distance")) OR PFDoS OR PFBA OR PFPeA OR PFHxA OE PFHpA OR (PFOA NOT ("patellofemoral osteoarthritis" OR "perfringolysin")) OR (PFNA NOT ("proximal femoral nail" OR "proximal femoral nails" OR "femur" OR "trochanteric fractures")) OR PFDA OR PFUnA OR PFUA OR PFUDA OR PFUnDA OR PFDoA OR PFTrA OR PFTeA OR PFTA OR PFHxDA OR PFODA OR FTOH OR diPAP OR monoPAP OR SAmPAP OR diSAmPAP OR PFOSA OR MeFOSA OR EtFOSA OR MeFOSE OR EtFOSE OR FOSAA OR MeFOSAA OR EtFOSAA OR “Perfluorooctane sulfonamide” OR ((((((((((("perfluorobutyric acid" [Supplementary Concept]) OR "perfluoropentanoic acid" [Supplementary Concept]) OR "perfluorohexanoic acid" [Supplementary Concept]) OR "perfluoroheptanoic acid" [Supplementary Concept]) OR "perfluorooctanoic acid" [Supplementary Concept]) OR "perfluorononanoic acid" [Supplementary Concept]) OR "perfluorodecanoic acid" [Supplementary Concept]) OR "perfluoroundecanoic acid" [Supplementary Concept]) OR "perfluorododecanoic acid" [Supplementary Concept]) OR "perfluorooctane sulfonic acid" [Supplementary Concept]) OR “sulfluramid" [Supplementary Concept])»)
Cancer (N~251) Abstract read-through to be	((((((((("Neoplasms"[Mesh] OR "Uterine Cervical Neoplasms"[Mesh] OR "Urologic Neoplasms"[Mesh] OR "Liver Neoplasms"[Mesh] OR "Hereditary Breast and Ovarian Cancer Syndrome"[Mesh] OR "Early Detection of Cancer"[Mesh]) OR ("Urogenital Neoplasms"[Mesh] OR "Testicular Neoplasms"[Mesh] OR "Endometrial Neoplasms"[Mesh] OR "Vaginal

conducted by VITO	Neoplasms"[Mesh] OR "Uterine Neoplasms"[Mesh])) OR ("Prostatic Neoplasms"[Mesh] OR "Ovarian Neoplasms"[Mesh] OR "Endocrine Gland Neoplasms"[Mesh])) OR ("Breast Neoplasms"[Mesh] OR "Neoplasms, Germ Cell and Embryonal"[Mesh] OR "Tumor Microenvironment"[Mesh])) OR ("Thyroid Neoplasms"[Mesh] OR "Pituitary Neoplasms"[Mesh] OR "Brain Neoplasms"[Mesh]))) OR (Cancer OR hormone-dependent cancer OR neoplasm OR malignant tumor OR colon neoplasms OR tumor OR tumour))) AND full text[sb] AND "last 10 years"[PDat])) AND («(PFAS NOT (patellofemoral OR "pollen-food allergy syndrome" OR "formylglycineamide" OR"phosphoribosylformylglycinamidine synthase" OR "Patient/Family Advisors" OR "posterior fossa abnormalities" OR "purine biosynthesis enzymes" OR "fetal alcohol syndrome")) OR (PFAA NOT ("plasma free amino acid" OR "plasma-free amino acids" OR "precursor-free L-amino acid supplements" OR "Profunda femoris artery aneurysms" OR "pfa cluster members")) OR Perfluorinated OR Perfluoroalkyl OR perfluorosulfonic OR perfluorocarboxylic OR Polyfluorinated OR polyfluoroalkyl OR polyfluorocarboxylic OR fluorotelomer OR (PFBS NOT ("PFB" OR "peripheral fingerstick fasting blood sugar" OR "protofilament bundles" OR "Perceived Family Burden Scale" OR "proximal forward- backward splitting" OR "pulmonary fibroblasts")) OR PFPeS OR PFHxS OR PFHpS OR (PFOS NOT ("PFO" OR "foramen ovale" OR "ferredoxin oxidoreductase“)) OR PFNS OR (PFDS NOT ("PFD" OR "personal flotation devices" OR "personal flotation device" OR "platelet function disorders" OR "platelet function disorder" OR "pelvic floor diseases" OR "pelvic floor disease" OR "pelvic floor disorders" OR "pelvic floor disorder" OR "Prion Forming Domains" OR "Prion Forming Domain" OR "pore-forming domain" OR "photon fluence density" OR "prefoldins" OR "fluence" OR "density" OR "Pelvic floor dysfunctions" OR "pulsatile flow devices" OR "plasma focus devices" OR "partial functional differential equations" OR "pre-coded food diary" OR "penicillin fermentation dregs" OR "positive fluid discrepancy" OR "pubo-femoral distance")) OR PFDoS OR PFBA OR PFPeA OR PFHxA OE PFHpA OR (PFOA NOT ("patellofemoral osteoarthritis" OR "perfringolysin")) OR (PFNA NOT ("proximal femoral nail" OR "proximal femoral nails" OR "femur" OR "trochanteric fractures")) OR PFDAOR PFUnA OR PFUA OR PFUDA OR PFUnDA OR PFDoA OR PFTrA OR PFTeA OR PFTA OR PFHxDA OR PFODA OR FTOH OR diPAP OR monoPAP OR SAmPAP OR diSAmPAP OR PFOSA OR MeFOSA OR EtFOSA OR MeFOSE OR EtFOSE OR FOSAA OR MeFOSAA OR EtFOSAA OR “Perfluorooctane sulfonamide” OR ((((((((((("perfluorobutyric acid" [Supplementary Concept]) OR "perfluoropentanoic acid" [Supplementary Concept]) OR "perfluorohexanoic acid" [Supplementary Concept]) OR "perfluoroheptanoic acid" [Supplementary Concept]) OR "perfluorooctanoic acid" [Supplementary Concept]) OR "perfluorononanoic acid" [Supplementary Concept]) OR "perfluorodecanoic acid" [Supplementary Concept]) OR "perfluoroundecanoic acid" [Supplementary Concept]) OR "perfluorododecanoic acid" [Supplementary Concept]) OR "perfluorooctane sulfonic acid" [Supplementary Concept]) OR “sulfluramid" [Supplementary Concept])»)
Endocrine (N ~ 277)	((((("Endocrine System"[Mesh] OR "Endocrine Glands"[Mesh] OR "Endocrine System Diseases"[Mesh] OR "Hormones"[Mesh] OR "Gonadal

Abstract read-through to be conducted by DTU	Hormones"[Mesh] OR "Placental Hormones"[Mesh] OR "Pituitary Hormones"[Mesh] OR "Growth Hormone"[Mesh] OR "Thyroid Hormones"[Mesh] OR "Gastrointestinal Hormones"[Mesh] OR "Sex Hormone-Binding Globulin"[Mesh] OR "Adrenocorticotropic Hormone"[Mesh] OR "Adrenal Cortex Hormones"[Mesh] OR Endocrine system OR hypothyroidism OR hyperthyroidism OR adrenal)) AND full text[sb] AND "last 10 years"[PDat])) AND («(PFAS NOT (patellofemoral OR "pollen-food allergy syndrome" OR "formylglycineamide" OR "phosphoribosylformylglycinamidine synthase" OR "Patient/Family Advisors" OR "posterior fossa abnormalities" OR "purine biosynthesis enzymes" OR "fetal alcohol syndrome")) OR (PFAA NOT ("plasma free amino acid" OR "plasma-free amino acids" OR"precursor-free L-amino acid supplements" OR "Profunda femoris artery aneurysms" OR "pfa cluster members")) OR Perfluorinated OR Perfluoroalkyl OR perfluorosulfonic OR perfluorocarboxylic OR Polyfluorinated OR polyfluoroalkyl OR polyfluorocarboxylic OR fluorotelomer OR (PFBS NOT ("PFB" OR "peripheral fingerstick fasting blood sugar" OR "protofilament bundles" OR "Perceived Family Burden Scale" OR "proximal forward- backward splitting" OR "pulmonary fibroblasts")) OR PFPeS OR PFHxS OR PFHpS OR (PFOS NOT ("PFO" OR "foramen ovale" OR "ferredoxin oxidoreductase“)) OR PFNS OR (PFDS NOT ("PFD" OR "personal flotation devices" OR "personal flotation device" OR "platelet function disorders" OR "platelet function disorder" OR "pelvic floor diseases" OR "pelvic floor disease" OR "pelvic floor disorders" OR "pelvic floor disorder" OR "Prion Forming Domains" OR "Prion Forming Domain" OR "pore-forming domain" OR "photon fluence density" OR "prefoldins" OR "fluence" OR "density" OR "Pelvic floor dysfunctions" OR "pulsatile flow devices" OR "plasma focus devices" OR "partial functional differential equations" OR "pre-coded food diary" OR "penicillin fermentation dregs" OR "positive fluid discrepancy" OR "pubo-femoral distance")) OR PFDoS OR PFBA OR PFPeA OR PFHxA OE PFHpA OR (PFOA NOT ("patellofemoral osteoarthritis" OR "perfringolysin")) OR (PFNA NOT ("proximal femoral nail" OR "proximal femoral nails" OR "femur" OR "trochanteric fractures")) OR PFDA OR PFUnA OR PFUA OR PFUDA OR PFUnDA OR PFDoA OR PFTrA OR PFTeA OR PFTA OR PFHxDA OR PFODA OR FTOH OR diPAP OR monoPAP OR SAmPAP OR diSAmPAP OR PFOSA OR MeFOSA OR EtFOSA OR MeFOSE OR EtFOSE OR FOSAA OR MeFOSAA OR EtFOSAA OR “Perfluorooctane sulfonamide” OR ((((((((((("perfluorobutyric acid" [Supplementary Concept]) OR "perfluoropentanoic acid" [Supplementary Concept]) OR "perfluorohexanoic acid" [Supplementary Concept]) OR "perfluoroheptanoic acid" [Supplementary Concept]) OR "perfluorooctanoic acid" [Supplementary Concept]) OR "perfluorononanoic acid" [Supplementary Concept]) OR "perfluorodecanoic acid" [Supplementary Concept]) OR "perfluoroundecanoic acid" [Supplementary Concept]) OR "perfluorododecanoic acid" [Supplementary Concept]) OR "perfluorooctane sulfonic acid" [Supplementary Concept]) OR “sulfluramid" [Supplementary Concept])»)
Immune System AND Allergy	((((("Allergy and Immunology"[Mesh] OR "Hypersensitivity"[Mesh] OR "Rhinitis, Allergic, Seasonal"[Mesh] OR "Food Hypersensitivity"[Mesh] OR "Drug Hypersensitivity"[Mesh] OR "Shellfish Hypersensitivity"[Mesh] OR

(N ~ 159) Abstract read-through to be conducted by VITO	Allergy OR Hypersensitive OR respiratory allergy OR gastrointestinal allergy OR multiple chemical sensitivity OR allergic hypersensitivity disease OR contact allergy OR "Immune System"[Mesh] OR "Immune System Diseases"[Mesh] OR Immune system OR autoimmune disease OR cytokines OR white cells OR innate immune system OR adaptive immune system)) AND full text[sb] AND "last 10 years"[PDat])) AND («(PFAS NOT (patellofemoral OR "pollen-food allergy syndrome" OR "formylglycineamide" OR "phosphoribosylformylglycinamidine synthase" OR "Patient/Family Advisors" OR "posterior fossa abnormalities" OR "purine biosynthesis enzymes" OR "fetal alcohol syndrome")) OR (PFAA NOT ("plasma free amino acid" OR "plasma-free amino acids" OR "precursor-free L-amino acid supplements" OR "Profunda femoris artery aneurysms" OR "pfa cluster members")) OR Perfluorinated OR Perfluoroalkyl ORperfluorosulfonic OR perfluorocarboxylic OR Polyfluorinated OR polyfluoroalkyl OR polyfluorocarboxylic OR fluorotelomer OR (PFBS NOT ("PFB" OR "peripheral fingerstick fasting blood sugar" OR "protofilament bundles" OR "Perceived Family Burden Scale" OR "proximal forward- backward splitting" OR "pulmonary fibroblasts")) OR PFPeS OR PFHxS OR PFHpS OR (PFOS NOT ("PFO" OR "foramen ovale" OR "ferredoxin oxidoreductase“)) OR PFNS OR (PFDS NOT ("PFD" OR "personal flotation devices" OR "personal flotation device" OR "platelet function disorders" OR "platelet function disorder" OR "pelvic floor diseases" OR "pelvic floor disease" OR "pelvic floor disorders" OR "pelvic floor disorder" OR "Prion Forming Domains" OR "Prion Forming Domain" OR "pore-forming domain" OR "photon fluence density" OR "prefoldins" OR "fluence" OR "density" OR "Pelvic floor dysfunctions" OR "pulsatile flow devices" OR "plasma focus devices" OR "partial functional differential equations" OR "pre-coded food diary" OR "penicillin fermentation dregs" OR "positive fluid discrepancy" OR "pubo-femoral distance")) OR PFDoS OR PFBA OR PFPeA OR PFHxA OE PFHpA OR (PFOA NOT ("patellofemoral osteoarthritis" OR "perfringolysin")) OR (PFNA NOT ("proximal femoral nail" OR "proximal femoral nails" OR "femur" OR "trochanteric fractures")) OR PFDA OR PFUnA OR PFUA OR PFUDA OR PFUnDA OR PFDoA OR PFTrA OR PFTeA OR PFTA OR PFHxDA OR PFODA OR FTOH OR diPAP OR monoPAP OR SAmPAP OR diSAmPAP OR PFOSA OR MeFOSA OR EtFOSA OR MeFOSE OR EtFOSE OR FOSAA OR MeFOSAA OR EtFOSAA OR “Perfluorooctane sulfonamide” OR ((((((((((("perfluorobutyric acid" [Supplementary Concept]) OR "perfluoropentanoic acid" [Supplementary Concept]) OR "perfluorohexanoic acid" [Supplementary Concept]) OR"perfluoroheptanoic acid" [Supplementary Concept]) OR "perfluorooctanoic acid" [Supplementary Concept]) OR "perfluorononanoic acid" [Supplementary Concept]) OR "perfluorodecanoic acid" [Supplementary Concept]) OR "perfluoroundecanoic acid" [Supplementary Concept]) OR "perfluorododecanoic acid" [Supplementary Concept]) OR "perfluorooctane sulfonic acid" [Supplementary Concept]) OR “sulfluramid" [Supplementary Concept])»)
Obesity, Metabolic AND Cardiovascular (N ~ 1183)	((((("Metabolic Syndrome"[Mesh] OR "Nutritional and Metabolic Diseases"[Mesh] OR "Metabolic Diseases"[Mesh] OR "Metabolism"[Mesh] OR "Glucose Metabolism Disorders"[Mesh] OR "Acidosis"[Mesh] OR "Metabolome"[Mesh] OR "Metabolomics"[Mesh] OR "Receptor,

Abstract read-through to be conducted by RIVM	Insulin"[Mesh] OR "Lipolysis"[Mesh] OR "Gout"[Mesh] OR "Diabetes Mellitus, Type 2"[Mesh] OR "Acidosis, Renal Tubular"[Mesh] OR "Homocysteinemia" [Supplementary Concept] OR "Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha"[Mesh] OR "Obesity"[Mesh] OR "Pediatric Obesity"[Mesh] OR "Obesity, Abdominal"[Mesh] OR "Abdominal obesity metabolic syndrome" [Supplementary Concept] OR "Cardiovascular System"[Mesh] OR "Cardiovascular Abnormalities"[Mesh] OR "Pregnancy Complications, Cardiovascular"[Mesh] OR "Cardiovascular Diseases"[Mesh] OR "Myocardial Infarction"[Mesh] OR obesity OR abdominal obesity OR waist hip ratio OR adipose tissue OR adypokine OR visceral fat OR body fat OR overweight OR Metabolic OR Metabolic disorder OR Metabolic syndrome OR glucose homeostasis OR Hyperlipidemia OR Dyslipidemia OR hypertriglyceridemia OR HOMA-IR OR insulin resistance OR pancreas OR liver OR kidney)) AND full text[sb] AND "last 10 years"[PDat])) AND («(PFAS NOT (patellofemoral OR "pollen-food allergy syndrome" OR "formylglycineamide" OR "phosphoribosylformylglycinamidine synthase" OR "Patient/Family Advisors" OR "posterior fossa abnormalities" OR "purine biosynthesis enzymes" OR "fetal alcohol syndrome")) OR (PFAA NOT ("plasma free amino acid" OR "plasma-free amino acids" OR "precursor-free L-amino acid supplements" OR "Profunda femoris artery aneurysms" OR "pfa cluster members")) OR Perfluorinated OR Perfluoroalkyl OR perfluorosulfonic OR perfluorocarboxylic OR Polyfluorinated OR polyfluoroalkyl OR polyfluorocarboxylic OR fluorotelomer OR (PFBS NOT ("PFB" OR "peripheral fingerstick fasting blood sugar" OR "protofilament bundles" OR "Perceived Family Burden Scale" OR "proximal forward-backward splitting" OR "pulmonary fibroblasts")) OR PFPeS OR PFHxS OR PFHpS OR (PFOS NOT ("PFO" OR "foramen ovale" OR "ferredoxin oxidoreductase“)) OR PFNS OR (PFDS NOT ("PFD" OR "personal flotation devices" OR "personal flotation device" OR "platelet function disorders" OR "platelet function disorder" OR "pelvic floor diseases" OR "pelvic floor disease" OR "pelvic floor disorders" OR "pelvic floor disorder" OR "Prion Forming Domains" OR "Prion Forming Domain" OR "pore-forming domain" OR "photon fluence density" OR "prefoldins" OR "fluence" OR "density" OR "Pelvic floor dysfunctions" OR "pulsatile flow devices" OR "plasma focus devices" OR "partial functional differential equations" OR "pre-coded food diary" OR "penicillin fermentation dregs" OR "positive fluid discrepancy" OR "pubo-femoral distance")) OR PFDoS OR PFBA OR PFPeA OR PFHxA OE PFHpA OR (PFOA NOT ("patellofemoral osteoarthritis" OR "perfringolysin")) OR (PFNA NOT ("proximal femoral nail" OR "proximal femoral nails" OR "femur" OR "trochanteric fractures")) OR PFDA OR PFUnA OR PFUA OR PFUDA OR PFUnDA OR PFDoA OR PFTrA OR PFTeA OR PFTA OR PFHxDA OR PFODA OR FTOH OR diPAP OR monoPAP OR SAmPAP OR diSAmPAP OR PFOSA OR MeFOSA OR EtFOSA OR MeFOSE OR EtFOSE OR FOSAA OR MeFOSAA OR EtFOSAA OR “Perfluorooctane sulfonamide” OR ((((((((((("perfluorobutyric acid" [Supplementary Concept]) OR "perfluoropentanoic acid" [Supplementary Concept]) OR "perfluorohexanoic acid" Supplementary Concept]) OR "perfluoroheptanoic acid" [Supplementary Concept]) OR "perfluorooctanoic acid" [Supplementary Concept]) OR "perfluorononanoic acid" [Supplementary

	Concept]) OR "perfluorodecanoic acid" [Supplementary Concept]) OR "perfluoroundecanoic acid" [Supplementary Concept]) OR "perfluorododecanoic acid" [Supplementary Concept]) OR "perfluorooctane sulfonic acid" [Supplementary Concept]) OR “sulfluramid" [Supplementary Concept])»)
Reproductive (N ~ 563) Abstract read-through to be conducted by **AU**	((((reproductive OR puberty OR pregnancy OR infertility OR semen quality OR placenta OR anogenital distance OR hypospadia OR cryptorchidism OR "Reproductive Health"[Mesh] OR "Reproductive Medicine"[Mesh] OR "Reproduction"[Mesh] OR "Reproductive Techniques, Assisted"[Mesh] OR "Infertility"[Mesh])) AND full text[sb] AND "last 10 years"[PDat])) AND («(PFAS NOT (patellofemoral OR "pollen-food allergy syndrome" OR "formylglycineamide" OR "phosphoribosylformylglycinamidine synthase" OR "Patient/Family Advisors" OR "posterior fossa abnormalities" OR "purine biosynthesis enzymes" OR "fetal alcohol syndrome")) OR (PFAA NOT ("plasma free amino acid" OR "plasma-free amino acids" OR "precursor-free L-amino acid supplements" OR "Profunda femoris artery aneurysms" OR "pfa cluster members")) OR Perfluorinated OR Perfluoroalkyl OR perfluorosulfonic OR perfluorocarboxylic OR Polyfluorinated OR polyfluoroalkyl OR polyfluorocarboxylic OR fluorotelomer OR (PFBS NOT ("PFB" OR "peripheral fingerstick fasting blood sugar" OR "protofilament bundles" OR "Perceived Family Burden Scale" OR "proximal forward-backward splitting" OR "pulmonary fibroblasts")) OR PFPeS OR PFHxS OR PFHpS OR (PFOS NOT ("PFO" OR "foramen ovale" OR "ferredoxin oxidoreductase“)) OR PFNS OR (PFDS NOT ("PFD" OR "personal flotation devices" OR "personal flotation device" OR"platelet function disorders" OR "platelet function disorder" OR "pelvic floor diseases" OR "pelvic floor disease" OR "pelvic floor disorders" OR "pelvic floor disorder" OR "Prion Forming Domains" OR "Prion Forming Domain" OR "pore-forming domain" OR "photon fluence density" OR "prefoldins" OR "fluence" OR "density" OR "Pelvic floor dysfunctions" OR "pulsatile flow devices" OR "plasma focus devices" OR "partial functional differential equations" OR "pre-coded food diary" OR "penicillin fermentation dregs" OR "positive fluid discrepancy" OR "pubo- femoral distance")) OR PFDoS OR PFBA OR PFPeA OR PFHxA OE PFHpA OR (PFOA NOT ("patellofemoral osteoarthritis" OR "perfringolysin")) OR (PFNA NOT ("proximal femoral nail" OR "proximal femoral nails" OR "femur" OR "trochanteric fractures")) OR PFDA OR PFUnA OR PFUA OR PFUDA OR PFUnDA OR PFDoA OR PFTrA OR PFTeA OR PFTA OR PFHxDA OR PFODA OR FTOH OR diPAP OR monoPAP OR SAmPAP OR diSAmPAP OR PFOSA OR MeFOSA OR EtFOSA OR MeFOSE OR EtFOSE OR FOSAA OR MeFOSAA OR EtFOSAA OR “Perfluorooctane sulfonamide” OR ((((((((((("perfluorobutyric acid" [Supplementary Concept]) OR "perfluoropentanoic acid" [Supplementary Concept]) OR "perfluorohexanoic acid" [Supplementary Concept]) OR "perfluoroheptanoic acid" [Supplementary Concept]) OR "perfluorooctanoic acid" [Supplementary Concept]) OR "perfluorononanoic acid" [Supplementary Concept]) OR "perfluorodecanoic acid" [Supplementary Concept]) OR "perfluoroundecanoic acid" [Supplementary Concept]) OR "perfluorododecanoic acid" [Supplementary Concept]) OR "perfluorooctane sulfonic acid" [Supplementary Concept]) OR “sulfluramid")

6.5.2 Exploratory search

The table below displays a brief summary of the distribution of references among partners.

Table 11. Division of relevant references among partners from PFOs PFOAs chemical family

	Experimental (in vitro)	Experimental (animal)	Epidemiologic / clinic
OMICS	sorted by biomarker endpoint	sorted by biomarker endpoint	sorted by biomarker endpoint
Molecular / genetic testing	sorted by biomarker endpoint	sorted by biomarker endpoint	sorted by biomarker endpoint
Oxidative stress	sorted by biomarker endpoint	sorted by biomarker endpoint	sorted by biomarker endpoint
DNA damage	sorted by biomarker endpoint	sorted by biomarker endpoint	sorted by biomarker endpoint
Ex vivo cell based assays	sorted by biomarker endpoint	sorted by biomarker endpoint	sorted by biomarker endpoint
Epidemiological biomarkers			Sorted by outcome (e.g. anthropometric vs fertility) and population
In vitro cell based assays	sorted by type of biomarker and type of cell line		
Animal studies		sorted by type of animal and type of biomarker	
Other methods	sorted by biomarker endpoint	sorted by biomarker endpoint	sorted by biomarker endpoint

- OMICS / molecular or genetic testing (RIVM)
- Oxidative stress / DNA damage (VITO)
- Other epidemiological / ex vivo (AU)
- Other animal / other in vitro (DTU)
- Other methods (AU, VITO, RIVM)

- Not relevant

Table 12. Relevant references among ex-vivo studies

Table 13. Relevant references among ex-vivo studies

AU-DK	Epidemiology	Animal	*In vitro*	*Others*
Neurological	30	-	-	0
Cancer	14	-	-	0
Endocrine	41	-	-	0
Immunological	26	-	-	0
Metabolic / Cardiovascular	61	-	-	0
Reproduction	85	-	-	8
Total	**257**	**-**	**-**	**8**

AU-DK	Epidemiology	Animal	*In vitro*	*Others*
Neurological	0	0	0	0
Cancer	1	0	0	0
Endocrine	0	1	0	0
Immunological	0	0	0	0
Metabolic / Cardiovascular	0	0	0	0
Reproduction	2	0	0	0
Total	**3**	**1**	**0**	**0**

6.5.3 List of traditional effect biomarkers

6.5.3.1 Reproductive

- **Fetal growth /size at birth:**

Approximately 80% of studies (n= 40) on **PFAS exposure vs. birth weight (BW)** reported a significantly inverse relationship, 14 % an inverse but not significant relationship and 8% no relationship. Most studies were on PFOA and PFOS. There are fewer studies on the other fetal growth parameters (BL, LBW, HC, PI, PW, BC and AC), and the data are less consistent. See table *'"Fetal growth"* above for references. One study showed that the inverse relationship between PFAS exposure and BW was independent of gestational diabetes (Alkhalawi et al., 2016).

Interestingly, it was reported that PFOS and adiponectin in cord blood show a marginal dose-response relationship and that both PFOS and PFOA show a significant dose-response relationship with BW. Thus, fetal PFAS exposure might alter adiponectin levels and might decrease

BW. Adiponectin may be a novel biomarker of PFOS and PFOA environmental exposure releted with birth weight.

Below the most relevant studies relating PFAS and fetal growth are showed:

* Two studies showed that maternal PFOS (1 PFOA) concentrations are positively associated with GWG (Andersen et al., 2010).

* One study found PFAS exposure vs. decreased anogenital distance in girls (Antignac et al., 2013).

* Out of 5 studies on preeclampsia, 3 found positive association with PFAS exposure (Ashley-Martin et al., 2017; Ashley-Martin et al., 2016; Avanasi et al. 2016) 1 no association Bach et al., 2016).

* PFOS and PFOA were positive associated with pregnancy hypertension (Darrow et al., 2013).

* Three studies showed no association between PFAS exposure and cryptorchidism or hypospadias (Toft et al., 2016; Vesterholm et al., 2014; Nolan et al., 2010).

* One study found that prenatal exposure to PFAS increased risk of cerebral palsy in boys (Liew et al., 2014).

* No clear relationship between PFAS and miscarriage (Louis et al., 2016; Jensen et al., 2015.; Darrow et al,. 2014).

* High PFOA in China was inversely related to BW, GA, BL and APGAR scores (Wu et al., 2012).

* One study suggest dose-response between MeFOSAA EXPOSURE and reversal secondary sex ratio (SSR) with excess of females (Bae et al., 2015).

* Three studies suggest that maternal levels of PFAS might reduce months of breastfeeding (Timmermann et al., 2017; Romano et al., 2016; Fei et al., 2010).

* Two out of three studies showed that prenatal exposure to PFAS might delay menarche (doi: Kristersen et al., 2013; Lopez-Espinosa et al., 2011; Christensen et al., 2011).

* Three studies on PFAS exposure suggest that it might be related to abnormal menstruation and length (doi: Zhou et al., 2017; Lum et al., 2017; doi: Lyngsø et al., 2014).

* Five studies (63%) out of eight studies found no association between female fertility, the three studies that found an association might partly be explained by higher PFAS levels (Whitworth et al., 2016; Bach et al., 2015; Bach et al., 2015; doi: Vélez et al., 2015; Vestergaard et al., 2012; Whitworth et al., 2012; Fei et al., 2009; Jørgensen et al., 2014).

* Four studies on PFAS exposure related to varian/oocyte function/fertilization, in ART, were inconsistent.

* One study suggests that PFBS may increase risk of female infertility due to endometriosis (Wang et al., 2017).

* PFOSA was associated with reduced female fecundability (Buck et al., 2013)

- Male reproduction:

* Out of 7 studies, 4 (57%) showed an inverse relationship between PFAS exposure and semen quality/count (Louis et al., 2015; Vested et al., 2013; Toft et al., 2012) one found a non-significant

trend (Joensen et al., 2009) and two observed no association (Raymer et al., 2012; Den Hond et al., 2015).

* Interestingly, two studies found that PFAS exposure was associated with higher level of plasma/semen luteinizing hormone (Vested et al., 2013; Toft et al., 2012); and another reported that luteinizing hormone was positively correlated with plasma PFOA and PFOS, but not with semen PFOS (Raymer et al., 2012).

6.5.3.2 Biochemical/physiological biomarkers in reproduction

- Cholesterol /Lipid

PFOA and PFOA were positively associated with serum cholesterol in pregnancy (Skuladottir et al., 2015; Starling et al., 2014). PFOS and PFOA were negatively associated with fatty acids and total triglycerides (Kishi et al., 2015).

- Albumin

Most PFAS were significantly associated with albumin, suggesting they may be immunotoxic chemicals (Jiang et al. 2014).

- Sex chromosomes

A negative trend was found between Y:X ratio and PFOS exposure among Inuits (Kvist et al., 2012).

6.5.3.3 Diabetes

- Gestational diabetes

PFAS was negatively associated with triglycerides and positively asociated with total cholesterol. Hence, PFAS exposure during pregnancy can influence lipid metabolism and glucose tolerance and impact on maternal and child health (Matilla-Santander et al., 2017); impaired gestational glucose tolerance (Shapiro et al., 2016); gestational diabetes (Zhang et al., 2015).

- Metabolic Syndrome

Increased serum PFNA concentrations were associated with high blood pressure, obesity, hypertriglyceridemia, suggesting bodily retention of PFASs and their association with the metabolic syndrome (Yang et al., 2018).

- Cardio-metabolic risk

PFAS was negatively associated with bone parameters (children) and positively associated with increased LDL-cholesterol and total cholesterol (Khalil et al., 2018; Manzano-Salgado et al., 2017; Koshy et al., 2017).

- Kidney Function

Option: Reduced kidney function is the cause rather than result of increased serum PFOA, suggesting caution in using biomarkers for cross-sectional studies (Dhindra et al., 2017; Watkins et al., 2013).

Adults: PFAAs are associated with a reduction in kidney function and increased uric acid levels in otherwise healthy adolescents, and suggest that the study should be done in children.

Inconsistent results on the association between PFAS exposure and chronic kidney disease (Dhingra et al., 2016; Shankar et al., 2011).

Children: Serum levels of PFOA and PFOS in children positively associated with hyperuricemia (high level of uric acid in blood), independent of age, sex, race/ethnicity, body mass index, annual household income, physical activity, serum total cholesterol, and serum cotinine levels (Geiger et al., 2013), as well as in general US population (Shankar et al., 2011).

- Liver Function

Results consistent with previous cross-sectional studies showing association between PFOA and Alanine aminotransferase (ALT), a marker of hepatocellular damage (Darrow et al., 2016).

Evidence of associations of biomarkers of liver function and uric acid with PFHxS, PFOS, PFOA, and PFNA at levels found in the general U.S. population. PFOA was associated with uric acid, ALT, GGT, and total bilirubin. PFNA was linearly associated with ALT ($p < 0.001$), and there was a statistically significant increase (Gleason et al., 2015).

PFOS and PFOA show positive association with alanine aminotransferase (Gallo et al., 2012.

Gilbert syndrome (GS) might be associated with PFHxA. GS is a mild liver disorder in which the liver does not properly process bilirubin (Fan et al., 2014).

- Atherosclerosis / Coronary heart disease/ Cardiovascular disease

Atherosclerosis: a pronounced gender difference was observed in associations of some PFASs, especially the long-chain PFUnDA, with markers of atherosclerosis, whih were stronger in women. Coronary heart disease: previously reported cross-sectional relationship between PFAS levels and CHD risk, finding a significant association with PFHpA (Mattson et al., 2015).

Higher PFOA exposure was associated with medically controlled incident hypercholesterolemia but not with hypertension or coronary artery disease (Winquis et al., 2014).

Cardiovascular disease (CVD): exposure to PFOA is associated with CVD and peripheral arterial disease (PAD) independently of traditional cardiovascular risk factors (Shankar et al., 2012).

Stroke: only modest evidence of an association between PFOA and stroke incidence (Simpson et al., 2013).

Hypertension: background exposure to PFOA may be a risk factor for cardiovascular disease (Min et al., 2012).

- Bone density

Serum PFAS concentrations were associated with lower bone mineral density, which varied according to the specific PFAS and bone site. Most associations found in women from US adult population. Osteoporosis in women was also associated with PFAS exposure, based on a small number of cases (Khalil et al., 2016).

Osteoarthritis (OA): divergent associations observed with PFOA and PFOS (OA is a type of joint disease that results from breakdown of joint cartilage and underlying bone (Innes et al., 2011).

6.5.3.4 Biochemical/physiological biomarkers for metabolism

Below the most relevant studies relating PFAS and biochemical/physiological metabolism biomarkers are showed:

Cholesterol: positive association of PFOS and PFOA with cholesterol despite low exposure. Results for PFNA and PFHxS are novel (Nelson et al., 2010). In addition, positive associations of plasma PFOA and PFOS with total cholesterol were found in a middle-aged Danish population (Eriksen et al., 2013).

Aminotransferases: Growing evidence that exposure to per- and polyfluoroalkyl substances (PFASs) may disrupt lipid homeostasis and liver function. Prenatal and mid-childhood PFAS exposure may be associated with modest but somewhat conflicting changes in lipid profile and ALT levels in children (Mora et al., 2018; Mora et al., 2018).

Lipids: findings indicate that serum PFOA and PFOS are significantly associated with dyslipidemia in adolescents, even at the lower "background" exposure levels of the US general population (Geiser et al., 2014).

Lipid/Cholesterol: significant associations of PFHxS but not of PFOS or PFOS with cholesterol outcomes (LDL, TC, NON-HDL, TC/HDL ratio) (Fisher et al., 2013).

An increase in HDL was associated with an increase in PFOA, although the magnitude was small. No associations were found between PFOA or PFOS and non-HDL cholesterol, HDL, or clinical hepatic chemistries (Olsen et al., 2012).

Exposure to low PFOA levels during prenatal development may alter lipid metabolism later in life. Given the small sample size, this association must be verified in large independent cohorts (Maisinet et al., 2015).

Adiponectin: Higher serum PFNA concentrations are associated with elevated serum adiponectin concentration. Plasma adiponectin level is highly responsive to PPAR gamma agonist drugs, but it was not previously established whether adiponectin levels are related to serum PFC levels (Lin et al., 2011).

- Hepatic enzymes

Serum levels of hepatic enzymes (GOT, GPT) and ω-3 polyunsaturated fatty acids DHA and EPA, showed significant positive correlations with PFOS and PFOA in blood (Yamaguchi et al., 2013). Moreover, higher serum concentrations of PFOA may cause liver enzymes to increase abnormally in the general population, particularly in obese individuals (Lin et al., 2010).

6.5.3.5 Anthropometric biomarkers:

Below the most relevant studies relating PFAS and anthropometric biomarkers are showed:

- Adiposity

Prenatal exposure to PFOA and PFOS was associated with girls' %BodyFat within some strata of maternal education status. PFHxS and PFNA were not associated with %BF (Hartman et al., 2017).

Childhood exposure to PFOS and PFOA can predict adiposity at 15 and 21 years of age, as well as impaired β-cell function at 15 years of age (Domazet et al., 2016).

Prenatal exposure to PFASs was associated with small increases in adiposity measurements in mid-childhood, but only among girls (Mora et al., 2017).

Higher prenatal serum PFOA concentrations were associated with greater adiposity at 8 years and a more rapid increase in BMI between 2 and 8 years of age (Braun et al., 2016).

Increased PFC exposure in overweight 8- to 10-year-old children was associated with higher insulin and triglyceride concentrations. Chance findings may explain some of the results, and the cross-sectional design means that reverse causation cannot be ruled out. These findings therefore need to be confirmed in longitudinal studies (Timmenmann et al., 2014).

- Overweight/obesity

Positive associations were found between maternal serum PFAS concentrations and child overweight-obesity at 5-year follow-up, particularly among Norwegian participants. There was also some evidence among Swedish participants (Lauritzen et al., 2018)

In cross-sectional analyses, inverse associations were found between serum POPs and BMI z-scores at age of 5 years; the possibility of reverse causation should be tested in prospective studies. Findings in a recent cohort support a role for maternal exposure to endocrine disruptors in the childhood obesity epidemic. Significant data for PFOS – PFOA at ages 18 months and/or 5 years (Karlsen et al., 2017).

Prenatal PFOA and PFOS exposure may be associated with child waist-to-height ratio, but not with overweight (Høyer et al., 2015).

Elevated levels of PFOA exposure in early life were not associated with overweight or obesity risk in adulthood and results did not vary by sex (Barry et al., 2014).

Plasma levels of PFOS and PFOA in pregnant women did not seem to have any appreciable influence on their children's anthropometry (Andersen et al., 2013).

Low-dose developmental exposures to PFOA are in line with experimental results suggesting obesogenic effects in female offspring at 20 years of age (Halldorson et al., 2012).

6.5.3.6 Miscellaneous diseases

Ulcerative colitis and rheumatoid arthritis were positively linked to PFOA exposure in workers (Steenland et al., 2015).

Evidence of positive exposure-response relation for malignant and non-malignant renal disease has been published among 5,791 workers exposed to PFOA at a DuPont chemical plant in West Virginia. The results were limited by small numbers and restriction to mortality data (Steenland et al., 2012).

In male workers a significant association of total cholesterol and uric acid was found with serum PFOA levels. Likely interference by PFOA with intermediate metabolism deserves further investigation (Costa et al., 2009). Moreover, Ammonium perfluorooctanoate exposure was not associated with liver, pancreatic, or testicular cancer or cirrhosis of the liver. However, exposure was associated (albeit inconsistently) with prostate cancer, cerebrovascular disease, and diabetes, in 3993 employees of an ammonium perfluorooctanoate (APFO) manufacturing facility (Lundin et al., 2009).

6.5.3.7 Neurological effects

- Developmental milestones

Below the most relevant studies relating PFAS and neurological biomarkers are showed:

No convincing associations have been reported between developmental milestones in early childhood (6-18 months) and levels of PFOA or PFOS measured in maternal plasma early in pregnancy (Fei et al., 2008).

Prenatal exposure to PFOS but not PFOA, may affect children's development, especially gross-motor development, at 2 years of age (Chen et al., 2013).

Prenatal exposure to PFOS and PFOA may have a small to moderate effect on children's neurobehavioral development, specifically in terms of hyperactive behavior (5 year). The associations were strongest in Greenland, where the exposure was highest (Høyer et al., 2018; Høyer et al., 2015).

No association of perinatal PFOS and PFOA exposure with early neuropsychological development was found in a Norwegian birth cohort. Further longitudinal studies are needed (Forns et al., 2015).

Prenatal PFOA exposure may affect female mental scales of neurodevelopment at 6 months of age (Goudarzi et al., 2016).

No association found between exposure to PFAS and adult depression in two studies (Nerk et al., 2014; Shiue, 2015).

- Attention and behavior

Fifteen studies on ASD/ADHD and behavior at 18 months and at 5, 7, 9, 12, and 15 years were found. Some positive associations were observed, but no clear significant and convincing correlation was found between PFAS exposure and risk of ASD/ADHD. More studies are recommended.

One study suggests an association between PFAS exposure and child impulsivity (Gump et al., 2011)

Sleep trouble: Higher levels of urinary arsenic, phthalates, and polyfluoroalkyl compounds were associated with waking up at night in adults (Shiue, 2017).

- Cognition or IQ.

PFAS might be protective for elderly diabetes (Power et al., 2013; Shiue et al., 2015).

Children from Taiwan, with higher prenatal PFNA levels, had lower VIQ with an adjusted β of -2.1 (95% CI: -3.9, -0.2). Prenatal exposure to two long-chain PFASs were associated with decreased IQ test scores in children. Further research warranted on long-chain PFASs and child neurodevelopment (Wang et al., 2015)

Children (from US) in the highest versus lowest quartile of estimated in utero PFOA exposure showed negligible associations of PFOA with reading and math skills or neuropsychological functioning. These results do not suggest an adverse association between the PFOA exposure experienced by children in this cohort and their performance in neuropsychological tests (Stein et al., 2013).

6.5.3.8 Endocrine diseases

- Polycystic ovarian syndrome

PCOS patients may differ from controls in their environmental contaminant profile. PCOS subjects had higher serum concentrations of two PFCs, PFOA and PFOS, and lower urine concentrations of mBP and mBzP (Vagi et al., 2014).

Significant inverse association was found between PFOS and estradiol in perimenopausal (β = -3.65; $p < 0.0001$) and menopausal age groups (β = -0.83; $p = 0.007$) but not between PFOA and estradiol. The PFCs were associated with endocrine disruption in women, and further research on mechanisms is warranted (Knox et al., 2011).

- Thyroid disease

Higher PFOA exposure was associated with incident functional thyroid disease in this large cohort with high exposure (Winquist et al., 2014).

Higher concentrations of serum PFOA and PFOS are associated with current thyroid disease in the U.S. general adult population. More work is needed to establish the mechanisms involved and to rule out confounders and pharmacokinetic explanations. (Melzer et al., 2010).

There was no support for the view that PFOA and PFOS are actively concentrated in the thyroid. However, PFOA and PFOS are both found in surgical and autopsy thyroid specimens. Therefore, further studies are needed to determine whether they have disrupting effects in thyroid cells or tissue (Pirali et al., 2009).

Findings do not support a causal link between PFA exposure and maternal hypothyroxinemia in the studied population (Chan et al., 2011).

6.5.3.9 Biochemical/physiological biomarkers

- Reproductive hormones

Results suggest that the fetal synthesis and secretion of reproductive hormones may be affected by in utero exposure to measurable levels of PFOS and PFOA (Itoh et al., 2016).

One study reported that prenatal exposure to some PFAAs (PFOS, PFOA, PFHxS) may alter testosterone concentrations in females; however, findings were based on a small study sample and should be interpreted with caution (Maisonet et al., 2015).

Serum concentrations of PFOA, PFOS, and PFUA were negatively associated with serum levels of SHBG, FSH, and testosterone in a young Taiwanese population, and these effects were strongest in females aged 12-17 (Tsai et al., 2015).

PFOS and perfluorooctanesulfonic acid may be associated with decreased production of E2 and progesterone in reproductive age women (Barrett et al., 2015).

PFOS levels were negatively associated with testosterone (T), calculated free testosterone (FT), free androgen index (FAI), and T/LH, FAI/LH and FT/LH ratios. Other PFCs were found at lower levels versus PFOS and did not exhibit the same associations. PFC levels were not significantly associated with semen quality. PFOS levels in these samples, collected in 2008-2009, were lower than in a previous study of men in 2003 (Joensene t al., 2013).

Higher levels of PFASs were associated with lower testosterone and higher estradiol levels. More significant associations of PFASs with reproductive hormones were found in males than in females (Zhou et al., 2016)

- Thyroid hormones

PFOS and ∑8PFASs were significantly negatively correlated with FT3 and FT4 and positively correlated with TSH; while PFPeA and PFHxA were significantly positively correlated with TGAb and TMAb in all samples. Opposite associations of FT3 and TSH with PFOS, PFOA, and PFHxS were found in hypothyroidism and hyperthyroidism groups, indicating that PFOS, PFOA and PFHxS enhance negative feedback mechanisms of the thyroid gland (Chinese general population) (Li et al., 2017).

No evidence was found of mediation by THs of PFOA-neuropsychological function associations. However, T4 and fT4 partially mediated the protective effect of PFOS on BDT (Block Design Subtest) total scores. Findings do not suggest an association of PFASs with poor neuropsychological function. There was some evidence of mediation by THs in the association between PFASs and neuropsychological functions in “older adults” (Shrestha et al., 2017).

Cord blood thyroid hormone levels are affected by PFASs, finding a negative association between T4 and PFOS and a positive association between TSH and PFOS. The causal associations of thyroid hormones and PFASs require further exploration (Tsai et al., 2017).

Background exposure to POPs can alter maternal thyroid homeostasis. Several POPs were significantly associated with TSH and THs: a) PFOS was positively associated with TSH; b) PCBs, HCB, and nonachlors were inversely associated with T3, T4, and FT4; and, c) PFDA and PFUnDA were inversely associated with T3 and FT3. Upon adjustment, PFDA and PFUnDA remained significantly associated with T3 and FT3, respectively. Infants born to mothers within the highest TSH quartile had 10% higher mean concentrations of TSH compared with children born to mothers in the lowest TSH quartile (Berg et al., 2017).

Maternal PFOS levels were inversely correlated with maternal serum TSH and positively associated with infant serum TSH, whereas maternal PFOA showed no significant relationship with TSH or FT4 among mothers and infants. Findings suggest that PFOS may independently affect the secretion and balance of maternal and infant TSH even at low levels of environmental exposure (Kato et al., 2016).

PFAS levels were higher in cases versus controls (sera from infants with congenital hypothyroidism and a control infant group). Concentrations of serum perfluorooctanoic acid (PFOA, $p<0.01$), perfluorononanoic acid (PFNA, $p<0.001$), perfluorooctanoic acid (PFDA, $p<0.005$), and perfluoroundecanoic acid (PFUnDA, $p<0.005$) were significantly higher in cases than in controls group. Levels of certain PFASs (PFOA, perfluorotridecanoic acid [PFTrDA], and perfluorohexane sulfonate [PFHxS]) showed a moderate to weak correlation with relevant antibodies (Kim et al., 2016).

Maternal triiodothyronin (T3) and free T3 (FT3) showed negative correlations with most fetal PFASs ($r = -0.229$ to -0.165 for T3; $r = -0.293$ to -0.169 for FT3, all $P < 0.05$). Results suggest prenatal exposure of fetus to PFASs and potential associations between PFASs and thyroid hormone homeostasis in humans (Yang et al., 2016).

Evidence of PFAS-associated thyroid disruption in a subset of U.S. adults with high TPOAb (a marker of autoimmune hypothyroidism) and low iodine status, who may represent a vulnerable subgroup. However, the study is limited by small sample size, cross-sectional design, and the possibility of reverse causation (Webster et al., 2016).

Effect estimates from the majority of the adjusted models were not statistically significant. However, exposure to PFASs may be associated with increased FT3, TT3, and FT4 in female adults and with increased TSH in male adolescents and decreased TSH in female adolescents. No significant relationships were observed between PFASs and T in any model. These findings suggest that exposure to PFASs may disrupt thyroid hormone homeostasis (Lewis et al., 2015).

Findings suggest modifications of TH homeostasis by PFASs in a background-exposed maternal population. The variation in levels of THs between PFAS quartiles was within normal reference ranges and may not be of clinical relevance for pregnant woman. However, subtle individual changes in maternal THs may have significant consequences for fetal health (Berg et al., 2015)

DDE and perfluorinated alkyl acids may be associated with T4 in a sex-specific manner. However, these results should be interpreted with caution due to the relatively small study population (Cock et al., 2014).

Results in older adults suggested that PFASs are associated with subtle alterations in thyroid hormone levels in this population, and that these associations are likely to vary by age and by levels of other environmental compounds such as PCBs and PBDEs (Shrestha et al., 2015).

PFASs were positively associated with TSH, and weakly negatively associated with fT4 in a subset of pregnant women with high TPOAb, which occurs in 6-10% of pregnancies. PFASs may exacerbate the already high TSH and low fT4 levels of these women during early pregnancy, which is a critical time for thyroid hormone-mediated fetal brain development. The clinical significance of these findings is not clear. We propose a "multiple hit hypothesis" to explain these findings, to be tested in larger, more representative study samples (Webster et al., 2014).

Results (Taiwan) suggested that exposure to some PFASs during pregnancy may interfere with thyroid hormone homeostasis in pregnant women and fetuses (Wang et al., 2015).

Results suggested that circulating levels of POPs (including PFOS) in Inuit women of childbearing age were not suffucuently high to affect TTR-mediated thyroid hormone transport. The possibility of increased delivery of these compounds to the developing brain requires further investigation (Audet-Delage et al., 2013).

Association between thyroid profile and perfluoroalkyl acids: data from NHNAES 2007-2008. TSH levels increased with increase in levels of PFOA ($p<0.01$). There were no statistically significant associations of FT3 or FT4 levels with the levels of any of six PFAAs. Levels of TT3 were found to increase with higher PFOA levels ($p=0.01$) and TT4 levels with higher PFHxS levels ($p<0.01$). Males had statistically significantly higher levels of FT3 than females and females had statistically significantly higher levels of TT4 than males (Jain et al., 2018).

Results suggested an association between PFOS and TSH in pregnant women that is small and may be of no clinical relevance (Yan et al., 2013).

Higher serum concentrations of PFOA and PFHxS were associated with total T3, total T4, and free T4 in the U.S. general population. Further studies are warranted to clarify the causal relationship between PFCs and thyroid function (Wen et al., 2013).

Association between PFNA and free T4 was more significant in males aged 20-30 years, active smokers, and those with higher BMI in stratified analysis. Serum concentrations of PFNA were associated with serum free T4 levels in adolescents and young adults (Lin et al., 2013).

Among the studied PFCs, the concentrations of perfluorotridecanoic acid (PFTrDA) were negatively correlated with total T4 and positively correlated with TSH levels, especially among females (Lin et al., 2013).

This is the first large-scale report in children living near a chemical plant suggesting associations of serum PFOS and PFNA with thyroid hormone levels and of serum PFOA with hypothyroidism (Lopez-Espinosa et al., 2012).

Fetal PFOS, PFOA, PFTrDA and maternal PFTrDA were correlated with fetal total T4 concentrations; however, after adjusting for major covariates, most of the relationships were no longer statistically significant. However, the negative correlations of maternal PFOS with fetal T3, and of maternal PFTrDA with fetal T4 and T3 remained significant (Kim et al., 2011).

Both PFOA and perfluorooctane sulfonate (PFOS) were associated with significant elevations in serum thyroxine and a significant reduction in T3 uptake in all participants. Significant gender/PFOS interactions were found for T3 uptake and thyroxine and significant gender/PFOA interactions for T3 uptake. Results provide evidence of thyroid function disruption related to these common chemicals (Knox et al., 2011).

Findings indicated no observable bias from the use of the analog versus dialysis method, across the range of PFOS and PFOA concentrations in the study population (Lopez-Espinosa et al., 2012).

PFOS concentrations in Inuit adults were associated negatively with TSH, tT(3) and TBG and positively with fT(4) concentrations.. The effects of PFOS and BDE-47 on thyroid homeostasis require further investigation, because other human populations display similar or higher concentrations of these chemicals (Dallaire et al., 2009).

Exploratory assessment of perfluorinated compounds and human thyroid function. Preliminary studies did not indicate associations between non-occupational PFCs exposure and thyroid function. However, they raise the possibility of a weak association of FT(4) with PFDA and PFUnDA at low concentrations (Bloom et al., 2010).

Cord blood perfluoro n-pentanoic acid (PFPeA) was positively associated with cord blood T4 ($p=0.01$) levela. Gender-specific analysis showed that exposure to the prenatal PFCs PFPeA and Perfluorohexane sulfonic acid (PFHxS) significantly increased T4 ($p<0.01$) and T3 ($p=0.03$), respectively, while perfluorononanoic acid (PFNA) decreased TSH ($p=0.04$) concentrations in female newborns. Hence, prenatal PFC exposure may disrupt thyroid hormone homeostasis. (Shah.kulkarni et al., 2016).

- Insulin-like growth factors

A study suggested that PFAS are inverse associated with lower levels of IGF-1 and sex hormones in young children.

In boys, PFOA concentrations were significantly inversely associated with testosterone levels, PFOS was inversely associated with estradiol & testosterone and IGF-1; and PFNA was inversely associated with IGF-1. In girls, significant inverse associations were found between PFOS and testosterone (-6.6%; 95% CI: -10.1, -2.8%) and IGF-1 (-5.6%; -8.2, -2.9%); and between PFNA and IGF-1 (-3.8%; 95% CI: -6.4, -1.2%). In both sexes, the magnitudes of the associations decreased monotonically across quartiles for both testosterone and IGF-1 in relation to PFOS an, in girls, for IGF-1 in relation to PFNA (Lopez-Espinosa et al., 2016).

- Glucocorticoids & Androgenic hormones

Prenatal exposure to PFCs was significantly inversely associated with glucocorticoid and positively associated with DHEA levels in cord blood:

An in verse dose-response relationship was observed between exposure to prenatal PFOS but not PFOA and glucocorticoid levels after adjusting for potential confounders.

The highest quartile of prenatal PFOS exposure was positively associated and that of PFOA exposure negatively associated with the highest versus lowest level of androgenic hormone [dehydroepiandrosterone (DHEA) (Benjamin et al., 2007).

- Asthma, allergy, eczema

16-kDa club cell secretory protein (Clara) (CC16) level, a prominent biomarker of asthma, was studied in adolescents. Asthmatic participants had significantly higher serum PFAS concentrations overall versus healthy controls. After adjusting for confounding factors, urinary CC16 was significantly negatively associated with PFASs, notably PFOS, PFOA, PFDA and PFNA, especially

among males. Significant interaction effects ($p < 0.15$) on CC16 levels were found between asthma and PFOS, PFOA, PFBS and PFHxA in all participants. Overall results showed that serum PFASs were significantly inversely associated with CC16 levels. Associations were stronger among males (Yang et al., 2017).

Interaction effects of polyfluoroalkyl substances and sex steroid hormones on asthma in children. Findings suggested that reproductive hormones amplify the association between PFASs and asthma in adolescents. Results showed that, among asthmatics, PFASs were positively associated with estradiol levels and negatively associated with testosterone levels. However, the only significant association identified was between PFNA and estradiol in controls. After controlling for hormone levels, associations between PFAS exposure and asthma were consistently stronger in children with higher versus lower estradiol, with odds ratio (OR) for asthma of 1.25 for PFOS. Notably, the interactions between estradiol and PFASs were significant for PFOS ($p = 0.026$) and PFNA ($p = 0.043$) in girls. However, testosterone significantly attenuated the association between PFOS and asthma across sexes (Zhou et al., 2017).

Associations of perfluoroalkyl substance exposure with asthma and allergic disease in children were addressed, studying modifications due to MMR vaccination. In conclusion, PFAS exposure at age 5 was associated with increased risk of asthma in a small subgroup of non-MMR-vaccinated children but not in MMR-vaccinated children. While PFAS exposure may impact immune system functions, this study suggests that MMR vaccination might be a potential effect-modifier (Timmermann et al., 2017).

Prenatal exposure to environmental chemical contaminants and asthma and eczema was studied in school-age children (Greenland and Ukraine), offering limited evidence to support a link between prenatal exposure to environmental chemical contaminants and childhood asthma and eczema (Smit et al., 2015).

PFOA was associated with higher odds of having received a diagnosis of asthma, whereas there was an inverse relationship between PFOS and with both asthma and wheezing. No associations were observed between the other PFCs and any outcome. This cross-sectional study provides some evidence for associations between exposure to PFCs and asthma-related outcomes in children (Humblet et al., 2014).

Adjusted odds ratios for asthma among those with the highest versus lowest quartile of PFC exposure ranged from 1.81 (95% CI: 1.02, 3.23) for perfluorododecanoic acid (PFDoA) to 4.05 (95% CI: 2.21, 7.42) for perfluorooctanic acid (PFOA). PFOS, PFOA, and subsets of the other PFCs were positively associated with serum IgE concentrations, absolute eosinophil counts (AEC), eosinophilic cationic protein (ECP) concentrations, and asthma severity scores among asthmatics. Association was reported of PFC exposure with juvenile asthma. Because of widespread exposure to these chemicals, these findings may be of potential public health concern (Dong et al., 2013).

Conclusion, prenatal exposure to long-chain PFAAs, such as PFDoDa and PFTrDA may have an immunosuppressive effect on allergic diseases in 4-year-olds (Doudarzi et al., 2016).

Serum PFOA, PFOS, and PFHxS were statistically significantly associated with higher odds of self-reported food allergy in NHANES 2007-2010. When using IgE levels as a marker of food sensitization, serum PFNA was inversely associated with food sensitization (NHANES 2005-2006). Conclusion, serum levels of PFASs were associated with higher odds of self-reported food allergy. Conversely, adolescents with higher serum PFNA were less likely to be sensitized to food allergens (Buser et al., 2016).

At 24 months, the adjusted odds ratio (OR) (first vs. fourth quartiles) for eczema in association with higher maternal perfluorotridecanoic acid (PFTrDA) levels was 0.62 (95% confidence interval (CI) 0.45, 0.86). Resuts for female infants from mothers with higher maternal perfluoroundecanoic acid (PFUnDA) and PFTrDA levels were also statistically significant (PFUnDA: OR=0.50; 95% CI, 0.30, 0.81; PFTrDA: OR=0.39; 95% CI, 0.23, 0.64). Findings suggest that lower prenatal exposure to

PFTrDA may decrease the risk of developing eczema in early childhood in female infants alone. (Okada et al., 2014).

Cord blood IgE levels significantly decreased with high maternal PFOA concentrations in female infants. However, there were no significant associations of maternal PFOS or PFOA levels with food allergy, eczema, wheezing, or otitis media in 18-month-old infants (adjusted for confounders). Although cord blood IgE level decreased significantly with high maternal PFOA levels in female infants, no relationship was found between maternal PFOS or PFOA levels and infant allergies or infectious diseases at 18 months of age (Okada et al., 2012).

PFOA and PFOS levels were positively correlated with cord blood IgE levels. Analyses stratified by gender revealed that PFOA and PFOS levels positively correlated with cord blood IgE levels in boys alone (per ln-unit: β=0.206 KU/l, p=0.025 for PFOA; β=0.175 KU/l, p=0.053 for PFOS). When dividing cord blood serum PFCs into quartiles in the fully adjusted models, atopic dermatitis was not significantly associated with PFOS. Pre-natal PFOA and PFOS exposures were positively correlated with cord blood IgE levels (Wang et al., 2011).

- Infections

PFOS levels in the highest quartile were significant associated with increased odds ratios of total infectious diseases in all children. Perfluorohexane sulfonate (PFHxS) was associated with a higher risk of total infectious diseases in girls alone. Findings suggest that prenatal exposure to PFOS and PFHxS may be associated with the risk of infectious disease in early life. Therefore, prenatal exposure to PFAAs may be immunotoxic for the immune system in offspring (Goudarzi et al., 2017

Prenatal exposure to perfluoralkyl substances (PFASs) associated with respiratory tract infections but not with allergy- or asthma-related health outcomes in childhood Environ Res 160 518-523. Reported airway infections were significantly associated with cord blood concentrations of PFAS; (PFUnDA, PFOA, PFOS, PFOSA, PFNA, PFUnDA)., Several PFASs were associated with an increased number of respiratory tract infections in the first 10 years of life, suggesting immunosuppressive effects of PFASs (Nygaard, et al., 2018)

- Vaccine

Elevated exposure to PFCs was associated with reduced humoral immune response to routine childhood tetanus and diphtheria immunizations in children aged 5 and 7 years (Grandjean et al., 2012).

- Lung function.

Association of perfluoroalkyl substances exposure with impaired lung function in children. Serum PFASs levels were significantly negatively associated with three pulmonary function measurements (forced vital capacity: FVC; 1s: FEV1; FEF25-75) among children with asthma, PFASs were not, however, significantly associated with pulmonary function among children without asthma. In conclusion, this study suggests that serum PFASs are associated with decreased lung function in children with asthma (Qin et al., 2017).

- Inflammation

PFOA was associated with ulcerative colitis in a prospective analysis of ulcerative colitis diagnosed after the baseline 2005-2006 survey (n = 29 cases) suggested a positive but non-monotonic trend (p trend = 0.21), the first report of an association between this common environmental exposure and autoimmune diseases in humans (Steenland et al., 2013).

6.5.3.10 Biochemical or physiological

Prenatal exposure to OCs was marginally associated with decreases in neutrophil counts. In contrast, PFASs concentrations at 5 years were associated with higher basophil counts. Significantly reduced subpopulations of lymphocytes such as B cells, CD4-positive T helper cells and CD4-positive recent thymic emigrants may suggest cellular immunity effects and dysregulation of T-cell mediated immunity. Developmental exposure to environmental immunotoxicants appears to have different impacts on WBC counts in childhood (Oulhote et al., 2017).

Associations of serum perfluoroalkyl acid levels with T-helper cell-specific cytokines in children by gender and asthma status Asthmatics had significantly higher serum PFAA concentrations than healthy controls. Serum PFAAs were associated positively with TH2 cytokines and inversely with TH1 cytokines among male asthmatics. In conclusion, increased serum PFAAs levels may promote TH cell dysregulation and alter the availability of key TH1 and TH2 cytokines, ultimately contributing to the development of asthma that may impact on males to a greater degree than on females (Zhu et al., 2016).

Study in primarily urban Canadian population of pregnant women and their newborns, finding that maternal urinary and plasma concentrations of phthalate metabolites, BPA, and perfluoroalkyl substances were not associated with immunotoxic effects manifested as increased odds of elevated levels of IgE, TSLP, or IL-33 (Ashley-Martin et al., 2015).

At 7 years of age, blood mercury concentrations were positively associated with titers of multiple neural- and non-neural-specific antibodies, mostly of the IgM isotype. Additionally, prenatal blood-mercury and -PCBs were negatively associated with anti-keratin IgG and prenatal PFOS was negatively associated with anti-actin IgG. These exploratory findings demonstrate that autoantibodies can be detected in peripheral blood after exposure to environmental chemicals. The unexpected association of exposures with antibodies specific for non-neural antigens suggests that these chemicals may have toxicities that have not yet been recognized (Osuna et al., 2014).

6.5.3.11 Cancer

- Breast Cancer (BC):

Prospective study found weak positive and negative non-significant associations between BC risk and levels of perfluorooctane sulfonamide (PFOSA) and perfluorohexanesulfonate (PFHxS), respectively. Grouped into quintiles, BC cases showed significant positive association with PFOSA at the highest quintiles and a negative association with PFHxS. Sensitivity analyses excluding uncertain cases yielded stronger data for PFOSA and weaker data for PFHxS. No further significant associations were observed (Bonefeld-Jørgensen et al., 2014).

Breast cancer (BC) study of Inuits first reported a significant association between serum PFC levels and the risk of BC. BC cases also showed a significantly higher concentration of polychlorinated biphenyls for the highest quartile. Combined serum POP-induced agonistic AhR transactivity was significantly associated with BC risk. Cases had a higher proportion of samples with significant POP-related hormone-like agonistic ER transactivity. The AhR toxic equivalent was lowest in cases. Hormone disruption by combined serum POP-related xenoestrogenic and xenoandrogenic activities may contribute to the risk of developing breast cancer in Inuits (Bonefeld-Jorgensen et al., 2011)

- Colorectal cancer

In this large cross-sectional study, a strong inverse association was found between PFOS and likelihood of CRC diagnosis and a significant but more modest inverse association was found between PFOA and CRC. If confirmed in prospective investigations, these findings may aid in developing new strategies for CRC prevention and treatment and as background for future studies on the mechanisms underlying CRC pathogenesis (Innes et al., 2014).

- Liver Cancer

In a study of paired serum and liver samples, PFOS, PFHxS, PFDA, PFNA, and PFOA concentrations were generally higher in serum than in explanted liver from patients. These findings also suggest that pathological changes in diseased livers alter the distribution of PFASs between liver and serum. The study reports for the first time on the detection and comparison of a range of PFASs in the liver of patients with liver cancer and/or cirrhosis (Yeung et al., 2013).

- Prostate cancer

Only blood PFDA blood concentrations were significantly higher in cases than in controls. Analyses based on Gleason score and prostate specific antigen (PSA) level did not change the results. Heredity was a risk factor for prostate cancer, yielding odds ratio (OR)=1.8, 95% confidence interval (CI)=1.01-3.1. The analyzed PFAAs gave significantly higher ORs in cases with a first degree relative reporting prostate cancer, e.g., PFOA gave OR=2.6, 95% CI=1.2-6.0 and PFOS gave OR=2.7, 95% CI=1.04-6.8. The results showed a higher likelihood of prostate cancer in cases with heredity as risk factor (Hardell et al., 2014).

- Other

Sex-specific number, standardized mortality rates and rate ratios (RR) for drinking water in PFAS contaminated and uncontaminated areas were computed for each cause of death using the ENEA epidemiological database. In both sexes, statistically significant RRs were detected for all-causes mortality, diabetes, cerebrovascular diseases, myocardial infarction and Alzheimer's disease. In females, kidney and breast cancer, and Parkinson's disease. Increased risk, although not statistically significant, was observed for bladder cancer in both sexes, and for testicular cancer, pancreatic cancer and leukemia in males alone. Higher mortality levels for some causes of death, possibly associated with PFAS exposure, were detected in contaminated municipalities in comparison with uncontaminated ones with similar socioeconomic status and smoking habits. These results warrant further individual level analytic studies to delineate casual associations (Mastrantonio et al., 2018).

The HR for dying from the cancer and non-cancer outcomes of interest Occup Environ Med 2014;71: 500-6were not associated with APFO exposure. Similarly, there was little evidence that the incident cancers were associated with APFO exposure. Compared to the non-exposed population, modestly elevated, but imprecise HRs were observed in the higher-exposure quartiles for bladder cancer (HR=1.66, 95% CI 0.86 to 3.18) and pancreatic cancer (HR=1.36, 95% CI 0.59 to 3.11). No association was observed between APFO exposure and kidney, prostate or breast cancers. This analysis did not support an association between occupational APFO exposure and the evaluated health endpoints; however, the study had limited power to evaluate some conditions of interest (Raleigh et al., 2014).

Estimated cumulative serum PFOA concentrations were positively associated with kidney and testicular cancer was found among adults living near a chemical plant.. PFOA exposure was associated with kidney and testicular cancer in this population. Because this is largely a survivor cohort, findings must be interpreted with caution, especially for highly fatal cancers such as pancreatic and lung cancer (Barry et al., 2013).

There was a positive association between kidney cancer and the very high and high serum PFOA exposure categories and a null association with the other exposure categories compared with non-

exposure. The highest adjusted odds ratio (AOR) was for testicular cancer with the very high exposure category [2.8 (95% CI: 0.8, 9.2) n = 6], but there was an inverse association with the lower exposure groups, and all estimates were imprecise because of small number of cases. Results suggest that higher PFOA serum levels may be associated with testicular, kidney, prostate, and ovarian cancers and non-Hodgkin lymphoma. Strengths of this study include near-complete case ascertainment for state residents and well-characterized contrasts in predicted PFOA serum levels from six contaminated water supplies (Vieira et al., 2013).

No clear differences were found in incidence rate ratios for cancers in relation to plasma concentrations of perfluorooctanoate or perfluorooctanesulfonate in a middle-aged Danish population. A 30%-40% increase in risk estimates for prostate cancer was observed for the three upper quartiles of perfluorooctanesulfonate concentration compared with the lowest quartile. Plasma concentrations of perfluorooctanoate and perfluorooctanesulfonate in the general Danish population do not appear to be associated with risk of prostate, bladder, pancreatic, or liver cancer (Eriksen et al., 2013).

6.5.3.12 Biochemical/physiological biomarkers

Prostate specific antigen: Perfluoroalkyl acids are not consistently associated with PSA concentration in general, or with PSA >4.0. These findings do not provide evidence that PFAA exposure is associated with PSA.

6.5.3.13 Others

K. Fry and M. C. Power 2017 Persistent organic pollutants and mortality in the United States, NHANES 1999-2011 Environ Health 16 105. Serum measurements of PBDEs, PFASs, and PCBs were not clearly associated with increased all-cause or cause-specific mortality in older Americans.

6.5.3.14 Ex-vivo PFAS impact

- **Reproduction (human/pregnant women) (2 novel studies)**

Extraction of perfluorinated alkyl acids from human serum for determination of the combined xenoestrogenic transactivity: a method development. Analysis of the extracted PFAA serum fraction from three pregnant women showed that ER-active endogenous hormones were removed. The method was further documented by extraction of the PFAAs from the serum of 18 Danish pregnant women. The PFAA fraction from three of the 18 samples significantly induced ER-transactivity. Upon co-exposure with the natural ER-ligand 17β-estradiol (E2), 17 of the 18 PFAA fractions caused a significant further increase of E2-induced ER-transactivity. In conclusion, a method was developed to extract PFAAs from human serum, and the method documentation suggested that PFAAs at levels found in human serum can transactivate the ER (Bjerregaard-Olesen et al., 2015).

Activation of the estrogen receptor by human serum extracts containing mixtures of perfluorinated alkyl acids from pregnant women. PFAAs were extracted from human serum with simultaneous removal of endogenous hormones and interfering steroid metabolites. The xenoestrogenic activity of the PFAA extracts were analyzed by estrogen receptor (ER) transactivation using MVLN cells carrying an estrogen response element luciferase reporter vector. The results indicated that the serum extracts induced the ER in a non-monotonic concentration dependent manner. The median

EEQ of the extracts containing the PFAAs corresponds to the effect of 0.5pg E2 per mL serum. In conclusion, most of the extracts containing the PFAA mixtures from pregnant women's serum agonized the ER and enhanced E2-induced effects in a non-monotonic concentration-dependent manner (Bjerregaard-Olesen et al., 2016).

- **Breast Cancer: (1 novel study)**

Receptor activities of persistent pollutant serum mixtures and breast cancer risk. No association was found between the combined xenoestrogenic activities of serum lipPOPs or PFAAs extracts and breast cancer risk. Serum lipPOP mixtures are hormone disruptive and may influence breast cancer risk, whereas PFAAs seem to influence breast cancer risk through other pathways (Wielsøe et al., 2018).

- **Endocrine (Animal) / TTR-binding capacity. 1 novel (few studies)**

Blood plasma sample preparation method for the assessment of thyroid hormone-disrupting potency in effect-directed analysis. FAASs were extracted from human plasma with SPE-liquid-liquid extraction and simultaneous removal of endogenous TH hormones, eliminating their possible contribution to a binding assay response. This method can be used for extraction of a broad range of thyroid hormone (TH)-disruptors from plasma with high recoveries. In the extracts, the potency of the compound classes spiked to the cow plasma to competitively bind to transthyretin (TTR) was recovered by 84.9% +/- 8.8%. A first screening revealed TTR-binding potency in the polar bear plasma extracts, 60 - 85% of which could be explained for by the presence of OH-PCBs (Simon et al., 2011).

6.5.4 List of novel effect biomarkers found during the literature search

Table 14. List of novel effect biomarkers for PFAs PFOAs chemical family

Novel effect biomarker	**Brief description of the effect biomarker**	**Scoring table procedure** *(Please, specify the points assigned during the scoring procedure)*	**Final Score (max. 20 points)**
Adiponectin	A protein hormone which is involved in regulating glucose levels as well as fatty acid breakdown. In humans, it is encoded by the *ADIPOQ* gene and produced in adipose tissue Dose-response PFOS and adiponectin and related to decreased BW	*The biomarker was assessed in serum (3p). There is a plausible mechanism of action between exposure and biomarker (2p). No related AOP was found (0p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "high" (5p).*	15
Luteinizing hormone	**Luteinizing hormone** (**LH**, also known as **lutropin** and sometimes **lutrophin**) is a hormone produced by gonadotropic cells in the anterior pituitary gland. In females, an acute rise of LH ("**LH surge**") triggers ovulation and development of the corpus luteum. In males, where LH had also been called **interstitial cell–stimulating hormone** (**ICSH**), it stimulates Leydig cell production of testosterone. It acts synergistically with FSH. In men, PFAS is related to higher level of plasma and /or semen Luteinizing hormone	The biomarker was assessed in serum (3points). There is a plausible mechanism of action between exposure and biomarker (0p). No related AOP was found (1p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered "high" (5p).	14
Fatty acids /cholesterol	PFOA and PFOA positively associated with serum cholesterol in pregnancy (Skuladottir et al., 2015; Starling et al., 2014). PFOS & PFOA negatively associated with fatty acids and total triglycerides (Kishi et al., 2015).	Not novel but easily applied biomarker of PFASs exposure effect	

	Most PFAS have been significantly associated with albumin, which might indicate that it is immunotoxic (Jiang et al., 2014)	Not novel but easy biomarker of PFASs exposure effect	
CC16 protein	Report on an association between serum perfluoroalkyl substances (PFASs) and asthma. However, few studies have examined the possible associations between PFASs and the 16-kDa club cell secretory protein (Clara) (CC16), a prominent biomarker of asthma, in adolescents. Experimental studies suggest an important protective role of this protein in inflammation and oxidative stress. In humans and animals, the CC16 assay is a sensitive non-invasive test allowing detection of early damage to the respiratory epithelium caused by acute or chronic exposure	The biomarker was assessed in urine (5p). There is a plausible mechanism of action between exposure and biomarker (0p). No related AOP was found (0p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered “high” (5p).	15
Plasma PFAS extract for ex vivo TTR binding assays Method developed	FAAs were extracted from human plasma by SPE-liquid-liquid extraction with simultaneous removal of endogenous TH hormones, eliminating their possible contribution to a binding assay response. This extraction can be used for extraction of a broad range of thyroid hormone (TH)-disruptors from plasma with high recoveries. In the extracts, the potency of the compound classes spiked to the cow plasma to competitively bind to transthyretin (TTR) was recovered by 84.9% ± 8.8%. A first screening revealed TTR-binding potency in the polar bear plasma extracts, 60-85% ofwhich could be explained for by the presence of OH-PCBs	The biomarker was assessed in serum (1p). There is a plausible mechanism of action between exposure and biomarker (2p). No related AOP was found (0p). The biomarker has been implemented in epidemiologic studies (0p). The feasibility was considered “high” (2p).	5
Ex-vivo serum PFAS extract vs. ER transactivity Novel method documented	PFAAs were extracted from human serum with simultaneous removal of endogenous hormones and interfering steroid metabolites. The xenoestrogenic activity of the PFAA extracts was analyzed by estrogen receptor (ER) transactivation using MVLN cells carrying an estrogen response element luciferase reporter vector. Used to study pregnant women and their outcomes, as well as in a breast cancer study	The biomarker was assessed in serum (3p). There is a plausible mechanism of action between exposure and biomarker (2p). No/Yes related AOP was found (3p). The biomarker has been implemented in epidemiologic studies (5p). The feasibility was considered “high” (2p).	15

6.5.4.1 Omics, molecular/genetic effect biomarkers on human studies

The majority of publications detected on the molecular effects of PFASs published experimental data, while there were fewer human studies (Table 1). Overall, these human data provide an insight on the mechanistic aspects of PFASs’ toxicity, rather than pinpointing very discrete effect of biomarkers used directly for the purposes of this exercise. However, a concrete picture of the mechanism of action cannot be drawn solely from this information.

Table 15. Number of papers found for the corresponding health endpoint related to the adverse effects of PFASs

Health endpoint	**Epidemiology**	**Animal**	***In vitro***	**Others**
Neurological	0	14	8	0
Cancer	2	7	9	1
Endocrine	2	27	12	3
Immunological	2	22	5	2
Metabolic / Cardiovascular	3	59	27	0
Reproduction	6	35	12	0
Total	**15**	**166**	**79**	**6**

Three important findings are briefly discussed below:

- In **classical epidemiological data**, exposure to these compounds is often linked to increased levels of **total cholesterol** in the blood. Nevertheless, no clear association with cardiovascular diseases has been established to date. On a molecular level, some data (Fletcher et al., 2013) demonstrate associations between PFOS/PFOA blood concentrations and alterations in the levels of certain gene transcripts (e.g. *NR1H2* (**LXRB**), ***ABCG1*** & ***NPC1***) from whole blood samples. These genes are involved in cholesterol metabolism and transport, suggesting consistency with the commonly observed elevations in blood cholesterol.
- PFASs have been correlated with **immunotoxicity**, commonly manifested as decreased antibody response in humans, including in early childhood. At a transcriptomic level, associations were found between PFASs maternal blood concentrations and common cold incidence as well as **rubella antibody levels** in early childhood (Pennings et al., 2016). The analysis was performed in samples taken from the cord blood just after birth. The finding show **changes in the expression of a series of genes that can be linked to the immune system function (e.g. *CYTL1, IL27*).**
- PFAS are also suspected to possess endocrine disrupting properties. Their effects on a molecular level in humans were examined as part of a broader group of these compounds (EDCs). In these studies, the researchers attempted to underline any relationships between environmental exposure to EDCs and **infertility** (Caserta, et al., 2013; Caserta, et al., 2013; La Rocca et al., 2012; La Rocca et al., 2015; La Rocca et al., 2014). These data show some associations between PFOS/PFOA blood levels and the expression of certain **Nuclear Receptors** (e.g. *AR*, *PXR*), known cellular targets of EDCs. The examinations were performed in peripheral blood cells.

Given the limited epidemiological database on molecular effects of PFASs, it is uncertain whether these studies can be used for the identification of proper effect biomarkers. **Changes in these genes can constitute *per se* meaningful 'effect biomarkers' for measurement, for example, in blood**.

However, for the moment we cannot recommend their measurement within the HBM concept and they are therefore not included in the table below. Mining of the experimental studies would possibly assist in the interpretation of these results and the construction of a sounder mechanistic pathway of PFAS toxicity.

6.5.5 Preliminary proposal of effect biomarkers that could be implemented in HBM

The abovementioned table lists the proposed biomarkers, explaining why they can be used and are suitable for HBM implementation

6.5.6 Gaps in Knowledge

A brief description of those Gaps in Knowledge identified during the literature search and also based on your previous knowledge on the subject.

Endocrine:

- No studies on E1 (estrone), E3 (estriol); other hormones); Important to also cover hormone synthesis and metabolism.

- No studies on 3,3'-T2, 3,5-T2, rT3. Important to obtain an overview of the THs

- Only studies on 25-OH vitamin D – Studies about the relation of PFASs and other vitamins might also be important

Immunology/Infections:

- No studies on Bronchitis, although other lower respiratory tract infections are covered.

- No studies on skin infections

- No studies on IgD

6.5.7 Conclusions

Reproduction

Although often based on few studies, the following was observed: PFAS exposure positively associated with GWG, preeclampsia pregnancy hypertension, cerebral palsy in boys, and secondary sex ratio (SSR); prenatal exposure to PFAS might delay menarche, produce abnormal menstruation and menstruation length, and higher levels of plasma/ semen luteinizing hormone. PFAS exposure was negatively associated with decreased anogenital distance in girls, BW, GA, BL and APGAR scores; maternal levels of PFAS might reduce months of breastfeeding and an inverse relationship was observed between PFAS exposure and semen quality/count.

Biochemical/physiological biomarkers in reproduction

PFAS was associated positively with total cholesterol in pregnancy and negatively with fatty acids and total glycerides. Positively associated with albumin and a negative trend for exp and X:Y ratio.

Diabetes

Some studies found association of PFAS exposure with impaired glucose homeostasis-metabolism, dyslipidemia, increased high cholesterol, metabolic syndrome and risk of diabetes See also ref at Excel sheet for novel biomarkers: doi: 10.1016/j.scitotenv.2017.12.186; doi: 10.1016/j.envint.2016.03.012; doi: 10.1016/j.envint.2015.11.016; doi: 10.1007/s00125-013-3126-3

Biochemical/physiological biomarkers

PFAS exposure positively associated with total cholesterol and often inversely correlated with triglycerides; it may disrupt lipid homeostasis/alter lipid metabolism and liver function and cause liver enzymes to increase abnormally. PFNA /PFAS positively associated with adiponectin, a protein hormone involved in regulating glucose levels and in fatty acid breakdown.

Anthropometric biomarkers

Several studies (7 out of 10) found that prenatal exposure to PFAS was associated with greater adiposity in the child, resulting in obesogenic risk for offspring.

Developmental milestones

Out of 6 publications 3 found indication of an negative effect; 2 found no association with developmental milestones, 1 found a positive association with reading skills for some (PFOA, PFOS, PFNA but not for PFHxS). Data are inconsistent and further studies areneeded to investigate PFASs exposure and developmental skills.

Reproductive Hormones

PFASs can potential disrupt the level of reproductive hormones, with lower testosterone and estradiol found in fetuses and in young people and adults

Thyroid hormones

More than 27 studies have found that PFASs have thyroid hormone disruptive potential (influencing TH homeostasis) in fetus, children, and adults. TH factors involved are: FT3, T3, TT3, FT4, T4, TT¤, TSH, TPOAb (marker of autoimmune hypothyroidism).

Asthma, allergy, eczema

CC16 biomarker of asthma involved in anti-oxidative processes is suppressed by PFASs.

Some evidence for associations between exposure to PFCs and asthma-related outcomes in children. Reproductive hormones might amplify the association between PFASs and asthma among adolescent.

Association between perfluoroalkyl substance exposure and asthma and allergic disease in children as modified by MMR vaccination.

Association between PFC exposure and juvenile asthma; prenatal exposure to long-chain PFAAs, such as PFDoDa and PFTrDA, may have an immunosuppressive effect on allergic diseases in 4-year-old children.

Findings suggest that lower prenatal exposure to PFTrDA may decrease the risk of developing eczema in early childhood.

Cord blood IgE levels decreased significantly with high maternal PFOA concentrations in female infants but no relationship was found between maternal PFOS or PFOA levels and infant allergies and infectious diseases at the age of 18 months.

Pre-natal PFOA and PFOS exposures were positively correlated with cord blood IgE levels in boys.

Biochemical or physiological

PFAS related to asthma and T-cell specific cytokines and auto-antibodies

Ex-vivo PFAS impact

Extraction of the PFASs fraction from pregnant women –being free from endogenous hormones- elicits combined PFAS-induced xenoestrogenic activity. In a revision manuscript, authors demonstrate that combined PFAS xeno-estrogenicity is associated with significant reduced birth weight.

In a breast cancer study, combined lipoPOP and PFASs induced elicited no association between the combined xenoestrogenic activities of serum lipPOP or PFAA extracta and breast cancer risk.

Method developed for PFASs plasma extraction with simultaneously removal of endogenous THs for use in transthyretin (TTR) binding assay.

6.5.8 References

List of epidemiology publications sorted by topic

Reproduction

Alkhalawi et al., Perfluoroalkyl acids (PFAAs) and anthropometric measures in the first year of life: Results from the Duisburg Birth Cohort. J Toxicol Environ Health A 2016;79: 1041-1049.

Andersen et al., Prenatal exposures to perfluorinated chemicals and anthropometric measures in infancy. Am J Epidemiol 2010;172: 1230-7.

Antignac et al., Occurrence of perfluorinated alkylated substances in breast milk of French women and relation with socio-demographical and clinical parameters: results of the ELFE pilot study. Chemosphere 2013;91: 802-8.

Ashley-Martin et al., Maternal Concentrations of Perfluoroalkyl Substances and Fetal Markers of Metabolic Function and Birth Weight. Am J Epidemiol 2017;185: 185-193.

Ashley-Martin et al., Maternal and Neonatal Levels of Perfluoroalkyl Substances in Relation to Gestational Weight Gain. Int J Environ Res Public Health 2016;13(1).

Avanasi et al., Variability and epistemic uncertainty in water ingestion rates and pharmacokinetic parameters, and impact on the association between perfluorooctanoate and preeclampsia in the C8 Health Project population. Environ Res 2016;146: 299-307.

Bach et al., Perfluoroalkyl Acids in Maternal Serum and Indices of Fetal Growth: The Aarhus Birth Cohort. Environ Health Perspect 2016;124: 848-54.

Bach et al., Serum perfluoroalkyl acids and time to pregnancy in nulliparous women. Environ Res 2015;142: 535-41.

Bach et al., Perfluoroalkyl acids and time to pregnancy revisited: An update from the Danish National Birth Cohort. Environ Health 2015;14: 59.

Bae et al., Maternal and paternal serum concentrations of perfluoroalkyl and polyfluoroalkyl substances and the secondary sex ratio. Chemosphere 2015;133: 31-40.

Buck Louis et al., Persistent environmental pollutants and couple fecundity: the LIFE study. Environ Health Perspect 2013;121: 231-6.

Chen et al., Perfluorinated compounds in umbilical cord blood and adverse birth outcomes. PLoS One 2012;7: e42474.

Christensen et al., Exposure to polyfluoroalkyl chemicals during pregnancy is not associated with offspring age at menarche in a contemporary. British cohort Environ Int 2011;37: 129-35.

Crawford et al., Effects of perfluorinated chemicals on thyroid function, markers of ovarian reserve, and natural fertility. Reprod Toxicol 2017;69: 53-59.

Darrow et al., PFOA and PFOS serum levels and miscarriage risk. Epidemiology 2014;25: 505-12

Darrow et al., Serum perfluorooctanoic acid and perfluorooctane sulfonate concentrations in relation to birth outcomes in the Mid-Ohio Valley, 2005-2010. Environ Health Perspect 2013:121: 1207-13.

de Cock et al., Prenatal exposure to endocrine disrupting chemicals and birth weight-A prospective cohort study. J Environ Sci Health A Tox Hazard Subst Environ Eng 2016;51: 178-185.

Den Hond et al., Human exposure to endocrine disrupting chemicals and fertility: A case-control study in male subfertility patients. Environ Int 2015;84: 154-60.

Fei et al., Maternal levels of perfluorinated chemicals and subfecundity. Hum Reprod 2009;24: 1200-5,

Fei et al., Maternal concentrations of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) and duration of breastfeeding. Scand J Work Environ Health 2010;36: 413-21.

Fei et al., Fetal growth indicators and perfluorinated chemicals: a study in the Danish National Birth Cohort. Am J Epidemiol 2008;168: 66-72.

Govarts et al., 2016 Combined Effects of Prenatal Exposures to Environmental Chemicals on Birth Weight. Int J Environ Res Public Health 2016;13(1).

Governini et al., The impact of environmental exposure to perfluorinated compounds on oocyte fertilization capacity. J Assist Reprod Genet 2011;28: 415-8.

Hamm et al., Maternal exposure to perfluorinated acids and fetal growth. J Expo Sci Environ Epidemiol 2010;20: 589-97.

Holtcamp W. Pregnancy-induced hypertension "probably linked" to PFOA contamination. Environ Health Perspect 2012;120: a59.

Jensen et al., Association between perfluorinated compound exposure and miscarriage in Danish pregnant women. PLoS One 2015;10: e0123496.

Jiang et al., Serum levels of perfluoroalkyl acids (PFAAs) with isomer analysis and their associations with medical parameters in Chinese pregnant women. Environ Int 2014;64: 40-7.

Joensen et al., Do perfluoroalkyl compounds impair human semen quality? Environ Health Perspect 2009;117: 923-7.

Jorgensen et al., Perfluoroalkyl substances and time to pregnancy in couples from Greenland, Poland and Ukraine. Environ Health 2014;13: 116.

Jaacks et al., Pre-Pregnancy Maternal Exposure to Persistent Organic Pollutants and Gestational Weight Gain: A Prospective Cohort Study. Int J Environ Res Public Health 2016;13.

Kishi et al., The Association of Prenatal Exposure to Perfluorinated Chemicals with Maternal Essential and Long-Chain Polyunsaturated Fatty Acids during Pregnancy and the Birth Weight of Their Offspring: The Hokkaido Study. Environ Health Perspect 2015;123: 1038-45.

Kristensen et al., Long-term effects of prenatal exposure to perfluoroalkyl substances on female reproduction. Hum Reprod 2013;28: 3337-48.

Kvist et al., Serum levels of perfluorinated compounds and sperm Y:X chromosome ratio in two European populations and in Inuit from Greenland. Reprod Toxicol 2012;34: 644-50.

Lauritzen et al., Maternal serum levels of perfluoroalkyl substances and organochlorines and indices of fetal growth: a Scandinavian case-cohort study. Pediatr Res 2017;81: 33-42.

Lee et al., Association between perfluorinated compound concentrations in cord serum and birth weight using multiple regression models. Reprod Toxicol 2016;59: 53-9.

Lee et al., Concentrations of perfluoroalkyl compounds in maternal and umbilical cord sera and birth outcomes in Korea. Chemosphere 2013;90: 1603-9.

Lenters et al., Prenatal Phthalate, Perfluoroalkyl Acid, and Organochlorine Exposures and Term Birth Weight in Three Birth Cohorts: Multi-Pollutant Models Based on Elastic Net Regression. Environ Health Perspect 2016;124: 365-72.

Lenters et al., Phthalates, perfluoroalkyl acids, metals and organochlorines and reproductive function: a multipollutant assessment in Greenlandic, Polish and Ukrainian men. Occup Environ Med 2015;72: 385-93.

Li et al., Isomers of perfluorooctanesulfonate (PFOS) in cord serum and birth outcomes in China: Guangzhou Birth Cohort Study. Environ Int 2017;102: 1-8.

Lien et al., Neonatal-maternal factors and perfluoroalkyl substances in cord blood. Chemosphere 2013;92: 843-50.

Liew et al., Prenatal exposure to perfluoroalkyl substances and the risk of congenital cerebral palsy in children. Am J Epidemiol 2014;180: 574-81.

Lind et al., Prenatal exposure to perfluoroalkyl substances and anogenital distance at 3 months of age in a Danish mother-child cohort. Reprod Toxicol 2017;68: 200-206.

Lind et al., Prenatal exposure to perfluoroalkyl substances and anogenital distance at 3 months of age as marker of endocrine disruption. Reprod Toxicol 2016; pii: S0890-6238(16)30265-9.

Lopez-Espinosa et al., Association of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) with age of puberty among children living near a chemical plant. Environ Sci Technol 2011;45: 8160-6.

Louis et al., Perfluorochemicals and human semen quality: the LIFE study. Environ Health Perspect 2015;123: 57-63.

Louis et al., Preconception perfluoroalkyl and polyfluoroalkyl substances and incident pregnancy loss, LIFE Study. Reprod Toxicol 2016;65: 11-17.

Lum et al., Perfluoroalkyl Chemicals, Menstrual Cycle Length, and Fecundity: Findings from a Prospective Pregnancy Study. Epidemiology 2017;28: 90-98.

Lyngso et al., Menstrual cycle characteristics in fertile women from Greenland, Poland and Ukraine exposed to perfluorinated chemicals: a cross-sectional study. Hum Reprod 2014;29: 359-67.

Maekawa et al., Evidence of exposure to chemicals and heavy metals during pregnancy in Japanese women. Reprod Med Biol 2017;16: 337-348.

Maisonet et al., Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls. Environ Health Perspect 2012;120:1432-7

Manzano-Salgado et al., Prenatal exposure to perfluoroalkyl substances and birth outcomes in a Spanish birth cohort. Environ Int 2017;108: 278-284

McCoy et al., Associations between perfluorinated alkyl acids in blood and ovarian follicular fluid and ovarian function in women undergoing assisted reproductive treatment. Sci Total Environ 2017;605-606: 9-17.

Minatoya et al., Association of prenatal exposure to perfluoroalkyl substances with cord blood adipokines and birth size: The Hokkaido Study on environment and children's health. Environ Res 2017;156: 175-182.

Monroy et al., Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. Environ Res 2008;108: 56-62.

Nolan et al., The relationship between birth weight, gestational age and perfluorooctanoic acid (PFOA)-contaminated public drinking water. Reprod Toxicol 2009;27: 231-8.

Nolan et al., Congenital anomalies, labor/delivery complications, maternal risk factors and their relationship with perfluorooctanoic acid (PFOA)-contaminated public drinking water. Reprod Toxicol 2010;29: 147-55.

Petro et al., Perfluoroalkyl acid contamination of follicular fluid and its consequence for in vitro oocyte developmental competence. Sci Total Environ 2014;496: 282-288.

Raymer et al., Concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) and their associations with human semen quality measurements. Reprod Toxicol 2012;33: 419-27.

Robledo et al., Preconception maternal and paternal exposure to persistent organic pollutants and birth size: the LIFE study. Environ Health Perspect 2015;123: 88-94.

Romano et al., Maternal serum perfluoroalkyl substances during pregnancy and duration of breastfeeding. Environ Res 2016;149 239-246.

Sagiv et al., Early Pregnancy Perfluoroalkyl Substance Plasma Concentrations and Birth Outcomes in Project Viva: Confounded by Pregnancy Hemodynamics? Am J Epidemiol 2017.

Savitz et al., Perfluorooctanoic acid exposure and pregnancy outcome in a highly exposed community. Epidemiology 2012;23: 386-92.

Savitz et al., Relationship of perfluorooctanoic acid exposure to pregnancy outcome based on birth records in the mid-Ohio Valley. Environ Health Perspect 2012;120: 1201-7.

Shi et al., Occurrence of perfluoroalkyl substances in cord serum and association with growth indicators in newborns from Beijing. Chemosphere 2017;169: 396-402.

Skuladottir et al., Examining confounding by diet in the association between perfluoroalkyl acids and serum cholesterol in pregnancy. Environ Res 2015;143: 33-8.

Starling et al., Perfluoroalkyl Substances during Pregnancy and Offspring Weight and Adiposity at Birth: Examining Mediation by Maternal Fasting Glucose in the Healthy Start Study. Environ Health Perspect 2017;125: 067016.

Starling et al., Perfluoroalkyl substances during pregnancy and validated preeclampsia among nulliparous women in the Norwegian Mother and Child Cohort Study. Am J Epidemiol 2014;179: 824-33.

Starling et al., Perfluoroalkyl substances and lipid concentrations in plasma during pregnancy among women in the Norwegian Mother and Child Cohort Study. Environ Int 2014;62: 104-12.

Stein et al., Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome. Am J Epidemiol 2009;170: 837-46.

Timmermann et al., Shorter duration of breastfeeding at levated exposures to perfluoroalkyl substances. Reprod Toxicol 2017;68: 164-170.

Toft et al., Perfluorooctane Sulfonate Concentrations in Amniotic Fluid, Biomarkers of Fetal Leydig Cell Function, and Cryptorchidism and Hypospadias in Danish Boys (1980-1996). Environ Health Perspect 2016;124: 151-6.

Toft et al., Exposure to perfluorinated compounds and human semen quality in Arctic and European populations. Hum Reprod 2012;27: 2532-40.

Valvi et al., Gestational diabetes and offspring birth size at elevated environmental pollutant exposures. Environ Int 2017;107: 205-215

Velez et al., Maternal exposure to perfluorinated chemicals and reduced fecundity: the MIREC study. Hum Reprod 2015;30: 701-9.

Vested et al., Associations of in utero exposure to perfluorinated alkyl acids with human semen quality and reproductive hormones in adult men. Environ Health Perspect 2013;121: 453-8

Vestergaard et al., Association between perfluorinated compounds and time to pregnancy in a prospective cohort of Danish couples attempting to conceive. Hum Reprod 2012;27: 873-80.

Vesterholm Jensen et al., No association between exposure to perfluorinated compounds and congenital cryptorchidism: a nested case-control study among 215 boys from Denmark and Finland. Reproduction 2014;147: 411-7.

Wang et al., Perfluoroalkyl substances and endometriosis-related infertility in Chinese women. Environ Int 2017;102: 207-212

Wang et al., Prenatal Exposure to Perfluorocarboxylic Acids (PFCAs) and Fetal and Postnatal Growth in the Taiwan Maternal and Infant Cohort Study. Environ Health Perspect 2016;124: 1794-1800.

Washino et al., Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. Environ Health Perspect 2009;117: 660-7

Whitworth et al., Perfluorinated compounds in relation to birth weight in the Norwegian Mother and Child Cohort Study. Am J Epidemiol 2012;175: 1209-16

Whitworth et al., 2012 Perfluorinated compounds and subfecundity in pregnant women. Epidemiology 2012;23: 257-63.

Whitworth et al., Brief Report: Plasma Concentrations of Perfluorooctane Sulfonamide and Time-to-pregnancy Among Primiparous Women. Epidemiology 2016;27: 712-5.

Woods et al., Gestational exposure to endocrine disrupting chemicals in relation to infant birth weight: a Bayesian analysis of the HOME Study. Environ Health 2017;16: 115.

Wu et al., Can the observed association between serum perfluoroalkyl substances and delayed menarche be explained on the basis of puberty-related changes in physiology and pharmacokinetics? Environ Int 2015;82: 61-8.

Wu et al., Association between maternal exposure to perfluorooctanoic acid (PFOA) from electronic waste recycling and neonatal health outcomes. Environ Int 2012;48: 1-8.

Zhou et al., Plasma Perfluoroalkyl and Polyfluoroalkyl Substances Concentration and Menstrual Cycle Characteristics in Preconception Women. Environ Health Perspect 2017;125: 067012.

Metabolism / Cardiovascular

Andersen et al., Prenatal exposures to perfluorinated chemicals and anthropometry at 7 years of age. Am J Epidemiol 2013;178: 921-7.

Barry et al., Early life perfluorooctanoic acid (PFOA) exposure and overweight and obesity risk in adulthood in a community with elevated exposure. Environ Res 2014;132: 62-9.

Braun et al. Prenatal perfluoroalkyl substance exposure and child adiposity at 8 years of age: The HOME study. Obesity (Silver Spring) 2016;24: 231-7.

Cardenas et al. Plasma Concentrations of Per- and Polyfluoroalkyl Substances at Baseline and Associations with Glycemic Indicators and Diabetes Incidence among High-Risk Adults in the Diabetes Prevention Program Trial. Environ Health Perspect 2017;125: 107001.

Christensen et al., Perfluoroalkyl substances in older male anglers in Wisconsin. Environ Int 2016;91: 312-8.

Coperchini et al., Thyroid disruption by perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). J Endocrinol Invest 2017;40: 105-121.

Costa et al. Thirty years of medical surveillance in perfluooctanoic acid production workers J Occup Environ Med 2009;51: 364-72.

Dagnino et al., Identification of Biomarkers of Exposure to FTOHs and PAPs in Humans Using a Targeted and Nontargeted Analysis Approach. Environ Sci Technol 2016;50: 10216-25.

Darrow et al., Modeled Perfluorooctanoic Acid (PFOA) Exposure and Liver Function in a Mid-Ohio Valley Community. Environ Health Perspect 2016;124: 1227-33.

Dhingra et al., Perfluorooctanoic acid and chronic kidney disease: Longitudinal analysis of a Mid-Ohio Valley community. Environ Res 2016;145: 85-92.

Dhingra et al., A Study of Reverse Causation: Examining the Associations of Perfluorooctanoic Acid Serum Levels with Two Outcomes. Environ Health Perspect 2017;125: 416-421.

Domazet et al., Longitudinal Associations of Exposure to Perfluoroalkylated Substances in Childhood and Adolescence and Indicators of Adiposity and Glucose Metabolism 6 and 12 Years Later: The European Youth Heart Study. Diabetes Care 2016;39: 1745-51.

Eriksen et al., Association between plasma PFOA and PFOS levels and total cholesterol in a middle-aged Danish population. PLoS One 2013;8: e56969.

Fan et al., Perfluorocarbons and Gilbert syndrome (phenotype) in the C8 Health Study Population. Environ Res 2014;135: 70-5.

Fisher et al., Do perfluoroalkyl substances affect metabolic function and plasma lipids?-Analysis of the 2007-2009, Canadian Health Measures Survey (CHMS) Cycle 1. Environ Res 2013;121: 95-103.

Fleisch et al., Early-Life Exposure to Perfluoroalkyl Substances and Childhood Metabolic Function. Environ Health Perspect 2017;125: 481-487.

Gallo et al., Serum perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) concentrations and liver function biomarkers in a population with elevated PFOA exposure. Environ Health Perspect 2012;120: 655-60.

Geiger et al., The association between PFOA, PFOS and serum lipid levels in adolescents. Chemosphere 2014;98: 78-83.

Geiger et al., Positive association between perfluoroalkyl chemicals and hyperuricemia in children. Am J Epidemiol 2013;177: 1255-62.

Gleason et al., Associations of perfluorinated chemical serum concentrations and biomarkers of liver function and uric acid in the US population (NHANES), 2007-2010. Environ Res 2015;136: 8-14.

Halldorsson et al., Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: a prospective cohort study. Environ Health Perspect 2012;120: 668-73.

Hartman et al., Prenatal Exposure to Perfluoroalkyl Substances and Body Fatness in Girls. Child Obes 2017;13: 222-230.

He et al. PFOA is associated with diabetes and metabolic alteration in US men: National Health and Nutrition Examination Survey 2003-2012. Sci Total Environ 2017;625: 566-574.

Hoyer et al., Anthropometry in 5- to 9-Year-Old reenlandic and Ukrainian Children in Relation to Prenatal Exposure to Perfluorinated Alkyl Substances. Environ Health Perspect 2015;123: 841-6.

Innes et al., Association of osteoarthritis with serum levels of the environmental contaminants perfluorooctanoate and perfluorooctane sulfonate in a large Appalachian population. Am J Epidemiol 2011;174: 440-50.

Karlsen et al., Early-life exposures to persistent organic pollutants in relation to overweight in preschool children. Reprod Toxicol 2017;68: 145-153.

Karnes et al., Incidence of type II diabetes in a cohort with substantial exposure to perfluorooctanoic acid. Environ Res 2014;128: 78-83.

Kataria et al., Association between perfluoroalkyl acids and kidney function in a cross-sectional study of adolescents. Environ Health 2015;14: 89.

Khalil et al., Association of Perfluoroalkyl Substances, Bone Mineral Density, and Osteoporosis in the U.S. Population in NHANES 2009-2010. Environ Health Perspect 2016;124: 81-7.

Khalil et al., Perfluoroalkyl substances, bone density, and cardio-metabolic risk factors in obese 8-12 year old children: A pilot study. Environ Res 2018;160: 314-321.

Kim et al., The modifying effect of vitamin C on the association between perfluorinated compounds and insulin resistance in the Korean elderly: a double-blind, randomized, placebo-controlled crossover trial. Eur J Nutr 2016;55: 1011-20.

Koshy et al., Serum perfluoroalkyl substances and cardiometabolic consequences in adolescents exposed to the World Trade Center disaster and a matched comparison group. Environ Int 2017;109: 128-135.

Lauritzen et al., Prenatal exposure to persistent organic pollutants and child overweight/obesity at 5-year follow-up: a prospective cohort study. Environ Health 2018;17: 9.

Lin et al., Investigation of the associations between low-dose serum perfluorinated chemicals and liver enzymes in US adults. Am J Gastroenterol 2010;105: 1354-63.

Lin et al., Associations between levels of serum perfluorinated chemicals and adiponectin in a young hypertension cohort in Taiwan. Environ Sci Technol 2011;45: 10691-8.

Lind et al., Circulating levels of perfluoroalkyl substances and prevalent diabetes in the elderly. Diabetologia 2014;57: 473-9.

Lind et al., Circulating levels of perfluoroalkyl substances (PFASs) and carotid artery atherosclerosis. Environ Res 2017;152: 157-164.

Liu et al., Association among total serum isomers of perfluorinated chemicals, glucose homeostasis, lipid profiles, serum protein and metabolic syndrome in adults: NHANES, 2013-2014. Environ Pollut 2018;232: 73-79

Lundin et al., Ammonium perfluorooctanoate production and occupational mortality. Epidemiology 2009;20: 921-8.

MacNeil et al., A cross-sectional analysis of type II diabetes in a community with exposure to perfluorooctanoic acid (PFOA). Environ Res 2009;109: 997-1003

Maisonet et al., Prenatal exposures to perfluoroalkyl acids and serum lipids at ages 7 and 15 in females. Environ Int 2015;82: 49-60

Manzano-Salgado et al., Prenatal Exposure to Perfluoroalkyl Substances and Cardiometabolic Risk in Children from the Spanish INMA Birth Cohort Study. Environ Health Perspect 2017;125: 097018.

Matilla-Santander et al., Exposure to Perfluoroalkyl Substances and Metabolic Outcomes in Pregnant Women: Evidence from the Spanish INMA Birth Cohorts. Environ Health Perspect 2017;125: 117004.

Mattsson et al., Levels of perfluoroalkyl substances and risk of coronary heart disease: Findings from a population-based longitudinal study. Environ Res 2015;142: 148-54.

Min et al., Perfluorooctanoic acid exposure is associated with elevated homocysteine and hypertension in US adults Occup Environ Med 2012;69: 658-62.

Mora et al., Early life exposure to per- and polyfluoroalkyl substances and mid-childhood lipid and alanine aminotransferase levels. Environ Int 2018;111 1-13.

Mora et al., Prenatal Exposure to Perfluoroalkyl Substances and Adiposity in Early and Mid-Childhood. Environ Health Perspect 2017;125: 467-473.

Nelson et al., Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population. Environ Health Perspect 2010;118: 197-202.

Olsen et al., Longitudinal assessment of lipid and hepatic clinical parameters in workers involved with the demolition of perfluoroalkyl manufacturing facilities. J Occup Environ Med 2012;54: 974-83.

Shankar et al., Perfluoroalkyl chemicals and elevated serum uric acid in US adults. Clin Epidemiol 2011;3: 251-8.

Shankar et al., Perfluoroalkyl chemicals and chronic kidney disease in US adults. Am J Epidemiol 2011;174: 893-900.

Shankar et al. Perfluorooctanoic acid and cardiovascular disease in US adults. Arch Intern Med 2012;172: 1397-403.

Shapiro et al., Exposure to organophosphorus and organochlorine pesticides, perfluoroalkyl substances, and polychlorinated biphenyls in pregnancy and the association with impaired glucose tolerance and gestational diabetes mellitus: The MIREC Study. Environ Res 2016;147: 71-81.

Simpson et al., Relation between perfluorooctanoic acid exposure and strokes in a large cohort living near a chemical plant Environ Res 2013;127: 22-8.

Steenland & Woskie. Cohort mortality study of workers exposed to perfluorooctanoic acid. Am J Epidemiol 2012;176: 909-17.

Steenland et al., A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA). Occup Environ Med 2015;72: 373-80.

Su et al., Serum perfluorinated chemicals, glucose homeostasis and the risk of diabetes in working-aged Taiwanese adults. Environ Int 2016;88: 15-22.

Timmermann et al., Adiposity and glycemic control in children exposed to perfluorinated compounds. J Clin Endocrinol Metab 2014;99: E608-14.

Watkins et al., Exposure to perfluoroalkyl acids and markers of kidney function among children and adolescents living near a chemical plant. Environ Health Perspect 2013;121: 625-30.

Winquist & Steenland. Modeled PFOA exposure and coronary artery disease, hypertension, and high cholesterol in community and worker cohorts. Environ Health Perspect 2014;122: 1299-305

Yamaguchi et al., Consumption of seafood, serum liver enzymes, and blood levels of PFOS and PFOA in the Japanese population. J Occup Health 2013;55: 184-94.

Yang et al., Association of serum levels of perfluoroalkyl substances (PFASs) with the metabolic syndrome (MetS) in Chinese male adults: A cross-sectional study. Sci Total Environ 2018;621: 1542-1549.

Zhang et al., A prospective study of prepregnancy serum concentrations of perfluorochemicals and the risk of gestational diabetes. Fertil Steril 2015;103: 184-9.

Zong et al., Lactation history, serum concentrations of persistent organic pollutants, and maternal risk of diabetes. Environ Res 2016;150: 282-8.

Immunology

Ashley-Martin et al. Prenatal exposure to phthalates, bisphenol A and perfluoroalkyl substances and cord blood levels of IgE, TSLP and IL-33. Environ Res 2015; 140: 360-8

Brieger et al. Impact of perfluorooctanesulfonate and perfluorooctanoic acid on human peripheral leukocytes Toxicol In Vitro 2011; 25: 960-8

Buser and Scinicariello. Perfluoroalkyl substances and food allergies in adolescents. Environ Int 2016; 88: 74-79

Dalsager, N., et al. Association between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1-4years among 359 children in the Odense Child Cohort. Environ Int 2016; 96: 58-64

Dong et al. Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case-control study of Taiwanese children. Environ Health Perspect 2013; 121: 507-13

Fei et al. Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. Environ Res 2010; 110: 773-7

Goudarzi et al. Prenatal exposure to perfluoroalkyl acids and prevalence of infectious diseases up to 4years of age. Environ Int 2017; 104: 132-138

Goudarzi et al. Effects of prenatal exposure to perfluoroalkyl acids on prevalence ofallergic diseases among 4-year-old children. Environ Int 2016; 94: 124-132

Grandjean et al. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. Jama 2012; 307: 391-7

Grandjean et al. Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine antibody concentrations at age 5-years. J Immunotoxicol 2017; 14: 188-195

Granum et al. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. J Immunotoxicol 2013; 10: 373-9

Humblet et al. Perfluoroalkyl chemicals and asthma among children 12-19 years of age: NHANES (1999-2008). Environ Health Perspect 2014; 122: 1129-33

Impinen et al. Prenatal exposure to perfluoralkyl substances (PFASs) associated with respiratory tract infections but not allergy- and asthma-related health outcomes in childhood. Environ Res 2018; 160: 518-523

Lee et al. Association between perfluorooctanoic acid exposure and degranulation of mast cells in allergic inflammation. J Appl Toxicol 2017; 37: 554-562

Okada et al. Prenatal exposure to perfluoroalkyl acids and allergic diseases in early childhood. Environ Int 2014; 65: 127-34

Okada et al. Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants. Environ Res 2012; 112 :118-25

Osuna et al. Autoantibodies associated with prenatal and childhood exposure to environmental chemicals in Faroese children. Toxicol Sci 2014; 142: 158-66

Oulhote et al. Children's white blood cell counts in relation to developmental exposures to methylmercury and persistent organic pollutants. Reprod Toxicol 2017; 68: 207-214

Qin et al. Association of perfluoroalkyl substances exposure with impaired lung function in children. Environ Res 2017; 155: 15-21

Smit et al. Prenatal exposure to environmental chemical contaminants and asthma and eczema in school-age children. Allergy 2015; 70: 653-60

Steenland et al. Ulcerative colitis and perfluorooctanoic acid (PFOA) in a highly exposed population of community residents and workers in the mid-Ohio valley. Environ Health Perspect 2013; 121: 900-5

Stein et al. Perfluoroalkyl substance serum concentrations and immune response to FluMist vaccination among healthy adults. Environ Res 2016; 149: 171-178

Steinet al. Perfluoroalkyl and polyfluoroalkyl substances and indicators of immune function in children aged 12-19 y: National Health and Nutrition Examination Survey. Pediatr Res 2016; 79: 348-57

Timmermannet al. Association between perfluoroalkyl substance exposure and asthma and allergic disease in children as modified by MMR vaccination. J Immunotoxicol 2017; 14: 39-49

Tsai et al. Children's environmental health based on birth cohort studies of Asia. Sci Total Environ 2017; 609: 396-409

Wang et al. The effect of prenatal perfluorinated chemicals exposures on pediatric atopy. Environ Res 2011; 111: 785-91

Zhou Y. et al. Perfluoroalkyl substance exposure and urine CC16 levels among asthmatics: A case-control study of children. Environ Res 2017; 159: 158-163

Zhou Y., et al. Interaction effects of polyfluoroalkyl substances and sex steroid hormones on asthma among children. Sci Rep 2017; 7: 899

Zhu et al. Associations of serum perfluoroalkyl acid levels with T-helper cell-specific cytokines in children: By gender and asthma status. Sci Total Environ 2016; 559: 166-73

Endocrine

Audet-Delage et al. Persistent organic pollutants and transthyretin-bound thyroxin in plasma of Inuit women of childbearing age. Environ Sci Technol 2013; 47: 13086-92

Barrett et al. Perfluoroalkyl substances and ovarian hormone concentrations in naturally cycling women. Fertil Steril 2015; 103: 1261-70.

Berg et al. Assessing the relationship between perfluoroalkyl substances, thyroid hormones and binding proteins in pregnant women; a longitudinal mixed effects approach. Environ Int 2015; 77: 63-9

Berg et al. Persistent Organic Pollutants and the Association with Maternal and Infant Thyroid Homeostasis: A Multipollutant Assessment. Environ Health Perspect 2017; 125: 127-133

Bloom et al. Exploratory assessment of perfluorinated compounds and human thyroid function. Physiol Behav 2010; 99: 240-5

Chan et al. Perfluorinated acids and hypothyroxinemia in pregnant women. Environ Res 2011; 111: 559-64

Dallaire et al. Thyroid function and plasma concentrations of polyhalogenated compounds in Inuit adults. Environ Health Perspect 2009; 117: 1380-6

de Cock et al. Prenatal exposure to endocrine disrupting chemicals in relation to thyroid hormone levels in infants - a Dutch prospective cohort study. Environ Health 2014; 13: 106

Goudarzi et al. The Association of Prenatal Exposure to Perfluorinated Chemicals with Glucocorticoid and Androgenic Hormones in Cord Blood Samples: The Hokkaido Study. Environ Health Perspect 2017; 125: 111-118

Itoh et al. Association of perfluoroalkyl substances exposure in utero with reproductive hormone levels in cord blood in the Hokkaido Study on Environment and Children's Health. Environ Int 2016; 94: 51-59

Jain RB. Association between thyroid profile and perfluoroalkyl acids: data from NHNAES 2007-2008. Environ Res 2013; 126: 51-9

Kim, et al. Serum concentrations of major perfluorinated compounds among the general population in Korea: dietary sources and potential impact on thyroid hormones. Environ Int 2012; 45: 78-85

Joensen et al. PFOS (perfluorooctanesulfonate) in serum is negatively associated with testosterone levels, but not with semen quality, in healthy men. Hum Reprod 2018;28(3):599-608.

Kato et al. Association of perfluorinated chemical exposure in utero with maternal and infant thyroid hormone levels in the Sapporo cohort of Hokkaido Study on the Environment and Children's Health. Environ Health Prev Med 2016; 21: 334-344

Kim et al. Perfluoroalkyl substances in serum from South Korean infants with congenital hypothyroidism and healthy infants--Its relationship with thyroid hormones. Environ Res 2016; 147: 399-404

Kim et al. Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environ Sci Technol 2011; 45: 7465-72

Knox, S. et al. Perfluorocarbon exposure, gender and thyroid function in the C8 Health Project. J Toxicol Sci 2011; 36: 403-10

Knox et al. Implications of early menopause in women exposed to perfluorocarbons. J Clin Endocrinol Metab 2011; 96: 1747-53

Lewis et al. Serum Biomarkers of Exposure to Perfluoroalkyl Substances in Relation to Serum Testosterone and Measures of Thyroid Function among Adults and Adolescents from NHANES 2011-2012. Int J Environ Res Public Health 2015; 12: 6098-114

Li et al. Perfluorinated alkyl substances in serum of the southern Chinese general population and potential impact on thyroid hormones. Sci Rep 2017; 7: 43380

Lin et al. The associations between serum perfluorinated chemicals and thyroid function in adolescents and young adults. J Hazard Mater 2013; 244-245: 637-44

Lopez-Espinosa et al. Comparison between free serum thyroxine levels, measured by analog and dialysis methods, in the presence of perfluorooctane sulfonate and perfluorooctanoate. Reprod Toxicol 2012; 33: 552-5

Lopez-Espinosa et al. Thyroid function and perfluoroalkyl acids in children living near a chemical plant. Environ Health Perspect 2012; 120: 1036-41

Lopez-Espinosa et al. Perfluoroalkyl Substances, Sex Hormones, and Insulin-like Growth Factor-1 at 6-9 Years of Age: A Cross-Sectional Analysis within the C8 Health Project. Environ Health Perspect 2016; 124: 1269-75

Maisonet et al. Prenatal Exposure to Perfluoroalkyl Acids and Serum Testosterone Concentrations at 15 Years of Age in Female ALSPAC Study Participants. Environ Health Perspect 2015; 123: 1325-30

Melzer et al. Association between serum perfluorooctanoic acid (PFOA) and thyroid disease in the U.S. National Health and Nutrition Examination Survey. Environ Health Perspect. 2010;118(5):686-92.

Pirali et al. Perfluorooctane sulfonate and perfluorooctanoic acid in surgical thyroid specimens of patients with thyroid diseases. Thyroid 2009;19: 1407-12

Shah-Kulkarni et al. Prenatal exposure to perfluorinated compounds affects thyroid hormone levels in newborn girls. Environ Int 2016; 94: 607-613

Shrestha et al. Perfluoroalkyl substances, thyroid hormones, and neuropsychological status in older adults. Int J Hyg Environ Health 2017; 220: 679-685

Shrestha et al. Perfluoroalkyl substances and thyroid function in older adults. Environ Int 2015; 75: 206-14

Tsai et al. Perfluoroalkyl substances and thyroid hormones in cord blood. Environ Pollut 2017; 222: 543-548

Tsai et al. Association between perfluoroalkyl substances and reproductive hormones in adolescents and young adults. Int J Hyg Environ Health 2015; 218: 437-43

Vagi et al. Exploring the potential association between brominated diphenyl ethers, polychlorinated biphenyls, organochlorine pesticides, perfluorinated compounds, phthalates, and bisphenol A in polycystic ovary syndrome: a case-control study. BMC Endocr Disord 2014; 14: 86

Wang et al. Association between maternal serum perfluoroalkyl substances during pregnancy and maternal and cord thyroid hormones: Taiwan maternal and infant cohort study. Environ Health Perspect 2014; 122: 529-34

Wang et al. Association between perfluoroalkyl substances and thyroid stimulating hormone among pregnant women: a cross-sectional study. Environ Health 2013; 12: 76

Webster et al. Cross-Sectional Associations of Serum Perfluoroalkyl Acids and Thyroid Hormones in U.S. Adults: Variation According to TPOAb and Iodine Status (NHANES 2007-2008). Environ Health Perspect 2016; 124: 935-42

Webster et al. Associations between perfluoroalkyl acids (PFASs) and maternal thyroid hormones in early pregnancy: a population-based cohort study. Environ Res 2014; 133: 338-47

Wen et al. Association between serum perfluorinated chemicals and thyroid function in U.S. adults: the National Health and Nutrition Examination Survey 2007-2010. J Clin Endocrinol Metab 2013; 98: E1456-64

Winquist & Steenland. Perfluorooctanoic acid exposure and thyroid disease in community and worker cohorts. Epidemiology 2014; 25: 255-64

Yang et al. Placental Transfer of Perfluoroalkyl Substances and Associations with Thyroid Hormones: Beijing Prenatal Exposure Study. Sci Rep 2016;6: 21699

Zhou et al. Association of perfluoroalkyl substances exposure with reproductive hormone levels in adolescents: By sex status. Environ Int 2016; 94: 189-195

Cancer

Alexander & Olsen. Bladder cancer in perfluorooctanesulfonyl fluoride manufacturing workers. Ann Epidemiol 2007; 17: 471-8

Barry et al. Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. Environ Health Perspect 2013; 121: 1313-8

Bonefeld-Jorgensen et al. Perfluorinated compounds are related to breast cancer risk in Greenlandic Inuit: a case control study. Environ Health 2011; 10: 88

Bonefeld-Jorgensen et al. Breast cancer risk after exposure to perfluorinated compounds in Danish women: a case-control study nested in the Danish National Birth Cohort. Cancer Causes Control 2014; 25: 1439-48

Ducatman et al. Prostate-specific antigen and perfluoroalkyl acids in the C8 health study population. J Occup Environ Med 2015; 57: 111-4

Eriksen, et al. Perfluorooctanoate and perfluorooctanesulfonate plasma levels and risk of cancer in the general Danish population. J Natl Cancer Inst 2009; 101: 605-9

Fry & Power Persistent organic pollutants and mortality in the United States, NHANES 1999-2011. Environ Health 2017; 16 :105

Hardell et al. Case-control study on perfluorinated alkyl acids (PFAAs) and the risk of prostate cancer. Environ Int 2014; 63: 35-9

Innes et al. Inverse association of colorectal cancer prevalence to serum levels of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in a large Appalachian population. BMC Cancer 2014; 14: 45

Koskela et al. Effects of developmental exposure to perfluorooctanoic acid (PFOA) on long bone morphology and bone cell differentiation. Toxicol Appl Pharmacol 2016; 301: 14-21

Koskela et al. Perfluoroalkyl substances in human bone: concentrations in bones and effects on bone cell differentiation. Sci Rep 2017; 7: 6841

Mastrantonio et al. Drinking water contamination from perfluoroalkyl substances (PFAS): an ecological mortality study in the Veneto Region, Italy. Eur J Public Health 2018; 28: 180-185

Raleigh et al. Mortality and cancer incidence in ammonium perfluorooctanoate production workers. Occup Environ Med 2014; 71: 500-6

Vieira et al. Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: a geographic analysis. Environ Health Perspect 2013; 121: 318-23

Wielsoe et al. Serum levels of environmental pollutants is a risk factor for breast cancer in Inuit: a case control study. Environ Health 2017; 16: 56

Yeung et al. Profiles of perfluoroalkyl substances in the liver and serum of patients with liver cancer and cirrhosis in Australia. Ecotoxicol Environ Saf 2013; 96: 139-46

Neurological

Berk et al. Pop, heavy metal and the blues: secondary analysis of persistent organic pollutants (POP), heavy metals and depressive symptoms in the NHANES National Epidemiological Survey. BMJ Open 2014; 4: e005142

Braun et al. Gestational exposure to endocrine-disrupting chemicals and reciprocal social, repetitive, and stereotypic behaviors in 4- and 5-year-old children: the HOME study. Environ Health Perspect 2014; 122 513-20

Chen et al. Perfluorinated compound levels in cord blood and neurodevelopment at 2 years of age. Epidemiology 2013; 24: 800-8

Donauer et al. Prenatal exposure to polybrominated diphenyl ethers and polyfluoroalkyl chemicals and infant neurobehavior. J Pediatr 2015; 166 736-42

Fei et al. Prenatal exposure to perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy. Environ Health Perspect 2008; 116: 1391-5

Fei and Olsen. Prenatal exposure to perfluorinated chemicals and behavioral or coordination problems at age 7 years. Environ Health Perspect 2011; 119: 573-8

Forns et al. Perfluoroalkyl substances measured in breast milk and child neuropsychological development in a Norwegian birth cohort study. Environ Int 2015; 83: 176-82

Goudarzi et al. Prenatal exposure to perfluorinated chemicals and neurodevelopment in early infancy: The Hokkaido Study. Sci Total Environ 2016; 541: 1002-1010

Gump et al. Perfluorochemical (PFC) exposure in children: associations with impaired response inhibition. Environ Sci Technol 2011; 45: 8151-9

Hoffman et al. Exposure to polyfluoroalkyl chemicals and attention deficit/hyperactivity disorder in U.S. children 12-15 years of age. Environ Health Perspect 2010; 118: 1762-7

Hoyer B. et al. Exposure to perfluoroalkyl substances during pregnancy and child behaviour at 5 to 9years of age. Horm Behav. 2018;101:105-112

Hoyer B. et al. Pregnancy serum concentrations of perfluorinated alkyl substances and offspring behaviour and motor development at age 5-9 years-a prospective study. Environ Health 2015;14: 2

Jeddy et al. Prenatal concentrations of Perfluoroalkyl substances and early communication development in British girls. Early Hum Dev 2017; 109: 15-20

Lien et al. Perfluoroalkyl substances in cord blood and attention deficit/hyperactivity disorder symptoms in seven-year-old children. Chemosphere 2016; 156: 118-127

Liew et al. Attention deficit/hyperactivity disorder and childhood autism in association with prenatal exposure to perfluoroalkyl substances: a nested case-control study in the Danish National Birth Cohort. Environ Health Perspect 2015; 123: 367-73

Lyall et al. Prenatal Maternal Serum Concentrations of Per- and Polyfluoroalkyl Substances in Association with Autism Spectrum Disorder and Intellectual Disability. Environ Health Perspect 2018; 126: 017001

Ode et al. Fetal exposure to perfluorinated compounds and attention deficit hyperactivity disorder in childhood. PLoS One 2014; 9: e95891

Oulhote et al. Behavioral difficulties in 7-year old children in relation to developmental exposure to perfluorinated alkyl substances. Environ Int 2016; 97: 237-245

Power et al. Cross-sectional association between polyfluoroalkyl chemicals and cognitive limitation in the National Health and Nutrition Examination Survey. Neuroepidemiology 2013; 40: 125-32

Quaak et al. Prenatal Exposure to Perfluoroalkyl Substances and Behavioral Development in Children. Int J Environ Res Public Health 2016; 13

Shiue I. Urinary heavy metals, phthalates and polyaromatic hydrocarbons independent of health events are associated with adult depression: USA NHANES, 2011-2012. Environ Sci Pollut Res Int 2015; 22: 17095-103

Shiue I. Arsenic, heavy metals, phthalates, pesticides, hydrocarbons and polyfluorinated compounds but not parabens or phenols are associated with adult remembering condition: US NHANES, 2011-2012. Environ Sci Pollut Res Int 2015; 22: 6381-6

Shiue I. Urinary arsenic, pesticides, heavy metals, phthalates, polyaromatic hydrocarbons, and polyfluoroalkyl compounds are associated with sleep troubles in adults: USA NHANES, 2005-2006. Environ Sci Pollut Res Int 2017; 24: 3108-3116

Stein and Savitz. Serum perfluorinated compound concentration and attention deficit/hyperactivity disorder in children 5-18 years of age. Environ Health Perspect 2011; 119: 1466-71

Stein et al. Perfluorooctanoate and neuropsychological outcomes in children. Epidemiology 2013; 24: 590-9

Stein et al. Perfluorooctanoate exposure in a highly exposed community and parent and teacher reports of behaviour in 6-12-year-old children. Paediatr Perinat Epidemiol 2014; 28: 146-56

Strom et al. Persistent organic pollutants measured in maternal serum and offspring neurodevelopmental outcomes--a prospective study with long-term follow-up. Environ Int 2014; 68: 41-8

Vuong et al. Prenatal polybrominated diphenyl ether and perfluoroalkyl substance exposures and executive function in school-age children. Environ Res 2016; 147: 556-64

Wang et al. Prenatal exposure to perfluroalkyl substances and children's IQ: The Taiwan maternal and infant cohort study. Int J Hyg Environ Health 2015; 218: 639-44

Zhang et al. Prenatal and childhood perfluoroalkyl substances exposures and children's reading skills at ages 5 and 8years. Environ Int 2018;111: 224-231

Ex vivo abstracts

Bjerregaard-Olesen et al. Extraction of perfluorinated alkyl acids from human serum for determination of the combined xenoestrogenic transactivity: a method development. Chemosphere. 2015;129:232-8.

Bjerregaard-Olesen et al. Activation of the estrogen receptor by human serum extracts containing mixtures of perfluorinated alkyl acids from pregnant women. Environ Res. 2016;151:71-79.

Wielsoe et al. Receptor activities of persistent pollutant serum mixtures and breast cancer risk. Endocr Relat Cancer. 2018;25(3):201-215.

Simon E, Bytingsvik J. et al. Blood plasma sample preparation method for the assessment of thyroid hormone-disrupting potency in effect-directed analysis. Environ Sci Technol. 2011;45(18):7936-44.

List of novel effect biomarkers found during the literature search.

Caserta et al., The influence of endocrine disruptors in a selected population of infertile women', Gynecol Endocrinol, 2013;29: 444-7.

Caserta et al., Correlation of endocrine disrupting chemicals serum levels and white blood cells gene expression of nuclear receptors in a population of infertile women. Int J Endocrinol, 2013;2013: 510703.

Fletcher et al., Associations between PFOA, PFOS and changes in the expression of genes involved in cholesterol metabolism in humans. Environ Int, 2013;57-58: 2-10.

La Rocca et al., Exposure and effective dose biomarkers for perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) in infertile subjects: preliminary results of the PREVIENI project. Int J Hyg Environ Health, 2012;215: 206-11.

La Rocca et al., Exposure to Endocrine Disruptors and Nuclear Receptors Gene Expression in Infertile and Fertile Men from Italian Areas with Different Environmental Features. Int J Environ Res Public Health, 2015;12: 12426-45.

La Rocca et al., Exposure to endocrine disrupters and nuclear receptor gene expression in infertile and fertile women from different Italian areas. Int J Environ Res Public Health, 2014;11: 10146-64.

Pennings et al., Cord blood gene expression supports that prenatal exposure to perfluoroalkyl substances causes depressed immune functionality in early childhood. J Immunotoxicol, 2016;13: 173-80.

6.6 Brominated Flame Retardants (coordinated by MU)

6.6.1 Exploratory search

In the literature search for biomarkers of effects (biochemical/molecular) used in epidemiology studies, linking BFRs exposure to adverse health effects, 170 were selected. Abstracts and some whole articles were studied in an initial screening step.

This first screening for biomarkers resulted in:

- 87 epidemiology studies that did not use biomarkers of effects (molecular/biochemical), considering adverse effects of BFR exposure at tissue/organism (cohort) level.
- 9 “non-standard” studies (e.g., placental explants, SNPs…).
- 74 “relevant” references: epidemiology studies linking exposure to BFRs to molecular/biochemical biomarkers.

Among these 74 references, 58 molecular/biochemical biomarkers were found, of which:

- ✓ 35 were found in one study alone, making it difficult to establish the link to exposure. These are therefore considered as traditional but less studied effect biomarkers.
- ✓ 23 biomarkers were found in at least two studies.
- ✓ Of these 23 biomarkers.
- ✓ 19 can be considered as “traditional”. These were analyzed in the large majority of these studies.
- ✓ 4 can be considered as “novel”.

6.6.2 List of traditional effect biomarkers

The table below summarizes the traditional effect biomarkers related to BFR exposure together with the number of scientific references retrieved in the literature search.

Table 16. List of traditional effect biomarkers for Brominated flame retardants chemicals

Biomarker	No. Of studies	Type	Health endpoint
T4	40	traditional	Thyroid
TSH	37	traditional	Thyroid
T3	32	traditional	Thyroid
thyroglobulin antibodies	2	traditional	Thyroid
Thyroid stimulating immunoglobulin	2	traditional	Thyroid
TBG/thyroxin Binding Globulin/Thyroid Binding globulin	4	traditional	Thyroid
LH	9	traditional	Reprod
FSH	8	traditional	Reprod
Testosterone	7	traditional	Reprod
estradiol(E2)	6	traditional	Reprod
Lipids	8	traditional	Metab
Glucose	2	traditional	Metab
Hemoglobin	2	traditional	Blood
white blood cells	2	traditional	Blood
Inhibin-B	5	traditional	Reprod
SHBG	5	traditional	Reprod & Metab
FAI	2	traditional	Reprod
HbA1c	4	traditional	Metab
Anti-TPO	3	traditional	Thyroid
BMI:leptin	1	Traditional but less studied	Obesity/ Cardio-metabolic
Body fat	1	Traditional but less studied	Obesity/ Cardio-metabolic

cardiovascular response	1	Traditional but less studied	Obesity/ Cardio-metabolic
Cortisol	1	Traditional but less studied	Neurodevelopment
Cotinine	1	Traditional but less studied	Allergy/Immune
Creatinine	1	Traditional but less studied	Metabolic
CRP	1	Traditional but less studied	Oxidative stress
Cyp17A1	1	Traditional but less studied	Neurodevelopment/ Reproductive
Cyp19A1	1	Traditional but less studied	Neurodevelopment/ Reproductive
Cyp1A1	1	Traditional but less studied	Neurodevelopment/ Reproductive
Cyp2E1	1	Traditional but less studied	Neurodevelopment/ Reproductive
Cyp2J2	1	Traditional but less studied	Neurodevelopment/ Reproductive
DIO3	1	Traditional but less studied	Thyroid/ OMICs
DNA damage	1	Traditional but less studied	Oxidative stress/ Metabolism
Epstein-Barr virus IgG	1	Traditional but less studied	Immune System
ESR1	1	Traditional but less studied	Systemic / Signaling
ESR2	1	Traditional but less studied	Systemic / Signaling
Fas	1	Traditional but less studied	Systemic / Signaling
GGT	1	Traditional but less studied	Metabolism/ Hepatic Function
GH	1	Traditional but less studied	Reproductive
Cholesterol	1	Traditional but less studied	Cardio-metabolic/ Obesity
Insulin	1	Traditional but less studied	Cardio-metabolic/ Obesity
NR3C1	1	Traditional but less studied	Systemic / Signaling
Platelets	1	Traditional but less studied	Blood
Protein complement (C3, 3a, 4)	1	Traditional but less studied	Immune System
SHBG-bound testosterone	1	Traditional but less studied	Reproductive
TH SULT activities	1	Traditional but less studied	Reproductive
Thrombopoietin	1	Traditional but less studied	Blood
TNFa	1	Traditional but less studied	Immune System
TUNEL	1	Traditional but less studied	DNA damage

6.6.3 List of novel effect biomarkers found during the literature search

A brief list of novel effect biomarkers related to brominated flame retardants (BFR) exposure is shown in the table below.

Table 17. List of novel effect biomarkers for Brominated flame retardants.

Novel effect Biomarker	No. of studies	Type	**Health Endpoint**
Global DNA methylation (LINE1, Alu)	2	novel	Systemic / Signaling
IGF-1	3	novel	Systemic / Signaling
IGF2	2	novel	Systemic / Signaling
IGFBP-3	2	novel	Systemic / Signaling

6.6.4 References

References were not provided

6.7 Organophosphate retardants (coordinated by EASP)

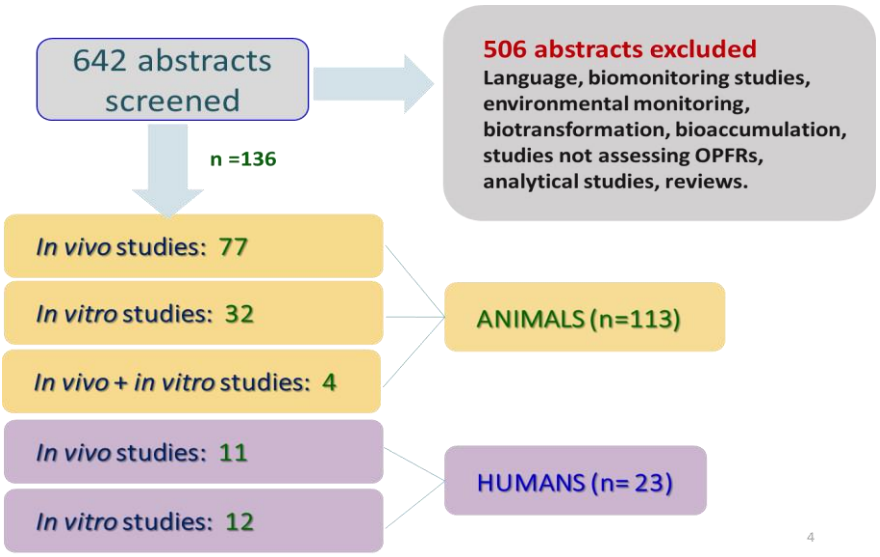
6.7.1 Terms used for the search

Two search strategies were used to identify references on effect biomarkers for organophosphate flame retardants (OPFRs). First, we used a specific syntax including MeSH terms, individual names of OPFRs compounds, and terms for health endpoints. Because fewer references were retrieved than expected, we then conducted a broader and more general search, using only the terms (organophosphate OR organophosphorus) AND (“Flame Retardants [Mesh]), thereby generating a larger number of studies of potential interest.

6.7.2 Exploratory search

The figure below depicts the selection and nature of studies found on organophosphate flame retardants exposure in relation to health outcomes.

Figure 6. Exploratory search used for organophosphate flame retardants chemical family



6.7.3 List of traditional effect biomarkers

The few human studies identified that met the selection criteria assessed only traditional biomarkers of effect, and those related to neurodevelopment used functional evaluations of neuropsychological skills. In some cases, effect biomarkers (e.g., SM, Sph, CER, for cardiac function) are not consistently used in clinical settings or in preclinical evaluations of chemicals. In other cases, such as allergy, outcomes can be assessed by using clinical end points or diagnostic criteria which do not fully meet the definition of biomarkers of effect. The same applies to a few reproductive outcomes of in vitro fertilization such as those related to oocyte or embryo quality. However, these outcomes are used in a similar way than biomarkers of effect.

A list of traditional effect biomarkers identified in our systematic review is shown in the table below:

Table 18. List of effect biomarkers found for OPFRs.

Health Outcome	Biomarker
Neurodevelopment	Social Behavior (Skills Improvement Rating scale)
Neurodevelopment	WISC-IV
Neurodevelopment	Standard Progressive matrices test
Endocrine Disruption	fT4, TT4, TT3, TSH
Endocrine Disruption	Sexual and Thyroid hormones; Semen quality
Reproductive Diseases	Oocyte (retrieval, fertilization), embryo quality, implantation, pregnancy, birth
Oxidative Stress	8-OHdG

Cardiovascular Function	SM, Cer, Sph, S1P
Allergy and Immune Diseases	Allergy (cutaneous and respiratory)

6.7.4 List of novel effect biomarkers found during the literature search

The systematic review did not identify any epidemiological studies that used novel effect biomarkers for OPFRs.

6.7.5 Proposal of effect biomarkers for their implementation in HBM

One, two, or several biomarkers are proposed for their implementation in HBM together with an explanation of their selection.

Based on the results of the systematic search, the following biomarkers of effect for OPFR are proposed for possible implementation in human biomonitoring studies, based on their feasibility and standardized utilization in epidemiological studies:

• Thyroid hormones: TSH TT3, TT4, FT3, FT4 (serum).

• Sexual hormones: estradiol, prolactin, testosterone, inhibin-B, Follicle-Stimulating Hormone (FSH), and Luteinizing Hormone (LH) (serum).

6.7.6 Gaps in Knowledge

A brief description of the Gaps in Knowledge identified in the literature search and based on the knowledge of OPFRS partners.

• Most of the biomarkers of effect of OPFR have been used in in vitro studies but not in epidemiologic research.

• Only a few AOPs have been properly developed (most human studies assess AO instead of KE, although modern epidemiology can make use of -omics as health outcomes, some of which can be late downstream KE). These AOPs can be found in the AOP wiki. For instance, AOP 42: "inhibition of thyroperoxidase and subsequent adverse neurodevelopmental outcomes in mammals" (https://aopwiki.org/wiki/index.php/Aop:42), and AOP 8: "Upregulation of thyroid hormone catabolism via activation of hepatic nuclear receptors, and subsequent adverse neurodevelopmental outcomes in mammals (https://aopwiki.org/wiki/index.php/Aop:8). In both cases, TSH and T3 or T4 are considered KE. The same applies for sexual hormones (AOP 23).

• There is paucity of data for assessing the feasibility of biomarkers of effect for OPFRs.

• More research is needed to evaluate whether in vitro biomarkers are suitable for use in epidemiological studies.

- More research is needed to evaluate if *in vitro* biomarkers are amenable for use in epidemiological studies.

6.7.7 Conclusions

• The available epidemiological information on biomarkers of effect for OPFRs is limited.

• There are human biomonitoring studies measuring OPFRs in urine and environmental monitoring (home dust) but not accompanied by biomarkers of effect.

• Novel information is available from in vitro studies (animal and human systems), but potential biomarkers have not been translated to human epidemiological research.

6.7.8 References

Araki A, Saito I, Kanazawa A, Morimoto K, Nakayama K, Shibata E, Tanaka M, Takigawa T, Yoshimura T, Chikara H, Saijo Y, Kishi R. Phosphorus flame retardants in indoor dust and their relation to asthma and allergies of inhabitants. Indoor Air. 2014; 24:3-15. doi: 10.1111/ina.12054.

Ballesteros-Gómez A, Erratico C, Eede NV, Ionas AC, Leonards PE, Covaci A. In vitro metabolism of 2-ethylhexyldiphenyl phosphate (EHDPHP) by human liver microsomes. Toxicol Lett. 2015;232:203-12. doi: 10.1016/j.toxlet.2014.11.007.

Belcher SM, Cookman CJ, Patisaul HB, Stapleton HM. In vitro assessment of human nuclear hormone receptor activity and cytotoxicity of the flame retardant mixture FM 550 and its triarylphosphate and brominated components. Toxicol Lett. 2014; 228:93-102. doi: 10.1016/j.toxlet.2014.04.017.

Carignan CC, Mínguez-Alarcón L, Butt CM, Williams PL, Meeker JD, Stapleton HM, Toth TL, Ford JB, Hauser R; EARTH Study Team. Urinary Concentrations of Organophosphate Flame Retardant Metabolites and Pregnancy Outcomes among Women Undergoing in Vitro Fertilization. Environ Health Perspect. 2017; 25; 125:087018. doi: 10.1289/EHP1021.

Castorina R, Bradman A, Stapleton HM, Butt C, Avery D, Harley KG, Gunier RB, Holland N, Eskenazi B. Current-use flame retardants: Maternal exposure and neurodevelopment in children of the CHAMACOS cohort. Chemosphere. 2017; 189:574-580. doi: 10.1016/j.chemosphere.2017.09.037.

Chapman DE, Michener SR, Powis G. Metabolism of the flame retardant plasticizer tris(2-chloroethyl) phosphate by human and rat liver preparations. Fundam Appl Toxicol. 1991;17: 215-24.

Esa AH, Warr GA, Newcombe DS. Immunotoxicity of organophosphorus compounds. Modulation of cell-mediated immune responses by inhibition of monocyte accessory functions. Clin Immunol Immunopathol. 1988; 49:41-52.

Gutter B, Rosenkranz HS. The flame retardant tris (2, 3-dibromopropyl) phosphate: alteration of human cellular DNA. Mutat Res. 1977; 56:89-90.

Hu W, Gao F, Zhang H, Hiromori Y, Arakawa S, Nagase H, Nakanishi T, Hu J. Activation of Peroxisome Proliferator-Activated Receptor Gamma and Disruption of Progesterone Synthesis of 2-Ethylhexyl Diphenyl Phosphate in Human Placental Choriocarcinoma Cells: Comparison with Triphenyl Phosphate. Environ Sci Technol. 2017; 51:4061-4068. doi: 10.1021/acs.est.7b00872.

Hutter HP, Haluza D, Piegler K, Hohenblum P, Fröhlich M, Scharf S, Uhl M, Damberger B, Tappler P, Kundi M, Wallner P, Moshammer H. Semivolatile compounds in schools and their influence on cognitive performance of children. Int J Occup Med Environ Health. 2013; 26:628-35. doi: 10.2478/s13382-013-0125-z.

Jin Y, Chen G, Fu Z. Effects of TBEP on the induction of oxidative stress and endocrine disruption in Tm3 Leydig cells. Environ Toxicol. 2016; 31:1276-86. doi: 10.1002/tox.22137.

Li F, Cao L, Li X, Li N, Wang Z, Wu H. Affinities of organophosphate flame retardants to tumor suppressor gene p53: an integrated in vitro and in silico study. Toxicol Lett. 2015; 232:533-41. doi: 10.1016/j.toxlet.2014.12.006.

Li F, Wang L, Ji C, Wu H, Zhao J, Tang J. Toxicological effects of tris(2-chloropropyl) phosphate in human hepatic cells. Chemosphere. 2017; 187:88-96. doi: 10.1016/j.chemosphere.2017.08.083.

Li L, Jiang S, Li K, Lin B, Wang Z, Zhang Z, Fang Y. Assessment of tris (1, 3-dichloro-2-propyl) phosphate toxicology in PC12 cells by using digital gene expression profiling. Chemosphere. 2017; 183:353-360. doi: 10.1016/j.chemosphere.2017.05.108.

Li R, Zhou P, Guo Y, Zhou B. The involvement of autophagy and cytoskeletal regulation in TDCIPP-induced SH-SY5Y cell differentiation. Neurotoxicology. 2017; 62:14-23. doi: 10.1016/j.neuro.2017.05.002.

Lipscomb ST, McClelland MM, MacDonald M, Cardenas A, Anderson KA, Kile ML. Cross sectional study of social behaviors in preschool children and exposure to flame retardants. Environ Health. 2017; 16(1):23. doi: 10.1186/s12940-017-0224-6.

Lu SY, Li YX, Zhang T, Cai D, Ruan JJ, Huang MZ, Wang L, Zhang JQ, Qiu RL. Effect of E-waste Recycling on Urinary Metabolites of Organophosphate Flame Retardants and Plasticizers and Their Association with Oxidative Stress. Environ Sci Technol. 2017; 51:2427-2437. doi: 10.1021/acs.est.6b05462.

Meeker JD, Stapleton HM. House dust concentrations of organophosphate flame retardants in relation to hormone levels and semen quality parameters. Environ Health Perspect. 2010 ;118: 318-23. doi: 10.1289/ehp.0901332.

Phillips AL, Chen A, Rock KD, Horman B, Patisaul HB, Stapleton HM. Editor's Highlight: Transplacental and Lactational Transfer of Firemaster® 550 Components in Dosed Wistar Rats. Toxicol Sci. 2016; 153:246-57. doi: 10.1093/toxsci/kfw122.

Preston EV, McClean MD, Claus Henn B, Stapleton HM, Braverman LE, Pearce EN, Makey CM, Webster TF. Associations between urinary diphenyl phosphate and thyroid function. Environ Int. 2017; 101:158-164. doi: 10.1016/j.envint.2017.01.020.

Van den Eede N, Maho W, Erratico C, Neels H, Covaci A. First insights in the metabolism of phosphate flame retardants and plasticizers using human liver fractions. Toxicol Lett. 2013; 223: 9-15. doi: 10.1016/j.toxlet.2013.08.012.

Xiang P, Liu RY, Li C, Gao P, Cui XY, Ma LQ. Effects of organophosphorus flame retardant TDCPP on normal human corneal epithelial cells: Implications for human health. Environ Pollut. 2017; 230:22-30. doi: 10.1016/j.envpol.2017.06.036.

Zhang J, Williams TD, Chipman JK, Viant MR. Defensive and adverse energy-related molecular responses precede tris (1,3-dichloro-2-propyl) phosphate cytotoxicity. J Appl Toxicol. 2016 ;36: 649-58. doi: 10.1002/jat.3194.

Zhao F, Wan Y, Zhao H, Hu W, Mu D, Webster TF, Hu J. Levels of Blood Organophosphorus Flame Retardants and Association with Changes in Human Sphingolipid Homeostasis. Environ Sci Technol. 2016; 50: 8896-903. doi: 10.1021/acs.est.6b02474.

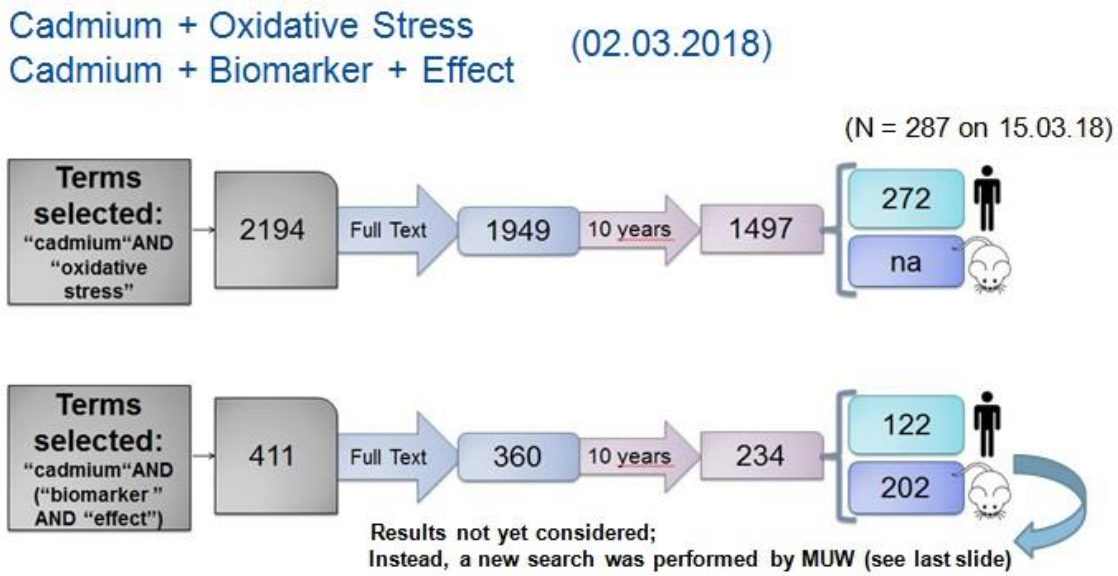
6.8 Metals: Cadmium (Coordinated by MUW)

6.8.1 Terms Used and Exploratory search

The search was divided into two main parts: The first, for which MUW was responsible, focused on cadmium exposure and i) renal function/ nephrotoxicity. The second, coordinated by BfR, focused on cadmium exposure and the following health outcomes: ii) oxidative stress, iii) Allergy and Immune system, iv) Epigenetics and OMICs.

MU: It was used the search terms “Cadmium“ AND “Effect marker“ filtered for species by “human” and for publication date by “10 years”. The resulting 84 abstracts were reviewed for specific information on biomarkers of kidney dysfunction associated with Cd exposure levels. Abstracts of 'related' articles were also included in case they yielded additional relevant information. 25 abstracts were finally selected for this summary.

Figure 7. Strategy used by MUW for the exploratiry search

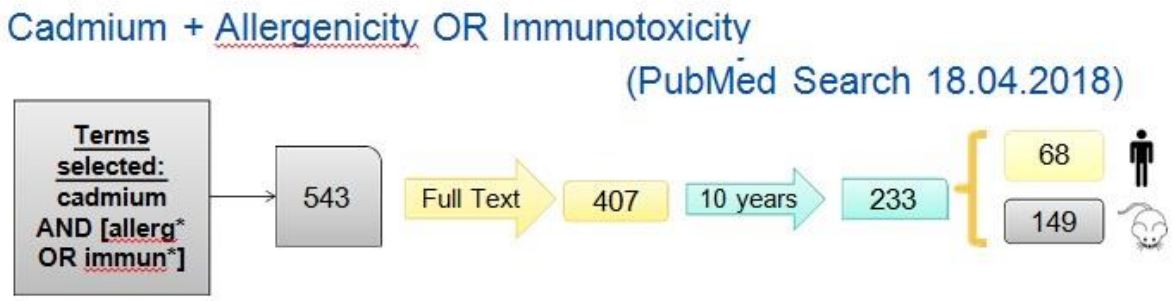


BfR used the search terms: “Cadmium“ AND “Oxidative Stress“ In this search, filtered by the category “human”, 287 Abstracts were found. Oxidative stress is well known to be associated with multiple environmental exposures (mostly PAH and heavy metals). Accordingly, many human studies found in this search related to multi-exposure scenarios. Compared with the first search, only a small number of relevant abstracts were obtained (N = 9).

Figure 8. Strategy used by BfR for the exploratory search

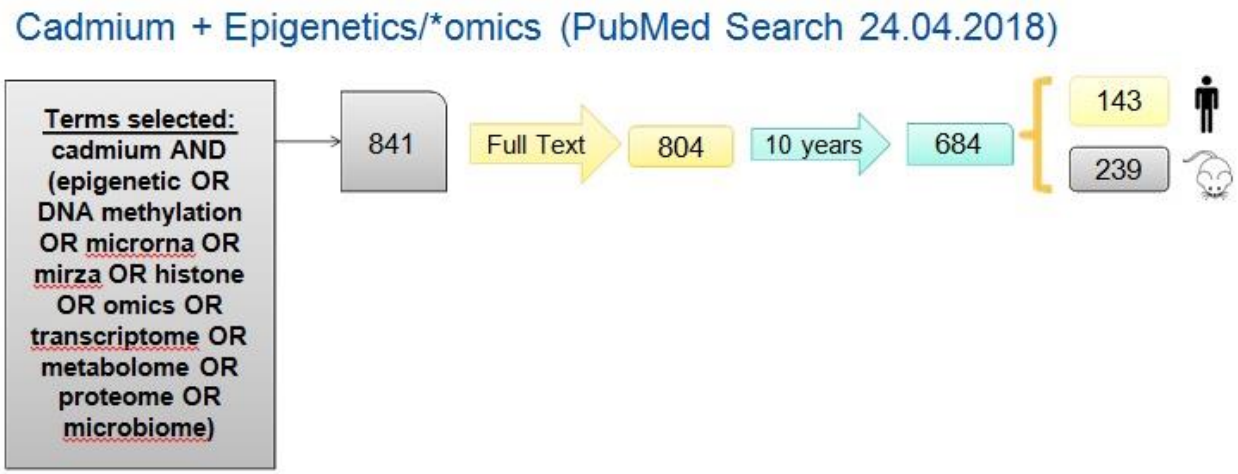
PubMed search: “Allergenicity“ AND “Immunotoxicity“ (BfR). Out of 189 screened abstracts (filter “Human” and “Animal”), 15 references were considered as potentially relevant. Generally, only a few references addressed the effects of Cd on immune functions in an epidemiological setting; therefore, data from in vitro and animal studies were also considered.

Figure 9. Strategy used for allergy/immunmology health outcomes and cadmium exposure



“Epigenetics“ AND “Omics-related methods“ (BfR). After screening the 347 abstracts obtained (search filtered for “human” and “animal”), 55 references were selected and reviewed. The results are presented in different sections according to the search terms used, covering the different fields of omics approaches.

Figure 10. Strategy used for Epigenetics/omics outcomes and cadmium exposure



6.8.2 List of traditional effect biomarkers

6.8.2.1 Renal Function Effect Biomarkers (MUW):

The earliest manifestations of cadmium nephrotoxicity, with increased urinary excretion of low and high molecular weight proteins such as β2-microglobulin, α1-microglobulin, retinol-binding protein, albumin, transferrin, IgG and others, have been characterized (Bernard & Lauwerys, 1990).

Urinary Beta-2 microglobulin (B2MG) and N-acetyl-β-D glucosaminidase (NAG) are well established and sensitive, and are the most frequently used biomarkers relating Cd exposure to renal tubular dysfunction. Kidney dysfunction is indicated by enhanced urinary B2MG and NAG levels. While B2MG is a microprotein (12 kDa) necessary for cell surface expression of MHC class I molecules and the HFE protein, NAG is a large lysosomal brush border enzyme (>130 kDa) of the proximal tubular cells not filtered through the glomerular membrane (Argyropoulos et al., 2017; Moriguchi et al., 2009). Despite their wide use in HBM studies and their entirely different functions, surprisingly little is known in about the way in which Cd-imnduced damage to kidney cells causes a simultaneously increase in urinary B2MG and NAG levels.

The role of metallothionein (MT) in Cd metabolism is more than well-known (Nordberg et al.,2009). To establish MT expression levels of lymphocytes on the protein and/or mRNA as biomarker of Cd exposure is challenging for practical reasons but merits further investigation.
Other markers of kidney failure including creatinine, urinary albumin, urinary total protein, urinary calcium, urinary retinol binding protein (RBP) as well as the glomerular filtration rate were examined together with B2MG and/or NAG in most of the reviewer studies.

6.8.2.2 Oxidative stress:

Most of the markers presented in the papers were also related to early effects of tubular dysfunction in the kidney, which is the main target organ of cadmium-induced toxicity. Accordingly, only a few markers related to oxidative stress were detected and were not retrieved in the first search. These are glutathione peroxidase (GPx), selenium, and glutathione (GSH), reduced in blood and urine of Cd-exposed human subjects (Cabral et al.,2015); and protein carbonylation

related to changes in oxidative stress-associated enzymes GPx, glutathione reductase (GR), SOD and CAT in breast milk, reported by (Castillo-Castaneda et al., 2017). Metabolic changes induced by Cd, other heavy metals and PAH in 252 study subjects were associated with oxidative stress biomarkers 8-OHdG, HNE-MA, 8- isoPGF2alpha, and 8-NO2Gua, using 2D-GC-ToF mass spectrometry. Urine metabolomics identified age-dependent biological pathways [33]. Additionally, associations of Cd exposure with lipid peroxidation (e.g., malondialdehyde/MDA) have been described [Cabral et al., 2015; Cuypers et al., 2010; Krueger & Wade, 2016].

The search retrieved no additional references to those in a review on Cd-related biomarkers of oxidative stress (Cuypers et al., 2010). At this preliminary stage of the analysis, our search does not yield any effect biomarker.

6.8.2.3 Allergy and Immune system.

The results of a cross-sectional human health survey (NHANES data) suggest that the immunological effects of lead and cadmium toxicity may result in an increased susceptibility to chronic infections (Krueger & Wade, 2016).

A study in urban workers demonstrated an association between airborne Cd exposure and values of blood counts (Ciarrocca et al., 2015).

In a birth cohort study, the relationship between prenatal arsenic and cadmium exposures and a variety of T cell subpopulations was analyzed in cord blood of 63 participants. An effect of prenatal exposure to As and Cd on the distribution of T cell populations at birth was postulated (Nygaard et al., 2017).

A review on immunotoxic effects of heavy metals (Ohsawa M, 2009) reports an enhanced autoantibody production due to oral exposure to Cd at environmental levels. Immunostimulation, including induction of autoantibodies, was concluded to be the primary immunotoxic effect of Cd, because of the dose-sensitivity and the association with polyclonal B cell activation (PBA). Based on the similarity among PBAs induced by inorganic salts of Cd, mercury and lead, a common effect of the metals and their involvement in pathogenesis of nephritis was hypothesized.

In vitro and animal studies confirmed significant effects of Cd on the immune system. However, due to the nature of the data, no effect biomarkers related to immune responses can be recommended.

6.8.3 List of novel effect biomarkers found during the literature search

6.8.3.1 Renal Function

The biomarkers urinary citrate (known to protect against urinary stone formation), Kidney injury molecule-1 (Kim-1) (indicating early renal proximal tubule injury), and Cystatin C (CysC) (a marker of the glomerular filtration rate), are promising novel biomarkers [24-28]. The available knowledge on Cd-induced alterations of Kim-1 and CysC levels stems from experiments on male adult rats. It is substantiated by a thorough description of the potential underlying pathomechanisms [29]. However, considering the large differences in Cd nephrotoxicity between females and males, more research is required to confirm these findings in (female) humans. There is no clear evidence for

involvement of the biomarker proteins in AOP 257 (https://aopwiki.org/aops/257), i.e., disturbance of lysosomal function also induced by Cd, which results in proximal tubule cell toxicity.

6.8.3.2 Epigenetics and OMICs

In general, many explorative references we found, mainly for *in vitro* or animal studies or in reference to ecotoxicology. There were few epidemiological/human studies, and the results were often difficult to interpret because of the low sample sizes. These limitations especially apply to the transcriptomics and proteomics studies.

- Epigenetics

A review published 6 years ago focuses on epigenetic targets of toxicologically relevant metals including Cd (Cheng et al., 2012). Cd is well known to induce DNA hyper- and hypomethylation in a diverse set of genes and to affect non-coding RNA expression. No human studies were referenced.

Epigenetic effects focusing on differential methylation of both maternal and fetal genomes associated with maternal Cd burden are reviewed in (Dharmadasa et al., 2017). Authors conclude that research on methylation changes to the fetal genome due to prenatal Cd exposure is scarce. Effects of maternal Cd exposure on fetal growth, and the possible role of the PCDHAC1 gene on this association are described in (Everson et al., 2016). Findings suggest that maternal Cd affects fetal growth even at very low concentrations, and some of these effects may be due to the differential expression and methylation of PCDHAC1, which encodes for the cell-adhesion protein protocadherin alpha-C1. Environmental cadmium exposure was associated with DNA hypomethylation in peripheral blood (Hossain et al., 2012). Another preliminary study supports infant sex-specific placental Cd-DNA methylation association, possibly accounting for previously reported differences in Cd-fetal growth associations across fetal sex (Mohanty et al., 2015). In addition, (Sanders et al., 2014) provides evidence of distinct patterns of DNA methylation or "footprints" in fetal and maternal DNA associated with exposure to Cd. A larger cohort study showed that DNA methylation of Cd-related markers (DNMT3B, MGMT, LINE-1, MT2A) was clearly affected by increased Cd exposure (Virani et al., 2016). With reference to an association of the tumor suppressor RAS protein activator like 1 (RASAL1) gene and KLOTHO (KL) with renal fibrogenesis, the degree of methylation of RASAL1 and KLOTHO in peripheral blood DNA was analyzed in 81 residents of Cd- polluted and non-polluted areas (Zhang et al., 2013). It was shown that levels of BCd and UCd positively correlated with levels of DNA methylation in RASAL1 and KLOTHO. Interestingly, the well-known proteohormone KLOTHO inhibits phosphate resorption in proximal tubules of the kidney.

- Transcriptomics and Proteomics

A transcriptomic study based on human data highlighted genotype-dependent differences in transcriptome responses exposed to environmental carcinogens, including Cd (Espin-Perez et al., 2015). No effect biomarkers for Cd were suggested. A proteomics study using human placenta samples showed 32 differentially-expressed proteins, which were predominantly involved in protein translocation, cytoskeletal structure, and energy metabolism. Fumarate hydratase was down-regulated in exposed placenta tissues as validated by ELISA. Alterations in placental proteome suggested that imbalances in placental mitochondria respiration might be a vital pathway for fetal growth restriction induced by exposure to Cd (Xu et al., 2016).

- MicroRNA (miRNA)

In a review on miRNA in metal-induced cell transformation and tumorigenesis (Humphries et al., 2016), only one study related to Cd was referred (Liu et al., 2015) and it was not relevant for HBM requirements. No further papers of relevance were identified.

- Metabolomics

In this field, several human studies were conducted in relevant epidemiological settings. Detection of metabolites used H NMR or LC/MS, mostly in urine but also in blood.

Exposure of volunteers to environmental Cd was confirmed by urinary levels of 8-OHdG and correlated with six biomolecules/metabolites, three related to mitochondrial metabolism (Ellis et al., 2012). This pathway was also affected in a rat study (Lee et al., 2014) and in the above-mentioned proteomics study (Humphries et al., 2016). Among others, citrate levels retained a significant correlation with urinary cadmium and smoking status after controlling for age and sex. Results were confirmed in other relevant metabolomics studies, which also reported changes in amino acids and related pathways.

Accordingly, profiling of amino acids in urine may be used as a metabolomics effect marker. Further research is required on associations among metal exposures, traditional effect markers and metabolic profiles and on the elucidation of more mechanistic causalities between AO and molecular markers.

6.8.4 Preliminary proposal of effect biomarkers that could be implemented in HBM

Several biomarkers are proposed for implementation in HBM and their selection is explained. B2MG and NAG should be examined in combination with novel biomarkers such as Kim-1 and/or CysC.

6.8.5 Gaps in Knowledge

• Description of the MoAs underlying the association between Cd exposure and enhanced B2MG and NAG levels in urine

• Confirmation of the importance of Kim-1 and CysC as biomarkers of Cd exposure in humans.

6.8.6 Conclusions

Overall conclusion: In HBM studies dealing with Cd-induced nephrotoxicity, the well-established biomarkers B2MG and NAG should be examined in combination with novel biomarkers such as Kim-1 and/or CysC.

6.8.7 References

Akerstrom et al., The relationship between cadmium in kidney and cadmium in urine and blood in an environmentally exposed population. Toxicol Appl Pharmacol 2013;268: 286-293.

Argyropoulos et al., Rediscovering beta-2 microglobulin as a biomarker across the spectrum of kidney diseases. Frontiers in Medicine 2017; 4.

Bernard & Lauwerys. Early markers of cadmium nephrotoxicity: Biological significance and predictive value. Toxicol Environ Chem 1990;27(1-3): 65-72.

Boonprasert et al., Study on the association between environmental cadmium exposure, cytochrome p450-mediated 20-hete, heme-oxygenase-1 polymorphism and hypertension in Thai population residing in a malaria endemic area with cadmium pollution. Environ Toxicol Pharmacol 2011;31: 416-426.

Buser et al., Urinary and blood cadmium and lead and kidney function: NHANES 2007–2012. Int J Hyg Environ Health 2016;219: 261-267.

Cabral et al., Effects of environmental cadmium and lead exposure on adults neighboring a discharge: Evidences of adverse health effects. Environ Pollut 2015;206: 247-255.

Castillo-Castaneda et al., Oxidative damage to proteins related to metals and antioxidant defenses in breastmilk. Nutr Hosp 2017;34(1): 59-64.

Chen et al., Linking sources to early effects by profiling urine metabolome of residents living near oil refineries and coal-fired power plants. Environ Int 2017;102: 87-96.

Cheng et al., Epigenetic targets of some toxicologically relevant metals: a review of the literature. J Appl Toxicol 2012;32(9): 643-653.

Ciarrocca et al., Correlation between cadmium and blood counts in workers exposed to urban stressor. Arch Environ Occup Health 2015;70(2): 70-76.

Cuypers et al., Cadmium stress: an oxidative challenge. Biometals 2010;23(5): 927-940.

Dharmadasa et al., Maternal cadmium exposure and impact on foetal gene expression through methylation changes. Food Chem Toxicol 2017;109(Pt 1): 714-720.

Dudka et al., Metabonomic analysis of serum of workers occupationally exposed to arsenic, cadmium and lead for biomarker research: a preliminary study. Environ Int 2014;68: 71-81.

Ellis et al., Metabolic profiling detects early effects of environmental and lifestyle exposure to cadmium in a human population. BMC Med 2012;10: 61.

Eom et al., Low-level environmental cadmium exposure induces kidney tubule damage in the general population of Korean adults. Arch Environ Contam Toxicol 2017;73: 401-409.

Espin-Perez et al., Distinct genotype-dependent differences in transcriptome responses in humans exposed to environmental carcinogens. Carcinogenesis 2015;36(10): 1154-1161.

Everson et al., Maternal cadmium, placental PCDHAC1, and fetal development. Reprod Toxicol 2016;65: 263-271.

Fay et al., Cadmium Nephrotoxicity Is Associated with Altered MicroRNA Expression in the Rat Renal Cortex. Toxics 2018;6(1).

Gao et al., Identifying early urinary metabolic changes with long-term environmental exposure to cadmium by mass-spectrometry-based metabolomics. Environ Sci Technol 2014;48(11): 6409-6418.

Hambach et al., Co-exposure to lead increases the renal response to low levels of cadmium in metallurgy workers. Toxicol Lett 2013;222: 233-238.

Hossain et al., Low-level environmental cadmium exposure is associated with DNA ypomethylation in Argentinean women." Environ Health Perspect 2012;120(6): 879-884.

Huang et al., Evaluation of factors associated with cadmium exposure and kidney function in the general population. Environ Toxicol 2013;28: 563-570.

Huang et al., Risk assessment of low-level cadmium and arsenic on the kidney. J Toxicol Environ Health A 2009;72(21-22):1493-8.

Humphries et al., The role of microRNAs in metal carcinogen-induced cell malignant transformation and tumorigenesis. Food Chem Toxicol 2016;98(Pt A): 58-65.

Ikeda et al., Closer correlation of cadmium in urine than that of cadmium in blood with tubular dysfunction markers in urine among general women populations in Japan. Int Arch Occup Environ Health 2011;84: 121-129.

Ke et al. (2015). Estimation of the benchmark dose of urinary cadmium as the reference level for renal dysfunction: A large sample study in five cadmium polluted areas in china. BMC Public Health 2015;15: 656.

Krueger & Wade. Elevated blood lead and cadmium levels associated with chronic infections among non-smokers in a cross-sectional analysis of NHANES data. Environ Health 2016;15: 16.

Lee et al., Evaluation of cadmium-induced nephrotoxicity using urinary metabolomic profiles in sprague-dawley male rats. J Toxicol Environ Health A 2014;77(22-24): 1384-1398.

Liu et al., MicroRNAs-mRNAs Expression Profile and Their Potential Role in Malignant Transformation of Human Bronchial Epithelial Cells Induced by Cadmium. Biomed Res Int 2015: 902025.

Mohanty et al., Infant sex-specific placental cadmium and DNA methylation associations. Environ Res 2015;138: 74-81.

Moriguchi et al., N-acetyl-β-d-glucosaminidase (NAG) as the most sensitive marker of tubular dysfunction for monitoring residents in non-polluted areas. Toxicol Lett 2009;19: 1-8.

Nishijo et al., Risk assessment for Thai population: Benchmark dose of urinary and blood cadmium levels for renal effects by hybrid approach of inhabitants living in polluted and non-polluted areas in Thailand. BMC Public Health 2014;14: 702.

Nogawa et al., Benchmark dose of cadmium concentration in rice for renal effects in a cadmium-polluted area in Japan. J Appl Toxicol 2015;35: 24-28.

Nordberg et al., Kidney dysfunction and cadmium exposure – factors influencing dose–response relationships. J Trace Elem Med Biol 2012;26: 197-200.

Nordberg et al., Prevalence of kidney dysfunction in humans – relationship to cadmium dose, metallothionein, immunological and metabolic factors. Biochimie 2009;91: 1282-1285.

Nygaard et al., Cord blood T cell subpopulations and associations with maternal cadmium and arsenic exposures. PLoS One 2017;12(6): e0179606.

Ohsawa, M. Heavy metal-induced immunotoxicity and its mechanisms. Yakugaku Zasshi 2009: 129(3): 305-319.

Prozialeck & Edwards. Mechanisms of cadmium-induced proximal tubule injury: New insights with implications for biomonitoring and therapeutic interventions. Journal of Pharmacology and Experimental Therapeutics 2012, 343, 2.

Prozialeck et al., Evaluation of cystatin C as an early biomarker of cadmium nephrotoxicity in the rat. BioMetals 2016;29: 131-146.

Prozialeck et al., Expression of kidney injury molecule-1 (Kim-1) in relation to necrosis and apoptosis during the early stages of cd-induced proximal tubule injury. Toxicol Appl Pharmacol 2009;238, 306-314.

Prozialeck et al., Preclinical evaluation of novel urinary biomarkers of cadmium nephrotoxicity. Toxicol Appl Pharmacol 2009;238: 301-305.

Sanders et al., Cadmium exposure and the epigenome: Exposure-associated patterns of DNA methylation in leukocytes from mother-baby pairs. Epigenetics 2014;9(2): 212-221.

Sarma et al., Effects of long-term cadmium exposure on urinary metabolite profiles in mice. J Toxicol Sci 2018;43(2): 89-100.

Sughis et al., Bone resorption and environmental exposure to cadmium in children: A cross - sectional study. Environ Health 2011;10: 104.

Sun et al., Association of cadmium in urine and blood with age in a general population with low environmental exposure. Chemosphere 2016;156: 392-397.

Suvagandha et al., A biomarker found in cadmium exposed residents of Thailand by metabolome analysis. Int J Environ Res Public Health 2014;11: 3661.

Swaddiwudhipong et al., An association between urinary cadmium and urinary stone disease in persons living in cadmium-contaminated villages in northwestern Thailand: A population study. Environ Res 2011;111: 579-583.

Swaddiwudhipong et al., Progress in cadmium-related health effects in persons with high environmental exposure in northwestern Thailand: A five-year follow-up. Environ Res 2012;112: 194-198.

Trzcinka-Ochocka et al., The effects of low environmental cadmium exposure on bone density. Environ Res 2010;110: 286-293.

Virani et al. DNA methylation is differentially associated with environmental cadmium exposure based on sex and smoking status. Chemosphere 2016;145: 284-290.

Wang et al., Tubular and glomerular kidney effects in the Chinese general population with low environmental cadmium exposure. Chemosphere 2016;147: 3-8.

Weaver et al., Differences in urine cadmium associations with kidney outcomes based on serum creatinine and Cystatin c. Environ Res 2011;111: 1236-1242.

Xu et al., Differential proteomic expression of human placenta and fetal development following e-waste lead and cadmium exposure in utero. Sci Total Environ 2016;550: 1163-1170.

Xu et al., Urine metabolomics of women from small villages exposed to high environmental cadmium levels. Environ Toxicol Chem 2016;35(5): 1268-1275.

Zhang et al., Hypermethylations of RASAL1 and KLOTHO is associated with renal dysfunction in a Chinese population environmentally exposed to cadmium. Toxicol Appl Pharmacol 2013;271(1): 78-85.

Zhang et al., Long-term effect of cadmium exposure on residents' renal dysfunction: An epidemiologic study. Zhonghua Yu Fang Yi Xue Za Zhi 2015;49(7): 638-43.

7 Limitations

The field of “biomarkers of effect” represents an incredibly broad area, thus being incredibly difficult to categorize, systematize and analyze. For this reason, we decided to organize the literature searches based on the previous expertise of teams participating in WP14. Thus, the different teams chose the chemical families they were experts on to participate in the searches. This means that often a different number of resources, teams and expertise were available for each chemical family. Indeed, no teams with previous knowledge on chromium and anilines could be found in WP14, and searches on effect biomarkers for these compounds are now being conducted through interactions of WP14 with other WPs. As a result, the literature searches for some chemical families have been developed more in depth than others.

Additionally, scientific limitations were found in some cases. For example, in the case of the PAHs and Cadmium searches, more than 40.000-45.000 references were found, and this number could not be reduced following the established methodology. Therefore, the PAHs group searched only for review articles about PAHs-related effect biomarkers (≈ 1000 references), and further reviewed the biomarkers proposed by the IARC monograph. The Cadmium group focused on kidney-related effect biomarkers and oxidative stress, based on previous knowledge of its specific toxicity (≈400 references).

Although efforts have been done to homogenize all the chapters, due to logistic and scientific limitations, all the searches cannot be equally compared. However, since the selection process of effect biomarkers will continue through the WP13-WP14 interaction, if any other relevant effect biomarker not contemplated in D14.2 was identified, it could be easily included and analyzed later on.

8 Summary of the literature search.

After reviewing all of the work completed by the different WP14 groups, all biomarkers of effect were grouped into three broad categories:

8.1 Traditional and studied effect biomarkers

Reproductive hormones, Thyroid hormones; Glucose metabolism, Serum lipids (total cholesterol, LDL, HDL, TG), Blood pressure and anthropometric measures, among others.

The literature search highlighted the role of reproductive, thyroid, and adrenal hormones as well as metabolic parameters such as serum lipids, glucose and insulin as key effect biomarkers for most of the chemical families studied. These biomarkers are largely related to hormonal and metabolic pathways; therefore, it is important to take into account the entire hormonal or metabolic cascade. In other words, the whole pathway must be followed in order to provide obtain the most complete picture possible.

8.2 Traditional but less studied effect biomarkers

Hypothalamic-pituitary-adrenal Axis; Adipokines; Inflammatory markers; Liver enzymes; Renal function; Others (IgE and vitamin D); urinary 8-OHdG and 8-isoprostane, among others.

8.3 Novel effect biomarkers:

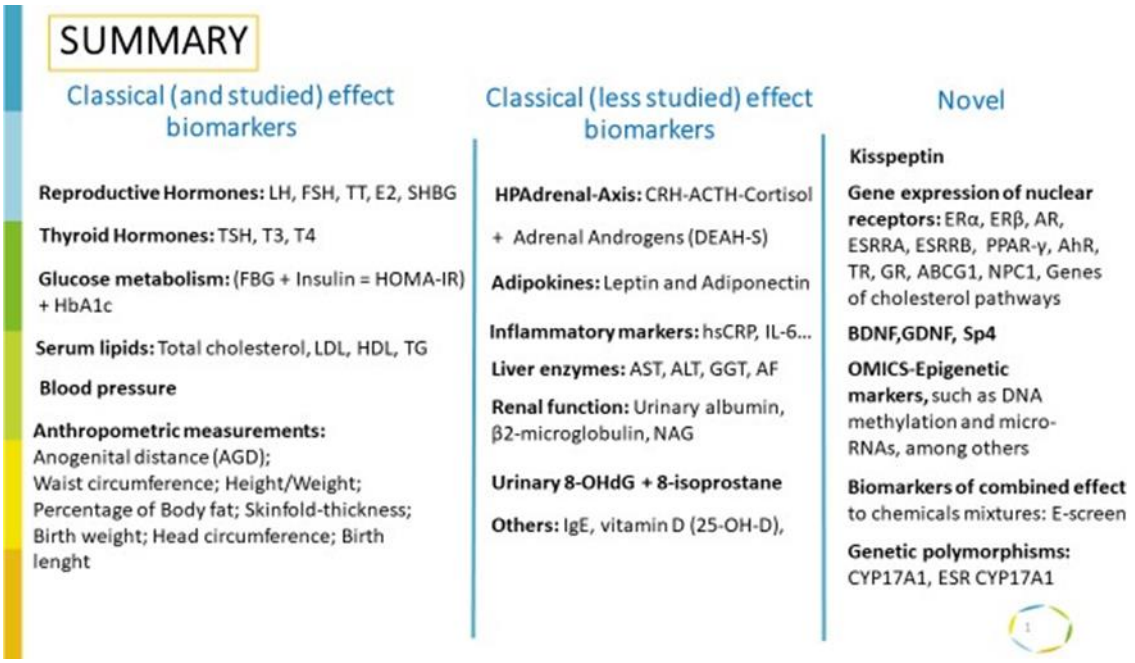
- Gene expression of nuclear receptors in peripheral blood mononuclear or lympochyte cells. Gene expression of nuclear receptors measured in cell populations in serum could provide novel information to improve the mechanistic understanding of environmental chemicals: ERα, ERβ, AR, PPARγ, AhR, TR, GR, and genes of cholesterol pathways, among others.

- BNDF and GDNF. Proposed as a novel effect biomarker to cover the gap in knowledge on neurodevelopment.

- Omics/epigenetic markers. Several chemical families were associated with omics or epigenetic markers, including DNA methylation and micro-RNAs among others. This field warrants further investigation. One handicap is that the feasibility of these biomarkers for HBM is not clear.

- Kisspeptin. Proposed as a novel effect biomarker for studying reproductive health both during pregnancy and puberty.

Table 19. Summary of preliminary effect biomarkers obtained from the bisphenols literature search.



8.4 List of proposed effect biomarkers

The table below shows the list of effect biomarkers identified in the literature search for the first set of prioritized substances. Two types of effect biomarker [Traditional (studied and less studied) *vs.* Novel] are shown and four levels of complexity are considered (Biochemical/Physiological, In vitro/ex vivo, Omics and anthropometrics). Colors indicate the group of diseases to which they are commonly associated.

Table 20. Summary list of effect biomarkers found in the literatire search.

Type of effect Biomarker	Biochemical/ Physiological	In vitro-Ex vivo	OMICs	Anthropometrical
Traditional Studied and Less studied	**Reproductive Hormones:** LH, FSH, TT, E2, SHBG **Thyroid Hormones:** TSH, T3,T4 **Serum lipids:** Total cholesterol, LDL, HDL, TG **HP-Adrenal Axis:** CRH-ACTH-Cortisol Adrenal Androgens (DEAH-S) **Adipokines:** Leptin, Adiponectin **Inflammatory markers:** hsCRP, IL-6 … **Liver enzymes:** AST, ALT, GGT, AF **Kidney Function:** Urinary albumin, NAG, β-2 microglobulin; Urinary 8-OHdG and 8-isoprostane **Others:** IgE, Vitamin D (25-OH-D)			Blood pressure Anogenital distance (AGD) Waist Circumference Height/Weight; Body fat percentage, Skinfold-thickness; Birth weight, Head circumference, Birth length
Novel	Kisspeptin BDNF, GDNF, Sp4	**Combined effect of chemical mixtures:** E-Screen	**Gene expression of nuclear receptors in PBMC:** ERα, ERβ, AR, ESRRα, ESRRβ, PPAR-γ, AhR, TR, GR, ABCG1, NPC1 **Genes of cholesterol pathways** **Epigenetic markers:** Diverse DNA methylation and micro-RNAs Comet Assay **Genetic polymorphisms:** CYP17A1, ESR CYP17A1	

Neurodevelopment; Reproductive; Endocrine; Oxidative Stress
Cardiometabolic and Obesity; Allergy-Immune; Cancer

8.5 Gaps in knowledge

a. Biochemical/physiological effect biomarkers for neurodevelopment and neurodegenerative diseases.
b. Biomarkers of combined effect of chemical mixtures. There are several ex vivo cell-based effect biomarkers implemented in the epidemiologic literature, including the estrogenic screen (E-Screen) and the anti-androgenic screen (A-Screen). These and other biomarkers of combined effect to chemical mixtures will be extensively studied in the Proof of concept (D14.4).
c. Interaction between markers of nutritional status and exposure to environmental contaminants.
d. There is a need to understand how different chemical families could converge to generate oxidative stress, and whether this is mainly related to detoxification pathways in the liver and kidneys.
e. Although most effect biomarkers studied are not specific to chemical families, some omics or epigenetic effect biomarkers, such as DNA methylation and expression of nuclear receptor genes, could be more specific and warrant further study.
f. There is a lack of knowledge on biomarkers of effect related to PFAS exposure and immune system.
g. In general, few effect biomarkers for cancer endpoints were found.

9 Conclusions

a. Most of the effect biomarkers identified in the literature search were not specific for environmental substances, being more commonly related to physiological function and/or disease rather than a specific chemical substance exposure. However, some omics or epigenetic effect biomarkers, such as DNA methylation and expression of nuclear receptor genes, may be more specific for some chemical families and warrant further study.
b. Gene expression of nuclear receptors measured in cell populations in serum could be used to provide novel information to improve our mechanistic understanding of environmental chemicals. Moreover, in some cases, the interaction of chemicals with nuclear receptors could be considered in the AOP framework as KEs or even MIEs.
c. Some chemical families, including bisphenols and phthalates, were associated with higher genomic instability and epigenetic alterations. However, the feasibility of these biomarkers is not clear.
d. All chemical families were associated with urinary markers of oxidative stress in several epidemiologic studies (8-OHdG and/or 8-isoprostane), suggesting a converging role of these environmental contaminants in the redox status. However, these markers are limited by their unespecificity to a given adverse outcome.
e. An important gap in knowledge was highlighted in relation to neurodevelopment and neurological function. While many epidemiological associations were found between environmental chemicals and neuropsychological function test results, no biochemical or physiological effect biomarkers were found for neurological function. The following neurotrophins and transcription factors are proposed as novel or potential effect biomarkers: BNDF, GDNF, Sp4.

f. A further gap in knowledge are biomarkers of combined effect to chemical mixtures: In this line, the proof of concept (D14.4) will provide data on the implementation of several ex-vivo cell-based effect biomarkers to assess the combined effect of chemical mixtures isolated from human placenta samples. Among these bioassays, combined estrogenic, anti-androgenic, anti-thyroid, and aryl-hydrocarbon activities will be assessed.
g. Associations between several chemical families and vitamin D levels were identified. The study of vitamin D and other vitamins and markers of nutritional status will be useful to address the innovative area of nutritional-chemical interactions. An increasing number of experimental studies show that some nutrients could counteract the effect of environmental chemicals, and further investigation on this topic is warranted.
h. Associations were found between renal function and most of the chemical families studied.
i. Genetic polymorphisms of oxidative stress, estrogen metabolism…etc., can modify exposure- health associations. Although these biomarkers of susceptibility could be useful to identify susceptible subpopulations, the feasibility of their implementation is considered challenging.
j. Regarding classic and studied effect biomarkers, mainly hormonal and metabolic effect biomarkers, it is very important to take into account the entire hormonal or metabolic cascade, following the whole pathway, in order to provide the most complete picture and interpretation possible.

10 Future Steps

In D14.2, an inventory of effect biomarkers has been created based on the information collected through all the literature searches. However, a thorough process is needed to go from this initial list or inventory of effect biomarkers, to the final selection of the effect biomarkers that will be implemented in the HBM4EU aligned studies. D14.3, “Report on available biomarkers of effect of utility in human epidemiological studies for the first set of prioritized substances”, will continue the selection process, narrowing down the number of possible effect biomarkers, taking into account: the chemical family of interest, the adverse outcomes of highest concern, and the period of development in which the chemical will be assessed in the HBM4EU aligned studies (children, adolescents and/or adults). Finally, specific working groups from both WP13 (Adverse Outcomes Pathways-AOPs) and WP14 (Effect biomarkers) will discuss and propose the final list of effect biomarkers to be implemented in HBM4EU aligned studies, which will take into account experimental studies and AOPs, the adverse outcomes of highest concern for each chemical family, the critical period of development in which exposure is assessed in the aligned studies (children, adolescents and/or adults), the biological matrix available (urine or serum), the validity of each particular effect biomarker, as well as logistic and feasibility variables.