ENDOCRINE DISRUPTION ASSESSMENT: EXPOSURE, BIOMONITORING AND POTENTIAL ACTIVITY OF WIDESPREAD ENDOCRINE DISRUPTORS DURING PREGNANCY AND EARLY STAGES OF LIFE

María Ángeles Martínez Rodríguez

UNIVERSITAT ROVIRA i VIRGILI

ENDOCRINE DISRUPTION ASSESSMENT: EXPOSURE, BIOMONITORING AND POTENTIAL ACTIVITY OF WIDESPREAD ENDOCRINE DISRUPTORS DURING PREGNANCY AND EARLY STAGES OF LIFE.

MARÍA ÁNGELES MARTÍNEZ RODRÍGUEZ

DOCTORAL THESIS

2020

UNIVERSITAT ROVIRA I VIRGILI
ENDOCRINE DISRUPTION ASSESSMENT: EXPOSURE, BIOMONITORING AND POTENTIAL ACTIVITY OF WIDESPREAD ENDOCRINE DISRUPTORS DURING
PREGNANCY AND EARLY STAGES OF LIFE
María Ángeles Martínez Rodríguez

María Ángeles Martínez Rodríguez

Endocrine disruption assessment: exposure, biomonitoring and potential activity of widespread endocrine disruptors during pregnancy and early stages of life.

DOCTORAL THESIS

Supervised by

Prof. Dr. Marta Schuhmacher Ansuategui

Dr. Joaquim Rovira Solano

Dr. Vikas Kumar

Department of Chemical Engineering

UNIVERSITAT ROVIRA i VIRGILI

2020

UNIVERSITAT ROVIRA I VIRGILI
ENDOCRINE DISRUPTION ASSESSMENT: EXPOSURE, BIOMONITORING AND POTENTIAL ACTIVITY OF WIDESPREAD ENDOCRINE DISRUPTORS DURING
PREGNANCY AND EARLY STAGES OF LIFE
María Ángeles Martínez Rodríguez

UNIVERSITAT ROVIRA i VIRGILI

FAIG CONSTAR que aquest treball, titulat **"Endocrine disruption assessment: exposure, biomonitoring and potential activity of widespread endocrine disruptors during pregnancy and early stages of life."** que presenta María Ángeles Martínez Rodríguez per a l'obtenció del títol de Doctor, ha estat realitzat sota la meva direcció al Departament d'Enginyeria Química d'aquesta universitat.

HAGO CONSTAR que el presente trabajo, titulado "**Endocrine disruption assessment: exposure, biomonitoring and potential activity of widespread endocrine disruptors during pregnancy and early stages of life**.", que presenta María Ángeles Martínez Rodríguez para la obtención del título de Doctor, ha sido realizado bajo mi dirección en el Departamento de Ingeniería Química de esta universidad.

I STATE that the present study, entitled "**Endocrine disruption assessment: exposure, biomonitoring and potential activity of widespread endocrine disruptors during pregnancy and early stages of life."**, presented by María Ángeles Martínez Rodríguez for the award of the degree of Doctor, has been carried out under my supervision at the Chemical Engineering Department of this university.

Tarragona, a 11 de marzo de 2020

El/s director/s de la tesi doctoral
El/los director/es de la tesis doctoral
Doctoral Thesis Supervisor/s

Prof. Dr. Marta Schuhmacher Ansuategui Dr. Joaquim Rovira Solano Dr. Vikas Kumar

UNIVERSITAT ROVIRA I VIRGILI
ENDOCRINE DISRUPTION ASSESSMENT: EXPOSURE, BIOMONITORING AND POTENTIAL ACTIVITY OF WIDESPREAD ENDOCRINE DISRUPTORS DURING
PREGNANCY AND EARLY STAGES OF LIFE
María Ángeles Martínez Rodríguez

A ti mamá.

UNIVERSITAT ROVIRA I VIRGILI
ENDOCRINE DISRUPTION ASSESSMENT: EXPOSURE, BIOMONITORING AND POTENTIAL ACTIVITY OF WIDESPREAD ENDOCRINE DISRUPTORS DURING
PREGNANCY AND EARLY STAGES OF LIFE
María Ángeles Martínez Rodríguez

AKNOWLEDGEMENTS

UNIVERSITAT ROVIRA I VIRGILI
ENDOCRINE DISRUPTION ASSESSMENT: EXPOSURE, BIOMONITORING AND POTENTIAL ACTIVITY OF WIDESPREAD ENDOCRINE DISRUPTORS DURING
PREGNANCY AND EARLY STAGES OF LIFE
María Ángeles Martínez Rodríguez

Me gustaría agradecer a todas las personas que han hecho posible la realización de esta Tesis Doctoral.

En primer lugar, me gustaría agradecer a la Doctora Marta Schuhmacher la oportunidad que me brindó al abrirme las puertas al grupo. Aún recuerdo como si fuera ayer ese viaje Valencia-Reus con ganas y nervios por hacer la entrevista. Que ilusión me hizo cuando supe que iba a formar parte de este grupo y del proyecto de investigación HEALS. Esa ilusión la sigo teniendo hoy en día, me encanta lo que hago, y me siento muy afortunada de poder formar parte del grupo AGA-TecnATox.

Creo que las oportunidades de las que he podido disfrutar aquí son inmensas. Hemos podido publicar los resultados obtenidos, he podido apuntarme a muchos cursos formativos y charlas científicas y, en especial, he podido hacer divulgación científica en muchos congresos a nivel nacional e internacional. Por todo ello, me gustaría volver a agradecerte Marta que siempre me hayas propuesto nuevos retos y que de cierta forma hayas hecho que mi curiosidad nunca se estancase. Creo que hay muchos jefes y jefas, pero muy pocos líderes que te guíen y te aconsejen sobre las decisiones que debes tomar para un futuro.

Seguidamente, me gustaría agradecer al Doctor Joaquim Rovira su dedicación, esfuerzo, paciencia y ganas por estar ahí en cada momento. Desde que nos conocimos en el primer seminario del grupo siempre has estado súper atento, haciendo que las dudas y los posibles problemas se solucionasen rápido. Gracias por tu gran apoyo Quim, eres todo un ejemplo a seguir.

También quiero darle las gracias al Doctor Vikas Kumar, sin ti todas las estancias que he realizado y todas las experiencias vividas en otros laboratorios no habrían sido posible. Siempre has intentado darme la oportunidad de conocer a otros investigadores con los que poder colaborar y te has preocupado mucho para que pudiera tener otras visiones fuera de la investigación que hacemos. Muchas gracias Vikas por tu dedicación y paciencia.

He tenido la gran suerte de que seáis los directores de mi Tesis, muchísimas gracias por confiar en mí y por haber contado conmigo.

Quisiera reconocer, asimismo, a todas las personas que han estado conmigo durante este tiempo. En Reus me he sentido como en casa, tanto dentro como fuera del laboratorio. Sinceramente creo que eso es posible cuando estás rodeada de personas que tienen la capacidad de generar una atmosfera especial.

Me gustaría agradecer al Doctor José Luis Domingo y al Doctor Martí Nadal por acogerme aquí y por estar pendientes de todo lo que he necesitado.

Durante el doctorado he podido trabajar con gente maravillosa con la que he convivido, incluso vivido un montón de momentos especiales. Sin ellas la atmosfera de la que hablaba no hubiera sido tan especial. Gracias por ser cada una a su manera CASA. Gracias también a toda la gente que he conocido durante estos cuatro años. Juntos formamos un grupo muy especial, una pequeña familia aquí en Reus. También quiero dar las gracias a las personas que SUMAN y que hacen que te ilusiones y que guste todavía más estar aquí.

Y como no podía ser de otra manera, quería agradecer a a las amigas de toda la vida, las que hacen que te llegue a doler la cabeza de felicidad al verlas y que están ahí siempre que lo necesitas.

Finalmente, quiero agradecer a mis padres y a mi hermana por su capacidad de impulsarme hacia delante, por su apoyo infinito, y por esa manera tan positiva de ver las cosas siempre. Gracias por hacer que sienta que puedo con todo lo que me proponga y que además disfrute haciéndolo.

Gracias a mi familia, a toda ella, vuestra fuerza y ánimos han sido claves, en especial gracias a mis abuelos, mis personas favoritas, las más sabias y transparentes a la hora de darte cualquier consejo, iaios “la catalana” os quiere muchísimo.

Gracias por hacerlo posible.

ABBREVIATIONS

UNIVERSITAT ROVIRA I VIRGILI
ENDOCRINE DISRUPTION ASSESSMENT: EXPOSURE, BIOMONITORING AND POTENTIAL ACTIVITY OF WIDESPREAD ENDOCRINE DISRUPTORS DURING
PREGNANCY AND EARLY STAGES OF LIFE
María Ángeles Martínez Rodríguez

A

AA: acetic anhydride
ADME: absorption, distribution, metabolism and excretion
AOPs: adverse outcome pathways
ABS: dermal absorption factor

B

BBZP: butyl benzyl phthalate
BMI: body mass index
BPA: bisphenol A
BPF: bisphenol F
BPS: bisphenol S
BFR: brominated flame retardants
BW: body weight
BW_{20GW}: body weight at 20 gestational weeks

C

C: creatinine corrected
$C_{BPA/DEHP}$: BPA or DEHP concentration
C_c: concentration of BPA or DEHP in PCPs
C_c^{indoor} concentration of BPA or DEHP in the indoor air
$C_c^{outdoor}$ concentration of BPA or DEHP in the outdoor air
Cd: cadmium
C/EBPα: CCAAT/enhancer binding protein α
CLA: 9c,11t-octadecadienoic acid
cx-MiNP: Mono-4-methyl-7-carboxyoctyl phthalate

D

DEHP: di (2-ethylhexyl) phthalate
DEP: diethyl phthalate
DHA: docosahexaenoic acid
DI: dietary intake
DiBP: diisobutyl phthalate
DINCH: 1,2-cyclohexane dicarboxylic acid diisononyl ester
DiNP: diisononyl phthalate
DnBP: di-n-butyl phthalate
DMSO: dimethyl sulfoxide
DPHP: di(2-propylheptyl) phthalate

$Dust_{Ing}$: dust ingestion

E

ECHA: European Chemical Agency
ED: endocrine disruptor
pl. **EDs**: endocrine disruptors
EFSA: European Food Safety Authority
EI: electron ionization
EPA: Environmental Protection Agency
ESI: electrospray ionization
EU: European Union

F

FABP4: Fatty acid-binding protein 4
FAO: Food and Agriculture Organization
F_f: food frequency consumption
FFQ: food frequency questionnaire
*pl.***FFQs**: food frequency questionnaires
F_r: food ingestion ration
FUE: urinary excretion factor

G

G: gamma distribution
GW: gestational weeks
GC-MS: gas chromatography–mass spectrometry

H

HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
Hg: mercury
HMWP: high-molecular-weight phthalates
HPLC: high-performance liquid chromatography

I

IARC: International Agency for Research on Cancer
ICP-OES: inductively coupled plasma optical emission spectrometry
ICP-MS: inductively coupled plasma mass spectrometry
$Ih_{r\ moderate}$: inhalation rate during moderate activities
$Ih_{r\ sedentary}$: inhalation rate while sedentary activities

$Ih_{r\ sleep:}$: inhalation rate during sleep
Ing_f: ingestion factor
Inh: inhalation rate
IQR: interquartile range
I_r: ingestion rate
IS: internal standards

L

LA: linolenic acid
LC-MS/MS: liquid chromatography–tandem mass spectrometry
LMWP: low-molecular-weight phthalates
LOD: limit of detection
LOQ: limit of quantification
LN: log-normal distribution

M

mAb: primary monoclonal antibodies
MBzP: mono benzyl phthalate
MCFA: medium-chained fatty acids
MeCN: acetonitrile
MEHHP: mono-2-ethyl-5-hydroxyhexyl phthalate
MEHP: mono (2-ethylhexyl) phthalate
MiBP: mono-iso-butyl phthalate
MEOHP: mono-2-ethyl-5-oxohexyl phthalate
MECPP: mono-2-ethyl-5-carboxypentyl phthalate
MMCHP: mono-2-carboxymethyl hexyl phthalate
MnBP: mono-n-butyl phthalate
MRM: multiple reaction monitoring
MS: mass spectrometry
MTT: metabolic dye [4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium
MUSFA: monounsaturated fatty acids

N

N: normal distribution
NA: not available
NaOH: sodium hydroxide
NIST: National Institute of Standards and Technology
NTP: national toxicology program

O

OD: optical density
OECD: Organisation for Economic Cooperation and Development
oh-MINCH: 2-(((Hydroxy-4methyloctyl) oxy) carbonyl) cyclohexanecarboxylic Acid
oh-MiNP: mono-4-methyl-7-hydroxyoctyl phthalate
oh-MPHP: 6-Hydroxy Monopropylheptylphthalate
oxo-MINCH: 2-(((4-Methyl-7-oxyooctyl) oxy) carbonyl) cyclohexanecarboxylic Acid
oxo-MiNP: mono-4-methyl-7-oxooctyl phthalate

P

P-AKT: phosphorylated AKT
Pb: lead
PUFA: polyunsaturated fatty acids
POPs: persistent organic pollutants
PBPK: physiologically based pharmacokinetic (model)
PC: polycarbonate
PCPs: personal care products
PVC: polyvinyl chloride
PFOS: perfluorooctane sulfonic acid
PFOA: perfluorooctanoic acid
PFASs: perfluoroalkylated substances
PPARγ: peroxisome proliferator-activated receptor gamma γ
PBS: phosphate-buffered saline
P: punctual
PCP_a amount per application of personal care products
PCP_{fr}: frequency application of personal care products
P5: percentile 5
P50: percentile 50
P 95: percentile 95
PUFA: poliunsaturated fatty acids

R

R_f: retention factor for rinse-off products

S

SFA: saturated fatty acids
SPSS: Statistical Package for the Social Sciences software
SD: standard deviation

SG: specific gravity corrected
SIM: selected ion monitoring
SFA: saturated fatty acids

T

T: triangular distribution
TBBPA: tetrabromobisphenol A
TCA: trichloroacetic acid
TDI: tolerable daily intake
TFA: trans fatty acids
t_{indoor} : time spending indoor (at work and at home)
$t_{outdoor}$: time spending in doing activity outdoor
t_{sleep} : time sleeping
TWI: tolerable weekly intake
T4CE: tetrachloroethylene

U

U: uniform distribution

W

WHO: World Health Organization
WMP: whole milk powder

UNIVERSITAT ROVIRA I VIRGILI
ENDOCRINE DISRUPTION ASSESSMENT: EXPOSURE, BIOMONITORING AND POTENTIAL ACTIVITY OF WIDESPREAD ENDOCRINE DISRUPTORS DURING
PREGNANCY AND EARLY STAGES OF LIFE
María Ángeles Martínez Rodríguez

INDEX

UNIVERSITAT ROVIRA I VIRGILI
ENDOCRINE DISRUPTION ASSESSMENT: EXPOSURE, BIOMONITORING AND POTENTIAL ACTIVITY OF WIDESPREAD ENDOCRINE DISRUPTORS DURING
PREGNANCY AND EARLY STAGES OF LIFE
María Ángeles Martínez Rodríguez

INDEX

SUMMARY

UNIVERSITAT ROVIRA I VIRGILI
ENDOCRINE DISRUPTION ASSESSMENT: EXPOSURE, BIOMONITORING AND POTENTIAL ACTIVITY OF WIDESPREAD ENDOCRINE DISRUPTORS DURING
PREGNANCY AND EARLY STAGES OF LIFE
María Ángeles Martínez Rodríguez

SUMMARY

Endocrine disruptors (EDs) are exogenous substances that alter function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations. There is a group of EDs that takes an important place due to their widespread distribution and presence in food-packaging or food contact materials. The packaging is an essential element in food manufacture. However, packaging could be a source of contamination as migration can occur from these materials with the transfer of chemicals or particles into the food. According to that, bisphenol A (BPA) and two analogues of it, bisphenol S (BPS) and bisphenol F (BPF); phthalate and non-phthalate plasticizers; perfluorooalkilated substances (PFAS) specifically perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) and tetrabromobisphenol A (TBBPA) were selected in this thesis.

The general hypothesis of this thesis is that, there is exposure to EDs during the prenatal period and early stages of life and this exposure can cause adverse outcomes. Therefore, the general objective is to assess the exposure levels to different EDs from pregnant women of EXHES cohort in Tarragona, Spain and to study how these chemicals can affect normal cellular development. This main goal has been divided into four different specific objectives: (I) to estimate the exposure to EDs for pregnant women and their babies. (II) Biomonitoring the levels of EDs in urine, blood and breast milk from pregnant women. (III) To reconstruct the exposure to EDs using biomonitored levels and the physiologically based pharmacokinetic (PBPK) model. (IV) To determine the endocrine potential activity of some widely used EDs.

In Chapter 1 (publication 1 and 2), exposure to two high distributed EDs, BPA and DEHP from the EXHES Tarragona, Spain, cohort of pregnant women and their babies were calculated. Exposure assessments based on data obtained from questionnaires and personal interviews were used. Subsequently, the contribution of each item to the total exposure for the EXHES cohort was evaluated. Results showed that diet had the highest contribution to total exposure with >99.9% for BPA and 63% for DEHP. However, with this

method, based on data obtained from questionnaires and interviews, it is possible to underestimate the exposure due to a lack of knowledge of all possible sources that would also contribute to total exposure. Consequently, another possible approach for investigating human exposure to different chemicals could be to reconstruct the exposure through biomonitoring data. For that reason, it was compared the previously estimated exposure with the reconstructed exposure using the DEHP in urine as a case study (chapter 2, publication 3). Apart from phthalate metabolites determination, DINCH metabolites were also measured in urine. Surprisingly, phthalates and DINCH metabolites were detected in almost all (94 %) pregnant women's urine samples. Results showed a correlation between lower birth weights with higher levels of DINCH metabolites in maternal urine ($p<0.05$).

Both approaches, the estimated and the reconstructed, were combined with PBPK modelling to predict the amount of BPA and MEHP (DEHP metabolite) that can reach the fetus. The results showed that both values obtained from the PBPK model using estimated or reconstructed data were close to each other. However, the PBPK simulated exposure for reconstructed dose was closer to the extrapolated 24 h biomonitoring data. In general terms, BPA and MEHP stay longer in the fetal body, which may cause a higher risk to fetuses and makes the fetus more vulnerable to the exposure compared with the mothers. Consequently, PBPK results from dose reconstruction were more accurate compared with the previously estimated PBPK-simulation. These results suggested that is possible to estimate the exposure of a chemical from their metabolites concentrations in spot urine. Also, these results from reconstructed data are closer to a real exposure scenario. Our values for BPA and DEHP estimated and reconstructed were far away from the tolerable daily intake (TDI) values of the EFSA (4 $\mu g/kg_{bw}$/day) and ECHA (50 $\mu g/kg_{bw}$/day) for BPA and DEHP, respectively.

The same methodology applied in chapters 1 and 2 to assess the estimated exposure was used again for PFASs, a group of synthetic chemicals, which include PFOS and PFOA (chapter 2, publication 4). Besides, to verify the accuracy of this method, the estimated exposure was compared with biomonitoring data for the same cohort. In agreement with many other

studies, results showed that again, dietary intake was the main route of exposure to PFOS (>99%) and PFOA (>96%). Concerning the prenatal exposure, the model simulation results obtained for plasma were compared with biomonitoring data from the same cohort. To achieve this goal, PFOA and PFOS were biomonitored in maternal blood. It was detected traces of PFOS in all samples. PFOA and PFOS plasma levels in the first trimester were significantly higher than those at delivery and in cord blood. Decreases of 69% and 25% in PFOA and PFOS plasma levels, respectively, between the first trimester and at delivery were registered. This fact could be due to both a placental transfer and a dilution process caused by the increase of blood plasma volume. In this study, around 70% and 60% of maternal plasma levels in the first trimester were found in cord blood for PFOA and PFOS, respectively. Modelled levels were higher than those obtained by biomonitoring. The performance of the PBPK model can be further improved by introducing temporal dynamics of exposure concentration and physiological parameters for long-term exposure. Despite this, the PBPK model was able to validate analytical data from biomonitoring samples, even with a small cohort population. Dietary exposure of PFOA in our study were close to, but below, the provisional tolerable weekly intake (TWI) set by EFSA (13 ng/kg_{bw}/week). In turn, the dietary intake of PFOS was calculated to be well above the most updated provisional TWI (6 ng/kg_{bw}/week). Therefore, more studies regarding the exposure of PFOS are needed.

Apart from estimate the prenatal exposure to EDs (publications from 1 to 4), the exposure to BPA during the early stages of life through breast and infant formula milk (chapter 2, publication 5) was established. To assess that, it was used the concentration levels of BPA in breast and formula milk. The presence of some pollutants like BPA, TBBPA, fatty acids and a wide spectrum of major and trace elements were analysed in human milk and infant formula. The concentration of BPA was significantly higher in infant formula samples than in breast milk, which also contained significantly lower values of some essential elements. The fatty acid profiling also revealed major differences between human milk and infant formulas, which should be taken into account in the development of new formulas as well as in specific recommendations for the diet of breastfeeding mothers. Results showed that the estimated exposure to BPA was higher in formula-fed infants than in breastfed infants.

However, BPA intake was far below the TDI threshold set by EFSA (4 μg/kg_{bw}/day) for both formula-fed and breastfed infants. Consequently, the results of this study reinforce that breastfeeding should be always the first feeding option in early life.

Finally, in this thesis, it was also evaluated the effects of endocrine-disrupting activity. BPA and its analogues, BPS and BPF, were selected as a case study. The European Union (EU) banned the use of BPA in plastic materials that can be in contact with food intended for children (0–3 years). However, the principal analogues of BPA, BPS, and BPF are still being used. Then, in publication 6 (chapter 3) we wanted to clarify if BPA has equal or different effects than its analogues, BPS and BPF, in cell toxicity and on the adipocyte differentiation process. This was assessed by *in vitro* experiments with preadipocytic 3T3-L1 cell line. Results suggest that BPA has a greater toxic effect at equal and even lower concentrations than its analogues. However, BPS followed by BPF has higher endocrine-disrupting activity compared with BPA and the control. Further studies on these analogues would be necessary to corroborate these results, and consequently, changes in the legislation on the bisphenols' family would be necessary.

As a general conclusion, the exposure to selected EDs (BPA, and its analogues, BPS and BPF; phthalate and non-phthalate plasticizers; PFAS, specifically PFOS and PFOA, and TBBPA) is a common phenomenon in adult populations and during early stages of life, such as prenatal and newborn children. This exposure can cause adverse outcomes. For that reason, more studies regarding endocrine potential activity are crucial to reevaluate the statements and the TDI exposure including dietary and non-dietary sources. Regulations based on the assessment of the safety of the most vulnerable exposure population, pregnant women and their children, should be established in the endocrine disruption context.

RESUM

Els disruptors endocrins (DEs) són substàncies exògenes que alteren les funcions del sistema endocrí i, per tant, provoquen efectes adversos sobre la salut en un organisme intacte, o la seva descendència, o (sub) poblacions. Hi ha un grup DEs que tenen una gran rellevància a causa de la seva distribució generalitzada i de la seva presència en envasos o materials en contacte amb aliments. Els envasos són un element essencial en la fabricació d'aliments. Tot i això, aquestes envasos poden ser una font de contaminació, ja que es poden produir migracions de productes químics o partícules al menjar. D'acord amb això, en aquesta tesi s'han seleccionat: el bisfenol A (BPA) i dos anàlegs d'aquest, el bisfenol S (BPS) i el bisfenol F (BPF); plastificants no ftalats i ftalats; substàncies perfluoroalcanilades (PFAS) específicament àcid sulfònic perfluorooctà (PFOS) i àcid perfluorooctanoic (PFOA) i tetrabromobisfenol A (TBBPA).

La hipòtesi general d'aquesta tesi doctoral és que hi ha exposició als DEs durant el període prenatal i les primeres etapes de la vida i aquesta exposició pot causar efectes adversos. Per tant, l'objectiu general és avaluar els nivells d'exposició a diferents DEs en una població d'estudi de dones embarassades –EXHES- a Tarragona, Espanya, i estudiar com aquests productes químics poden afectar el desenvolupament cel·lular normal. Aquest objectiu principal s'ha dividit en quatre objectius específics diferents: (I) estimar l'exposició als DEs en dones embarassades i en els seus nadons. (II) Biomonitoritzar els nivells d'EDs en orina, sang i llet materna de dones embarassades. (III) Reconstruir l'exposició a DEs utilitzant nivells en les mostres biològiques i mitjançant el model farmacocinètic (PBPK). (IV) Determinar l'activitat l endocrina d'alguns DEs àmpliament distribuïts i utilitzats.

Al capítol 1 (publicacions 1 i 2), es va calcular l'exposició a dos DEs altament distribuïts, BPA i DEHP de la cohort EXHES de dones embarassades i els seus nadons. Es va avaluar l'exposició mitjançant dades obtingudes a partir de qüestionaris i entrevistes personals. Posteriorment, es va avaluar la contribució de cada producte (alimentari i no alimentari) a l'exposició total. Els resultats van mostrar que la dieta va tenir la major contribució a l'exposició total amb> 99,9% per a BPA i 63% per a DEHP. Però amb aquest mètode, a partir

de dades obtingudes dels qüestionaris i entrevistes personals, possiblement es subestima l'exposició pel desconeixement de totes les fonts possibles que també contribuirien a l'exposició total. En conseqüència, un altre enfocament possible per investigar l'exposició humana a diferents productes químics podria ser reconstruir l'exposició mitjançant la biomonitorització. Per això, es va comparar l'exposició prèviament estimada (capítol 1, publicació 1 i 2) amb l'exposició reconstruïda utilitzant el DEHP en orina com a cas d'estudi (capítol 2, publicació 3). A part de la determinació dels metabòlits de ftalats, també es van mesurar els metabòlits del DINCH en l'orina. Sorprenentment, es van detectar gairebé en totes (94%) les mostres d'orina de dones embarassades. Els resultats van mostrar una correlació entre pesos inferiors dels nadons al part amb nivells més elevats de metabòlits del DINCH en l'orina materna.

Ambdós enfocaments, l'estimació i la reconstrucció, es van combinar amb el model PBPK per predir la quantitat de BPA i MEHP (metabòlit DEHP) que poden arribar al fetus. Els resultats van mostrar que tots dos valors obtinguts del model PBPK mitjançant dades estimades o reconstruïdes eren propers. No obstant això, l'exposició simulada per PBPK per a dosis reconstruïdes es va apropar a les dades de biomonitorització extrapolades de 24 hores. En termes generals, el BPA i el MEHP es mantenen més temps en el fetus, cosa que pot causar un risc més elevat als fetus i fa que el fetus sigui més vulnerable a l'exposició en comparació amb les mares. Els resultats del PBPK considerant els valors reconstruïts, van ser més precisos en comparació amb la simulació prèviament estimada del PBPK. Aquests resultats suggereixen que és possible estimar l'exposició d'una substància química a partir de les concentracions dels seus metabòlits en una mostra d'orina puntual. A més, aquests resultats de les dades reconstruïdes s'aproximen més a un escenari d'exposició real. Els valors per a BPA i DEHP estimats i reconstruïts en el nostre estudi estaven lluny dels valors tolerables d'ingesta (TDI) l'EFSA i ECHA, 4 $\mu g/kg_{bw}$/dia i 50 $\mu g/kg_{bw}$/dia per a BPA i DEHP, respectivament.

La mateixa metodologia aplicada als capítols 1 i 2 per avaluar l'exposició estimada es va tornar a utilitzar per PFASs, un grup de productes químics sintètics, que inclouen PFOS i

PFOA (capítol 2, publicació 4). A més, per verificar la precisió d'aquest mètode, es va comparar l'exposició estimada amb les dades de biomonitorització del mateix grup d'estudi. D'acord amb molts altres estudis, els resultats van demostrar que de nou, la ingesta dietètica va ser la principal via d'exposició a PFOS (> 99%) i PFOA (> 96%). Quant a l'exposició prenatal, es van comparar els resultats de la simulació del model obtinguts per plasma amb les dades de biomonitorització. Per assolir aquest objectiu, PFOA i PFOS van ser biomonitoritzadese n sang materna. Es van detectar traces de PFOS en totes les mostres. Els nivells de plasma PFOA i PFOS al primer trimestre van ser significativament superiors als del moment del part i en sang de cordó. Es va registrar una disminució del 69% i del 25% en els nivells de plasma PFOA i PFOS, respectivament, entre el primer trimestre i el moment del part. Aquest fet es podria deure tant a una transferència placentària com a un procés de dilució causat per l'augment del volum de plasma sanguini. En aquest estudi, al voltant del 70% i el 60% dels nivells de plasma matern del primer trimestre es van trobar en sang de cordó per PFOA i PFOS, respectivament. Els nivells modelats van ser superiors als obtinguts per biomonitorització. El rendiment del model PBPK es pot millorar mitjançant la introducció temporal dinàmica de concentració d'exposició i paràmetres fisiològics per a exposició a llarg termini. Malgrat això, el model PBPK va ser capaç de validar dades analítiques de mostres de biomonitorització. L'exposició dietètica de PFOA al nostre estudi es va aproximar a la ingesta provisional tolerable setmanal (TWI) fixada per EFSA (13 ng/kg_{bw}/setmana). Al seu torn, la ingesta dietètica de PFOS va resultar per sobre del TWI provisional més actualitzat (6 ng/kg_{bw}/setmana). Per tant, es necessiten més estudis sobre l'exposició de PFOS. Si escau, l'EFSA hauria de realitzar una reavaluació dels valors d'exposició a PFOS.

A part de l'estimació de l'exposició prenatal als DEs (publicacions de l'1 al 4), es va establir l'exposició a BPA durant les primeres etapes de la vida a través de llet de fórmula i materna (capítol 2, publicació 5). Es va utilitzar els nivells de concentració de BPA a la llet materna i de fórmula i es va analitzar la presència d'alguns contaminants com el BPA, TBBPA, àcids grassos i un ampli espectre d'elements principals i traces en la llet humana i en la de fórmula infantil. En conseqüència, pel que fa a l'exposició, la concentració de BPA va ser

significativament més elevada en mostres de fórmula infantil que en la llet materna, que també contenia valors significativament inferiors d'alguns elements essencials. Els àcids grassos també van ser diferents entre les dues llets, per això s'han de tenir en compte en l'elaboració de noves fórmules així com en recomanacions específiques per a la dieta de les mares que alleten. Els resultats van demostrar que el BPA estimat era més elevada en els nadons alimentats amb fórmules que en els lactants. No obstant això, la ingesta de BPA estava molt per sota del TDI establert per EFSA (4 μg/kg_{bw}/dia) tant per a lactants alimentats amb fórmules com per a lactants. En conseqüència, els resultats d'aquest estudi reforcen que l'alletament matern hauria de ser sempre la primera opció d'alimentació a la vida primerenca.

Finalment, en aquesta tesi també es van avaluar els efectes de l'activitat disruptora endocrina. Es va seleccionar BPA i els seus anàlegs, BPS i BPF com a estudi de cas. La Unió Europea (UE) va prohibir l'ús de BPA en materials plàstics que podrien estar en contacte amb aliments destinats a nens (0-3 anys). Tot i això, encara s'utilitzen els principals anàlegs de BPA, BPS i BPF. Després, a la publicació 6 (capítol 3) volíem aclarir si la BPA té els mateixos efectes que els seus anàlegs, en la toxicitat cel·lular i en el procés de diferenciació adiposa. Per això es va avaluar mitjançant experiments *in vitro* amb la línia cel·lular preadipocítica 3T3-L1. Els resultats suggereixen que el BPA té un efecte tòxic major a concentracions iguals i fins i tot inferiors que els seus anàlegs. Tot i això, el BPS seguit de BPF té una activitat endocrina més elevada en comparació amb el BPA i el control. Serien necessaris més estudis sobre els anàlegs per corroborar aquests resultats obtinguts i, si escau, serien necessaris canvis en la legislació de la família dels bisfenols.

Com a conclusió general, l'exposició als DEs seleccionats (BPA, i els seus anàlegs, BPS i BPF; ftalats i plastificants no ftalats; PFAS, específicament PFOS i PFOA, i TBBPA) és un fenomen habitual en poblacions adultes i també durant les etapes primerenques de la vida, com els nens durant la gestació i en les primeres etapes del desenvolupament. Aquesta exposició pot provocar efectes adversos. Per això, són crucials més estudis sobre l'activitat endocrina per revaluar els informes i l'exposició diària tolerable incloent les fonts no dietètiques. Les

regulacions basades en l'avaluació de la seguretat de la població d'exposició més vulnerable, les dones embarassades i els seus fills, haurien d'establir-se en el context de la alteració endocrina.

INTRODUCTION

1. Endocrine disruptors: history and regulation

In the last few decades, it has been demonstrated that pollutants present in the environment (soil, air, water) and in food coming directly from human sources (such as industrial manufacturing, agricultural run-off, wastewater discharges, chemical additives in consumer goods) or from natural sources can interfere with the endocrine system of humans and wildlife and produce adverse effects. The natural and anthropogenic chemicals that interfere with the body's hormonal system are known as Endocrine Disruptors (EDs). "*EDs are exogenous substances or mixtures that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*" (WHO, 2012). The potential of the EDs to exhibit hormone activity is not a recent discovery. In 1985, Dr. Colborn showed during her PhD studies how in different species the transfer of persistent human-made chemicals to their offspring was possible, which undermined the development and programming of their youngsters' organs before they were born or hatched (Kwiatkowski et al., 2016). In light of this evidence, in 1991, she convened 21 international scientists from 15 different disciplines to share their research relevant to transgenerational health impacts. During that historic meeting at the Wingspread Conference Center in Racine, Wisconsin, the term -endocrine disruption- was coined (Kwiatkowski et al., 2016). From this meeting, laws were being drafted to identify endocrine-disrupting chemicals. This led to the creation, in 1996, of the Endocrine Disruptor Screening and Testing Advisory Committee, a group of multiple stakeholders tasked with advising the U.S. Environmental Protection Agency (EPA, 1998). In addition, in 1999, the European Commission established a legislative-based strategy for EDs, to address the potential impacts of endocrine disruption on the health of humans and wildlife and to establish an agreed integrated plan for future research (EC, 1999). Dr. Colborn's work has prompted the enactment of new laws around the world and redirected the research of academicians, governments, and the private sector.

Nowadays, there is a growing concern in the European Union (EU) and worldwide about negative human health and environmental impacts possibly caused by EDs. These substances have been associated with a wide variety of disorders in humans and/or wildlife,

including cancer, reproductive and neurological developmental and metabolic diseases (Weihrauch-Blüher and Wiegand, 2018). The legislative and approval procedures for active substances in biocides and plant protection products are described in the Biocidal Product Regulation (No 528/2012, 2012) (EP, 2012) and Plant Protection Products Regulation (No 1107/2009, 2009) (EP, 2009), respectively. These regulations state that EDs must be excluded from the market on the basis of hazard identification (although there are some derogations such as negligible exposure or risk) (Daston et al., 2003). In the regulation on industrial chemicals (Registration, Evaluation, Authorization, and restriction of Chemicals, EC 1907/2006, REACH), a chemical identified as an ED may be banned and subject to authorisation based on risk assessment and socio-economic analysis. However, with the currently available tests that are included in the OECD (Organisation for Economic Cooperation and Development)-Test Guidelines and describe which tests should be used for hazard identification of EDs, they cannot be properly identified (EFSA and ECHA, 2017). Additionally, not all of the available tests can discriminate between general toxicological effects of chemical substances and effects that are specifically caused by interference of the chemical substance with the endocrine system (EP, 2019). These limitations pose the risk that chemical substances with endocrine-disrupting activity will be approved for market introduction based on false-positive test results, or that chemical substances which are not EDs are excluded from the market on basis of false-negative results. Because of the urgent need to adequately assess EDs, the EU Commission presented in 2016 a first draft of the scientific criteria for the identification of EDs (EC, 2016). According to these criteria, an exogenous substance will be identified as an ED if it obeys the following:

- It shows an adverse effect in an intact organism or its progeny, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism system or (sub)population, that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences.
- It has an endocrine mode of action, i.e. it alters the function(s) of the endocrine system.

- The adverse effect is a consequence of the endocrine mode of action; therefore, a biologically plausible link between adverse effects and endocrine mode of action has to be shown.

European Food Safety Authority (EFSA) and European Chemicals Agency (ECHA) have recently developed a guidance document that describes how to implement the scientific criteria for the identification of endocrine-disrupting properties of chemicals pursuant to the Biocidal Products Regulation and the Plant Protection Products Regulation (EFSA and ECHA, 2017). These guidances refer to lines of evidence, or adverse outcome pathways (AOPs), which are structured representations of biological events leading to adverse effects. Although EDs are heavily studied and knowledge on AOPs is rapidly increasing, a robust and comprehensive picture of AOPs is from far complete. More knowledge on the biological relevant link between a mode of action and adversity must become available for the hazard identification and risk assessment of EDs (EFSA and ECHA, 2017). In addition, this proposal version highlighted the following urgent and lacking points:

- Regarding the endocrine mode of action, there is only a limited number of OECD-accepted in-vitro and/or in-vivo tests available that qualify for assessing endocrine disrupting activities. There is an urgent need for additional OECD accepted tests to cover a broader range of endocrine activities for regulatory decision making.
- Risk assessment is explicitly not included in the guidance.
- The in vivo tests in the OECD Test Guidelines do not consider all adverse effects of EDs. For example, female reproductive disturbances which are related to the human ovulation cycle and placental development/function and result from maternal exposure to EDs cannot be assessed up till now.

2. Vulnerability to EDs

Nowadays, it is known that there are two important windows of exposure, the pregnancy and early postnatal life (infants and toddlers); these are exceedingly sensitive periods for EDs exposure (Heindel and Vandenberg, 2015). This is not surprising, given the fact that it is during these periods (especially early pregnancy) that all the organs (brain, liver, muscles,

skeleton) are formed and that certain endocrine feedback mechanisms are not yet mature (Bourguignon et al., 2018).

Several lines of evidence show that many childhood and adult diseases, including cardiovascular disease, obesity and metabolic disorders including type-2 diabetes, certain reproductive cancers, and neurodevelopmental disease, are consequences of EDs exposure during pregnancy (Bourguignon et al., 2018). Even though there is less data on childhood and adolescence, given their marked dependence on the endocrine system, adverse ED effects are suspected, and have been documented on animal models and in epidemiological studies (Cohn et al., 2007**;** Sergeyev et al., 2017).

These two windows of exposure are also important because during pregnancy, not only the mother and the fetus are exposed, even the next generation via the germinal cells (the eggs and the sperm) that are forming in the unborn child are affected (EP, 2019). During fetal development, significant changes in methylation patterns in the germ cells occur, with complex waves of DNA demethylation and re-methylation occurring modulating gene expression through epigenetic changes. These may contribute to the extreme sensitivity of the developing fetus (EP, 2019).

3. Selected EDs

Close to 800 chemicals are known or suspected to be capable of interfering with hormone receptors, hormone synthesis or hormone conversion (WHO, 2012; 2018). Among these EDs, some take an important place due to their widespread presence in food-packaging or food contact materials.

The packaging is an essential element in the food manufacture which protects foods from physical, chemical and microbiological alterations and promotes the safety and marketing strategy of the product. However, according to EFSA (2006), the packaging may be a source of contamination as migration can occur from these materials with the transfer of chemicals or particles into the food. Food packaging components like paper, glue, inks, and cardboard or the epoxy resin from canned food and teflon coating from non-stick cookware are important sources of EDs. According to that, the following EDs were selected in this thesis:

bisphenol A (BPA) and two analogues of it, bisphenol S (BPS) and bisphenol F (BPF); phthalate and non-phthalate plasticizers; perfluorooalkilated substances (PFAS) specifically perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) and tetrabromobisphenol A (TBBPA). These EDs selected have also been used for many industrial applications including textiles, paints, waxes, polishes, electronics, personal care products (PCPs) and adhesives. Consequently, all of them are related to oral exposure sources and with other non-dietary sources such as dermal, dust and air.

These EDs are considered as a particularly high concern for human health (Fromme et al., 2009; Groh et al., 2019; Muncke, 2011; Vandermeersch et al., 2015).

The exposure to pregnant mothers to these EDs is directly related to the fetus' exposure, due to a bi-directional transfer of chemicals through the placenta (Li et al., 2013). Consequently, this prenatal window exposure is related to some potential diseases, obesity and concomitant diseases in midlife such as type 2 diabetes, cardiovascular disease, or arterial hypertension (Weihrauch-Blüher and Wiegand, 2018).

3.1. Bisphenol A and two analogues, BPS and BPF

BPA is a phenolic chemical that has been used for over 50 years in the manufacture of polycarbonate plastics and epoxy resins, in thermal paper production, and as a polymerization inhibitor in the formation of some polyvinyl chloride plastics. Polycarbonates are used to make products such as compact discs, automobile parts, baby bottles, plastic dinnerware, eyeglass lenses, toys, and impact-resistant safety equipment (Geens et al., 2009). Epoxy resins containing BPA are used in protective linings of some canned food containers, wine vat linings, epoxy resin-based paints, floorings, and some dental composites (Geens et al., 2012). Around 3 billion kilograms of BPA are produced annually worldwide and over 100,000 kg of this compound are released annually into the atmosphere (Myridakis et al., 2016).

The exposure to bisphenol, in general population, mostly occurs through the ingestion of foods in contact with bisphenol containing materials (Wilson et al., 2007). Exposures from indoor air and dermal contact are smaller compared with dietary exposure sources (Wilson

et al., 2007). BPA is well absorbed orally and is glucuronidated in the liver. However, free bisphenol from dermal and little free bisphenol after oral absorption can circulate and accumulate in adipose tissue (Vandenberg et al., 2012). The glucuronidated BPA is excreted in the urine within 24 hours, but the human exposure to this chemical is chronic (Volkel et al., 2002). Consequently, regarding biomonitoring, BPA can be accurately measured without contamination in human urine as the best matrix and it can also be measured in serum. The conjugated and unconjugated forms reflect recent exposure to the chemical (Vom Saal and Welshons, 2014).

BPA is a well-known ED and has an estrogenic effect. The adverse effects of BPA are largely related to its estrogenic activity and they can disturb reproductive function (Hiroi et al., 1999; Kurosawa et al., 2002). In addition, recent epidemiologic evidence has also shown that BPA is implicated in cardiovascular disease, type 2 diabetes mellitus, and obesity (Giulivo et al., 2016). Furthermore, the effects of prenatal and early exposure to BPA may be manifested at any time in life (Giulivo et al., 2016).

Therefore, BPA has been regulated in many countries. BPA was banned in baby bottles in Canada in 2008, in France in 2010, and in the European Union in 2011 (Cano-Nicolau et al., 2016). However, the principal analogues of BPA, BPS, and BPF are still in them (Martínez et al., 2018). Both have similar chemical structures to BPA (**Figure 1**) and have been suggested to be endocrine-disrupting chemicals. There are some studies that confirm that they are involved in the processes of preadipocytes differentiation and lipid accumulation as BPA (Liu et al., 2019).

The tolerable daily intake (TDI) of BPA is 4 $\mu g/kg_{bw}$/day (EFSA, 2015). However, other studies have demonstrated that dosages below the current TDI could cause significant effects in animal models (Rezg et al.,2014). In the context of developmental risk, some authors affirm that BPA can affect the reproductive system and adipocyte differentiation (Myridakis et al., 2016). Especially for children, exposure to these EDs appears to be related to altered birth weight, male genital abnormalities, and behavioral and neurodevelopmental problems (Rochester, 2013; Tewar et al., 2016).

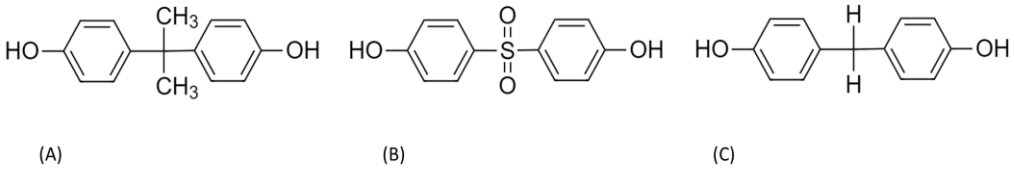


Figure 1. Chemical structures of (A) bisphenol A, (B) bisphenol S, and (C) bisphenol F.

3.2. Phthalate and non-phthalate plasticizers

Phthalates are ubiquitous environmental contaminants. They are a chemical family of several dozen derivative compounds (salts or esters) of phthalic acid. They all share a common chemical structure (**Figure 2(A)**). They are composed of a benzene ring and of two carboxylate groups placed in ortho (Grinbaum et al., 2019). The size of the carbon alkyl chain may be longer or shorter. Phthalates can thus be classified according to their molecular weight. Consequently, high-molecular-weight phthalates (HMWP) can be found in tubing, vinyl flooring, and wall covering (Mallozzi et al., 2016). Low-molecular-weight phthalates (LMWP) more commonly can be present in PCPs (shampoo, cosmetics, fragrances, and nail polish) (Mallozzi et al., 2016).

Human exposure to phthalates is widespread and occurs mostly through ingestion, inhalation, and dermal contact (Giovanoulis et al., 2018; Wormuth et al., 2006). Phthalates are easily released from the products that contain them to the environment. In general, they do not persist due to rapid biodegradation, photodegradation, and anaerobic degradation (Rudel and Perovich, 2009). It is known that food is the major source of exposure to di (2-ethylhexyl) (DEHP) phthalate (**Figure 2**), it is very widespread (Wormuth et al., 2006). The exposure to DEHP, in general population, mostly occurs through the ingestion of foods in contact with this chemical. However, other sources such as dermal contact with products that contain them, dust ingestion and inhalation, are also potential contributors to human exposure (Arbuckle et al., 2016). Non-dietary sources can contribute more to the total DEHP exposure than other EDs (Wormuth et al., 2006). After ingestion, DEHP is metabolized to more than 30 metabolites which are rapidly eliminated in the urine

(Albro et al., 1982; Albro and Lavenhar, 1989). Regarding biomonitoring, urine is the best matrix to measure phthalates, but it can also be detected in blood and sweat (Genuis et al., 2012).

Mono-(2-ethyl-5-hexyl) phthalate (MEHP) is primarily formed by the hydrolysis of DEHP in the gastrointestinal tract and then absorbed. It is the most toxic and active one among the metabolites (Gobas et al., 2016).

Phthalates are considered as EDs; they can mimic or block naturally occurring hormones in our body, changing the final cellular response (Zoeller et al., 2012). Prenatal exposure to these EDs is associated with potential diseases, diabetes and insulin resistance, breast cancer, obesity, metabolic disorders, and immune function (Bansal et al., 2018). Despite being metabolized quickly, they are able to reach the fetus by crossing the placenta barrier (Li et al., 2013). For that reason, DEHP has been removed from or replaced in most children toys and food packaging in the United States and EU (Hutzinger, 1982).

The ECHA established a TDI of 50 $\mu g/kg_{bw}$/day for DEHP (ECHA, 2010). Nowadays, due to the increasing evidence for adverse health effects on humans, there is a need for an alternative. One extended non-phthalate plasticizer is 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH), which is used as an alternative for some phthalate plasticizers (**Figure 2**). People are exposed to DINCH through ingestion and possibly, through dermal contact, indoor air and dust (Fromme et al., 2016; Nagorka et al., 2011). However, dietary sources have been considered the major exposure route (Fromme et al., 2016; Nagorka et al., 2011). Children may also ingest DINCH when they mouth toys or other DINCH-containing plastics. Intravenous or parenteral exposure can occur in persons undergoing medical procedures involving devices or materials containing DINCH. Researchers detected that the best matrix to measure their metabolites is urine, but serum can be used too (Silva et al., 2012).

Currently available toxicological data suggest that DINCH does not induce reproductive toxicity or endocrine disruption (EFSA, 2008). DINCH has not been evaluated for human carcinogenicity by the International Agency for Research on Cancer (IARC), National

Toxicology Program (NTP), or the U.S. Environmental Protection Agency (U.S. EPA). The European Union has established a TDI for DINCH at 1 mg/kg_{bw}/day (EFSA, 2006).

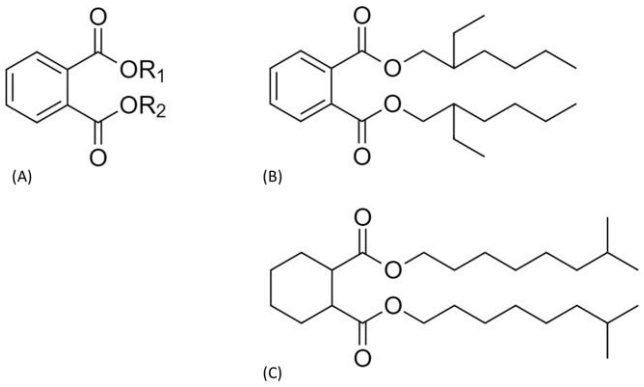


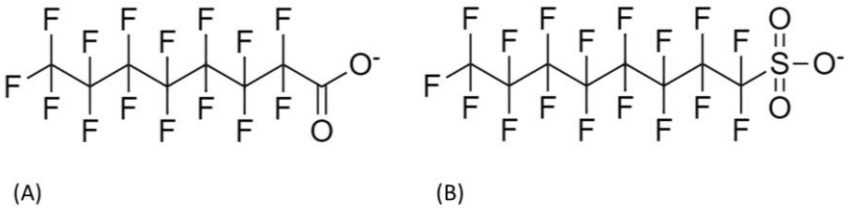
Figure 2. Chemical structures of general phthalates (A), DEHP (B) and DINCH (C).

3.3. Perfluorinated alkylated substances (PFASs)

PFASs include a wide group of synthetic compounds, such as, PFOS and PFOA among others (**Figure 3**), that are water- and oil-repellents and are able to reduce surface tension (Mancini et al., 2019). Due to these characteristics, PFASs have gained an important commercial value, which resulted in their use in a large number of industrial and consumer applications. The dramatic amount of PFASs produced in the last 60 years, combined with their high resistance to biodegradation, resulted in their global dissemination in the environment (Lindstrom et al., 2011). The PFASs have applications in waterproofing and protective coatings of clothes, furniture, and other products; and as constituents of floor polish, adhesives, fire retardant foam, and insulation of the electrical wire. A major application has been the heat-resistant non-stick coatings used on cooking ware and other protected surfaces. Because of their properties, they are used in a wide range of industries including aerospace, automotive, building/construction, chemical processing, electrical and electronics, semiconductor, and textiles (Lindstrom et al., 2011). Many chemicals in this group, including PFOS and PFOA, have been a concern because they do not break down in

the environment, can move through soils and contaminate drinking water sources, and they build up (bioaccumulate) in fish and wildlife. PFASs have been found in rivers and lakes and in many types of animals on land and in the water (Kannan et al., 2005; Keller et al., 2005). All sources of human exposure are uncertain, but probably dietary sources are the most important (Kannan et al., 2005; Prevedouros et al., 2006; Tittlemier et al., 2007). The ingestion of contaminated food or the migration of these substances from food packaging or cookware has been identified as key sources of human exposure to PFASs (Begley et al., 2005; Domingo, 2012; Ericson et al., 2012; Fromme et al., 2009). In contrast, the indoor environment (dust and air) has a minor contribution, with less than 1 % and 2 % for PFOS and PFOA, respectively (Ericson et al., 2012). PFOA and PFOS do not tend to accumulate in fat tissue but still can have long residence times in the body. PFOA is mostly excreted in the urine in animal studies, but limited observations in humans suggest that only one-fifth of the total body clearance is renal (Harada et al., 2004). The elimination half-life of PFOA in humans is roughly estimated to be 3.5 years and for PFOS, approximately 4.8 years (Olsen et al., 2007). So, regarding biomonitoring, the amount of PFASs that have entered people's bodies can be detected by measuring PFASs in serum (Ericson et al., 2007). Concerning the exposure of pregnant women, the placental barrier is not impermeable to the passage of PFOS and PFOA (Liu et al., 2011; Kim et al., 2011). Furthermore, these toxic substances can be also transferred from mother to child during breastfeeding (Kärrman et al., 2010; Motas Guzmàn et al., 2016).

PFOS and PFOA have been recognized as endocrine-disrupting chemicals (WHO, 2013). Moreover, prenatal and early exposure to PFASs is associated with a decreased birth weight and gestational age (Meng et al., 2018), developmental problems (Chen et al., 2013), reproductive system problems (Lyngsø et al., 2014), and increased susceptibility to disease in adulthood (Tsai et al., 2015; Zhang et al., 2018). Finally, according with the regulation, recently, EFSA (2018) updated the values regarding the provisional tolerable weekly intake (TWI) of PFOA at 13 ng/kg_{bw}/week ($1.86 \cdot 10^{-3}$µg/ kg_{bw}/day) and of PFOS at 6 ng/kg_{bw}/week ($8.6 \cdot 10^{-4}$ µg/ kg_{bw}/day).



(A) (B)

Figure 3. Chemical structures of (A) PFOA and (B) PFOS.

3.4. Tetrabromobisphenol A (TBBPA)

Tetrabromobisphenol A (TBBPA) is also an analog of BPA, however, the presence of bromine gives it the property of a flame retardant. It is used in a variety of products, including epoxy and polycarbonate resins (Hays and Kirman, 2019) (**Figure 4**). TBBPA is the most widely produced brominated flame retardant (Kacew and Hayes, 2019), representing around 60 % of the total brominated flame retardants (Vandermeersch et al., 2015). TBBPA is employed as a flame retardant in a variety of consumer products including printed circuit boards, communication and electronic equipment, appliances, transportation devices (Birnbaum and Staskal 2004; BSEF 2012).

Human exposure to TBBPA and TBBPA-containing products from all sources is estimated to be low, even in the case of specific occupational exposure scenarios (Kacew and Hayes, 2019). TBBPA is absorbed from the gastrointestinal tract and excreted rapidly after Phase II metabolism/conjugation to more water-soluble metabolites (Knudsen et al., 2014). The absorption of TBBPA by the dermal route is expected to be poor based on its physicochemical properties (EFSA, 2011; Lai et al., 2015). TBBPA does not accumulate in human fat tissue, it is not a neurotoxicant and the brain is not a target for this compound (Kacew and Hayes, 2019). However, limited data are available on the consequences of human exposure attributed to TBBPA (Kacew and Hayes, 2019). Regarding biomonitoring TBBPA is present in human breast milk and serum, relevant exposure to TBBPA has been assessed by measuring TBBPA in the blood (Liu et al., 2018). TBBPA exposure can have adverse health effects, affecting thyroid hormones, the neurological function, and the reproductive system (Cruz et al., 2009).

The TDI established by the EFSA, 2011 of TBBPA and its derivatives in food, was 1 $mg/kg_{bw}/day$.

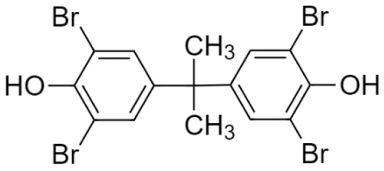


Figure 4. Chemical structure of TBBPA.

HYPOTHESIS AND OBJECTIVES

HYPOTHESIS AND OBJECTIVES

The general hypothesis of this thesis is that, there is exposure to EDs during the prenatal period and early stages of life and this exposure can cause adverse outcomes.

Therefore, the general objective of this doctoral thesis is to assess the exposure levels to different EDs and to study how these chemicals can affect normal cellular development. This main goal has been divided into four different specific objectives:

- To estimate the exposure to EDs for pregnant women cohort and their babies.
- Biomonitoring the levels of EDs in urine, blood and breast milk from pregnant women.
- To reconstruct the exposure to EDs using biomonitored levels and the physiologically based pharmacokinetic (PBPK) model.
- To determine the endocrine potential activity of some widespread EDs.

MATERIALS AND METHODS

4. The EXHES study population

The EXHES study population, comprises a Tarragona, Spain cohort of pregnant women and birth cohort. Pregnant women were recruited during the first trimester of pregnancy as part of the European "HEALS" project. The recruitment of pregnant mothers started in March 2016 and currently 176 mother-child pairs from Reus (Tarragona, Spain) were included. Women were informed of the study during their first visit at 12th gestational weeks (GWs) to the University Hospital "Sant Joan de Reus", in Reus (Tarragona, Spain). Women were eligible to participate according to the following inclusion criteria: ≥ 16 years old, intention to deliver at the reference hospital, and no problems with the communication language. This study was approved by the Ethical Committee of Clinical Research of the Hospital and a written informed consent was obtained from the participants.

4.1. Face-to-face interviews schedule

At 12th, 20th, 32nd GWs and after birth, face-to-face interviews were done in order to know their habits, diet, lifestyle and general characteristics data of the pregnant women. More detail information about these questionnaires is described in Martínez et al. 2017, 2018.

4.2. Biological samples: collection schedule and storage

Protocols for the collection of biological samples was applied to pregnant women and babies. Five blood samples were taken at different times (at 12th, 24th, 34th GW, at delivery and one extraction from the umbilical cord). In all of the blood extractions, fasting was required. Aliquots of plasma, serum and total blood were kept frozen at -80 °C, until analytical determination. In addition, during their visits to the hospital (at 12th, 20th and 32nd GW), urine samples were collected and stored at -80 °C until analytical determination. Furthermore, at delivery, samples of placenta and umbilical cord were taken and stored at -80 °C. Finally, breast milk samples were taken after one month of breastfeeding and they were also stored at -80 °C. **Figure 5** shows the schedule of the visits and the sampling period of the pregnant women cohort.

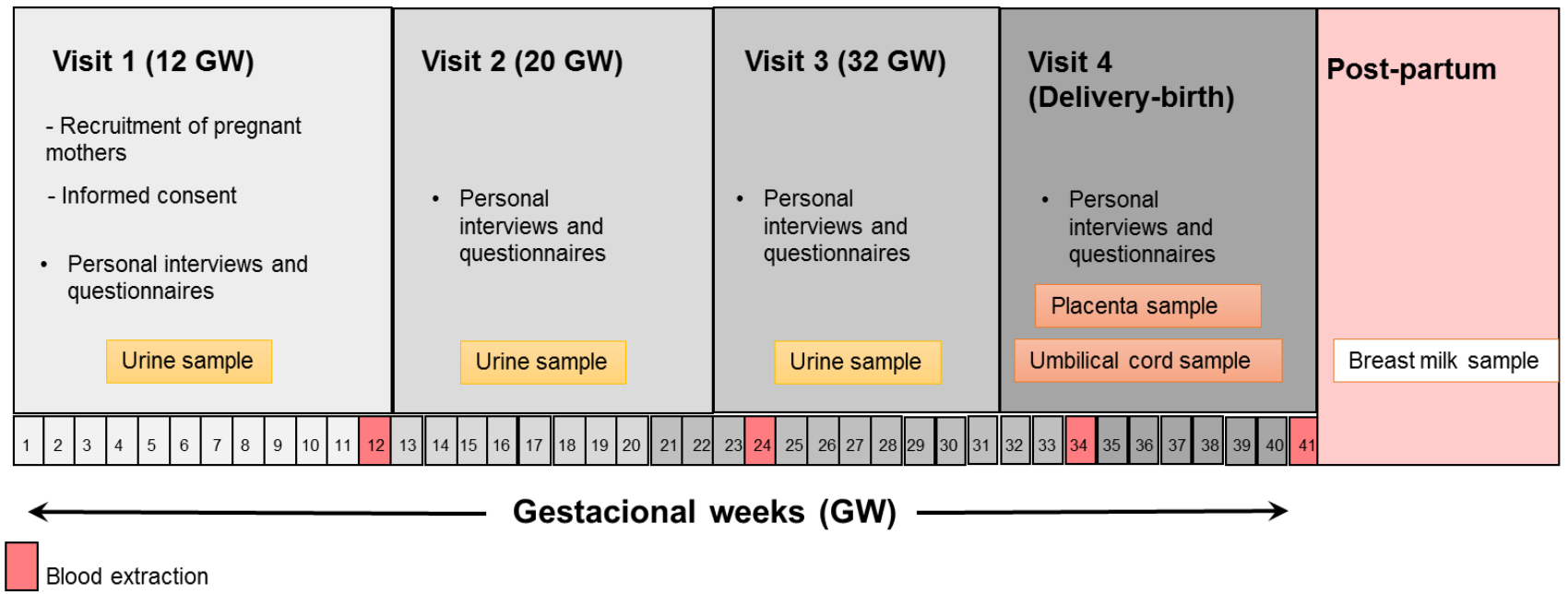


Figure 5. Schedule of the visits and the sampling period of the pregnant women cohort.

5. Exposure assessment

The maternal, prenatal and postnatal exposure to EDs was assess through the external and internal exposure dosimetry. To assess the exposure, dietary and non-dietary sources (dermal contact, non-dietary ingestion, and inhalation) were included.

External exposure

The contribution of each pathway, dietary or not (dermal, inhalation, dust ingestion) to the total exposure to EDs for the EXHES Tarragona, Spain cohort was assessed in a probabilistic way. To assess that, questionnaires from the interviews, general characteristic data and the concentration levels of the chemical in different food items, dust, PCPs and in the air were taken from the literature, applying as preference rule, data from Tarragona country > Catalonia > Europe. To deal with the variability and uncertainty of parameters, the dietary and non-dietary exposure was estimated in a probabilistic way by Monte-Carlo simulation, this is a common approach used to incorporate variability and uncertainty of the parameters used into the estimation of human exposure.

Internal exposure

The PBPK model was used to estimate the internal exposure. This model can predict the absorption, distribution, metabolism and excretion (ADME) of synthetic or natural chemical substances in humans and other animal species. The chemicals' dose inputs considered to run the PBPK, were probabilistically estimated by Monte-Carlo (external exposure data). Simulation was performed for pregnant women and fetuses for 24 h during the 24^{th}GW. This period was selected because at this time fetus organs are more developed and able to incorporate the right biological process. The outputs generated after running the model were selected considering the metabolites generated, their toxicity, gestational period and ability to reach the fetus.

6. Biomonitoring analyses

In the present study (the EXHES Tarragona, Spain cohort), EDs were biomonitored in maternal biological samples such as urine, breast milk, and blood. The biomonitoring data from just a single biological sample can be used to calculate the reconstructed exposure; this is the exposure to pregnant women to each parent compound calculated from the concentration levels of its metabolites found in a biological matrix.

Reconstructed exposure and estimated exposure data (from the same cohort, obtained from the method based on questionnaires and interviews) were used as an input to run the PBPK model to estimate the internal exposure. Estimated PBPK simulation exposure and reconstructed PBPK simulation exposure of pregnant women per day were compared. In addition, both simulations were compared with the extrapolated 24 h biomonitoring data.

7. Endocrine disruption activity. *In vitro* experiment

The toxicity effects regarding cell viability and the obesogenic activity of different widespread EDs were assessed by *in vitro* assays with preadipocytic 3T3-L1 cell line. To proceed with that, MTT assay was performed to compare the viability and to select the optimal concentration, which did not show a cell viability decrease. The cell phenotype (the mechanism of neutral lipid storage) were determined in differentiated 3T3-L1 adipocytes by red oil O staining. The expression levels of different adipocyte marker proteins, such as the transcription factors CCAAT/enhancer-binding protein α (C/EBPα) and peroxisome proliferator-activated receptor γ (PPARγ) and the fatty acid-binding protein 4 (FABP4) adipokine were determined with Western Blot test. Cells were seeded in 60-mm culture dishes and treated at the beginning of the adipocyte differentiation process. After 48 h, the differential growth medium with treatments was removed, and the cells were washed twice with phosphate-buffered saline (PBS). During the remainder of the differentiating process, no additional treatment was added. **Figure 6** illustrates the treatment schedule and maturation stages of 3T3-L1 preadipocytes.

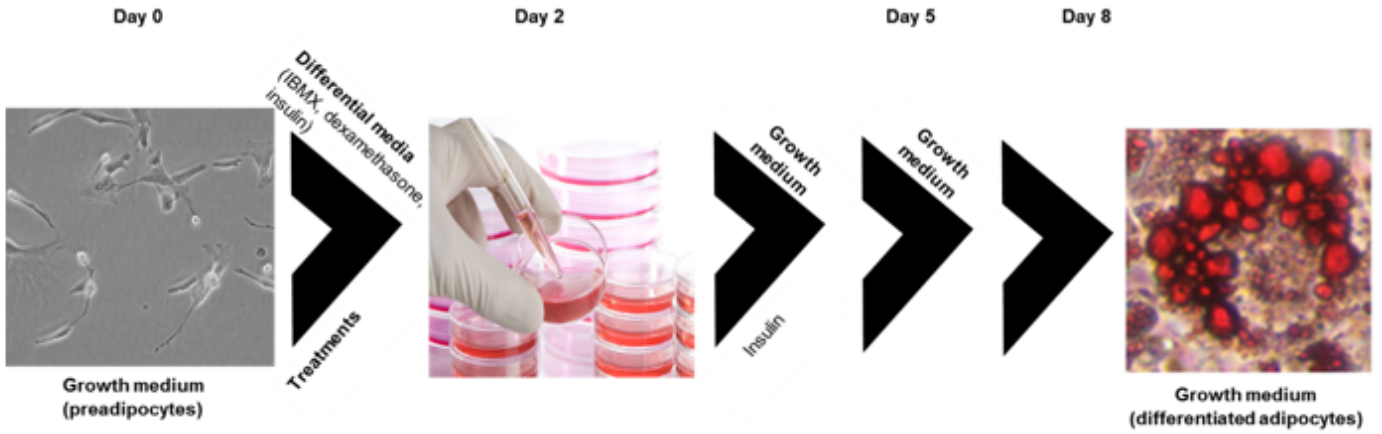


Figure 6. Treatment schedule. Cell culture, growth and differentiation

RESULTS

CHAPTER 1. EXPOSURE ASSESSMENT

PUBLICATION 1.

M.A. Martínez; J. Rovira; R. Prasad Sharma; M. Nadal; M. Schuhmacher; V. Kumar. **Prenatal exposure estimation of BPA and DEHP using integrated external and internal dosimetry: A case study**. *Environmental Research,* 158 (2017) 566 – 575.

PUBLICATION 2.

M. A. Martínez; J. Rovira; R. Prasad Sharma; M. Nadal; M. Schuhmacher; V. Kumar. **Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure: A Catalonia (Spain) case study.** *Environmental Research*, 166 (2018) 25 – 34.

PUBLICATION 1

Prenatal exposure estimation of BPA and DEHP using integrated external and internal dosimetry: A case study

M. A. Martínez[a], J. Rovira[a,b], R. Prasad Sharma[a], M. Nadal[b], M. Schuhmacher[a,b], V. Kumar[a,b]

[a]Environmental Engineering Laboratory, Departament d'Enginyeria Quimica, Universitat Rovira i Virgili, Av. Països Catalans 26, 43007 Tarragona, Catalonia, Spain. [b]Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Catalonia, Spain.

Environmental Research, 158 (2017) 566 – 575.

Abstract

Exposure to Endocrine disruptors (EDs), such as Bisphenol A (BPA) and di (2-ethylhexyl) phthalate (DEHP), has been associated with obesity and diabetes diseases in childhood, as well as reproductive, behavioral and neurodevelopment problems. The aim of this study was to estimate the prenatal exposure to BPA and DEHP through food consumption for pregnant women living in Tarragona Country (Spain). Probabilistic calculations of prenatal exposure were estimated by integrated external and internal dosimetry modelling, physiologically based pharmacokinetic (PBPK) model, using a Monte-Carlo simulation. Physical characteristic data from the cohort, along with food intake information from the questionnaires (concentrations of BPA and DEHP in different food categories and the range of the different food ratios), were used to estimate the value of the total dietary intake for the Tarragona pregnancy cohort. The major contributors to the total dietary intake of BPA were canned fruits and vegetables, followed by canned meat and meat products. In turn, milk and dairy products, followed by ready to eat food, were the most important contributors to the total dietary intake of DEHP. Despite the dietary variations among the participants, the intakes of both chemicals were considerably lower than their respective current tolerable daily intake (TDI) values established by the European Food Safety Authority (EFSA). Internal dosimetry estimates suggest that the plasma concentrations of free BPA and the most important DEHP metabolite, mono (2-ethylhexyl) phthalate (MEHP), in pregnant women were characterized by transient peaks (associated with meals) and short half-lives (<2 h). In contrast, fetal exposure was characterized by a low and sustained basal BPA and MEHP concentration due to a lack of metabolic activity in the fetus. Therefore, EDs may have a greater effect on developing organs in young children or in the unborn child.

Keywords

Endocrine disruptors; Bisphenol A (BPA); di (2-ethylhexyl) phthalate (DEHP); mono (2-ethylhexyl) phthalate (MEHP); physiologically based pharmacokinetic (PBPK) model; Prenatal exposure.

1. Introduction

The endocrine system secretes hormones which regulate the metabolic functions of the body. Endocrine disruptors (EDs) are substances that can mimic or partly mimic naturally occurring hormones in the body like estrogens, androgens, and thyroid hormones (Matsui, 2008). EDs can also bind to a receptor within a cell and block the endogenous hormone from binding. (Sharma et al., 2016 a). Therefore, EDs can interfere or block the way natural hormones or their receptors are made or controlled (Thomson and Grounds, 2005). Bisphenol A (BPA) and di (2-ethylhexyl) phthalate (DEHP), among others, are very important EDs due to the widespread distribution of products that contain them. According to the World Health Organization (WHO), both of these chemicals can cause adverse health effects in an intact organism, or its progeny (Hughes et al., 2006; Meeker, 2012; WHO, 2012). The effects of prenatal and early exposures to EDs may be manifested any time in life (Giulivo et al., 2016; Sharma et al., 2016 a).

Around 3 billion kilograms of BPA are produced annually worldwide and over 100,000 kilograms of this compound are released annually into the atmosphere (Myridakis et al., 2016). BPA is used in industry for the production of resins and polycarbonate plastic. Although the use of BPA in Europe is banned for the manufacture of plastic materials in contact with food intended for children (0-3 years) (European-Parliament, 2011), it is not banned in polycarbonate (PC) plastics for other uses. It can be found in food and beverage processing, and in many other commercial products such as epoxy resin cans, dental sealants, personal care products (PCPs), baby bottles, building materials, flame retardant materials, optical lenses, materials for the protection of window glazing, DVDs, and household electronics (Geens et al., 2012; Myridakis et al., 2016). Although the ingestion of BPA from food or water is the predominant route of exposure (Lorber et al., 2015), there are other nonfood routes, such as inhalation of free BPA (concentrations in indoor and outdoor air), indirect ingestion (dust, soil, and toys), and dermal route (contact with thermal papers and application of dental treatment), which contributes to the total BPA exposure (Myridakis et al., 2016). In addition, recent studies (De Coensel et al., 2009; Sungur et al., 2014) have seen that temperature has a major impact on the BPA migration level into water;

an increase from 40 ºC to 60 ºC can lead to a 6 - 10 fold increase in the migration level (De Coensel et al., 2009). The TDI of BPA is 4 μg/kg_{bw}/day (EFSA, 2015). However, other studies have demonstrated that dosages below the current TDI could cause significant effects in animal models (Rezg et al., 2014). In the context of developmental risk, some authors affirm that BPA can affect the reproductive system and adipocyte differentiation (Myridakis et al., 2016). Especially for children, exposure to these EDs appears to be related to altered birth weight, male genital abnormalities, and behavioral and neurodevelopmental problems (Rochester, 2013; Tewar et al., 2016).

Phthalates are ubiquitous environmental contaminants made up of dialkylesters or alkyl and aryl esters oforthophthalic acid (1,2-dicarboxylic acid). High-molecular-weight phthalates (HMWP) can be found in tubing, vinyl flooring, and wall covering (Mallozzi et al., 2016). Low-molecular-weight phthalates (LMWP) more commonly can be present in personal care products (shampoo, cosmetics, fragrances and nail polish) (Mallozzi et al., 2016). Phthalates are also found as both inert and active ingredients in some pesticide formulations (EFSA, 2015). It is known that food is the major source of exposure to diisobutyl (DiBP), di-n-butyl (DnBP), and di (2-ethylhexyl) (DEHP) phthalate (Wormuth et al., 2006). However, other sources such as dermal contact with products that contain them, dust ingestion and inhalation, are also potential contributors to human exposure (Arbuckle et al., 2016). An additional exposure route for young children is through mouthing toys, childcare articles and other products containing phthalates. Through mouthing of these products, phthalates can dissolve in saliva and finally be absorbed into the bloodstream. (De Coensel et al., 2009). Once absorbed, phthalate diesters are quickly metabolized into monoesters (as MEHP), which are biologically active and ultimately excreted in urine (Genuis et al., 2012). DEHP metabolite, the mono (2-ethylhexyl) phthalate (MEHP), is the most toxic and active one among these phthalates (Gobas et al., 2016). The EFSA and the European Chemical agency (ECHA) established a TDI of 50 μg/kg_{bw}/day for DEHP (EFSA, 2015; ECHA, 2010). In the context of risk, DEHP and its metabolite MEHP, mainly affect estrogen production and action in granulosa cells, resulting in hypo-estrogenic, polycystic ovary and anovulatory cycles. This leads to infertility and affects the reproductive

development of the fetus (Das et al., 2014; Davis et al., 1994; Lovekamp-Swan and Davis, 2003; Wang et al., 2015).

BPA and phthalates are considered "non-persistent" EDs because they are rapidly eliminated from the human body. Despite their short biological half-lives, exposure is prevalent and continuous because of their widespread use in food and everyday products, leading to consistent detection of these EDs in human biological matrices like urine and blood. BPA undergoes glucuronidation and sulfation producing BPAG and BPAS in the liver, respectively (Hanioka et al., 2008; Kim et al., 2003). These metabolites are not toxic in comparison to BPA (Gramec Skledar and Peterlin Mašič, 2016). Instead, DEHP is metabolized into mono (2-ethylhexyl) phthalate (MEHP), which is more toxic than DEHP (Gobas et al., 2016; Latini, 2005).

Optimal development and health in early life are key factors for health and wellbeing during later childhood and adulthood. It has been hypothesized that adult health and disease have their origin in the prenatal and early postnatal environment, a concept referred to as the Developmental Origins of Health and Disease (Hanson and Gluckman, 2011). There are various parameters early in life, which are indicators for development later in life. The exposition to these EDs in the early period of life conditions to suffer and develop illnesses like obesity and type 2 diabetes in childhood and adulthood (Casas et al., 2011; De Cock et al., 2014; Myridakis et al., 2016).

The aim of this study is to estimate the prenatal exposure to EDs (BPA and DEHP) through the dietary intake of pregnant women using integrated external and internal dosimetry estimation. To assess the prenatal exposure, we used a mathematical physiologically based pharmacokinetic model (PBPK) adapted for pregnancy, in order to know the internal dosimetry levels of EDs in the fetus. PBPK models are mathematical representations of the human body aimed at describing the time course distribution of chemicals in human tissues (Fàbrega et al., 2016). In recent years, PBPK models have been used in human health risk assessment to estimate the burdens of chemicals in human tissues, thus avoiding the analysis of this kind of samples (Fàbrega et al., 2014; Fàbrega et al., 2015; Schuhmacher et

al., 2014). The present study is in the framework of the "HEALS" project (FP7-603946), Health and environmental-wide associations based on large population surveys.

2. Materials and Methods

2.1. Study Population cohort

The study population comprises a cohort of pregnant women and ongoing birth cohort. The pregnant women were recruited during the first trimester of pregnancy as part of the European "HEALS" project. The recruitment of pregnant mothers has started in March 2016 and in the present study 45 mother-child pairs were included. Women were informed of the study during their first prenatal visit to the University Hospital "Sant Joan de Reus", in Reus, Catalonia, Spain. Women were eligible to participate according to the following inclusion criteria: ≥16 years, intention to deliver at the reference hospital, and no problems with the communication language. This study was approved by the Ethical Committee of Clinical Research of the University Hospital "Sant Joan de Reus". Written informed consent was obtained from the participants.

2.2. Pregnancy and diet

Diet has been considered the primary source of BPA and phthalates exposure (Lakind and Naiman, 2010; Maffini et al., 2006; Welshons et al., 2006). Therefore, face-to-face food frequency questionnaires (FFQ) and personal interviews were used in order to determine the pregnant women's dietary intake of BPA and DEHP, like other authors had done it before (Casas et al., 2011; Myridakis et al., 2016). Apart from food frequency questions, the questionnaires also included a set of questions targeting to know other sources of these compounds.

Dietary factors were assessed using FFQ (times per week), the questionnaires give information about general food intakes by mothers during pregnancy trimesters. These questionnaires were originally designed to assess average dietary intakes during two phases of pregnancy: the 1st FFQ covered the year before pregnancy and the 2nd FFQ covered the

whole pregnancy including the last period until birth. Intake frequency for each food item was converted to an average daily intake for each participant and then expressed like servings/week. Different food items from the FFQs administered during pregnancy study were classified in 8 general food groups: a) Grains and grain-based products (cereals, pasta, and bread), b) Milk and dairy products (milk, yogurt, hard cheese and fresh cheese), c) Meat and meat products (chicken, turkey, beef, pork, lamb and minced meat), d) Fish and other seafood (white fish, blue fish and seafood), e) Fruits and vegetables (salad, green beans, swiss chard, spinach, garnish vegetables, potatoes, and), f) Legumes (lentils, chickpeas, and white beans), g) Ready to eat (pre-cooked and canned food) and h) Water. In addition, questions potentially relevant to EDs exposure were asked: type and frequency of water consumption (bottled water or tap water), organic food consumption, heating and use of plastic microwave food containers and consumption of plastic packaged food or canned food. Especially canned food is considered as the predominant source of BPA and DEHP (Hartle et al., 2016; Schecter et al., 2013).

Face-to-face interviews were conducted with mothers during pregnancy about habits and lifestyle, in order to know relevant information related to the exposure to EDs, such us smoking or alcohol drinking, hobbies or activities that they usually do, place of living and work environment.

2.3. BPA and DEHP total dietary intake assessment

The estimation of the total dietary intake of BPA and DEHP for pregnant women was calculated according to equation A.1.

$$\text{Total dietary intake} = (C_{BPA/DEHP} \cdot F_r \cdot F_f) / BW / 7 \qquad \text{Eq. (A.1)}$$

Where $C_{BPA/DEHP}$ is the BPA or DEHP concentration found in the different food categories (in µg/kg); F_r is the food ingestion ration (in kg/ration); F_f is the food frequency consumption (in ration/week), and BW is the body weight (in kg). The total dietary intake is given in µg/kg bw/day. Data used to assess the total dietary intake of BPA and DEHP is shown in Table 1.

Concentrations of BPA and DEHP in the different food categories were taken from the literature with a preference rule of Spanish > Mediterranean > European average > other available data. The range of the different food rations was taken from Spanish Society of Community Nutrition (Serra Majem, 2011). Finally, the food frequency and body weight were taken from the cohort of the present study. To deal with variability and uncertainty of parameters mentioned, probabilistic estimation of the total dietary intake was performed using Monte-Carlo simulation. Monte-Carlo simulation is a common approach used to incorporate variability and uncertainty of the parameters mentioned into the estimation of human health exposure (Mari et al., 2009; May et al., 2002; Rovira et al., 2016; Schuhmacher et al., 2001). Table 1 includes the probabilistic distribution of parameters for the calculation of human health exposure. In this study, Monte-Carlo simulation was carried out by Oracle Crystal Ball©. This program is able to calculate risk based on the propagation variable of variability and uncertainty given by each parameter probability function until a certain number of iterations. An iteration size of 100,000 was used. Appropriate probabilistic distributions were used according to the input parameters (concentrations of BPA and DEHP in the different food categories, food fraction, food frequency, and body weight): Log-normal, triangular and uniform distribution (Table 1). In general, we used triangular distribution when the literature data was limited; in these cases, the minimum, maximum and mean values of the parameter were considered. We used log-normal distributions only for positive values and when literature data was available (mean and standard values). Finally, we used the uniform distribution when the information available was only the min-max range assuming equal probability of occurrence. To simulate different exposure scenarios, detailed data from the cohort study (food frequency and the body weight of the mothers) has also been considered. A complementary aspect of the Monte-Carlo study is the possibility of creating sensitivity charts, which show information about how much each predictor variable (each food item) contributes to the uncertainty or variability of prediction (Shade and Jayjock, 1997).

Table 1. Monte-Carlo parameter description to assess the total dietary intake of BPA and DEHP

Parameter	Symbol	Units	Type	Distribution [a]	Reference
BPA concentration in[b]	C_{BPA}	–	–	–	–
Grains and grain-based products	–	µg/kg	T	18.0(0-47.5)	EFSA, 2015
Fruits and vegetables	–	µg/kg	T	9.93 (0-116)	EFSA, 2015
Legumes	–	µg/kg	T	51.5 (0-103)	EFSA, 2015
Meat and meat products	–	µg/kg	T	36.9 (0-394)	EFSA, 2015
Fish and other seafood	–	µg/kg	T	20.7 (0-169)	EFSA, 2015
Milk and dairy products	–	µg/kg	T	1.45 (0-15.2)	EFSA, 2015
Ready to eat (including canned dinner)	–	µg/kg	T	5.80 (2.90-8.70)	Sakhi et al., 2014
Bottle water	–	µg/L	T	0.20 (0-4.40)	EFSA, 2015
Tap water	–	µg/L	P	0	EFSA, 2015
DEHP concentration in	C_{DEHP}	–	–	–	–
Grains and grain-based products	–	µg/kg	T	43 (18-61)	Sakhi et al., 2014
Fruits and vegetables	–	µg/kg	T	4.80 (0.05-9.50)	Sakhi et al., 2014
Meat and meat products	–	µg/kg	T	0 (0-64)	Sakhi et al., 2014
Fish and other seafood	–	µg/kg	T	0 (0-35)	Sakhi et al., 2014
Milk and dairy products	–	µg/kg	T	126 (19-173)	Sakhi et al., 2014
Ready to eat (including canned dinners)	–	µg/kg	T	136 (37-235)	Sakhi et al., 2014
Bottle water	–	µg/L	LN	0.11 ± 0.05	Santana et al., 2014
Tap water	–	µg/L	LN	0.16 ± 0.04	Santana et al., 2014
Food ration	Fr	–	–	–	–
Grains and grain-based products	–	kg/ration	U	0.05-0.07	Dapcich et al., 2004
Fruits and vegetables	–	kg/ration	U	0.15-0.20	Dapcich et al., 2004
Legumes	–	kg/ration	U	0.06-0.08	Dapcich et al., 2004
Meat and meat products	–	kg/ration	U	0.10-0.13	Dapcich et al., 2004
Fish and other seafood	–	kg/ration	U	0.13-0.15	Dapcich et al., 2004
Milk and dairy products	–	kg/ration	U	0.26-0.34	Dapcich et al., 2004
Ready to eat (including canned dinners)	–	kg/ration	U	0.21-0.41	Dapcich et al., 2004

Parameter	Symbol	Units	Type	Distribution [a]	Reference
Food frequency	Ff	–	–	–	–
Grains and grain-based products	–	ration/week	LN	9.60 ± 3.57	Present study
Fruits and vegetables	–	ration/week	LN	21.1 ± 7.09	Present study
Legumes	–	ration/week	LN	1.80 ± 1.38	Present study
Meat and meat products	–	ration/week	LN	5.13 ± 2.81	Present study
Fish and other seafood	–	ration/week	LN	2.87 ± 1.74	Present study
Milk and dairy products	–	ration/week	LN	6.86 ± 4.59	Present study
Ready to eat (including canned dinners)	–	ration/week	LN	3.09 ± 1.82	Present study
Bottle Water	–	L/day	LN	1.40 ± 0.67	Present study
Tap water	–	L/day	LN	1.02 ± 0.50	Present study
Conversion factor	-	day/week	-	7	
Bodyweight	BW	kg	LN	65.5 ± 14.0	Present study

[a]Mean, minimum, and maximum values were used for triangular distributions; Mean and standard deviation were used for log-normal; minimum, and maximum values for uniform distributions. [b]Including canned and non-canned food. LN= Log-normal; T= Triangular; U= Uniform; P= Punctual

2.4. Cohort Characteristics

A description of the characteristics of the study population is shown in Table 2. 43 % of mothers had university studies and 25 % had more than 12 years of education. Almost 75 % of the mothers were between 30 and 39 years old and 15 % were actively smoking during pregnancy. Regarding water consumption, most of the mothers drink bottled water (70 %) and most of them never eat organic products (56 %). Almost 50% of our cohort eats fast-food once a week and 70 % of them eat canned food between 1 and 3 times per week. This data can be directly related to the cohort's complexion (around 50 % of the pregnant mothers were overweight, and 15 % of them were obese).

Table 2. Characteristics of the study population from Reus, Tarragona (Spain) (n=45).

Characteristics of the study population (n = 45)	**%**		**%**
Maternal age at delivery (years)		*Mother's diet*	
< 20	0	Omnivorous	96
20-29	10	Vegetarians	4
30-39	73	Vegans	0
>40	17	*Water consumption (liters)*	
Twin pregnancy	9	< 1	4
*Maternal pre-pregnancy BMI**		1-2	85
Underweight (<19 kg/m2)	11	>2	11
Normal (19-25 kg/m^2)	52	*Kind of water consumption*	
Overweight (>25 kg/m^2)	26	Tap water	16
Obese (>30 kg/m^2)	11	Bottled water	71
*Maternal pregnancy (20 GW) BMI**		Both	13
Underweight (<19kg/m2)	0	*Eat in a plastic recipient (times/week)*	
Normal (19-25 kg/m^2)	41	Never	69
Overweight (>25 kg/m^2)	44	1-3	4
Obese (>30 kg/m^2)	15	4-6	20
Maternal education		> 6	7
Primary	25	*Eat canned food (times/ week)*	
Secondary	32	Never	18
University	43	1-3	71
Social economic status		4-6	7
High level (> 35000 €/year)	25	> 6	4
Median level (19000-35000 €/year)	57	*Eat Fast-food*	
Low level (< 9000-19000 €/year)	18	Never	29
Maternal country of origin		1 a week	47
Spain	81	>1 a week	24
Other	19	*Eat organic products*	

Characteristics of the study population (n = 45)	**%**		**%**
Marital Status		Never	56
Living with the father	98	Hardly ever	18
Not living with the father	2	Sometimes	20
Maternal smoking		Very often	7
Never smoke	74		
Not during pregnancy	11		
During pregnancy	15		
**BMI= Body mass index*			

2.5. Tissue dosimetry model (PBPK)

A previously developed and validated adult PBPK model of BPA (Sharma et al., 2016 b, unpublished) and of DEHP (Sharma et al., 2016 c, unpublished) was adapted for the pregnancy-PBPK model and was used to estimate internal dosimetry of mothers and fetuses for the present cohort study. The basic structure of adult human PBPK model (which included plasma, liver, kidneys, filtrate, fat, brain, gonads and a rest of the body compartment for the remaining tissues) (**Figure** 1), has been adapted for pregnant women model. In addition, compartments of placenta and fetus were considered as a sub-model in order to predict the internal dosimetry for the fetus. It was subcategorized again into liver, brain, and plasma (**Figure** 1). The physiological and chemical-specific parameters were adapted from the adult human model and modified for the fetuses and mothers as a function of the gestational period. The metabolism capacity in the fetus was scaled from the adult data. The source of exposure to fetuses was through free fraction of chemicals into mothers placenta, considering that fetuses exposure is directly related to mother's exposure. The placental-fetal unit assumes a bidirectional transfer process describing chemical transfer between mother's placenta to fetus plasma and fetus plasma to the mother. A detailed description of standard and pregnancy specific model equations are provided in supplementary material (Annex-I). All physiological parameters were considered as a function of gestational day and model equations were adapted from different literature sources and are provided in Annex-I. Metabolic kinetic parameters namely Vmax (maximum rate of reaction) and Km (affinity of the substrate for the enzyme),

for mothers and fetuses, were taken from in-vitro studies and were scaled to in-vivo. The chemical-specific parameters are also provided in supplementary material (Annex-I).

PBPK model inputs were the outputs of the Monte-Carlo simulation used previously for the exposure assessment. We considered three total dietary intake scenarios of BPA and DEHP: 5th percentile, mean and 95th percentile. In addition, a biologically active metabolite of DEHP, MEHP was considered as relevant internal exposure chemical and was used as an input in the PBPK simulation model to estimate fetus exposure. DEHP is rapidly metabolized into MEHP (Latini, 2005) and normally stay in the systemic circulation of mother's body and pass to the fetuses.

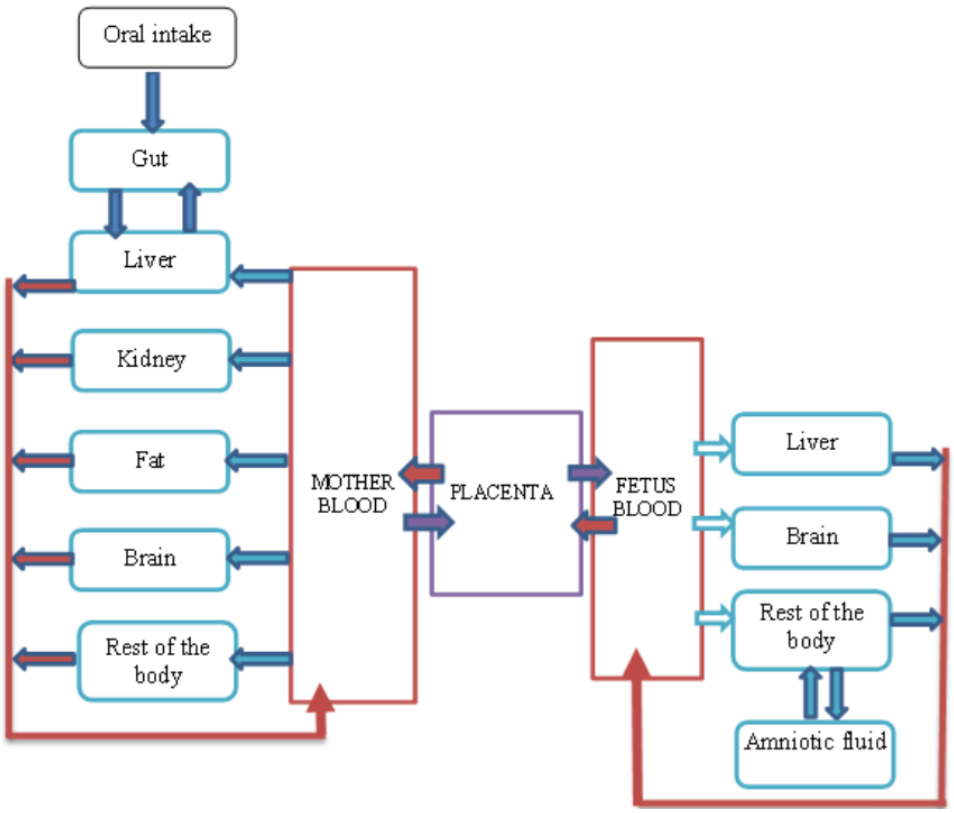


Figure 1. Conceptual structure of pregnancy PBPK model for BPA and DEHP. Adapted PBPK model for pregnant women and fetus which included the body organs compartments for both. The compartments like placenta and fetus compartments were considered as a sub-model in order to predict the internal dosimetry for the fetus.

3. Results and Discussion

3.1 BPA and DEHP total dietary intake and food categories contribution

The contribution of each food item to the total dietary intake for the Tarragona population cohort was assessed in a probabilistic way using a Monte-Carlo simulation. **Figure** 2, summarizes the food categories contributing to the total dietary intake of BPA (**Figure** 2, A.1) and DEHP (**Figure** 2, A.2)

Regarding BPA (**Figure** 2, A.1), the total dietary intake mean value was 0.72 μg/kg_{bw}/day (0.28 and 1.42 μg/kg_{bw}/day for 5^{th} and 95^{th} percentile, respectively). The variable showing the greatest contribution to the total dietary intake mean value was "fruits and vegetables" with 49 %, followed by "meat and meat products" with 26 %. The contribution of the remaining food categories were 8 %, 5 %, 4 %, 4 %, 2 % and 2 % corresponding to "fish and other seafood", "water consumption" (bottled water and tap water were considered, but only bottled water added risk of exposure to BPA), "grain and grain-base products", "milk and dairy products", "ready to eat (including canned food)" and "legumes", respectively.

The high contribution (49 %) of "fruits and vegetables" to the total dietary intake was due to the high consumption of this food item (an average of 21.1 servings per week), typical of a Mediterranean diet. The concentration of BPA in fruits and vegetables was not excessively high compared with other food items, with an average concentration of 9.92 μg/kg, although there was a maximum value of 116 μg/kg due to canned fruits and vegetables. It should be noted that fruits and vegetables are also packaged in plastic and in these cases, migration of BPA to the products occurs (Lakind and Naiman, 2010). The next major contributor to the total dietary intake was "meat and meat products" with a contribution of 26 % and an average concentration of BPA of 36.9 μg/kg and a maximum value of 395 μg/kg (canned). In this case, unlike the group of fruits and vegetables, although the frequency of consumption is lower, the levels of BPA in this category are higher.

EFSA (2015) published its comprehensive re-evaluation of BPA exposure and toxicity, in January 2015 it established a TDI of 4 μg/kg_{bw}/day for BPA. In the present study, although

the maximum value estimated was 4.40 μg/kg_{bw}/day, 95% of the population were under 1.41 μg/kg_{bw}/day. In addition, the present study data matches with the established values, which FAO (Food and Agriculture Organization)/WHO set during the last expert meeting in order to review the toxicological and health aspects of BPA. For adults, the highest exposure estimates did not exceed 1.4 μg/kg_{bw} per day at the mean and 4.2 μg/kg_{bw}/day at the 95th percentile (FAO/WHO, 2010).

Regarding DEHP, the total dietary intake mean value for our cohort was 1.00 μg/kg_{bw}/day (0.41 μg/kg_{bw}/day and 2.01 μg/kg_{bw}/day for 5th and 95th percentile, respectively) (**Figure** 2, A.2). The maximum contribution to this exposure comes from "milk and dairy products" with 56 %, followed by "ready to eat (including canned food)" with 30 %. The other food items "grain and grain-base products", "meat and meat products", "fruits and vegetables", "fish and other seafood" and "water consumption" (bottled water and tap water were considered) contributed to 6 %, 4 %, 3 %, 1 %, and 1 %, respectively.

On the one hand, the high contribution (56 %) of "milk and dairy products" category to the total dietary intake of DEHP in the present study is due to the high DEHP levels in milk and dairy products (with a mean and maximum of 126 and 173 μg/kg, respectively) in comparison to other categories. DEHP contamination of milk and dairy products occurs in several stages: contaminated DEHP feed, mechanical milking process, and migration from packaging material used in milk and dairy products (Fierens et al., 2013). Milk and dairy products were the second most consumed food item during pregnancy (an average of 6.86 servings per week), which can also be related to the general recommendation for a pregnant woman of maintaining optimal levels of calcium in the body in order to prevent adverse gestational outcomes (WHO, 2013). Also, the high concentration of DEHP in this food group is due to lipophilic nature of phthalates; and for this reason, it is assumed that high-fat foods contain more phthalates than low-fat food products (Fierens et al., 2013). Various authors (Page and Lacroix, 1989; Sharman et al., 1994) reported that there is a positive relationship between the fat content of a dairy product and the DEHP content in that product. The second most contributed food item to the total dietary intake of DEHP

was ready to eat food (30 %). It has been found a strong correlation between fast food intake and phthalates exposure but not with BPA exposure. This evidence coincides with another study from the USA, in which they observe the same evidence of a positive dose-response relationship between fast food intake and DEHP exposure but not for BPA (Zota et al., 2016).

The EFSA and the ECHA established the total daily intake for DEHP to 50 $\mu g/kg_{bw}$/day (EFSA, 2015; ECHA, 2010). In this study, both, the maximum (11.4 $\mu g/kg_{bw}$/day) and the 95^{th} percentile (2.01 $\mu g/kg_{bw}$/day) were far below this threshold.

Finally, the concentration of BPA and DEHP in bottle water was found in the literature data. However, in tap water, only levels of DEHP was found (Table 1). The presence of DEHP in tap water is due to leaching from PVC tubes and others materials from the pipes (Santana et al., 2014).

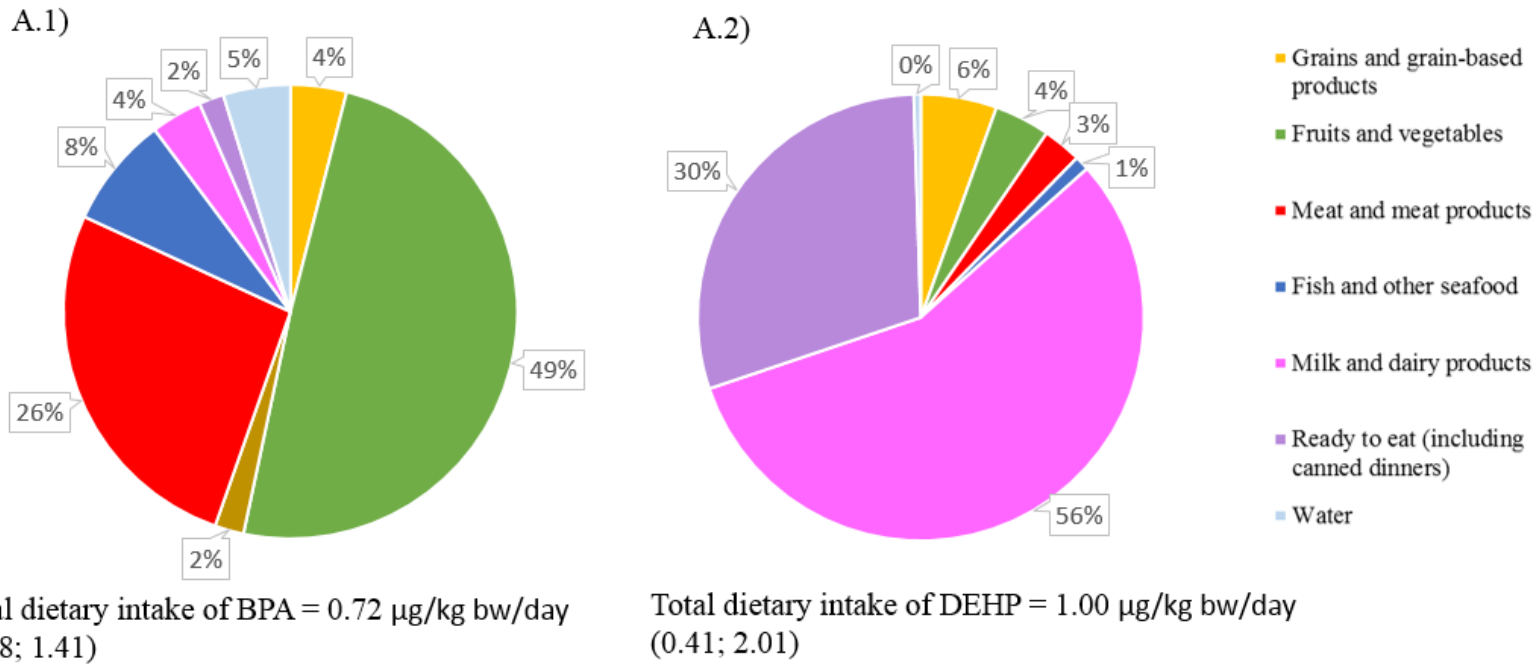


Figure 2. Food categories contribution to the total dietary intake of BPA (A.1) and DEHP (A.2) in µg/kg$_{bw}$/day. Results are given in mean (5th percentile; 95thpercentile

3.2 Dietary exposure compared to other countries

Table 3 shows the BPA and DEHP total dietary intake in adult populations in different countries. All data from the studies in Table 3 were experimentally analyzed in different food items.

Regarding BPA, it can be observed that the mean daily intake of it in the Tarragona cohort (Spain) was in the same order of magnitude as data presented for the Spanish cohort in EFSA report (EFSA b) (EFSA, 2013) and it was slightly below the European mean dietary intake of previous EFSA report (EFSA, 2006). Total dietary intake of BPA in Tarragona was also in the same order of magnitude as in Taiwan (Chen et al., 2016). However, data from countries such as France (Bemrah et al., 2014), Belgium (Geens et al., 2010), and USA (Lorber et al., 2015) were one order of magnitude lower; whereas, countries such as New Zealand (Thomson and Grounds, 2005), and Norway (Sakhi et al., 2014) were two orders of magnitude lower than the Tarragona study.

Regarding DEHP, it can be observed that the mean daily intake in the Tarragona cohort (Spain) was in the same order of magnitude as data presented from other European studies such as Belgium (Sioen et al., 2012), France (Martine et al., 2013) and Switzerland (Dickson-Spillmann et al., 2009). The present study estimations were in the same order of magnitude as Norway (Sakhi et al., 2014), USA (Schecter et al., 2013), Germany (Fromme et al., 2007) and China (Sui et al., 2014). However, DEHP exposure in countries like Cambodia (Cheng et al., 2013) and Germany (Heinemeyer et al., 2013) were presented one order of magnitude higher than the Tarragona's results.

It should be noted that dietary preference and food sources in different regions might lead to variability of the estimated daily intakes of EDs. In addition, it is important to mention that not all studies have considered exactly the same items and that could lead to differences in results. Despite this, estimated daily dietary exposure to DEHP and BPA in our study is comparable with other studies worldwide (Table 3)

Table 3. BPA and DEHP total dietary intake in adult populations found in the recent scientific literature.

	Year		Total dietary intake ($\mu g/kg_{bw}/day$)	Reference
BPA		*		
Belgium	2004	Mean	0.015	Geens et al.,2010
Europe	2006	Mean	1.5	EFSA, 2006
France	2014	Mean range (P50 range; P95 range)	0.038-0.040 (0.033-0.035; 0.077-0.0087)	Bemrah et al.,2014
New Zealand	2004	Mean (P50; P95)	0.008 (0.00; 0.041)	Thomson and Grounds, 2005
Norway	2014	Mean (P50; P95)	0.004 (0.003; 0.01)	Sakhi et al., 2014
Spain	2013	Mean (P95)	0.061 (0.099)	EFSA, 2013 [a]
Spain	2013	Mean (P95)	0.18 (0.33)	EFSA, 2013[b]
Taiwan	2015	Mean (P50;P95)	0.64 (0.27;2.29)	Chen et al., 2016
USA	2010	Mean	0.012	Lorber et al., 2015
Tarragona, Spain	2016	Mean (P5; P95)	0.72 (0.28; 1.41)	Present study
DEHP				
Belgium	2012	Mean	1.59	Sioen et al., 2012
Cambodia	2016	Mean	11.67	Cheng et al., 2013
China	2011-2012	Mean (P97.5)	2.03 (3.64)	Sui et al., 2014
France	2008	Mean	1.46	Martine et al., 2013
Germany	2005	Mean (P50;P95)	2.5 (2.4;4.0)	Fromme et al., 2007
Germany	2005-2006	Mean (P95)	14 (28.5)	Heinemeyer et al., 2013
Norway	2014	Mean	0.42	Sakhi et al., 2014
Switzerland	2009	Mean	1.90	Dickson-Spillmann et al.,2009
USA	2013	Mean	0.67	Schecter et al., 2013
Tarragona, Spain	2016	Mean (P5; P95)	1.00 (0.41; 2.01)	Present study

[a]Only foods specifically codified as canned in the dietary survey are assigned the corresponding occurrence level for BPA. [b]Any food category (at the lowest available level of food category classification) which has been codified as canned in at least one survey is always considered to be consumed as canned in all dietary surveys included in the Comprehensive Database.
* P5, P50, P95 and P97.5 are 5^{th}, 50^{th}, 95^{th} and 97.5^{th} percentile, respectively.

3.3 Internal dosimetry

The chemicals' dose inputs considered to run the PBPK, were probabilistically estimated by Monte-Carlo simulation (section 3.1). From probabilistic distribution, three total dietary intake reference scenarios were selected for BPA and DEHP: the 5th percentile, the mean and the 95th percentile. The outputs generated after running the model were selected considering the metabolites generated, their toxicity, gestational period and ability to reach the fetus. For this reason, only free BPA and MEHP (a metabolite of DEHP) were considered.

The simulation was performed for pregnant women and fetus for 24 hours during the 24th gestational week. This period was selected because at this time fetus organs are more developed and able to incorporate right biological process. This helps us to explain the difference in metabolic processes in mothers and fetuses. Normally, at the early stage of pregnancy, for both BPA and MEHP, fetus plasma concentration level is higher due to low or no metabolic activities in the fetus (Gauderat et al., 2016; Latini et al., 2003). In order to understand the elimination profile of the chemicals (BPA and MEHP) in the body, single dose simulation for all three exposure scenarios (5th percentile; mean; 95th percentile) was simulated. Time versus plasma concentration (for mothers and fetuses) of BPA and MEHP are shown in **Figure** 3 and 4, respectively. Due to the fast absorption properties of BPA and DEHP, simulated concentration curves show a sharp peak concentration observed within 1 hour of intake. Both, BPA and MEHP are fast elimination chemicals, with a half-life of fewer than 2 hours and complete elimination within 24 hours in adult (mother). The elimination of BPA and MEHP in the fetuses is slower than mothers as the fetal metabolic activity is lower comparing mother's metabolism. In general, it was observed that BPA and MEHP stay longer in the fetal body, which may cause higher risk to fetuses compare with the mothers even for lower exposure scenario (**Figure** 3 and 4). Similar results have been observed by Sharma et al., (2016 b, c, unpublished) for BPA and MEHP, respectively. In reality, the oral exposure has multiple intakes and in that case, the residence time of the chemical in the human body increases. However, absorption and elimination profile of chemical after three intakes have little or no effect. **Figure** 5 summarizes the levels concentration of BPA in

plasma in mothers and fetuses considering three oral intakes. To simulate three doses scenario, the single intake was divided into three with 8 hours of interval. The area under the curve for each day has increased significantly with higher residence time but lower peak compares to one oral dose scenario. In multiple dose scenarios, absorption peak concentration for each intake time and the half-life of elimination are similar to single dose scenario with 1 hour for the peak and less than 2 hours for the half-life. However, in multiple dose scenarios, as each intake is lower than single-dose intake, peak concentrations for the corresponding intake are lower. For example, the peak concentration of BPA (95^{th} percentile) for mothers and fetuses considering only one dose were 0.047 µg/L and 0.039 µg/L, respectively and considering multiple doses, were 0.015 µg/L (95^{th} percentile) and 0.018 µg/L (95^{th} percentile) for mothers and fetuses, respectively; this peaks concentrations were around 1/3 of the value for one dose. Although, in the case of fetus, the peak concentration was slightly more than 1/3 due to his low metabolic capacity. In the case of MEHP, the profile was the same as the BPA. For only one dose the plasma concentration peak was 11 µg/L (P95) in the mothers and 9 µg/L (P95) in the fetuses and considering three doses, it was obtained values that were the third part of the previous ones mentioned. It was observed that the concentration peaks of DEHP in plasma were higher compared with BPA. However, it should be noted that the probabilistic total dietary intake of DEHP obtained by Monte-Carlo was higher than the total dietary intake obtained for BPA.

Despite their short biological half-lives, exposure is prevalent and detectable in blood matrix at any time. Mothers are able to decrease much more the basal levels of these chemicals compared to the fetuses due to her metabolic activity. For that reason, fetuses are always subject to a risk of constant exposure. The results of the present study were not comparable with biomonitoring studies for multiple reasons. Firstly, in the present case study, only oral exposure was estimated whereas, in reality, both BPA and DEHP have multi-route exposure with significant contribution from coming from dermal exposure (Myridakis et al., 2016). Secondly, both BPA and DEHP show high variability in their internal dosimetry with no steady state concentration, which makes the timing of biomonitoring sampling very relevant. Which means, the concentration levels of the EDs obtained from plasma are

subject to different conditions such as the diet of each patient, the time of sampling (it will not be the same concentration if it is collected after longer period without any exposure or closer to peak hour of exposure) and the routes of exposure (oral vs dermal).

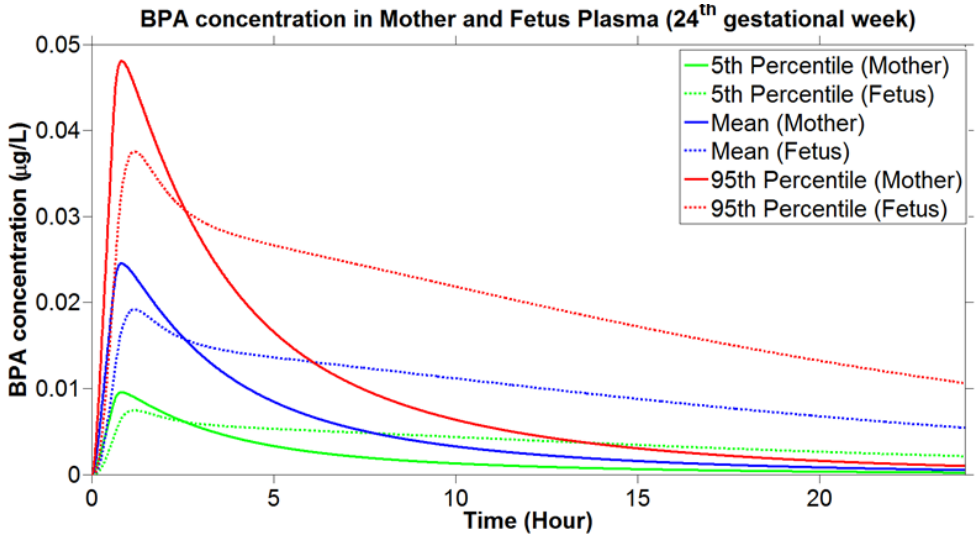


Figure 3. Time versus BPA plasma concentration for mothers and fetuses, considering different exposure scenarios (5th percentile; mean; 95th percentile) and only one food intake dose.

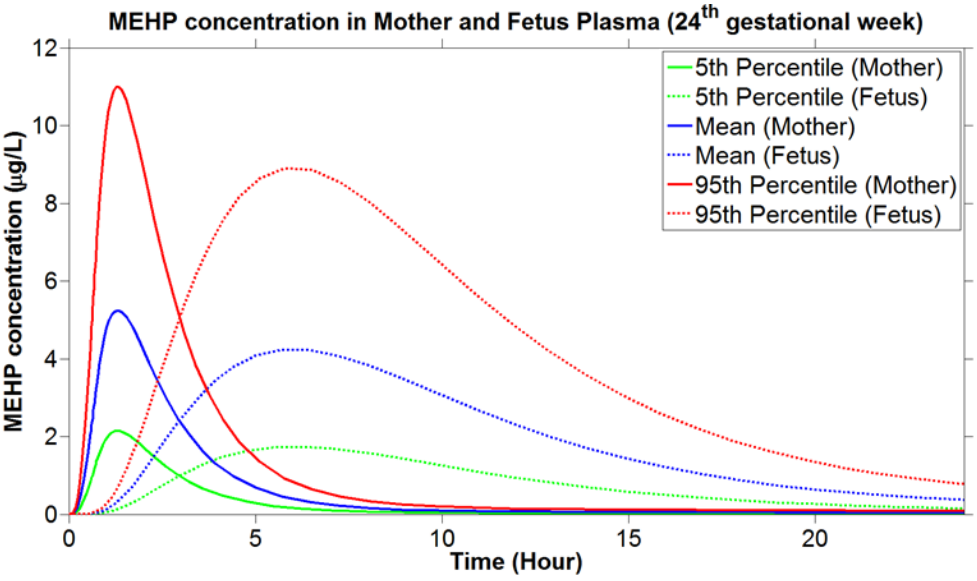


Figure 4. Time versus MEHP plasma concentration for mothers and fetuses, considering different exposure scenarios (5th percentile; mean; 95th percentile) and only one food intake dose.

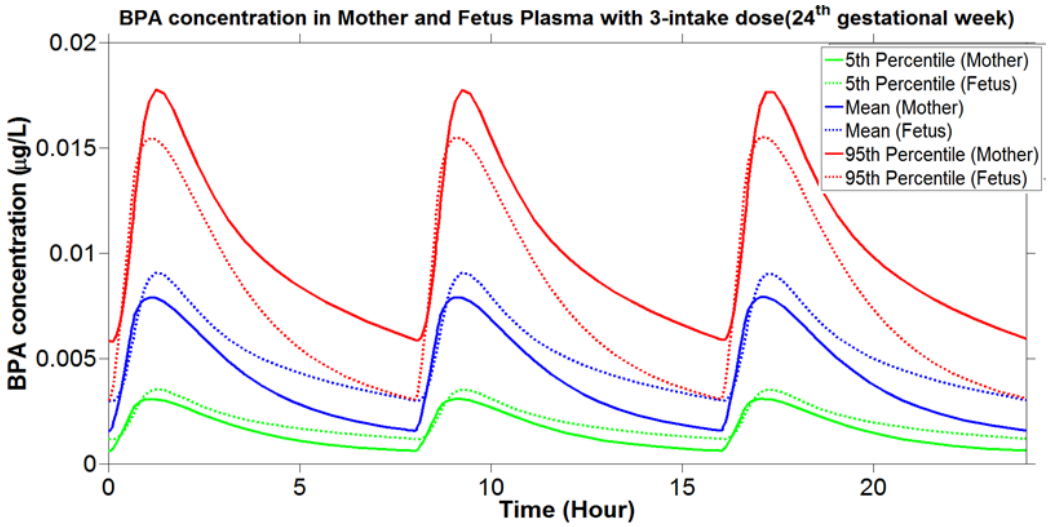


Figure 5. Time versus BPA plasma concentration for mothers and fetuses, considering different exposure scenarios (5th percentile; mean; 95th percentile) and three-food intake dose.

4. Conclusions

The aim of this study was to estimate the prenatal exposure to EDs (BPA and DEHP) through the dietary intake of pregnant women using the interview-based method, in order to improve the knowledge about the risks that they pose to prenatal health. To assess the early exposure, integrated external and internal dosimetry estimate was performed.

Canned fruits and vegetables followed by canned meat and meat products were the major contributors to the dietary exposure to BPA in pregnant women population in Tarragona (Spain). For DEHP, milk and dairy products followed by ready to eat food (included canned dinners) were the most important contributors to the estimated dietary exposure. In spite of dietary variation and resulting differences in exposure, the total dietary intake estimate for BPA and DEHP was considerably lower than their respective current TDI values established by EFSA (4 and 50 µg/kg$_{bw}$/day, respectively) (EFSA, 2015). Internal dosimetry simulations carried out in this study suggest that free BPA and MEHP plasma concentrations in women were characterized by transient peaks (associated with meals). In contrast, fetal

exposure was characterized by a low but sustained basal BPA and MEHP concentration due to a lack of metabolic activity in the fetus.

The ongoing research is to validate the PBPK model with biological samples from this cohort and demonstrate that this methodology allows the determination of BPA and MEHP for monitoring in plasma and urine biological matrices and the PBPK model can predict the prenatal exposure of the child/fetus to EDs.

Finally, the health implications of this fetal exposure to BPA and MEHP should be addressed because they are associated with infertility issues and reproductive development of the fetus. Therefore, a strategy to reduce their exposure is to regulate their production and restrict their use in articles specially meant for childcare and pregnant women.

Acknowledgement

We would like to thank all pregnant women who participate in this study. The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement No. 603946-2 (HEALS project). J. Rovira receives funds from Health Department of Catalonia Government, trough "Pla Estratègic de Recerca i Innovació en salut" (PERIS 2016-2020) fellowship.

References

Arbuckle, T.E., Davis, K., Boylan, K., Fisher, M., Fu, J., 2016. Processed data for CHMS 2007-2009: Bisphenol A, phthalates and lead and learning and behavioral problems in Canadian children 6-19 years of age. Data Br. 8, 784–802. doi:10.1016/j.dib.2016.06.017

Bemrah, N., Jean, J., Rivière, G., Sanaa, M., Leconte, S., Bachelot, M., Deceuninck, Y., Bizec, B. Le, Dauchy, X., Roudot, A.C., Camel, V., Grob, K., Feidt, C., Picard-Hagen, N., Badot, P.M., Foures, F., Leblanc, J.C., 2014. Assessment of dietary exposure to bisphenol A in the French population with a special focus on risk characterisation for pregnant French women. Food Chem. Toxicol. 72, 90–97. doi:10.1016/j.fct.2014.07.005

Casas, L., Fernández, M.F., Llop, S., Guxens, M., Ballester, F., Olea, N., Irurzun, M.B., Rodríguez, L.S.M., Riaño, I., Tardón, A., Vrijheid, M., Calafat, A.M., Sunyer, J., 2011. Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. Environ. Int. 37, 858–866. doi:10.1016/j.envint.2011.02.012

Chen, W.Y., Shen, Y.P., Chen, S.C., 2016. Assessing bisphenol A (BPA) exposure risk from long-term dietary intakes in Taiwan. Sci. Total Environ. 543, 140–146. doi:10.1016/j.scitotenv.2015.11.029

Cheng, Z., Wang, H.-S., Du, J., Sthiannopkao, S., Xing, G.-H., Kim, K.-W., Yasin, M.S.M., Hashim, J.H., Wong, M.-H., 2013. Dietary exposure and risk assessment of mercury via total diet study in Cambodia. Environ. Res. 92, 143–9. doi:10.1016/j.chemosphere.2013.02.025

Dapcich, V., Salvador, G., Ribas, L., Pérez, C., Aranceta, J., 2004. Consejos para una alimentación saludable de la Sociedad Española de Nutrición Comunitaria (SENC). Ed Spanish Soc. Community Nutr. 105.

Das, M.T., Ghosh, P., Thakur, I.S., 2014. Intake estimates of phthalate esters for South Delhi population based on exposure media assessment. Environ. Pollut. 189, 118–125. doi:10.1016/j.envpol.2014.02.021

Davis, B.J., Maronpot, R.R., Heindel, J.J., 1994. Di (2-ethylhexyl) phthalate suppresses estradiol and ovulation in cycling rats. Toxicol. Appl. Pharmacol. 128, 216–23. doi:10.1006/taap.1994.1200

De Cock, M., de Boer, M.R., Lamoree, M., Legler, J., van de Bor, M., 2014. First year growth in relation to prenatal exposure to endocrine disruptors - A dutch prospective cohort study. Int. J. Environ. Res. Public Health 11, 7001–7021. doi:10.3390/ijerph110707001

De Coensel, N., David, F., Sandra, P., 2009. Study on the migration of bisphenol-A from baby bottles by stir bar sorptive extractionthermal desorption-capillary GC-MS. J. Sep. Sci. 32, 3829–3836. doi:10.1002/jssc.200900349

Dickson-Spillmann, M., Siegrist, M., Keller, C., Wormuth, M., 2009. Phthalate exposure through food and consumers' risk perception of chemicals in food. Risk Anal. 29, 1170–1181. doi:10.1111/j.1539-6924.2009.01233.x

ECHA, 2010. Review of new available information for Bis (2-Ethylhexyl) Phthalate (DEHP). Evaluation of new scientific evidence concerning the restrictions contained in Annex XVII to Regulation (Ec) No 1907/2006 (Reach). ECHA 2006, 1–24.

EFSA, 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: Executive summary. EFSA J. 13, 1–621. doi:doi:10.2903/j.efsa.2015.3978

EFSA, 2013. Draft Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs– Part: exposure assessment. EFSA J. 314. doi:No EFSA-Q-2012-00423

EFSA, 2006. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to Maltitol Syrup E 965 (ii) new production process. Change of specification Question number E. EFSA J. 428, 75. doi:http://www.efsa.europa.eu/en/science/afc/afc_opinions.html

European-Parliament, 2011. Comission Directive 2011/8/EU. Off. J. Eur. Communities 29–32.

Fàbrega, F., Kumar, V., Schuhmacher, M., Domingo, J.L., Nadal, M., 2014. PBPK modeling for PFOS and PFOA: Validation with human experimental data. Toxicology Letters 230(2), 224-251.

Fàbrega, F., Kumar, V., Schuhmacher, M., Domingo, J.L., Nadal, M., 2015. Physiologically based pharmacokinetic (PBPK) modeling of perfluoroalkyl substances (PFASs) in the human body, Archives of Environmental Contamination and Toxicology 97(6), 814-827.

Fàbrega, F., Nadal, M., Schuhmacher, M., Domingo, J.L., Kumar, V., 2016. Influence of the uncertainty in the validation of PBPK models: A case-study for PFOS and PFOA. Regul. Toxicol. Pharmacol. 77, 230–239. doi:10.1016/j.yrtph.2016.03.009

FAO/WHO, 2010. Joint FAO / WHO Expert meeting to review toxicological and health aspects of Bisphenol A Canada , October 2010 Call for data. WHO 1–2.

Fierens, T., Van Holderbeke, M., Willems, H., De Henauw, S., Sioen, I., 2013. Transfer of eight phthalates through the milk chain — A case study. Environ. Int. 51, 1–7. doi:10.1016/j.envint.2012.10.002

Fromme, H., Gruber, L., Schlummer, M., Wolz, G., Böhmer, S., Angerer, J., Mayer, R., Liebl, B., Bolte, G., 2007. Intake of phthalates and di (2-ethylhexyl) adipate: Results of the Integrated Exposure Assessment Survey based on duplicate diet samples and biomonitoring data. Environ. Int. 33, 1012–1020. doi:10.1016/j.envint.2007.05.006

Gauderat, G., Picard-Hagen, N., Toutain, P.L., Corbel, T., Viguié, C., Puel, S., Lacroix, M.Z., Mindeguia, P., Bousquet-Melou, A., Gayrard, V., 2016. Bisphenol A glucuronide deconjugation is a determining factor of fetal exposure to bisphenol A. Environ. Int. 86, 52–59. doi:10.1016/j.envint.2015.10.006

Geens, T., Aerts, D., Berthot, C., Bourguignon, J.P., Goeyens, L., Lecomte, P., Maghuin-Rogister, G., Pironnet, A.M., Pussemier, L., Scippo, M.L., Van Loco, J., Covaci, A., 2012. A review of dietary and non-dietary exposure to bisphenol-A. Food Chem. Toxicol. doi:10.1016/j.fct.2012.07.059

Geens, T., Apelbaum, T.Z., Goeyens, L., Neels, H., Covaci, A., 2010. Intake of bisphenol A from canned

beverages and foods on the Belgian market. Food Addit. Contam. Part A. Chem. Anal. Control. Expo. Risk Assess. 27, 1627–37. doi:10.1080/19440049.2010.508183

Genuis, S.J., Beesoon, S., Lobo, R. a., Birkholz, D., 2012. Human Elimination of Phthalate Compounds: Blood, Urine, and Sweat (BUS) Study. Sci. World J. 2012, 1–10. doi:10.1100/2012/615068

Giulivo, M., Lopez, M., Alda, D., Capri, E., Barceló, D., 2016. Human exposure to endocrine disrupting compounds : Their role in reproductive systems , metabolic syndrome and breast cancer . A review. Environ. Res. 151, 251–264. doi:10.1016/j.envres.2016.07.011

Gobas, F., Otton, V., Tupper-Ring, L., Crawford, M., Clark, K., Ikonomou, M., 2016. Chemical activity-based environmental risk analysis of the plasticizer p di-ethylhexyl phthalate and its main metabolite mono-ethylhexyl phthalate. Environ. Toxicol. Chem. 31. doi:10.1002/etc.3689

Gramec Skledar, D., Peterlin Mašič, L., 2016. Bisphenol A and its analogs: Do their metabolites have endocrine activity? Environ. Toxicol. Pharmacol. 47, 182–199. doi:10.1016/j.etap.2016.09.014

Hanioka, N., Naito, T., Narimatsu, S., 2008. Human UDP-glucuronosyltransferase isoforms involved in bisphenol A glucuronidation. Chemosphere 74, 33–36. doi:10.1016/j.chemosphere.2008.09.053

Hanson, M.A., Gluckman, P.D., 2011. Developmental origins of health and disease: Moving from biological concepts to interventions and policy. Int. J. Gynecol. Obstet. 115, S3–S5. doi:10.1016/S0020-7292(11)60003-9

Hartle, J.C., Navas-Acien, A., Lawrence, R.S., 2016. The consumption of canned food and beverages and urinary Bisphenol A concentrations in NHANES 2003-2008. Environ. Res. 150, 375–382. doi:10.1016/j.envres.2016.06.008

Heinemeyer, G., Sommerfeld, C., Springer, A., Heiland, A., Lindtner, O., Greiner, M., Heuer, T., Krems, C., Conrad, A., 2013. Estimation of dietary intake of bis(2-ethylhexyl)phthalate (DEHP) by consumption of food in the German population. Int. J. Hyg. Environ. Health 216, 472–480. doi:10.1016/j.ijheh.2013.01.001

Hughes, I.A., Martin, H., Jaaskelainen, J., 2006. Genetic mechanisms of fetal male undermasculinization : A background to the role of endocrine disruptors. Int. J. Environ. Res. Public Heal. 100, 44–49. doi:10.1016/j.envres.2005.07.001

Kim, Y.H., Kim, C.S., Park, S., Han, S.Y., Pyo, M.Y., Yang, M., 2003. Gender differences in the levels of bisphenol A metabolites in urine. Biochem. Biophys. Res. Commun. 312, 441–448. doi:10.1016/j.bbrc.2003.10.135

Lakind, J.S., Naiman, D.Q., 2010. Daily intake of bisphenol A and potential sources of exposure: 2005-

2006 National Health and Nutrition Examination Survey. J. Expo. Sci. Environ. Epidemiol. 21, 272–9. doi:10.1038/jes.2010.9

Latini, G., 2005. Monitoring phthalate exposure in humans. Clin. Chim. Acta 361, 20–29. doi:10.1016/j.cccn.2005.05.003

Latini, G., De Felice, C., Presta, G., Del Vecchio, A., Paris, I., Ruggieri, F., Mazzeo, P., 2003. Exposure to Di(2-ethylhexyl)phthalate in humans during pregnancy: A preliminary report. Biol. Neonate 83, 22–24. doi:10.1159/000067012

Lorber, M., Schecter, A., Paepke, O., Shropshire, W., Christensen, K., Birnbaum, L., 2015. Exposure assessment of adult intake of bisphenol A (BPA) with emphasis on canned food dietary exposures. Environ. Int. 77, 55–62. doi:10.1016/j.envint.2015.01.008

Lovekamp-Swan, T., Davis, B.J., 2003. Mechanisms of phthalate ester toxicity in the female reproductive system. Environ. Health Perspect. 111, 139–145. doi:10.1289/ehp.5658

Maffini, M. V., Rubin, B.S., Sonnenschein, C., Soto, A.M., 2006. Endocrine disruptors and reproductive health: The case of bisphenol-A. Mol. Cell. Endocrinol. 254–255, 179–186. doi:10.1016/j.mce.2006.04.033

Mallozzi, M., Bordi, G., Garo, C., Caserta, D., 2016. Review The Effect of Maternal Exposure to Endocrine Disrupting Chemicals on Fetal and Neonatal Development: A Review on the Major Concerns. Wiley Period. Inc. 19. doi:10.1002/bdrc.21137

Mari, M., Nadal, M., Schuhmacher, M., Domingo, J.L., 2009. Exposure to heavy metals and PCDD/Fs by the population living in the vicinity of a hazardous waste landfill in Catalonia, Spain: Health risk assessment. Environ. Int. 35, 1034–1039. doi:10.1016/j.envint.2009.05.004

Martine, B., Marie-Jeanne, T., Cendrine, D., Fabrice, A., Marc, C., 2013. Assessment of adult human exposure to phthalate esters in the urban centre of paris (France). Bull. Environ. Contam. Toxicol. 90, 91–96. doi:10.1007/s00128-012-0859-5

Matsui, S., 2008. Endocrine Disruptors. Encycl. Toxicol. 1259–1260. doi:http://dx.doi.org/10.1016/B978-008045405-4.00402-X

May, L.M., Gaborek, B., Pitrat, T., Peters, L., 2002. Derivation of risk based wipe surface screening levels for industrial scenarios. Sci. Total Environ. 288, 65–80. doi:10.1016/S0048-9697(01)01117-2

Meeker, J., 2012. Exposure to environmental endocrine disruptors and child development. Arch. Pediatr. Adolesc.166, E1–E7. doi:10.1001/archpediatrics.2012.241.Exposure

Myridakis, A., Chalkiadaki, G., Fotou, M., Kogevinas, M., Chatzi, L., Stephanou, E.G., 2016. Exposure

of Preschool-Age Greek Children (RHEA Cohort) to Bisphenol A, Parabens, Phthalates, and Organophosphates. Environ. Sci. Technol. 50, 932–941. doi:10.1021/acs.est.5b03736

Page, B.D., Lacroix, G.M., 1989. The occurrence of phthalate ester and di-2-ethylhexyl adipate plasticizers in Canadian packaging and food sampled in 1985-1989: a survey. Food Addit. Contam. 12, 129–151. doi:10.1080/02652039509374287

Rezg, R., El-Fazaa, S., Gharbi, N., Mornagui, B., 2014. Bisphenol A and human chronic diseases: Current evidences, possible mechanisms, and future perspectives. Environ. Int. 64, 83–90. doi:10.1016/j.envint.2013.12.007

Rochester, J.R., 2013. Bisphenol A and human health: A review of the literature. Reprod. Toxicol. 42, 132–155. doi:10.1016/j.reprotox.2013.08.008

Rovira, J., Roig, N., Nadal, M., Schuhmacher, M., Domingo, J.L., 2016. Human health risks of formaldehyde indoor levels: An issue of concern. J. Environ. Sci. Heal. Part a-Toxic/Hazardous Subst. Environ. Eng. 51, 357–363. doi:10.1080/10934529.2015.1109411

Sakhi, A.K., Lillegaard, I.T.L., Voorspoels, S., Carlsen, M.H., Løken, E.B., Brantsæter, A.L., Haugen, M., Meltzer, H.M., Thomsen, C., 2014. Concentrations of phthalates and bisphenol A in Norwegian foods and beverages and estimated dietary exposure in adults. Environ. Int. 73, 259–269. doi:10.1016/j.envint.2014.08.005

Santana, J., Giraudi, C., Marengo, E., Robotti, E., Pires, S., Nunes, I., Gaspar, E.M., 2014. Preliminary toxicological assessment of phthalate esters from drinking water consumed in Portugal. Environ. Sci. Pollut. Res. 21, 1380–1390. doi:10.1007/s11356-013-2020-3

Schecter, A., Lorber, M., Guo, Y., Wu, Q., Yun, S.H., Kannan, K., Hommel, M., Imran, N., Hynan, L.S., Cheng, D., Colacino, J.A., Birnbaum, L.S., 2013. Phthalate concentrations and dietary exposure from food purchased in New York state. Environ. Health Perspect. 121, 473–479. doi:10.1289/ehp.1206367Schuhmacher, M., Fàbrega, F., Kumar, V., García, F., Nadal, M., Domingo, J.L., 2014. A PBPK model to estimate PCDD/F levels in adipose tissue: Comparison with experimental values of residents near a hazardous waste incinerator. Environ. Int. 73, 150–157. doi:10.1016/j.envint.2014.07.020

Schuhmacher, M., Meneses, M., Xifr, A., Domingo, J.L., 2001. The use of Monte-Carlo simulation techniques for risk assessment: study of a municipal waste incinerator. Chemosphere 43, 787–799.

Serra Majem, L., 2011. Objetivos nutricionales para la población española: consenso de la Sociedad Española de Nutrición Comunitaria 2011. Rev. española Nutr. comunitaria (Spanish J.

community Nutr). 17, 178–199.

Shade, W.D., Jayjock, M. a, 1997. Monte Carlo uncertainty analysis of a diffusion model for the assessment of halogen gas exposure during dosing of brominators. Am. Ind. Hyg. Assoc. J. 58, 418–24. doi:10.1080/15428119791012658

Sharma, R.P., Schuhmacher, M., Kumar, V., 2016. Review on crosstalk and common mechanisms of endocrine disruptors: Scaffolding to improve PBPK/PD model of EDC mixture. Environ. Int. 14. doi:10.1016/j.envint.2016.09.016

Sharman, M., Read, W. a, Castle, L., Gilbert, J., 1994. Levels of di (2-ethylhexyl) phthalate and total phthalate esters in milk, cream, butter and cheese. Food Addit. Contam. 11, 375–385. doi:10.1080/02652039409374236

Sioen, I., Fierens, T., Van Holderbeke, M., Geerts, L., Bellemans, M., De Maeyer, M., Servaes, K., Vanermen, G., Boon, P.E., De Henauw, S., 2012. Phthalates dietary exposure and food sources for Belgian preschool children and adults. Environ. Int. 48, 102–108. doi:10.1016/j.envint.2012.07.004

Sui, H.-X., Zhang, L., Wu, P.-G., Song, Y., Yong, L., Yang, D.-J., Jiang, D.-G., Liu, Z.-P., 2014. Concentration of di(2-ethylhexyl) phthalate (DEHP) in foods and its dietary exposure in China. Int. J. Hyg. Environ. Health 217, 695–701. doi:10.1016/j.ijheh.2014.02.006

Sungur, Ş., Koroğlu, M., Özkan, A., 2014. Determination of bisphenol a migrating from canned food and beverages in markets. Food Chem. 142, 87–91. doi:10.1016/j.foodchem.2013.07.034

Tewar, S., Auinger, P., Braun, J.M., Lanphear, B., Yolton, K., Epstein, J.N., Ehrlich, S., Froehlich, T.E., 2016. Association of Bisphenol A exposure and Attention-De fi cit / Hyperactivity Disorder in a national sample of U.S. children. Environ. Res. 150, 112–118. doi:10.1016/j.envres.2016.05.040

Thomson, B.M., Grounds, P.R., 2005. Bisphenol A in canned foods in New Zealand: an exposure assessment. Food Addit. Contam. 22, 65–72. doi:10.1080/02652030400027920

Wang, Y., You, L., Zeng, Q., Sun, Y., Huang, Y., Wang, C., 2015. Phthalate exposure and human semen quality : Results from an infertility clinic in China. Environ. Res. 142, 1–9. doi:10.1016/j.envres.2015.06.010

Welshons, W. V., Nagel, S.C., Vom Saal, F.S., 2006. Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. Endocrinology 147, 56–69. doi:10.1210/en.2005-1159

WHO, 2013. Guideline : Calcium supplementation in pregnant women 1–35.

WHO, 2012. Endocrine disrupters and child health. Possible developmental early effects of endocrine

disrupters on child health. World Health Organization, Geneva.

Wormuth, M., Scheringer, M., Vollenweider, M., Hungerbühler, K., 2006. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? Risk Anal. 26, 803–824. doi:10.1111/j.1539-6924.2006.00770.x

Zota, A., Phillips, C., Mitro, S., 2016. Recent fast food consumption and Bisphenol A and Pthalates exposure among the U.S. population in NHANES, 2003-2010. Environ. Health Perspect. 124, 1521–1528. doi:http://dx.doi.org/10.1289/ehp.1510803

CHAPTER 1 EXPOSURE ASSESSMENT

PUBLICATION 1.

M.A. Martínez; J. Rovira; R. Prasad Sharma; M. Nadal; M. Schuhmacher; V. Kumar. **Prenatal exposure estimation of BPA and DEHP using integrated external and internal dosimetry: A case study** (2017). *Environmental Research.* 158 (2017) 566 – 575.

SUPPLEMENTARY MATERIAL

Supplementary material

Standard ordinary differential equations used in tissue dosimetry model for the development of P-PBPK model for DEHP and BPA

$$\frac{d}{dt}(stomach) = input1 - K * Astomach - GE * Astomach$$

- $\frac{d}{dt}(stomach)$ is the rate of change of chemical amount in Stomach (nmol)
- $input1$is the oral dose exposure (nmol/day) ;3 equal divided dose per day
- K is the absorption rate constant in the stomach (1/hr)
- GE is the gastric emptying time

$$\frac{d}{dt}(gut) = GE * Astomach - Vmaxgut_{glu} * Cgut * fu/(Cgut * fu + Kmgut\_glu) - K1 * AGut$$

- $Vmaxgut\_glu$ is the scaled maximum rate of glucuronidation in gut (nM/hr)
- $cgut$ is the concentration of chemical in gut
- fu is the plasma fractional unbound
- $Kmgut\_glu$ is the concentration in nmol/liter to produce half maximum reaction
- $K1$ is uptake rate of chemical from oral to liver

$$\frac{d}{dt}(Liver) = Qliver * \left(Cplasma * fu - Cliver * \left(\frac{fu}{K_{liver_{plasma}}} \right) \right) + K1 * AGut - Vmaxliver_{glu} * Cliver * \frac{fu}{Cliver * fu + Kmliver_{glu}} - Vmaxliver\_sulf * Cliver * fu/(Cliver * fu + Kmliver\_sulf)$$

- $Qliver$ is the cardiac blood flow to liver
- $Cplasma$ is the plasma concentration of chemical
- $Cliver$ is the concentration of chemical in liver
- $K_{liver_{plasma}}$ is the liver plasma partition coefficient

- $Vmaxliver_{glu}$ is the maximal glucuronidation rate of chemical in liver
- $Kmliver_{glu}$ is the concentration at which half maximal reaction occurs for glucuronidation in liver
- $Vmaxliver\_sulf$ is the maximal sulfation rate of chemical in liver
- $Kmliver\_sulf$ is the concentration at which half maximal reaction sulfation occurs in liver

$$\frac{d}{dt}(Brain) = Qbrain * \left(Cplasma * fu - \ Cbrain * \left(\frac{fu}{K_{brain_{plasma}}} \right) \right)$$

- $Qbrain$ is the blood flow to brain
- $K_{brain_{plasma}}$ brain plasma partition coefficient

$$\frac{d}{dt}(Kidney) = QKidney * \left(Cplasma * fu - \ Ckidney * \left(\frac{fu}{K_{kidney_{plasma}}} \right) \right) - Kurine * Ckidney$$

- $Qkidney$ blood flow to kidney
- $K_{kidney_{plasma}}$ kidney plasma partition coefficient
- $Kurine$ is the excretion rate of chemical to urine

$$\frac{d}{dt}(fat) = Qfat * \left(Cplasma * fu - \ Cfat * \left(\frac{fu}{K_{fat_{plasma}}} \right) \right)$$

- $Qfat$ blood flow to the fat
- $K_{fat_{plasma}}$ fat plasma partition coefficient

$$\frac{d}{dt} skin = Qskin * (Cplasma * fu - Cskin * \frac{fu}{K\_skin\_plasma})$$

- $QSkin$ blood flow to the skin
- K_skin_plasma is the skin plasma partition coefficient

- Cskin is the concentration of BPA in skin

$$\frac{d}{dt}(gonads) = Qgonads * \left(Cplasma * fu - \ Cgonads * \left(\frac{fu}{K_{gonads_{plasma}}} \right) \right)$$

- $Qgonads$ is the gonads blood flow
- $K_{fat_{plasma}}$ brain plasma partition coefficient

$$\frac{d}{dt}(restbody) = Qrestbody * \left(Cplasma * fu - \ Crestbody * \left(\frac{fu}{K_{restbody_{plasma}}} \right) \right)$$

- $Qrestbody$ is the blood flow to rest of the body
- $K_{restbody_{plasma}}$ restbody plasma partition coefficient

$$\frac{d}{dt}(placenta) = (Qplacenta \ * (Cplasma * fu \ - \ Cplacenta * (fu/K\_placenta\_plasma))) \ - \ K\_t1 * Cplacenta * (fu/K\_placenta\_plasma) \ + \ K\_t2 * Cplasma\_fetus * fu \ - \ Vmaxplacenta\_glu * Cplacenta * fu/(Cplacenta * fu + Kmplacenta\_glu)$$

- $Qplacenta$ is the blood flow to placenta
- $Cplacenta$ is the concentration of chemical in placenta
- $K\_placenta\_plasma$ placenta plasma partition coefficient
- K_t1 is the transfer rate of chemical to the fetus from placenta
- K_t2 is the transfer of chemical to placenta from fetus
- $Vmaxplacenta\_glu$ is the glucuronidation of chemical in the placenta (similar with liver scaled assuming 10 percent of microsomal protein (MSP) in comparison to liver MSP)
- $Kmplacenta\_glu$ is the concentration of chemical producing half maximal reaction

$$\frac{d}{dt}(plasma) = Qfat * Cfat * (fu/K\_fat\_plasma) + Qliver * Cliver * (fu/ K\_liver\_plasma) + (Qbrain * Cbrain * (fu/K\_brain\_plasma)) + \left(Qkidney * Ckidney * \left(\frac{fu}{K_{kidney_{plasma}}}\right)\right) + \left(Qrestbody * Crestbody * \left(\frac{fu}{K_{restbody_{plasma}}}\right)\right) - (QCplasma * Cplasma * fu) + (QSkin * Cskin * (fu/K\_skin\_plasma)) + \left(Qgonads * Cgonads * \left(\frac{fu}{K_{gonads_{plasma}}}\right)\right) + Qplacenta * Cplacenta * (fu/K\_placenta\_plasma)$$

- $Qplasma$ is the blood flow to plasma

%%

Fetus model equation

%%

$$\frac{d}{dt} liver\_fetus = Qliver\_fetus * \left(Cplasma_{fetus} * fu - Cliver_{fetus} * \left(\frac{fu}{K_{liver_{fetus_{plasma}}}}\right)\right) - Vmaxliver\_glu\_fetus * \frac{Cliver_{fetus}}{Cliver_{fetus} + Kmliver_{glu}}$$

- $Qliver\_fetus$ is the blood flow to the fetal liver
- $Cplasma_{fetus}$ is the chemical fetus plasma concentration
- $Cliver_fetus$is the liver chemical concentration
- $K_{liverfetus}: plasma$ is the fetus liver plasma concentration

- $Vmaxliver\_glu\_fetus$ is the scaled maximum rate of reaction for chemical metabolism from in-vitro data considering fetal liver volume and fetus liver microsomal protein content
- $Kmliver$_glu is the concentration of substrate (chemical) producing half maximal reaction

$$\frac{d}{dt} fetus\_brain = Qbrain\_fetus * (Cplasma\_fetus * fu - Cbrain\_fetus * \left(\frac{fu}{K_{brain_{fetus_{plasma}}}}\right)$$

- Qbrain_fetus is the blood flow to the fetal brain
- Cbrain_fetusis the brain chemical concentration

$$\frac{d}{dt} restbody\_fetus = Qrestbody\_fetus * (Cplasma\_fetus * fu - Crestbody\_fetus * \left(\frac{fu}{K_{restbody\_fetus\_plasma}}\right)$$

- Qrestbody_fetus is the blood flow to the rest of the body
- $Crestbody\_fetus$ is the concentration in rest of the body of fetus

$$\frac{d}{dt} amnioticfluid = K\_t3 * Cliver\_fetus * fu - Camnioticfluid * K\_t4$$

- K_t3 is chemical transfer rate from fetus to amniotic fluid
- k_t4 is the chemical transfer rate from amniotic fluid to fetus
- Camnioticfluidis the concentration of BPA in amniotic fluid

$$\frac{d}{dt}plasma\_fetus = (Qliver\_fetus * (Cliver\_fetus * (fu/K\_liver\_fetus\_plasma))) + (Qbrain\_fetus * (Cbrain\_fetus * (fu/K\_brain\_fetus\_plasma))) + Qrestbody\_fetus * (Crestbody\_fetus * (fu/K\_restbody\_fetus\_plasma)) - (QCplasma\_fetus * Cplasma\_fetus * fu) + (K\_t1 * Cplacenta * (fu/K\_placenta\_plasma)) - K\_t2 * Cplasma\_fetus * fu + Kde * Cplasma\_fetusBPAG$$

Equations for scaling physiological parameter of fetus

1. $\mathrm{V\_fetus} = 3.779 * \left(e^{-16.08*e^{-5.67*e^{-4}*GD*24}}\right) + \left(e^{-140.78*e^{-7.01*e^{-4}*24*GD}}\right)$
   - V_fetus = fetal volume
   - GD= Gestational day (T/24)
2. $\mathrm{V\_Aminiotic\ fluid} = 0 + 1.9648 * GA - 1.2056 * GA^2 + 0.2064 * GA^3 - 0.0061 * GA^4 + 0.00005 * GA^5$
3. $\mathrm{Vbldfet} = F_{vldfet} * \mathrm{Vfet}$
   - Vbldfet = fetal blood volume in L
   - F_{vldfet} = fetal blood volume as a fraction of body weight, L/kg = 0.085
   - Vfet = fetal body weight in kg
4. $\mathrm{Vliverfet} = F_{liverfet} * \mathrm{Vfet}$
   - Vliverfet = fetal liver volume in L
   - $F_{liverfet}$ = fetal liver volume as a fraction of body weight = 0.04 (ICRP, 2002)
   - Vfet = fetal body weight in kg
5. $\mathrm{Vkidneyfet} = F_{kidneyfet} * \mathrm{Vfet}$
   - Vkidneyfet = fetal kidney volume in L
   - $F_{kidneyfet}$ = fetal kidney volume as a fraction of body weight = 0.0072
   - Vfet = fetal body weight in kg

6. $\mathrm{Vbrainfet} = \mathrm{F_{brainfet}} * \mathrm{Vfet}$
   - Vbrainfet = fetal brain volume in L
   - $\mathrm{F_{brainfet}}$ = fetal brain volume as a fraction of body weight = 0.11
   - Vfet = fetal body weight
7. $\mathrm{Qfet} = \mathrm{F_{Qfet}} * \mathrm{Vbldfet}$
   - Qfet = fetal cardiac output L/h
   - $\mathrm{F_{Qfet}}$ = fetal cardiac output as fraction of blood weight in kg (L/h/Kg) = 54
   - Vbldfet = fetal blood volume in kg
8. $\mathrm{QLiv\ fet} = \mathrm{F_{Qlive_m}} * \mathrm{Qfet}$
   - QLiv fet = fetal liver flood flow in L/h
   - $\mathrm{F_{Qlive_m}}$ = maternal liver blood flow as fraction of cardiac output
   - Qfet = fetal cardiac output in L/h
9. $\mathrm{Qkidney\ fet} = \mathrm{F_{Qkidney_m}} * \mathrm{Qfet}$
   - Qkidney fet = fetal kidney flood flow in L/h
   - $\mathrm{F_{Qkidney_m}}$ = maternal kidney blood flow as fraction of cardia output
   - Qfet = fetal cardiac output in L/h
10. $\mathrm{Qbrain\ fet} = \mathrm{F_{Qbrain_m}} * \mathrm{Qfet}$
    - Qbrain fet = fetal brain blood flow in L/h
    - $\mathrm{F_{Qbrain_m}}$ = maternal brain blood flow as fraction of cardiac output
    - Qfet = fetal cardiac output

Table I-1: General Physiology parameters for PBPK model

Paramter	Symbol	Value (L/h/kg)	References
Cardiac blood output	QCC	20	(Brown et al., 1997) (Davies and Morris, 1993)
Fractional liver blood flow	FQliver	0.25	(Brown et al., 1997)
Fractional brain blood flow	FQbrain	0.117	(Brown et al., 1997)
Fractional kidney blood flow	FQkidney	0.177	(Brown et al., 1997)
Fractional fat blood flow	FQfat	0.052	(Brown et al., 1997)
Fractional skin blood flow	FQskin	0.058	(Brown et al., 1997)
Fractional gonads blood flow	FQgonads	0.0002	(Brown et al., 1997)
Constant Fraction of organs volume to body weight			
Fractional liver volume	Fliver	0.026	(Brown et al., 1997)
Fractional brain volume	Fbrain	0.021	(Brown et al., 1997)
Fractional kidney volume	Fkidney	0.004	(Brown et al., 1997)
Fractional fat volume	Ffat	0.187	(Brown et al., 1997)
Fractional gonads volume	Fgonads	0.0027	(Brown et al., 1997)
Fractional skin volume	Fskin	0.0371	(Brown et al., 1997)
Fractional plasma volume	Fplasma	0.0428	(Davies and Morris, 1993)
Fractional gut volume	Fgut	0.016	(Brown et al., 1997)
Haematocrit	HCT	0.45	(Davies and Morris, 1993)
fetal cardiac output as fraction of blood weight in kg (L/h/Kg)	FQblood_fetus	54	(Clewell et al., 1999)
Fetal haematocrit	HCT_fetus	0.5	(Sisson et al., 1959)
Fractional liver volume of fetus	Fliver_fetus	0.04*	(ICRP, 2002)

*Fractional organ weight for the fetus was estimated from ICRP (2002) data. Blood flow is scaled by multiplying fractional blood flow to tissue in a mother with the volume of fetus tissue.

Table I-2 Pharmacokinetic parameters of BPA used for the P-PBPK

Parameters	Symbol/Unit	Mean value	References
Gastric emptying time	GE (L/h/kg$^{-0.25}$)	3.5	(Kortejärvi et al., 2007)
Oral absorption rate	K_oral (L/h/kg$^{-0.25}$)	9	Optimize
BPAG uptake to the liver	KGlin_BPAG (L/h/kg$^{-0.25}$)	50	(Fisher et al., 2011)
Fraction of BPAS transferred to plasma from liver	Fbpasliver	1	(Fisher et al., 2011)
Enterohepatic recirculation of BPAG	Kentero_BPAG (L/h/kg$^{-0.25}$)	0.2	(Fisher et al. 2011)
BPA urinary excretion rate	Kurine_BPA (L/h/kg$^{0.75}$)	0.1	Optimize
BPAG urinary excretion rate	Kurine_bpag (L/h/kg$^{0.75}$)	0.40	Optimize
BPAS urinary excretion rate	Kurine_bpas (L/h/kg$^{0.75}$)	0.025	Optimize
BPAG fraction volume of distribution	Vdbpag [a] (L)	0.0435	set equal to plasm fraction volume
BPAS fraction volume of distribution	Vdbpas[a] (L)	0.0435	set equal to plasm fraction volume
fractional constant placental transfer from mother to fetus	FK_t1 [b]	5.2e-05	(Kawamoto et al., 2007)
fractional constant for placental transfer from fetus to mother	FK_t2[b]	2.0e-05	(Kawamoto et al., 2007)
fractional constant for chemical transfer from fetus to amniotic fluid	FK_t3[b]	0.008	Visually fit against (Ikezuki et al., 2002) data
fractional constant for chemical from amniotic fluid to fetus	FK_t4[b]	0.001	Visually fit against (Ikezuki et al., 2002) data
fractional constant for placental transfer from mother to fetus	FK_t1_BPAG[b]	3.3e-6	(Kawamoto et al., 2007)

Parameters	Symbol/Unit	Mean value	References
fractional constant for placental transfer from fetus to mother	FK_t2_BPAG[b]	6.6e-14	(Kawamoto et al., 2007)
Glucuronidation of BPA in liver [c]			
Vmax (nmol/min/mg of protein)	Km (µmole)	Vmax (nmol/hr/kg $BW^{.75}$)	(Coughlin et al., 2012)
4.71	45.8	680,095.6	(Coughlin et al., 2012)
Glucuronidation of BPA in gut [c]			
1.4	58	22750	(Trdan Lusin et al., 2012)
Sulfation of BPA in liver [c]			
149 nmol/h/g liver	10.1	11657	(Kurebayashi et al., 2010)
Partition coefficient			
Liver/blood PC	k_liver_plasma	0.73	(Doerge et al., 2011)
Brain/blood PC	k_brain_plasma	2.8	(Doerge et al., 2011)
Kidney/blood PC	k_kidney_plasma	0.858	(Kawamoto et al., 2007)
Fat/blood PC	k_fat_plasma	5.0	(Doerge et al., 2011)
Skin/blood PC	k_skin_plasma	5.7	(Mielke et al., 2011)
Rest of the PC body/blood	k_restbody_plasma	2.7	Assumed similar to brain
Placenta/blood PC	k_placenta_plasma	1.43	(Csanády et al., 2002)
Placenta/blood BPAG PC	k_placentaBPAG_plasma	0.680	(Kawamoto et al., 2007)
Fetus Liver/blood PC	k_liver_fetus_plasma	0.73	set equal to mother
Fetus rest of the PC body/blood	k_restbody_fetus_plasma	2.7	set equal to mother

a = parameter set to plasma volume, **b**= value need to scale ($Vfetus^{0.75}$) to use in P-PBPK modeling, c = mean experimental value has used for scaling to in-vivo

Table I-3 Pharmacokinetic parameters of MEHP used for the P-PBPK

Parameters	Symbol/Unit	Mean value	References
Oral absorption rate	K_oral ($L/h/kg^{-0.25}$)	7	(Adachi et al., 2015)
Plasma unbound fraction	Fum	0.007	(Adachi et al., 2015)
Fraction unbound in microsomes	Fumi	0.786	(Adachi et al., 2015)
Partition coefficient for MEHP			
Liver/blood PC	k_liver_plasma	1.7	(Keys et al., 1999)
gonads/blood PC	k_gonads_plasma	1.6	(Keys et al., 1999)
restbody/blood PC	k_restbody_plasma	.38	Set to slow perfused organ (muscle) (Keys et al., 1999)
Fat/blood PC	k_fat_plasma	0.12	(Keys et al., 1999)
Placenta/blood PC	k_placenta_plasma	0.88	Estimated
Fetus Liver/blood PC	k_liver_fetus_plasma	1.7	set equal to mother
Fetus rest of the PC body/blood	k_restbody_fetus_plasma	.38	set equal to mother
fractional constant for placental transfer from mother to fetus	FK_t1[a]	5.2e-3	Estimated
fractional constant for placental transfer from fetus to mother	FK_t2[a]	2.0e-3	Estimated
Partition coefficient for DEHP			
Liver/blood PC	k_liver_plasma	21.8	(Keys et al., 1999)
Restbody/blood PC	k_restbody_fetus_plasma	6.1	Set to slow perfused organ (muscle) (Keys et al., 1999)

Note: [a] parameters was optimized based on assumption of distribution of MEHP to mother and fetus 1:1 (parameters should be used cautiously until validation of model done)

Table I-4. Hepatic and intestinal in –vitro metabolic data of DEHP and its metabolites

Human liver (HLM) binding affinity (DEHP to MEHP)	Km	30,3 ± 3,0	microMolar	(Choi et al., 2013)
Human liver (HLM) metabolic capacity (DEHP to MEHP)	Vmax	286,1 ± 7,0	pmol mg protein-1 min-1	(Choi et al., 2013)
Human Intestine (HINT) binding affinity (DEHP to MEHP)	Km	17,8 ± 2,0	microMolar	(Choi et al., 2013)
Human Intestine (HINT) metabolic capacity (DEHP to MEHP)	Vmax	277,8 ± 5,0	pmol mg protein-1 min-1	(Choi et al., 2013)
Human liver (HLM) binding affinity 5-OH MEHP (M2)	Km	28,4 ± 5,0	microMolar	(Choi et al., 2013)
Human liver (HLM) metabolic capacity 5-OH MEHP (M2)	Vmax	611,4 ± 24,0	pmol mg protein-1 min-1	(Choi et al., 2013)
Human Intestine (HINT) binding affinity 5-OH MEHP (M2)	Km	80,1 ± 13,0	microMolar	(Choi et al., 2013)
Human Intestine (HINT) metabolic capacity 5-OH MEHP (M2)	Vmax	4,1 ± 0,2	pmol mg protein-1 min-1	(Choi et al., 2013)
Human liver (HLM) binding affinity 5-Carboxy MEPP (M3)	Km	4,0 ± 3,3	microMolar	(Choi et al., 2013)
Human liver (HLM) metbolic capacity 5-Carboxy MEPP (M3)	Vmax	8,3 ± 0,4	pmol mg protein-1 min-1	(Choi et al., 2013)
Human liver (HLM) binding affinity 5-Oxo MEHP (M4)	Km	78,9 ± 14,0	microMolar	(Choi et al., 2013)
Human liver (HLM) metabolic capacity 5-Oxo MEHP (M4)	Vmax	10,3 ± 1,0	pmol mg protein-1 min-1	(Choi et al., 2013)
Human Intestine (HINT) binding affinity 5-Oxo MEHP (M4)	Km	747,7 ± 221	microMolar	(Choi et al., 2013)
Human Intestine (HINT) metabolic capacity 5-Oxo MEHP (M4)	Vmax	4,2 ± 1,0	pmol mg protein-1 min-1	(Choi et al., 2013)
Human liver (HLM) binding affinity PA (M5)	Km	502,9 ± 298,0	microMolar	(Choi et al., 2013)
Human liver (HLM) metabolic capacity PA (M5)	Vmax	312,8 ± 86,0	pmol mg protein-1 min-1	(Choi et al., 2013)

Note: all parameters was scaled using IVIVE methodology prior to use in PBPK model

References

Adachi, K., Suemizu, H., Murayama, N., Shimizu, M., Yamazaki, H., 2015. Human biofluid concentrations of mono(2-ethylhexyl)phthalate extrapolated from pharmacokinetics in chimeric mice with humanized liver administered with di(2-ethylhexyl)phthalate and physiologically based pharmacokinetic modeling. Environ. Toxicol. Pharmacol. doi:10.1016/j.etap.2015.02.011

Brown, R.P., Delp, M.D., Lindstedt, S.L., Rhomberg, L.R., Beliles, R.P., 1997. Physiological parameter values for physiologically based pharmacokinetic models. Toxicol. Ind. Health 13, 407–484.

Choi, K., Joo, H., Campbell, J.L., Andersen, M.E., Clewell, H.J., 2013. In vitro intestinal and hepatic metabolism of Di(2-ethylhexyl) phthalate (DEHP) in human and rat. Toxicol. Vitr. 27, 1451–1457. doi:10.1016/j.tiv.2013.03.012

Clewell, H.J., Gearhart, J.M., Gentry, P.R., Covington, T.R., VanLandingham, C.B., Crump, K.S., Shipp, A.M., 1999. Evaluation of the uncertainty in an oral reference dose for methylmercury due to interindividual variability in pharmacokinetics. Risk Anal. 19, 547–558. doi:10.1023/A:1007017116171

Coughlin, J.L., Thomas, P.E., Buckley, B., 2012. Inhibition of genistein glucuronidation by bisphenol A in human and rat liver microsomes. Drug Metab. Dispos. 40, 481–485. doi:10.1124/dmd.111.042366

Csanády, G., Oberste-Frielinghaus, H., Semder, B., Baur, C., Schneider, K., Filser, J., 2002. Distribution and unspecific protein binding of the xenoestrogens bisphenol A and daidzein. Arch. Toxicol. 76, 299–305. doi:10.1007/s00204-002-0339-5

Davies, B., Morris, T., 1993. Physiological parameters in laboratory animals and humans. Pharm. Res. doi:10.1023/A:1018943613122

Doerge, D.R., Twaddle, N.C., Vanlandingham, M., Brown, R.P., Fisher, J.W., 2011. Distribution of bisphenol A into tissues of adult, neonatal, and fetal Sprague–Dawley rats. Toxicol. Appl. Pharmacol. 255, 261–270. doi:10.1016/j.taap.2011.07.009

Fisher, J.W., Twaddle, N.C., Vanlandingham, M., Doerge, D.R., 2011. Pharmacokinetic modeling: Prediction and evaluation of route dependent dosimetry of bisphenol A in monkeys with extrapolation to humans. Toxicol. Appl. Pharmacol. 257, 122–136. doi:10.1016/j.taap.2011.08.026

ICRP, 2002. Basic anatomical and physiological data for use in radiological protection: reference

values. Ann. ICRP 32, 1–277. doi:10.1016/S0146-6453(03)00002-2

Ikezuki, Y., Tsutsumi, O., Takai, Y., Kamei, Y., Taketani, Y., 2002. Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. Hum. Reprod. 17, 2839–2841. doi:10.1093/humrep/17.11.2839

Kawamoto, Y., Matsuyama, W., Wada, M., Hishikawa, J., Chan, M.P.L., Nakayama, A., Morisawa, S., 2007. Development of a physiologically based pharmacokinetic model for bisphenol A in pregnant mice. Toxicol. Appl. Pharmacol. 224, 182–191. doi:10.1016/j.taap.2007.06.023

Keys, D.A., Wallace, D.G., Kepler, T.B., Conolly, R.B., 1999. Quantitative evaluation of alternative mechanisms of blood and testes disposition of di(2-ethylhexyl) phthalate and mono(2-ethylhexyl) phthalate in rats. Toxicol. Sci. 49, 172–85. doi:10.1093/toxsci/49.2.172

Kortejärvi, H., Urtti, A., Yliperttula, M., 2007. Pharmacokinetic simulation of biowaiver criteria: The effects of gastric emptying, dissolution, absorption and elimination rates. Eur. J. Pharm. Sci. 30, 155–166. doi:10.1016/j.ejps.2006.10.011

Kurebayashi, H., Okudaira, K., Ohno, Y., 2010. Species difference of metabolic clearance of bisphenol A using cryopreserved hepatocytes from rats, monkeys and humans. Toxicol. Lett. 198, 210–215. doi:10.1016/j.toxlet.2010.06.017

Mielke, H., Partosch, F., Gundert-Remy, U., 2011. The contribution of dermal exposure to the internal exposure of bisphenol A in man. Toxicol. Lett. 204, 190–198. doi:10.1016/j.toxlet.2011.04.032

Sisson, T.R., Lund, C.J., Whalen, L.E., Telek, A., 1959. The blood volume of infants. I. The full-term infant in the first year of life. J. Pediatr. 55, 163–79. doi:10.1016/S0022-3476(59)80084-6

Trdan Lusin, T., Roskar, R., Mrhar, A., 2012. Evaluation of bisphenol A glucuronidation according to UGT1A1*28 polymorphism by a new LC-MS/MS assay. Toxicology 292, 33–41. doi:10.1016/j.tox.2011.11.015

PUBLICATION 2

Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure: A Catalonia (Spain) case study

M. A. Martínez[a], J. Rovira[a,b], R. Prasad Sharma[a], M. Nadal[b], M. Schuhmacher[a,b], V. Kumar[a,b]

[a]Environmental Engineering Laboratory, Departament d'Enginyeria Quimica, Universitat Rovira i Virgili, Av. Països Catalans 26, 43007 Tarragona, Catalonia, Spain. [b]Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Catalonia, Spain.

Environmental Research, 166 (2018) 25 – 34.

Abstract

Bisphenol A (BPA) and Di-(2-ethylhexyl) phthalate (DEHP) are two widespread chemicals classified as endocrine disruptors (ED). The present study aims to estimate the non-dietary (dermal, non-dietary ingestion and inhalation) exposure to BPA and DEHP for a pregnant women cohort. In addition, to assess the prenatal exposure for the fetus, a physiologically based pharmacokinetic (PBPK) model was used. It was adapted for pregnancy in order to assess the internal dosimetry levels of EDs (BPA and DEHP) in the fetus. Estimates of exposure to BPA and DEHP from all pathways along with their relative importance were provided in order to establish which proportion of the total exposure came from diet and which came from non-dietary exposures. In this study, the different oral dosing scenarios (dietary and non-dietary) were considered keeping inhalation as a continuous exposure case. Total non-dietary mean values were 0.002 $\mu g/kg_{bw}$/day (0.000; 0.004 $\mu g/kg_{bw}$/day for 5^{th} and 95^{th} percentile, respectively) for BPA and 0.597 $\mu g/kg_{bw}$/day (0.116 $\mu g/kg_{bw}$/day and 1.506 $\mu g/kg_{bw}$/day for 5^{th} and 95^{th} percentile, respectively) for DEHP. Indoor environments and especially dust ingestion were the main non-dietary contributors to the total exposure of BPA and DEHP with 60% and 81%. However, as expected, diet showed the higher contribution to total exposure with >99.9% for BPA and 63% for DEHP. Although diet was considered the primary source of exposure to BPA and phthalates, it must be taken into account that with non-dietary sources the first-pass metabolism is lacking, so these may be of equal or even higher toxicological relevance than dietary sources.

The present study is in the framework of “Health and environmental-wide associations based on large population surveys” (HEALS) project (FP7-603946).

Keywords

Bisphenol-A; Di-(2-ethylhexyl) phthalate (DEHP); PBPK modeling; exposure assessment.

1. Introduction

Bisphenol A (BPA) and Di-(2-ethylhexyl) phthalate (DEHP) are two high volume industrial chemicals used in a wide variety of consumer products. These compounds are defined as non-persistent Endocrine Disrupters (EDs) and are categorized as chemicals of concern by the World Health Organization (WHO, 2010). The exposure to EDs plays a key role in the epigenome shaping of many aspects of the endocrine function (Casati, 2013; Chen et al., 2018). The evidences present in the literature indicate that EDs can affect the different levels of epigenetic control (Sharma et al., 2017) and in some cases can act transgenerationally, if the exposure to EDs occurs during "critical windows of exposure", especially, the prenatal and the early life period (Sharma et al., 2016; Volle et al., 2015; Watkins et al., 2017). Furthermore, some studies have shown that exposure to these chemicals in the early period of life may cause functional impairment of development and reproduction (Dodson et al., 2012; Meeker, 2012; Sakhi et al., 2014), increase the risk of allergy/asthma (Robinson and Miller, 2015; Sakhi et al., 2014) and can also develop obesity and type 2 diabetes (Casas et al., 2011; De Cock et al., 2014; Myridakis et al., 2016). It is known that fetal exposure is directly related to the mother's exposure, due to a bi-directional transfer of chemicals between the placenta and fetal plasma (Sharma et al. 2018). Normally placental barrier is considered protective layer against harmful compounds, however, a recent study has found poor barrier mechanism of placenta against some common EDs (Go et al., 2007; Li et al., 2013; Ribeiro et al., 2017).

Phthalates such as DEHP are industrial chemicals, which are used in polyvinyl chloride (PVC) plastics, found in products such as shoes, gloves and packing materials as well as in building materials, floorings and wall coverings (Giovanoulis et al.,2018). In addition, they are used in pharmaceuticals products, personal care products (PCPs), paints and adhesives (Bao et al., 2015). All of these applications are related to dermal contact, non-dietary ingestion or inhalation exposure sources. Some studies confirm that DEHP is an important contaminant in dust household; people can be exposed to it via dust ingestion, the exposure through this will be higher for workers in PVC industries (Fromme et al., 2004). It is known that babies

and young children are the most vulnerable groups with respect to phthalates due to their developmental status (Giovanoulis G et al., 2018; Sathyanarayana et al., 2008; Zhu et al., 2018).

BPA is currently used in polycarbonate plastics, found in materials intended to come into contact with food, like reusable plastic bottles, feeding-bottles, plates, cups, microwave and ovenware (Geens et al., 2009). In addition, we can find BPA in storage containers and epoxy resin linings for food and beverage containers. Furthermore, they are used in thermal papers and paper currencies, medical devices, dental sealants, and PCPs which are related with dermal exposure sources (Geens et al., 2012; Lv et al., 2017). Some studies showed that BPA exposure via dermal route can highly contribute to overall internal exposure (Biedermann et al., 2010; Mielke et al., 2011). Other studies affirm that people who work in offices will be more exposed via dust ingestion or inhalation than others because the levels of BPA in dust offices were almost 5–10 times higher than dust from particular homes (Geens et al., 2009).

The human exposure routes to EDs are multiple (Giulivo et al., 2016). Although the major human route of exposure to BPA and DEHP has been shown by several assessments, including the European Food Safety Authority (EFSA), to be the dietary pathway (EFSA, 2013; Geens et al., 2012; Guo et al., 2013). However, some studies confirm that non-dietary sources need to be more thoroughly characterized (EFSA, 2015; Geens et al., 2012). Estimates of exposure to DEHP and BPA from all pathways along with their relative importance should be provided in order to establish which proportion of the total exposure comes from diet and which comes from non-dietary exposures. Human exposure to EDs from non-dietary sources, their toxicity, as well as their combined effects, are poorly understood (Larsson et al., 2014).

In this study, occupational risk, lifestyle and the use of different PCPs were considered in order to assess the exposure to different pathways (dermal contact, non-dietary ingestion, and inhalation). Sharma et al., (2018) developed a P-PBPK model for BPA including specific pregnancy physiology and both oral and dermal route of exposure. The simulation results

were presented to compare the reported data from different cohorts presuming the collection of samples can be from at different time points, in order to explain the inconsistency in biomonitoring data. Moreover, some authors compared the results obtained between real measurements concentrations levels of EDs in the blood reported and the exposure estimates based on PBPK models (Mielke and Gundert-Remy, 2009); the intake estimated were several orders of magnitude lower than the real values in blood reported in the literature. One way to explain this abnormality could be that in the PBPK model they only considered the dietary source, so this could have led to an underestimation of the exposure to these chemicals through non-dietary routes like dermal, inhalation or dust ingestion. However, there are other contributing factors for this difference such as genetic variability, biomonitoring sampling strategy and contamination of sample during analysis.

The present study aims to estimate the non-dietary (dermal, non-dietary ingestion and inhalation) exposure to BPA and DEHP for a pregnant women cohort. In addition, to assess the prenatal exposure for the fetus, through all routes (diet and non-dietary) a physiologically based pharmacokinetic (PBPK) model was used. The pregnancy PBPK model structure was adapted from Sharma et al., (2018). Previous work has been extended to estimate the aggregate exposure of these EDs to humans to understand the relative importance of non-dietary exposure. Parameters and structure of the models were kept same as our previous publications (Sharma et al., 2018; Martínez et al., 2017), except nondietary routes (inhalation and dermal) were included. The present study is in the framework of “Health and environmental-wide associations based on large population surveys” (HEALS) project (FP7-603946) and part of the study has been completed in MODELBIS project (MINECO funded with ref no AGL2016-78942-R).

2.Materials and methods

2.1 Study population

The sudy population comprises a cohort of pregnant women and ongoing birth cohort. The pregnant women were recruited during the first trimester of pregnancy as part of the European "HEALS" project. The recruitment of pregnant mothers has started in March 2016 and in the present study 72 mother-child pairs from Reus (Tarragona, Spain) were included. Women were informed of the study during their first visit (12th gestational week) to the University Hospital "Sant Joan de Reus", in Reus (Catalonia, NE Spain). Women were eligible to participate according to the following inclusion criteria: ≥16 years old, intention to deliver at the reference hospital, and no problems with the communication language. This study was approved by the Ethical Committee of Clinical Research of the Hospital and a written informed consent was obtained from the participants.

2.2 Questionnaires and data acquisition

At 20th gestational weeks (GW), a PCPs frequency questionnaire was filled in a face-to-face interview. Different PCPs were included in the questionnaire: a) makeup (face cream, eyeshadow and liquid foundation), b) lipstick, c) body lotion, d) shampoo, e) shower gel, f) hair conditioner, g) toothpaste, h) deodorant and i) spray perfume. In addition, the questionnaires also included in one hand, general characteristics data of the study population, such as maternal age at delivery, twin pregnancy, maternal body mass index (BMI), maternal education, social economic status, country of origin, and marital status. On the other hand, a set of questions targeting to know other sources of these compounds are included, such as maternal smoking, lifestyle, hours spend outdoors and indoors and occupational risk. A description of the characteristics of the study population is shown in Table 1.

Table 1. Characteristics of the study population from Reus cohort, Tarragona (Spain) (n=72).

Characteristics of the study population (n = 72)	**%**
Maternal age at delivery (years)	
< 20	1
20-29	14
30-39	72
>40	13
Twin pregnancy	8
*Maternal pre-pregnancy BMI**	
Underweight (<19kg/m^2)	6
Normal (19-25 kg/m^2)	50
Overweight (>25 kg/m^2)	25
Obese (>30 kg/m^2)	19
*Maternal pregnancy (20 GW) BMI**	
Underweight (<19kg/m^2)	1
Normal (19-25 kg/m^2)	41
Overweight (>25 kg/m^2)	37
Obese (>30 kg/m^2)	21
Maternal education	
Primary	28
Secondary	31
University	41
Social economic status	
Low level (< 9000-19000 €/year)	24
Median level (19000-35000 €/year)	49
High level (> 35000 €/year)	27
Maternal country of origin	
Spain	76
Other	24
Marital Status	
Living with the father	99
Not living with the father	1
Maternal smoking	
Never smoke	73
Not during pregnancy	9
During pregnancy	18
**BMI= Body mass index*	

2.3 BPA and DEHP non-dietary assessment

2.3.1 Dermal contact exposure

The assessment of exposure of BPA and DEHP through dermal contact for pregnant women population was calculated according to equation 1. We considered all PCPs previously mentioned.

$$\text{Dermal exposure} = \sum(C_c \times PCP_{fr} \times PCP_a \times ABS \times R_f)/BW_{20GW} \quad \text{Eq. 1}$$

Where C_c is the concentration of BPA or DEHP in PCPs (in µg/g); PCP_{fr} is the frequency application (in application/day); PCP_a is the amount per application (in g/application); ABS is the dermal absorption factor (non-dimensional); R_f is the retention factor for rinse-off products (non-dimensional); and BW_{20GW} is the body weight at 20 gestational weeks (in kg). Dermal exposure is given in $\mu g/kg_{bw}/day$. Data used to assess the dermal exposure of BPA and DEHP are summarized in Table 2.

Table 2. Monte-Carlo parameter description to assess the total dermal contribution of BPA and DEHP.

Parameter	Symbol	units	Type	Distribution	Reference
DEHP concentration in	C_{DEHP}	-	-	-	-
Lipstick	-	µg/g	T	1.79 (0-6.45)	Guo and Kannan, 2013
Body lotion	-	µg/g	T	0.96 (0-11.3)	Guo and Kannan, 2013
Face cream	-	µg/g	T	0.4 (0-2.45)	Guo and Kannan, 2013
Shampoo	-	µg/g	T	0.1 (0-1.1)	Esteve et al., 2016
Shower gel	-	µg/g	U	9.53-32.4	Guo et al., 2013
Deodorant	-	µg/g	T	4.98 (0-65.3)	Guo and Kannan, 2013
Hair conditioner	-	µg/g	T	0.18 (0-0.39)	Guo and Kannan, 2013
Spray perfume	-	µg/g	T	15 (7-130)	Wormuth et al., 2006
Eye shadow	-	µg/g	T	0.64 (0-1.46)	Guo and Kannan, 2013
BPA concentration in	C_{BPA}	-	-	-	-
Body lotion	-	µg/g	LN[a]	$3.54 \cdot 10^{-04}$, $1.18 \cdot 10^{-02}$, $1.67 \cdot 10^{-01}$	Liao and Kannan, 2014
Face cream	-	µg/g	LN	0.03 ± 0	Cacho et al., 2013
Liquid foundation	-	µg/g	LN[a]	0,0.02,0.04	Liao and Kannan, 2014
Shampoo	-	µg/g	LN	0.09 ± 0	Cacho et al., 2013
Shower gel	-	µg/g	LN	0.07 ± 0	Cacho et al., 2013
PCP frequency	PCP_{fr}	-	-	-	-
Lipstick	-	Application/day	N	0.18 ± 0.34	Present study
Body lotion	-	Application/day	N	0.78 ± 0.41	Present study
Face cream	-	Application/day	N	0.72 ± 0.44	Present study
Liquid foundation	-	Application/day	N	0.42 ± 0.44	Present study
Shampoo	-	Application/day	N	0.62 ± 0.37	Present study
Shower gel	-	Application/day	N	0.92 ± 0.31	Present study
Deodorant	-	Application/day	N	0.94 ± 0.27	Present study
Hair conditioner	-	Application/day	N	0.35 ± 0.28	Present study
Spray perfume	-	Application/day	N	0.68 ± 0.45	Present study

Parameter	Symbol	units	Type	Distribution	Reference
Eye shadow	-	Application/day	N	0.42 ± 0.44	Present study
PCP amount	PCP_a	-	-	-	-
Parameter	Symbol	units	Type	Distribution	Reference
Lipstick	-	g/application	LN^g	0.01±3.29	Loretz et al., 2005
Body lotion	-	g/application	LN^g	3.26 ± 2.25	Loretz et al., 2005
Face cream	-	g/application	LN^g	0.80 ± 2.55	Loretz et al., 2005
Liquid foundation	-	g/application	LN^g	0.33 ± 2.99	Loretz et al., 2006
Shampoo	-	g/application	G	0.38,5.79,2.15	Loretz et al., 2006
Shower gel	-	g/application	G	0.67,4.89,2.84	Loretz et al., 2006
Deodorant	-	g/application	LN^g	0.56 ± 2.41	Loretz et al., 2006
Hair conditioner	-	g/application	LN^g	10.28 ± 2.20	Loretz et al., 2006
Spray perfume	-	g/application	LN^g	0.30 ± 3.36	Loretz et al., 2006
Eye shadow	-	g/application	LN^g	0.01± 3.61	L. J. Loretz et al., 2008
Body weight	BW_{20GW}	kg	LN	71.42 ± 17.15	Present study
Retention factor (rinse off PCP)	R_f	-	-	-	-
Shampoo	-	-	U	0-0.02	EFSA, 2015
Shower gel	-	-	U	0-0.02	EFSA, 2015
Hair conditioner	-	-	U	0-0.02	EFSA, 2015
Ingestion factor lipstick	1-(Ing_f)	-	LN	0.20 ± 0.04	Franzen et al., 2016
DEHP dermal absorption factor	$ABS_{(DEHP)}$	**-**	U	0.05-0.15	EPA, 2011
BPA dermal absorption factor	$ABS_{(BPA)}$	-	U	0.08-0.10	Demierre et al., 2012

LN = Log-normal; T = Triangular; U = Uniform; G = Gamma; N= Normal distribution. Mean, minimum, and maximum values were used for triangular distributions; Mean and standard deviation were used for log-normal distributions; Geometrical mean and geometrical standard deviation were used in log-normal^g distributions; minimum and maximum values were used for uniform distributions; Percentile 50,95 and maximum were used in log-normal^a distributions and location, scale and shape were used for gamma distribution.

2.3.2 Non-dietary ingestion exposure

Non-dietary ingestion pathways include, on the one hand, dust ingestion that was calculated according to equation 2.a. On the other hand, exposure through PCPs ingestion was considered. Lipstick and toothpaste ingestion was assessed according to equation 2.b.

$$\text{Non} - \text{dietary ingestion exposure } (Dust_{\text{ingestion}}) = (C_c \times I_r)/BW_{20GW} \quad \text{Eq. 2.a.}$$

$$\text{Non} - \text{dietary ingestion exposure } (PCP_{\text{ingestion}}) = (C_c \times PCP_{fr} \times PCP_a \times Ing_f)/BW_{20GW} \quad \text{Eq. 2.b.}$$

Where C_c is the concentration of BPA or DEHP in homes dust (in µg/kg); I_r is the Ingestion rate (in kg/day) and $BW_{20\,GW}$ is the body weight at 20 gestational weeks (in kg). PCP_{fr} is the frequency application (in application/day); PCP_a is the amount per application (in g/application) and Ing_f is the ingestion factor (non-dimensional). The total non-dietary exposure is given in $\mu g/kg_{bw}$/day. Table 3 provides data used to assess the non-dietary ingestion exposure of BPA and DEHP.

Table 3. Monte-Carlo parameter description to assess the total non-dietary ingestion contribution of BPA and DEHP.

Parameter	Symbol	units	Type	Distribution	Reference
DEHP concentration in	C_{DEHP}	-	-		-
Lipstick	-	µg/g	T	1.79 (0-6.45)	Guo and Kannan,2013
Dust indoor	-	µg/kg dust	LN[b]	$1.20 \cdot 10^6$	Wormuth et al., 2006
BPA concentration in	C_{BPA}	-	-		-
Toothpaste	-	µg/g	LN[c]	0.35,0.83	Liao and Kannan,2014
Dust indoor	-	µg/kg dust	LN	$2 \cdot 10^3 \pm 2.1 \cdot 10^3$	Geens et al., 2009
PCP frequency	PCP_{fr}	-	-		-
Lipstick	-	Application/day	N	0.18 ± 0.34	Present study
Toothpaste	-	Application/day	N	1.82 ± 0.76	Present study
PCP amount	PCP_a	-	-	-	-
Lipstick	-	g/application	LN[g]	0.01 ± 3.29	Loretz et al., 2005
Toothpaste	-	g/application	U	0.79-1.20	McNamara et al., 2007
Dust ingestion rate	I_r	kg/day	N	$3 \cdot 10^{-5} \pm 3 \cdot 10^{-6}$	EPA, 2011
Ingestion factor	Ing_f	-	-	-	-
Lipstick	-	-	LN	0.20 ± 0.04	Franzen et al., 2016
Toothpaste	-	-	U	0-0.10	Angerer et al., 2010
Body weight	BW_{20GW}	kg	LN	71.42 ± 17.15	Present study

LN = Log-normal; T = Triangular; U = Uniform. Mean, minimum, and maximum values were used for triangular distributions; Mean and standard deviation were used for log-normal distributions; Geometrical mean and geometrical standard deviation were used in log-normal[g] distributions; minimum and maximum values were used for uniform distributions; Mean and P95 were used for log-normal[b] distributions; Percentile 50 and 95 were used in log-normal[c] distributions.

2.3.3 Inhalation exposure

The exposure assessment of BPA and DEHP through inhalation for pregnant women was calculated according to equation 3. We considered levels of BPA and DEHP in the outdoor and indoor air. In this case, three different scenarios were assessed: sleeping (3.a), indoors (3.b) and outdoors (3.c) scenarios.

$$\text{Inhalation exposure}_{sleeping} = (C_c^{indoor} \times Ihr_{sleep} \times t_{sleep})/BW_{20GW}$$

Eq. 3.a

$$\text{Inhalation exposure}_{indoor} = (C_c^{indoor} \times Ihr_{sedentry} \times t_{indoor})/BW_{20GW}$$

Eq.3.b

$$\text{Inhalation exposure}_{outdoor} = (C_c^{outdoor} \times Ihr_{moderate} \times t_{outdoor})/BW_{20GW}$$

Eq.3.c

Where C_c^{indoor} is the concentration of BPA or DEHP in the indoor air (in μg/m^3); $C_c^{outdoor}$ is the concentration of BPA or DEHP in the outdoor air (in μg/m^3); $Ih_{r\ sleep}$ is the inhalation rate during sleep (in m^3/min); $Ih_{r\ sedentary}$ is the inhalation rate while sedentary activities (in m^3/min); $Ih_{r\ moderate}$ is the inhalation rate during moderate activities (in m^3/min); t_{sleep} is the mean of time sleeping (in min); t_{indoor} is the mean of time spending indoor (at work and at home) (in min); $t_{outdoor}$ is the time spending in doing activity outdoor (in min) and BW_{20GW} is the body weight at 20 gestational weeks (in kg). The total inhalation exposure is given in μg/kg$_{bw}$/day. Table 4 contains the data used to assess the inhalation exposure of BPA and DEHP.

The concentration levels of BPA and DEHP in different PCPs, in dust and air, were taken from the literature with a preference rule of Spanish values> European values> other available data. To deal with variability and uncertainty of parameters used, probabilistic estimation of the dermal, non-dietary ingestion and inhalation exposure was performed in a probabilistic way. Monte-Carlo simulation is a common approach used to incorporate variability and uncertainty of the parameters used into the estimation of human health

exposure (Mari et al., 2009; May et al., 2002; Rovira et al., 2016; Schuhmacher et al., 2001). Table 2, 3 and 4 includes the probabilistic distribution of parameters for the calculation of human health exposure. Monte-Carlo simulation was carried out by Oracle Crystal Ball© software. Exposures were calculated based on the propagation variable of variability and uncertainty given by each parameter probability function until 100,000 iterations.

Table 4. Monte-Carlo parameter description to assess the total inhalation contribution of BPA and DEHP.

Parameter	Symbol	units	Type	Distribution	Reference
DEHP concentration in	C_{DEHP}	-	-	-	-
Air indoor	-	$\mu g/m^3$	T	0.3 (0.05-0.62)	Wormuth et al., 2006
Air outdoor	-	$\mu g/m^3$	T	0.01 (0-0.05)	Wormuth et al., 2006
BPA concentration in	C_{BPA}	-	-	-	-
Air indoor	-	$\mu g/m^3$	T	0 (0-0.01)	EFSA, 2015
Air outdoor	-	$\mu g/m^3$	LN	0.01 ± 0.01	Salapasidou et al.,2011
Inhalation rate					
sleeping	$Ih_{r\ sleep}$	m^3/min	LN[b]	0,0.01	EPA, 2011
sedentary activity	$Ih_{r\ sedentary}$	m^3/min	LN[b]	0,0.01	EPA, 2011
moderate activity	$Ih_{r\ moderate}$	m^3/min	LN[b]	0.02,0.03	EPA, 2011
Time sleeping	t_{sleep}	min	N	521 ± 52.10	IEC, 2012
Time outdoor	$t_{outdoor}$	min	N	106 ± 10.60	IEC, 2012
Time indoor	t_{indoor}	min	-	1440	-
Body weight	BW_{20GW}	kg	LN	71.42 ± 17.15	Present study

Time indoor= 24 hours – (T_{sleep} + $T_{outdoor}$). LN = Log-normal; T = Triangular. Mean, minimum, and maximum values were used for triangular distributions; Mean and standard deviation were used for log-normal distributions; Mean and P95 were used for log-normal[b] distributions.

2.4 Tissue dosimetry model (PBPK).

The basic structure of pregnant PBPK model has been adapted from Sharma et al., (2018) in the current study in order to assess dietary and non-dietary exposure. It comprises plasma, liver, kidneys, fat, brain, skin, placenta, a rest of the body and a fetus compartment. Fetus compartment was subcategorized again into liver, brain, and plasma. All the Physiological parameters during pregnancy are considered to be dynamic parameters that change due to the growth of mother organs (Abduljalil et al., 2012; Gentry et al., 2003; Loccisano et al., 2013). The source of exposure to fetuses was via a free fraction of chemicals into mother's placenta, considering that fetuses' exposure is directly related to mother's exposure. The placental-fetal unit assumes a bidirectional transfer process describing chemical transfer between mothers' placenta to fetuses' plasma and fetuses' plasma to the mothers. Detailed descriptions of standard and pregnancy-specific model equations are adapted form Sharma et al., (2018). Metabolic kinetic parameters for both mothers and fetuses were previously estimated from in-vitro studies (Martínez et al., 2017; Sharma et al., 2018).

Two different sources of exposure were considered for the current study, dietary exposure and the combination of dietary with non-dietary exposure. The dosing considered being inputs for the PBPK model was estimated using Monte Carlo technique for the exposure assessment. It has been considered the six following exposure scenarios of BPA and DEHP: 5^{th} percentile diet; 5^{th} percentile diet + non-diet; Mean diet; Mean diet+ non-diet; 95^{th} percentile diet, and 95^{th} percentile diet + non-diet. For the current study, the routes of exposure were the following: ingestion and dermal exposure that were divided into three equal doses (with 8 hours of the interval). On the other hand, continuous exposure for inhalation was presumed, considering three different inhalation rates (sleeping time, doing sedentary activities and doing moderate activities).

3. Results and discussion

3.1 Non-dietary (dermal, non-dietary ingestion and inhalation) exposure to BPA and DEHP.

The contribution of dermal contact, non-dietary ingestion, and inhalation to the total non-dietary intake from Reus pregnant mothers' cohort was assessed in a probabilistic way using Monte-Carlo simulation. **Figure** 1, summarizes the contribution of each non-dietary source to the total exposure of BPA and DEHP.

Regarding BPA (**Figure** 1), the total non-dietary mean value was 0.002 $\mu g/kg_{bw}$/day (0.000 and 0.004 $\mu g/kg_{bw}$/day for 5^{th} and 95^{th} percentile, respectively). Relative mean contributions were 60%, 36% and 4% for non-dietary ingestion, inhalation, and dermal routes, respectively. For DEHP (**Figure** 1), the total non-dietary mean exposure was 0.597 $\mu g/kg_{bw}$/day (0.116 $\mu g/kg_{bw}$/day and 1.506 $\mu g/kg_{bw}$/day for 5^{th} and 95^{th} percentile, respectively). The maximum mean contribution was, again, non-dietary ingestion with 81%, followed by dermal route and inhalation with 15% and 4%, respectively.

For both chemicals, BPA and DEHP, non-dietary ingestion was the highest mean relative contributor with 60% and 81%, respectively, of the total non-dietary exposure. These represented a mean non-dietary ingestion exposure of $9.62 \cdot 10^{-4}$ and 0.485 $\mu g/kg_{bw}$/day for BPA and DEHP, respectively. Non-dietary ingestion route considered the levels of both compounds in homes dust and in PCPs that could be accidentally ingested during their use (lipstick and toothpaste). In both cases, the major contribution (>99.9%) to the total non-dietary ingestion exposure to BPA and DEHP came from home dust ingestion. The average concentration of BPA and DEHP in dust were very high, $2 \cdot 10^3$ and $1.20 \cdot 10^6$ $\mu g/kg_{dust}$, respectively. BPA levels in dust were obtained from Belgian houses (Geens et al., 2009) and phthalate levels in dust came from different European homes (Wormuth et al., 2006). However, similar BPA and DEHP levels in indoor dust were found worldwide (Das et al., 2014; Fromme et al., 2004; Ginsberg and Belleggia, 2017; Kubwabo et al., 2016; Langer et al., 2014; Loganathan and Kannan, 2011). The high contribution of dust in the total DEHP non-dietary ingestion exposure is due to phthalates, which are used as plasticizers in

numerous consumer products, commodities, and building materials. Consequently, phthalates are found in human residential and occupational environments in high concentrations (Wormuth et al., 2006). As well as DEHP, the high contribution of dust in the total BPA non-dietary ingestion exposure is due to BPA is used in a variety of household applications. Through manufacture and usage, these contaminants can leach into the environment and can be deposited in the indoor dust (Geens et al., 2009). It was assumed that consumers accidentally ingest small amounts of PCPs. So, it was estimated the scenario for non-dietary ingestion using information about the amounts cosmetics ingested daily (Table 3), and the DEHP and BPA concentrations in PCP. No such information was available on how much PCPs are ingested daily and it was not much literature data about concentration levels of these two EDs in different cosmetic products. Only data regarding DEHP in lipstick and BPA in toothpaste content were found. Therefore, it was only considered the accidental ingestion of these two cosmetics, lipstick and toothpaste, during their use. Results showed that the contribution to this kind of ingestion to the total DEHP and BPA non-dietary ingestion was insignificant (0.07% and 0.01% for BPA and DEHP, respectively) compared to total non-dietary ingestion and with the dietary total intake. However, more bibliographic data is needed to be able to carry out a good exposure assessment.

According to BPA, inhalation was the second greatest contributor to the total exposure with an exposure of $5.90 \cdot 10^{-4}$ $\mu g/kg_{bw}/day$, that meant the 36% of the total non-dietary exposure. In this case, three different scenarios were assessed: indoor, outdoor and sleeping inhalation exposure that showed a contribution to total BPA inhalation exposure of 37%, 51%, and 12%, respectively. Inhalation exposure was lower than the dust exposure; this can be due to BPA has a comparatively low vapour pressure. As a result, concentrations of BPA in the air can be expected to be low and it will be present mainly in the particulate phase, adsorbed to dust (EFSA, 2013). Finally, dermal contact was the exposure route that contributed the least (4%) to the total mean non-dietary BPA exposure, with a dose of $6.39 \cdot 10^{-5}$ $\mu g/kg_{bw}/day$. Among all the PCPs, face cream (39%), shower gel (20%) and body lotion (18%) have the higher contribution. In Europe, BPA is not allowed as an ingredient in

cosmetics (Regulation (EC) no. 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products). However, if BPA is present in the packaging (e.g. polycarbonates plastic (PC) packaging), it could migrate into the cosmetic products (EFSA, 2013). It must be taken into account that dermal absorption of BPA can reach 95-100% if BPA is applied dissolved in ethanol, because ethanol may act as a transport mediator for BPA into the skin, thus enhancing the absorption fraction. In addition, his property of dissolving in ethanol can be found in similar compounds in the formulation of creams and body lotions (EFSA, 2013).

Regarding DEHP, dermal contact with a mean value of 0.087 $\mu g/kg_{bw}/day$, was the second greatest contributor to the total non-dietary exposure (15%). In this exposure assessment, perfume and deodorant were the items which contribute more to the total DEHP dermal exposure, with 36% and 33%. The quite high presence of these ED is due to phthalates in general, are added as humectants, emollients, or skin penetration enhancers, which are very common in perfumes and fragrances (Koo and Lee, 2004). Finally, DEHP inhalation (0.025 $\mu g/kg_{bw}/day$) was the item which contributed less (4%) to the DEHP mean non-dietary exposure. Indoor exposure and sleeping inhalation exposure had a relative contribution of 61% and 36%, respectively. Other authors (Wormuth et al., 2006) found that accidental ingestion of PCPs are the major sources of exposure to DEHP in all consumer groups that we estimated. Although the food is the dominating source of exposure to DEHP in all consumer groups (Wormuth et al., 2006).

Indoor environment (home dust ingestion and inhalation (indoor and sleeping)) were the principal source of BPA and DEHP of non-dietary exposure with a relative contribution of 78% and 85%, respectively. PCPs contribute with 4% and 15% to total mean non-dietary exposure of BPA and DEHP, respectively, almost exclusively through dermal contact. Finally, outdoor environment (trough outdoor inhalation) showed a contribution of 18% and <0.1% to total mean non-dietary exposure for BPA and DEHP, respectively.

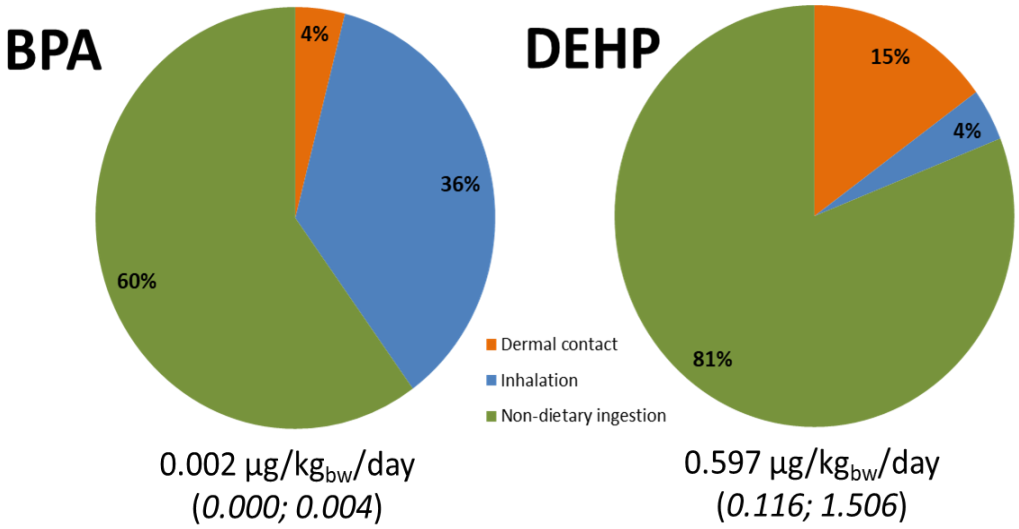


Figure 1. Non-dietary exposure (dermal contact, non-dietary ingestion and inhalation) Reus (Tarragona, Spain) pregnant women cohort exposure to BPA and DEHP exposure. Results are given in mean (*5th; 95th percentile*).

3.2 Dietary exposure vs non-dietary exposure

Figure 2, shows the comparison between total dietary exposure and non-dietary (dermal, non-dietary ingestion and inhalation) exposure to BPA and DEHP. Data from the dietary exposure was previously estimated using the same cohort population (Martínez et al., 2017).

Regarding BPA, mean dietary daily intake from Reus (Tarragona, Spain) cohort was 0.715 µg/kg$_{bw}$/day (Martínez et al., 2017), and the mean exposure estimated for non-dietary ingestion, inhalation, and dermal contact were $9.62 \cdot 10^{-4}$, $5.90 \cdot 10^{-4}$, $6.39 \cdot 10^{-5}$ µg/kg$_{bw}$/day, respectively. In general, in the present study according to non-dietary exposure, the maximum exposure estimated for BPA was 0.0072 µg/kg$_{bw}$/day and the 95% of the population were under 0.0040 µg/kg$_{bw}$/day. Non-dietary exposure practically did no contribute to the total exposure (0.2%). In other words, diet was the greatest contributor to the total exposure (99.8%) (**Figure** 2). However, it is important to know that in this study

thermal paper was not considered in dermal exposure estimation, which is considered as a potential exposure source for BPA in the EU by the EFSA, 2015.

BPA is conjugated in the liver by glucuronidation and sulfation, "total BPA" stands for the sum of conjugated and unconjugated forms. For further risk assessment, these two forms need to be distinguished, the unconjugated BPA is more toxicologically relevant. The contribution of dermal and inhalation sources to internal exposure to total BPA is considerably smaller compared to oral sources. However, with dermal and inhalation exposure the first-pass metabolism is lacking, regardless of the small contribution of non-dietary sources to total BPA, their contribution to the plasma concentration levels of unconjugated BPA may be considerable. Kinetic studies have shown that in monkeys only around 1% of orally absorbed BPA becomes systemically bioavailable as unconjugated BPA (Fisher et al., 2011), whereas after dermal absorption, practically all absorbed BPA (around 10% of the external dermal dose, Demierre et al., 2012) initially becomes bioavailable as unconjugated BPA. For that reason, non-dietary sources may be of equal or even higher toxicological relevance than dietary sources (Lu et al., 2017; Völkel et al., 2002; von Goetz et al., 2017). Considering diet and non-diet sources the mean of the total exposure was 0.72 $\mu g/kg_{bw}$/day and the 5^{th} and 95^{th} percentile of the total exposure were 0.28 and 1.41 $\mu g/kg_{bw}$/day (**Figure** 2).

Regarding DEHP, **Figure** 2 shows that non-dietary sources contribute with 37 % of the total exposure. The mean dietary daily intake of DEHP exposure from Reus cohort was 1.00 $\mu g/kg_{bw}$/day (Martínez et al., 2017), and the mean exposure estimated for non-dietary ingestion, inhalation, and dermal contact were 0.485, 0.025, 0.087 $\mu g/kg_{bw}$/day respectively. According to total non-dietary exposure, the maximum dose was 3.86 $\mu g/kg_{bw}$/day and the 95^{th} percentile was 1.51 $\mu g/kg_{bw}$/day, and mean value was 0.60 $\mu g/kg_{bw}$/day. Considering diet and non-diet sources the mean of the total exposure was 1.60 $\mu g/kg_{bw}$/day and the 5^{th} and 95^{th} of the total exposure were 0.52 and 3.52 $\mu g/kg_{bw}$/day, respectively (**Figure** 2).

EFSA published its comprehensive re-evaluation of BPA exposure and toxicity, in January 2015, and established a tolerable daily intake (TDI) of 4 μg/kg_{bw}/day for BPA (EFSA, 2015). On the other hand, EFSA and the European Chemicals Agency (ECHA) established the TDI for DEHP to 50 μg/kg_{bw}/day (ECHA, 2010; EFSA, 2015). Only the non-dietary ingestion estimated data from this study can be compared with this EFSA and ECHA tolerable values because the TDI values are concerned about "daily intake". Therefore, in this study, the maximum value estimated for BPA non-dietary ingestion exposure was 0.0052 μg/kg_{bw}/day and the 95% of the population were below 0.0028 μg/kg_{bw}/day. Whereas, for DEHP, the maximum value estimated for non-dietary ingestion exposure was 3.39 μg/kg_{bw}/day and the 95% of the population were under 1.24 μg/kg_{bw}/day. These values for BPA and DEHP estimated in our study were far away from the tolerable values of the EFSA and ECHA. Although BPA and DEHP non-dietary ingestion exposure assessment values were under the tolerable established, it is important to take into account that non-dietary ingestion and, in general, non-dietary levels must be added to the total dietary exposure assessment, in order to make a good exposure estimation.

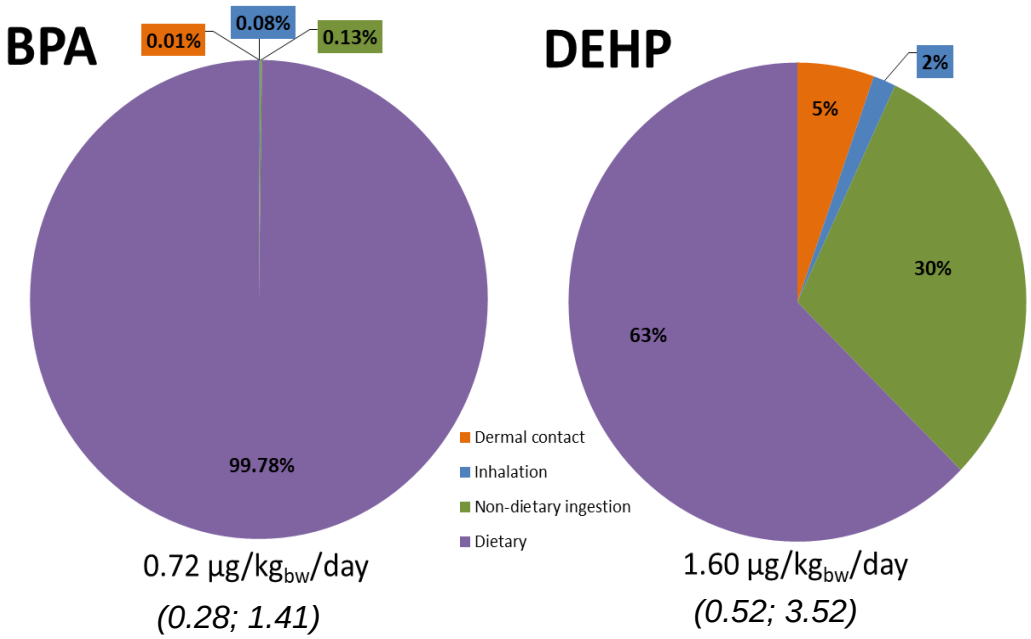


Figure 2. Total mean exposure dietary (Martínez et al., 2017) and non-dietary (dermal, non-dietary ingestion and inhalation) to BPA and DEHP for Reus pregnant women cohort. Results are given in mean (*5th; 95th percentile*).

3.3 Internal dosimetry

The chemicals' dose inputs considered to run the P-PBPK, were probabilistically estimated by Monte-Carlo simulation (Section 2.4). From probabilistic distribution, six total scenarios were selected for BPA and DEHP: the 5th percentile diet; the 5th percentile diet + non-diet; mean diet; mean diet + non-diet; the 95th percentile diet and the 95th percentile diet + non-diet. The outputs from the model simulation were selected considering the metabolites generated, their toxicity, gestational period and ability to reach the fetus. For this reason, only free BPA and MEHP (a metabolite of DEHP) were considered. The simulation data were taken from pregnant women and fetus for 24 h during the 24th gestational week. This period was selected because at this time fetus organs are more developed and able to incorporate right biological process. This helps us to explain the difference in metabolic processes in mothers and fetuses. Normally, at the early stage of pregnancy, for both BPA

and MEHP, fetus plasma concentration level is higher due to low or no metabolic activities in the fetus (Gauderat et al., 2016; Latini et al., 2003). In order to be near to a real scenario, a dietary, and non-dietary (dermal and ingestion) exposure were divided into three equal doses, along with continuous exposure of non-dietary source (inhalation) and were simulated (**Figure** 3) in the case of BPA. On the other hand, DEHP metabolite MEHP time plasma concentration profile in case of both mother and fetus is showed in **Figure** 4, the result of single-dose intake of dietary and non-dietary. In this case, inhalation was considered again as continuous exposure, the simulated concentration curves show a sharp peak concentration o within 1 h of intake. It is known that metabolic activity in the fetus is lower compared to mother's metabolism (Heindel et al., 2017). For that reason, concentration levels of both chemicals in the fetus' plasma were higher than in the mother. Therefore, BPA and MEHP stay longer in the fetal body, which may cause higher risk to fetuses and makes the fetus more vulnerable to the exposure. A similar trend has been observed by Sharma et al., (2018).

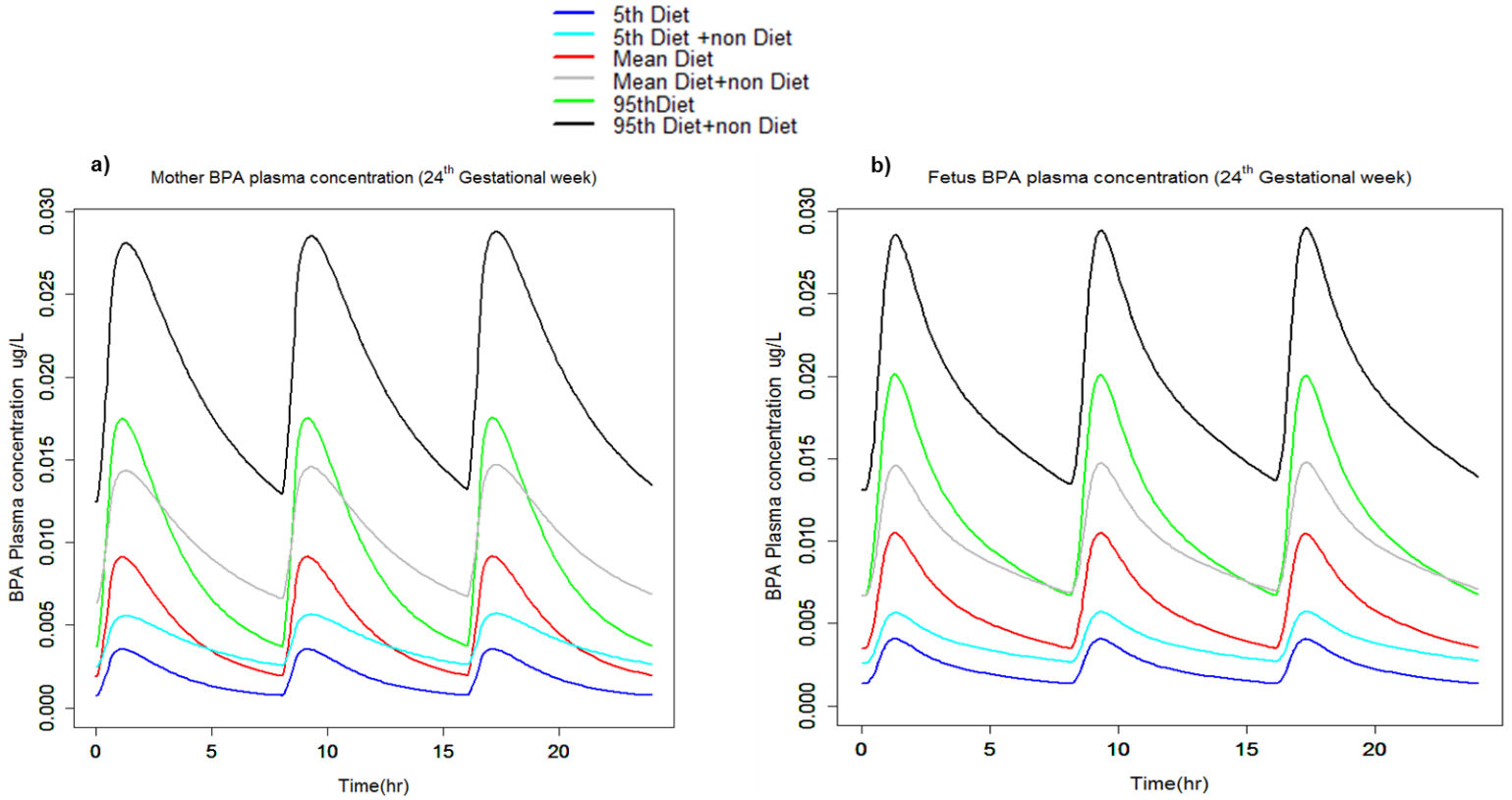


Figure 3. Time versus BPA plasma concentration for mothers a), and fetuses b), considering six different exposure scenarios (the 5th percentile diet; the 5th percentile diet + non-diet; mean diet; mean diet + non-diet; the 95th percentile diet and the 95th percentile diet + non-diet). It was considered three-food intake dose for diet and non-diet (dermal and dust ingestion) keeping inhalation as a continuous exposure

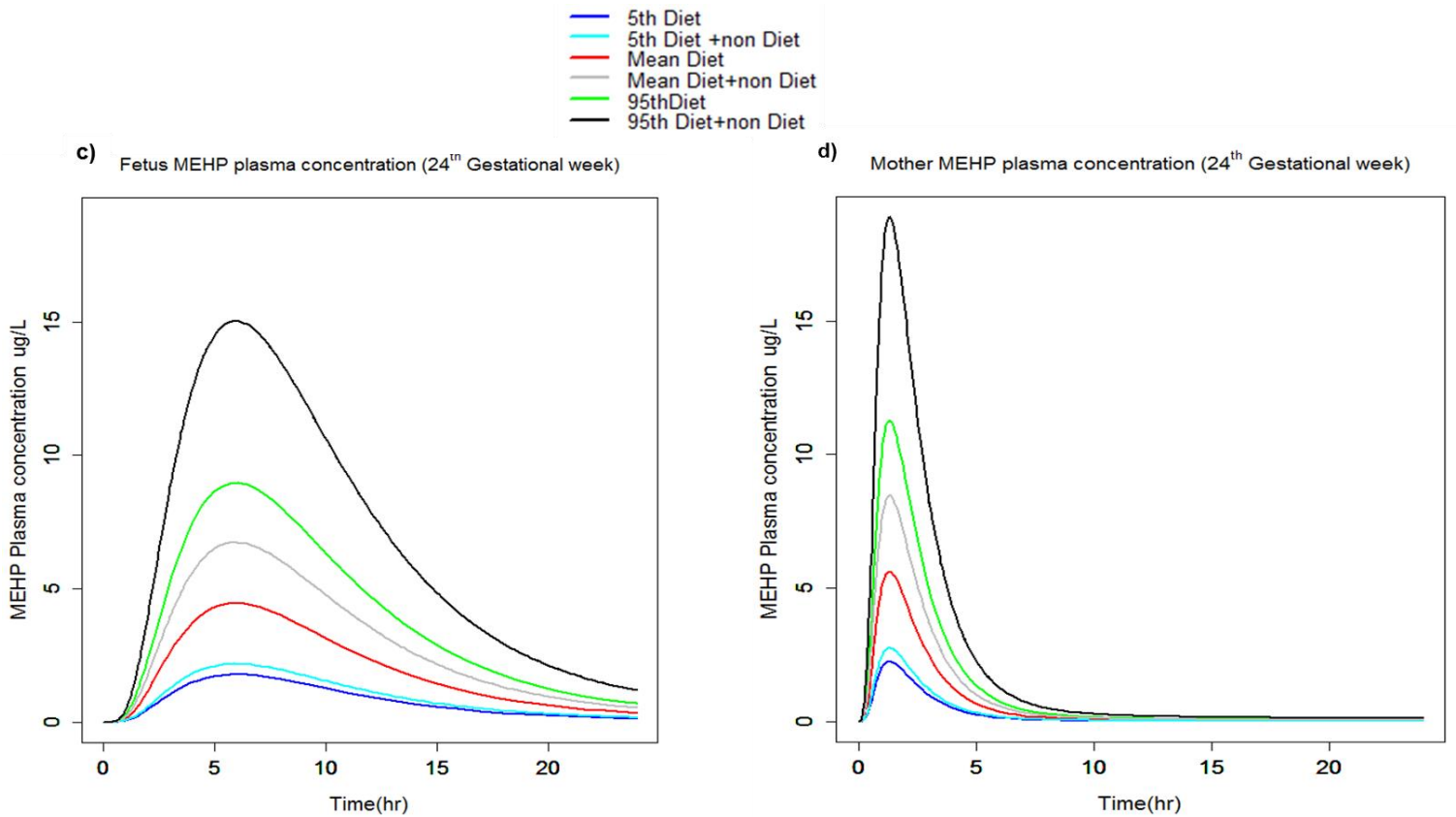


Figure 4. Time versus MEHP plasma concentration for mothers c) and fetuses d), considering six different exposure scenarios (the 5th percentile diet; the 5th percentile diet + non-diet; mean diet; mean diet + non-diet; the 95th percentile diet and the 95th percentile diet + non-diet). It was considered one-food intake dose keeping inhalation as a continuous exposure

4.Conclusions

Regarding BPA non-dietary exposure was 0.002 µg/kgbw/day, with the greatest contribution coming from non-dietary ingestion with 60%, followed by inhalation with 36%. Finally, dermal exposure was the one that contributed the least with 4%. However, in this study, the thermal paper was not considered in dermal exposure estimation, which is considered as a potential exposure source for the general population (EFSA, 2015). According to DEHP non-dietary exposure (0.597 µg/kgbw/day), the maximum contributor was non-dietary ingestion with 81%, followed by dermal contact with 15% and inhalation with 4%. As expected, diet was the main contributor to total exposure to both chemicals. Regarding DEHP, non-dietary sources contribute 37% of the total exposure. The non-dietary exposure to BPA practically did no contribute to the total exposure (0.22%). Indoor environment, dust ingestion, and indoor air inhalation was the main contributor to non-dietary exposure to both ED (78% for BPA and 85% for DEHP) meanwhile PCPs contribute in 4% and 15%, for BPA and DEHP, respectively. However, with dermal absorption that passes the first-pass metabolism, dermal sources may be of equal or even higher toxicological relevance than dietary sources (Völkel et al., 2002; von Goetz et al., 2017). Only the non-dietary ingestion estimated data in combination with other dietary exposure from this study can be comparable with EFSA and ECHA tolerable values because the TDI values are concerned about “daily intake”. Although BPA and DEHP non-dietary ingestion exposure assessment values were under the tolerable established, it is important to take into account that non-dietary exposure levels must be added to the total dietary exposure assessment, in order to make a good exposure estimation.

According to internal dosimetry, six different scenarios were considered in order to run the PBPK model. When the simulation considered diet + non-diet scenarios, the concentration levels of BPA and MEHP (main metabolite of DEHP) increased considerably in plasma. In addition, in fetus’ plasma, the concentration of both chemicals reached levels much higher than those seen previously in mothers. The low metabolic activity in fetus led to maintain a continuous concentration in time. Therefore, this can make the fetus more vulnerable to

the exposure compared with their mothers. The ongoing research is to validate the PBPK model with biological samples from this cohort and demonstrate that this methodology allows the determination of BPA and MEHP for monitoring in biological matrices, such as plasma and urine. Finally, demonstrate that PBPK model can predict the prenatal exposure of the child/fetus to EDs. To conclude, on the one hand, strategies must be presented in order to reduce their exposure. Restrictions must be imposed to regulate the production and use of products related especially with childcare and pregnant women.

Acknowledgements

Authors thank all pregnant women who participate in this study. The research leading to these results has partially funded from the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement No. 603946-2 (HEALS project) and the Spanish Ministry of Economy and Competitiveness for the MODELBIS project (Ref. No. AGL2016-78942-R). V. Kumar and J. Rovira received funds from Health Department of Catalonia Government trough "Pla Estratègic de Recerca i Innovació en salut" (PERIS 2016-2020).

References

Abduljalil, K., Furness, P., Johnson, T.N., Rostami-Hodjegan, A., Soltani, H., 2012. Anatomical, Physiological and Metabolic Changes with Gestational Age during Normal Pregnancy. Clin. Pharmacokinet. 51, 365–396. doi:10.2165/11597440-000000000-00000

Bao, J., Wang, M., Ning, X., Zhou, Y., He, Y., Yang, J., Gao, X., Li, S., Ding, Z., Chen, B., 2015. Phthalate concentrations in personal care products and the cumulative exposure to female adults and infants in Shanghai. J. Toxicol. Environ. Health. A 78, 325–41. doi:10.1080/15287394.2014.968696

Biedermann, S., Tschudin, P., Grob, K., 2010. Transfer of bisphenol A from thermal printer paper to the skin. Anal. Bioanal. Chem. 398, 571–576. doi:10.1007/s00216-010-3936-9

Cacho, J.I., Campillo, N., Viñas, P., Hernández-Córdoba, M., 2013. Stir bar sorptive extraction with EG-Silicone coating for bisphenols determination in personal care products by GC-MS. J. Pharm.

Biomed. Anal. doi:10.1016/j.jpba.2013.02.023

Casas, L., Fernández, M.F., Llop, S., Guxens, M., Ballester, F., Olea, N., Irurzun, M.B., Rodríguez, L.S.M., Riaño, I., Tardón, A., Vrijheid, M., Calafat, A.M., Sunyer, J., 2011. Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. Environ. Int. 37, 858–866. doi:10.1016/j.envint.2011.02.012

Casati, L., 2013. Epigenetics and PCBs. Endocr. Disruptors 1, e27347-1-e27347-4. doi:10.4161/endo.27347

Chen, C.-H., Jiang, S.S., Chang, I.-S., Wen, H.-J., Sun, C.-W., Wang, S.-L., 2018. Association between fetal exposure to phthalate endocrine disruptor and genome-wide {DNA} methylation at birth. Environ. Res. 162, 261–270. doi:https://doi.org/10.1016/j.envres.2018.01.009

Das, M.T., Ghosh, P., Thakur, I.S., 2014. Intake estimates of phthalate esters for South Delhi population based on exposure media assessment. Environ. Pollut. 189, 118–125. doi:10.1016/j.envpol.2014.02.021

De Cock, M., de Boer, M.R., Lamoree, M., Legler, J., van de Bor, M., 2014. First year growth in relation to prenatal exposure to endocrine disruptors - A dutch prospective cohort study. Int. J. Environ. Res. Public Health 11, 7001–7021. doi:10.3390/ijerph110707001

Demierre, A.L., Peter, R., Oberli, A., Bourqui-Pittet, M., 2012. Dermal penetration of bisphenol A in human skin contributes marginally to total exposure. Toxicol. Lett. 213, 305–308. doi:10.1016/j.toxlet.2012.07.001

Dodson, R.E., Nishioka, M., Standley, L.J., Perovich, L.J., Brody, J.G., 2012. Review Endocrine Disruptors and Asthma-Associated Chemicals in Consumer Products 120, 935–944.

ECHA, 2010. Review of new available information for Bis (2-Ethylhexyl) Phthalate (DEHP). Evaluation of new scientific evidence concerning the restrictions contained in Annex XVII to Regulation (Ec) No 1907/2006 (Reach). ECHA 2006, 1–24.

EFSA, 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: Executive summary. EFSA J. 13, 1–621. doi:doi:10.2903/j.efsa.2015.3978

EFSA, 2013. Draft Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs– Part: exposure assessment. EFSA J. 314. doi:No EFSA-Q-2012-00423

Esteve, C., Herrero, L., Gómara, B., Quintanilla-López, J.E., 2016. Fast and simultaneous determination of endocrine disrupting compounds by ultra-high performance liquid chromatography-tandem mass spectrometry. Talanta 146, 326–334. doi:10.1016/j.talanta.2015.08.064

Fisher, J.W., Twaddle, N.C., Vanlandingham, M., Doerge, D.R., 2011. Pharmacokinetic modeling : Prediction and evaluation of route dependent dosimetry of bisphenol A in monkeys with extrapolation to humans. Toxicol. Appl. Pharmacol. 257, 122–136. doi:10.1016/j.taap.2011.08.026

Franzen, A., Van Landingham, C., Greene, T., Plotzke, K., Gentry, R., 2016. A global human health risk assessment for Decamethylcyclopentasiloxane (D5). Regul. Toxicol. Pharmacol. 74, S25–S43. doi:10.1016/j.yrtph.2015.10.023

Fromme, H., Lahrz, T., Piloty, M., Gebhart, H., Oddoy, A., Ruden, H., 2004. Occurrence of phthalates and musk fragrances in indoor air and dust from apartments and kindergartens in Berlin (Germany). Indoor Air 14, 188–195. doi:10.1111/j.1600-0668.2004.00223.x

Gauderat, G., Picard-Hagen, N., Toutain, P.L., Corbel, T., Viguié, C., Puel, S., Lacroix, M.Z., Mindeguia, P., Bousquet-Melou, A., Gayrard, V., 2016. Bisphenol A glucuronide deconjugation is a determining factor of fetal exposure to bisphenol A. Environ. Int. 86, 52–59. doi:10.1016/j.envint.2015.10.006

Geens, T., Aerts, D., Berthot, C., Bourguignon, J.P., Goeyens, L., Lecomte, P., Maghuin-Rogister, G., Pironnet, A.M., Pussemier, L., Scippo, M.L., Van Loco, J., Covaci, A., 2012. A review of dietary and non-dietary exposure to bisphenol-A. Food Chem. Toxicol. doi:10.1016/j.fct.2012.07.059

Geens, T., Roosens, L., Neels, H., Covaci, A., 2009. Assessment of human exposure to Bisphenol-A, Triclosan and Tetrabromobisphenol-A through indoor dust intake in Belgium. Chemosphere 76, 755–760. doi:10.1016/j.chemosphere.2009.05.024

Gentry, P.R., Covington, T.R., Clewell, H.J., 2003. Evaluation of the potential impact of pharmacokinetic differences on tissue dosimetry in offspring during pregnancy and lactation. Regul. Toxicol. Pharmacol. 38, 1–16. doi:10.1016/S0273-2300(03)00047-3

Ginsberg, G.L., Belleggia, G., 2017. Use of Monte Carlo analysis in a risk-based prioritization of toxic constituents in house dust. Environ. Int. 1–13. doi:10.1016/j.envint.2017.06.009

Giovanoulis G., Bui T., Xu F., Papadopoulou E., Padilla-Sanchez J.A., Covaci A., Haug L.S., Cousins A.P., Magnér J., Cousins I.T., de W.C.A., 2018. Multi-pathway human exposure assessment of phthalate esters and DINCH Georgios Giovanoulis. Environ. Int. 112, 115–126. doi:10.1016/j.envint.2017.12.016

Giulivo, M., Lopez, M., Alda, D., Capri, E., Barceló, D., 2016. Human exposure to endocrine disrupting compounds : Their role in reproductive systems , metabolic syndrome and breast cancer . A review. Environ. Res. 151, 251–264. doi:10.1016/j.envres.2016.07.011

Go, B., Herrero, L., Ramos, J.J., Mateo, J.R., Ferna, M.A., Gonza, M.J., 2007. Distribution of Polybrominated Diphenyl Ethers in Human Umbilical Cord Serum , Paternal Serum , Maternal Serum , Placentas , and Breast Milk from Madrid Population , Spain 41, 6961–6968.

Guo, Y., Kannan, K., 2013. A survey of phthalates and parabens in personal care products from the United States and its implications for human exposure. Environ. Sci. Technol. 47, 14442–9. doi:10.1021/es4042034

Guo, Y., Wang, L., Kannan, K., 2013. Phthalates and parabens in personal care products from China: Concentrations and human exposure. Arch. Environ. Contam. Toxicol. 66, 113–119. doi:10.1007/s00244-013-9937-x

Heindel, J.J., Blumberg, B., Cave, M., Machtinger, R., Mantovani, A., Mendez, M.A., Nadal, A., Palanza, P., Panzica, G., Sargis, R., Vandenberg, L.N., vom Saal, F., 2017. Metabolism disrupting chemicals and metabolic disorders. Reprod. Toxicol. 68, 3–33. doi:10.1016/j.reprotox.2016.10.001

Institut d'Estadística de Catalunya, 2012. Enquesta de l'ús del temps 2010-2011: principals resultats. Estadística Soc. 155.

Koo, H.J., Lee, B.M., 2004. Estimated Exposure To Phthalates in Cosmetics and Risk Assessment. J. Toxicol. Environ. Heal. Part A 67, 1901–1914. doi:10.1080/15287390490513300

Kubwabo, C., Rasmussen, P.E., Fan, X., Kosarac, I., Grenier, G., Coleman, K., 2016. Simultaneous quantification of bisphenol A, alkylphenols and alkylphenol ethoxylates in indoor dust by gas chromatography-tandem mass spectrometry and a comparison between two sampling techniques. Anal. Methods 8, 4093–4100. doi:10.1039/C6AY00774K

Langer, S., Bekö, G., Weschler, C.J., Brive, L.M., Toftum, J., Callesen, M., Clausen, G., 2014. Phthalate metabolites in urine samples from Danish children and correlations with phthalates in dust samples from their homes and daycare centers. Int. J. Hyg. Environ. Health. doi:10.1016/j.ijheh.2013.03.014

Larsson, K., Ljung, K., Palm, B., Wennberg, M., Kaj, L., Lindh, C.H., J??nsson, B.A.G., Berglund, M., 2014. Exposure determinants of phthalates, parabens, bisphenol A and triclosan in Swedish mothers and their children. Environ. Int. 73, 323–333. doi:10.1016/j.envint.2014.08.014

Latini, G., De Felice, C., Presta, G., Del Vecchio, A., Paris, I., Ruggieri, F., Mazzeo, P., 2003. Exposure to Di(2-ethylhexyl)phthalate in humans during pregnancy: A preliminary report. Biol. Neonate 83, 22–24. doi:10.1159/000067012

Li, L., Chen, L., Meng, X., Chen, B., Chen, S., Zhao, Y., Zhao, L., 2013. Exposure Levels of Environmental

Endocrine Disruptors in Mother-Newborn Pairs in China and Their Placental Transfer Characteristics 8, 1–9. doi:10.1371/journal.pone.0062526

Liao, C., Kannan, K., 2014. A survey of alkylphenols, bisphenols, and triclosan in personal care products from China and the United States. Arch. Environ. Contam. Toxicol. 67, 50–59. doi:10.1007/s00244-014-0016-8

Loccisano, A.E., Longnecker, M.P., Campbell, J.L., Andersen, M.E., Clewell, H.J., 2013. Development of Pbpk Models for PFOA and PFOS for Human Pregnancy and Lactation Life Stages. J. Toxicol. Environ. Heal. Part A 76, 25–57. doi:10.1080/15287394.2012.722523

Loganathan, S.N., Kannan, K., 2011. Occurrence of bisphenol a in indoor dust from two locations in the Eastern United States and implications for human exposures. Arch. Environ. Contam. Toxicol. 61, 68–73. doi:10.1007/s00244-010-9634-y

Loretz, L., Api, A.M., Barraj, L., Burdick, J., Davis, D.A., Dressler, W., Gilberti, E., Jarrett, G., Mann, S., Laurie Pan, Y.H., Re, T., Renskers, K., Scrafford, C., Vater, S., 2008. Exposure data for personal care products: Hairspray, spray perfume, liquid foundation, shampoo, body wash, and solid antiperspirant. Food Chem. Toxicol. 44, 2008–2018. doi:10.1016/j.fct.2006.06.029

Loretz, L.J., Api, A.M., Babcock, L., Barraj, L.M., Burdick, J., Cater, K.C., Jarrett, G., Mann, S., Pan, Y.H.L., Re, T.A., Renskers, K.J., Scrafford, C.G., 2008. Exposure data for cosmetic products: Facial cleanser, hair conditioner, and eye shadow. Food Chem. Toxicol. 46, 1516–1524. doi:10.1016/j.fct.2007.12.011

Loretz, L.J., Api, A.M., Barraj, L.M., Burdick, J., Dressler, W.E., Gettings, S.D., Han Hsu, H., Pan, Y.H.L., Re, T.A., Renskers, K.J., Rothenstein, A., Scrafford, C.G., Sewall, C., 2005. Exposure data for cosmetic products: Lipstick, body lotion, and face cream. Food Chem. Toxicol. 43, 279–291. doi:10.1016/j.fct.2004.09.016

Lu, S., Yu, Y., Ren, L., Zhang, X., Liu, G., Yu, Y., 2017. Estimation of intake and uptake of bisphenols and triclosan from personal care products by dermal contact. Sci. Total Environ. 1–8. doi:10.1016/j.scitotenv.2017.10.088

Lv, Y., Lu, S., Dai, Y., Rui, C., Wang, Y., Zhou, Y., Li, Y., Pang, Q., Fan, R., 2017. Higher dermal exposure of cashiers to BPA and its association with DNA oxidative damage. Environ. Int. 98, 69–74. doi:10.1016/j.envint.2016.10.001

Mari, M., Nadal, M., Schuhmacher, M., Domingo, J.L., 2009. Exposure to heavy metals and PCDD/Fs by the population living in the vicinity of a hazardous waste landfill in Catalonia, Spain: Health risk assessment. Environ. Int. 35, 1034–1039. doi:10.1016/j.envint.2009.05.004

Martínez, M.A., Rovira, J., Sharma, R.P., Nadal, M., Schuhmacher, M., Kumar, V., 2017. Prenatal exposure estimation of BPA and DEHP using integrated external and internal dosimetry: A case study. Environ. Res. 158, 566–575. doi:10.1016/j.envres.2017.07.016

May, L.M., Gaborek, B., Pitrat, T., Peters, L., 2002. Derivation of risk based wipe surface screening levels for industrial scenarios. Sci. Total Environ. 288, 65–80. doi:10.1016/S0048-9697(01)01117-2

McNamara, C., Rohan, D., Golden, D., Gibney, M., Hall, B., Tozer, S., Safford, B., Coroama, M., Leneveu-Duchemin, M.C., Steiling, W., 2007. Probabilistic modelling of European consumer exposure to cosmetic products. Food Chem. Toxicol. 45, 2086–2096. doi:10.1016/j.fct.2007.06.037

Meeker, J., 2012. Exposure to environmental endocrine disruptors and child development. Arch. Pediatr. Adolesc. … 166, E1–E7. doi:10.1001/archpediatrics.2012.241.Exposure

Mielke, H., Gundert-Remy, U., 2009. Bisphenol A levels in blood depend on age and exposure. Toxicol. Lett. 190, 32–40. doi:10.1016/j.toxlet.2009.06.861

Mielke, H., Partosch, F., Gundert-Remy, U., 2011. The contribution of dermal exposure to the internal exposure of bisphenol A in man. Toxicol. Lett. 204, 190–198. doi:10.1016/j.toxlet.2011.04.032

Myridakis, A., Chalkiadaki, G., Fotou, M., Kogevinas, M., Chatzi, L., Stephanou, E.G., 2016. Exposure of Preschool-Age Greek Children (RHEA Cohort) to Bisphenol A, Parabens, Phthalates, and Organophosphates. Environ. Sci. Technol. 50, 932–941. doi:10.1021/acs.est.5b03736

Ribeiro, E., Ladeira, C., Viegas, S., 2017. EDCs Mixtures: A Stealthy Hazard for Human Health? Toxics 5, 5. doi:10.3390/toxics5010005

Robinson, L., Miller, R., 2015. The Impact of Bisphenol A and Phthalates on Allergy, Asthma, and Immune Function: a Review of Latest Findings. Curr. Environ. Heal. reports 2, 379–87. doi:10.1007/s40572-015-0066-8

Rovira, J., Roig, N., Nadal, M., Schuhmacher, M., Domingo, J.L., 2016. Human health risks of formaldehyde indoor levels: An issue of concern. J. Environ. Sci. Heal. Part a-Toxic/Hazardous Subst. Environ. Eng. 51, 357–363. doi:10.1080/10934529.2015.1109411

Sakhi, A.K., Lillegaard, I.T.L., Voorspoels, S., Carlsen, M.H., Løken, E.B., Brantsæter, A.L., Haugen, M., Meltzer, H.M., Thomsen, C., 2014. Concentrations of phthalates and bisphenol A in Norwegian foods and beverages and estimated dietary exposure in adults. Environ. Int. doi:10.1016/j.envint.2014.08.005

Salapasidou, M., Samara, C., Voutsa, D., 2011. Endocrine disrupting compounds in the atmosphere

of the urban area of Thessaloniki, Greece. Atmos. Environ. 45, 3720–3729. doi:10.1016/j.atmosenv.2011.04.025

Sathyanarayana, S., Calafat, A.M., Liu, F., Swan, S.H., 2008. Maternal and infant urinary phthalate metabolite concentrations: Are they related? Environ. Res. 108, 413–418. doi:10.1016/j.envres.2008.07.002

SCCS, 2010. The SCCS's notes of guidance for the testing of cosmetic ingredients. Sci. Comm. Consum. Saf. SCCS 112.

Schuhmacher, M., Meneses, M., Xifr, A., Domingo, J.L., 2001. The use of Monte-Carlo simulation techniques for risk assessment: study of a municipal waste incinerator. Chemosphere 43, 787–799.

Sharma, R.P., Schuhmacher, M., Kumar, V., 2018. The development of a pregnancy PBPK Model for Bisphenol A and its evaluation with the available biomonitoring data. Sci. Total Environ. 624, 55–68. doi:10.1016/j.scitotenv.2017.12.023

Sharma, R.P., Schuhmacher, M., Kumar, V., 2017. Developing Integrated PBPK/PD Coupled mechanistic pathway model (miRNA-BDNF): an approach towards System toxicology. Toxicol. Lett. 280, 79–91. doi:10.1016/j.toxlet.2017.08.003

Sharma, R.P., Schuhmacher, M., Kumar, V., 2016. Review on crosstalk and common mechanisms of endocrine disruptors: Scaffolding to improve PBPK/PD model of EDC mixture. Environ. Int. 14. doi:10.1016/j.envint.2016.09.016

US Environmental Protection Agency, 2011. Exposure Factors Handbook: 2011 Edition. U.S. Environ. Prot. Agency EPA/600/R-, 1–1466. doi:EPA/600/R-090/052F

Völkel, W., Colnot, T., Csanády, G.A., Filser, J.G., Dekant, W., 2002. Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. Chem. Res. Toxicol. 15, 1281–1287. doi:10.1021/tx025548t

Volle, D.H., Maggi, A., Casati, L., Sendra, R., Sibilia, V., Celotti, F., 2015. Endocrine disrupters: the new players able to affect the epigenome Epigenetics: What are the Mechanisms? Front. Cell Dev. Biol. 3. doi:10.3389/fcell.2015.00037

von Goetz, N., Pirow, R., Hart, A., Bradley, E., Poças, F., Arcella, D., Lillegard, I.T.L., Simoneau, C., van Engelen, J., Husoy, T., Theobald, A., Leclercq, C., 2017. Including non-dietary sources into an exposure assessment of the European Food Safety Authority: The challenge of multi-sector chemicals such as Bisphenol A. Regul. Toxicol. Pharmacol. 85, 70–78. doi:10.1016/j.yrtph.2017.02.004

Watkins, D.J., Sánchez, B.N., Téllez-Rojo, M.M., Lee, J.M., Mercado-García, A., Blank-Goldenberg, C., Peterson, K.E., Meeker, J.D., 2017. Phthalate and bisphenol A exposure during in utero windows of susceptibility in relation to reproductive hormones and pubertal development in girls. Environ. Res. 159, 143–151. doi:10.1016/j.envres.2017.07.051

WHO, F. and A.O. of the U.N., 2010. Toxicological and Health Aspects of Bisphenol A. World Heal. Organ. 60.

Wormuth, M., Scheringer, M., Vollenweider, M., Hungerbühler, K., 2006. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? Risk Anal. 26, 803–824. doi:10.1111/j.1539-6924.2006.00770.x

Zhu, Y., Gao, H., Huang, K., Zhang, Y., Cai, X., Yao, H., Mao, L., Ge, X., Zhou, S., Xu, Y., Jin, Z., Sheng, J., Yan, S., Pan, W., Hao, J., Zhu, P., Tao, F., 2018. Prenatal phthalate exposure and placental size and shape at birth: A birth cohort study. Environ. Res. 160, 239–246. doi:10.1016/j.envres.2017.09.012

CHAPTER 2 BIOMONITORING

PUBLICATION 3.

M.A. Martínez, J. Rovira, R. Prasad Sharma, V. Kumar, M. Schuhmacher. **Reconstruction of phthalate exposure and DINCH metabolites from biomonitoring data from the EXHES cohort of Tarragona, Spain: a case study on Estimated vs Reconstructed DEHP using the PBPK model.** *Environmental Research. In press.*

PUBLICATION 4.

J. Rovira; M.A. Martínez; T. espuis; M. Nadal.; V. kumar; D. Costopoulou; I. Vassiliadou; L. Leondiadis; J.L. Domingo; M. Schuhmacher. **Prenatal exposure to PFOS and PFOA in a Catalan pregnant women cohort**. *Environmental Research, 175* (2019) 384 – 392.

PUBLICATION 5.

M.A. Martínez; I. Castro; J. Rovira; S. Ares; J.M. Rodríguez; S.C. Cunha; S. Casal; J. Olivera-Fernandes; M. Schuhmacher; M. Nadal. **Early-life intake of major trace elements, bisphenol A, tetrabromobisphenol A and fatty acids: Comparing human milk and commercial infant formulas.** *Environmental Research,* 169 (2018) 246 – 255

PUBLICATION 3.

Reconstruction of phthalate exposure and DINCH metabolites from biomonitoring data from the EXHES cohort of Tarragona, Spain: a case study on Estimated vs Reconstructed DEHP using the PBPK model

M. A. Martínez[a], J. Rovira[a,b], R. Prasad Sharma[a], V. Kumar[a,b], M. Schuhmacher[a,b].

[a]Environmental Engineering Laboratory, Departament d'Enginyeria Quimica, Universitat Rovira i Virgili, Av. Països Catalans 26, 43007 Tarragona, Catalonia, Spain.
[b]Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Catalonia, Spain.

Environmental Research,

In press

Abstract

Phthalates are known endocrine disruptors (EDs) and are associated with potential diseases, such as obesity and diabetes. As an alternative to phthalates, in 2002 the plasticizer 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH) was introduced in the European market. The objective of this study was to evaluate the total exposure to phthalate and DINCH metabolites from EXHES Tarragona, Spain cohort of pregnant women. On the one hand, the analytical determination of phthalate and DINCH metabolites in urine was carried out. On the other hand, the reconstructed exposure was calculated for phthalates and DINCH using their metabolites concentration measured in the urine. Thirteen different phthalate metabolites and two metabolites of DINCH were measured and detected in almost all pregnant women's urine samples (n=60). The statistical analysis showed positive significant Pearson's correlations between metabolites that came from the same parent compound, and also between DEHP and MBzP metabolites, between DiNP and BBZP metabolites, and between DEHP and DiNP metabolites. The exposure of pregnant women to phthalate and DINCH parent compounds were also backcalculated using the levels of each metabolite found in pregnant women urine (reconstructed exposure). In addition, to demonstrate the utility of this approach, the physiologically based pharmacokinetic (PBPK) model was used to predict the cumulative amount of MEHP (a principal metabolite of DEHP in urine). To proceed with that, DEHP reconstructed exposure and estimated exposure from the same cohort (previously studied by the same authors) were simulated using the PBPK model. Results showed that the reconstructed-PBPK simulation was closer to the 24 h biomonitoring data than the estimated PBPK-simulation. Consequently, the combination of reconstructed exposure with the PBPK model is a good tool to predict chemicals exposure. However, some discrepancies between simulated and biomonitored values were found. This can be associated with other sources that contribute to the total exposure and emphasises the need to consider multi-routes exposure for the widely distributed chemicals like phthalates and DINCH.

Keywords

Phthalates, DINCH, Reconstructed exposure, Estimated exposure, PBPK model, pregnant biomonitoring.

1. Introduction

Phthalates are esters of phthalic acid and they are mainly used as plasticizers, substances added to plastics to increase their flexibility, transparency, durability, and longevity (Guo, et. al., 2011). They are used primarily to soften polyvinyl chloride (PVC) (Arbuckle et al., 2016). Human exposure to phthalates is widespread and occurs mostly through ingestion, inhalation, and dermal contact (Martínez et al., 2018). Phthalates are easily released from the products that contain them to the environment. In general, they do not persist due to rapid biodegradation, photodegradation, and anaerobic degradation (Rudel and Perovich, 2009).

Phthalates are known endocrine disruptors (EDs); they can mimic or block naturally occurring hormones in our body, changing the final cellular response (Thomas Zoeller et al., 2012). Prenatal exposure to these EDs is associated with potential diseases, diabetes and insulin resistance, breast cancer, obesity, metabolic disorders, and immune function (Bansal et al., 2018). Despite a quick metabolization, they can reach the fetus by crossing the placenta barrier (Li et al., 2013). As an alternative to phthalates, in 2002 the plasticizer 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH) was introduced in the European market. This is a plasticizer for the manufacture of flexible plastic articles in sensitive application areas such as toys, medical devices, and food packaging. It belongs to the group of aliphatic esters (Giovanulis et al., 2018). Currently available toxicological data suggest that DINCH does not induce reproductive toxicity or endocrine disruption (EFSA,2008). However, human exposure to this chemical rises considerably in the last years, and exposure data of DINCH are limited (Giovanoulis et al., 2018).

The diet is believed to be the main source of bis (2-ethylhexyl) phthalate (DEHP) and other phthalates in the general population (Martínez et al., 2018). Low-molecular-weight phthalates such as diethyl phthalate (DEP), di-n-butyl phthalate (DBP), and benzylbutyl phthalate (BBzP) may be dermally absorbed, and the more volatile phthalates, such as dimethyl phthalate (DMP) and dibutyl phthalate (DBP) may be air inhaled or ingested through dust (Heudorf and Mersch-sundermann, 2007). DEP and dimethyl phthalate (DMP) are present in higher concentrations in the air in comparison with the heavier and less volatile DEHP. Outdoor air concentrations are higher in urban and suburban areas than in rural and remote areas (Rudel and Perovich, 2009). In addition, higher concentrations of these phthalates in the air are related to higher temperatures (Diamanti-kandarakis et al., 2009). Nowadays, phthalates are also found very widely in PVC flooring. The most common phthalates found are butyl benzyl phthalate (BBP) and DEHP, which are more prevalent in the dust (Fromme et al., 2004).

In other previous studies from our group (Martínez et al., 2017, 2018), we calculated the total estimated exposure to phthalates through the method based on questionnaires and personal interviews. This method considered the personalised data like the diet habits, uses of personal care products, and lifestyle. In addition, the inventory of concentration levels of DEHP in dietary and non-dietary items from the literature were also considered. More detailed information can be found in Martínez et al. (2018). However, limited information on other routes of exposure (such as dermal and inhalation) can underestimate the total exposure and internal dose. The most ideal approach for investigating human exposure to different chemicals is biomonitoring. As biomonitoring data integrates all routes (oral, inhalation, dermal and dust ingestion) and sources of exposure (i.e. including occupational, environmental and lifestyle factors such as diet, smoking and hobbies), it can provide a valuable perspective to help evaluate aggregate exposure to chemicals (Angerer et al., 2006, 2007; Pirkle et al., 1995). Human biomonitoring can be an effective tool for assessing exposure i.e. measuring chemicals and their metabolites in usually, blood or urine and then use this data to reconstruct the exposure amount by using either a simple approach or a reverse physiologically based pharmacokinetic (PBPK) model (Diamanti-

kandarakis et al., 2009; Johns et al., 2015). The compounds like phthalates and DINCH have several metabolites and they have very short half-lives between 3 and 18 h (Johns et al., 2015). For such cases, it has been recommended that these metabolites concentrations in urine should also be measured instead of only parent compound concentrations.

This study aimed to evaluate the total exposure to phthalates and DINCH from EXHES Tarragona, Spain cohort of pregnant women. On the one hand, analytical determination of phthalate and DINCH metabolites in urine was carried out. On the other hand, the reconstructed exposure was calculated for phthalates and DINCH using their metabolite concentrations measured in the urine. To demonstrate the utility of such a simple deterministic reconstruction approach, a PBPK model was used to predict the cumulative amount of MEHP (a principal metabolite of DEHP in urine).

Thus, the objective of the current study comprised the following tasks: the first task was to determine the phthalate and DINCH metabolites in spot urine at 12^{th} and 32^{nd} gestational weeks (GW) for each person. The second task was to reconstruct the exposure of different phthalates and DINCH from the spot urine concentrations. The third task was to run the forward PBPK model to calculate the MEHP cumulative amounts per day in the urine. The fourth task was to compare the current PBPK simulated MEHP cumulative concentration for reconstructed dose with the previously PBPK simulated MEHP cumulative concentration for an estimated dose (Martínez et al., 2018). Subsequently, both simulations, reconstructed and estimated were compared with the measured MEHP in the current biomonitoring study.

2. Materials and Methods

2.1 EXHES Tarragona, Spain cohort study

In the EXHES Tarragona, Spain, cohort, pregnant women were recruited during the first trimester of pregnancy as part of the European “HEALS” project. The recruitment of pregnant mothers started in March 2016 and, in the present study, 60 mother-child pairs were included. Women were informed of the study during their first visit (12^{th} GW) to the

University Hospital "Sant Joan de Reus", in Reus (Catalonia, Spain). Women were eligible to participate according to the following inclusion criteria: ≥16 years old, intention to deliver at the reference hospital, and no problems with the communication language. This study was approved by the Ethical Committee of Clinical Research of the Hospital and written informed consent was obtained from the participants.

2.2 Questionnaires data acquisition and sampling

At 12th and 32nd GW, face-to-face interviews were done in order to know their habits, diet, lifestyle and general characteristics data of the pregnant mothers. More detail information about these questionnaires is described in Martínez et al. (2018). During their visits to the hospital, urine samples were collected in the first and third trimester of pregnancy between 2016 and 2017 and stored at -80 ˚C until analytical determination. The summary of the characteristics of the study population is shown in **Table 1**.

Table 1. Characteristics of the study population from from EXHES Tarragona, Spain, cohort (n=60).

Characteristics of the study population (n = 60)	**%**
Maternal age at delivery (years)	
< 20	1
20-29	14
30-39	72
>40	13
Twin pregnancy	8
*Maternal pre-pregnancy BMI**	
Underweight (<19 kg/m^2)	6
Normal (19-25 kg/m^2)	50
Overweight (>25-30 kg/m^2)	25
Obese (>30 kg/m^2)	19
*Maternal pregnancy (20 GW) BMI**	
Underweight (<19 kg/m^2)	1
Normal (19-25 kg/m^2)	41
Overweight (>25-30 kg/m^2)	37
Obese (>30 kg/m^2)	21
Maternal education	
Primary	28
Secondary	31
University	41

Social economic status	
Low level (<9,000-19,000 €/year)	24
Median level (19,000-35,000 €/year)	49
High level (>35,000 €/year)	27
Maternal country of origin	
Spain	76
Other	24
Marital Status	
Living with the father	99
Not living with the father	1
Characteristics of the study population (n = 60)	**%**
Maternal smoking	
Never smoke	73
Not during pregnancy	9
During pregnancy	18

**BMI= Body mass index*

2.3. Analytical determination of phthalate and DINCH metabolites

Thirteen different phthalate metabolites and two DINCH metabolites were determined in a total of 120 urine samples (60 from 12th GW and 60 from 32nd GW) from the 60 participants, using an on-line column switching liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) as described elsewhere (Sabaredzovic et al., 2015). In brief, labeled internal standard solution and enzyme solution (beta-glucuronidase in ammonium acetate buffer, pH = 6.5) were added to the urine sample (300 µL). The samples were incubated for 1.5 h at 37 °C. After 1.5 h, formic acid was added, the samples were centrifuged, and the supernatant was injected into the system. The limits of detection (LOD) were between 0.07 and 0.7 ng/mL. The accuracy of the method ranged from 70 % to 120 %. In-house pooled urine samples and standard reference material from the National Institute of Standards and Technology (NIST) were analysed along with the samples, and the margin of error was below 20 % for the phthalate and DINCH metabolites. **Table 2** provides information about the specific compounds measured. The phthalate and DINCH metabolites concentrations were adjusted by creatinine, measured by the Jaffé's method, using a Cobas autoanalyser. Sample replicates and standards were analysed. Correction of urinary phthalates and DINCH content by creatinine was proceeded to correct for variable

dilutions among spot samples (Barr et al., 2005). Some studies recognized that urinary creatinine is affected by age, sex, diet - particularly protein intake - and specific conditions like pregnancy (James et al., 1988). Consequently, adjustment by creatinine may provide higher values for urinary metabolites concentrations ($\mu g/g_{crea}$) than did the corresponding data expressed as µg/L. For this reason, we reported both values of urinary phthalates; for non-creatinine and creatinine adjusted levels.

Table 2. Phthalate and DINCH metabolites and their parent compound analysed

Compounds	**Abbreviation**	***Parent Compound**
Monoethyl phthalate	MEP	DEP
Mono-iso-butyl phthalate	MiBP	DiBP
Mono-n-butyl phthalate	MnBP	DnBP/ BBZP
Mono benzyl phthalate	MBzP	BBZP
Mono-ethyl phthalate	MEHP	DEHP
Mono-2-ethyl-5-hydroxyhexyl phthalate	MEHHP	DEHP
Mono-2-ethyl-5-oxohexyl phthalate	MEOHP	DEHP
Mono-2-ethyl 5-carboxypentyl phthalate	MECPP	DEHP
Mono-2-carboxymethyl hexyl phthalate	MMCHP	DEHP
Mono-4-methyl-7-hydroxyoctyl phthalate	OH-MiNP	DiNP
Mono-4-methyl-7-oxooctyl phthalate	oxo-MiNP	DiNP
Mono-4-methyl-7-carboxyoctyl phthalate	cx-MiNP	DiNP
6-Hydroxy Monopropylheptylphthalate	OH-MPHP	DPHP
2-(((Hydroxy-4methyloctyl) oxy) carbonyl) cyclohexanecarboxylic Acid	OH-MINCH	DINCH
2-(((4-Methyl-7-oxyooctyl) oxy) carbonyl) cyclohexanecarboxylic Acid	oxo-MINCH	DINCH

*DEP= Diethyl phthalate; DiBP= Diisobutyl phthalate; DnBP= Di-n-butyl phthalate; BBZP= Butyl benzyl phthalate; DEHP= bis (2-ethylhexyl) phthalate; DiNP= Diisononyl phthalate; DINCH= 1,2-Cyclohexane dicarboxylic acid diisononyl ester; DPHP= Di(2-propylheptyl) phthalate.

2.4. Reconstructed exposure

In the present study phthalate and DINCH metabolites were biomonitored in maternal urine from 60 pregnant women at the first and third trimester of pregnancy. The biomonitoring data were used to calculate the reconstructed exposure, this is the exposure to pregnant women to each parent compound calculated from the concentration levels of their metabolites found in urine.

To calculate the reconstructed exposure of each parent compound, the pharmacokinetic model of one compartment (the total body) was considered. The pharmacokinetic model can be reduced to a mathematical equation, which describes the transit of the compound throughout the body, a net balance sheet from absorption and distribution to metabolism and excretion. To calculate the reconstructed exposure of each parent compound, all their metabolites were considered. The following equation was used (Eq.1).

$$Reconstructed\ exposure\ (i) = \sum \left(\frac{C_m \times MW_{PC}}{MW_m} \right) \times \frac{Crea_d}{FUE} \qquad Eq.1$$

Where reconstructed exposure (i) is the individual exposure of the parent compound (phthalates and DINCH) for each person calculated from the levels of metabolites found in the urine (µg/kg_{bw}/d); C_m is the concentration of each metabolite from phthalates or DINCH adjusted by creatinine (in µg/$g_{creatinine}$); MW_{PC} is the molecular weight of each parent compound (in µg/mol); MW_m is the molecular weight of each metabolite (in µg/mol); $Crea_d$ is the creatinine excreted per day per weight (in g/µg/kg_{bw}/d) (Koppen et al., 2019); FUE is the Urinary Excretion Factor for each parent compound (**Table 3**).

Table 3. FUE for phthalate and DINCH parent compounds

Phthalate and DINCH parent compounds	**MW***	**FUE****	**Reference**
DEP	222.24	0.69	Anderson et al., 2001; Koch et al., 2003
BBzP	312.4	0.73	Anderson et al., 2001
DiBP	278.34	0.90	Koch et al., 2012
DnBP	278.34	0.91	Koch et al., 2012
DEHP	390.57	0.47	Anderson et al., 2011
DiNP	418.609	0.30	Anderson et al., 2011
DINCH	424,666	0.30	Analogy to DiNP

*MW= molecular weight (g/mol); **FUE= Urinary Excretion Factor (non-dimensional)

2.5. Physiologically-based pharmacokinetic (PBPK) modelling

The DEHP PBPK model was adapted from a previous study (Sharma et al., 2018; Martinez et al., 2017). All the physiological parameters during pregnancy are considered to be dynamic parameters that change due to the growth of mother organs (Abduljalil et al., 2012; Gentry et al., 2003; Loccisano et al., 2013). More detail of the model is described in (Martínez et al., 2017, 2018; Sharma et al., 2018).

The objective is to compare the predicted cumulative amount of MEHP in urine for both the reconstructed exposure (calculated in 2.4 section) and the previously estimated exposure calculated in Martinez et al., (2018) against the biomonitoring data using a forward PBPK. **Figure 1,** shows a flowchart with the different steps that were followed. All of these steps are explained below.

The spot urine sampling was carried out in the morning. This urine collected was used to calculate the total DEHP reconstructed dose (following the Eq.1, section 2.4). Then the total dose DEHP reconstructed exposure was divided considering the percentage of the contribution from the different routes based on a previous calculation by Martinez et al., (2018) i.e. 63 % oral, 5 % dermal, 2 % dust ingestion and 30 % inhalation.

Then the calculated oral, dermal, ingestion and inhalation routes' exposure data were used to run the PBPK model. Afterward, the PBPK simulated MEHP cumulative amount in urine for reconstructed exposure was obtained (Figure 1, letter a). The PBPK model was also simulated to predict the MEHP cumulative amount in urine for the estimated exposure (Martinez et al., 2018) (Figure 1, letter b).

The spot MEHP urine concentration was extrapolated to MEHP cumulative amount for 24 hours following equation 2 (Eq.2) (Mann and Gerber, 2010) (Figure 1, letter c).

$$24\ h\ MEHP\ excreted = \left(\frac{Spot\ urine\ [MEHP]}{Spot\ urine\ [crea]}\right) \times 24\ h\ crea\ excretion \qquad Eq.2$$

In this way, the PBPK simulated MEHP cumulative concentration for estimated dose (b) and PBPK simulated MEHP cumulative concentration for reconstructed (a) were compared with extrapolated MEHP cumulative concentration (biomonitoring data).

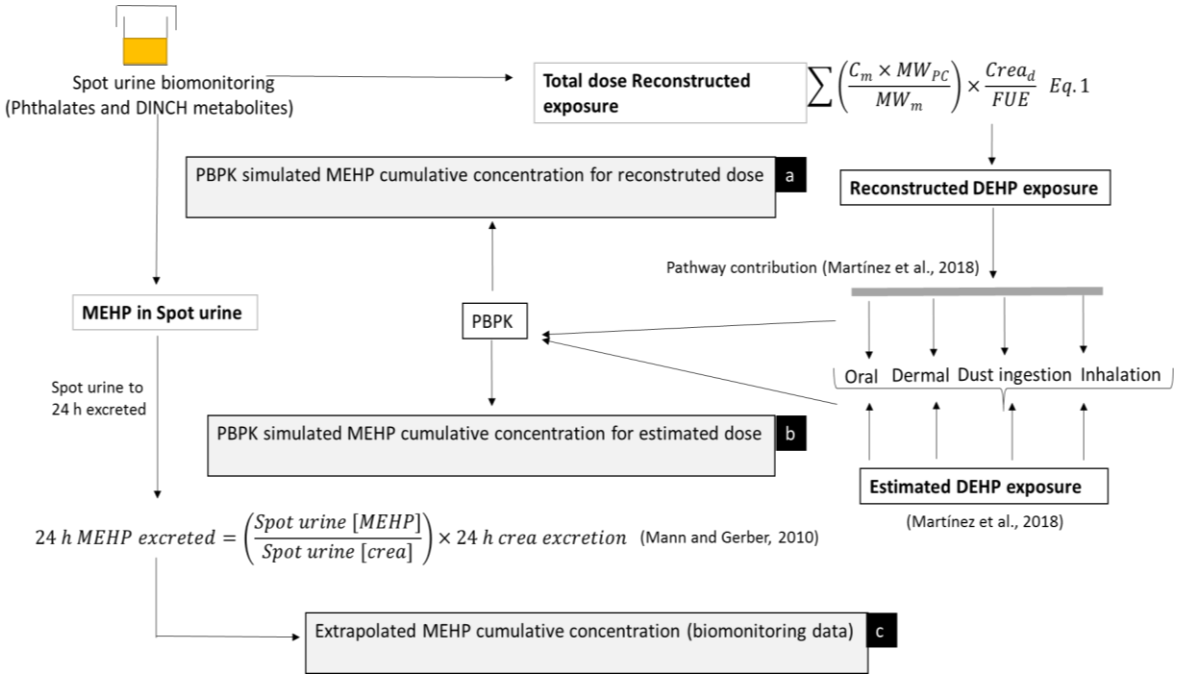


Figure 1. Flowchart with the different parts that include the simulation exposure of MEHP through the PBPK model.

2.6. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences software (SPSS v.25). Subsequently, Student's t-test, ANOVA (parametric data) or Kruskal-Wallis (non-parametric data) test were applied. A significance level of 0.05 ($p<0.05$) was established.

3. Results and discussion

3.1 Levels of Phthalate and DINCH metabolites and parent compounds

A total of 13 phthalate metabolites and 2 DINCH metabolites from urine samples from 12th and 32nd GW from 60 pregnant mothers were measured. **Table 4** summarizes the phthalate and DINCH levels non-corrected (µg/L) and corrected (µg/g_{crea}) by the creatinine levels. Mean, standard deviation (SD), percentile 5, 50 and 95 (P5, P50, and P95, respectively) are given. All phthalate and DINCH metabolites were found in almost all (94%) urine samples. Only MEHP levels were found below LOD (0.20 µg/L) in one sample from the 1st trimester and seven samples from the 3rd trimester. The most abundant individual metabolite was MEP, a DEP metabolite with a mean concentration of 248 and 714 µg/L in 12th and 32nd gestational weeks, respectively. For both periods, 12th and 32nd gestational week, the highest levels after MEP correspond to MECPP (16.6 and 24 µg/L), cx-MiNP (15.9 and 19.91 µg/L), MiBP (15.2 and 13.7µg/L) and MnBPP (13.4 and 20.5 µg/L). There were no significant differences ($p>0.05$) in any phthalate and DINCH metabolite urine levels (creatinine corrected or not) between the first (12nd GW) and the third trimester (32nd GW).

Significant positive Pearson's correlations, around or above 0.9 ($p<0.01$), were found between DEHP metabolites (MEHP, MEHHP, MEOHP, and MECPP), between DiNP metabolites (OH-MiNP, oxo-MiNP, cx-MiNP) and between DINCH metabolites (OH-MINCH and oxo-MINCH) (Figure 2). These correlations were expected because each metabolite family shares the same parent compound. However, other significant positive correlations were found between DEHP and BBZP metabolites, DiNP and BBZP metabolites, and DEHP and DiNP metabolites with a Pearson's coefficient between 0.6 and 0.8 at $p<0.05$. Regarding

these correlations, these results are in agreement with other studies (Wang et al., 2019), that affirmed that in most commercial products, it is easy to find mixtures of DEHP, DiNP, and MBzP (a metabolite of the BBZP). These compounds are used as additives, and they can easily migrate into the environment through evaporation, leaching, and abrasion. DINCH is being introduced as a substitute for phthalates, so it is being used for the same kind of containers or plastic recipients due to their similar physicochemical properties. Consequently, it is possible to find some metabolites of them in similar products as mixtures. In addition, it could be possible to find these significant correlations because the high molecular weight phthalates are used in many applications (PVC polymers and plastisol applications, plastics, food packaging, and food processing materials, vinyl toys and vinyl floor coverings, and building products); in this group are included DEHP, DiNP, and MBzP among others (North et al., 2014). These products are in our day to day life and for that reason, the exposure to these chemicals is reflected in the detection of mixtures in the urine.

Phthalate and DINCH metabolites levels in urine were compared with maternal characteristic (BMI, education, income, etc.), new-borns characteristics (birth weights and height at birth), dietary habits (fast-food, milk, yogurt, tetra brick consumption, etc.) and lifestyle habits (like smoking or alcohol consumption). No significant differences ($p>0.05$) were found in phthalate and DINCH metabolites according to maternal characteristics or lifestyle habits. However, significant differences ($p<0.05$) were found in OH-MPHP urine levels across yogurt consumption groups; and in OH-MINCH and oxo-MINCH urine levels regarding the weight of new-borns at the delivery. Zero servings per week and between 1 to 3 servings per week of yogurt were related to higher ($p<0.05$) urinary levels of OH-MPHP (3.88 and 4.18 $\mu g/g_{crea}$, respectively) than moderate servings (4 to 6 servings a week) or high number of servings (7 or more servings a week) (2.11 and 2.38 $\mu g/g_{crea}$, respectively). In addition, higher maternal urine levels of OH-MINCH (16.3 vs. 2.38 $\mu g/g_{crea}$) and oxo-MINCH (10.5 vs. 1.48 $\mu g/g_{crea}$) were related with lower weight at birth (1600-2500 g) than those with a higher weight (3600-4500 g). However, to our knowledge, there was no information in the literature about DINCH metabolites and weight at delivery. Meanwhile, some studies

affirm that higher levels of phthalates for pregnant women are related to lower weight at birth (Song et al., 2018; Zhao et al., 2014). According with that, in this study, some differences were found between low and high birth weight for MiBP (26.5 vs. 17.5 $\mu g/g_{crea}$), MEHP (10.5 vs. 5.52 $\mu g/g_{crea}$), MEHHP (34.5 vs. 12.0 $\mu g/g_{crea}$), MEOHP (24.0 vs. 8.30 $\mu g/g_{crea}$), MECCP (78.9 vs. 19.3 $\mu g/g_{crea}$), MMCHP (39.7 vs. 13.3 $\mu g/g_{crea}$), and OH-MPHP (5.93 vs. 3.19 $\mu g/g_{crea}$), but without statistical significance ($p>0.05$). Nowadays it is known that phthalates are potential endocrine disruptors and for that reason, they are being replaced by DINCH chemicals (Giovanulis et al., 2018). Therefore, it could be quite early to know what are going to be the side effects of them. Regarding yogurt consumption, the results show that greater consumption of yogurts during the week was related to lower levels of OH-MPHP in urine samples. There is no information about that in the bibliography; for that reason, more exhaustive studies are needed in order to understand that new finding. Some authors associate the consumption of food in a plastic container with a higher presence of phthalates in the urine. However, DEHP used to be the most frequently related to that and can be detected in urine (Dong et al., 2017; Petersen and Jensen, 2010). Otherwise, OH-MPHP is recently increasing its production volume and its wide field of application, for that reason, this must be analysed more rigorously. In addition, it is well-known that the dairy-products are recommended for a pregnant woman to maintain optimal levels of calcium in the body in order to prevent adverse gestational outcomes (WHO, 2013). For that reason, they try to increase the consumption of dairy products, which are frequently in plastic containers. Consequently, further study on this will be extremely necessary to confirm and interpret the results obtained. Finally, it is known that milk products should not be avoided (even by lactose-intolerant individuals) as they have important health benefits, including a potential beneficial impact on gut microbiota; so maybe another possibility could be that the microbiota can be related with some enzymes which metabolized the parent compound of DPHP to OH-MPHP. Anyway, to affirm this interpretation, going deeper into the study about microbiota behaviour and more scientific evidence would be necessary.

Levels of phthalate and DINCH metabolites in the urine of pregnant women found in several studies performed around the world are summarized in **Table 5**. Afterward, comparing the

levels of the metabolites of the present study with other studies, it was found that our results were in the same order of magnitude as the results of a previous study from Spain (Casas et al., 2016). However, some metabolites were higher in this previous study. It could be very interesting if DINCH metabolites from Casas et al., (2016) were available, in that way we could see if those higher levels of phthalate that they found are reflected in our study as lower due to an increase in DINCH metabolites. In general terms, it can be observed that our results are in the same line as other results from other countries over the world. Anyway, it is important to say that it is not usual to find studies with more than 3 or 4 metabolites measured in urine, and this is also more complicated regarding only those who consider pregnant women's urine.

Table 4. Phthalate metabolites (13) and DINCH metabolites (2) levels in urine. Non-creatinine adjusted (in µg/L) and creatinine-adjusted (in $\mu g/g_{crea}$) levels

12th GW	(n=60)														
Non-crea. adjusted	**MEP**	**MiBP**	**MnBP**	**MBzP**	**MEHP**	**MEHHP**	**MEOHP**	**MECPP**	**MMCHP**	**OH-MPHP**	**OH-MiNP**	**oxo-MiNP**	**cx-MiNP**	**OH-MINCH**	**oxo-MINCH**
% Detected	99	100	100	100	99	100	100	100	98	100	100	100	100	100	100
Mean	248	15.2	13.4	2.60	2.96	8.61	6.17	16.6	9.10	2.09	9.65	4.13	15.9	3.30	1.94
SD	639	18.7	18.9	3.19	5.07	14.5	9.88	36.0	14.2	2.70	14.0	5.29	21.9	9.22	5.79
P5	3.80	2.21	1.82	0.29	0.65	1.50	1.00	2.83	3.24	0.54	1.13	0.53	3.48	0.45	0.27
P50	60.8	8.55	6.62	1.66	1.69	5.34	3.83	9.52	6.31	1.38	5.09	2.59	8.07	1.49	0.88
P95	1422	63.6	39.1	10.8	11.7	24.3	16.8	36.9	21.2	4.57	34.7	18.3	74.79	8.03	4.27
32nd GW															
Non-crea. adjusted															
% Detected	98	100	100	100	100	100	100	100	99	100	100	100	100	100	100
Mean	714	13.7	20.5	4.51	4.36	11.2	9.71	24.0	14.7	3.42	15.5	8.98	19.91	3.22	1.91
SD	2714	15.8	38.8	13.5	8.70	22.3	18.4	49.9	29.5	3.43	41.3	27.8	65.7	3.46	2.74
P5	5.79	2.25	2.07	0.34	0.27	1.16	1.11	4.26	3.21	0.71	1.50	0.73	4.19	0.56	0.35
P50	51.1	7.53	9.90	2.05	1.57	4.73	4.17	9.32	6.96	2.41	8.03	4.06	9.03	2.19	1.23
P95	2503	44.7	86.5	10.2	16.7	52.5	54.2	123	62.8	11.6	51.4	24.0	26.2	10.0	5.32
12th GW															
Crea. adjusted	**MEP**	**MiBP**	**MnBP**	**MBzP**	**MEHP**	**MEHHP**	**MEOHP**	**MECPP**	**MMCHP**	**OH-MPHP**	**OH-MiNP**	**oxo-MiNP**	**cx-MiNP**	**OH-MINCH**	**oxo-MINCH**
Mean	255	23.7	19.9	4.24	5.52	14.5	10.4	25.1	16.7	3.29	14.6	6.65	25.9	4.68	2.80
SD	355	31.1	21.3	3.92	7.85	17.5	12.2	32.7	16.1	2.45	14.0	7.66	26.7	9.01	5.49
P5	16.9	4.99	4.37	0.70	0.72	2.85	2.27	5.65	4.04	0.92	2.64	1.17	4.51	0.88	0.50
P50	83.2	16.6	13.3	2.59	3.14	8.76	6.25	16.0	10.9	2.67	9.30	3.98	16.6	2.60	1.53
P95	874	58.5	69.2	14.0	27.0	62.4	44.8	70.0	48.5	8.69	43.6	22.0	75.1	14.2	8.45
32nd GW															
Crea. adjusted															
Mean	574	14.9	20.5	5.97	4.78	13.5	11.3	29.1	17.8	4.02	21.8	13.2	30.8	4.04	2.24
SD	1835	10.9	23.7	22.9	10.7	33.9	25.4	72.6	37.4	3.47	70.4	48.2	114	5.00	2.44
P5	16.1	3.85	4.65	0.83	0.55	2.42	2.00	6.10	4.49	1.30	2.71	1.30	5.71	1.24	0.54
P50	67.6	11.5	12.8	2.47	2.27	6.78	5.86	13.6	9.51	3.09	8.72	4.44	12.8	2.92	1.49
P95	3177	32.9	82.0	11.4	11.2	34.3	32.4	63.9	38.6	9.60	36.9	33.8	50.2	9.90	8.34

P5= Percentile 5; P50= Percentile 50; P 95= Percentile 95; SD= Standard deviation

	MEP	MiBP	MnBP	MBzP	MEHP	MEHHP	MEOHP	MECPP	MMCHP	oh_MiNP	oxo_MiNP	cx_MiNP	oh_MINCH	oxo_MINCH	oh_MPHP
MEP	1	0.227*	0.303**	0.037	0.183*	0.200*	0.277**	0.408**	0.617**	0.435	0.0210	0.046	0.032	0.051	0.260**
MiBP		1	0.410**	0.092	0.187*	0.200*	0.210*	0.192*	0.185*	0.165	0.0750	0.082	0.238**	0.231*	0.463**
MnBP			1	0.112	0.499**	0.381**	0.488**	0.285**	0.235*	0.105	0.079	0.068	0.102	0.253**	0.382**
MBzP				1	0.605**	0.730**	0.697**	0.669**	0.613**	0.913**	0.943**	0.923**	0.014	00.031	0.439**
MEHP					1	0.916**	0.941**	0.826**	0.726**	0.621**	0.608**	0.631**	0.376**	0.541**	0.480**
MEHHP						1	0.984**	0.943**	0.828**	0.751**	0.735**	0.755**	0.486**	0.566**	0.498**
MEOHP							1	0.927**	0.830**	0.704**	0.693**	0.706**	0.427**	0.542**	0.510**
MECPP								1	0.947**	0.679**	0.663**	0.687**	0.541**	0.596**	0.441**
MMCHP									1	0.627**	0.608**	0.638**	0.391**	0.444**	0.488**
oh_MiNP										1	0.981**	0.971**	0.081	0.070	0.569**
oxo_MiNP											1	0.974**	0.008	0.005	0.467**
cx_MiNP												1	0.043	0.041	0.477**
oh_MINCH													1	0.943**	0.233*
oxo_MINC														1	0.250**
oh_MPHP															1

Figure 2. Pearson's correlation between 13 phthalate and 2 DINCH metabolites detected in pregnant urine. ** The correlation is significant at the 0.01 level (2 tails). *The correlation is significant at the 0.05 level (2 tails).

Table 5. Phthalate and DINCH metabolites urinary levels in studies worldwide.

Ref	Country	n		MEP	MiBP	MnBP	MBzP	MEHP	MEHHP	MEOHP	MECPP	MMCHP	oh-MiNP	oxo-MiNP	cx-MiNP	oh-MPHP	oh-MINCH	oxo-MINCH
Casas et al., 2016	Spain	390	C	389.1 (353.4,428.4)	33.0 (31.0,35.1)	32.7 (30.4,35.2)	12.6 (11.6,13.6)	11.3 (10.6,12.1)	29.0 (27.1,31.0)	21.7 (20.3,23.1)	41.4 (38.9,44.1)							
Rodríguez-Carmona et al., 2018	Mexico	155 (T1)	SG	143.4 (116.8,176.0)	1.04 (0.85,1.27)	62.6 (51.8,75.7)	2.83 (2.34,3.42)	5.15 (4.46,5.94)	16.61 (14.3,19.2)	8.88 (7.67,10.3)	30.64 (27.3,34.4)							
		153 (T2)	SG	134.26 (112.5,160.2)	0.9 (0.74,1.09)	52.61 (43.8,63.2)	2.74 (2.33,3.23)	5.36 (4.68,6.14)	18.61 (16.1,21.5)	11.08 (9.59,12.8)	34.82 (30.9,39.2)							
		175 (T3)	SG	138.66 (113.6,169.2)	2.2 (1.92,2.52)	63.78 (54.6,74.4)	5.17 (4.50,5.93)	6.47 (5.67,7.37)	22.89 (19.9,26.3)	13.87 (12.1,15.9)	38.18 (33.8,43.1)							
Shin et al., 2018	USA	188	SG	26.7 (5.6,184)	7.2 (2,23.9)	12 (3.4,41.1)	6.4 (1.2,37.7)	2.7 (<LOD,23.1)	13 (2.5,98.4)	10 (2,71.2)	21.8 (4.8,156.9)							
Noor et al., 2018	USA	277	U	121.5 (106.9,138.0)	6.5 (6.1,7.0)	15.2 (14.2,16.3)	6.0 (5.5,6.7)											
Evre et al., 2019	USA	758	U	41.7 (-, 642)	7.2 (-,34.1)	9.8 (-,42.9)	4.8 (-,36.3)	1.9 (-,9.3)	6.7 (-,31.0)	5.2 (-,22.4)	12.0 (-,51.3)			5.3 (-,78.5)	15.9 (-,129)		0.3 (-,1.3)	0.4 (-,1.2)
Polanska et al., 2014	Poland	165	C	81.3 (-,336.4)	106.8 (-,563.2)	23.0 (-,73.8)	0.2 (-, 0.5)	0.5 (-,1.6)	24.1 (-,97.0)	10.6 (-,72.6)			4.8 (-,20.3)	0.5 (-,1.7)				
Koch et al., 2016	Germany	594 Male	U	23.9	17.1	11.1	2.6	2.3	7.4	4.5	6.9		2.5	1.2	2.5			
		569 Female	U	23.8	15.5	12.5	2.7	2.0	6.8	4.9	7.3		2.4	1.2	2.4			
Shu et al., 2017	Sweden	1651	U	69 (65,72)		68 (66,71)	16 (15,17)	3.8 (3.6,4.0)	16 (15,17)	11.1 (10.6,11.6)	15.8 (15.2,16.5)	6.3 (6.1,6.6)	6.2 (5.9,6.6)	2.9 (2.7,3.1)	9.8 (9.3,10.3)	1.2 (1.16,1.28)		0.30 (0.28,0.32)
Sabaredzovic et al., 2015	Norway	116	SG	88{97}	32{44}	31{21}	13{12}	11{18}	35{68}	25{44}	49{114}	82{159}	1.0{0.65}	1.0{0.93}	3.3{1.4}			
Tefre de Renzy-Martin et al., 2014	Denmark	200	C	18.9(355))	35.3(98.3)	13.9(37.5)	2.32(17.5)	1.12(3.82)	5.70(13.4)	3.72(9.22)	4.75(10.1)		1.47(11.5)	1.02(8.90)	3.65(34.9)			
Machtinger et al., 2018	Israel	50	U	56.7 (803)	12.5(94.1)	11.1(45.2)	0.8(4.7)	1.5(9.9)	6.2(28.1)	5.7(25.8)	9.9(51.9)			2.3(14.3)	4.0(21.7)		0.6(3.7)	<LD(1.7)
Suzuki et al., 2012	Japan	50	C	10.9 [1.25,1430]		66.9 [11.1,211]	5.74 [0.69,106]	4.63 [<LD,67.8]	12.9 [3.90,164]	13.7 [3.46,174]								
Shapiro et al., 2015	Canada	1152	U	38.8 {4.1}		13.3 {2.2}	13.3 {2.2}	2.6 {2.5}	10.6 {2.5}	7.4 {2.3}								
Ye et al., 2008	Netherlands	99	C	173.0 (13.6,1810)	64.1 (20.9,327.0)	67.1 (21.7,228.0)	13.9 (3.6,84.7)	10.8 (1.2,88.7)	22.2 (7.5,99.5)	23.3 (8.4,126.0)	30.1 (10.5,155.0)	9.7 (3.3,36.1)	4.6 (1.1,53.0)	3.9 (0.8,43.9)				
Present study	Spain	60+60 T1+T3	U	73.7 (4.4,1831)	9.1 (2.3,48.8)	9.0 (2,55.3)	1.8 (0.3,10.1)	1.9 (0.4,11.5)	5.5 (1.4,31.7)	4.4 (1.1,28.3)	11.0 (3.1,53.8)	7.4 (3.3,30.3)	6.6 (1.2,34.3)	3.2 (0.6,20.7)	10.3 (3.6,39.3)	1.9 (0.6,10.5)	1.9 (0.5,9.2)	1.1 (0.3,5.3)
		60+60 T1+T3	C	113.5 (16.9,1914)	14.1 (4.2,57.6)	14.0 (4.6,90.5)	2.7 (0.8,14.5)	2.9 (0.7,25.8)	8.6 (2.8,57.2)	6.8 (2.3,42.9)	17.0 (5.7,75.7)	11.7 (4.5,68.8)	10.2 (2.7,55)	4.9 (1.3,33.9)	15.9 (5.5,99.2)	2.9 (0.9,11.5)	2.9 (1.1,26)	1.7 (0.6,11)

T1, T2, T3: First, second and third trimester of pregnancy, respectively. C: Creatinine corrected (µg /g_{crea}); SG: Specific gravity corrected (µg /L); U: Uncorrected (µg /L). Results expressed in: Geometric Mean (P5, P95); mean; median (P95); mean [range]; or mean {SD}.

3.2. Reconstructed exposure for each parent compound

Table 6 summarizes the total reconstructed exposure to phthalate and DINCH at 12th and 32nd GW. No statistical differences between the exposures at the two different periods were found. Pregnant women from the present study were highly exposed to DEHP (3.92 and 3.80 µg/kg$_{bw}$/d at 12th and 32nd GW, respectively) and DiNP (3.90 and 4.96 µg/kg$_{bw}$/d at 12th and 32nd GW, respectively). In addition, DINCH presented the third highest exposure level with 0.69 and 0.51 µg/kg$_{bw}$/d at 12th and 32nd GW, respectively.

Table 6. Reconstructed exposure (µg/kg$_{bw}$/d) to each parent compound at 12th and 32nd GW

	DEHP	DiNP	DEP	DiBP	DnBP	BBZP	DPHP	DINCH
12th GW								
Mean	3.92	3.90	$8.44\cdot10^{-3}$	$6.54\cdot10^{-4}$	$5.42\cdot10^{-4}$	$1.42\cdot10^{-4}$	$1.21\cdot10^{-4}$	0.69
SD	4.73	3.99	$1.15\cdot10^{-2}$	$9.18\cdot10^{-4}$	$5.81\cdot10^{-4}$	$1.34\cdot10^{-4}$	$9.74\cdot10^{-5}$	1.40
P5	0.90	0.74	$5.00\cdot10^{-4}$	$1.45\cdot10^{-4}$	$1.23\cdot10^{-4}$	$2.48\cdot10^{-5}$	$2.95\cdot10^{-5}$	0.13
P50	2.80	2.38	$2.90\cdot10^{-3}$	$4.82\cdot10^{-4}$	$3.34\cdot10^{-4}$	$8.21\cdot10^{-5}$	$9.25\cdot10^{-5}$	0.37
P95	12.9	12.1	$3.12\cdot10^{-2}$	$1.55\cdot10^{-3}$	$2.07\cdot10^{-3}$	$4.93\cdot10^{-4}$	$3.57\cdot10^{-4}$	2.35
32nd GW								
Mean	3.80	4.96	$1.77\cdot10^{-2}$	$3.46\cdot10^{-4}$	$3.43\cdot10^{-4}$	$1.78\cdot10^{-4}$	$1.38\cdot10^{-4}$	0.51
SD	8.19	16.5	$5.64\cdot10^{-2}$	$2.09\cdot10^{-4}$	$2.07\cdot10^{-4}$	$6.32\cdot10^{-4}$	$1.16\cdot10^{-4}$	0.61
P5	0.84	0.81	$4.98\cdot10^{-4}$	$9.64\cdot10^{-5}$	$9.53\cdot10^{-5}$	$2.38\cdot10^{-5}$	$3.55\cdot10^{-5}$	0.13
P50	1.98	2.10	$1.82\cdot10^{-3}$	$2.94\cdot10^{-4}$	$2.91\cdot10^{-4}$	$6.96\cdot10^{-5}$	$1.06\cdot10^{-4}$	0.35
P95	11.3	12.1	$9.91\cdot10^{-2}$	$7.27\cdot10^{-4}$	$7.19\cdot10^{-4}$	$3.76\cdot10^{-4}$	$3.79\cdot10^{-4}$	1.32

P5= Percentile 5; P50= Percentile 50; P 95= Percentile 95; SD= Standard deviation

3.3. Estimated DEHP exposure vs Reconstructed DEHP exposure.

In a previous study (Martínez et al., 2018), the estimated exposure to DEHP in pregnant women was assessed. Estimated DEHP exposure was calculated considering both, dietary and non-dietary (inhalation, dermal and dust ingestion) routes. The total mean estimated exposure was set at 1.60 ± 0.11 µg/kg$_{bw}$/d. Subsequently, in the present study, the total mean reconstructed DEHP exposure for the same cohort was 3.92 ± 4.73 µg/kg$_{bw}$/d. These values were in the same order of magnitude. However, discrepancies between both exposures can be associated with other sources that also contribute to the total exposure, but were not taken into account. For instance, phthalates are also found in medications,

where they are used as inactive ingredients in the production of enteric coatings. In that case, health risks exposures associated with taking these medications have to be more evaluated in vulnerable segments population, including pregnant women and children (Hernández-Díaz et al., 2009). In addition, the exposure from flooring materials or cleaning products (Jeon et al., 2016) can also contribute to the total exposure (Jeon et al., 2016). It is important to highlight that this is fastly eliminated chemical. For that reason, the best way to obtain an accurate reconstructed exposure value will be considering 24 h urine. However, it is quite complicated to obtain 24 h urine especially, with large-populations. Furthermore, the determination of different metabolites concentration in urine could be quite expensive and time-consuming. For that reason, spot urine was used in the current study. Finally, discrepancies could also be due to the high variability among individuals and uncertainty in the experiments.

MEHP cumulative amounts for both, the reconstructed dose and the estimated dose (Martínez et al., 2018) were plotted. **Figure 3** shows the results of predicted mean cumulative MEHP in urine from the estimated DEHP exposure (a), the reconstructed DEHP exposure (b), and the biomonitoring data of the extrapolated MEHP cumulative concentration in urine during 24 h (c) (obtained from equation 2, section 2.5). Consequently, this information suggests that there was an underestimation in the previously estimated exposure (Martínez et al., 2018). These results indicate that there might be chances of more unknown sources that contribute to the total DEHP exposure. These sources could come from the lifestyle or habits that pregnant women can follow during the pregnancy or maybe with food habits consumption or personal care habits applications which would be challenging to take into account via questionaries.

Comparing (a) and (b) plots with the biomonitoring data (c), it is clear that reconstructed exposure (b) is closer biomonitoring data (c). This would demonstrate that reconstructed-PBPK simulation is a better method than estimated-PBPK simulation to predict the chemical amount that a population might have been exposed to. In addition, with these results, we can suggest that it is possible to predict the total internal exposure of a chemical close to

the real internal exposure by using reconstructed exposure data of the most abundant parent/metabolite compound from spot urine human with the implication of a PBPK model. This might be obvious as the reconstructed dose takes into account all the sources of exposure which are unknown while considering the information of the questionnaires and interviews to estimate the routes of exposure (ingestion, dermal and inhalation). For that reason, as it was mentioned before, deep studies about missing known sources that could contribute to the concentration levels of DEHP are needed. The finding of these new sources and inclusion of this in questionaries would make the exposure estimated PBPK-simulations even closer to biomonitoring data.

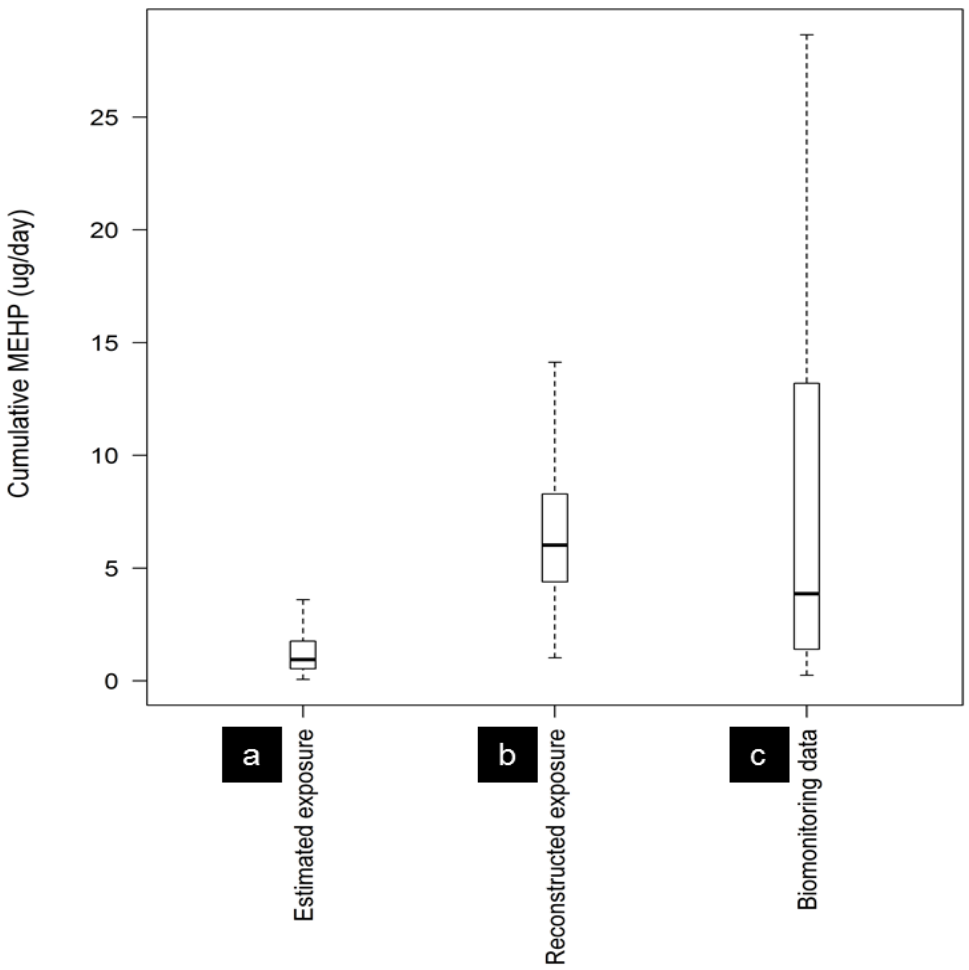


Figure 3. PBPK simulated MEHP cumulative concentration for estimated dose (a), PBPK simulated MEHP cumulative concentration for reconstructed dose (b) and extrapolated MEHP cumulative concentration in urine (c). Mean MEHP levels were plotted considering oral, dermal, dust ingestion and inhalation routes in (a) and (b). Mean MEHP levels in urine (extrapolated as 24 h) were considered in (c). Exposure and biomonitoring data were in $\mu g/kg_{bw}/d$.

4. Conclusions

Phthalate and DINCH metabolites were detected in almost all (94%) urine samples in both periods, first and third trimester. Expected significant positive Pearson's correlations were found between DEHP, DiNP and DINCH metabolites. In addition, other significant positive correlations were found between DEHP and MBzP metabolites, DiNP and BBZP metabolites, and DEHP and DiNP metabolites. According to habits, lifestyle and physiological characteristics data from pregnant women and for their babies at birth, significant differences between some of these characteristics and levels of phthalate and DINCH metabolites in urine were found. Firstly, an increase of yoghurt consumption during the week was related significantly with lower levels of OH-MPHP in urine samples. Secondly, a decrease in the weight of the babies at birth was associated significantly with a higher presence of OH-MINCH and oxo-MINCH metabolites.

Comparisons were made between other studies around the world and our results were in the same line as these studies. However, it is important to clarify that it is not easy to find scientific articles with more than 3 or 4 metabolites measured in urine, and this was also more complicated when only pregnant women's urine is considered. The information about the levels of different phthalate and DINCH metabolites in pregnant women urine is lacking in the bibliography. The prenatal and early life exposure to these chemicals is a critical window of exposure. For that reason, more information regarding this population is needed.

MEHP estimated vs reconstructed exposure was compared and the values were close to each other. However, the PBPK simulated MEHP cumulative amount for reconstructed dose was closer to the extrapolated MEHP cumulative concentration (24 h biomonitoring data). So, PBPK simulation from dose reconstruction was more accurate than the previously estimated PBPK simulated. Therefore, PBPK model is a good tool to predict chemical exposure, especially if it is combined with a reconstructed dose. In addition, it has been demonstrated that using only spot urine is possible to obtain very representative exposure

values. However, further studies about new possible sources are needed and achieving the accuracy between simulated and biomonitoring values is still challenging.

Acknowledgements

The authors thank all pregnant women who participate in this study. The research leading to these results has partially funded from the European Community's Seventh Framework Programme (FP7/ 2007–2013) under Grant Agreement No. 603946-2 (HEALS project) and the Spanish Ministry of Economy and Competitiveness for the MODELBIS Project (Ref. no. AGL2016-78942-R). V. Kumar and J. Rovira received funds from the Health Department of Catalonia Government trough "Pla Estratègic de Recerca i Innovació en salut" (PERIS 2016–2020).

References

Abduljalil, K., Furness, P., Johnson, T.N., Rostami-Hodjegan, A., Soltani, H., 2012. Anatomical, Physiological and Metabolic Changes with Gestational Age during Normal Pregnancy. Clin. Pharmacokinet. 51, 365–396. https://doi.org/10.2165/11597440-000000000-00000

Anderson, W.A., Castle, L., Scotter, M.J., Massey, R.C., Springall, C. A., 2001. Biomarker approach to measuring human dietary exposure to certain phthalate diesters. Food Addit Contam. 18, 1068–1074. https://doi.org/10.1080/02652030110050113

Anderson, W.A., Castle, L., Hird, S., Jeffery, J., Scotter, M.J., 2011. A twenty-volunteer study using deuterium labelling to determine the kinetics and fractional excretion of primary and secondary urinary metabolites of di-2-ethylhexylphthalate and di-iso-nonylphthalate. Food Chem Toxicol. 49 (9) 2022-9. https://doi.org/10.1016/j.fct.2011.05.013. Epub 2011 May 15.

Angerer, J., Bird, M.G., Burke, T.A., Doerrer N.G., Needham L., Robison, S.H., Sheldon, L., Zenick, H., 2006. Strategic biomonitoring initiatives: moving the science forward. Toxicol Sci. 93, 3–10. https://doi.org/10.1093/toxsci/kfl042

Angerer, J., Ewers, U., Wilhelm, M., 2007. Human biomonitoring: state of the art. Int J Hyg Environ Health. 210, 201–28. https://doi.org/10.1016/j.ijheh.2007.01.024

Arbuckle, T.E., Fisher, M., Macpherson, S., Lang, C., Provencher, G., Leblanc, A., Hauser, R., Feeley, M., Ayotte, P., Neisa, A., Ramsay, T., Tawagi, G., 2016. Science of the Total Environment

Maternal and early life exposure to phthalates : The Plastics and Personal-care Products use in Pregnancy (P4) study. Sci. Total Environ. 551–552, 344–356. https://doi.org/10.1016/j.scitotenv.2016.02.022

Bansal, A., Henao-mejia, J., Simmons, R.A., 2018. Immune System: An Emerging Player in Mediating Effects of Endocrine Disruptors on Metabolic Health 159, 32–45. https://doi.org/10.1210/en.2017-00882

Barr, D.B., Wilder, L.C., Caudill, S.P., Gonzalez, A.J., Needham, L.L., Pirkle, J.L., 2005. Urinary Creatinine Concentrations in the U . S . Population : Implications for Urinary Biologic Monitoring Measurements. Environ. Health Perspect. 113, 192-200. https://doi.org/10.1289/ehp.7337

Casas, M., Valvi, D., Ballesteros-Gomez, A., Gascon, M., Fernández, M.F., Garcia-Esteban, R., Iñiguez, C., Martínez, D., Murcia, M., Monfort, N., Luque, N., Rubio, S., Ventura, R., Sunyer, J., Vrijheid, M., 2016. Exposure to Bisphenol A and Phthalates during Pregnancy and Ultrasound Measures of Fetal Growth in the INMA-Sabadell Cohort. Environ. Health Perspect. 124, 521–528. https://doi.org/10.1289/ehp.1409190

Diamanti-kandarakis, E., Bourguignon, J., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T., Gore, A.C., 2009. Endocrine-Disrupting Chemicals : An Endocrine 30, 293–342. https://doi.org/10.1210/er.2009-0002

Dong, R.H., Zhang, H., Zhang, M.R., Chen, J.S., Wu, M., Li, S.G., Chen, B., 2017. Association between Phthalate Exposure and the Use of Plastic Containers in Shanghai Adults. Biomed. Environ. Sci. 30, 727–736. https://doi.org/10.3967/bes2017.098

EFSA, 2008. Opinion of the Scientific Panel on food additives , flavourings , processing aids and materials in contact with food (AFC) on a request related to a 17th list of substances for food contact materials 1–24. 10.2903/j.efsa.2008.601

Erve, T.J., Rosen, E.M., Barrett, E.S., Nguyen, R.H.N., Sathyanarayana, S., Milne, G.L., Calafat, A.M., Swan, S.H., Ferguson, K.K., 2019. Phthalates and Phthalate Alternatives Have Diverse Associations with Oxidative Stress and Inflammation in Pregnant Women. Environ. Sci. Technol. 53, 3258–3267. https://doi.org/10.1021/acs.est.8b05729

Fromme, H., Lahrz, T., Piloty, M., Gebhart, H., Oddoy, A., Ruden, H., 2004. Occurrence of phthalates and musk fragrances in indoor air and dust from apartments and kindergartens in Berlin (Germany). Indoor Air 14, 188–195. http://dx.doi.org/10.1111/j.1600-0668.2004.00223.x.

Gentry, P.R., Covington, T.R., Clewell, H.J., 2003. Evaluation of the potential impact of

pharmacokinetic differences on tissue dosimetry in offspring during pregnancy and lactation. Regul. Toxicol. Pharmacol. 38, 1–16. https://doi.org/10.1016/S0273-2300(03)00047-3

Giovanoulis, G., Bui, T., Xu, F., Papadopoulou, E., Padilla-Sanchez, J.A., Covaci, A., Haug, L.S., Cousins, A.P., Magnér, J., Cousins, I.T., de Wit, C.A., 2018. Multi-pathway human exposure assessment of phthalate esters and DINCH. Environ. Int. 112, 115–126. https://doi.org/10.1016/j.envint.2017.12.016

Giovanulis, G., Bui, T., Xu, F., Papadopoulou, E., Padilla-sanchez, J.A., Covaci, A., Haug, L.S., Palm, A., Magnér, J., Cousins, I.T., Wit, C.A. De, 2018. Multi-pathway human exposure assessment of phthalate esters and DINCH Georgios Giovanoulis. Environ. Int. 112, 115–126. https://doi.org/10.1016/j.envint.2017.12.016

Guo, Y., Wu, Q., Kannan, K., 2011. Phthalate metabolites in urine from China, and implications for human exposures. Environment International, 37(5), 893–898. https://doi.org/10.1016/j.envint.2011.03.005

Hernández-díaz, S., Mitchell, A.A., Kelley, K.E., Calafat, A.M., Hauser, R., 2009. Medications as a Potential Source of Exposure to Phthalates in the U.S. Population. Environ. Health Perspect. 117, 185–189. https://doi.org/10.1289/ehp.11766

Heudorf, U., Mersch-sundermann, V., 2007. Phthalates: Toxicology and exposure 210, 623–634. https://doi.org/10.1016/j.ijheh.2007.07.011

James, G.D., Sealey, J.E., Alderman, M., Ljungman, S., Mueller, F.B., Pecker, M.S., Laragh, J.H., 1988. A longitudinal study of urinary creatinine and creatinine clearance in normal subjects. Am. J. Hypertens. 1, 124–131. https://doi.org/10.1093/ajh/1.2.124

Jeon, S., Kim, K.-T., Choi, K., 2016. Migration of DEHP and DINP into dust from PVC flooring products at different surface temperature. Sci. Total Environ. 547, 441–446. https://doi.org/10.1016/j.scitotenv.2015.12.135

Johns, L.E., Cooper, G.S., Galizia, A., Meeker, J.D., 2015. Exposure assessment issues in epidemiology studies of phthalates. Environ. Int. 85, 27–39. doi:10.1016/j.envint.2015.08.005

Koch, H.M., Rüther, M., Schütze, A., Conrad, A., Pälmke, C., Apel, P., Brüning, T., Kolossa-Gehring, M., 2016. Phthalate metabolites in 24-h urine samples of the German Environmental Specimen Bank (ESB) from 1988 to 2015 and a comparison with US NHANES data from 1999 to 2012. Int. J. Hyg. Environ. Health 220, 130–141. https://doi.org/10.1016/j.ijheh.2016.11.003

Koch, H.M., Gonzalez-Reche, L.M., Angerer J., 2003. On-line clean-up by multidimensional liquid chromatography-electrospray ionization tandem mass spectrometry for high throughput

quantification of primary and secondary phthalate metabolites in human urine. Chromatogr. B Analyt. Technol. Biomed. Life Sci., 784 (1), 169-182 https://doi.org/10.1016/S1570-0232(02)00785-7

Koch, H.M., Christensen, K.L.Y., Harth, V., Lorber, M., Brüning, T., 2012. Di-n-butyl phthalate (DnBP) and diisobutyl phthalate (DiBP) metabolism in a human volunteer after single oral doses. Arch Toxicol 86, 1829–1839 https://doi.org/10.1007/s00204-012-0908-1

Koppen, G., Govarts, E., Vanermen, G., Voorspoels, S., Govindan, M., Dewolf, M.C., Den Hond, E., Biot, P., Casteleyn, L., Kolossa-Gehring, M., Schwedler, G., Angerer, J., Koch, H.M., Schindler, B.K., Castaño, A., López, M.E., Sepai, O., Exley, K., Bloemen, L., Knudsen, L.E., Joas, R., Joas, A., Schoeters, G., Covaci, A., 2019. Mothers and children are related, even in exposure to chemicals present in common consumer products. Environ. Res. 175, 297–307. https://doi.org/10.1016/j.envres.2019.05.023

Li, L., Chen, L., Meng, X., Chen, B., Chen, S., Zhao, Y., Zhao, L., 2013. Exposure Levels of Environmental Endocrine Disruptors in Mother-Newborn Pairs in China and Their Placental Transfer Characteristics. PLoS One 8, 1–9. https://doi.org/10.1371/journal.pone.0062526

Loccisano, A.E., Longnecker, M.P., Campbell, J.L., Andersen, M.E., Clewell, H.J., 2013. Development of Pbpk Models for PFOA and PFOS for Human Pregnancy and Lactation Life Stages. J. Toxicol. Environ. Heal. Part A 76, 25–57. https://doi.org/10.1080/15287394.2012.722523

Machtinger, R., Berman, T., Adir, M., Mansur, A., Baccarelli, A.A., Racowsky, C., Calafat, A.M., Hauser, R., Nahum, R., 2018. Urinary concentrations of phthalate metabolites, bisphenols and personal care product chemical biomarkers in pregnant women in Israel. Environ. Int. 116, 319–325. https://doi.org/10.1016/j.envint.2018.04.022

Mann, S.J., Gerber, L.M., 2010. Estimation of 24-Hour Sodium Excretion from Spot Urine Samples. J. Clin. Hypertens. 12, 174–180. https://doi.org/10.1111/j.1751-7176.2009.00241.x

Martínez, M.A., Rovira, J., Sharma, R.P., Nadal, M., Schuhmacher, M., Kumar, V., 2018. Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure : A Catalonia (Spain) case study. Environ. Res. 166, 25–34. https://doi.org/10.1016/j.envres.2018.05.008

Martínez, M.A., Rovira, J., Sharma, R.P., Nadal, M., Schuhmacher, M., Kumar, V., 2017. Prenatal exposure estimation of BPA and DEHP using integrated external and internal dosimetry: A case study. Environ. Res. 158, 566–575. https://doi.org/10.1016/j.envres.2017.07.016

Noor, N., Ferguson, K.K., Meeker, J.D., Seely, E.W., Hauser, R., James-Todd, T., McElrath, T.F., 2018.

Pregnancy phthalate metabolite concentrations and infant birth weight by gradations of maternal glucose tolerance. Int. J. Hyg. Environ. Health 222, 395–401. https://doi.org/10.1016/j.ijheh.2018.12.005

North, M.L., Takaro, T.K., Diamond, M.L., Ellis, A.K., 2014. Effects of phthalates on the development and expression of allergic disease and asthma. Ann. Allergy, Asthma Immunol. 112, 496–502. https://doi.org/10.1016/j.anai.2014.03.013

Petersen, J.H., Jensen, L.K., 2010. Phthalates and food-contact materials: Enforcing the 2008 European Union plastics legislation. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 27, 1608–1616. https://doi.org/10.1080/19440049.2010.501825

Pirkle, J.L., Sampson, E.J., Needham, L.L., Patterson, D.G., Ashley, D.L., 1995. Using biological monitoring to assess human exposure to priority toxicants. Environ Health Perspect. 103, 45–8. https://doi.org/10.1289/ehp.95103s345

Polanska, K., Ligocka, D., Sobala, W., Hanke, W., 2014. Phthalate exposure and child development: The Polish Mother and Child Cohort Study. Early Hum. Dev. 90, 477–485. https://doi.org/10.1016/j.earlhumdev.2014.06.006

Rodríguez-Carmona, Y., Cantoral, A., Trejo-Valdivia, B., Téllez-Rojo, M.M., Svensson, K., Peterson, K.E., Meeker, J.D., Schnaas, L., Solano, M., Watkins, D.J., 2018. Phthalate exposure during pregnancy and long-term weight gain in women. Environ. Res. 169, 26–32. https://doi.org/10.1016/j.envres.2018.10.014

Rudel, R.A., Perovich, L.J., 2009. Endocrine disrupting chemicals in indoor and outdoor air. Atmos Environ 43, 170–181. https://doi.org/10.1016/j.atmosenv.2008.09.025

Sabaredzovic, A., Sakhi, A.K., Brantsæter, A.L., Thomsen, C., 2015. Determination of 12 urinary phthalate metabolites in Norwegian pregnant women by core – shell high performance liquid chromatography with on-line solid-phase extraction, column switching and tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 1002, 343–352. https://doi.org/10.1016/j.jchromb.2015.08.040

Shapiro, G.D., Dodds, L., Arbuckle, T.E., Ashley-Martin, J., Fraser, W., Fisher, M., Taback, S., Keely, E., Bouchard, M.F., Monnier, P., Dallaire, R., Morisset, A., Ettinger, A.S., 2015. Exposure to phthalates, bisphenol A and metals in pregnancy and the association with impaired glucose tolerance and gestational diabetes mellitus: The MIREC study. Environ. Int. 83, 63–71. https://doi.org/10.1016/j.envint.2015.05.016

Sharma, R.P., Schuhmacher, M., Kumar, V., 2018. Development of a human physiologically based

pharmacokinetic (PBPK) model for phthalate (DEHP) and its metabolites: A bottom up modeling approach. Toxicol. Lett. 296, 152–162. https://doi.org/10.1016/j.toxlet.2018.06.1217

Shin, H.-M., Bennett, D.H., Barkoski, J., Ye, X., Calafat, A.M., Tancredi, D., Hertz-Picciotto, I., 2018. Variability of urinary concentrations of phthalate metabolites during pregnancy in first morning voids and pooled samples. Environ. Int. 122, 222–230. https://doi.org/10.1016/j.envint.2018.11.012

Shu, H., Jönsson, B.A., Gennings, C., Svensson, Å., Nånberg, E., Lindh, C.H., Knutz, M., Takaro, T.K., Bornehag, C.-G., 2017. Temporal trends of phthalate exposures during 2007–2010 in Swedish pregnant women. J. Expo. Sci. Environ. Epidemiol. 28, 437–447. https://doi.org/10.1038/s41370-018-0020-6

Song, Q., Li, R., Zhao, Y., Zhu, Q., Xia, B., Chen, S., Zhang, Y., 2018. Evaluating effects of prenatal exposure to phthalates on neonatal birth weight: Structural equation model approaches. Chemosphere 205, 674–681. https://doi.org/10.1016/j.chemosphere.2018.04.063

Suzuki, Y., Yoshinaga, J., Mizumoto, Y., Serizawa, S., Shiraishi, H., 2012. Foetal exposure to phthalate esters and anogenital distance in male newborns. Int. J. Androl. 35, 236–244. https://doi.org/10.1111/j.1365-2605.2011.01190.x

Tefre de Renzy-Martin, K., Frederiksen, H., Christensen, J.S., Boye Kyhl, H., Andersson, A. M., Husby, S., Barington, T., Main, K.M., Jensen, T.K., 2014. Current exposure of 200 pregnant Danish women to phthalates, parabens and phenols. Reproduction 147, 443–453. https://doi.org/10.1530/REP-13-0461

Thomas Zoeller, R., Brown, T.R., Doan, L.L., Gore, A.C., Skakkebaek, N.E., Soto, A.M., Woodruff, T.J., Vom Saal, F.S., 2012. Endocrine-disrupting chemicals and public health protection: A statement of principles from the Endocrine Society. Endocrinology 153, 4097–4110. https://doi.org/10.1210/en.2012-1422

Wang, Y., Zhu, H., Kannan, K., 2019. A Review of Biomonitoring of Phthalate Exposures. Toxics 7, 21. https://doi.org/10.3390/toxics7020021

WHO, 2013. Guideline : Calcium supplementation in pregnant women. Geneva, World Health Organization. ISBN 978 92 4 150537 6 ©

Wormuth, M., Scheringer, M., Vollenweider, M., Hungerbühler, K., 2006. What are the sources of exposure to eight frequently used phthalic acid esters in europeans?. Risk analysis, 26 (3), 803-824. https://doi.org/10.1111/j.1539-6924.2006.00770.x

Ye, X., Pierik, F.H., Hauser, R., Duty, S., Angerer, J., Park, M.M., Burdorf, A., Hofman, A., Jaddoe, V.W.V., Mackenbach, J.P., Steegers, E.A.P., Tiemeier, H., Longnecker, M.P., 2008. Urinary metabolite concentrations of organophosphorous pesticides, bisphenol A, and phthalates among pregnant women in Rotterdam, the Netherlands: The Generation R study. Environ. Res. 108, 260–267. https://doi.org/10.1016/j.envres.2008.07.014

Zhao, Y., Chen, L., Li, L.X., Xie, C.M., Li, D., Shi, H.J., Zhang, Y.H., 2014. Gender-specific relationship between prenatal exposure to phthalates and intrauterine growth restriction. Pediatr. Res. 76, 401–408. https://doi.org/10.1038/pr.2014.103

PUBLICATION 4

Prenatal exposure to PFOS and PFOA in a Catalan pregnant women cohort.

J. Rovira[a,b], M. A. Martínez[a] , T. Espuis[a], M. Nadal[b], V. Kumar[a,b], D. Costopoulou[c]; I. Vassiliadou[c]; L. Leondiadis[c]; J.L. Domingo[b]; M. Schuhmacher[a,b]

[a]Environmental Engineering Laboratory, Departament d'Enginyeria Quimica, Universitat Rovira i Virgili, Av. Països Catalans 26, 43007 Tarragona, Catalonia, Spain.
[b]Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Catalonia, Spain.
[c]Mass Spectrometry and Dioxin Analysis Laboratory, INRASTES, NCSR"Demokritos", Neapoleos 27, 15341, Athens, Greece.

Environmental Research 175 (2019) 384 – 392

Abstract

This study was aimed at assessing the prenatal exposure to perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) in a cohort of pregnant women living in Reus (Tarragona County, Catalonia, Spain). These chemicals were biomonitored in maternal plasma during the first trimester of pregnancy, at delivery, and in cord blood. The dietary exposure of PFOS and PFOA was estimated by using questionnaires of food frequency and water intake, as well as data on food levels previously reported in the same area. In addition, the exposure through air inhalation and indoor dust ingestion was also calculated. Finally, a physiologically-based pharmacokinetic (PBPK) model was applied in order to establish the prenatal exposure of the fetus/child and to adjust exposure assessment vs. biomonitoring results. Probabilistic calculations of fetal exposure were performed by forward internal dosimetry and Monte-Carlo simulation. Mean plasma levels of PFOA were 0.45, 0.13 and 0.12 ng/mL at the first trimester, at delivery and in cord plasma, while those of PFOS were 2.93, 2.21, and 1.17 ng/mL, respectively. Traces of PFOS were found in all samples in the first trimester and at delivery, and almost in all cord blood samples. Transplacental transfers of PFOS and PFOA were estimated to be around 70% and 60%, respectively. A temporal decrease trend in plasma levels of PFOS and PFOA was noticed when comparing current values with data obtained 10 years ago in the same area. In agreement with many other studies, dietary intake was the main route of exposure to PFOS and PFOA in our cohort of pregnant women. It is an important issue to establish the exposure in critical windows periods such as fetal development to perfluoroalkylated substances, but also to other endocrine-disrupting chemicals.

Keywords

PFOS, PFOA, prenatal exposure, PBPK modeling, cord blood, plasma, dietary intake

1. Introduction

Perfluoroalkylated substances (PFASs) are a group of synthetic chemicals, which include perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). They share a common structure, as they own a long hydrophobic carbon chain saturated with fluorine atoms with a hydrophilic functional group at the end. This structure gives oil and water repellence, chemical stability and reduces surface tension (OECD, 2002). Since 1940s, these substances have been used in a wide range of industrial and commercial proposes, such as textiles, cosmetics, adhesives, electronic and photographic devices, cleaning agents, fire-fighting foams, paper, board, cookware and food packing materials, among others (Domingo and Nadal, 2017; Hekster et al., 2001).

Due to the high volume of production, demand and consumption of these products, PFASs have been detected in a number of environmental matrices, including air, dust and water (Fromme et al., 2009). Moreover, they have also been found in remote areas of the planet, such as the Tibetan Plateau and the Arctic (Cai et al., 2012; Shi et al., 2010; Shoeib et al., 2006). In addition, PFASs are highly resistant to degradation in the environment and tend to bioaccumulate in living organisms (Suja et al., 2009). As a result of this occurrence, traces of PFASs have been largely detected not only in wildlife but also in humans (Butt et al, 2010; Kannan et al., 2004; Kärrman et al., 2010; Letcher et al., 2010; Morales et al., 2015).

The food chain, as well as the water and indoor environments, are polluted with PFASs, meaning a worldwide health problem (Arrebola et al., 2018; Islam et al., 2018). The ingestion of contaminated food or the migration of these substances from food packaging or cookware have been identified as key sources of human exposure to PFASs (Begley et al., 2005; Domingo, 2012; Ericson et al., 2012; Fromme et al., 2009). In an exhaustive study performed in Tarragona County (Catalonia, Spain), it was established that the dietary intake is the main exposure pathway to PFOS and PFOA, followed by the consumption of drinking water (Ericson et al., 2008). In contrast, the indoor environment (dust and air) has a minor contribution, with less than 1% and 2% for PFOS and PFOA, respectively (Ericson et al., 2012). Among food, fish and seafood was pointed out as the most important contributor to

total dietary intake of both chemicals, while high levels of PFOS and PFOA were also found in eggs and meat, respectively (Domingo and Nadal, 2017; Sungur, 2018).

Concerning the exposure of pregnant women, the placental barrier is not impermeable to the passage of PFOS and PFOA (Liu et al., 2011; Kim et al., 2011). Furthermore, these toxic substances can also be transferred from mother to child during breastfeeding (Kärrman et al., 2010; Motas Guzmàn et al., 2016). PFOS and PFOA have been recognized as endocrine disrupting chemicals (WHO, 2013). Moreover, prenatal and early exposure to PFASs is associated with a decreased birth weight and gestational age (Meng et al., 2018), developmental problems (Chen et al., 2013), reproductive system problems (Lyngsø et al., 2014), and increased susceptibility to disease in adulthood (Tsai et al., 2015; Zhang et al., 2018). However, some controversy exists in the association between PFASs and decrease in birth weight. A meta-analysis (Steenland et al, 2018) showed little or no association with decreased birthweight when PFASs were measured during early pregnancy where potential confounding exposure by the glomerular filtration rate would be minimal. However, Meng et al. (2018) study performed in early pregnancy, not included in this meta-analysis, found association between PFASs and a decrease of birth weight (Verner et al., 2015).

This study aimed at assessing the prenatal exposure to PFOA and PFOS from a cohort of pregnant women in Tarragona County (Catalonia, Spain). To achieve this goal, PFOA and PFOS were biomonitored in maternal blood during the first trimester of pregnancy, at delivery and in cord blood. In addition, the intake of PFOS and PFOA though food and drinking water consumption for this cohort was also estimated, while the potential contribution of indoor dust ingestion and air inhalation were also calculated according to the activity profile of these pregnant women.

2. Materials and methods

2.1. Subjects

The study population comprised a cohort of pregnant women and an ongoing birth cohort. Pregnant women were recruited during the first trimester of pregnancy as part of the HEALS

European project. The recruitment of pregnant women started in March 2016 and ended in September 2017 and, in the present survey, 50 mother-child pairs from Reus (Tarragona, Spain) were included. Women were informed about the investigation during their first visit (12^{th} gestational week (GW)) to the University Hospital "Sant Joan" in Reus (Catalonia, NE Spain). Women were eligible to participate according to the following inclusion criteria: ≥16 years old, intention to deliver at the reference hospital, and no problems with the communication language. This study was approved by the Ethical Committee of Clinical Research of the Hospital and a written informed consent was obtained from the participants. A description of the characteristics of the study population is shown in Table 1.

Table 1 Characteristics of the participants in the study: 50 pregnant women living in Reus (Catalonia, Spain).

Maternal age at delivery (years)		**Water consumption**	
<20	0%	<1 L	6%
20–29	15%	1–2 L	87%
30–39	71%	>2 L	7%
>40	14%	**Type of water consumed**	-
Twin pregnancy	7%	Tap water	6%
Number of children		Bottled water	77%
Primiparous	35%	Both	17%
1	48%	**Fish and seafood consumption in pre-pregnancy**	
2	15%	<2 servings/week	19%
>2	2%	2 to 4 servings/week	53%
Maternal pre-pregnancy BMI		>4 servings/week	28%
		Fish and seafood consumption during	
Underweight (< 19 kg/m^2)	6%	**pregnancy**	
Normal (19–25 kg/m^2)	50%	<2 servings/week	27%
Overweight (>25-30 kg/m^2)	28%	2 to 4 servings/week	55%
Obese (>30 kg/m^2)	19%	>4 servings/week	18%
Maternal pregnancy (32 GW) BMI		**Milk consumption in pre-pregnancy**	
Underweight (<24 kg/m^2)	13%	<1 serving/day	25%
Normal (24–28 kg/m^2)	31%	1 to 2 servings/day	64%
Overweight (>28-32 kg/m^2)	35%	>2 servings/day	11%
Obese (>32 kg/m^2)	21%	**Milk consumption during pregnancy**	
Maternal education		<1 serving/day	24%
Primary	23%	1 to 2 servings/day	60%
Secondary	29%	>2 servings/day	16%
University	48%	**Eat Fast-food in pre-pregnancy**	
Social economic status (€/year)		Never	38%
Low level (<9000)	10%	≤ 1 serving/week	52%
Mid-Low level (9000-19,000)	41%	> 1 serving/week	10%
Mid-High level (>19,000-35,000)	20%	**Eat Fast-food during pregnancy**	
High level (>35,000)	29%	Never	66%
Maternal country of origin		≤ 1 serving/week	31%
Spain	69%	>1 serving/week	3%
Other	31%	**Eat organic products in pre-pregnancy**	
Marital Status		Never	49%
Living with the father	98%	Sometimes	29%
Not living with the father	2%	Frequently	18%
Smoking 1 year before pregnancy		Almost daily	4%
Non-smoker	71%	**Eat organic products during pregnancy**	
Active smoker	29%	Never	48%
1-5 cigarettes/day	20%	Sometimes	36%
6-10 cigarettes/day	4%	Frequently	10%
>10 cigarettes/day	5%	Almost daily	6%

[a] BMI: Body mass index; GW: gestational week

2.2. Questionnaires for data acquisition and sampling

Face-to-face and personal interviews were used in order to determine the pregnant women's dietary intake of PFOS and PFOA. Dietary factors were assessed using food frequency questionnaires (FFQs), which were originally designed to evaluate the average dietary intake in two phases: the 1st FFQ covered the year before pregnancy, and the 2nd FFQ covered the whole pregnancy including the last period until birth. Food items were classified into 9 general groups: a) grains and grain-based products (cereals, pasta, rice, and bread), b) milk and dairy products (milk, yogurt, hard cheese, and fresh cheese), c) meat (chicken, turkey, beef, pork, lamb, and minced meat), d) fish and seafood (white fish, blue fish and seafood), e) fruits and vegetables (salad, green beans, Swiss chard, spinach, and garnish vegetables), f) legumes and potatoes (lentils, chickpeas, white beans, and potatoes), g) eggs, h) sugar and confectionary (cupcakes, biscuits, stuffed cookies, donuts, sweets, cream or chocolate cakes, chocolate, and ice cream), and i) drinking water (tap water and bottle water). As well as dietary factors, the questionnaires also included information on other non-dietary sources such as smoking, lifestyle, time spent outdoors/indoors, and occupational risk. Therefore, based on this information, dietary (food) and non-dietary (inhalation and dust ingestion) values of exposure were estimated individually. Blood samples were collected at the end of the first trimester (around the 12th GW), at delivery, as well as from the umbilical cord. Blood was centrifuged at 3500 rpm for 15 minutes to separate the plasma. The aliquots of plasma (2 mL) were stored at -80°C until the determination of PFOS and PFOA.

2.3. Analysis of PFOS and PFOA

PFOA and PFOS analyses were performed as previously described (Vassiliadou et al., 2010). Briefly, 2 mL of blood plasma, 200 µL of the internal standard working solution (containing 100 ng/mL of $^{13}C_4$-labelled PFOS and 100 ng/mL of $^{13}C_4$-labelled PFOA in methanol) and 20 mL of acetonitrile were added and vortex-mixed for 1 min. The sample was centrifuged at 4000 rpm for 5 min to clarify the supernatant. The organic phase was evaporated until dryness in a flash evaporator and the residue obtained was dissolved in 5 mL of phosphate

buffer solution 0.05 M, pH = 7.8. A solid-phase extraction was performed conditioning a C18 cartridge with 2.5 mL of methanol and 5 mL of water. The sample was passed through, and then the cartridge was washed with 5 mL of water. Finally, PFOS and PFOA were eluted from the cartridge with 5 mL of methanol. The flow rate of the cartridge was approximately 1–2 drops per second. The organic phase was evaporated until dryness in a flash evaporator and re-suspended in 200 µL of methanol:ammonium acetate (5 mM) (20:80, v/v).

Sample extracts were analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) with electrospray ionization (ESI) operating in negative mode. The method was validated for accuracy, repeatability, robustness, recovery and sensitivity with spiked samples at three different levels (n = 3) of 1, 5 and 10 ng/mL. The limits of detection (LoD) and quantification (LoQ) were determined as signal/noise (S/N) ratio of 3 and 10, respectively. For both, PFOS and PFOA, the LoD was calculated at 0.1 ng/mL.

2.4. Exposure assessment

Pregnant women exposure to PFOA and PFOS was assessed at the 12th and 32nd gestational weeks (GW) in a probabilistic way using a Monte Carlo simulation. Equations, data and parameters were adapted and described elsewhere (Martínez et al., 2017, 2018). The total exposure to PFOS and PFOA for pregnant women was considered as the sum of dietary intake (DI), dust ingestion ($Dust_{Ing}$) and inhalation (Inh), being calculated by applying the following equations:

$$DI = \sum(C_{PFOA/PFOS}(i) \cdot F_r(i) \cdot F_f(i)) / BW / 7 \quad \text{Eq. (1)}$$

$$Dust_{ing} = (C_{PFOA/PFOS}dust \cdot Ir) / BW \quad \text{Eq. (2)}$$

$$Inh = (C_{PFOA/PFOS}air \cdot Ihr) / BW \quad \text{Eq. (3)}$$

Where DI is the total dietary intake of PFOA or PFOS (in µg/kg bw/day); $C_{PFOA/PFOS}$ (i) is the concentration of PFOS or PFOA in the food category *i* (in µg/kg); F_r(i) is the food ingestion rate of food category *i* (in kg/ration); F_f(i) is the food consumption frequency of category i

(in rate/week); BW is the body weight (in kg); $C_{PFOA/PFOS}$dust is the level of PFOS or PFOA in homes dust (in µg/kg); Ir is the Ingestion rate (in kg/day); $C_{PFOA/PFOS}$air is the concentration of PFOA or PFOS in air (in µg/m^3); and Ihr is the inhalation rate (in m^3/day).

The concentration levels of PFOS and PFOA in different food items, in dust and in air were taken from the literature, applying as preference rule, data from Tarragona County > Catalonia > Europe. To deal with the variability and uncertainty of parameters, the dietary and non-dietary (dust ingestion and inhalation) exposure was estimated in a probabilistic way. Monte-Carlo simulation is a common approach used to incorporate variability and uncertainty of the parameters used into the estimation of human health exposure (Linares et al., 2010; Nadal et al., 2004; Rovira et al., 2016). The Monte-Carlo simulation was carried out by Oracle Crystal Ball© software. Exposures were calculated based on the propagation of variability and uncertainty given by each parameter probability function until 100,000 iterations. Data and probabilistic distributions used to assess total exposure of PFOA and PFOS are shown in the supplementary material (Table S1, Supplementary Information). To calculate the exposure, levels below the LoD were assumed to be zero.

2.5. Physiologically-based pharmacokinetic (PBPK) modelling

The PBPK model of PFASs was adapted from previously published studies (Fàbrega et al., 2014, 2015; Sharma et al. 2018a). It comprises plasma, fat, brain, lung, gut, liver, kidneys, filtrate, mammary gland, bone marrow, placenta, and the rest of the body. As the target population was pregnant women, a fetal compartment was included. Furthermore, a renal resorption process was inserted in the model. In the filtrate compartment, chemicals are reabsorbed back in the plasma comportment by a saturable process (Andersen et al., 2006; Tan et al., 2008). The fetal compartment was subcategorized again into liver, kidney, brain, and plasma. All the physiological parameters during pregnancy were considered as dynamic, so they changed due to the growth of maternal organs (Abduljalil et al., 2012; Gentry et al., 2003; Loccisano et al., 2013). The source of exposure to fetuses was via a free fraction of chemicals into mother's placenta, considering that fetuses' exposure is directly

related to mother's exposure. In the plasma compartment, more than 90% of PFOA and PFOS is bound to albumin, and only less than 10% is free to move to other tissues (Han et al., 2003). Detailed descriptions of standard and pregnancy-specific model equations were adapted from those published by Sharma et al. (2018b). Metabolic kinetic parameters for both mothers and fetuses were previously estimated from *in vitro* studies.

2.6. Statistical analysis

For statistical analysis, the software package IBM SPSS Statistics (version 25.0) was used. To elucidate whether data presented a parametric distribution, a Levene test was performed. Subsequently, Student's t-test, ANOVA (parametric data) or Kruskal-Wallis (non-parametric data) test were applied. A significance level of 0.05 (p<0.05) was established.

3. Results and discussion

3.1. Levels of PFOS and PFOA

Plasma levels of PFOS and PFOA in maternal blood at the first trimester, at delivery and in cord blood are depicted in Fig. 1. PFOA mean values were 0.45, 0.13 and 0.12 ng/mL, respectively, with only four samples above the LOD (0.1 ng/mL) in maternal plasma at delivery. Plasma levels in the first trimester were significantly higher than those at delivery, and in cord blood ($p<0.001$). PFOS presented higher levels than PFOA, with mean concentrations of 2.93, 2.21, and 1.17 ng/mL at the first trimester, at delivery and in cord plasma, respectively. PFOS was detected in all plasma samples, except in a single sample of cord blood. Furthermore, significant concentrations ($p<0.001$) were found according to the pregnancy period, with a decreasing trend with time. High positive Pearson's correlations were found in PFOS levels when comparing the values of the first trimester and those at delivery (0.855), between the first trimester and in cord blood (0.720), and between those at delivery and in cord blood (0.708). Regarding PFOA levels, no significant correlations were found due to the low ratio of detected samples in cord blood, and especially in mother plasma at delivery. Finally, a low but significant ($p< 0.05$) Pearson's correlation coefficient

was found between PFOS and PFOA levels during the first trimester (0.349) (Table S2, Supplementary Information).

Decreases of 69% and 25% in PFOA and PFOS plasma levels, respectively, between the first trimester and at delivery were registered. Our results are in agreement with others from the scientific literature, where a decreasing trend during pregnancy has also been reported. Reduction rates of PFOS in plasma have been observed to range 11-30%, while those of PFOA are even higher (16-30%) (Glynn et al. 2012; Kato et al. 2014). This fact could be due to both a placental transfer and a dilution process caused by the increase of blood plasma volume (Mitro et al., 2015; Glynn et al. 2012). Moreover, Caserta et al. (2018) also observed the occurrence of transplacental transfer of PFOS and PFOA. In this study, around 70% and 60% of maternal plasma levels in the first trimester were found in cord blood for PFOA and PFOS, respectively. Similar trends have been observed, especially for PFOS, in several studies worldwide (Buck et al. 2018; Cariou et al., 2015; Han et al., 2018; Kim et al., 2011).

The levels of PFOS and PFOA in blood of pregnant women and in cord blood in several studies performed around the world are summarized in Table 2. Plasma concentrations of both chemicals in pregnant women living in Reus (Spain) were notably lower compared to other studies in Germany, Denmark, USA, as well as in some Asian countries (e.g., China, Korea, and Japan). They are also slightly lower but in the same order of magnitude as those found in other residential areas in Spain (Valencia, Sabadell, and Gipuzkoa) (Matilla-Santander et al., 2017). On the other hand, our findings were in line with levels found in studies from France (Cariou et al., 2015; Dereumeaux et al., 2016), Italy (Caserta et al., 2018), Australia (Callan et al., 2016), and USA (Morello-Frosch et al. 2016). In the latter studies, samples were collected more recently, being this an important issue to be considered when comparing different studies. Decreasing temporal trends in PFOS and PFOA blood levels were found in pregnant women in Sweden from 1996 to 2010 (Glynn et al., 2012), in Australia between 2002 and 2011 (Toms et al., 2014), and in an adult population of USA between 2000 to 2015 (Olsen et al., 2017). In 2006, our group performed a biomonitoring study of PFASs in an adult female population in the same area (Tarragona County). PFOS and PFOA levels in blood were 6.81 and 1.57 ng/mL, respectively (Ericson et

al., 2007). This means that in 10 years (from 2006 to 2016/17), plasma levels of PFASs have decreased to about one third in this area, being reduced from 6.81 to 2.93 ng/mL, for PFOS, and from 1.57 to 0.45 ng/mL, for PFOA. It should be finally remarked that only in some specific studies from China, PFOA levels are higher than PFOS levels in maternal and cord blood (Tian et al., 2018; Han et al., 2018).

Table 3 shows the levels of PFOA and PFOS in maternal plasma at the first trimester, at delivery and cord blood plasma according to maternal characteristics. Only few significant ($p<0.05$) differences were noted. PFOS levels in cord blood and PFOA levels in the first trimester of pregnancy were significantly higher ($p<0.05$) in primiparous than multiparous women. Underweight in pre-pregnancy (BMI<18.5 kg/m^2) and medium-high annual income (>19,000 €) is associated with higher levels ($p<0.05$) of PFOA at the first trimester of pregnancy. In contrast, a lower annual income (<9,000-19,000 €) is associated with higher ($p<0.05$) levels of PFOS at the first trimester and at delivery, but not in cord blood. Despite not reaching levels of significance ($p<0.05$), an increasing trend of PFOS and PFOA plasma levels was detected with the increase of maternal age. This fact has been largely reported elsewhere (Tsai et al. 2018; Manzano-Salgado et al., 2016). Significantly ($p<0.05$) higher PFOA levels were found in Spanish mothers. In an investigation of pregnant women conducted in Spain, Manzano-Salgado et al. (2016) pointed out that maternal PFAS concentrations during pregnancy were mainly associated with maternal country of birth (higher in Spanish mothers), parity (decreasing trend of PFAS levels with number of children) and age (lower levels in younger mothers).

Regarding the food intake, Table 4 summarizes the levels of PFOS and PFOA in maternal plasma at the first trimester, at delivery and in cord blood plasma in relation to food frequency intakes. The chosen food groups were selected to be relevant to PFAS exposure according to levels found in Catalonia and international previous studies (Ericson et al., 2008; Domingo and Nadal, 2017; Sungur, 2018). Concerning mother characteristics, few differences were found between groups. Significant differences ($p<0.05$) in PFOS levels at the first trimester and in cord blood were found only between groups reporting high milk consumption (>1 serving/day) and medium consumption (1 serving/day). Higher levels of

PFOA at the first trimester were also found in the group reporting high egg consumption (>7 servings/week). Despite that no significant differences ($p>0.05$) were found between high- and medium-fish and seafood consumer groups, an increasing trend in PFOS and PFOA was noted when the consumption edible marine species increases. The same tendency was pointed out in PFOA levels, especially in the first trimester, when milk consumption increased. Manzano-Salgado et al. (2016) found that fish and shellfish consumption was the main factor associated with higher PFAS levels in a Spanish cohort.

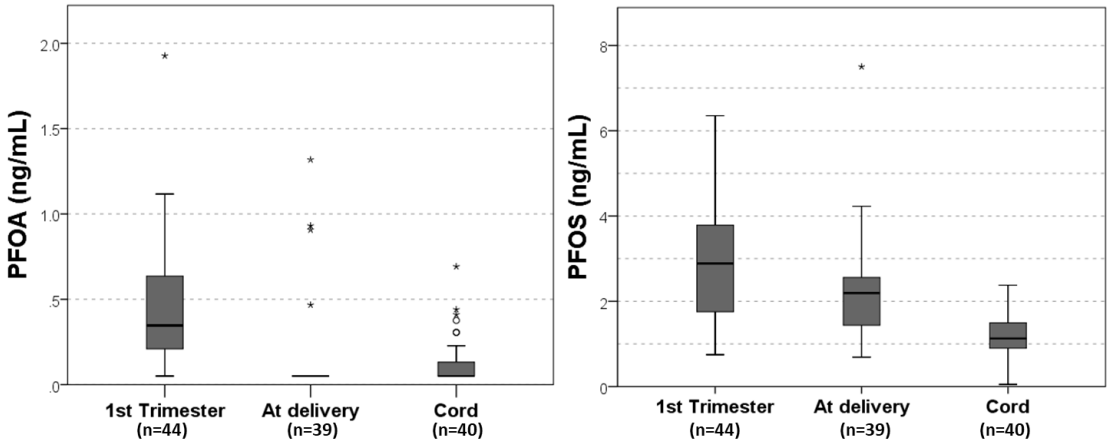


Fig. 1. PFOA and PFOS levels in plasma of pregnant women during the first trimester, at delivery and in cord blood.

Table 2 PFOA and PFOS levels in maternal and cord blood recently published in the scientific literature.

					PFOA (ng/mL)				PFOS (ng/mL)			
					Maternal		Cord		Maternal		Cord	
	Country	Year	Sampling	n	Mean (P 95%)	n	Mean (P 95%)	n	Mean (P 95%)	n	Mean (P 95%)
Europe												
Present study	Spain	2016/17	T1 At del.	44 39	0.45 (P95: 1.08) 0.14 (P95: 0.93)	40	0.12 (0.45)	44 39	2.93 (P95: 5.48) 2.20 (P95: 4.23)	40	1.17 (P95: 2.03)
Caserta et al., 2018	Italy	2016	At del.	29	1.05 [0.45-1.9][a]	29	0.98 [0.30-2.50] [a]	29	1.54 [0.02-4.7][a]	29	1.75 [0.02-6.00][a]
Dereumeaux et al., 2016	France	2011	T3	277	1.49 (1.39, 1.59)[b]	-	-	277	3.07 (2.87, 3.27)[b]	-	-
Cariou et al., 2015	France	2010/13	At del.	100	1.22 [0.31-7.31][a]	94	0.92 [0.31-7.06][a]	100	3.67 [0.32-24.5][a]	94	1.28 [<LD-8.04][a]
Glynn et al., 2012	Sweden	2009/10	After del.	pool	[1.39-2.40] ng/g[c]	-	-	pool	[5.11-8.89] ng/g[c]	-	-
Matilla-Santander et al., 2017	Spain	2003/08	T1	1240	2.31 (P95: 5.23)	-	-	1240	5.77 (P95: 11.4)	-	-
Wilhelm et al. 2015	Germany	2000/02	T3	81	2.69 (P95: 5.09)	104	1.02 (P95: 2.09)	81	9.01 (P95: 15.4)	104	1.74 (P95: 3.10)
Meng et al. 2018	Denmark	1996/02	T1/T2	3535	4.6 (3.3-6.0)[d]	-	-	3535	30.1 (22.9-39.0) [d]	-	-
Impinen et al. 2018	Norway	1992/93	-	-	-	641	1.8 [0.1-11][a]	-	-	641	5.6 [0.5-21][a]
Asia												
Han et al., 2018	China	2010/13	At del.	369	39.3 (P95: 144)	369	31.8 (P95: 110)	369	4.25 (P95: 10.1)	369	1.33 (P95: 3.30)
Tian et al., 2018	China	2012	T2	981	19.6 (P95: 25.4)	-	-	981	10.8 (P95: 26.8)	-	-
Tsai et al., 2018	Japan	2003/12	T3	2123	2.06 [0.25-24.9][e]	-	-	2123	4.96 [0.81-30.3][e]	-	-
Kim et al., 2011	Korea	2008/09	T3.	44	1.46 (1.15-1.91) [d]	43	1.15 (0.95-1.86)[d]	44	2.93 (2.08-4.36) [d]	43	1.26 (0.81-1.82)[d]
America												
Morello-Frosch et al. 2016	USA	2010/11	T2/T3	77	0.47 (P95: 2.14)	64	<LD (P95: 1.68)	77	2.55 (P95 7.25)	64	2.27 (P95: 4.35)
Buck et al. 2018	USA	2003/06	T1 At del.	71	T1: 4.91(4.32, 5.59)[d]	71	2.85 (2.51, 3.24)[d]	71	T1: 11.57(9.90, 13.53)[d]	71	3.32 (2.84, 3.89)[d]

				PFOA (ng/mL)	PFOS (ng/mL)							
					Maternal		Cord		Maternal		Cord	
	Country	Year	Sampling	n	Mean (P 95%)	n	Mean (P 95%)	n	Mean (P 95%)	n	Mean (P 95%)	
Europe												
					3.43(3.01-3.90)[d]				8.20(7.01-9.58)[d]			
Woodruff et al. 2011	USA	2003/04	T1 to T3	76	2.39 (P95: 5.6)	-	-	-	12.3 (P95: 21.8)	-	-	
Africa and Oceania												
Callan et al., 2016	Australia	2008/11	T3	98	1.00 [0.21-3.1]	-	-	98	2.32 [0.45-8.1]	-	-	
Hanssen et al. 2010	South Africa	2005/06	At delivery	71	1.3 [0.17-8.5][e]	58	1.3 [<LD-10.5][e]	71	1.6 [<LD-15.9][e]	58	0.7 [<LD-10.8][e]	

T1, T2, and T3: first, second, and third trimester of pregnancy, respectively. At del.: at delivery. NA: Not available.
[a] mean [Range]; [b] geometric mean (95% confidence interval); [c] [Range] in ng/g; [d] median (Interquartile range (IQR)); [e] median [Range].

Table 3 PFOA and PFOS plasma levels (in ng/mL*)* according to mother and pregnancy characteristics. Data given as mean (standard deviation).

	PFOA			PFOS		
	First trimester	**At delivery**	**Cord**	**First trimester**	**At delivery**	**Cord**
Mothers age						
<30 years old	0.32(0.26)	<0.10	<0.10	2.40(1.11)	1.63(0.90)	1.17(0.39)
30-35 years old	0.44(0.46)	0.12(0.31)	0.15(0.18)	2.81(1.26)	2.17(0.88)	1.16(0.48)
>35 years old	0.51(0.27)	0.18(0.30)	0.13(0.14)	3.19(1.41)	2.37(1.54)	1.16(0.46)
Body mass index (pre-pregnancy)						
<18.5 kg/m^2	0.84(0.27)^a	<0.10	0.16(0.19)	3.86(1.48)	1.86(1.00)	1.11(0.51)
18.5-25 kg/m^2	0.44(0.40)^{ab}	0.16(0.34)	0.15(0.18)	2.65(1.07)	2.02(0.76)	1.11(0.42)
>25 kg/m^2	0.38(0.29)^b	0.12(0.22)	0.10(0.11)	3.09(1.49)	2.48(1.58)	1.20(0.50)
Nationality						
Spanish	0.52(0.38)^a	0.15(0.31)	0.14(0.16)	3.08(1.28)	2.24(1.21)	1.21(0.46)
Other	0.26(0.20)^b	<0.10	0.07(0.06)	2.53(1.30)	2.03(1.19)	0.89(0.30)
Education						
Primary	0.32(0.32)	0.10(0.14)	<0.10	3.26(1.60)	2.89(2.03)	1.37(0.62)
Secondary	0.41(0.31)	0.17(0.32)	0.10(0.12)	2.48(1.33)	1.81(0.66)	0.97(0.43)
University	0.55(0.42)	0.13(0.32)	0.19(0.19)	3.06(1.02)	2.17(0.80)	1.22(0.33)
Annual income						
<9.000-19.000€	0.24(0.20)^a	0.10(0.15)	<0.10	3.53(1.56)^a	3.24(2.05)^a	1.40(0.59)
19.001-35.000€	0.43(0.26)^b	0.19(0.39)	0.16(0.20)	2.39(1.07)^a	1.76(0.81)^b	1.03(0.42)
>35.000€	0.66(0.50)^b	0.12(0.24)	0.14(0.12)	3.27(1.17)^{ab}	2.21(0.53)^{ab}	1.20(0.43)
Parity						
Primiparous	0.61(0.42)^a	0.08(0.12)	0.10(0.12)	3.16(1.22)	2.27(0.92)	1.38(0.52)^a
1	0.35(0.23)^b	0.18(0.37)	0.17(0.19)	2.83(1.38)	2.36(1.60)	1.08(0.46)^b
≥2	0.26(0.32)^b	0.18(0.33)	0.08(0.07)	2.38(1.13)	1.88(0.84)	0.93(0.13)^b
Breastfeeding last child						
Yes	0.34(0.27)	0.23(0.43)	0.13(0.19)	2.89(1.40)	2.60(1.72)	0.97(0.38)
No	0.32(0.27)	0.14(0.27)	0.19(0.16)	2.48(1.24)	1.87(0.88)	1.14(0.44)
Years form last child						
No previous child	0.61(0.42)	0.08(0.12)	0.10(0.12)	3.16(1.22)	2.27(0.92)	1.38(0.52)
≤ 3 years	0.37(0.28)	0.23(0.42)	0.17(0.21)	2.64(1.23)	2.14(0.86)	1.10(0.43)
>3 to 10 years	0.25(0.12)	0.16(0.31)	0.13(0.13)	2.49(1.12)	1.66(0.86)	0.95(0.40)
>10 years	0.51(0.65)	<0.10	0.13(0.16)	2.55(0.24)	2.22(0.39)	1.00(0.13)
Smoking						
Never	0.41(0.29)	0.18(0.33)	0.14(0.17)	3.04(1.38)	2.44(1.30)	1.15(0.47)
Not during pregnancy	0.57(0.52)	<0.10	0.09(0.11)	2.74(1.06)	1.68(0.79)	1.22(0.50)
Yes	0.40(0.50)	<0.10	0.13(0.15)	2.17(1.01)	1.68(0.63)	1.00(0.14)

Different superscripts indicate significant differences at 0.05 level.

Table 4 PFOA and PFOS plasma levels (in ng/mL) according to the mother's food frequency. Data given as mean (standard deviation).

	PFOA			PFOS		
	first trimester	**At delivery**	**Cord**	**first trimester**	**At delivery**	**Cord**
Fish and seafood consumption						
<2 times/week	0.38(0.21)	<0.10	0.15(0.15)	3.05(1.16)	2.04(1.11)	1.17(0.53)
2 to 4 times/week	0.41(0.32)	0.21 (0.37)	0.13(0.16)	2.76(1.17)	2.10(0.80)	1.14(0.44)
>4 times/week	0.54(0.51)	<0.10	0.08(0.09)	3.20(1.73)	2.56(1.86)	1.18(0.43)
Meat consumption						
<7 times/week	0.34(0.30)	0.08(0.11)	0.09(0.09)	3.45(1.49)	2.65(1.72)	1.26(0.53)
7 to 14 times/week	0.52(0.40)	0.20(0.37)	0.14(0.18)	2.84(1.14)	2.04(0.79)	1.11(0.39)
>14 times/week	0.35(0.23)	<0.10	0.12(0.11)	2.01(0.98)	1.79(0.72)	1.16(0.54)
Eggs consumption						
<2 times/week	0.34(0.29)[a]	0.24(0.43)	0.14(0.21)	2.85(1.35)	2.40(1.07)	1.16(0.61)
2 times/week	0.38(0.26)[a]	0.15(0.28)	0.12(0.12)	2.89(1.46)	2.23(1.51)	1.09(0.30)
>2 times/week	0.62(0.48)[b]	<0.10	0.13(0.15)	3.05(1.14)	2.05(0.82)	1.23(0.48)
Milk consumption						
<1 time a day	0.48(0.31)	0.26(0.46)	0.21(0.24)	3.47(1.12)[a]	2.36(0.80)	1.30(0.41)[a]
1 time a day	0.37(0.25)	0.09(0.18)	0.11(0.11)	2.57(1.16)[b]	2.07(1.34)	1.04(0.38)[b]
>1 time a day	0.73(0.78)	0.13(0.19)	0.05(0.01)	3.81(1.87)[a]	2.58(1.21)	1.46(0.67)[a]
Cheese consumption						
<3 times/week	0.30(0.29)	0.17(0.29)	0.13(0.13)	2.91(1.43)	2.13(0.99)	1.21(0.51)
3 to 7 times/week	0.48(0.42)	0.09(0.18)	0.11(0.12)	2.85(1.39)	2.22(1.35)	1.13(0.46)
>7 times/week	0.50(0.23)	0.26(0.52)	0.16(0.26)	3.17(0.98)	2.28(1.02)	1.16(0.36)
Fast food consumption						
0 times a week	0.43(0.27)	0.26(0.46)	0.14(0.21)	2.60(1.11)	1.99(0.95)	1.12(0.55)
1 time/week	0.51(0.43)	<0.10	0.11(0.12)	3.23(1.26)	2.54(1.42)	1.17(0.34)
>1 time/week	0.40(0.32)	0.16(0.30)	0.19(0.16)	2.72(1.33)	1.88(0.60)	1.05(0.41)
Water consumption						
≤1 litter /day	0.47(0.46)	0.12(0.22)	0.10(0.10)	2.77(1.16)	2.12(0.80)	1.21(0.52)
>1 litter/day	0.42(0.27)	0.15(0.33)	0.14(0.18)	3.05(1.43)	2.28(1.47)	1.11(0.37)

Different superscripts indicate significant differences at 0.05 level.

3.2. Exposure assessment

The total exposure to PFOS and PFOA, as well as the contribution of each pathway (dietary intake, inhalation and dust ingestion), are depicted in Fig. 2. The global dietary intake of PFOA and PFOS, including drinking water, at the first trimester of pregnancy was estimated in $1.3 \cdot 10^{-4}$ (CI 95%: $1.1 \cdot 10^{-4}$; $1.7 \cdot 10^{-4}$) and $3.3 \cdot 10^{-3}$ µg/(kg·day) (CI 95%: $1.7 \cdot 10^{-3}$; $6.6 \cdot 10^{-3}$), respectively. In the 32nd GW, the global dietary intake of PFOA and PFOS was calculated in $9.8 \cdot 10^{-5}$ (CI 95%: $8.2 \cdot 10^{-5}$; $1.2 \cdot 10^{-4}$) and $2.5 \cdot 10^{-3}$ µg/(kg·day) (CI 95%: $1.4 \cdot 10^{-3}$; $4.6 \cdot 10^{-3}$), respectively. The slight decrease in both, PFOS and PFOA, exposure levels between 12th and 32nd GW was due to an increase of the body weight, while no significant changes in the dietary intake patterns were reported. The global mean inhalation exposure at the 12th GW for PFOA and PFOS was $2.2 \cdot 10^{-6}$ (CI 95%: $9.0 \cdot 10^{-7}$; $4.3 \cdot 10^{-6}$) and $5.0 \cdot 10^{-6}$ (CI 95%: $4.6 \cdot 10^{-7}$; $1.6 \cdot 10^{-5}$) µg/(kg·day), respectively. Finally, the mean dust ingestion at 12th GW of PFOA and PFOS was estimated in $2.0 \cdot 10^{-6}$ (CI 95%: $6.2 \cdot 10^{-7}$; $4.9 \cdot 10^{-6}$) and $1.9 \cdot 10^{-6}$ (CI 95%: $6.2 \cdot 10^{-7}$; $4.8 \cdot 10^{-6}$) µg/(kg·day), respectively.

Recently, the European Food Safety Authority (EFSA) published its scientific opinion on the risk of the presence of PFOA and PFOS in food (EFSA, 2018). EFSA estimated a dietary exposure for the adult population between 0.22 and 3.28 ng/(kg·day) for PFOA and between 0.29 and 4.08 ng/(kg·day) for PFOS. In a previous study conducted in Catalonia, the adult female dietary ingestion was estimated to be $2.3 \cdot 10^{-3}$ and $2.2 \cdot 10^{-3}$ µg/(kg·day) for PFOA and PFOS, respectively (Domingo et al., 2012b). Furthermore, when analyzing the role of inhalation as a contributive exposure pathway of PFOA and PFOS for the Catalan population (Ericson et al., 2012), an estimated exposure of $5.7 \cdot 10^{-4}$ µg/(kg·day) for PFOA and $1.4 \cdot 10^{-3}$ µg/(kg·day) for PFOS was reported, with a contribution of <1% for inhalation and dust ingestion (Ericson et al., 2012). In Latvia, very similar exposure levels were obtained in a cohort of pregnant women, with dietary exposure levels of 0.32 (P95: 0.62) and 0.43 (P95: 0.99) ng/(kg·day) for PFOA and PFOS, respectively (EFSA, 2018).

In 2008, EFSA set a provisional tolerable daily intake (TDI) for PFOA and PFOS at 1.5 and 0.15 µg/(kg·day), respectively (EFSA, 2008). These values were well above the current levels for

the population here assessed. However recently, EFSA (2018) updated the values regarding the provisional tolerable weekly intake (TWI) of PFOA at 13 ng/(kg·week) ($1.86 \cdot 10^{-3}$ µg/(kg·day)) and of PFOS at 6 ng/(kg·week) ($8.6 \cdot 10^{-4}$ µg/(kg·day)). Taking into account these new provisional tolerable intake limits, which are almost 1000-fold lower than the previous ones, the dietary exposure of PFOS for most pregnant women in the studied cohort would be higher than the provisional TWI. In addition, the PFOA exposure would be close to the provisional threshold. It must be highlighted that the current provisional TWI is based on epidemiological studies that have shown an increase in serum total cholesterol for PFOA and increased serum total cholesterol (adults) and decreased antibody response (children) for PFOS (EFSA, 2018). However, some recent findings, involving humans in a phase 1 clinical trial with PFOA (Convertino et al., 2018), genetically engineered "humanized" mice fed with PFOA (Pouwer et al., 2019) and a six-month feeding study of primates with PFOS (Chang et al., 2017), disagree with the results of the epidemiologic studies considered by EFSA in its opinion. This means an important issue of discussion before the publication of EFSA final report. In any case, despite TWIs are still provisional, it is important to state the current position of the regulatory body.

Fish and seafood showed the highest contribution in the total dietary intake for PFOS (86%) and the second highest for PFOA (42%), after milk and dairy products (48%) (Fig. 2). According to EFSA (2012), fish and other seafood is the highest contributor to PFOS (50-80%), while fruits and fruit products (8-27%), as well as meat and meat products (5-8%) have also some importance. With respect to PFOA, the food categories that mostly contribute to the total exposure are fruit and vegetables (18-39%), followed by fish and seafood (8-27%), eggs and eggs products (10-15%), and meat and meat products (2-11%).

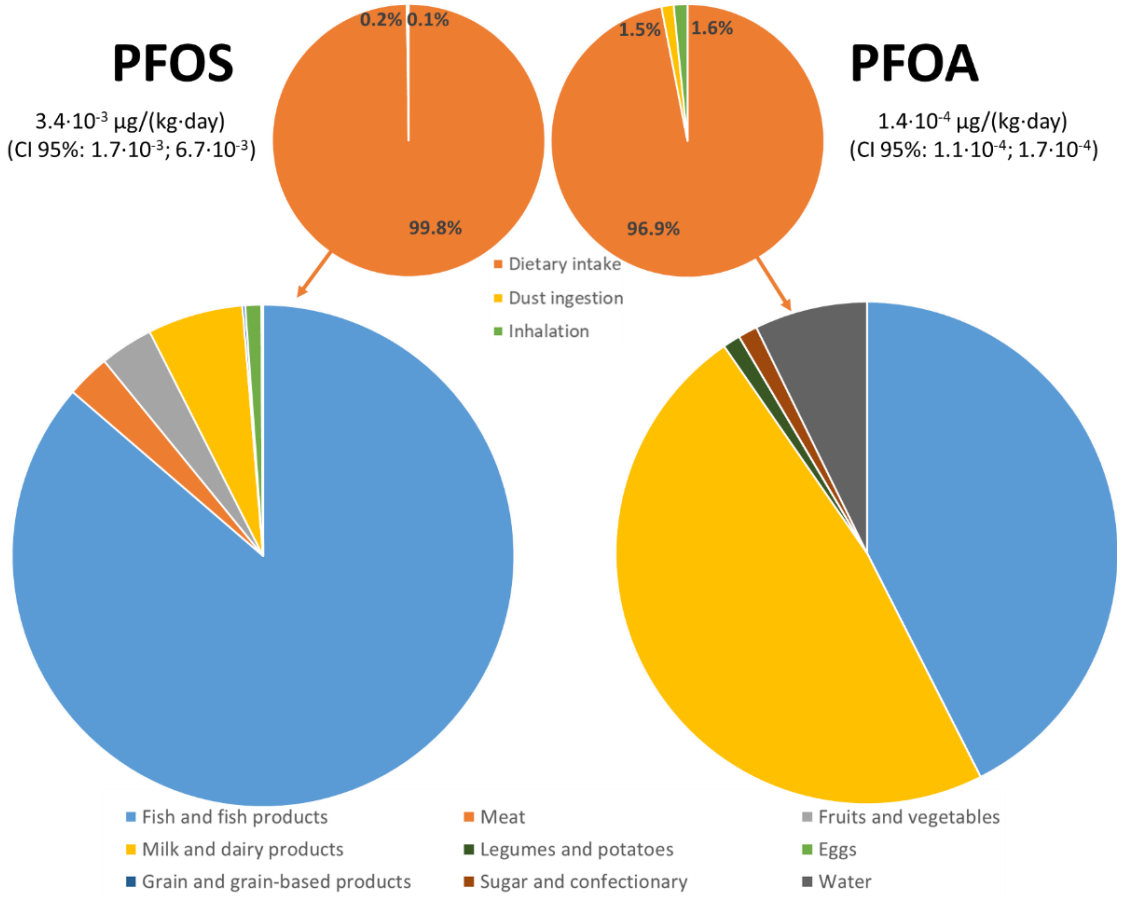


Fig. 2. Total PFOS and PFOA exposure: Main pathways (dietary intake, dust ingestion, and air inhalation) and contribution of each food category to the dietary intake.

3.3. Fetal exposure (PBPK modelling)

Pregnancy PBPK models of PFOA and PFOS were applied for individual participants with their respective physiology, age and exposure dose. We assumed that lifetime exposure of PFOA and PFOS was constant for each subject. The estimated mean plasma concentrations for the mother in the first trimester of pregnancy of PFOA and PFOS were 0.34 (CI 95%: 0.29; 0.38) and 3.14 ng/mL (CI 95%: 2.83; 3.46), respectively. Estimated mean plasma concentrations for the fetus during the first trimester of pregnancy (6-12th GW) were 0.33 (CI 95%: 0.28; 0.38) and 1.39 ng/mL (CI 95%: 1.25; 1.53) for PFOA and PFOS, respectively. At the time of delivery (38-39th GW), the estimated mean plasma concentrations of PFOA

and PFOS for the mother were 0.33 ng/mL (CI 95%: 0.28; 0.38) and 2.99 ng/mL (CI 95%: 2.71; 3.27), respectively. For the fetus (38-39th GW), they were 0.32 ng/mL (CI 95%: 0.27; 0.37) and 1.34 ng/mL (CI 95%: 1.22; 1.47), respectively (Fig. 3).

The model simulation results obtained for plasma were mostly in line with results from biomonitoring in samples for both PFOS and PFOA, excepting for plasma concentration of PFOA in the mother at delivery. In general terms, modelled levels were higher than those obtained by biomonitoring. This could be due to the fact that data used for exposure assessment were obtained from studies performed in the study area some years ago (Ericson 2008a,b, Domingo et al. 2012a). It is well known that levels of PFOS and PFOA have decreased in food during the last years (Johansson et al. 2014). In addition, the use of FFQ results and food ratios from the literature instead of food models could underestimate the real intake during pregnancy.

The amount of total PFOA and PFOS in the fetal body, in terms of body burden, significantly increased by the time of delivery. However, the relative concentrations of both chemicals showed a slight decrease due to the increased body weight of the fetus. In comparison to previous biomonitoring studies in the same area (Pérez et al. 2013; Fàbrega et al, 2016), a significantly lower concentration of PFOA has been observed in the present study.

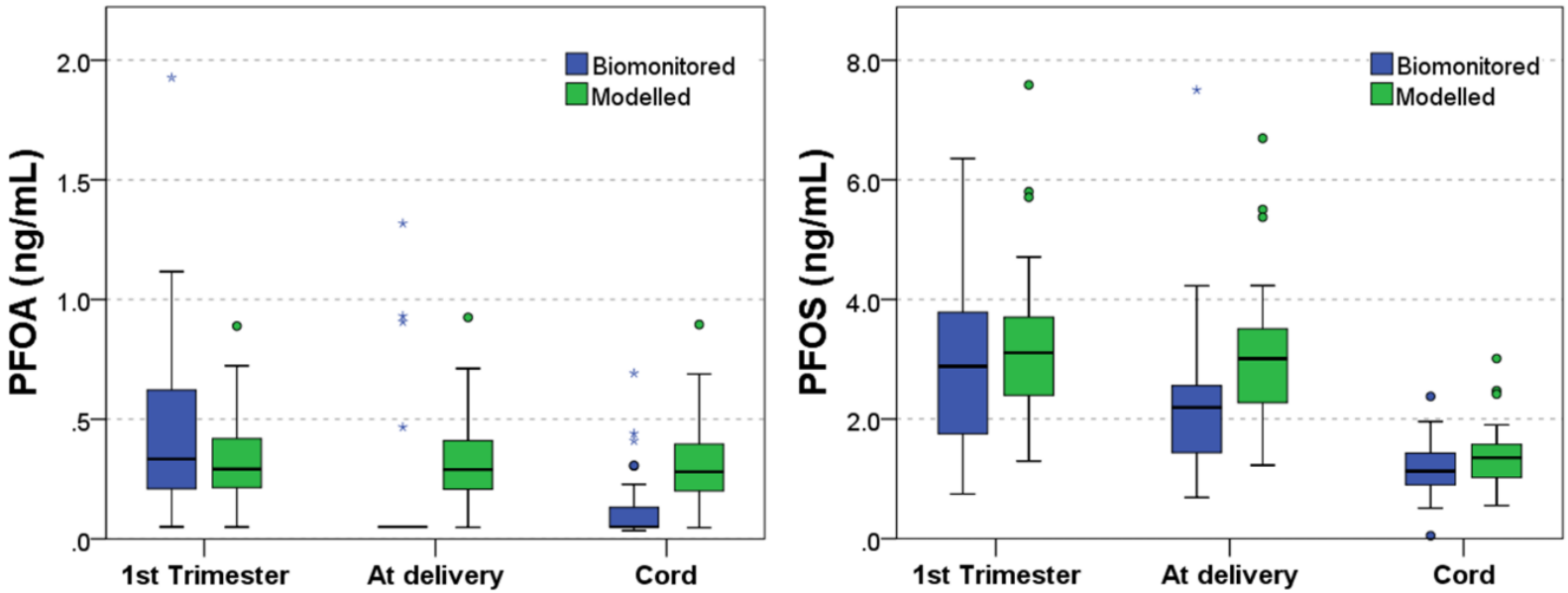


Fig. 3. Modelled and biomonitored levels of PFOA and PFOS in plasma of pregnant women at the first trimester, at delivery and in cord blood

4. Conclusions

Perfluoroalkylated substances are ubiquitous pollutants, especially PFOS, which were found in all plasma samples in the first trimester and at delivery, and in most cord blood samples. Mean plasma levels were 0.45, 0.13 and 0.12 ng/mL for PFOA and 2.93, 2.21, and 1.17 ng/mL for PFOS at the first trimester, at delivery and in cord blood, respectively. A transplacental transfer of PFOS and PFOA of around 70% and 60%, respectively, was observed. A decreasing trend of PFOS and PFOA in plasma was found when data from the current study are compared with values obtained in the past for the same adult population. The dietary intake was the main route of exposure to PFOS (>99%) and PFOA (>96%) for pregnant women in Tarragona County. In addition, the consumption of fish and seafood was the main contributor to PFOS intake (86%), and the second contributor to PFOA (42%), after milk and dairy products (48%). The results of PFAS dietary intakes are in line with other national and international studies, including a recently published assessment conducted by EFSA. Dietary exposures of PFOA in our study were close to, but below, the provisional TWI set by EFSA (13 ng/(kg·week)). In turn, the dietary intake of PFOS was calculated to be well above the most updated provisional TWI (6 ng/(kg·week)). Pregnancy PBPK models of PFOA and PFOS were adapted and used to simulate mother and fetus internal exposure. Even with a small cohort population, models were able to validate analytical data from biomonitoring samples. However, assumptions for exposure scenarios and intake assessment were simplistic with high uncertainty, meaning this issue needs a clear improvement. Moreover, the performance of the PBPK model can be further improved by introducing temporal dynamics of exposure concentration and physiological parameters for long-term exposure.

Acknowledgements

The authors wish to thank all participants in this study and the professional team of University Hospital “Sant Joan” in Reus. The research received funding from the European Union's Seventh Programme for research, technological development and demonstration

under grant agreement No. 603946 (Health and Environment-wide Associations based on Large population Surveys, HEALS), and from the Spanish Ministry of Economy and Competitiveness (MODELBIS Project, Ref. no. AGL2016-78942-R). V. Kumar and J. Rovira received funds from Health Department of Catalonia Government, through Pla Estratègic de Recerca i Innovació en Salut (PERIS 2016–2020) fellowship.

Reference

Andersen, M.E., Clewell Iii, H.J., Tan, Y.M., Butenhoff, J.L., Olsen, G.W., 2006. Pharmacokinetic modeling of saturable, renal resorption of perfluoroalkylacids in monkeys-Probing the determinants of long plasma half-lives. Toxicology 227, 156-164.

Arrebola, J.P., Castaño, A., Esteban, M., Bartolomé, M., Pérez-Gómez, B., Ramos, J.J., Es, B., Ramos, J.J., Aleixandre, J.L., Aragonés, N., Cañas, A.I., Calvo, E., Cervantes-Amat, M., Cortés, M. V, Díaz, G., Gómez, S., González, S., Huetos, O., Jimenez, J.A., López-Abente, G., López-Herranz, A., Mayor, J., Méndez, C.F., Molina, M.A., Pollán, M., Pastor, R., Rodriguez, C., Rosado, M., Ruiz-Moraga, M., Román, J., 2018. Differential contribution of animal and vegetable food items on persistent organic pollutant serum concentrations in Spanish adults. Data from BIOAMBIENT.ES project. Sci. Total Environ. 634, 235–242.

Begley, T.H., White, K., Honigfort, P., Twaroski, M.L., Neches, R., Walker, R.A., 2005. Perfluorochemicals: Potential sources of and migration from food packaging. Food. Addit. Contam. 22, 1023-1031.

Buck, C.O., Eliot, M.N., Kelsey, K.T., Calafat, A.M., Chen, A., Ehrlich, S., Lanphear, B.P., Braun, J.M. 2018. Prenatal exposure to perfluoroalkyl substances and adipocytokines: the HOME Study. Pediatr. Res. 84, 854-860.

Butt, C.M., Berger, U., Bossi, R., Tomy, G.T., 2010. Levels and trends of poly- and perfluorinated compounds in the arctic environment. Sci. Total Environ. 408, 2936-2965.

Cai, M., Zhao, Z., Yin, Z., Ahrens, L., Huang, P., Cai, M., Yang, H., He, J., Sturm, R., Ebinghaus, R., Xie, Z., 2012. Occurrence of Perfluoroalkyl Compounds in Surface Waters from the North Pacific to the Arctic Ocean. Environ. Sci. Technol. 46, 661–668.

Callan, A.C., Rotander, A., Thompson, K., Heyworth, J., Mueller, J.F., Odland, J.Ø., Hinwood, A.L., 2016. Maternal exposure to perfluoroalkyl acids measured in whole blood and birth outcomes

in offspring. Sci. Total Environ. 569-570, 1107-1113.

Cariou, R., Veyrand, B., Yamada, A., Berrebi, A., Zalko, D., Durand, S., Pollono, C., Marchand, P., Leblanc, J.-C., Antignac, J.-P., Le Bizec, B. 2015. Perfluoroalkyl acid (PFAA) levels and profiles in breast milk, maternal and cord serum of French women and their newborns. Environ. Int. 84, 71-81.

Caserta, D., Pegoraro, S., Mallozzi, M., Di Benedetto, L., Colicino, E., Lionetto, L., Simmaco, M., 2018. Maternal exposure to endocrine disruptors and placental transmission: a pilot study. Gynecol. Endocrinol. 34, 1001-1004.

Chang, S., Allen, B.C., Andres, K.L., Ehresman, D.J., Falvo, R., Provencher, A., Olsen, G.W., Butenhoff, J.L., 2017. Evaluation of serum lipid, thyroid, and hepatic clinical chemistries in association with serum perfluorooctanesulfonate (PFOS) in cynomolgus monkeys after oral dosing with potassium PFOS. Toxicol. Sci. 156, 387-401.

Chen, M.H., Ha, E.H., Liao, H.F., Jeng, S.F., Su, Y.N., Wen, T.W., Lien, G.W., Chen, C.Y., Hsieh, W.S., Chen, P.C., 2013. Perfluorinated compound levels in cord blood and neurodevelopment at 2 years of age. Epidemiol. 24, 800-808.

Convertino, M., Church, T.R., Olsen, G.W., Liu, Y., Doyle, E., Elcombe, C.R., Barnett, A.L., Samuel, L.M., MacPherson, I.R., Evans, T.R.J., 2018. Stochastic pharmacokinetic-pharmacodynamic modeling for assessing the systemic health risk of perfluorooctanoate (PFOA). Toxicol. Sci. 163, 293-306.

Dereumeaux, C., Saoudi, A., Pecheux, M., Berat, B., de Crouy-Chanel, P., Zaros, C., Brunel, S., Delamaire, C., le Tertre, A., Lefranc, A., Vandentorren, S., Guldner, L. 2016. Biomarkers of exposure to environmental contaminants in French pregnant women from the Elfe cohort in 2011. Environ. Int. 97, 56-67.

Domingo, J.L., 2012. Health risks of dietary exposure to perfluorinated compounds. Environ. Int. 40, 187-195.

Domingo, J.L., Ericson-Jogsten, I., Perelló, G., Nadal, M., van Bavel, B., Kärrman, A., 2012a. Human Exposure to Perfluorinated Compounds in Catalonia, Spain: Contribution of Drinking Water and Fish and Shellfish. J. Agric. Food Chem. 60, 4408–4415.

Domingo, J.L., Jogsten, I.E., Eriksson, U., Martorell, I., Perelló, G., Nadal, M., van Bavel, B., 2012b. Human dietary exposure to perfluoroalkyl substances in Catalonia, Spain. Temporal trend. Food Chem. 135, 1575-1582.

Domingo, J.L., Nadal, M., 2017. Per- and polyfluoroalkyl substances (PFASs) in food and human dietary intake: A review of the recent scientific literature. J. Agric. Food. Chem. 65, 533-543.

EFSA, European Food Safety Authority, 2018. Risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food. EFSA J. 16, 5194.

EFSA, European Food Safety Authority, 2012. Perfluoroalkylated substances in food: occurrence and dietary exposure. EFSA J. 10, 2743-2798.

EFSA, European Food Safety Authority, 2008. Opinion of the Scientific Panel on Contaminants in the Food chain on Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. EFSA J. 653, 1-131

Ericson, I., Gómez, M., Nadal, M., van Bavel, B., Lindström, G., Domingo, J.L., 2007. Perfluorinated chemicals in blood of residents in Catalonia (Spain) in relation to age and gender: A pilot study. Environ. 33, 616-623.

Ericson, I., Martí-Cid, R., Nadal, M., Van Bavel, B., Lindström, G., Domingo, J.L., 2008. Human exposure to perfluorinated chemicals through the diet: Intake of perfluorinated compounds in foods from the Catalan (Spain) market. J. Agric. Food Chem. 56, 1787–1794.

Ericson, I., Nadal, M., van Bavel, B., Lindström, G., Domingo, J.L., 2012. Per- and polyfluorinated compounds (PFCs) in house dust and indoor air in Catalonia, Spain: Implications for human exposure. Environ. Int. 39, 172-180.

Fàbrega, F., Kumar, V., Schuhmacher, M., Domingo, J.L., Nadal, M., 2014. PBPK modeling for PFOS and PFOA: Validation with human experimental data. Toxicol. Lett. 230, 244-251.

Fàbrega, F., Kumar, V., Benfenati, E., Schuhmacher, M., Domingo, J.L., Nadal, M., 2015. Physiologically based pharmacokinetic modeling of perfluoroalkyl substances in the human body. Toxicol. Environ. Chem. 97, 814-827.

Fàbrega, F., Nadal, M., Schuhmacher, M., Domingo, J.L., Kumar, V., 2016. Influence of the uncertainty in the validation of PBPK models: A case-study for PFOS and PFOA. Regul. Toxicol. Pharmacol. 77, 230-239.

Fromme, H., Tittlemier, S.A., Völkel, W., Wilhelm, M., Twardella, D., 2009. Perfluorinated compounds - Exposure assessment for the general population in western countries. Int. J. Hyg. Environ. Health 212, 239-270.

Glynn, A., Berger, U., Bignert, A., Ullah, S., Aune, M., Lignell, S., Darnerud, P.O. 2012. Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: Serial sampling during pregnancy and nursing, and temporal trends 1996-2010. Environ. Sci. Technol. 46, 9071-9079.

Han, X., Snow, T.A., Kemper, R.A., Jepson, G.W., 2003. Binding of perfluorooctanoic acid to rat and human plasma proteins. Chemical Research in Toxicology 16, 775-781.

Han, W., Gao, Y., Yao, Q., Yuan, T., Wang, Y., Zhao, S., Shi, R., Bonefeld-Jorgensen, E.C., Shen, X., Tian, Y. 2018. Perfluoroalkyl and polyfluoroalkyl substances in matched parental and cord serum in Shandong, China. Environ. Int. 116, 206-213.

Hanssen, L., Röllin, H., Odland, J.O., Moe, M.K., Sandanger, T.M. 2010. Perfluorinated compounds in maternal serum and cord blood from selected areas of South Africa: Results of a pilot study. J. Environ. Monit. 12, 1355-1361.

Hekster, F.M., Laane, R.W.P.M., de Voogt, P., 2001. Environmental and toxicity effects of perfluoroalkylated substances. Rev. Environ. Contam. Toxicol. 179, 99–121.

Impinen, A., Nygaard, U.C., Lødrup Carlsen, K.C., Mowinckel, P., Carlsen, K.H., Haug, L.S., Granum, B. 2018. Prenatal exposure to perfluoralkyl substances (PFASs) associated with respiratory tract infections but not allergy- and asthma-related health outcomes in childhood. Environ. Res. 160, 518-523.

Islam, R., Kumar, S., Karmoker, J., Kamruzzaman, M., Rahman, M.A., Biswas, N., Tran, T.K.A., Rahman, M.M., 2018. Bioaccumulation and adverse effects of persistent organic pollutants (POPs) on ecosystems and human exposure: A review study on Bangladesh perspectives. Environ. Tech. Innovation 12, 115–131.

Johansson, J.H., Berger, U., Vestergren, R., Cousins, I.T., Bignert, A., Glynn, A., Darnerud, P.O., 2014. Temporal trends (1999-2010) of perfluoroalkyl acids in commonly consumed food items. Environ. Pollut. 188, 102-108.

Kannan, K., Corsolini, S., Falandysz, J., Fillman, G., Kumar, K.S., Loganathan, B.G., Mohd, M.A., Olivero, J., van Wouwe, N., Yang, J.H., Aldoust, K.M., 2004. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. Environ. Sci. Technol. 38, 4489–4495.

Kärrman, A., Domingo, J.L., Llebaria, X., Nadal, M., Bigas, E., van Bavel, B., Lindström, G., 2010. Biomonitoring perfluorinated compounds in Catalonia, Spain: Concentrations and trends in human liver and milk samples. Environ. Sci. Pollut. Res. 17, 750-758.

Kato, K., Wong, L.-Y., Chen, A., Dunbar, C., Webster, G.M., Lanphear, B.P., Calafat, A.M. 2014. Changes in serum concentrations of maternal poly- and perfluoroalkyl substances over the course of pregnancy and predictors of exposure in a multiethnic cohort of Cincinnati, Ohio pregnant women during 2003-2006. Environ. Sci. Technol. 48, 9600-9608

Kim, S., Choi, K., Ji, K., Seo, J., Kho, Y., Park, J., Kim, S., Park, S., Hwang, I., Jeon, J., Yang, H., Giesy, J.P., 2011. Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environ. Sci. Technol. 45, 7465-7472.

Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jørgensen, E.H., Sonne, C., Verreault, J., Vijayan, M.M., Gabrielsen, G.W., 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. Sci. Total Environ. 408, 2995-3043.

Linares, V., Perelló, G., Nadal, M., Gómez-Catalán, J., Llobet, J.M., Domingo, J.L., 2010. Environmental versus dietary exposure to POPs and metals: A probabilistic assessment of human health risks. J. Environ. Monitor. 12, 681-688.

Liu, J., Li, J., Liu, Y., Chan, H.M., Zhao, Y., Cai, Z., Wu, Y., 2011. Comparison on gestation and lactation exposure of perfluorinated compounds for newborns. Environ. Int. 37, 1206-1212.

Lyngsø, J., Ramlau-Hansen, C.H., Høyer, B.B., Støvring, H., Bonde, J.P., Jönsson, B.A.G., Lindh, C.H., Pedersen, H.S., Ludwicki, J.K., Zviezdai, V., Toft, G., 2014. Menstrual cycle characteristics in fertile women from Greenland, Poland and Ukraine exposed to perfluorinated chemicals: A cross-sectional study. Hum. Reprod. 29, 359-367.

Martínez, M.A., Rovira, J., Prasad Sharma, R., Nadal, M., Schuhmacher, M., Kumar, V., 2018. Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure: A Catalonia (Spain) case study. Environ. Res. 166, 25-34.

Martínez, M.A., Rovira, J., Sharma, R.P., Nadal, M., Schuhmacher, M., Kumar, V., 2017. Prenatal exposure estimation of BPA and DEHP using integrated external and internal dosimetry: A case study. Environ. Res. 158, 566-575.

Matilla-Santander, N., Valvi, D., Lopez-Espinosa, M.-J., Manzano-Salgado, C.B., Ballester, F., Ibarluzea, J., Santa-Marina, L., Schettgen, T., Guxens, M., Sunyer, J., Vrijheid, M. 2017. Exposure to Perfluoroalkyl Substances and Metabolic Outcomes in Pregnant Women: Evidence from the Spanish INMA Birth Cohorts. Environ. Health Perspect. 125, 117004.

Manzano-Salgado, C.B., Casas, M., Lopez-Espinosa, M.J., Ballester, F., Martinez, D., Ibarluzea, J., Santa-Marina, L., Vioque, J., Sunyer, J., Vrijheid, M., 2016. Variability of perfluoroalkyl substance concentrations in pregnant women by socio-demographic and dietary factors in a Spanish birth cohort. Environ. Int. 92-93, 357-365.

Meng, Q., Inoue, K., Ritz, B., Olsen, J., Liew, Z. 2018. Prenatal exposure to perfluoroalkyl substances and birth outcomes; An updated analysis from the Danish National Birth Cohort. Int. J. Environ. Res. Public Health 15, 1832, .

Mitro, S.D., Johnson, T., Zota, A.R. 2015. Cumulative Chemical Exposures During Pregnancy and Early Development. Curr. Environ. Health Rep. 2, 367-378.

Morales, L., Martrat, M.G., Olmos, J., Parera, J., Vicente, J., Bertolero, A., Ábalos, M., Lacorte, S.,

Santos, F.J., Abad, E., 2012. Persistent Organic Pollutants in gull eggs of two species (Larus michahellis and Larus audouinii) from the Ebro delta Natural Park. Chemosphere 88, 1306-1316.

Morello-Frosch, R., Cushing, L.J., Jesdale, B.M., Schwartz, J.M., Guo, W., Guo, T., Wang, M., Harwani, S., Petropoulou, S.-S.E., Duong, W., Park, J.-S., Petreas, M., Gajek, R., Alvaran, J., She, J., Dobraca, D., Das, R., Woodruff, T.J. 2016. Environmental chemicals in an urban population of pregnant women and their newborns from San Francisco. Environ. Sci. Technol. 50 12464-12472.

Motas Guzmàn, M., Clementini, C., Pérez-Cárceles, M.D., Jiménez Rejón, S., Cascone, A., Martellini, T., Guerranti, C., Cincinelli, A., 2016. Perfluorinated carboxylic acids in human breast milk from Spain and estimation of infant's daily intake. Sci. Total Environ. 544, 595-600.

Nadal, M., Schuhmacher, M., Domingo, J.L., 2004. Probabilistic human health risk of PCDD/F exposure: A socioeconomic assessment. J. Environ. Monitor. 6, 926-931.

OECD, 2002. Hazard assessment of perfluorooctane sulfonate (PFOS) and its salts. Co-operation on existing chemicals. Organization for Economic Co-operation and Development (OECD), ENV/JM/RD (2002) 17/FINAL. November 21 2002.

Olsen, G.W., Mair, D.C., Lange, C.C., Harrington, L.M., Church, T.R., Goldberg, C.L., Herron, R.M., Hanna, H., Nobiletti, J.B., Rios, J.A., Reagen, W.K., Ley, C.A., 2017. Per- and polyfluoroalkyl substances (PFAS) in American Red Cross adult blood donors, 2000–2015. Environ. Res. 157, 87-95.

Pouwer, M.G., Pieterman, E.J., Chang, S.C., Olsen, G.W., Caspers, M.P.M., Verschuren, L., Wouter Jukema, J., Princen, H.M.G., 2019. Dose Effects of Ammonium Perfluorooctanoate on Lipoprotein Metabolism in APOE∗3-Leiden.CETP Mice. Toxicol. Sci. 168, 519-534.

Rovira, J., Roig, N., Nadal, M., Schuhmacher, M., Domingo, J.L., 2016. Human health risks of formaldehyde indoor levels: An issue of concern. J. Environ. Sci. Heal. A Toxic/Hazardous Subst. Environ. Eng. 51, 357–363.

Sharma, R.P., Schuhmacher, M., Kumar, V., 2018a. Development of a human physiologically based pharmacokinetic (PBPK) model for phthalate (DEHP) and its metabolites: A bottom up modeling approach. Toxicol. Lett. 296, 152-162.

Sharma, R.P., Schuhmacher, M., Kumar, V., 2018b. The development of a pregnancy PBPK Model for Bisphenol A and its evaluation with the available biomonitoring data. Sci. Total Environ. 624, 55-68.

Shi, Y., Pan, Y., Yang, R., Wang, Y., Cai, Y., 2010. Occurrence of perfluorinated compounds in fish from Qinghai-Tibetan Plateau. Environ. Int. 36, 46–50.

Shoeib, M., Harner, T., Vlahos, P., 2006. Perfluorinated chemicals in the Arctic atmosphere. Environ. Sci. Technol. 40, 7577-7583.

Steenland, K., Barry, V., Savitz, D., 2018. Serum perfluorooctanoic acid and birthweight an updated meta-analysis with bias analysis. Epidemiology, 29, 765-776.

Suja, F., Pramanik, B.K., Zain, S.Md., 2009. Contamination, bioaccumulation and toxic effects of perfluorinated chemicals (PFCs) in the water environment: A review paper. Water Sci. Technol. 60, 1533-1554.

Sungur, Ş, 2018. Dietary exposure to perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). Review of recent literature. Toxin Rev. 37, 106-116.

Tan, Y.M., Clewell Iii, H.J., Andersen, M.E., 2008. Time dependencies in perfluorooctylacids disposition in rat and monkeys: A kinetic analysis. Toxicology Letters 177, 38-47

Tian, Y., Zhou, Y., Miao, M., Wang, Z., Yuan, W., Liu, X., Wang, X., Wang, Z., Wen, S., Liang, H. 2018. Determinants of plasma concentrations of perfluoroalkyl and polyfluoroalkyl substances in pregnant women from a birth cohort in Shanghai, China. Environ. Int. 119, 165-173.

Toms, L.M.L., Thompson, J., Rotander, A., Hobson, P., Calafat, A.M., Kato, K., Ye, X., Broomhall, S., Harden, F., Mueller, J.F. 2014. Decline in perfluorooctane sulfonate and perfluorooctanoate serum concentrations. in an Australian population from 2002 to 2011. Environ. Int. 71, 74-80.

Tsai, M.S., Lin, C.Y., Lin, C.C., Chen, M.H., Hsu, S.H.J., Chien, K.L., Sung, F.C., Chen, P.C., Su, T.C., 2015. Association between perfluoroalkyl substances and reproductive hormones in adolescents and young adults. Int. J. Hyg. Environ. Health, 218, 437-443.

Tsai, M.-S., Miyashita, C., Araki, A., Itoh, S., Bamai, Y.A., Goudarzi, H., Okada, E., Kashino, I., Matsuura, H., Kishi, R. 2018. Determinants and Temporal Trends of Perfluoroalkyl Substances in Pregnant Women: The Hokkaido Study on Environment and Children's Health. Int. J. Environ. Res. Public Health 15, 989.

Vassiliadou, I., Costopoulou, D., Ferderigou, A., Leondiadis, L., 2010. Levels of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) in blood samples from different groups of adults living in Greece. Chemosphere 80, 1199-1206.

Verner, M.A., Loccisano, A.E., Morken, N.H., Yoon, M., Wu, H., McDougall, R., Maisonet, M., Marcus, M., Kishi, R., Miyashita, C., Chen, M.-H., Hsieh, W.-S., Andersen, M.E., Clewell, H.J., III, Longnecker, M.P., 2015. Assfociations of perfluoroalkyl substances (PFAS) with lower birth

weight: An evaluation of potential confounding by glomerular filtration rate using a physiologically based pharmacokinetic model (PBPK) Environ. Health Perspec. 123, 1317-1324.

WHO, 2013. State of the Science of Endocrine Disrupting Chemicals – 2012. WHO Library Cataloguing-in-Publication Data. ISBN: 978-92-807-3274-0.

Wilhelm, M., Wittsiepe, J., Völkel, W., Fromme, H., Kasper-Sonnenberg, M., 2015. Perfluoroalkyl acids in children and their mothers: Association with drinking water and time trends of inner exposures-Results of the Duisburg birth cohort and Bochum cohort studies. Int. J. Hyg. Environ. Health, 218, 645-655.

Woodruff, T.J., Zota, A.R., Schwartz, J.M. 2011. Environmental chemicals in pregnant women in the United States: NHANES 2003- 2004. Environ. Health Perspec. 119, 878-885.

Zhang, H., Yolton, K., Webster, G.M., Ye, X., Calafat, A.M., Dietrich, K.N., Xu, Y., Xie, C., Braun, J.M., Lanphear, B.P., Chen, A., Chen, A., 2018. Prenatal and childhood perfluoroalkyl substances exposures and children's reading skills at ages 5 and 8 years. Environ. Int. 111, 224–231.

PUBLICATION 5.

Early-life intake of major trace elements, bisphenol A, tetrabromobisphenol A and fatty acids: Comparing human milk and commercial infant formulas.

M.A. Martínez[a]; I. Castro[c]; J. Rovira[a,b]; S. Ares[d]; J.M. Rodríguez[c]; S.C. Cunha[e]; S. Casal[e]; J. Olivera-Fernandes[e]; M. Schuhmacher[a,b]; M. Nadal[b].

[a] Environmental Engineering Laboratory, Departament d'Enginyeria Quimica, Universitat Rovira I Virgili, Av. Països Catalans 26, 43007 Tarragona, Catalonia, Spain.
[b] Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Catalonia, Spain.
[c] Dpt. Nutrition and Food Science, Complutense University of Madrid, Avda. Puerta de Hierro s/n, 28040 Madrid, Spain.
[d] Department of Neonatology, Universitary Hospital La Paz, Pº de la Castellana, 261. 28046 Madrid, Spain.
[e] LAQV-REQUIMTE, Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Portugal

Environmental Research 169 (2018) 246 – 255.

Abstract

In the present study, the presence of a wide spectrum of major and trace elements (As, Ag, Al, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni, Sr, Sb, Se, Sn, Pb, V, and Zn), fatty acids, as well as some pollutants like free and total BPA and tetrabromobisphenol A (TBBPA), was analysed in human milk (n=53) and infant formula (n=50) samples. In addition, the infant exposure to these chemicals was assessed. The content of free BPA and several elements (Al, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Sn, Sr, and Zn) was higher ($p<0.01$) in infant formula samples. Furthermore, human milk contained levels of BPA and elements that, in almost all cases, were well below their respective EFSA and/or WHO thresholds, is also independent of the maternal characteristics (e.g., age, BMI or breastfeeding period). The fatty acid profiling also revealed major differences between human milk and infant formulas, which should be taken into account in the development of new formulas as well as in specific recommendations for the diet of breastfeeding mothers. Anyway, the results of this study reinforce that breastfeeding should be always the first feeding option in early life.

Keywords

Bisphenol A, chemical elements, human milk, formula milk, fatty acids

1. Introduction

Since it is adapted to the nutritional requirements of babies, human milk is the gold standard for infant nutrition during the first months of life (Ballard and Morrow, 2013). Breast milk contains a wide spectrum of biologically active components, including immunoglobulins, chemokines, growth factors, cytokines, bioactive lipids, oligosaccharides, microRNAs, hormones, immune cells and microorganisms, among others compounds (Hennet and Borsig, 2016; Oftedal, 2012). The concentrations of human milk components can vary among individuals, being also dependent of several factors, such as mother's genotype, geographical location, gestational age, maternal health status, diet and time of lactation (Gómez-Gallego et al., 2017; Inthavong et al., 2017; Shi et al., 2009).

Globally, the complex and dynamic composition of breast milk promotes a healthy infant growth and development (Mosca and Giannì, 2017). Therefore, the World Health Organization (WHO) recommends exclusive breast-feeding during the first six months of life; thereafter, infants should receive nutritionally adequate and safe complementary foods while breastfeeding continues for up to two years of age or beyond (WHO, 2003). In European countries, the exclusive breastfeeding at six months of age ranged from 0.7% to 37.0% in Greece and Hungary, respectively, whereas in Spain was 28.5% (WHO, 2013). Despite the short- and long-term health benefits that breastfeeding provides to mother-infant pairs, many breastfed neonates are, exclusively or partly, fed with cow's milk-derived infant formulas. As a consequence, a wide range of infant powdered milks have been developed over the last few years, with a great variety in terms of nutritional content, taste, digestibility of digestion and energy (O'Connor, 2009).

Unfortunately, neither human milk nor infant formula are pristine, and they can contain chemical contaminants depending on mother's diet and lifestyle, including persistent organic pollutants (POPs), pesticides, heavy metals and other well-known endocrine disruptors, such as bisphenol A (BPA) (Cardoso et al., 2014; Mead, 2008; Mendonca et al., 2015; Soleimani et al., 2014; Vela-Soria et al., 2016). These pollutants can be easily transferred during infant feeding (Klein et al., 2017). The confluence of different abiotic

contaminants in human milk and in infant formula milk, and its potential impact on the infant's health, has been largely investigated (Cruz et al., 2009; Winiarska-Mieczan and Tupaj, 2009; Soleimani et al., 2014). BPA is a raw material for many manufactured goods, including food and beverages packaging materials and medical devices. In fact, diet is considered the major source of BPA exposure (Mendonca et al., 2015). Prenatal exposure to BPA has been associated with obesity and diabetes diseases in childhood, as well as reproductive, behavioral and neurodevelopment problems (Martínez et al., 2017). Brominated Flame Retardants (BFR) include a variety of substances frequently applied to industrial and household products to make them less flammable. Among the large group of BFR, tetrabromobisphenol A (TBBPA) is known to be produced in high amounts, representing around 60% of the total BFR market (Vandermeersch et al., 2015). TBBPA exposure can have adverse health effects, affecting thyroid hormones, the neurological function, and the reproductive system (Cruz et al., 2009). Cadmium (Cd), lead (Pb) or mercury (Hg), among other heavy metals, are widely dispersed in the environment and have bioaccumulative features, being also described as neurotoxic substances (Karri et al., 2017; Mead, 2008; Tchounwou et al., 2012). Therefore, the presence/absence of these chemicals in human milk and infant formulas must be a priority to assure that the intake of milk in early-life does not mean an additional exposure to pollutants, whose effects are sometimes not observed until long-term.

The main polyunsaturated fatty acids are arachidonic, eicosapentaenoic, and docosahexaenoic acids. They are important for regulating growth, inflammatory responses, immune function, playing key role in neural tissue structure and function, cell membrane structure, cognitive development, and motor systems in newborns (Barreiro et al., 2018). For that reason, the WHO joint expert committee published the intake recommendations for these fatty acids and linolenic acid, considering human milk as the reference (WHO, 2018). A correct diet of the mother is also important to provide these essential fatty acids, first to the fetus, and later to the newborn through breast milk (Martin et al., 2016).

This study was firstly aimed at analyzing the presence of a wide spectrum of components (e.g., major and trace elements, fatty acids, free and total BPA and TBBPA) in human milk and infant formula samples. Subsequently, the newborn exposure to these chemicals through the intake of milk, either breast or formula, was evaluated.

2. Materials and methods

2.1 Participating women, collection and preparation of the samples

Human milk samples (n=53) were collected from healthy Spanish mothers (with healthy infants) at La Paz University Hospital (Madrid). Samples were immediately placed on ice until their arrival to the laboratory where they were frozen (-20 °C). Mothers' age ranged from 25 to 43 years old, being classified into 3 groups: (a) <30 years old (n=6); (b) from 30 to 35 years old (n=20); and (c) >35 years old (n=23). The mean body mass index (BMI) was 24.5 kg/m^2 and, according to this parameter, women were also grouped into 3 categories: (a) <18.5 kg/m^2 (n=1); (b) between 18.5 and 25 kg/m^2 (n=27); and (c) >25 kg/m^2 (n=22). Depending on the months of breastfeeding (ranging between 1 and 18 months), women were divided into 3 different groups: (a) first month (n=18); (b) 1 to 6 months (n=20); and (c) >6 months (n=5). Data relative to some specific samples were missing. All volunteers gave written informed consent to the protocol (C.P.-C.I. 10/017-E), which had been previously approved by the Ethical Committee of Clinical Research of La Paz University Hospital (Madrid, Spain).

Several samples of infant formula milk (n=50) of different commercial brands and types, including first infant (n=25), follow-on (n=14), and growing-up (n=11) formulas, were purchased in pharmacies and supermarkets from Spain. Infant formula samples were prepared according to manufacturer instructions in clean and BPA-free materials, using bottled water (Aquarel®), whose content of BPA and trace elements was also determined.

2.2 BPA and TBBPA analysis

2.2.1. Standards and reagents for BPA and TBBPA

BPA (99% purity) and TBBPA (99% purity) were purchased from Sigma-Aldrich (West Chester, PA, USA). Tetrabromobisphenol A ring-$^{13}C_{12}$ (TBBPA$^{13}C_{12}$; 99% purity) and d16-bisphenol A (BPAd_{16}; 98 atom % D), used as internal standards (I.S.), were purchased from Sigma-Aldrich and Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA) respectively. Individual standard solutions and internal standards were prepared in methanol (HPLC grade from Sigma-Aldrich) at concentrations of 2000 µg/L. Acetonitrile (MeCN, gradient grade for HPLC), acetic anhydride (AA; >99% purity), trichloroacetic acid (TCA, >99% purity), tetrachloroethylene (T4CE, >99% purity), anhydrous magnesium sulfate (anhydrous $MgSO_4$; 99.5% purity) and β-glucuronidase (Type 1 from Helix pomatia, ≥3000,000 U/g solid glucuronidase and ≥10,000 U/g solid sulfatase) were purchased from Sigma-Aldrich. MeOH (MeOH, for HPLC LC-MS grade), hydrochloric acid (HCl, 32%) and potassium carbonate (K_2CO_3, analytical grade) were purchased from Merck (Darmstadt, Germany). Sodium chloride (NaCl; 99.5% purity), sodium hydroxide (NaOH) and ammonium acetate ($C_2H_3O_2NH_4$, 97% purity) were purchased from AppliChem Panreac ITW Companies (Barcelona, Spain). Ultra-pure Milli-Q water was obtained using a Millipore Milli-Q system (Millipore, Bedford, MA, USA). Ultra-high-purity helium (99.999%) for GC-MS was obtained from Gasin (Maia, Portugal).

2.2.2. Instrument and analytical conditions for analysis of TBBPA

A high-performance liquid chromatography (HPLC) system Waters Alliance 2695 (Waters, Milford) was interfaced to a Quattro Micro triple quadrupole mass spectrometer (Waters, Manchester, UK). The chromatographic separation was achieved using a Kinetex C18 2.6 µ particle size analytical column (150 × 4.6 mm) with a Phenomenex pre-column (Tecnocroma, Portugal), at a flow-rate of 200 µL/min. The column was kept at 30ºC and the sample manager was kept at ambient temperature (± 25ºC). The mobile phase consisted of

methanol (90%) and an aqueous solution of 5 mM ammonium acetate (pH <5), isocratic (10%). Total run time was 15 min, while the sample injection volume was 20 µL.
MS/MS acquisition was operated in negative-ion mode with multiple reaction monitoring (MRM); the collision gas was Argon 99.995% (Gasin, Portugal) with a pressure of 2.9×10^{-3} mbar in the collision cell. Capillary voltages of 3.0 KV were used in the negative ionization mode. Nitrogen was used as desolvation gas and cone gas being the flows of 350 and 60 L/h, respectively. The desolvation temperature was set to 350ºC and the source temperature to 150ºC. Dwell times of 0.1 s/scan were selected. The data were collected using the software programme MassLynx4.1.

For each analyte, two transitions were selected for identification, and the corresponding cone voltage and collision energy were optimized for maximum intensity as described (Cunha et al., 2016). The quantification was made in multiple reaction monitoring (MRM), 524.87 >419.87, 542.87 > 446 for TBBPA and 554.92 > 428.84 and 554.92 > 457.92 for TBBPA$^{13}C_{12}$.

2.2.3. Instrument and analytical conditions for analysis of BPA

A gas chromatograph 6890 (Agilent, Little Falls, DE, USA) equipped with a Combi-PAL autosampler (CTC Analytics, Zwingen, Switzerland) and an electronically controlled split/splitless injection port, was interfaced to a single quadrupole inert mass selective detector (5975B, Agilent) with electron ionization (EI) chamber, was used. GC separation was performed on a DB-5MS column (30 m × 0.25 mm I.D. × 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA). Helium was the carrier gas with a constant flow of 1 mL min^{-1}. The injection was made in splitless mode (purge-off time, 60 s) at 280°C. The oven temperature program was as follows: 100°C held for 1 min, ramped to 280°C at 30°C min^{-1} held for 5.0 min. Total run time was 12 min. The MS transfer line was held at 280°C. Mass spectrometric parameters were set as follows: electron ionization with 70 eV energy; ion source temperature, 230°C and MS quadrupole temperature, 150°C. Agilent ChemStation (version D.0200SP1) was used for data collection/processing and GC–MS control. The

quantification was made in selected ion monitoring (SIM), m/z 213, 228 and 270 for BPA and 224, 242 and 284 for $BPAd_{16}$. The ion m/z 213 and 224 was used for quantification of BPA and $BPAd_{16}$, respectively, and the others for confirmation.

2.2.4. Sample extraction for TBBPA analysis

Sample preparation for extraction of TBBPA entailed the following steps: 1 g of homogenized sample (or 1 g of sample previous hydrolyzed with 20 µL β-glucuronidase solution -20,000 U/ml in 1 M ammonium acetate buffer pH 5.0, overnight at 37°C - Total TBBPA) spiked with 25 µL of $TBBPA^{13}C_{12}$ (IS, 1000 µg/L) was added with 10 µL of NaOH 2.5 M until pH ≥10. The mixture was added with 1 mL of hexane shake by hand 1 min centrifuge at 4736 *g* for 1 min. The supernatant was discarded and repeat the previous step again. Then, add 20 µL of HCl 3N until pH<5 followed by 2.5 mL of MeCN, 1 g of anhydrous $MgSO_4$ and 0.25 g of NaCl, shake vigorously by hand for 5 and centrifuge the tube at 4736 *g* for 3 min. One point five mL of extract was evaporated until dryness using a gentle nitrogen stream at room temperature. Finally, the dry extract was re-dissolved in 100 µL of mobile phase and 20 µL was injected in the LC-MS/MS system.

2.2.5. Sample extraction for BPA analysis

BPA extraction was performed according to Cunha et al. (2011), with some minor modifications adopted to human milk. Briefly, 2 g of homogenized sample (or 2 g of sample previous hydrolyzed with 40 µL β-glucuronidase solution -20,000 U/ml in 1 M ammonium acetate buffer pH 5.0, overnight at 37°C - Total BPA) was spiked with 40 µL of BPAd (250 µg L^{-1}) and mixed with 5 mL TCA solution (10% in MeOH) in a vortex for 2 min. The sample was centrifuged at 2,750 *g* for 5 min and the uplayer was added with 5% K_2CO_3 solution until pH >10. Then 4 ml of the extract were transferred to a tube with a conical bottom and a mixture of MeCN (210 mL), T4CE (60 µL) and AA (60 µl) was rapidly injected. The tube was closed and hand-shaken gently for 1 min. After that it was centrifuged at 2,750 *g* for 5 min and 50 µL of the sedimented phase were transferred for a vial and 1 µL of the extract was injected in the GC-MS system.

2.2.6. Quality assurance and control for TBBPA and BPA

In order to avoid any kind of contamination, nitrile plastic gloves were used throughout the analytical work and the use of plastic materials was avoided. Amber glass vials were heated (400°C) overnight prior to use. Using these precautions, no problems concerning levels in analytical blank samples were observed.

Due to the extent matrix effects exhibited by these particular samples, matrix-matched calibration curves were performed using powered infant formula free of TBBPA and BPA. Linearity was evaluated in the range of 1 to 100 µg/L for TBBPA and 0.1 to 100 µg/L range for BPA, using 7 calibration points for both analytes. Good correlation coefficients (>0.998) were obtained in both matrix-matched curves, confirming method reliability. The precision of the method was determined by repeatability (intraday precision) on a positive sample, using three replicates on each day. The relative standard deviations for TBBPA and BPA were lower than 18%. Recovery studies were performed by adding two known concentrations of TBBPA (5 and 25 µg/L) and BPA (0.5 and 10 µg/L) to a negative sample before the extraction and purification steps. Average recoveries of 88.5% (± 15.8) for TBBPA and 88% for BPA (± 5.1) were achieved, supporting the efficiency of the method. The detection and quantification limits (LOD and LOQ, respectively) were determined as the amount corresponding to signal-to-noise ratios of 3 and 10, respectively, from the analysis of a blank powered infant formula sample. LOQ was 1 µg/L and 0.05 µg/L TBBPA and BPA, respectively. LOD were 0.04 µg/L and 0.02 µg/L for TBBPA and BPA, respectively.

2.3 Main and trace elements analysis

Samples were submitted to a pre-treatment as follows: 0.50 mL of the milk sample was treated with 5 mL of 65% nitric acid (Suprapur, E. Merck, Darmstadt, Germany) in hermetic Teflon. Digestion was firstly performed at room temperature for 8 h and, then, at 80ºC for an additional period of 8 h. After cooling, the extracts were filtered and made up to 25 mL with MiliQ water.

The content of most elements (As, Ag, Al, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni, Sr, Sb, Se, Sn, Pb, V, and Zn) was determined by induction plasma coupled to a mass detector (ICP-MS, Perkin Elmer Elan 6000). Rhodium was used as internal standard. In turn, the levels of Ca, Fe, K, Mg and Na were determined by induction coupled plasma optical detector (ICP-OES, Perkin Elmer Optima 3200RL). LOD were: 0.03 mg/L Ba, Cd, Co, Cu, Mn, Sr, Se, Sn and Zn; 0.05 mg/L for As, Hg, Ni, Sb, and Pb; 0.13 mg/L for Ag, Cr and V; 0.25 mg/L for Al; 1.25 mg/L for Fe; 12.5 mg/L for Ca; 25.0 mg/L for Na; 50.0 mg/L for Mg and 125 mg/L for K.

For quality control, duplicate samples and blanks were also analysed. Three reference patterns were also used: whole milk powder (WMP), lobster hepatopancreas (TORT-2) and trace elements in spinach leaves (TES 1), obtaining recoveries ranging from 75% to 101%.

2.4 Fat and fatty acids analysis

For FA analysis, triundecanoin was used as internal standard for quantification (Larodan; Sweden). The fatty acid methyl esters standards used for quantitative and identification purposes were from diverse suppliers (Nu-Chek Prep, USA; Matreya, USA; and Supelco Inc., USA). Heptane, 2-propanol and cyclohexane, all <99%, were from Carl Roth (Germany). Boron trifluoride solution (14% in methanol) was purchased from Sigma-Aldrich. Dichloromethane (DCM, >99.5%) and anhydrous sodium sulphate (Na_2SO_4, analytical grade) were purchased from Merck (Darmstadt, Germany). Sodium chloride (NaCl; 99.5% purity), and potassium hydroxide (KOH) were purchased from AppliChem Panreac ITW Companies (Barcelona, Spain).

Fat extraction was achieved by liquid extraction, following the method described by Feng et al. (2004) with minor modifications, and the combined derivatization method (López-López et al., 2002), also with minor adjustments. Briefly, an accurate amount of 500 microliters of homogenised milk was spiked with 100 µL of internal standard solution (triundecanoin, 10 mg/ml) and mixed sequentially with 1.6 mL of 2-propanol, 2ml of cyclohexane and 2.2 mL of NaCl aqueous solution (1%), with 1 min. vortex mixture between steps. After centrifugation (2,750 *g* for 5 min), the upper layer was transferred to a second

vial and the extraction repeated with 2 ml of cyclohexane. The two organic phases were combined, dehydrated with anhydrous sodium sulphate, and taken to dryness under a gentle nitrogen stream at 40ºC. The extracted fat was dissolved in dichloromethane and the fatty acids were converted into their methyl esters first with hot alkaline derivatization with KOH (0.5 M in methanol; 80ºC) followed by addition the BF3 reagent. After cooling, phase separation was achieved by addition of heptane and NaCl aqueous solution (1%), with the upper layer collected for GC analysis. This derivatization method was previously compared with plain alkaline derivatization and a global increase of recovered lipids mass was achieved with combined derivatization, without an increase or alteration of the trans fatty acids.

The fatty acid composition was determined by gas chromatography on a Chrompack (CP 9001), equipped with a FAME CP-Select CB column (100 m x 0.25 mm x 0.2 µm; JW), with helium as carrier gas at 0.7 ml/min, and a temperature gradient from 100 ºC to 240 ºC, in a total of 60 min. Injection port was at 250 ºC, with a 1:100 split ratio, and the detector was at 270 ºC. Each peak was identified using known standards of fatty acid methyl esters (FAME, Nu-Chek Prep, Elysian, MN, USA; Matreya, Pleasant Gap, PA, USA; and Supelco 37 Component FAME mix, Supelco Inc.). A total of 80 fatty acids, from 6 to 24 carbon atoms, were quantified. Fatty acids contents were recorded as % weight of total fatty acids after external calibration with individual standards, and on a milk basis (mg/100 mL) using triundecanoin as internal standard that is also used to estimate the milk fat content (g/100 mL).

2.5 Exposure assessment

Equation 1 (see below) was used to establish the daily intake of BPA and elements. Three periods of exposure were considered: (a) <1-month-old; (b) between 1- and 6-month-old; and (c) between 6 and 12-month-old infants fed exclusively with either human milk or infant formula.

$DI_{i,p} = C_{i,p} \cdot I_{milk,\,p}$ (Equation 1)

Where $DI_{i,p}$ is the daily intake of the chemical *i* in the period *p* (in µg/kg_{bw}/day), $C_{i,p}$ is the concentration of the chemical *i* in milk in the feeding period (*p*) (in µg/mL), and $I_{milk,p}$ is the daily amount of milk ingested by body weight in each period (mL/day/kg_{bw}). Similar milk intakes between the breastfed and the formula-fed groups were assumed. Data on milk intake were obtained from the US EPA exposure handbook (EPA, 2011) with monthly temporal resolution

2.6 Statistics

For the statistical analysis of results, the items with values below the detection limit (LD), were assumed to be equal to one-half of that limit (ND = ½ LD). Statistical significance was established using firstly the Levene test to establish whether the data showed parametric distribution, or not. Subsequently, the ANOVA test for data following a parametric distribution, or the Kruskal-Wallis for non-parametric data were applied. A difference was considered as statistically significant when the probability was lower than 0.05 ($p<0.05$). Principal component analysis (PCA) was applied to reduce the number of variables extracting as much information as possible. PCA was performed with fatty acid relative contribution. For statistical analysis and PCA, IBM SPSS Statistics was used.

3. Results and discussion

Mean levels of BPA (free and total), TBBPA, elements and fatty acids in the human milk and infant formula samples analysed in this study are shown in Table 1. Tables 2 to 5 present the levels of the same chemicals, according to different parameters: feeding-period, mother's BMI and maternal age.

Table 1. Levels of BPA (free and total), TBBPA, elements and fat content in samples of human milk and infant formulas.

		By volume of milk			By value of fat		
	% detected	**Formula (n=50)**	**Human (n=53)**	***p-value***	**Formula (n=50)**	**Human (n=53)**	***p-value***
BPA (free)	38	0.88 ± 1.01	0.26 ± 0.81	**<0.001**	23.5 ± 29.5	6.38 ± 24.0	**<0.001**
Total BPA	76	3.85 ± 4.19	1.30 ± 4.24	**0.003**	106 ± 127	59.0 ± 270	0.270
TBBPA	8	0.57 ± 0.27	0.58 ± 0.34	0.970	14.6 ± 8.62	18.7 ± 16.7	0.129
Elements							
Ag	0	<0.13	<0.13	-	-	-	-
Al	17	0.64 ± 1.85	0.28 ± 0.67	**0.015**	16.8 ± 46.3	5.71 ± 8.98	0.212
As	0	<0.05	<0.05	-	-	-	-
Ba	25	0.04 ± 0.06	0.02 ± 0.03	0.167	0.76 ± 1.62	0.42 ± 0.80	0.494
Ca	100	511 ± 177	273 ± 73.9	**<0.001**	13457 ± 5087	8648 ± 6132	**<0.001**
Cd	8	<0.03	0.02 ± 0.03	**0.004**	-	0.60 ± 1.19	**<0.001**
Co	1	<0.03	0.02 ± 0.09	0.334	-	0.17 ± 0.43	**0.017**
Cr	100	0.38 ± 0.13	0.32 ± 0.05	**0.001**	9.98 ± 3.92	9.48 ± 4.15	0.534
Cu	98	0.49 ± 0.29	0.34 ± 0.24	**0.005**	13.1 ± 8.19	10.3 ± 8.04	0.086
Fe	53	7.50 ± 3.01	1.63 ± 5.84	**<0.001**	197 ± 84.0	43.5 ± 133	**<0.001**
Hg	2	0.03 ± 0.00	0.03 ± 0.01	0.614	0.96 ± 0.20	0.94 ± 0.61	0.379
K	100	727 ± 208	509 ± 88.0	**<0.001**	19792 ± 8818	16136 ± 9617	**0.048**
Mg	40	55.1 ± 18.9	26.1 ± 5.43	**<0.001**	1483 ± 684	800 ± 494	**<0.001**
Mn	51	0.15 ± 0.09	0.02 ± 0.02	**<0.001**	3.98 ± 2.53	0.27 ± 0.60	**<0.001**
Na	100	208± 69.1	172 ± 144	**<0.001**	5696 ± 2934	5323 ± 5279	**0.006**
Ni	44	1.22 ± 4.56	0.04 ± 0.04	**<0.001**	34.6 ± 138	1.13 ± 1.07	**<0.001**
Pb	4	0.03 ± 0.01	0.03 ± 0.01	0.992	1.00 ± 0.29	1.00 ± 0.82	0.199
Sb	0	<0.05	<0.05	-	-	-	-
Se	51	0.03 ± 0.03	0.07 ± 0.16	0.151	0.72 ± 1.11	2.33 ± 7.06	0.115

		By volume of milk	By value of fat				
	% detected	**Formula (n=50)**	**Human (n=53)**	***p-value***	**Formula (n=50)**	**Human (n=53)**	***p-value***
Elements							
Sn	12	0.02 ± 0.04	0.01 ± 0.00	**0.001**	0.40 ± 1.21	0.17 ± 0.43	0.358
Sr	93	0.25 ± 0.09	0.05 ± 0.04	**<0.001**	6.58 ± 2.59	1.65 ± 1.36	**<0.001**
V	0	<0.13	<0.13	-	-	-	-
Zn	83	5.73 ± 3.00	2.30 ± 3.54	**<0.001**	154 ± 86.6	62.1 ± 86.9	**<0.001**
% Fat	100	3.88 ± 0.74	3.86 ± 1.63	0.319	-	-	
Fatty acids							
SFA		14.0 ± 3.56	15.2 ± 6.97	0.748	35.9 ± 5.30	38.5 ± 4.18	**0.006**
MUFA		15.5 ± 3.20	14.6 ± 6.25	0.061	40.1 ± 4.68	37.8 ± 3.56	**0.005**
PUFA		6.97 ± 1.87	6.06 ± 3.30	**0.005**	17.7 ± 2.08	15.2 ± 2.98	**<0.001**
ω-3 PUFA		0.93 ± 0.24	0.42 ± 0.20	**<0.001**	2.38 ± 0.47	1.13 ± 0.52	**<0.001**
ω-6 PUFA		6.04 ± 1.71	5.60 ± 3.20	**0.040**	15.3 ± 2.08	14.0 ± 3.11	**0.012**
TFA		0.12 ± 0.07	0.43 ± 0.20	**<0.001**	0.33 ± 0.18	0.80 ± 0.31	**<0.001**
LA		5.90 ± 1.68	5.05 ± 2.95	**0.002**	15.1 ± 2.08	12.6 ± 2.99	**<0.001**
ALA		0.81 ± 0.22	0.20 ± 0.11	**<0.001**	2.08 ± 0.40	0.53 ± 0.25	**<0.001**
AA		0.08 ± 0.09	0.18 ± 0.09	**<0.001**	0.19 ± 0.20	0.46 ± 0.12	**0.001**
EPA		0.02 ± 0.03	0.02 ± 0.02	0.946	0.04 ± 0.05	0.07 ± 0.06	0.303
DHA		0.08 ± 0.09	0.16 ± 0.09	**<0.001**	0.22 ± 0.14	0.41 ± 0.26	**<0.001**
CLA		0.00 ± 0.01	0.04 ± 0.02	**<0.001**	0.01 ± 0.03	0.10 ± 0.05	**<0.001**

Results presented as mean ± standard deviation. SFA: Saturated fatty acids; MUSFA: Monounsaturated fatty acids; PUFA: Poliunsaturated fatty acids; TFA: Trans fatty acids; EPA: Eicosapentaenoic acid (20:5n-3); DHA: Docosahexaenoic acid (22:6 n-3); CLA: 9c,11t-octadecadienoic acid; AA: Arachidonic acid (eicosatetraenoic acid (20:4 n-6)).
Units (by volume of milk): Total BPA, Free BPA and TBBPA, in µg/L; elements, in mg/L; % fat in g/100 mL; Fatty acids in g/L
Units (by content of fat): Total BPA, Free BPA and TBBPA, in ng/g of fat; elements, in µg/g of fat; Fatty acids in g/100 g of fat

Table 2. Levels of BPA (free and total), TBBPA, elements and fat content in formula milk according to the feeding period. Results are shown by volume of milk.

	Formula milk					
	First infant **<1-6months (n=25)**		***Follow-on*** **>6 month (n=14)**		***Growing-up*** **>12 month (n=11)**	
BPA (free)	0.98 ± 1.03		0.82 ± 1.03		0.77 ± 1.01	
Total BPA	4.13 ± 4.01		3.01 ± 3.11		4.33 ± 5.93	
TBBPA	0.50 ± 0.02		0.71 ± 0.46		0.56 ± 0.20	
Elements						
Ag	<0.13		<0.13		<0.13	
Al	0.74 ± 2.54		0.69 ± 0.89		0.38 ± 0.49	
As	<0.05		<0.05		<0.05	
Ba	0.04 ± 0.07		0.04 ± 0.06		0.04 ± 0.04	
Ca	433 ± 162	a	555 ± 163	b	631 ± 148	b
Cd	<0.03		<0.03		<0.03	
Co	<0.03		<0.03		<0.03	
Cr	0.38 ± 0.12		0.40 ± 0.13		0.33 ± 0.13	
Cu	0.52 ± 0.35		0.51 ± 0.22		0.41 ± 0.25	
Fe	5.85 ± 3.11	a	9.35 ± 1.93	b	8.89 ± 1.67	b
Hg	0.03 ± 0.01		<0.05		<0.05	
K	646 ± 182	a	730 ± 91.9	b	910 ± 260	c
Mg	49.7 ± 20.3	a	57.8 ± 19.2	ab	64.0 ± 10.8	b
Mn	0.15 ± 0.08		0.15 ± 0.09		0.14 ± 0.11	
Na	183 ± 61.0	a	218 ± 43.7	b	251 ± 90.9	b
Ni	1.73 ± 6.43		0.83 ± 0.93		0.55 ± 0.50	
Pb	0.03 ± 0.01		0.03 ± 0.01		<0.05	
Sb	<0.05		<0.05		<0.05	

	Formula milk		
	First infant **<1-6months (n=25)**	***Follow-on*** **>6 month (n=14)**	***Growing-up*** **>12 month (n=11)**
Elements			
Se	0.04 ± 0.04	0.03 ± 0.03	0.02 ± 0.02
Sn	0.01 ± 0.00 [a]	0.05 ± 0.08 [b]	0.02 ± 0.01 [b]
Sr	0.25 ± 0.10	0.25 ± 0.07	0.26 ± 0.09
V	<0.13	<0.13	<0.13
Zn	5.26 ± 2.68	6.6 ± 3.45	5.69 ± 3.12
% Fat	3.84 ± 0.86	4.08 ± 0.56	3.69 ± 0.67
Fatty acids			
SFA	14.2 ±4.06 [ab]	15.3 ± 2.37 [a]	11.7 ± 2.66 [b]
MUFA	15.2 ± 3.59	15.7 ± 2.31	15.9 ± 3.41
PUFA	6.69 ± 1.85	7.41 ± 1.51	7.06 ± 2.33
ω-3 PUFA	0.91 ±0.24	0.97 ± 0.18	0.93 ± 0.32
ω-6 PUFA	5.78 ±1.68	6.43 ± 1.42	6.13 ± 1.71
TFA	0.11 ± 0.05	0.15 ±0.09	0.13 ± 0.08
LA	5.63 ± 1.62	6.29 ± 1.41	6.05 ±2.11
ALA	0.77 ± 0.22	0.83 ± 0.14	0.85 ±0.29
AA	0.09 ± 0.09 [a]	0.09 ± 0.11 [ab]	0.02 ± 0.03 [b]
EPA	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.03
DHA	0.10 ± 0.04 [a]	0.09 ± 0.07 [ab]	0.05 ± 0.07 [b]
CLA	0.00 ± 0.00	0.01 ± 0.02	0.00 ± 0.01

Results presented as mean ± standard deviation.
Units: Total BPA, Free BPA and TBBPA, in µg/L; elements, in mg/L; fat, in g/100 mL; fatty acids, in g/L
Significant differences at $p<0.05$ are indicated with different superscripts

Table 3. Levels of BPA (free and total), TBBPA, elements and fat content in human milk according to the time of breastfeeding. Results are shown by volume of milk.

	Human feeding period					
	<1month		**1-6 month**		**6 month**	
	n=18		**n=20**		**n=5**	
BPA (free)	0.37 ± 1.08		0.30 ± 0.84		<0.05	
Total BPA	3.01 ± 7.20		0.36 ± 0.36		0.69 ± 1.31	
TBBPA	0.6 ± 0.42		0.61 ± 0.39		<1.00	
Elements						
Ag	<0.13		<0.13		<0.13	
Al	0.35 ± 0.73		0.32 ± 0.86		0.20 ± 0.15	
As	<0.05		<0.05		<0.05	
Ba	0.02 ± 0.04		0.03 ± 0.03		0.02 ± 0.01	
Ca	266 ± 63.1		273 ± 87.3		283 ± 59.2	
Cd	0.02 ± 0.03		0.03 ± 0.04		<0.03	
Co	0.05 ± 0.15		<0.03		<0.03	
Cr	0.31 ± 0.04		0.31 ± 0.06		0.33 ± 0.08	
Cu	0.40 ± 0.19	[a]	0.25 ± 0.24	[b]	0.18 ± 0.11	[b]
Fe	3.32 ± 9.96		0.81 ± 0.79		0.88 ± 0.55	
Hg	<0.05	[a]	<0.05	[a]	0.04 ± 0.03	[b]
K	543 ± 93.3		491 ± 93.1		492 ± 73.9	
Mg	26.6 ± 6.85		26.4 ± 6.12		<50.0	
Mn	0.02 ± 0.02		0.01 ± 0.01		<0.02	
Na	215 ± 146		136 ± 155		102 ± 15.5	
Ni	0.05 ± 0.06		0.03 ± 0.02		<0.05	
Pb	0.03 ± 0.01		<0.05		<0.05	
Sb	<0.05		<0.05		<0.05	
Se	0.12 ± 0.27		0.04 ± 0.04		0.01 ± 0.00	

	Human feeding period		
	<1month	**1-6 month**	**6 month**
	n=18	**n=20**	**n=5**
Sn	<0.01	0.01 ± 0.00	<0.01
Sr	0.04 ± 0.02	0.06 ± 0.04	0.06 ± 0.02
Sr	0.04 ± 0.02	0.06 ± 0.04	0.06 ± 0.02
V	<0.13	<0.13	<0.13
Zn	2.77 ± 2.47	1.60 ± 3.95	0.24 ± 0.32
% Fat	3.54 ± 1.37	3.99 ± 2.02	4.48 ± 1.11
Fatty acids			
SFA	14.1 ± 5.71	15.3 ± 8.67	18.8 ± 6.25
MUFA	13.4 ± 5.66	15.3 ± 7.70	15.9 ± 2.46
PUFA	5.16 ± 2.36	6.35 ± 3.94	7.23 ± 3.11
ω-3 PUFA	0.41 ± 0.19	0.48 ± 0.25	0.45 ± 0.09
ω-6 PUFA	4.71 ± 2.27	5.82 ± 3.78	6.75 ± 3.18
TFA	0.32 ± 0.16	0.33 ± 0.22	0.34 ±0.13
LA	4.13 ± 2.03	5.29 ± 3.46	6.22 ±2.99
ALA	0.18 ± 0.08	0.23 ± 0.13	0.20 ±0.07
AA	0.18 ± 0.08	0.18 ± 0.12	0.17 ± 0.04
EPA	0.02 ± 0.02	0.03 ± 0.02	0.03 ± 0.02
DHA	0.16 ± 0.10	0.16 ± 0.09	0.16 ± 0.07
CLA	0.04 ± 0.02	0.04 ± 0.04	0.04 ± 0.02

Results presented as mean ± standard deviation.
Units: Total BPA, Free BPA and TBBPA, in µg/L; elements, in mg/L; fat, in g/100 mL; fatty acids, in g/L
Significant differences at $p<0.05$ are indicated with different superscripts

Table 4. Levels of BPA (free and total), TBBPA, elements and fat content in human milk according to the body mass index (BMI) of the mother. Results are shown by volume of milk.

	BMI		
	<18.5 kg/m^2 **n=1**	**18.5-25 kg/m^2** **n=27**	**>25 kg/m^2** **n=22**
BPA (free)	<0.05	0.38 ± 1.08	0.13 ± 0.24
Total BPA	<0.05	1.11 ± 3.30	1.75 ± 5.57
TBBPA	<1.00	0.58 ± 0.34	0.58 ± 0.38
Elements			
Ag	<0.13	<0.13	<0.13
Al	3.98	0.14 ± 0.07	0.31 ± 0.66
As	<0.05	<0.05	<0.05
Ba	<0.05	0.02 ± 0.02	0.03 ± 0.04
Ca	186	282 ± 64.7	272 ± 85.5
Cd	<0.03	0.02 ± 0.02	0.03 ± 0.04
Co	<0.03	<0.03	0.04 ± 0.14
Cr	0.37	0.31 ± 0.05	0.33 ± 0.05
Cu	0.23	0.31 ± 0.24	0.38 ± 0.26
Fe	4.17	2.28 ± 8.16	0.86 ± 0.8
Hg	<0.05	<0.05	0.03 ± 0.01
K	470	511 ± 79.3	505 ± 105
Mg	52.4	<50.0	26.3 ± 6.20
Mn	0.05	0.02 ± 0.02	0.01 ± 0.00
Na	741	143 ± 81.4	168 ± 139
Ni	0.08	0.03 ± 0.02	0.04 ± 0.05
Pb	<0.05	<0.05	0.03 ± 0.01
Sb	<0.05	<0.05	<0.05
Se	<0.03	0.08 ± 0.23	0.05 ± 0.04

	BMI		
	<18.5 kg/m²	**18.5-25 kg/m²**	**>25 kg/m²**
	n=1	**n=27**	**n=22**
Sr	<0.13	0.06 ± 0.04	0.05 ± 0.04
V	<0.13	<0.13	<0.13
Zn	4.98	2.29 ± 3.33	2.45 ± 4.04
% Fat	6.57	3.48 ± 1.10	4.41 ± 1.98
Fatty acids			
SFA	24.7	13.8 ± 5.30	17.2 ± 8.34
MUFA	24.8	13.1 ± 3.75[a]	16.8 ± 7.82[b]
PUFA	13.1	5.22 ± 2.31	7.10 ±3.86
ω-3 PUFA	0.83	0.38 ± 0.15[a]	0.49 ± 0.23[b]
ω-6 PUFA	12.3	4.80 ± 2.28	6.56 ± 3.75
TFA	0.36	0.30 ± 0.14	0.36 ± 0.21
LA	1.16	4.34 ± 2.16	5.88 ± 3.42
ALA	0.34	0.18 ± 0.07	0.23 ±0.13
AA	0.24	0.15 ± 0.06	0.21 ± 0.12
EPA	0.04	0.02 ± 0.02	0.03 ± 0.02
DHA	0.36	0.13 ± 0.09	0.16 ± 0.08
CLA	0.04	0.04 ± 0.02	0.05 ± 0.03

Results presented as mean ± standard deviation.
Units: Total BPA, Free BPA and TBBPA, in µg/L; elements, in mg/L; fat, in g/100 mL; fatty acids, in g/L
Significant differences at $p<0.05$ are indicated with different superscripts

Table 5. Levels of BPA (free and total), TBBPA, elements and fat content in human milk according to the maternal age. Results are shown by volume of milk.

	<30 years n=6	**>30-35 years n=20**	**>35 years n=23**
BPA (free)	0.75 ± 1.62	0.12 ± 0.26	0.28 ± 0.89
Total BPA	0.66 ± 1.32	1.71 ± 5.64	1.23 ± 3.60
TBBPA	<1.00	<1.00	0.67 ± 0.51
Elements			
Ag	<0.13	<0.13	<0.13
Al	<0.25	0.15 ± 0.08	0.43 ± 1.00
As	<0.05	<0.05	<0.05
Ba	0.03 ± 0.04	0.02 ± 0.01	0.03 ± 0.04
Ca	279 ± 54.0	270 ± 68.1	273 ± 81.2
Cd	<0.03	0.03 ± 0.04	0.02 ± 0.03
Co	<0.03	<0.03	0.04 ± 0.14
Cr	0.33 ± 0.03	0.31 ± 0.05	0.33 ± 0.06
Cu	0.36 ± 0.16	0.36 ± 0.34	0.30 ± 0.16
Fe	7.83 ± 17.3	0.78 ± 0.48	0.93 ± 0.99
Hg	<0.05	<0.05	0.03 ± 0.01
K	500 ± 64.3	506 ± 98.6	505 ± 86.4
Mg	<50.0	<50.0	27.5 ± 8.13
Mn	0.03 ± 0.04	0.01 ± 0.00	0.02 ± 0.01
Na	141 ± 82.1	171 ± 144	165 ± 148
Ni	<0.05	0.03 ± 0.02	0.04 ± 0.05
Pb	<0.05	0.03 ± 0.01	<0.05
Sb	<0.05	<0.05	<0.05
Se	0.04 ± 0.03	0.05 ± 0.04	0.09 ± 0.25
Sn	<0.03	<0.03	0.01 ± 0.00

	<30 years n=6	**>30-35 years n=20**	**>35 years n=23**
Elements			
Sr	0.05 ± 0.02	0.06 ± 0.04	0.05 ± 0.04
V	<0.13	<0.13	<0.13
Zn	2.59 ± 3.45	1.93 ± 3.35	2.46 ± 3.71
% Fat	4.33 ± 1.01	3.40 ± 1.37	4.33 ± 1.90
Fatty acids			
SFA	17.3 ±5.91	13.2 ± 6.38	17.1 ± 7.62
MUFA	15.4 ± 2.46	13.0 ± 5.09	16.4 ± 7.58
PUFA	7.61 ± 2.40[a]	5.00 ± 2.26[b]	6.82 ± 4.09[ab]
ω-3 PUFA	0.37 ± 0.11	0.42 ± 0.24	0.47 ± 0.19
ω-6 PUFA	7.20 ± 2.38[a]	4.55 ± 2.15[b]	6.30 ± 3.98[ab]
TFA	0.35 ±0.10	0.27 ± 0.14	0.37 ± 0.21
LA	6.52 ± 2.23[a]	4.12 ± 1.99[b]	5.67 ± 3.67[ab]
ALA	0.19 ± 0.07	0.20 ± 0.13	0.21 ± 0.09
AA	0.22 ± 0.08	0.15 ± 0.07	0.20 ± 0.11
EPA	0.01 ± 0.01	0.03 ± 0.02	0.03 ± 0.02
DHA	0.11 ± 0.04	0.14 ± 0.08	0.17 ± 0.11
CLA	0.04 ± 0.02	0.03 ± 0.02	0.05 ± 0.03

Results presented as mean ± standard deviation.
Units: Total BPA, Free BPA and TBBPA, in µg/L; elements, in mg/L; fat, in g/100 mL; fatty acids, in g/L
Significant differences at $p<0.05$ are indicated with different superscripts

3.1. Free and total BPA content

Free and total BPA (free plus conjugated BPA) were respectively detected in 38 and 76% of the samples of breast milk, with mean levels of 0.26 and 1.30 µg/L, respectively. In turn, the concentration of free BPA in infant formula was 0.88 µg/L, while that of total BPA was 3.85 µg/L (Table 1). The concentrations of both free BPA and total BPA in formula samples were significantly higher (p<0.001) than those in human milk (Table 1). When the ratios between the concentrations of free or total BPA and the fat content were calculated, means of 23.5 and 106 ng/g of fat, respectively, were found for formula samples, while values in breast milk were 6.4 and 59.0 ng/g of fat, respectively, Although both parameters were lower in human milk samples, only the ratio BPA:fat content showed a statistically significant (p<0.001) difference (Table 1) according to the kind of milk. No differences (p>0.05) in the BPA (free or total) levels were observed according to formula type, breastfeeding period, maternal age or mother's BMI (Tables 2 to 5). In the scientific literature, a wide range of values regarding BPA levels in human milk have been reported, ranging from 0.7 µg/L (Otaka et al., 2003) to as high as 42.6 µg/L (Yi et al., 2010). However, important differences between the studies have also been noted, including the fact of monitoring only free or total (free and conjugated) BPA. More recently, Cao et al. (2015) analysed the presence of both free and total BPA in human milk samples, observing similar results to those observed in our study: free BPA was detected in fewer samples than total BPA (16.5% *vs.* 25.9%), with amounts ranging from <0.036 to 2.3 ng/g. In agreement to our findings, the same authors also stated that the dietary exposure to BPA for breastfed infants was expected to be lower compared to that of formula-fed infants (Cao et al., 2015). However, the presence of BPA in infant formulas could have decreased in the last years due to the general tendency of using BPA-free coatings for canned formulas (Adesman et al., 2017; Cao et al., 2015). As for the presence of BPA in human milk, it is surely associated with the mother's ingestion of contaminated foods (Martinez et al. 2017, 2018).

3.2. Concentrations of TBBPA

In this study, only few samples (5 out of 50 among formula samples, and 3 out of 53 among human milk ones) presented TBBPA levels above the detection limit (0.50 μg/L). In those cases, the mean levels were 0.57 μg/L (14.6 ng/g of fat) and 0.58 μg/L (18.7 ng/g of fat) for formula and human milk samples, respectively, being the difference not significant (p>0.05; Table 1). Moreover, TBBPA concentrations were not statistically significant (p>0.05) according to the maternal characteristics (age, BMI, and breastfeeding period) (Tables 2 to 5). TBBPA was only detected in some samples corresponding to mothers aged >35 years, while it was not detected in the group with more than 6 months of breastfeeding. In any case, TBBPA concentrations were similar to those observed in other studies performed in France (Inthavong et al., 2017). In the Czech Republic, Lankova et al. (2013) found that TBBPA and α-hexabromocyclododecane (α-HBCD) were the only brominated flame retardants detected in human milk samples. However, they could be only detected in a low percentage of such samples and in none of the tested infant formulas samples (Lankova et al., 2013). These results should be taken with caution since TBBPA is usually detected and quantified either by gas or liquid chromatography coupled to mass spectrometry (MS) (Cariou et al., 2008), and the acidification and chloroformate derivatization steps required for GC-MS analysis may be responsible for a low recovery rate of this compound (Covaci et al., 2009).

3.3 Content of main and trace elements in the samples

The levels of Ag, As, Sb and V in all the samples and the levels of Cd and Co in the formula samples were below their respective LOD (Table 1). The levels of Al, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Sn, Sr, and Zn were significantly higher (p<0.05) in the infant formula samples, while Cd was the only element with a significantly higher concentration in breast milk (p<0.05) (Table 1). In relation to the formula type, the levels of Ca, Fe, K, Mg, Na, and Sn in first infant formulas were statistically lower (p<0.05) than in follow-on and growing-up milk samples (Table 2). In relation to the breastfeeding period, the levels of Cu were higher

among women during their first month (0.40 mg/L) than in the other women groups (0.25 and 0.18 mg/L for >1-6 and >6 month of breastfeeding, respectively) (Tables 2 to 5). Mercury (Hg) was detected only in a single human milk sample from a woman within the >6-months group. No statistically significant (p>0.05) differences were found for the levels of any element depending on maternal BMI or age (Tables 4 and 5, respectively).

Minerals and trace elements accounting for about 4% of total human body mass play an important role in bone structure, regulate certain body functions, and help maintain the body's water balance (WHO and FAO, 2004). It is known that apart from essential elements, human milk can also transfer potentially toxic metals, such as Pb, As or Cd, with varied concentrations depending on the environmental exposure, the diet or bad habits (Klein et al., 2017). Rapid growth and development may put infants at risk of deficiencies of certain essential minerals in vulnerable populations. Micronutrient deficiencies are associated with a higher frequency of infections in the short-term and increased rates of chronic diseases. However, excessive amounts of these elements can also be detrimental for health (Klein et al., 2017). For example, high levels of iron in formulas may increase the infant risk of infection by increasing nutrient bioavailability to pathogenic bacteria (Quinn, 2014), while high Mn exposure in children has been associated with impaired cognitive development and motor coordination (O'Neal and Zheng, 2015). Therefore, it is essential that infant formula and milk products intended for use by infants contain minerals in amounts that satisfy their nutritional requirements without leading to adverse effects (Poitevin, 2016).

3.4. Fat and fatty acid content in the samples

The fat content was similar among the human milk and the infant formula samples (3.86 and 3.88%, respectively) (Table 1). For this parameter, no statistically significant differences (p>0.05) were found according to the formula milk type (first infant, follow-on, and growing-up formula), breastfeeding period (<1, 1-6, and >6 month), maternal age (>30, 30-35, and >35 years old) or mother's BMI (18.5-25 kg/m^2 and >25 kg/m^2) (Tables 2 to 6). However, it is known that breastfed infants absorb fat better than formula-fed infants due to the

presence of lipases in human milk that are not present in cow's milk (Fields and Demerath, 2012). It is generally considered that a breastfed infant consumes less calories (around 85 kcal/kg body weight/day) during the first months of life than a formula-fed infant (100 kcal/kg/day) (Committee on the Evaluation of the Addition of and Ingredients New to Infant Formula, 2004). The breastfed infant has a lower total energy expenditure and a slower rate of weight gain (Butte et al., 1990). In addition, the gastro-esophageal reflux is usually lower in breastfed infants, most likely due to a more rapid gastric emptying time, resulting in lower loss of ingested food (Committee on the Evaluation of the Addition of and Ingredients New to Infant Formula, 2004).

Non-identified fatty acids ranged from 2.9 to 24.8% with a mean of 6.4% for all samples analysed. Globally, saturated fatty acids (SFA) contribution was higher in formula milks than in human milk, while the opposite was observed for unsaturated fatty acids (Table 1). The fatty acids that contributed the most to the total content were oleic acid (18:1), palmitic acid (16:0), linoleic acid (18:2), lauric acid (12:0), myristic acid (14:0), and stearic acid (18:0), with an overall contribution of 87.0% and 84.2% in formula and human milk samples, respectively (Table S1). Palmitic acid (16:0), γ-linolenic acid (18:3n-6), gondoic acid (20:1n-9), octadecatetraenoic acid (18:4n-3) and eicosapentaenoic acid (EPA) (C20:5n-3) showed a similar (p>0.05) contribution (in %, g/100 g of fat) between human milk and formula samples (Table S1). When levels (g/L of milk) of fatty acids were taken into account, decanoic acid (10:0), lauric acid (12:0), erucis acid (22:1n-9), and tetracosenoic acid (24:1n-9) presented also similar ($p<0.05$) levels between formula and human milk (Table S1). In contrast, the comparison of the composition of fatty acid between formulas and human milk revealed several differences. The main differences in the fat composition were a higher ($p<0.05$) contents of octanoic acid (8:0) lauric acid (12:0), linolenic acid (LA) (18:2n-6) and α linolenic acid (18:3n-3) (ALA) in the formula samples (Table S1), in opposition to a higher content ($p<0.05$) of myristic (14:0), palmitoleic (16:1), and stearic (18:0) in the human milk ones. In addition, human milk samples contained a higher ($p<0.05$) percentage of total SFA (35.9 vs 38.5 %), omega 7 fatty acids (0.3 vs. 2.2%), eicosatetraenoic acid (AA) (0.19 vs. 046%), docosahexaenoic acid (DHA) (0.22 vs. 0.41%) and conjugated linoleic acid (CLA) (0.01

vs 0.10%) and a lower ($p<0.05$) contributions of total MUFA (40.1 vs 37.8%) and polyunsaturated fatty acids (PUFA) (17.7 vs. 15.2 %), including omega 3 (2.38 vs. 1.13%) and omega 6 (15.3 vs. 14.0%) fatty acids, when they are compared to formula samples (Table 1). Similar significant differences in fatty levels (in g/L) were noted, except for SFA and MUFA that not reach the significance between formula and human milk.

In relation to the type of formula (Table 2), lower ($p<0.05$) levels of SFA were found in growing up formula than Follow –on which shown similar ($p>0.05$) levels than first infant formulas. Levels of AA and DHA were higher ($p<0.05$) in first formula levels than growing up milk formula. Regarding contribution of each fatty acid, differences were found regarding the percentages of low-chain FA (8:0 to 14:0), docosahexaenoic acid (DHA) and araquidonic acid (AA) with higher ($p<0.05$) in first formulas than in growing-up formulas, while the percentages of palmitic (16:0) and γ-linolenic were lower ($p<0.05$). Finally, higher ($p<0.05$) percentages of omega 7 and 9 were found in growing-up formulas (>12-month formula milk) when compared to the other formula milks (first infant: 0-6 month; follow-on: >6 month).

Regarding to the breastfeeding period (Table 3), no differences in the content and levels of all the fatty acids classes, both saturated and unsaturated, were found. Similar findings were reported from López-López et al., 2002. Only some unsaturated FA with low representativeness (>0.5%) were higher during the first period (<1 month) than after 6-months of breastfeeding.

Only minor significant difference observed in relation to the maternal BMI (Table 4), higher levels of MUFA and ω-3 PUFA were higher ($p<0.05$) in women with BMI>25 kg/m^2 compared with the group of BMI18.5-25 kg/m^2. Regarding individual fatty acids, few differences were noted; higher ($p<0.05$) amounts of 18:0 and 20:3n-6 in the milk fat of the group of women with a BMI>25 kg/m^2 when compared to the group comprising women with a BMI ranging from 18.5 to 25 kg/m^2.

No clear tendencies were found according to the maternal age (Table 5). Samples from mothers below 30 years old had a higher ($p<0.05$) levels of PUFA and total omega-6 fatty

acids than those from mothers between 30-35 years old, but in contrast, their content in such fatty acids were similar ($p>0.05$) to that found among women >35 years-old (Table 5). Additionally, some individual FA presented higher contributions in the mothers whose age was below 30 years ($p<0.05$) as linoleic acid, C18:3t, C18:2n6c, C20:2n6 and C22:2 than those older than 35.

Data on the fatty acid composition of the samples (in g/100mg of fat) were submitted to PCA (**Figure** 1). The results are represented as a graph with two principal components (PC) that explain 50 and 23 % of the data variance. The first PC (PC1) showed a highly positive correlation with MUFA and highly negative correlation with SFA, while PC2 was highly positively correlated with ω-6 PUFA and negatively correlated with TFA. In addition, PC1 and PC2 were positively correlated with ω-6 PUFA. PCA results revealed two main clusters that were differentiated through PC1 according the milk type (infant formula or human milk). This difference seems to be more relevant if PCA was conducted with fatty acids contributions instead of categorizing fatty acids (**Figure** S1). However, the human milk cluster showed bigger differences among samples (**Figure** 1), while formula milk samples appeared as a more homogeneous cluster, regardless of the type of formula (first infant, follow-on and growing-up formula).

Although the development of infant formulas can be traced to the nineteenth century, the addition of nucleotides in 1999 and long-chain polyunsaturated fatty acids in 2002 marked a new era in infant formula (Barreiro et al., 2018). In this work, first-stage formulas showed a lipid profile closer to that of the human milk samples, in terms of monounsaturated acids. New born do not have a fully developed ability to convert essential fatty acids into their long-chain metabolites and, for this reason, supplementation with DHA is critical in infant formulas (Barreiro et al., 2018). PCA performed in this work clearly showed that human milk is not a static fluid and changes over time; in contrast, infant formulas seem to be uniform with no clear fatty acid differences despite being marketed as intended for different stages of the infant development. It is important to mention that human milk fat includes medium-chained fatty acids (MCFA) and triacylglycerols emulsified by a sphingomyelin-rich

phospholipid membrane (milk phospholipids, MPL) while the sphingomyelin is lacking in infant formulas. Both the sphingomyelin content and the saturated level of phospholipids affect gut lipase activity, which alters the concentrations of lipid hydrolysis products in the ileum and colon, as a consequence, differences in phospholipid and fatty acid composition may modulate the acquisition and development of the infant gut microbiota (Nejrup et al., 2017).

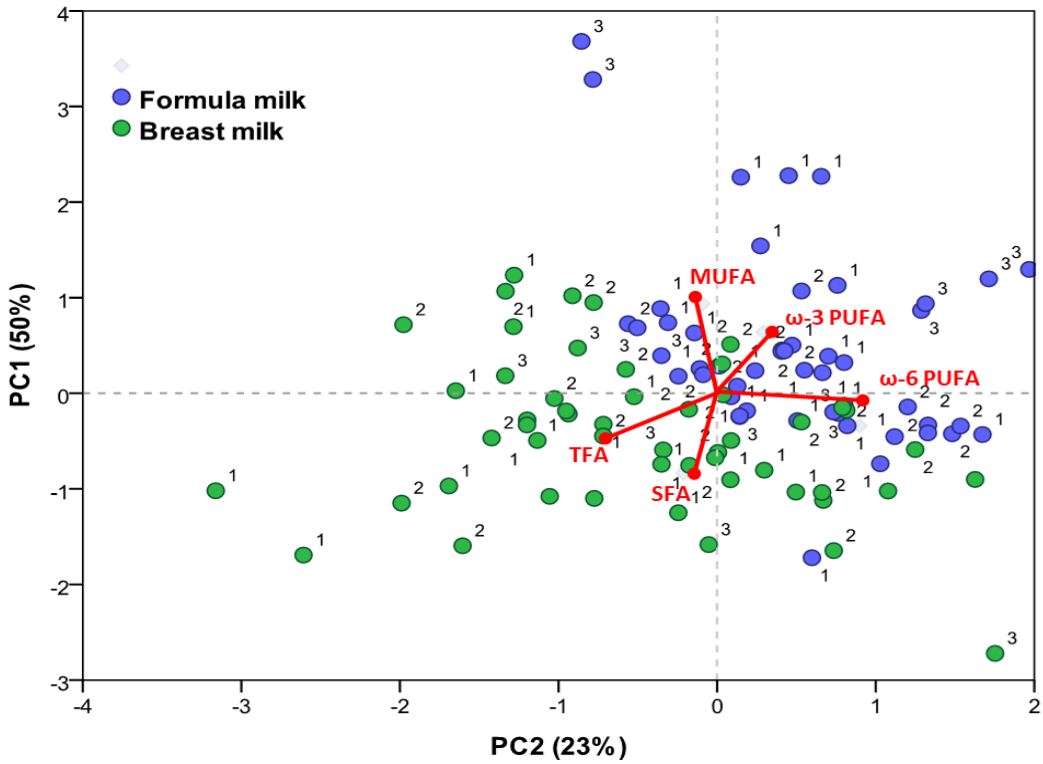


Figure 1. Principal component analysis (PC1 vs. PC2) of the fatty acid content. Each number represent the period of breast feeding (1: <1 month; 2: 1-6 month; and 3: >6 month) or type of formula milk (1: first infant; 2: follow-on; and 3: growing-up formula)

3.6 Exposure assessment

In general terms, the exposure to the compounds analysed in this study was estimated to be higher in formula-fed infants than in breastfed infants. Furthermore, the intake of BPA and most trace elements showed a similar decreasing trend in the different feeding period groups (< 1 month, 1-6 months and >6-12 months) (Table 6). Such decreasing trend may be

due to the fact that the milk intake:body weight ratio decreases with age. As abovementioned, the levels of BPA and elements were generally higher in infant formula samples than in human milk. Free BPA intake was far below the TDI threshold set by EFSA (4 µg/kg/day) for both formula-fed and breastfed infants. Our results on BPA exposure are in agreement with those obtained by Sarigiannis et al. (2016) for breastfed infants. However, they are lower for formula-fed infants (Sarigiannis et al., 2016). The fact that only BPA-free material was used in this study may account for the lower BPA exposure values estimated for formula-fed infants. The exposure to Al, Cr, Cu and Sn was below the respective tolerable intake values (2 mg/kg/week, 300 µg/kg/day, 500 µg/kg/day, and 14 mg/kg/week for Al, Cr, Cu and Sn, respectively) set by EFSA (2015a, 2015b, 2014, 2011, 2010ab) or WHO (2018). On the other hand, the Pb exposure (4.50 µg/kg/day) was higher than the provisional tolerable weekly intake (PTWI) set at 25 µg/kg (EFSA, 2010a) in the first period group (<1-month-old). This could be due to the low weight of the babies in their first weeks of life. Similarly, Ni exposure was higher than its corresponding TDI (2.80 µg/kg/day) (EFSA, 2015a) in all periods and milk types except for the group of infants who had been breastfed from 6 to 12 months. It should be noted that a conservative scenario was here considered by assuming non-detected values as one-half of the LOD.

Dietary reference values for fatty acids were referenced as energy ingested. Only fatty acid with a reference value by quantity is DHA for children between 6 and 24 month. These reference values are set between 10-12 mg/kg/day by WHO (WHO, 2008) and 100 mg/day by EFSA (EFSA, 2010b) (Table 6). These reference intakes were reached by breast feeding infants older than 6 months (11.8 mg/kg/day (109 mg/day)), but not by formula feeding infants of same age (6.62 mg/kg/day (60.9 mg/day)). Essential fatty acids (LA and ALA) shown higher intake levels, especially for LA in <1 month group and ALA in the three ages groups.

Table 6. Mean exposure to BPA (free and total), TBBPA, and elements through exclusive formula and breast feeding scenarios, and tolerable daily intake thresholds.

	<1 month		1-6month		>6-12month		TDI WHO	TDI EFSA
µg/kg/day	**Formula**	**Human**	**Formula**	**Human**	**Formula**	**Human**		
BPA (free)	0.15	0.06	0.12	0.04	0.06	NA		4
Total BPA	0.62	0.45	0.51	0.04	0.22	0.05		
TBBPA	0.08	0.09	0.06	0.07	0.05	NA		
µg/kg/day								
Al	111	52.5	90.5	39.1	50.7	14.7	286[a]	
Ba	6.00	3.00	4.89	3.67	2.94	1.47		
Cd	NA	3.00	NA	3.67	NA	NA	0.83[a]	0.36[a]
Co	NA	7.50	NA	1.22	NA	NA		
Cr	57.0	46.5	46.5	37.9	29.4	24.3		300[b]
Cu	78.0	60.0	63.6	30.6	37.5	13.2	500	
Hg	4.50	NA	NA	NA	NA	2.94		
Mn	22.5	3.00	18.4	1.22	11.0	0.74		
Ni	86.6	7.50	70.6	3.67	61.0	2.21		2.80
Pb	4.50	4.50	3.67	NA	2.21	NA	3.57[a]	3.57[a]
Se	6.00	18.00	4.89	4.89	2.21	0.74		
Sn	NA	NA	NA	NA	3.68	NA	2000[a]	
Sr	37.5	6.00	30.6	7.34	18.4	4.41		
mg/kg/day								
Ca	64.9	39.9	53.0	33.4	40.8	20.8		
K	96.9	81.5	79.0	60.1	53.7	36.2		
Fe	0.88	0.50	0.72	0.10	0.69	0.06		
Mg	7.46	3.99	6.08	3.23	4.25	1.84		
Na	27.5	32.3	22.4	16.6	16.0	7.50		
Zn	0.79	0.42	0.64	0.20	0.49	0.02		

	<1 month		1-6month		>6-12month		TDI WHO	TDI EFSA
g/kg/day	**Formula**	**Human**	**Formula**	**Human**	**Formula**	**Human**		
SFA	2.13	2.12	1.74	1.87	1.12	1.38		ALAP
MUFA	2.28	2.01	1.86	1.87	1.15	1.17		
PUFA	1.00	0.77	0.82	0.78	0.54	0.53		
ω-3 PUFA	0.14	0.06	0.11	0.06	0.07	0.03		
ω-6 PUFA	0.87	0.71	0.71	0.71	0.47	0.50		
TFA	0.02	0.05	0.01	0.04	0.01	0.02		ALAP
mg/kg/day								
LA	845	620	689	647	462	457		
ALA	116	27.0	94.2	28.1	61.0	14.7		
AA	13.5	27.0	11.0	22.0	6.62	12.5		
EPA	3.00	3.00	2.45	3.67	1.47	2.21		
DHA	15.0	24.0	12.2	19.6	6.62	11.8	10-12[c]	100[d]
CLA	0.00	6.00	0.00	4.89	0.74	2.94		

Exposure expressed in µg/kg/day except for Ca, K, Fe, Mg, Na and Zn expressed in *mg/kg/day*. NA: Not assessed due all samples were below detection limit. ALAP: as low as possible

Ag, As, Sb, and V not assessed due all samples were below their respective detection limits.

TDI: Tolerable daily intake. [a] Derived from provisional weekly or monthly intake (PWTI or PMTI). [b]Expressed as Cr(III). [c] month recommended daily intake 10-12 mg/kg/day for children between 6 and 24 month. [d]Acceptable intake 100 mg/d for >6 month <24 month children

TDI were obtained from WHO (2008, 2018) and EFSA (2010a, 2010b, 2011, 2014, 2015a, 2015b)

4. Conclusions

In this study, human milk samples contained levels of BPA and well below the EFSA/WHO thresholds, being also irrespective of the maternal characteristics (i.e., age, BMI and breastfeeding period). Actually, the concentration of free BPA was significantly higher in infant formula samples than in breast milk, which also contained significantly lower values of some essential elements, such as Al, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Sn, Sr, and Zn. The fatty acid profiling also revealed major differences between human milk and infant formulas, which should be taken in account in the development of new formulas a well as in specific recommendation for the diet of breastfeeding mothers. DHA acceptable intake limits set by EFSA and WHO were not reached by formula feeding infants in 6-12 months group. Anyway, the results of this study reinforces that breastfeeding should be always the first feeding option in early life.

Acknowledgements

This research was supported by the “HEALS” project (FP7-603946), Health and environmental-wide associations based on large population surveys, as well as by the Spanish Ministry of Economy and Competitiveness through grants AGL2016-75476-R and AGL2016-78942-R. J. Rovira received funds from Health Department of Catalonia Government trough "Pla Estratègic de Recerca i Innovació en Salut" (PERIS 2016-2020). Sara C. Cunha. Susana Casal and José O. Fernandes thanks REQUIMTE, FCT (Fundação para a Ciência e a Tecnologia) and FEDER through the project UID/QUI/50006/2013 – POCI/01/0145/FEDER/007265 with financial support from FCT/MEC through national funds and co-financed by FEDER, under the Partnership Agreement PT2020. Sara C. Cunha acknowledges FCT for the IF/01616/2015 contract.

References

Adesman, A., Soled, D., Rosen, L., 2017. Formula Feeding as a Risk Factor for Attention-

Deficit/Hyperactivity Disorder. J. Dev. Behav. Pediatr. 38, 545–551. doi:10.1097/DBP.0000000000000468

Ballard, O., Morrow, A.L., 2013. Human Milk Composition: Nutrients and Bioactive Factors. Pediatr. Clin. North Am. 60, 49-74. doi:10.1016/j.pcl.2012.10.002.

Barreiro, R., Regal, P., López-Racamonde, O., Cepeda, A., Fente, C.A., 2018. Comparison of the fatty acid profile of Spanish infant formulas and Galician women breast milk. J. Physiol. Biochem. 74, 127–138. doi:10.1007/s13105-017-0580-2

Butte, N.F., Wong, W.W., Ferlic, L., Smith, E.O., Klein, P.D., Garza, C., 1990. Energy Expenditure and Deposition of Breast-Fed and Formula-Fed Infants during Early Infancy. Pediatr. Res. 28, 631–640. doi:10.1203/00006450-199012000-00019

Cao, X.-L., Popovic, S., Arbuckle, T.E., Fraser, W.D., 2015. Determination of free and total bisphenol A in human milk samples from Canadian women using a sensitive and selective GC-MS method. Food Addit. Contam. Part A 32, 120–125. doi:10.1080/19440049.2014.980855

Cardoso, O.O., Julião, F.C., Alves, R.I.S., Baena, A.R., Díez, I.G., Suzuki, M.N., Celere, B.S., Nadal, M., Domingo, J.L., Segura-Muñoz, S.I., 2014. Concentration profiles of metals in breast milk, drinking water, and soil: Relationship between matrices. Biol. Trace Elem. Res. 160, 116-122. doi: 10.1007/s12011-014-0030-8

Committee on the Evaluation of the Addition of, Ingredients New to Infant Formula, 2004. Infant Formula: Evaluating the Safety of New Ingredients. The National Academies Press. Washington, DC. doi:10.17226/10935.

Covaci, A., Voorspoels, S., Abdallah, M.A.-E., Geens, T., Harrad, S., Law, R.J., 2009. Analytical and environmental aspects of the flame retardant tetrabromobisphenol-A and its derivatives. J. Chromatogr. A 1216, 346–363. doi:10.1016/j.chroma.2008.08.035

Cruz, G.C., Din, Z., Feri, C.D., Balaoing, A.M., Marie, E., Navidad, H.M., Margot, M., Schlaaff, F., Winter, J., 2009. Analysis of Toxic Heavy Metals (Arsenic , Lead , and Mercury) in Selected Infant Formula Milk Commercially Available in the Philippines By Aas. Int. Sci. Res. J. Int. Sci. Res. J. 1, 40–51.

Cunha, S.C., Almeida, C., Mendes, E., Fernandes, J.O., 2011. Simultaneous determination of bisphenol A and bisphenol B in beverages and powdered infant formula by dispersive liquid-liquid micro-extraction and heart-cutting multidimensional gas chromatography-mass spectrometry. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 28, 513–526. doi:10.1080/19440049.2010.542551

Cunha, S.C., Oliveira, C., Fernandes, J.O., 2016. Development of QuEChERS-based extraction and liquid chromatography-tandem mass spectrometry method for simultaneous quantification of bisphenol A and tetrabromobisphenol A in seafood: fish, bivalves, and seaweeds. Anal. Bioanal. Chem. 409, 151–160. doi:10.1007/s00216-016-9980-3

EFSA, 2015a. Scientific Opinion on the risks to public health related to the presence of nickel in food and drinking water. Panel on Contaminants in the Food Chain. EFSA J. 13, 4002. doi:10.2903/j.efsa.2015.4002

EFSA, 2015b. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs : Executive summary. EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids 13, 3978. doi:10.2903/j.efsa.2015.3978

EFSA, 2014. Scientific Opinion on the risks to public health related to the presence of chromium in food and drinking water. Panel on Contaminants in the Food Chain (CONTAM). EFSA J. 12, 3595. doi:10.2903/j.efsa.2014.3595

EFSA, 2011. Statement on tolerable weekly intake for cadmium. Panel on Contaminants in the Food Chain (CONTAM). EFSA J. 9, 1975. doi:10.2903/j.efsa.2011.1975.

EFSA, 2010a. Scientific Opinion on Lead in Food. Panel on Contaminants in the Food Chain. EFSA J. 8, 1570. doi:10.2903/j.efsa.2010.1570.

EFSA, 2010b. Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. Panel on Dietetic Products, Nutrition and Allergies. EFSA J. 8, 1461. doi:10.2903/j.efsa.2010.1461

EPA, 2011. Exposure Factors Handbook: 2011 Edition. U.S. Environmental Protection Agency EPA/600/R-, 1–1466. doi:EPA/600/R-090/052F

Feng, S., Lock, A.L., Garnsworthy, P.C., 2004. Technical Note: A Rapid Lipid Separation Method for Determining Fatty Acid Composition of Milk. J. Dairy Sci. 87, 3785–3788. doi:10.3168/jds.S0022-0302(04)73517-1

Fields, D.A., Demerath, E.W., 2012. Relationship of insulin, glucose, leptin, IL-6 and TNF-a in human breast milk with infant growth and body composition. Pediatr. Obes. 7, 304–312. doi:10.1016/j.pcl.2012.10.002.Human

Gómez-Gallego, C., Kumar, H., García-Mantrana, I., Du Toit, E., Suomela, J.P., Linderborg, K.M., Zhang, Y., Isolauri, E., Yang, B., Salminen, S., Collado, M.C., 2017. Breast Milk Polyamines and Microbiota Interactions: Impact of Mode of Delivery and Geographical Location. Ann. Nutr.

Metab. 70, 184–190. doi:10.1159/000457134

Hennet, T., Borsig, L., 2016. Breastfed at Tiffany's. Trends Biochem. Sci. 41, 508–518. doi:10.1016/j.tibs.2016.02.008

Inthavong, C., Hommet, F., Bordet, F., Rigourd, V., Guérin, T., Dragacci, S., 2017. Simultaneous liquid chromatography–tandem mass spectrometry analysis of brominated flame retardants (tetrabromobisphenol A and hexabromocyclododecane diastereoisomers) in French breast milk. Chemosphere 186, 762–769. doi:10.1016/j.chemosphere.2017.08.020

Karri, A.V., Kumar, V., Ramos, D., Oliveira, E., Schuhmacher, M., 2017. An In vitro Cytotoxic Approach to Assess the Toxicity of Heavy metals and their Binary Mixtures on Hippocampal HT-22 Cell line. Toxicol. Lett. 282, 25-36. doi:10.1016/j.toxlet.2017.10.002

Klein, L.D., Breakey, A.A., Scelza, B., Valeggia, C., Jasienska, G., Hinde, K., 2017. Concentrations of trace elements in human milk: Comparisons among women in Argentina, Namibia, Poland, and the United States. PLoS One 12, e0183367. doi:10.1371/journal.pone.0183367

Lankova, D., Lacina, O., Pulkrabova, J., Hajslova, J., 2013. The determination of perfluoroalkyl substances, brominated flame retardants and their metabolites in human breast milk and infant formula. Talanta 117, 318–325. doi:10.1016/j.talanta.2013.08.040

López-López, A., López-Sabater, M.C., Campoy-Folgoso, C., Rivero-Urgell, M., Castellote-Bargalló, A.I., 2002. Fatty acid and sn-2 fatty acid composition in human milk from Granada (Spain) and in infant formulas. Eur. J. Clin. Nutr. 56, 1242–1254. doi:10.1038/sj.ejcn.1601470

Martin, C., Ling, P.-R., Blackburn, G., 2016. Review of Infant Feeding: Key Features of Breast Milk and Infant Formula. Nutrients 8, 279. doi:10.3390/nu8050279

Martínez, M.A., Rovira, J., Sharma, R.P., Nadal, M., Schuhmacher, M., Kumar, V., 2018. Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure: A Catalonia (Spain) case study. Environ. Res. 166, 25-34. doi: 10.1016/j.envres.2018.05.008.

Martínez, M.A., Rovira, J., Sharma, R.P., Nadal, M., Schuhmacher, M., Kumar, V., 2017. Prenatal exposure estimation of BPA and DEHP using integrated external and internal dosimetry: A case study. Environ. Res. 158, 566–575. doi:10.1016/j.envres.2017.07.016

Mead, N.M., 2008. Contaminants in Human Milk.Weighing the risks against the benefts of breastfeeding. Environ. Health Perspect. 116, A427–A434. doi:10.1007/s13398-014-0173-7.2

Mendonca, R., Hauser, A.M., Calafat, T.E., Arbuckle, S.M.D., 2015. Bisphenol A concentrations in maternal breast milk and infant urine. Int. Arch. Occup. Env. Heal. 87, 13–20. doi: 10.1007/s00420-012-0834-9

Mosca, F., Giannì, M.L., 2017. Human milk: composition and health benefits. La Pediatr. Medica e Chir. 39, 47–52. doi:10.4081/pmc.2017.155

Nejrup, R.G., Licht, T.R., Hellgren, L.I., 2017. Fatty acid composition and phospholipid types used in infant formulas modifies the establishment of human gut bacteria in germ-free mice. Sci. Rep. 7, 3975. doi:10.1038/s41598-017-04298-0

O'Connor, N.R., 2009. Infant formula. Am. Fam. Physician. 79, 565–570.

O'Neal, S.L., Zheng, W., 2015. Manganese Toxicity Upon Overexposure: a Decade in Review. Curr. Environ. Heal. Reports 2, 315–328. doi:10.1007/s40572-015-0056-x

Oftedal, O.T., 2012. The evolution of milk secretion and its ancient origins. Animal 6, 355–368. doi:10.1017/S1751731111001935

Otaka, H., Yasuhara, A., Morita, M., 2003. Determination of Bisphenol A and 4-Nonylphenol in Human Milk Using Alkaline Digestion and Cleanup by Solid-Phase Extraction. Anal. Sci. 19, 1663–1666. doi:10.2116/analsci.19.1663

Poitevin, E., 2016. Official Methods for the Determination of Minerals and Trace Elements in Infant Formula and Milk Products: A Review. J. AOAC Int. 99, 42–52. doi:10.5740/jaoacint.15-0246

Quinn, E.A., 2014. Too much of a good thing: Evolutionary perspectives on infant formula fortification in the United States and its effects on infant health. Am. J. Hum. Biol. 26, 10–17. doi:10.1002/ajhb.22476

Sarigiannis, D.A., Karakitsios, S.P., Handakas, E., Simou, K., Solomou, E., Gotti, A., 2016. Integrated exposure and risk characterization of bisphenol-A in Europe. Food Chem. Toxicol. 98, 134–147. doi:10.1016/j.fct.2016.10.017

Shi, Z.X., Wu, Y.N., Li, J.G., Zhao, Y.F., Feng, J.F., 2009. Dietary exposure assessment of Chinese adults and nursing infants to tetrabromobisphenol-A and hexabromocyclododecanes: Occurrence measurements in foods and human milk. Environ. Sci. Technol. 43, 4314–4319. doi:10.1021/es8035626

Soleimani, S., Shahverdy, M.R., Mazhari, N., Abdi, K., Nejad, S.G., Shams, S., Alebooyeh, E., Khaghani, S., 2014. Lead concentration in breast milk of lactating women who were living in Tehran, Iran. Acta Med. Iran. 52, 56–59.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy Metal Toxicity and the Environment. Molecular, Clinical and Environmental Toxicology. 101, 133–164. doi:10.1007/978-3-7643-8340-4

Vandermeersch, G., Lourenço, H.M., Alvarez-Muñoz, D., Cunha, S., Diogène, J., Cano-Sancho, G.,

Sloth, J.J., Kwadijk, C., Barcelo, D., Allegaert, W., Bekaert, K., Fernandes, J.O., Marques, A., Robbens, J., 2015. Environmental contaminants of emerging concern in seafood – European database on contaminant levels. Environ. Res.143, 29-45. doi:10.1016/j.envres.2015.06.011.

Vela-Soria, F., Jiménez-Díaz, I., Díaz, C., Pérez, J., Iribarne-Durán, L.M., Serrano-López, L., Arrebola, J.P., Fernández, M.F., Olea, N., 2016. Determination of endocrine-disrupting chemicals in human milk by dispersive liquid-liquid microextraction. Bioanalysis 8, 1777–91. doi:10.4155/bio-2016-0073

Winiarska-mieczan, A., Tupaj, M., 2009. Evaluation of the Mineral Composition of Infant Formulas. J. Elementol. 14, 583–592. doi: 10.5601/jelem.2009.14.3.17

WHO, 2018. Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Available from: http://apps.who.int/food-additives-contaminants-jecfa-database/search.aspx Last accessed: June 8th, 2018

WHO, 2013. Country profiles on nutrition, physical activity and obesity in the 53 WHO European Region Member States. Methodology and summary. WHO Regional Office for Europe. Available from: http://www.euro.who.int/__data/assets/pdf_file/0004/243337/Summary-document-53-MS-country-profile.pdf Last accessed: November 14th, 2018

WHO, 2008. Interim Summary of Conclusions and Dietary Recommendations on Total Fat & Fatty Acids. From the Joint FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition, 10-14 November, 2008, WHO, Geneva. available from: http://www.who.int/nutrition/topics/FFA_summary_rec_conclusion.pdf. Last accesed: June 8th, 2018

WHO, 2003. Global strategy for infant and young child feeding. Report 1–30. doi:ISBN 92 4 156221 8

WHO and FAO, 2004. Vitamin and mineral requirements in human nutrition. WHO Library Cataloguing-in-Publication Data. ISBN 92 4 154612 3

Yi, B., Kim, C., Yang, M., 2010. Biological monitoring of bisphenol A with HLPC/FLD and LC/MS/MS assays. J. Chromatogr. B 878, 2606–2610. doi:10.1016/j.jchromb.2010.02.008

CHAPTER 2 BIOMONITORING

PUBLICATION 5.

M.A. Martínez[a]; I. Castro[c]; J. Rovira[a,b]; S. Ares[d]; J.M. Rodríguez[c]; S.C. Cunha[e]; S. Casal[e]; J. Olivera-Fernandes[e]; M. Schuhmacher[a,b]; M. Nadal[b]. **Early-life intake of major trace elements, bisphenol A, tetrabromobisphenol A and fatty acids: Comparing human milk and commercial infant formulas.** *Environmental Research* 169 (2018) 246 – 255

SUPPLEMENTARY MATERIAL

Supplementary material

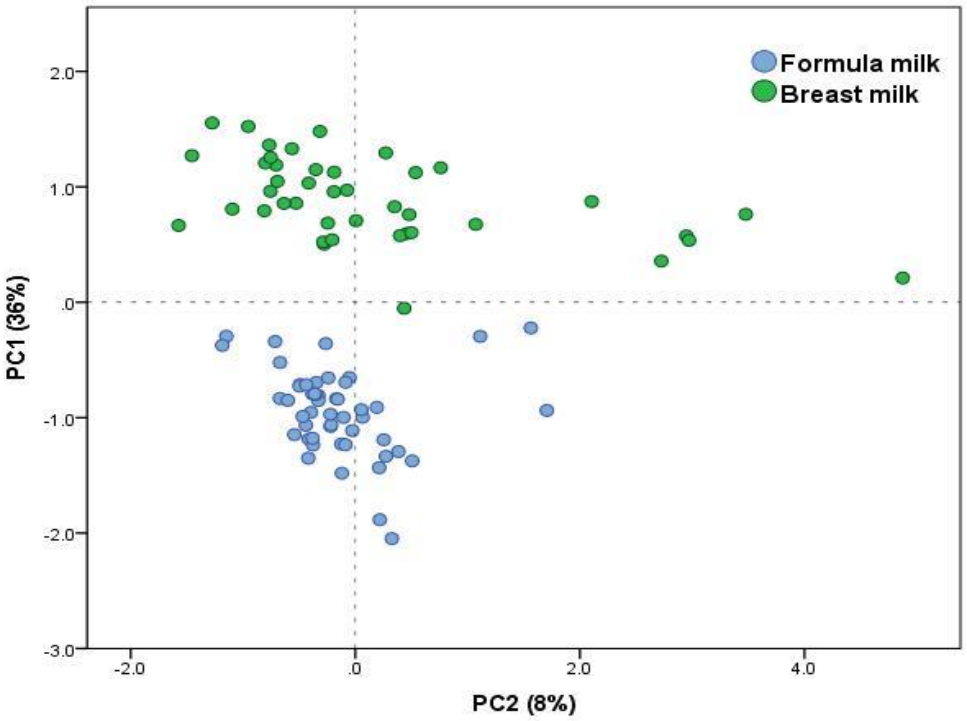


Figure S1. Principal component analysis (PC1 vs.PC2 and PC1 vs PC3) of the fatty acid content. The first PC (PC1) showed a highly positive correlation with C16:1n9, C16:1n7, C20:2n6; C20:3n6, C22:4, and C22:5n3, and a highly negative correlation with C18:3n3; PC2 was positively correlated with C14:1, C14:1t, C15:0, and C18:1t while PC3 was correlated with C18:4, C22:2, and C24:1.

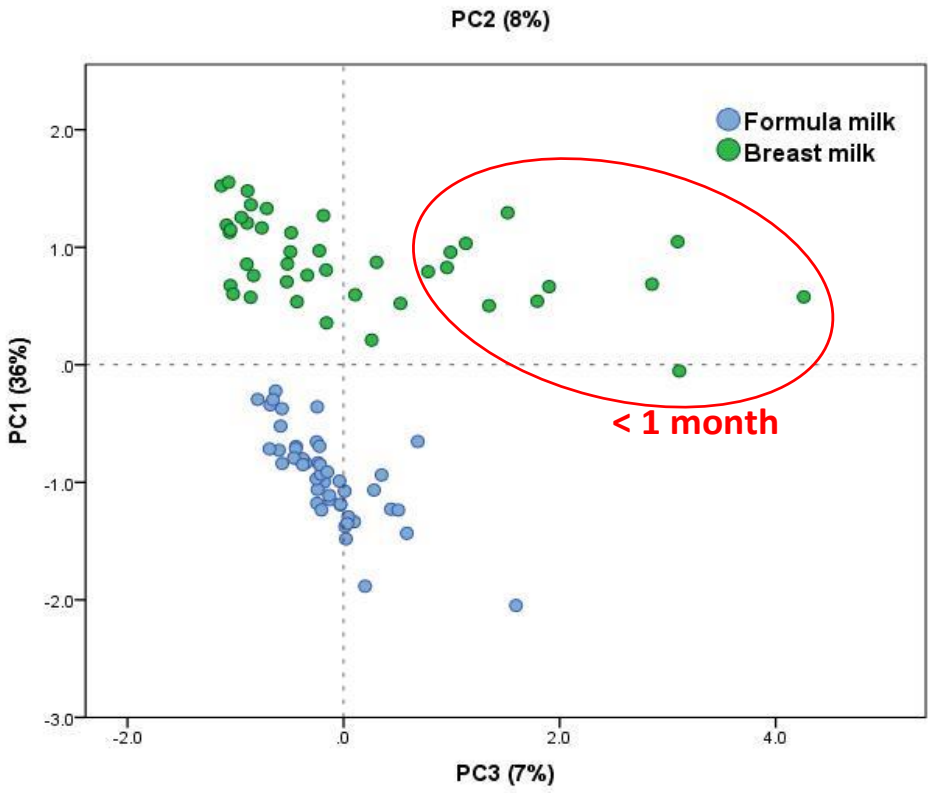

PC2 (8%)
Formula milk
Breast milk
< 1 month
PC1 (36%)
PC3 (7%)
2.0
1.0
.0
-1.0
-2.0
-3.0
-2.0
.0
2.0
4.0

Figure S1.

Table S1: Fatty acids codification and classification

		Formula	Human		Formula	Human	
		(n=50)	(n=53)	*p-value*	(n=50)	(n=53)	*p-value*
		g/L of milk			g/100g of fat		
Saturated fatty acids (SFA)							
Hexanoic acid	6:0	0.07±0.06	0.02±0.01	***<0.001***	0.19±0.16	0.06±0.07	***<0.001***
Octanoic acid	8:0	0.56±0.69	0.03±0.01	***<0.001***	1.41±1.62	0.16±0.05	***<0.001***
Decanoic acid	10:0	0.40±047	0.54±0.29	*0.067*	1.01±1.11	1.35±0.36	***0.036***
Lauric acid (dodecanoic acid)	12:0	2.65±1.40	2.36±1.29	*0.269*	6.84±3.32	5.85±1.64	***0.003***
Myristic acid (tetradecanoic acid)	14:0	1.27±0.58	2.42±1.42	***<0.001***	3.26±1.33	6.00±1.90	***<0.001***
Pentadecanoic acid	15:0	0.02±0.00	0.10±006	***<0.001***	0.06±0.06	0.25±0.10	***<0.001***
Palmitic acid (hexadecanoic acid)	16:0	7.33±2.33	7.34±3.31	*0.977*	18.8±4.41	18.9±2.07	*0.342*
Heptadecanoic acid	17:0	0.03±0.01	0.09±0.04	***<0.001***	0.09±0.03	0.24±0.05	***<0.001***
Stearic acid	18:0	1.41±0.32	2.12±0.96	***<0.001***	3.63±0.45	5.50±090	***<0.001***
Eicosanoic acid	20:0	0.07±0.02	0.05±0.03	***<0.001***	0.19±0.07	0.13±0.04	***<0.001***
Docosanoic acid	22:0	0.10±0.04	0.02±0.01	***<0.001***	0.25±0.09	0.05±0.01	***<0.001***
Tetracosanoic acid	24:0	0.05±0.01	0.02±0.01	***0.028***	0.12±0.03	0.05±0.02	***<0.001***
Monounsaturated fatty acids (MUFA)							
Tetradecenoic acid	14:1 n-5	0.01±0.02	0.06±0.05	***<0.001***	0.03±0.06	0.14±0.08	***<0.001***
Pentadecenoic acid	15:1 n-5	0.00±0.00	0.02±0.01	***<0.001***	0.01±0.01	0.05±0.03	***<0.001***
Palmitoleic acid (hexadecenoic acid)	16:1 n-7	0.08±0.04	0.66±0.39	***<0.001***	0.21±0.11	1.70±0.49	***<0.001***
Heptadecenoic acid	17:1 n-7	0.02±0.01	0.08±0.15	***<0.001***	0.04±0.02	0.23±0.55	***0.006***
Oleic acid (octadecenoic acid)	18:1 n-9	15.2±3.16	13.6±5.87	***0.008***	39.4±4.63	35.4±3.63	***<0.001***
Gondoic acid	20:1 n-9	0.13±0.04	0.15±0.06	*0.327*	0.35±0.08	0.38±0.09	*0.062*
Erucis acid (docosanoic acid)	22:1 n-9	0.03±0.02	0.03±0.01	*0.896*	0.07±0.05	0.10±0.03	***0.004***
Tetracosenoic acid	24:1 n-9	0.01±0.01	0.02±0.01	*0.189*	0.03±0.01	0.05±0.03	***0.002***
Polyunsaturated fatty acids (PUFA)							
PUFA n-6							

		Formula	**Human**	***p-value***	**Formula**	**Human**	
		(n=50)	**(n=53)**		**(n=50)**	**(n=53)**	
		g/L of milk			g/100g of fat		
Polyunsaturated fatty acids (PUFA)		**(n=50)**	**(n=53)**		**(n=50)**	**(n=53)**	
PUFA n-6							
LA Linoleic acid (octadecadienoic acid)	18:2 n-6	5.90±1.68	5.05±2.95	***0.002***	15.1±2.08	12.6±2.99	***<0.001***
γ-Linolenic acid (octadecatrienoic acid)	18:3 n-6	0.03±0.02	0.04±0.02	*0.254*	0.08±0.04	0.10±0.05	*0.243*
Eicosadienoic acid	20:2 n-6	0.01±0.00	0.12±0.07	***<0.001***	0.02±0.01	0.30±0.10	***<0.001***
Eicosatrienoic acid	20:3 n-6	0.00±0.00	0.15±0.10	***<0.001***	0.01±0.01	0.38±0.13	***<0.001***
Arachidonic acid (eicosatetraenoic acid)	20:4 n-6	0.08±0.09	0.18±0.09	***<0.001***	0.19±0.20	0.46±0.12	***0.001***
Docosadienoic acid	22:2 n-6	0.00±0.01	0.02±0.01	***<0.001***	0.01±0.03	0.04±0.02	***0.004***
Docosatetraenoic acid	22:4 n-6	0.00±0.00	0.04±0.02	***<0.001***	0.00±0.00	0.09±0.04	***0.001***
Docosapentaenoic acid	22:5 n-6	0.00±0.00	0.01±0.01	***<0.001***	0.01±0.01	0.03±0.02	***0.007***
PUFA n-3							
ALA α-Linolenic acid (octadecatrienoic acid)	18:3 n-3	0.81±0.22	0.20±0.11	***<0.001***	2.08±0.40	0.53±0.25	***<0.001***
Octadecatetraenoic acid	18:4 n-3	0.00±0.00	0.01±0.00	*0.061*	0.01±0.02	0.04±0.01	*0.054*
Eicosatrienoic acid	20:3 n-3	0.02±0.06	0.00±0.00	***<0.001***	0.05±0.13	0.00±0.00	***0.010***
EPA (Eicosapentaenoic acid)	20:5 n-3	0.02±0.03	0.02±0.02	*0.946*	0.04±0.05	0.07±0.06	*0.303*
Docosapentaenoic acid	22:5 n-3	0.00±0.00	0.04±0.02	***<0.001***	0.01±0.01	0.11±0.05	***<0.001***
DHA (docosahexaenoic acid)	22:6 n-3	0.08±0.09	0.15±0.09	***<0.001***	0.22±0.14	0.41±0.26	***<0.001***
***Trans* fatty acids (TFA)**							
total *trans*-tetradecenoic acid	C14:1 n-5t	0.00±0.00	0.01±0.01	***<0.001***	0.00±0.00	0.02±0.02	***0.025***
total *trans*-hexadecenoic acid	C16:1 n-7t	0.00±0.00	0.02±0.01	***<0.001***	0.00±0.01	0.05±0.02	***<0.001***
total *trans*-Heptadecenoic acid	C17:1 n-7t	0.00±0.00	0.01±0.01	***<0.001***	0.00±0.00	0.03±0.01	***0.046***
9-*trans*-octadecanoic acid	C18:1 n-9t	0.05±0.05	0.18±0.11	***<0.001***	0.13±0.13	0.48±0.20	***<0.001***
11-*trans*-octadecanoic acid	C18:1 n-7t						
total *trans*-octadecadienoic acid	C18:2 n-6t	0.07±0.04	0.10±0.05	***0.011***	0.19±0.10	0.25±0.06	***0.002***
CLA (9c,11t-octadecadienoic acid)	CLA	0.00±0.01	0.04±0.02	***<0.001***	0.01±0.03	0.10±0.05	***<0.001***

CHAPTER 3 ENDOCRINE DISRUPTION POTENTIAL

PUBLICATION 6

M.A. Martínez, J. Blanco, J. Rovira, V. Kumar, J.L. Domingo, M. Schuhmacher.**Bisphenol A analogues (BPS and BPF) present a greater obesogenic capacity in 3T3-L1 cell line**. *Food Chem. Toxicol. In press*

PUBLICATION 6

Bisphenol A analogues (BPS and BPF) present a greater obesogenic capacity in 3T3-L1 cell line.

M.A. Martínez[1], J. Blanco[2], J. Rovira[1,3], V. Kumar[1], J.L. Domingo[3], M. Schuhmacher[1,3].

[1]Environmental Engineering Laboratory, Departament d'Enginyeria Quimica, Universitat Rovira i Virgili, Av. Països Catalans 26, 43007 Tarragona, Catalonia, Spain.
[2] Research in Neurobehavior and Health (NEUROLAB), Universitat Rovira i Virgili,Tarragona, Spain; Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Catalonia, Spain.
[3]Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Catalonia, Spain.

Food Chem. Toxicol,

In press

Abstract

This study was aimed at comparing the toxicity effects on cell viability and the obesogenic activity of Bisphenol A (BPA) and its analogues, Bisphenol S (BPS) and Bisphenol F (BPF), by *in vitro* assays with a preadipocytic 3T3-L1 cell line. To compare the toxic potential and select the concentrations of each chemical not showing a decrease in cell viability, MTT assay was performed. The cell phenotype was determined in differentiated 3T3-L1 adipocytes by red oil O staining. To determine the expression levels of the different adipogenic proteins the Western Blot test was performed. The results from MTT assay showed a greater toxic effect of BPA - at equal and even lower concentrations- than its analogues. However, BPS followed by BPF showed a greater neutral lipid storage capacity than BPA, which was reflected in the increase of the protein expression of CCAAT/enhancer binding protein α (C/EBPα), peroxisome proliferator-activated receptor gamma γ (PPARγ) and acid-binding protein 4 (FABP4). In summary, these BPA analogues -especially BPS- present a greater endocrine potential activity than BPA.

Keywords

Bisphenol A (BPA); analogues; adipogenesis; cell viability; endocrine potential activity.

1. Introduction

Endocrine disruptors (EDs) are exogenous substances that can mimic, totally or partially, naturally occurring hormones such as estrogens, androgens, and thyroid hormones (Matsui, 2008). EDs can also bind to a cellular receptor and block the endogenous hormone action. Therefore, EDs can alter or block the signal transmission of natural hormones (Thomson and Grounds, 2005). One of the most important EDs is Bisphenol A (BPA). It is widely distributed in products that contain it, being of concern its relation with several relevant diseases such as obesity and reproductive, behavioural and neurodevelopmental adverse effects (WHO, 2012). The effects of prenatal and early exposure to EDs may appear at any time of life (Giulivo et al., 2016; Mercogliano and Santonicola, 2018). BPA is used to produce epoxy-resins and polycarbonate plastics. The EU has banned its use in plastic materials that can contact with food intended for children (0–3 years) (EC, 2011). However, the principal analogues of BPA, Bisphenol S (BPS) and Bisphenol F (BPF) are still being used (Martínez et al., 2018). These chemicals can be found in food and beverage processing, as well as in many other commercial products such as epoxy resin cans, dental sealants, personal care products, baby bottles, building materials, flame retardant materials, optical lenses, materials for the protection of window glazing, DVDs, and household electronics (Geens et al., 2012; González et al., 2019a; Mariscal-Arcas et al., 2009; Myridakis et al., 2016; Otaka et al., 2003). Moreover, BPA has also been found in breast and formula milk (Martínez et al., 2019). Furthermore, BPA and its analogue BPS have been found in hair from pregnant women (Katsikantami et al., 2020) and children (Karzi et al., 2018), and in amniotic fluid (Katsikantami et al., 2018).

BPA is considered as an obesogen. These substances can alter the system of hormonal equilibrium by mediating lipid homeostasis and promoting adipogenesis in an organism or its progeny (Muscogiuri et al., 2017; Veiga-Lopez et al., 2018). However, the potential adverse health effects of BPS and BPF remain still unclear (González et al., 2019b). Both have similar chemical structures than BPA, having been suggested that they are endocrine-disrupting chemicals. Some studies are confirming that they are involved in the processes

of preadipocytes differentiation and lipid accumulation (Ahmed and Atlas, 2016; Liu et al., 2019). However, data on cell viability effects along with the determination of the endocrine-disrupting activity through protein expression and neutral lipid storage are not available in the scientific literature.

According to the World Health Organization (WHO), obesity has considerably increased in the last four decades. It is a chronic disease related to excessive lipid accumulation in adipose tissue (WHO, 2017). Exposure to pregnant women to these EDs is directly related to the fetus' exposure due to a bi-directional transfer of the chemicals through the placenta (Li et al., 2013). Consequently, prenatal exposure to BPA is related to obesity and concomitant diseases in midlife, such as type 2 diabetes, cardiovascular diseases, and arterial hypertension (Mornagui et al., 2019; Weihrauch-Blüher and Wiegand, 2018). Moreover, BPS has also been found to have potential risk for cardiovascular diseases in rats (Pal et al., 2017).

The primary function of adipocyte cells is to store excess energy from consumed food, making it available to cells and tissues when circumstances require more energy input. However, bad lifestyle such as excessive caloric intake and lack of exercise in the long term, are important factors that lead to adipose tissue proliferation. Consequently, the size (hypertrophy) and number (hyperplasia) of adipocytes increases (Ghaben and Scherer, 2019).

The present study aimed to compare to compare the toxic effects (cell viability) and the obesogenic activity of BPA and its analogues BPS and BPF with a preadipocytic 3T3-L1 cell line. To achieve this goal, the MTT assay was performed to compare the cell viability between BPA and its analogues, as well as to select the optimal concentration to proceed with the cell's treatment. Phenotypic changes in differentiated 3T3-L1 adipocytes and their ability to storage neutral lipids were determined by red oil O staining. Furthermore, the expression levels of different adipogenic marker proteins were quantified. It includes transcription factors CCAAT/enhancer-binding protein α (C/EBPα), peroxisome proliferator-activated receptor γ (PPARγ), and the fatty acid-binding protein 4 adipokine (FABP4)

(Cowherd et al., 1999). The results should clarify if BPA has similar or different effects than its analogues BPS and BPF in cell toxicity and on the adipose differentiation process.

2. Material and Methods

2.1. 3T3-L1 Cell culture, growth, and differentiation

Undifferentiated 3T3-L1 mouse fibroblasts (ATCC CL-173) were available in host laboratory. Cells were grown during passages in growth complete medium composed of Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (v/v) foetal bovine serum (Gibco BRL, Life Technologies, Paisley, UK), 2 mM glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin, and 25 mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Sigma, St. Louis, MO, USA). Cultures were maintained in 5% CO_2 at 37 °C with 95% humidity. 3T3-L1 pre-adipocytes were treated with 32 µM of BPA, 32 µM of BPS or 32 µM of BPF along with differentiation medium [DMEN complete medium, insulin (5 mg/mL), dexamethasone 0.25 mM and 3-isobutyl-1-methylxanthine (IBMX) 0.1 M]. After 48h of exposition, the medium was removed and DMEM complete medium supplemented with insulin (5 mg/mL) was added. Finally, at day 6 of differentiation, the medium was removed and only DMEM complete medium was added. Treated and untreated (only exposed to solvent) cells were collected and analysed at day 8, after the differentiation process (Fig. 1).

Fig. 1. Treatment schedule. Cell culture, growth and differentiation.

2.2. Treatment conditions of 3T3-L1 cells

BPA, BPS, and BPF were purchased from Sigma (St. Louis, MO, USA). Media and antibiotics for cell culture were from Lonza (Lonza Group Ltd, Basel, Switzerland). The chemicals were dissolved in dimethyl sulfoxide (DMSO) and diluted in DMEM to make a stock solution, which was added to the culture medium at a range of concentrations below specified. In all treatments, the volume of DMSO was less than 0.1% of the total volume. Control cells were exposed to the same DMSO vehicle volume than that used to expose the treated cells. To assess cell viability, undifferentiated 3T3-L1 preadipocytes were seeded in 96-well plates and treated with a range of chemical concentrations: 0, 10, 50, 100, 200 and 400 µM BPA, BPS or BPF over 24 h and 48 h. For the oil red O staining, cells were seeded in 60-mm culture dishes and treated at the beginning of the adipocyte differentiation process with 32 µM BPA, 32 µM BPS and 32 µM BPF, according to Ahmed and Atlas (2016). After 48 h, the differential growth medium with treatments was removed, being the cells washed twice with phosphate-buffered saline (PBS). During the rest of the differentiating process, no additional treatment was added. To determine protein expression, cells were treated at the same concentrations. **Figure** 1 depicts the treatment schedule and maturation stages of 3T3-L1 preadipocytes.

2.3. Cell viability

Cell viability was colorimetrically quantified using the metabolic dye [4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium (MTT) (Sigma, St. Louis, MO, USA). 3T3-L1 preadipocytes were placed into 96-well plates at a density of 1×10^4 cells/well in 200 µL of complete medium and cultured overnight. Following treatment for 24 h, 20 µL MTT (5 mg/mL in PBS) were added to each well, being the cells incubated for 3 h at 37 °C. During incubation, MTT was reduced to produce a dark blue formazan product. The medium was removed, and the formazan crystals were solubilized with DMSO, being the absorbance determined by measuring the change in absorbance at 595 nm using a microplate reader (BioTek Power Wave XS). The

results of the cell viability assay are expressed as percentages, while the absorbance measured in the control cells was considered to be 100%.

2.4. Lipid accumulation

Eight days following the initiation of differentiation, the neutral lipid storage in cells determined by red oil O staining was evaluated as representative of the degree of adipocyte differentiation. Cells were rinsed twice with PBS and, fixed in 10% formaldehyde in a phosphate-buffered saline for 1 h at room temperature. The formaldehyde was then removed and the cells were washed with 60% isopropanol solution, and stained with 0.21% oil red O working solution (w/v, 60% isopropanol) for 30 min at room temperature. Cells were subsequently rinsed several times with water. The lipid droplets stained with oil red O were observed, and representative pictures of the droplets were imaged. Finally, the dye incorporated by the cells was extracted with 100% isopropanol and the absorbance was measured at 518 nm.

2.5. Protein expression

Total protein was isolated from 3T3-L1 cells. Aliquots of cell lysate, containing 30 g of protein per sample, were analysed by Western blot analysis. Briefly, samples were placed in a buffer (0.5 M Tris–HCl, pH 6.8; 10% glycerol; 2% SDS (w/v); 5% 2-β-mercaptoethanol (v/v), and 0.05% bromophenol blue) and denatured by boiling at 95–100 °C for 5 min. Samples were then separated by electrophoresis on 10% acrylamide gels. The proteins were subsequently transferred to Immobilon-P PVDF sheets (Millipore Corp., Bedford, MA, USA) using a transblot apparatus (BioRad, Madrid, Spain). The membranes were blocked for 1 h with 5% non-fat milk dissolved in TBS-T buffer (50 mM Tris, 1.5% NaCl, 0.05% Tween 20 at pH 7.5). They were then incubated overnight with primary monoclonal antibodies (mAb) from Cell Signaling (New England Biolabs, USA) against to total PPARγ (molecular weight [MW]: 53 kDa), total C/EBPα (MW: 42 kDa), total FABP4 (MW: 15 kDa), Akt phosphorylated at Ser473 (MW: 60 kDa), total AKT (MW: 60 kDa) or β-actin (MW: 45 kDa). The blots were thoroughly washed in TBS-T buffer and incubated for 1 h with a peroxidase-conjugated IgG

antibody. Immunoreactive proteins were visualized using an Immun-Star Chemiluminescence kit (BioRad, Madrid, Spain) according to the manufacturer's instructions. Digital images were taken with a VersaDoc system (BioRad, Madrid, Spain), which permits semi-quantification of the band intensity. The protein load was periodically monitored via the immuno-detection of β-actin.

2.8. Statistics

Statistical analysis was performed using the Statistical Package for the Social Sciences software (SPSS v.22). The homogeneity of the variances was analysed with Levene's test. If variances were homogenous, ANOVA was then used followed by Bonferroni's test. The Kruskal-Wallis test was used when variances were not homogeneous. Significance was set at $p < 0.05$.

3. Results

3.1. Cell viability (MTT assay)

Preliminary experiments were performed to evaluate the effects of BPA, BPS, and BPF on cell viability. It was assessed after exposure of undifferentiated 3T3-L1 preadipocytes to BPA, BPS, and BPF at a range of concentrations between 0 and 400 µM of each bisphenol. **Figure** 2 depicts the effect of BPA and its analogues (BPS and BPF) on cell viability after 24 and 48 hours.

After 24 h of cell exposure, only a significant decrease in cell viability for the treatment with 400 µM of BPA (16 ± 3%, $p < 0.001$) was observed with respect to the control.

After 48 h of cell exposure, a significant decrease in cell viability for the groups treated with 200 µM of BPA (59 ± 1%, $p = 0.002$), and 400 of µM BPA (15 ± 1%, $p = 0.005$) was noted in relation to the control. However, non-significant ($p > 0.05$) decrease were found for the groups treated with BPS or BPF.

In general terms, at the same concentration, BPA showed higher decreases in cell viability than BPS and BPF. Consequently, the current results show that BPA is more toxic than BPS and BPF.

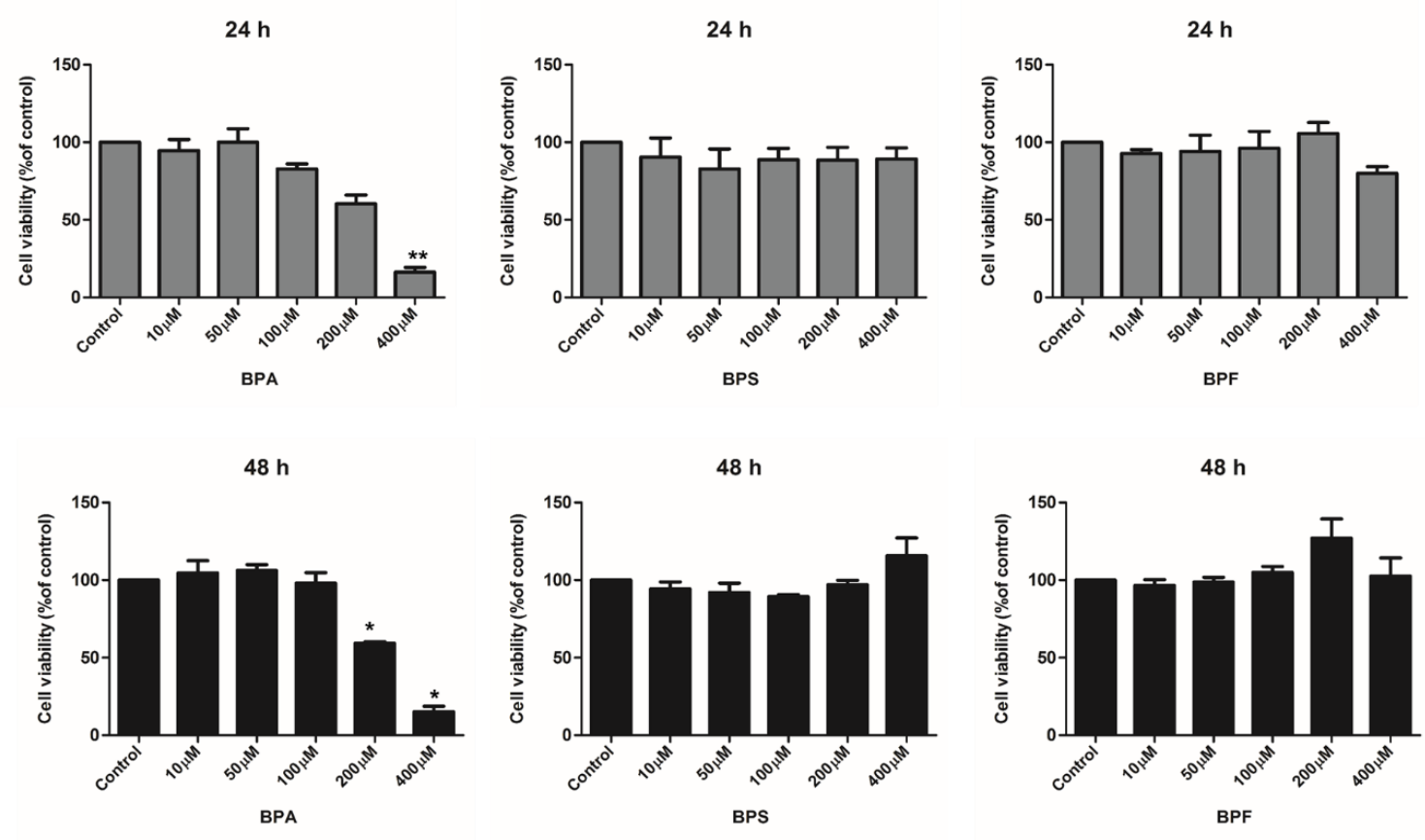


Fig. 2. Cell (3T3-L1) viability after treatment with BPA, BPS and BPF for 24 h (top) and for 48 h (bottom). Data are expressed as percentage regarding the control (mean ± SD) of 3 independent experiments (n = 3). Asterisks indicate significant differences with respect to control at $p < 0.05$ and $p < 0.001$, respectively.

3.2. Neutral lipid storage (red oil O cell staining)

The adipogenic differentiation capacity (neutral lipid storage) of BPA, BPS, and BPF (at 32 µM) was evaluated using red oil O cell staining. On day 8 of maturation, the cells were stained, and representative images of the droplets were captured at 10 X microscopic field magnification (Fig. 3). The fat-soluble dye was extracted from cells with isopropanol, being optical density (OD) measured at a wavelength of 518 nm The results show that addition of BPA, BPS or BPF to the differential media induced a significant increase in the number of differentiated 3T3-L1 adipocytes and in their ability to store neutral lipid droplets. More specifically, extractions with isopropanol after staining with red oil O showed that cells treated with 32 µM of BPS (199 ± 4%, $p < 0.001$) had a greater adipogenic effect compared with those treated with 32 µM of BPA (127 ± 3%, $p < 0.001$) and 32 µM of BPF (152 ± 3%, $p < 0.001$). Moreover, cells treated with bisphenols (BPA, BPS, or BPF) had a greater adipogenic effect than the controls. Thus, the adipogenic differentiation capacity (neutral lipid storage capacity) can be ordered as follows: BPS > BPF > BPA > control, being significant ($p < 0.001$) at all treatments.

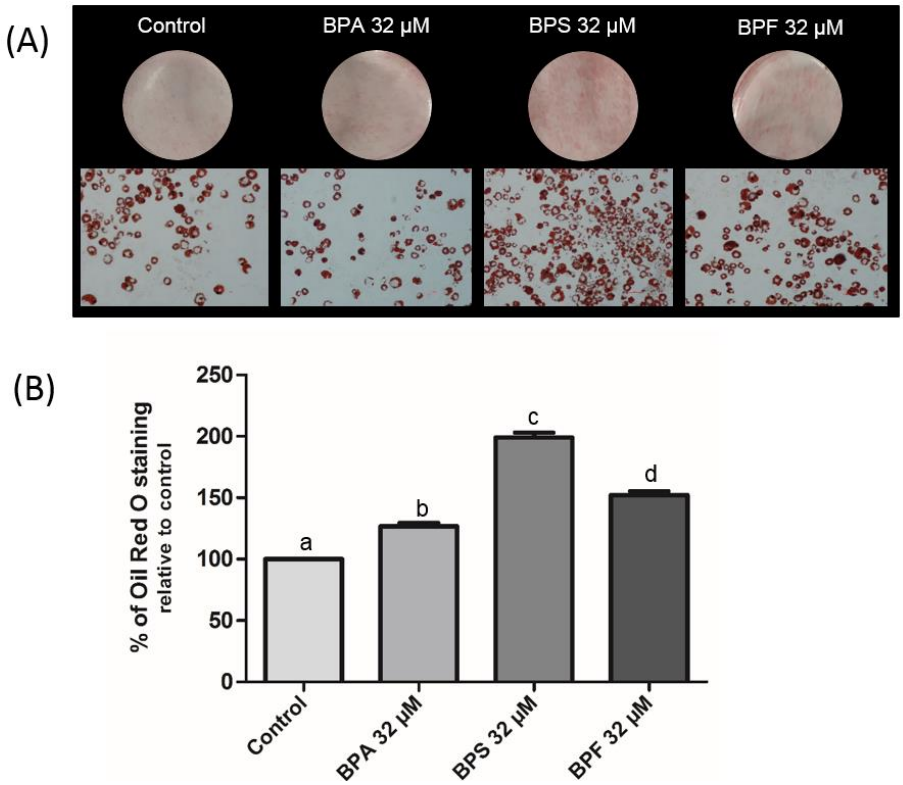


Figure. 3. Oil red O staining and quantification of neutral lipid accumulation of 3T3-L1 cells after treatment with BPA, BPS or BPF at 32 µM. 3T3-L1 preadipocytes were independently exposed to BPA, BPS, and BPF at 32 µM in the differential growth medium for two days. After differentiation process, images of the droplets were captured at 10 X microscopic field magnification (A). Data are expressed as the mean ± SD of 4 independent experiments (n = 4) (B). Different letters (a, b, c, d) indicate significant differences at $p < 0.001$.

3.3. Protein expression levels

Adipogenic markers at the end of the differentiation process were differently expressed in the treated cells compared with control cells (Fig. 4). Exposure of the 3T3-L1 preadipocytes to 32 µM of BPS had a great impact on the expression of C/EBPα ($p = 0.001$), of PPARγ ($p = 0.009$) and of FABP4 ($p = 0.023$), compared with the control. Based on these results, BPS was the only showing the ability to upregulate significantly ($p < 0.05$) C/EBPα, PPARγ and FABP4. In turn, BPA and BPF at 32 µM, upregulated protein expression compared with the control. However, it did not happened at a significant level ($p > 0.05$).

The active form of phosphorylated AKT (P-AKT) protein at Ser 473, in control cells and in cells treated with BPA, BPS or BPF, did not show significant changes. On the other hand, the P-AKT/AKT ratio did not show variations in treated and control cells.

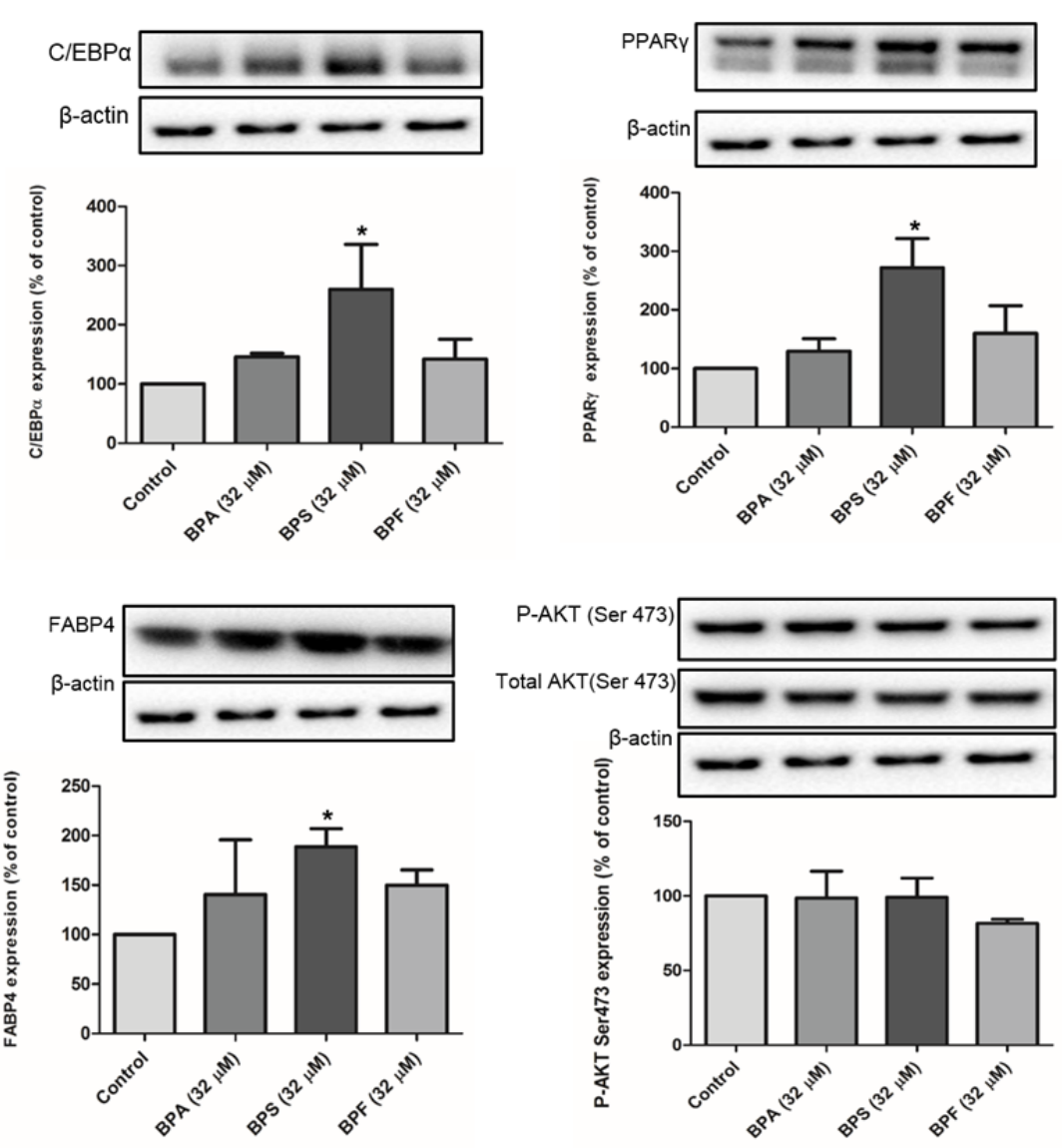


Figure 4. Adipogenic protein expressions of C/EBP*α*, PPAR*γ,* and FABP*4* after treatment of 3T3-L1 cells with BPS, BPF and BPA at 32 μM. Data were normalized using the *β*-actin protein as an internal control. Data are expressed as the mean ± SD of four independent experiments (n = 4). An asterisk indicates significant differences with respect to control at $p < 0.05$.

4. Discussion

The present investigation was focused on understanding the obesogenic capacity effects *in vitro* of BPA and two of its analogues, BPS and BPF. This study included a cell viability test (MTT assay), as well as, the determination of the cell phenotype (neutral lipid storage), protein (C/EBPα, PPARγ, and FABP4) expression (western blot) in 3T3-L1 cells, which were independently treated with BPA, BPS and BPF. The results showed that BPA is more toxic for 3T3-L1 preadepocitic cells than its analogues, BPS and BPF. Cell viability was not affected after 24 h or 48 h of treatment with BPS or BPF, even at the highest concentration (400 µM). There are no available data in the scientific literature that compare the effects of independently BPA, BPS and BPF treatments on cell viability in 3T3-L1 cells, being hard to find studies including all of the mentioned assays. To the best of our knowledge, the current study is the only one that has compared these three types of bisphenols (BPA, BPS and BPF).

The 3T3-L1 cells treated with bisphenols caused an increase in the number of differentiated preadipocytes, as well as an increase in the capacity to store lipid droplets compared with the control. BPS, followed by BPF and finally by BPA, showed a higher positive influence over the adipogenic process at the same concentration. This is related to the detected upregulation of transcription factors C/EBPα, PPARγ, and FABP4 adipokine. Moreover, the levels of phosphorylated Akt protein at Ser 473 remained constant, which indicates that mature adipocytes maintain their sensitivity to insulin signalling after the exposure to treatments (Zhang et al., 2009) .

The current results suggest that BPS has higher endocrine-disrupting activity compared with that of BPA and BPF. In agreement with this, various studies (Deceuninck et al., 2015; García-Córcoles et al., 2018; Van Leeuwen et al., 2019) report that the higher hormonal activity of BPS can likely be attributed to its strong polarity and the presence of a sulfonyl group. It was reported that most BPA analogues in *in vitro* studies had similar or higher estrogenic and antiandrogenic activity than BPA (Deceuninck et al., 2015; García-Córcoles et al., 2018; Van Leeuwen et al., 2019).

Several authors have reported that BPS promotes the accumulation of lipids and differentiation *in vitro* or *in vivo*. Regarding *in vitro* studies, the results of some investigators (Ahmed and Atlas 2016; Boucher et al., 2015; Boucher et al., 2016) were in agreement with the present results. Ahmed and Atlas (2016) exposed 3T3-L1 cells to a range of concentrations of 0.01–50 µM BPA or BPS during 48 h. It was also demonstrated that an increase in lipid accumulation, as well as in mRNA and protein expression of key adipogenic markers started after treatment with 25 µM, a similar value than that of our treatment conditions (32 µM). Ahmed and Atlas (2016) showed that treatment concentrations bellow 25 µM of BPS or BPA did not cause significant changes to the control. These authors also reported that treatment at the same concentration for each bisphenol (similarly to the current study), BPS, produced higher adipocyte differentiation and lipid accumulation than BPA, followed by higher upregulate expression levels of different adipogenic markers including PPARγ, FABP4 and C/EBPα (Ahmed and Atlas 2016). Regarding BPS, similar results were reported by Boucher et al. (2015, 2016) who exposed human preadipocytes cells to a range of concentrations between 0.1 nM and 25 µM of BPS during 14 days. It was observed that BPS -at equal concentration than BPA- induced higher lipid accumulation, while the mRNA and protein levels of several adipogenic markers were also increased. Regarding *in vivo* studies, Ivry Del Moral et al. (2016) administered to mice doses of 0.2, 1.5 and 50 µg/kg/day of BPS for 23 weeks. It was found that BPS potentiates obesity in high-fat diets by inducing lipid storage linked to faster lipid plasma clearance. Furthermore, a comprehensive study by Verbanck et al. (2017) revealed that the potency of BPS and BPF was of the same order of magnitude than BPA, showing similar hormonal effects *in vitro* and *in vivo*. They suggested that BPS and BPF have analogous potency and mechanisms of action to than those of BPA, with similar health effects (Verbanck et al., 2017).

In summary, the current results showed a greater toxic effect of BPA at equal and even lower concentrations than its analogues. At equal concentration, BPS followed by BPF showed a greater neutral lipid storage and adipocytes differentiation capacity than BPA. However, a significant increase of the protein expression levels in C/EBPα, PPARγ and FABP4, respect to the control was only found for BPS.

The endocrine-disrupting capabilities of BPA have led to the replacement of chemicals, such as BPS or BPF. However, the *in vitro* results here obtained suggest that the replacement by these analogues could not be safe. These analogues may contribute -as BPA- to diseases like obesity and diabetes in childhood. Unfortunately, the number of available studies based on the possible effects of BPA analogues is certainly limited (Andújar et al., 2019). Therefore, further investigations are clearly required. Regulations on the assessment of the safety of consumer products, especially considering the most vulnerable exposure windows, prenatal exposure and childhood, should be established.

Acknowledgements

This project has received funding from the European Unions´ Horizon 2020 research and innovation Programme under grant agreement No 733032 HBM4EU. V. Kumar and J. Rovira received funds from Health Department of Catalonia Government trough "Pla Estratègic de Recerca i Innovació en salut" (PERIS 2016–2020).

References

Ahmed, S., Atlas, E., 2016. Bisphenol S- and bisphenol A-induced adipogenesis of murine preadipocytes occurs through direct peroxisome proliferator-activated receptor gamma activation. Int. J. Obes. 40, 1566–1573. https://doi.org/10.1038/ijo.2016.95

Andújar, Gálvez-Ontiveros, Zafra-Gómez, Rodrigo, Álvarez-Cubero, Aguilera, Monteagudo, Rivas, 2019. Bisphenol A Analogues in Food and Their Hormonal and Obesogenic Effects: A Review. Nutrients 11, 2136. https://doi.org/10.3390/nu11092136

Boucher, J.G., Ahmed, S., Atlas, E., 2016. Bisphenol S induces adipogenesis in primary human preadipocytes from female donors. Endocrinology 157, 1397–1407. https://doi.org/10.1210/en.2015-1872

Boucher, J.G., Boudreau, A., Ahmed, S., Atlas, E., 2015. in Human and Murine Preadipocytes. Env. Heal. Perspect 123, 1287–1293.

Cowherd, R.M., Lyle, R.E., McGehee, R.E., 1999. Molecular regulation of adipocyte differentiation. Semin. Cell Dev. Biol. 10, 3–10. https://doi.org/10.1006/scdb.1998.0276

Deceuninck, Y., Bichon, E., Marchand, P., Boquien, C. Y., Legrand, A., Boscher, C., Antignac, J.P., Le Bizec, B., 2015. Determination of bisphenol A and related substitutes/analogues in human

breast milk using gas chromatography-tandem mass spectrometry. Analytical and Bioanalytical Chemistry, 407(9), 2485–2497. https://doi.org/10.1007/s00216-015-8469-9

European-Parliament, 2011. Comission Directive 2011/8/EU. Off. J. Eur. Communities 29–32.

García-Córcoles, M. T., Cipa, M., Rodríguez-Gómez, R., Rivas, A., Olea-Serrano, F., Vílchez, J. L., Zafra-Gómez, A., 2018. Determination of bisphenols with estrogenic activity in plastic packaged baby food samples using solid-liquid extraction and clean-up with dispersive sorbents followed by gas chromatography tandem mass spectrometry analysis. Talanta, 178, 441–448. https://doi.org/10.1016/j.talanta.2017.09.067

Geens, T., Aerts, D., Berthot, C., Bourguignon, J.P., Goeyens, L., Lecomte, P., Maghuin-Rogister, G., Pironnet, A.M., Pussemier, L., Scippo, M.L., Van Loco, J., Covaci, A., 2012. A review of dietary and non-dietary exposure to bisphenol-A. Food Chem. Toxicol. https://doi.org/10.1016/j.fct.2012.07.059

Ghaben, A.L., Scherer, P.E., 2019. Adipogenesis and metabolic health. Nat. Rev. Mol. Cell Biol. 20, 242–258. https://doi.org/10.1038/s41580-018-0093-z

Giulivo, M., Lopez de Alda, M., Capri, E., Barceló, D., 2016. Human exposure to endocrine disrupting compounds: Their role in reproductive systems, metabolic syndrome and breast cancer. A review. Environ. Res. 151, 251–264. https://doi.org/10.1016/j.envres.2016.07.011

González, N., Cunha, S.C., Ferreira, R., Fernandes, J.O., Nadal, M., Domingo, J.L., 2019a. Concentrations of nine bisphenol analogues in food purchased from Catalonia (Spain): Comparison of canned and non-canned foodstuffs. Food Chem. Toxicol. https://doi.org/10.1016/j.fct.2019.110992

González, N., Cunha, S.C., Monteiro, C., Fernandes, J.O., Marquès, M., Domingo, J.L., Nadal M., 2019b. Quantification of eight bisphenol analogues in blood and urine samples ofworkers in a hazardous waste incinerator Environ Res. 1-8. https://doi.org 10.1016/j.envres.2019.108576.

Ivry Del Moral, L., Le Corre, L., Poirier, H., Niot, I., Truntzer, T., Merlin, J.-F., Rouimi, P., Besnard, P., Rahmani, R., Chagnon, M.C., 2016. Obesogen effects after perinatal exposure of 4,4'-sulfonyldiphenol (Bisphenol S) in C57BL/6 mice. Toxicology 357–358, 11–20. https://doi.org/10.1016/j.tox.2016.05.023

Katsikantami, I., Karzi, V., Tzatzarakis, M. N., Sifakis, S., Xezonaki, P., Vakonaki, E. K., Rizos, A., Izotov, B., Burykina, T., & Tsatsakis, A. M., 2018. Biomonitoring of phthalate metabolites and bisphenols in amniotic fluid from pregnant women in Greece. Toxicol Lett. 295(1), S138. https://doi.org/10.1016/j.toxlet.2018.06.714

Katsikantami, I., Tzatzarakis, M. N., Karzi, V., Stavroulaki, A., Xezonaki, P., Vakonaki, E., Alegakis, A. K., Sifakis, S., Rizos, A. K., & Tsatsakis, A. M., 2020. Biomonitoring of bisphenols A and S and phthalate metabolites in hair from pregnant women in Crete. Sci Total Environ. 712, 135651. https://doi.org/ 10.1016/j.scitotenv.2019.135651

Karzi, V., Tzatzarakis, M. N., Vakonaki, E., Alegakis, T., Katsikantami, I., Sifakis, S., Rizos, A., & Tsatsakis, A. M., 2018. Biomonitoring of bisphenol A, triclosan and perfluorooctanoic acid in hair samples of children and adults. J Appl Toxicol.38(8), 1144–1152. https://doi.org/ 10.1002/jat.3627

Li, L., Chen, L., Meng, X., Chen, B., Chen, S., Zhao, Y., Zhao, L., 2013. Exposure Levels of Environmental Endocrine Disruptors in Mother-Newborn Pairs in China and Their Placental Transfer Characteristics. PLoS One 8, 1–9. https://doi.org/10.1371/journal.pone.0062526

Liu, B., Lehmler, H.J., Sun, Y., Xu, G., Sun, Q., Snetselaar, L.G., Wallace, R.B., Bao, W., 2019. Association of bisphenol A and its substitutes, bisphenol F and bisphenol S, with obesity in United States children and adolescents. Diabetes Metab. J. 43, 59–75. https://doi.org/10.4093/dmj.2018.0045

Mariscal-Arcas, M., Rivas, A., Granada, A., Monteagudo, C., Murcia, M.A., Olea-Serrano, F., 2009. Dietary exposure assessment of pregnant women to bisphenol-A from cans and microwave containers in Southern Spain. Food Chem. Toxicol. https://doi.org/10.1016/j.fct.2008.12.011

Martínez, M.A., Castro, I., Rovira, J., Ares, S., Rodríguez, J.M., Cunha, S.C., Casal, S., Fernandes, J.O., Schuhmacher, M., Nadal, M., 2019. Early-life intake of major trace elements, bisphenol A, tetrabromobisphenol A and fatty acids: Comparing human milk and commercial infant formulas. Environ. Res. 169, 246–255. https://doi.org/10.1016/j.envres.2018.11.017

Martínez, M.A., Rovira, J., Sharma, R.P., Nadal, M., Schuhmacher, M., Kumar, V., 2018. Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure : A Catalonia (Spain) case study. Environ. Res. 166, 25–34. https://doi.org/10.1016/j.envres.2018.05.008

Matsui, S., 2008. Endocrine Disruptors. Ecotoxicology 10, 1259–1260.

Mercogliano, R., Santonicola, S., 2018. Investigation on bisphenol A levels in human milk and dairy supply chain: A review. Food Chem. Toxicol. 114, 98–107. https://doi.org/10.1016/j.fct.2018.02.021

Mornagui, B., Rezg, R., Repond, C., Pellerin, L., 2019. Effects of bisphenol S, a major substitute of bisphenol A, on neurobehavioral responses and cerebral monocarboxylate transporters expression in mice. Food Chem. Toxicol. 132, 110670.

https://doi.org/10.1016/j.fct.2019.110670

Muscogiuri, G., Barrea, L., Laudisio, D., Savastano, S., Colao, A., 2017. Obesogenic endocrine disruptors and obesity: myths and truths. Arch. Toxicol. 91, 3469–3475. https://doi.org/10.1007/s00204-017-2071-1

Myridakis, A., Chalkiadaki, G., Fotou, M., Kogevinas, M., Chatzi, L., Stephanou, E.G., 2016. Exposure of Preschool-Age Greek Children (RHEA Cohort) to Bisphenol A, Parabens, Phthalates, and Organophosphates. Environ. Sci. Technol. 50, 932–941. https://doi.org/10.1021/acs.est.5b03736

Otaka, H., Yasuhara, A., Morita, M., 2003. Determination of Bisphenol A and 4-Nonylphenol in Human Milk Using Alkaline Digestion and Cleanup by Solid-Phase Extraction. Anal. Sci. 19, 1663–1666. https://doi.org/10.2116/analsci.19.1663

Pal, S., Sarkar, K., Nath, P. P., Mondal, M., Khatun, A., & Paul, G., 2017. Bisphenol S impairs blood functions and induces cardiovascular risks in rats. Toxicol Rep. 4, 560–565. https://doi.org/10.1016/j.toxrep.2017.10.006

Thomson, B.M., Grounds, P.R., 2005. Bisphenol A in canned foods in New Zealand: an exposure assessment. Food Addit. Contam. 22, 65–72. https://doi.org/10.1080/02652030400027920

van Leeuwen, S.P., Bovee, T.F., Awchi, M., Klijnstra, M.D., Hamers, A.R., Hoogenboom, R.L., Portier, L., Gerssen, A., 2019. BPA, BADGE and analogues: A new multi-analyte LC-ESI-MS/MS method for their determination and their in vitro (anti)estrogenic and (anti)androgenic properties. Chemosphere 221, 246–253. https://doi.org/10.1016/j.chemosphere.2018.12.189

Veiga-Lopez, A., Pu, Y., Gingrich, J., Padmanabhan, V., 2018. Obesogenic Endocrine Disrupting Chemicals: Identifying Knowledge Gaps. Trends Endocrinol. Metab. 29, 607–625. https://doi.org/10.1016/j.tem.2018.06.003

Verbanck, M., Canouil, M., Leloire, A., Dhennin, V., Coumoul, X., Yengo, L., Froguel, P., Poulain-Godefroy, O., 2017. Low-dose exposure to bisphenols A, F and S of human primary adipocyte impacts coding and non-coding RNA profiles. PLoS One 12, 1–20. https://doi.org/10.1371/journal.pone.0179583

Weihrauch-Blüher, S., Wiegand, S., 2018. Risk Factors and Implications of Childhood Obesity. Curr. Obes. Rep. 7, 254–259. https://doi.org/10.1007/s13679-018-0320-0

WHO, 2012. Endocrine disrupters and child health. Possible developmental early effects of endocrine disrupters on child health. World Health Organization, Geneva.

WHO, 2017. Obesity Update 2017. Der Diabetol.1–11. https://oecd.org/health/obesity-

update.htm© OECD 2017

Zhang, H.H., Huang, J., Düvel, K., Boback, B., Wu, S., Squillance, R.M., Wu, C.L., Manning, B.D., 2009. Insulin stimulates adipogenesis through the Akt-TSC2-mTORC1 pathway. PLoS One 4. https://doi.org/10.1371/journal.pone.0006189

OVERALL DISCUSSION

OVERALL DISCUSSION

In the present thesis, exposure of pregnant woman and their babies to different widespread EDs, mostly present in food-packaging or food contact materials, was evaluated. Subsequently, the endocrine potential activity of some of these EDs was determined. The EDs selected were BPA, and its analogues, BPS and BPF; phthalate and non-phthalate plasticizers; PFAS, specifically PFOS and PFOA, and TBBPA. These EDs are considered as a particularly high concern for human health (Fromme et al., 2009; Groh et al., 2019; Muncke, 2011; Vandermeersch et al., 2015). In addition, these EDs, present in food-packaging or food contact materials, may be a source of contamination via migration into food and beverage (EFSA 2006). Furthermore, these EDs were selected due to their capacity to cross the placental barrier and reach the fetus (Kawamoto et al., 2007; Li et al., 2013). The placenta does not act as an impermeable biological barrier for these chemicals (Gómara et al., 2007; Kim et al., 2011; Li et al., 2013; Ribeiro et al., 2017). Finally, pregnancy, prenatal and early postnatal stages are very critical windows of exposure to EDs, due to organs are developing and the endocrine system mechanisms are not yet mature (EP, 2019). Moreover, it is known that during pregnancy, not only the mother and the fetus are exposed, even the next generation via the germinal cells (EP, 2019).

Human exposure was assessed through two different approaches. On the one hand, it has used the method based on personal data through questionnaires and personal interviews. These questionnaires were originally designed to assess the whole pregnancy including the last period until birth. Information of participants in the study such as food frequency intake, PCPs application among others, were collected. Furthermore, it has combined all of the information mentioned with the levels of the selected EDs in dietary and non-dietary sources from the literature. On the other hand, biomonitored data in different biological matrices were used to calculate the reconstructed maternal exposure. Both approaches, the estimated, based on personal and habits data, and the reconstructed, based on biomonitored data were also combined with PBPK modelling in order to calculate the

exposure kinetics. All the exposure routes were assessed including oral, dermal, inhalation and non-dietary ingestion such as dust and some PCPs.

In Chapter 1, exposure to two high distributed EDs, BPA and DEHP from the EXHES Tarragona, Spain, cohort of pregnant women and their babies were calculated. Dietary and non-dietary routes (chapter 1, publications 1 and 2) were considered. The method based on data obtained from questionnaires and personal interviews was used. Subsequently, the contribution of each item to the total exposure for the EXHES cohort was evaluated in a probabilistic way using a Monte-Carlo simulation to be able to introduce variability and uncertainty to the different parameters considered.

Results showed that diet had the highest contribution to total exposure with >99.9% for BPA and 63% for DEHP, respectively. The major contributors to the total dietary intake of BPA were canned fruits and vegetables, followed by canned meat and meat products. This is due to BPA is an industrial compound used extensively to produce synthetic polymers, such as epoxy resins, which are incorporated into the inner coating of metal cans (Geens et al., 2012). In turn, milk and dairy products, followed by ready to eat food, were the most important contributors to the total dietary intake of DEHP. It is known that DEHP contamination of milk and dairy products occurs in several stages: contaminated DEHP feed, mechanical milking process, and migration from packaging material used in milk and dairy products (Fierens et al., 2013). It has been found a strong correlation between fast food intake and phthalates exposure but not with BPA exposure. This is due to DEHP is a widely used plasticizer to render poly (vinyl chloride) (PVC) soft and malleable and PVC is used, among others, in food wrapping (Erythropel et al., 2014). This evidence coincides with another study from the USA (Zota et al., 2016).

Although diet was considered the primary source of exposure to BPA and phthalates, it must be taken into account that with non-dietary sources (dermal, dust ingestion, and inhalation exposure) the first-pass metabolism is lacking (Lu et al., 2017; Völkel et al., 2002; Von Goetz et al., 2017). Therefore, these sources may be of equal or even higher toxicological relevance than dietary sources. BPA is conjugated in the liver by glucuronidation and

sulfation (from dietary sources); "total BPA" stands for the sum of conjugated and unconjugated forms. The unconjugated BPA (from non-dietary sources) is more toxicologically relevant (Vom Saal and Welshons, 2014).

However, with this method based on data obtained from questionnaires and interviews is possible to underestimate the exposure due to a lack of knowledge of all possible sources that would also contribute to total exposure (Angerer et al., 2006, 2007; Pirkle et al., 1995). Consequently, another possible approach for investigating human exposure to different chemicals could be to reconstruct exposure through biomonitoring. As biomonitoring data integrates all routes (oral, inhalation, dermal and dust ingestion) and sources of exposure (i.e. including occupational, environmental and lifestyle factors such as diet, smoking), it can provide valuable perspective to help evaluate aggregate exposure to chemicals (Angerer et al., 2006, 2007; Pirkle et al., 1995).

Human biomonitoring can be an effective tool for assessing exposure i.e. measuring chemicals and their metabolites, usually in blood or urine and then use this data to reconstruct the exposure amount using either a simple approach or reverse dosimetry. For that reason, the reconstructed exposure to DEHP, the most abundant parent/metabolite compound from the urine of the EXHES cohort (at first and third trimester of pregnancy) was calculated. In addition, thirteen different phthalate metabolites and two metabolites of DINCH were also determined in urine (chapter 2, publication 3). Surprisingly, they were detected in almost all (94 %) pregnant women's urine samples. Afterward, comparisons with other studies over the world were made. Our results were in the same line as other studies from Spain, Europe and the rest of the world (Casas et al., 2016; Koch et al., 2016; Machtinger et al., 2018; Van t' Evre et al., 2019). However, it is important to clarify that it is not usual to find articles with more than three or four metabolites measured in urine, and this was also more complicated regarding only those who consider pregnant women's urine.

No significant differences ($p>0.05$) were found in phthalate and DINCH metabolites according to maternal characteristics or lifestyle habits. However, lower birth weight was related to higher levels of DINCH metabolites in pregnant urine. To the best of our

knowledge, there was no information in the literature about DINCH metabolites and weight at delivery. Meanwhile, some studies affirm that higher levels of phthalates in pregnant women are related to lower weight at birth (Song et al., 2018; Zhao et al., 2014).

Both approaches, the estimated and the reconstructed, were combined with PBPK modelling to predict the amount of BPA and MEHP (DEHP metabolite) that can reach the fetus. These two EDs are fast elimination chemicals, with a half-life of fewer than 6 hours and between 3 and 18 h, respectively for BPA and DEHP, and complete elimination within 24 hours in adult (mother) (Johns et al., 2015; Völkel et al., 2002). The elimination of BPA and MEHP in the fetuses is slower than mothers as the fetal metabolic activity is lower comparing mother's metabolism (Gauderat et al., 2016; Latini et al., 2003). Subsequently, estimated exposure (chapter 1, publications 1 and 2) was compared with the reconstructed exposure using MEHP as a case study (chapter 2, publication 3). Results indicated that both values were close to each other, in the same order of magnitude. However, the PBPK simulated MEHP cumulative amount for reconstructed dose was closer to the extrapolated MEHP cumulative concentration (24 h biomonitoring data). Overall, results showed that BPA and MEHP stay longer in the fetal body, which may cause a higher risk to fetuses and makes the fetus more vulnerable to the exposure compared with the mothers. EDs exposure during these vulnerable periods can induce long-lasting changes, with adverse effects in the short and long terms; some of these effects are expected at very low doses (EP, 2019). Consequently, PBPK results from dose reconstruction were more accurate compared to the previously estimated PBPK-simulation. These results suggest that it is possible to estimate the exposure of a chemical from the concentration of their metabolites in spot urine. In addition, these results from reconstructed biomonitoring data are more close to a real exposure scenario than the results from the method based on questionnaires and personal interviews.

EFSA established a TDI of 4 $\mu g/kg_{bw}$/day for BPA (EFSA, 2015). On the other hand, ECHA established the TDI for DEHP to 50 $\mu g/kg_{bw}$/day (ECHA, 2010). Our values for BPA and DEHP estimated and reconstructed in our study were far away from the tolerable values of the

EFSA and ECHA. Although we were under the tolerable established, it is important to take into account that all sources (dietary and non-dietary) must be added to the total exposure assessment, in order to make an accurate exposure estimation. For that reason, a revision to establish a tolerable daily exposure is needed, including all routes of exposure. Currently, EFSA is undergoing a re-evaluation of the TDI for the BPA along with its exposure effects to the human population which is expected to be delivered in 2020.

The methodology applied in chapters 1 and 2 to assess the estimated exposure was used again for another ED family, the PFASs, a group of synthetic chemicals, which include PFOS and PFOA (chapter 2, publication 4). In addition, to verify the accuracy of this method, the estimated exposure was compared with biomonitoring data for the same cohort. PFASs have been detected in a number of environmental matrices, including air, dust and water (Fromme et al., 2009). PFASs are highly resistant to degradation in the environment and tend to bioaccumulate in living organisms including humans (Butt et al, 2010; Kannan et al., 2005; Kärrman et al., 2010; Letcher et al., 2010; Morales et al., 2012; Suja et al., 2009). Accordingly, the method based on questionnaires and personal interviews were applied, it was considered, among others, the intake of PFOS and PFOA through food and drinking water consumption for this cohort, while the potential contribution of indoor dust ingestion and air inhalation were also calculated according to the activity profile of these pregnant women. In agreement with many other studies (Domingo and Nadal, 2017; Ericson et al., 2008; Sungur, 2018), results showed that similar to BPA and DEHP, dietary intake was the main route of exposure to PFOS (>99%) and to PFOA (>96%). The consumption of fish and seafood was the main contributor to PFOS intake, and the second contributor to PFOA, after milk and dairy products. These results are in line with other national and international studies (Domingo and Nadal, 2017; Manzano-Salgado et al., 2016; Sungur, 2018).

Concerning the prenatal exposure, the placental barrier is not impermeable to PFOS and PFOA (Liu et al., 2011; Kim et al., 2011). Furthermore, these toxic substances can be transferred from mother to child during breastfeeding (Kärrman et al., 2010; Motas Guzmàn et al., 2016). Pregnancy PBPK models of PFOA and PFOS were adapted and used to simulate

and estimate mother and fetus internal exposure. The model simulation results obtained for plasma were compared with biomonitored data from the same cohort. To achieve this goal, PFOA and PFOS were biomonitored in maternal blood during the first trimester of pregnancy, at delivery and in cord blood. It was detected traces of PFOS in all samples. PFOA and PFOS plasma levels in the first trimester (0.45 and 2.93 ng/mL, respectively) were significantly higher ($p<0.001$) than those at delivery (0.13 and 2.21 ng/mL, respectively) and in cord blood (0.12 and 1.17 ng/mL, respectively). Decreases of 69% and 25% in PFOA and PFOS plasma levels, respectively, between the first trimester and at delivery were registered. In addition, around 70% and 60% of maternal plasma levels in the first trimester were found in cord blood for PFOA and PFOS, respectively. Our results are in agreement with others studies from the scientific literature, where a decreasing trend during pregnancy has also been reported (Glynn et al. 2012; Kato et al. 2014). This could be due the fact that there is a transplacental transfer and a dilution process caused by an increase in total blood volume (Caserta et al. 2018; Mitro et al., 2015; Glynn et al. 2012).

Modelled levels were almost in line with those obtained by biomonitoring. The estimated mean plasma concentrations for the mother in the first trimester of pregnancy of PFOA and PFOS were 0.34 and 3.14 ng/mL, respectively. Estimated mean plasma concentrations for the fetus during the first trimester of pregnancy (6-12th GW) were 0.33 and 1.39 ng/mL, respectively. At delivery, the estimated mean plasma concentrations of PFOA and PFOS for the mother were 0.33 ng/mL and 2.99 ng/mL, respectively. And for the fetus (38-39th GW), they were 0.32 ng/mL and 1.34 ng/mL, respectively. In general terms, modelled levels were always a little higher than those obtained by biomonitoring. This could be due to the fact that data used for exposure assessment were obtained from studies performed in the study area some years ago (Ericson et al., 2008; Domingo, 2012). It is well known that levels of PFOS and PFOA have decreased in food during the last years (Johansson et al. 2014). In addition, the use of food frequency questionnaires (FFQ) and food ratios from the literature instead of dietary records could not be the best accurate way. Despite this, the PBPK model was able to validate analytical data from biomonitoring samples, even with a small cohort

population. However, assumptions for exposure scenarios and intake assessment were simplistic with high uncertainty, meaning this issue needs a clear improvement. Moreover, the performance of the PBPK model can be further improved by introducing temporal dynamics of exposure concentration and physiological parameters for long-term exposure. Dietary exposures of PFOA in our study ($1.4 \cdot 10^{-4}$ µg/(kg·day)) were close to, but below, the provisional TDI set by EFSA ($1.86 \cdot 10^{-3}$ µg/(kg·day)). In turn, the dietary intake of PFOS was calculated to be well above ($3.4 \cdot 10^{-3}$ µg/(kg·day)) the most updated provisional TDI set by EFSA ($8.6 \cdot 10^{-4}$ µg/(kg·day)). Therefore, more studies regarding the exposure of PFOS are needed. Where appropriate, the EFSA should carried out a reassessment of the PFOS exposure values.

Apart from estimating the prenatal exposure to EDs (publications 1 to 4), the exposure to BPA and TBBPA during the early stages of life through breast and infant formula milk (chapter 2, publication 5) was established. Three periods of exposure were considered: (a) <1-month-old; (b) between 1- and 6-month-old; and (c) between 6 and 12-month-old infants, fed exclusively with either human milk or infant formula. To assess the exposure it was used the measured concentration levels of BPA and TBBPA in breast and formula milk and the literature data about the daily amount of milk ingested by body weight in each period previously mentioned. The study assumed similar milk intakes between the breastfed and the formula-fed groups. The presence of some pollutants like BPA, TBBPA, fatty acids and a wide spectrum of major and trace elements were analysed in human milk and infant formula. It was detected that BPA and most trace elements showed a similar decreasing trend in the different feeding period groups mentioned before (a,b and c). Such a decreasing trend may be due to the fact that the milk intake:body weight ratio decreases with age. The concentration of BPA was significantly higher in infant formula samples than in breast milk, which also contained significantly lower values of some essential elements. The fatty acid profiling also revealed major differences between human milk and infant formulas, which should be taken into account in the development of new formulas a well as in specific recommendations for the diet of breastfeeding mothers (EFSA, 2010; WHO

2008). In the case of TBBPA, only a few samples in formula milk and fewer in breast milk presented TBBPA levels above the detection limit (0.50 µg/L). These results should be taken with caution beacause the derivatization steps required for gas chromatography–mass spectrometry (GC-MS) analysis may be responsible for a low recovery rate of this compound (Covaci et al., 2009). In spite of this, our exposure values were far below the TDI threshold set by EFSA (1 mg/kg/day) for both formula-fed and breastfed infants. In any case, TBBPA concentrations were similar to those observed in other studies (Inthavong et al., 2017; Lankova et al., 2013). Results showed that the exposure to BPA was higher in formula-fed infants than in breastfed infants. However, BPA intake was far below the TDI threshold set by EFSA (4 µg/kg/day) for both formula-fed and breastfed infants. Our results on BPA exposure are in agreement with those obtained by Sarigiannis et al. (2016) for breastfed infants. But, they are lower for formula-fed infants (Sarigiannis et al., 2016). The fact that only BPA-free material was used in this study may account for the lower BPA exposure values estimated for formula-fed infants. Moreover, the presence of BPA in infant formulas could have decreased in the last years due to the general tendency of using BPA-free coatings for canned formulas (Adesman et al., 2017; Cao et al., 2015). Consequently, the results of this study reinforce that breastfeeding should be always the first feeding option in early life. WHO recommends exclusive breastfeeding up to 6 months of age with continued breastfeeding along with appropriate complementary foods up to 2 years of age or longer. The longer an infant is breastfed, the greater the protection from certain illnesses and long-term diseases. The more months or years a woman breastfeeds (combined breastfeeding of all her children), the greater the benefits to her health as well (Cosemans et al., 2020). Nevertheless, the milk quality is very important so, a good and healthy diet and lifestyle is the key to improve breast milk (Allen and Hampel, 2020).

As a final point, in this thesis, it was also evaluated the effects of the endocrine-disrupting activity. BPA and its analogues, BPS and BPF, were selected for the case study. They were selected because the EU banned the use of BPA in plastic materials that can contact with food intended for children (0–3 years) (EC, 2011). However, the principal analogues of BPA, BPS, and BPF are still being used. Then, in publication 6 (chapter 3) we wanted to clarify if

BPA has equal or different effects than its analogues, BPS and BPF, in cell toxicity and on the adipocyte differentiation process. This was assessed by *in vitro* experiments with preadipocytic 3T3-L1 cell line. The results showed that the 3T3-L1 cells treated with bisphenols caused an increase in the number of differentiated preadipocytes, as well as an increase in the capacity to store lipid droplets compared with the control. BPA was more toxic for 3T3-L1 preadipocytic cells than its analogues, BPS and BPF. Cell viability was not affected after 24 h or 48 h of the treatment with BPS or BPF. Therefore, the current results suggest that BPA has a greater toxic effect at equal and even lower concentrations than its analogues. But, BPS followed by BPF has higher endocrine-disrupting activity compared with BPA and the control. In agreement with this, various studies (Deceuninck et al., 2015; García-Córcoles et al., 2018; Van Leeuwen et al., 2019; Moon, 2019) report that the higher hormonal activity of BPS can likely be attributed to its strong polarity and the presence of a sulfonyl group. It was reported that most BPA analogues in *in vitro* studies had similar or higher estrogenic and antiandrogenic activity than BPA (Deceuninck et al., 2015; García-Córcoles et al., 2018; Van Leeuwen et al., 2019). A number of authors have reported that BPS promotes the accumulation of lipids and differentiation *in vitro* or *in vivo* (Ahmed and Atlas 2016; Boucher et al., 2015; Boucher et al., 2016). These authors also reported that treatment at the same concentration for each bisphenol (similar to the current study), BPS, produced higher adipocyte differentiation and lipid accumulation than BPA, followed by higher upregulate expression levels of different adipogenic markers including PPARγ, FABP4 and C/EBPα. Furthermore, Verbanck et al. (2017) revealed that the endocrine potency of BPS and BPF colud be in the same order of magnitude than BPA, showing similar hormonal effects *in vitro* and *in vivo*. They suggested that BPS and BPF have analogous potency and mechanisms of action to those of BPA, with similar health effects. To sum up, the *in vitro* results here obtained suggest that the replacement by these analogues could not be safe. Unfortunately, the number of available studies based on the possible effects of BPA analogues is certainly limited (Andújar et al., 2019). Further studies on these analogues would be necessary to corroborate these results, and consequently, changes in the legislation on the bisphenols' family would be necessary. Regulations on the assessment of

the safety of consumer products, especially considering the most vulnerable exposure windows, prenatal exposure and childhood, should be established. In addition, future *in vitro* experiments using mixtures of bisphenols or mixtures of different EDs will be very useful to understand the effect of synergy or antagonism of the mixture and to be more close to our real scenario.

With future studies, dealing with the current outstanding issues we could help to broaden the understanding in this endocrine disruption field and be more accurate in the regulations and legislative part.

CONCLUSIONS

CONCLUSIONS

This thesis project arises from the current problems of EDs exposure during the prenatal period and early stages of life, this exposure can cause adverse outcomes. Firstly, this thesis runs from recruitment and follow-up time of a cohort of pregnant women in the first weeks of pregnancy. Then, face-to-face questionnaires and personal interviews, sampling (blood, urine and breast milk) and analysis of different EDs in biological matrices were performed. Thirdly, to estimate the pregnant women and fetus exposure to selected EDs, the application of biological modeling tools (PBPK) was used. Finally, *in vitro* studies based on cell toxicity and adipose differentiation process by preadipocytic 3T3-L1 cell line were assessed for a well-known EDs family.

The main specific conclusions drawn from the present thesis were:

1. Diet was the main contributor to total BPA and DEHP exposure (99.9% and 63%, respectively). In the case of BPA, canned fruits and vegetables followed by canned meat and meat products were the major contributors. The non-dietary exposure to BPA practically did no contribute to the total exposure (0.22%). Regarding DEHP, milk and dairy products followed by ready to eat food were the most important contributors. Non-dietary sources contribute 37% of the total exposure. The total dietary intake estimated for BPA and DEHP was considerably lower than their respective current TDI values established by EFSA.
2. Internal dosimetry simulations showed that BPA and MEHP plasma concentrations in women were characterized by transient peaks (associated with meals). In contrast, in fetus' plasma, the concentration of both chemicals reach levels much higher than in mothers. Fetal exposure was characterized by a low but sustained basal BPA and MEHP concentrations due to a lack of metabolic activity in the fetus. Therefore, this can make the fetus more vulnerable to the exposure compared with their mothers.
3. Phthalate and DINCH metabolites were detected in almost all (94%) urine samples in both periods, first and third trimester of pregnancy. No significant differences

($p>0.05$) were found in phthalate and DINCH metabolites according to maternal characteristics or lifestyle habits. However, lower birth weight was related to higher levels of DINCH metabolites in pregnant urine.

4. Estimated vs reconstructed exposure to MEHP were compared and the values were close to each other. However, the PBPK simulated MEHP cumulative amount for reconstructed dose was closer to the extrapolated MEHP cumulative concentration (24 h biomonitoring data). In addition, it has been demonstrated that using only spot urine is possible to obtain very representative exposure values.
5. The dietary intake was again the main route of exposure to PFOS and PFOA. In addition, the consumption of fish and seafood was the main contributor to PFOS intake, and the second contributor to PFOA, after milk and dairy products. The non-dietary exposure to PFOS practically did no contribute to the total exposure (0.3%). Regarding PFOA, non-dietary sources contribute 3% of the total exposure. Dietary exposures of PFOA in our study were close to, but below, the provisional TWI set by EFSA. In turn, the dietary intake of PFOS was well above the most updated provisional TWI.
6. PFOS was found in all plasma samples in the first trimester and at delivery, and in most cord blood samples. A transplacental transfer of PFOS and PFOA of around 70% and 60%, respectively, was observed. A decreasing trend of PFOS and PFOA in plasma was found when data from the current study were compared with values obtained in the past for a similar adult population.
7. The model simulation results obtained for plasma were mostly in line with results from biomonitoring in samples for both PFOS and PFOA. PBPK model is a good tool to predict the chemicals exposure to these substances even with a small cohort population. This model was able to validate analytical data from biomonitoring samples.
8. Regarding human milk biomonitoring, the concentration of BPA was significantly higher in infant formula samples than in breast milk, which also contained significantly lower values of some essential elements. Subsequently, the exposure

to BPA was estimated to be higher in formula-fed infants than in breastfed infants. However, in both cases were lower than their respective current TDI values established by EFSA. The results of this study reinforces that human milk is the gold standard for infant nutrition during the first months of life.

9. The preadipocytic 3T3-L1 cells treated with bisphenols caused an increase in the number of differentiated cells, as well as an increase in the capacity to store lipid droplets compared with the control. Results showed that BPA was more toxic for 3T3-L1 preadepocitic cells than its analogues, BPS and BPF. However, BPS followed by BPF and finally by BPA, showed a higher positive influence over the adipogenic process at the same concentration.

As a general conclusion, the exposure to selected EDs (BPA, and its analogues, BPS and BPF; phthalate and non-phthalate plasticizers; PFAS, specifically PFOS and PFOA, and TBBPA) is a common phenomenon in adult populations and during early stages of life, such as prenatal and newborn children. This exposure can cause adverse outcomes. For that reason, more studies regarding endocrine potential activity are crucial to reevaluate the statements and the TDI exposure including dietary and non-dietary sources. Regulations based on the assessment of the safety of the most vulnerable exposure population, pregnant women and their children, should be established in the endocrine disruption context.

REFERENCES

REFERENCES

Adesman, A., Soled, D., Rosen, L., 2017. Formula Feeding as a Risk Factor for Attention-Deficit/Hyperactivity Disorder. J. Dev. Behav. Pediatr. 38, 545–551. https://doi.org/10.1097/DBP.0000000000000468.

Ahmed, S., Atlas, E., 2016. Bisphenol S- and bisphenol A-induced adipogenesis of murine preadipocytes occurs through direct peroxisome proliferator-activated receptor gamma activation. Int. J. Obes. 40, 1566–1573. https://doi.org/10.1038/ijo.2016.95

Andújar, Gálvez-Ontiveros, Zafra-Gómez, Rodrigo, Álvarez-Cubero, Aguilera, Monteagudo, Rivas, 2019. Bisphenol A Analogues in Food and Their Hormonal and Obesogenic Effects: A Review. Nutrients 11, 2136. https://doi.org/10.3390/nu11092136.

Angerer, J., Bird, M.G., Burke, T.A., Doerrer N.G., Needham L., Robison, S.H., Sheldon, L., Zenick, H., 2006. Strategic biomonitoring initiatives: moving the science forward. Toxicol Sci. 93, 3–10. https://doi.org/10.1093/toxsci/kfl042.

Angerer, J., Ewers, U., Wilhelm, M., 2007. Human biomonitoring: state of the art. Int J Hyg Environ Health. 210, 201–28. https://doi.org/10.1016/j.ijheh.2007.01.024.

Albro, P.W., Corbett, J.T., Schroeder, J.L., Jordan, S., Matthews, H.B., 1982. Pharmacokinetics, interactions with macromolecules and species differences in metabolism of DEHP. Environ Health Perspect, 45, 19-25. https://doi.org/ 10.1289/ehp.824519.

Albro, P.W., Lavenhar, S.R., 1989. Metabolism of di(2-ethylhexyl) phthalate. Drug Metab Rev, 21, 13-34. https://doi.org/10.3109/03602538909029953.

Allen, L.H., Hampel, D., 2020. Human Milk as the First Source of Micronutrients. Nestlé Nutr Inst Workshop Ser. 93, 67–76. https://doi.org/ 10.1159/000503359.

Arbuckle, T. E., Davis, K., Boylan, K., Fisher, M., Fu, J., 2016. Processed data for CHMS 2007-2009: Bisphenol A, phthalates and lead and learning and behavioral problems in Canadian children 6-19 years of age. NeuroToxicology 54, 89–98. https://doi.org/10.1016/j.dib.2016.06.017.

Bansal, A., Henao-mejia, J., Simmons, R. A., 2018. Immune System: An Emerging Player in Mediating Effects of Endocrine Disruptors on Metabolic Health. 159, 32–45. https://doi.org/10.1210/en.2017-00882.

Begley, T.H., White, K., Honigfort, P., Twaroski, M.L., Neches, R., Walker, R.A., 2005. Perfluorochemicals: Potential sources of and migration from food packaging. Food. Addit. Contam. 22, 1023-1031. https://doi.org/10.1080/02652030500183474.

Birnbaum L.S., Staskal, D.F., 2004. Brominated flame retardants: cause for concern? Environ Health Perspect 112, 9, https://doi.org/17. 10.1289/ehp.6559.

Boucher, J.G., Ahmed, S., Atlas, E., 2016. Bisphenol S induces adipogenesis in primary human preadipocytes from female donors. Endocrinology 157, 1397–1407. https://doi.org/10.1210/en.2015-1872

Boucher, J.G., Boudreau, A., Ahmed, S., Atlas, E., 2015. In Vitro Effects of Bisphenol A β-D-Glucuronide (BPA-G) on Adipogenesis in Human and Murine Preadipocytes. Env. Heal. Perspect 123, 1287–1293. https://doi.org/ 10.1289/ehp.1409143.

Bourguignon, J.P., Parent A.S., Kleinjans, C., Nawrot, T., Schoeters, G., Van Larebeke, N., 2018. Rationale for Environmental Hygiene towards global protection of fetuses and young children from adverse lifestyle factors. Environ Health, 17 (1), 42 https://doi.org/10.1186/s12940-018-0385-y.

BSEF, 2012. Tetrabromobisphenol A for printed circuit boards and ABS plastics. https ://www.bsef.com/wp-content/uploads/2015/06/Factsheet_TBBPA 25-10-2012.pdf Last accessed: 03-03-2020.

Butt, C.M., Berger, U., Bossi, R., Tomy, G.T., 2010. Levels and trends of poly- and perfluorinated compounds in the arctic environment. Sci. Total Environ. 408, 2936-2965. https://doi.org/10.1016/j.scitotenv.2010.03.015.

Cano-Nicolau, J., Vaillant, C., Pellegrini, E., Charlier, T.D., Kah, O., Coumailleau, P., 2016. Estrogenic effects of several BPA analogs in the developing zebrafish brain. Front Neurosci. 24, 10-112. https://doi.org/10.3389/fnins.2016.00112.

Cao, X.-L., Popovic, S., Arbuckle, T.E., Fraser, W.D., 2015. Determination of free and total bisphenol A in human milk samples from Canadian women using a sensitive and selective GC-MS method. Food Addit. Contam. Part A 32, 120–125. https://doi.org/10.1080/19440049.2014.980855.

Casas, M., Valvi, D., Ballesteros-Gomez, A., Gascon, M., Fernández, M.F., Garcia-Esteban, R., Iñiguez, C., Martínez, D., Murcia, M., Monfort, N., Luque, N., Rubio, S., Ventura, R., Sunyer, J., Vrijheid, M., 2016. Exposure to Bisphenol A and Phthalates during Pregnancy and Ultrasound Measures of Fetal Growth in the INMA-Sabadell Cohort. Environ. Health Perspect. 124, 521–528. https://doi.org/10.1289/ehp.1409190.

Caserta, D., Pegoraro, S., Mallozzi, M., Di Benedetto, L., Colicino, E., Lionetto, L., Simmaco, M., 2018. Maternal exposure to endocrine disruptors and placental transmission: a pilot study. Gynecol. Endocrinol. 34, 1001-1004. https://doi.org/10.1080/09513590.2018.1473362.

Cariou, R., Veyrand, B., Yamada, A., Berrebi, A., Zalko, D., Durand, S., Pollono, C., Marchand, P., Leblanc, J.-C., Antignac, J.-P., Le Bizec, B. 2015. Perfluoroalkyl acid (PFAA) levels and profiles in breast milk, maternal and cord serum of French women and their newborns. Environ. Int. 84, 71-81. https://doi.org/10.1016/j.envint.2015.07.014.

Chen, M.H., Ha, E.H., Liao, H.F., Jeng, S.F., Su, Y.N., Wen, T.W., Lien, G.W., Chen, C.Y., Hsieh, W.S., Chen, P.C., 2013. Perfluorinated compound levels in cord blood and neurodevelopment at 2 years of age. Epidemiol. 24, 800-808. https://doi.org/10.1097/EDE.0b013e3182a6dd46.

Cohn, B.A., Wolff, M.S., Cirillo, P.M., and Sholtz, R.I., 2007. DDT and breast cancer in young women: new data on the significance of age at exposure. Environ Health Perspect. 115 (10), 1406-14. https://doi.org/10.1289/ehp.10260.

Cosemans, C., Nawrot, T. S., Janssen, B. G., Vriens, A., Smeets, K., Baeyens, W., Bruckers, L.,Den Hond, E., Loots, I., Nelen, V., Van Larebeke, N., Schoeters, G., Martens, D., Plusquin, M., 2020. Breastfeeding predicts blood mitochondrial DNA content in adolescents. Scientific Reports, 10, 1–9. https://doi.org/10.1038/s41598-019-57276-z.

Covaci, A., Voorspoels, S., Abdallah, M.A.-E., Geens, T., Harrad, S., Law, R.J., 2009. Analytical and environmental aspects of the flame retardant tetrabromobisphenol-A and its derivatives. J. Chromatogr. A 1216, 346–363. doi:10.1016/j.chroma.2008.08.035

Cruz, G. C., Din, Z., Feri, C. D., Balaoing, A. M., Marie, E., Navidad, H. M., Winter, J., 2009. Analysis of Toxic Heavy Metals (Arsenic , Lead , and Mercury) in Selected Infant Formula Milk Commercially Available in the Philippines By Aas. *International Scientific Research Journal International Scientific Research Journal*, *1*(7600), 40–51. https://pdfs.semanticscholar.org/5aaa/f74e939ebd6c8f25773eb2e387de56d54f8d.pdf.

Daston, G.P., Cook, J. C., Kavlock, R. J., 2003. Systemic administration of Bordetella pertussis enhances pulmonary sensitization to house dust mite in juvenile rats. Toxicological Sciences, 72 (1), 113–121. https://doi.org/10.1093/toxsci/kfg015.

Deceuninck, Y., Bichon, E., Marchand, P., Boquien, C. Y., Legrand, A., Boscher, C., Antignac, J.P., Le Bizec, B., 2015. Determination of bisphenol A and related substitutes/analogues in human breast milk using gas chromatography-tandem mass spectrometry. Analytical and Bioanalytical Chemistry, 407(9), 2485–2497. https://doi.org/10.1007/s00216-015-8469-9.

Domingo, J.L., 2012. Health risks of dietary exposure to perfluorinated compounds. Environ. Int. 40, 187-195. https://doi.org/10.1016/j.envint.2011.08.001.

Domingo, J.L., Nadal, M., 2017. Per- and polyfluoroalkyl substances (PFASs) in food and human

dietary intake: A review of the recent scientific literature. J. Agric. Food. Chem. 65, 533-543. https://doi.org/10.1021/acs.jafc.6b04683.

EC,1999. European Comision. Community Strategy for Endocrine Disruptors. A range of substances suspected of interfering with the hormone systems of humans and wildlife. Brussels, 17.12.1999-706. Avaiable from: https://ec.europa.eu/environment/chemicals/endocrine/documents/index_en.htm#SubThemes2. Last accessed: 03-03-2020.

EC, 2016. European Comission. Defining criteria for identifying endocrine disruptors in the context of the implementation of the plant protection products regulation and biocidal products regulation. Brussels, 15.6.2016 SWD(2016) 211 final PART 1/16.

EC, 2011. European Comission. Union Guidance on Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food as regards information in the supply chain Brussels, 28.11.2013. Avaiable from: http://www.contactalimentaire.com/index.php?id=104&L=1&tx_ttnews[pointer]=2&tx_ttnews[tt_news]=825&tx_ttnews[backPid]=103&cHash=a91e577fa3. Last accessed: 03-03-2020.

ECHA, 2010. Review of new available information for Bis (2-Ethylhexyl) Phthalate (DEHP). Evaluation of new scientific evidence concerning the restrictions contained in Annex XVII to Regulation (Ec) No 1907/2006 (Reach),1–24. Avaiable from: http://www.echa.europa.eu/doc/reach/restrictions/dehp_echa_review_report_2010_6.pdf. Last accessed: 03-03-2020.

EFSA, 2015 Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs : Executive summary. EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids 13, 3978. https://doi.org/doi:10.2903/j.efsa.2015.3978.

EFSA, 2008. Opinion of the Scientific Panel on Contaminants in the Food chain on Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. *EFSA Journal,* 653, 1-131. Avaiable from: https://dare.uva.nl/search?identifier=418b8429-32dc-4a01-a735-e38d39a709be Last accessed: 03-03-2020.

EFSA, 2006. Opinion of the Scientific Panel on food additives, flavorings, processing aids and materials in contact with food (SFC). *EFSA Journal* 395, 1-221. Avaiable from: http://www.efsa.europa.eu/en/scdocs/doc/395.pdfpdf iconexternal icon. Last accessed: 03-03-2020.

EFSA, 2018. Risk to human health related to the presence of perfluorooctane sulfonic acid and

perfluorooctanoic acid in food. *EFSA Journal*, 16, 1-284. https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2018.5194

EFSA, 2011. Scientific Opinion on Tetrabromobisphenol A (TBBPA) and its derivatives in food. *EFSA Journal*, *9* (12), 1-67. https://doi.org/10.2903/j.efsa.2011.2477.

EFSA, 2010. Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. Panel on Dietetic Products, Nutrition and Allergies. EFSA J. 8, 1461. https://doi.org/10.2903/j.efsa.2010.1461.

EFSA, ECHA, 2017. Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. *EFSA Journal,* (528), 135. Avaiable from: https://www.efsa.europa.eu/en/press/news/171207. Last accessed: 03-03-2020.

EPA, 1998. Endocrine Disruptor Screening Program (EDSP). Fed Reg 53(154):42852–42855. Avaiable from: https://www.gpo.gov/fdsys/pkg/FR-1998-08-11/pdf/98- 21522.pdf. Last accessed: 03-03-2020.

EP, 2019. Endocrine Disruptors: from Scientific Evidence to Human Health Protection Policy Department for Citizens' Rights and Constitutional Affairs. *ISBN 978-92-846-4671-5*, 132. https://doi.org/10.2861/802173 | QA-01-19-272-EN-N.

EP, 2009. Regulation (EC) No 1107/2009. Concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC THE. *Official Journal of the European Union*, 1–50. Avaiable from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32009R1107. Last accessed: 03-03-2020.

EP, 2012. Regulation (EU) No 528/2012. Concerning the making available on the market and use of biocidal products. *Official Journal of the European Union*, 1–123. Avaiable from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32012R0528. Last accessed: 03-03-2020.

Ericson, I., Gómez, M., Nadal, M., van Bavel, B., Lindström, G., Domingo, J.L., 2007. Perfluorinated chemicals in blood of residents in Catalonia (Spain) in relation to age and gender: A pilot study. Environ. 33, 616-623. https://doi.org/10.1016/j.envint.2007.01.003.

Ericson, I., Nadal, M., van Bavel, B., Lindström, G., Domingo, J.L., 2012. Per- and polyfluorinated compounds (PFCs) in house dust and indoor air in Catalonia, Spain: Implications for human exposure. Environ. Int. 39, 172-180. https://doi.org/10.1016/j.envint.2011.09.004.

Erythropel, H., Maric, M., Nicell, J., Least, R., Yargeau, V., 2014. Leaching of the plasticizer di(2-ethylhexyl)phthalate (DEHP) from plastic containers and the question of human exposure.

Applied Microbiology and Biotechnology, 98 (24), 9967–9981. https://doi.org/10.1007/s00253-014-6183-8.

Fierens, T., Van Holderbeke, M., Willems, H., De Henauw, S., Sioen, I., 2013. Transfer of eight phthalates through the milk chain — A case study. Environ. Int. 51, 1–7. https://doi.org/10.1016/j.envint.2012.10.002

Fromme, H., Schutze, A., Lahrz, T., Kraft, M., Fembacher, L., Siewering, S., Burkardt, R., Dietrich, S., Koch, H.M., Volkel, W., 2016. Non-phthalate plasticizers in German daycare centrs and human biomonitoring of DINCH metabolites in children attending the centers (LUPE3). Int J Hyg Environ Health, 219, 33-9. https://doi.org/10.1016/j.ijheh.2015.08.002.

Fromme, H., Tittlemier, S.A., Völkel, W., Wilhelm, M., Twardella, D., 2009. Perfluorinated compounds -Exposure assessment for the general population in western countries. Int. J. Hyg. Environ. Health 212, 239-270. https://doi.org/10.1016/j.ijheh.2008.04.007.

Gauderat, G., Picard-Hagen, N., Toutain, P.L., Corbel, T., Viguié, C., Puel, S., Lacroix, M.Z., Mindeguia, P., Bousquet-Melou, A., Gayrard, V., 2016. Bisphenol A glucuronide deconjugation is a determining factor of fetal exposure to bisphenol A. Environ. Int. 86, 52–59. https://doi.org/10.1016/j.envint.2015.10.006.

García-Córcoles, M. T., Cipa, M., Rodríguez-Gómez, R., Rivas, A., Olea-Serrano, F., Vílchez, J. L., Zafra-Gómez, A., 2018. Determination of bisphenols with estrogenic activity in plastic packaged baby food samples using solid-liquid extraction and clean-up with dispersive sorbents followed by gas chromatography tandem mass spectrometry analysis. Talanta, 178, 441–448. https://doi.org/10.1016/j.talanta.2017.09.067.

Geens, T., Aerts, D., Berthot, C., Bourguignon, J.P., Goeyens, L., Lecomte, P., Maghuin-Rogister, G., Pironnet, A.M., Pussemier, L., Scippo, M.L., Van Loco, J., Covaci, A., 2012. A review of dietary and non-dietary exposure to bisphenol-A. Food Chem. Toxicol. 50, 3725-3740. https://doi.org/10.1016/j.fct.2012.07.059.

Geens, T., Roosens, L., Neels, H., Covaci, A., 2009. Assessment of human exposure to Bisphenol-A, Triclosan and Tetrabromobisphenol-A through indoor dust intake in Belgium. Chemosphere 76, 755–760. https://doi.org/10.1016/j.chemosphere.2009.05.024.

Genuis, S. J., Beesoon, S., Lobo, R. a., Birkholz, D., 2012. Human Elimination of Phthalate Compounds: Blood, Urine, and Sweat (BUS) Study. *The Scientific World Journal*, 1, 10. https://doi.org/10.1100/2012/615068.

Giovanoulis, G., Bui, T., Xu, F., Papadopoulou, E., Padilla-Sanchez, J.A., Covaci, A., Haug, L.S., Cousins,

A.P., Magnér, J., Cousins, I.T., de Wit, C.A., 2018. Multi-pathway human exposure assessment of phthalate esters and DINCH. Environ. Int. 112, 115–126. https://doi.org/10.1016/j.envint.2017.12.016.

Giulivo, M., Lopez, M., Alda, D., Capri, E., Barceló, D., 2016. Human exposure to endocrine disrupting compounds : Their role in reproductive systems, metabolic syndrome and breast cancer. A review. *Environmental Research*, *151*, 251–264. https://doi.org/10.1016/j.envres.2016.07.011.

Gobas, F., Otton, V., Tupper-Ring, L., Crawford, M., Clark, K., Ikonomou, M., 2016. Chemical activity-based environmental risk analysis of the plasticizer p di-ethylhexyl phthalate and its main metabolite mono-ethylhexyl phthalate. *Environmental Toxicology and Chemistry*, 1-31. https://doi.org/10.1002/etc.3689.

Gómara B., Herrero, L., Ramos, J.J., Mateo, J.R., Ferna, M.A., Gonza, M.J., 2007. Distribution of Polybrominated Diphenyl Ethers in Human Umbilical Cord Serum , Paternal Serum , Maternal Serum, Placentas, and Breast Milk from Madrid Population, Spain. Environ. Sci. Technol. 41, 6961–6968. https://doi.org/10.1021/es0714484.

Glynn, A., Berger, U., Bignert, A., Ullah, S., Aune, M., Lignell, S., Darnerud, P.O. 2012. Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: Serial sampling during pregnancy and nursing, and temporal trends 1996-2010. Environ. Sci. Technol. 46, 9071-9079. https://doi.org/10.1021/es301168c.

Grinbaum, M., Camponovo, A., Desseigne, J.-M., Poupault, P., Meisterman, E., Chatelet, B., Lempereur, V., 2019. Phthalates: Potential sources and control measures. *BIO Web of Conferences*, *12*, 1-7. https://doi.org/10.1051/bioconf/20191204008.

Groh, K.J., Backhaus, T., Carney-Almroth, B., Geueke, B., Inostroza, P., Lennquist, A., Leslie, H., Maffini, N., Slunge, D., Trasande, L., Warhursth, M., Muncke, J., 2019. Overview of known plastic packaging-associated chemicals and their hazards. Sci Total Environ, 651(2), 3253-3268. https://doi.org/10.1016/j.scitotenv.2018.10.015.

Harada K, Saito N, Inoue K, Yoshinaga T, Watanabe T, Sasaki S, Kamiyama S,Koizumi A., 2004. The influence of time, sex and geographic factors on levels of perfluorooctanesulfonate and perfluorooctanoate in human serum over the last 25 years. J Occup Health, 46 (2),141-147. https://doi.org/10.1539/joh.46.141.

Hays, S. M., & Kirman, C. R., 2019. Biomonitoring Equivalents (BEs) for tetrabromobisphenol A. *Regulatory Toxicology and Pharmacology*, *102*, 108–114. https://doi.org/10.1016/j.yrtph.2018.12.014.

Heindel, J., Vandenberg, L., 2015. Developmental origins of health and disease: a paradigm for understanding disease cause and prevention. Curr Opin Pediatr, 27 (2), 248-53. https://doi.org/10.1097/MOP.0000000000000191.

Hiroi, H., Tsutsumi, O., Momoeda, M., Takai, Y., Osuga, Y., Taketani, Y., 1999. Differential interactions of bisphenol A and 17beta-estradiol with estrogen receptor alpha (ERalpha) and ERbeta. Endocr J, 46, 773-8. https://doi.org/10.1507/endocrj.46.773.

Hutzinger, O., 1982. The handbook of environmental chemistry. In *Environmental Pollution Series A, Ecological and Biological*, 27 83-166. https://doi.org/10.1016/0143-1471(82)90111-8.

Inthavong, C., Hommet, F., Bordet, F., Rigourd, V., Guérin, T., Dragacci, S., 2017. Simultaneous liquid chromatography–tandem mass spectrometry analysis of brominated flame retardants (tetrabromobisphenol A and hexabromocyclododecane diastereoisomers) in French breast milk. Chemosphere 186, 762–769. https://doi.org/10.1016/j.chemosphere.2017.08.020.

Johansson, J.H., Berger, U., Vestergren, R., Cousins, I.T., Bignert, A., Glynn, A., Darnerud, P.O., 2014. Temporal trends (1999-2010) of perfluoroalkyl acids in commonly consumed food items. Environ. Pollut. 188, 102-108. https://doi.org/10.1016/j.envpol.2014.01.026.

Johns, L.E., Cooper, G.S., Galizia, A., Meeker, J.D., 2015. Exposure assessment issues in epidemiology studies of phthalates. Environ. Int. 85, 27–39. https://doi.org/10.1016/j.envint.2015.08.005.

Kacew, S., Hayes, A. W., 2019. Absence of neurotoxicity and lack of neurobehavioral consequences due to exposure to tetrabromobisphenol A (TBBPA) exposure in humans, animals and zebrafish. *Archives of Toxicology*, 1-8 https://doi.org/10.1007/s00204-019-02627-y.

Kannan, K., Yun, S.H., Evans, T.J., 2005. Chlorinated, brominated, and perfluorinated contaminants in livers of polar bears from Alaska. Environ Sci Technol, 39 (23), 9057-9063. https://doi.org/10.1021/es051850n.

Kärrman, A., Domingo, J.L., Llebaria, X., Nadal, M., Bigas, E., van Bavel, B., Lindström, G., 2010. Biomonitoring perfluorinated compounds in Catalonia, Spain: Concentrations and trends in human liver and milk samples. Environ. Sci. Pollut. Res. 17, 750-758. https://doi.org/10.1007/s11356-009-0178-5.

Kato, K., Wong, L.-Y., Chen, A., Dunbar, C., Webster, G.M., Lanphear, B.P., Calafat, A.M. 2014. Changes in serum concentrations of maternal poly- and perfluoroalkyl substances over the course of pregnancy and predictors of exposure in a multiethnic cohort of Cincinnati, Ohio pregnant women during 2003-2006. Environ. Sci. Technol. 48, 9600-9608. https://doi.org/10.1021/es501811k.

Kawamoto, Y., Matsuyama, W., Wada, M., Hishikawa, J., Chan, M.P.L., Nakayama, A., Morisawa, S., 2007. Development of a physiologically based pharmacokinetic model for bisphenol A in pregnant mice. Toxicol. Appl. Pharmacol. 224, 182–191. https://doi.org/10.1016/j.taap.2007.06.023

Keller, J.M., Kannan, K., Taniyasu, S., Yamashita, N., Day, R.D., Arendt, M.D., Segars, A.L., Kucklick, J.R., 2005. Perfluorinated compounds in the plasma of loggerhead and Kemp's ridley sea turtles from the southeastern coast of the United States. Environ Sci Technol, 39 (23), 9101-9108. https://doi.org/10.1021/es050690c.

Kim, S., Choi, K., Ji, K., Seo, J., Kho, Y., Park, J., Kim, S., Park, S., Hwang, I., Jeon, J., Yang, H., Giesy, J.P., 2011. Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environ. Sci. Technol. 45, 7465-7472. https://doi.org/10.1021/es202408a.

Koch, H.M., Rüther, M., Schütze, A., Conrad, A., Pälmke, C., Apel, P., Brüning, T., Kolossa-Gehring, M., 2016. Phthalate metabolites in 24-h urine samples of the German Environmental Specimen Bank (ESB) from 1988 to 2015 and a comparison with US NHANES data from 1999 to 2012. Int. J. Hyg. Environ. Health 220, 130–141. https://doi.org/10.1016/j.ijheh.2016.11.003.

Knudsen, G. A., Sanders, J. M., Sadik, A. M., Birnbaum, L. S., 2014. Disposition and kinetics of tetrabromobisphenol A in female Wistar Han rats. *Toxicology Reports*, *1*, 214–223. https://doi.org/10.1016/j.toxrep.2014.03.005.

Kurosawa, T., Hiroi, H., Tsutsumi, O., Ishikawa, T., Osuga, Y., Fuji-wara, T., Inoue, S., Muramatsu, M., Momoeda, M., Taketani, Y., 2002. The activity of bisphenol A depends on both the estrogen receptor subtype and the cell type. Endocr J 49, 465- https://doi.org/71. 10.1507/endocrj.49.465.

Kwiatkowski, C., Bolden, A., Liroff, R., Rochester,J., Vandenbergh, J., 2016. Twenty-Five Years of Endocrine Disruption Science: Remembering Theo Colborn *Environmental Health Perspectives*, 124, 151–154. http://dx.doi.org/10.1289/EHP746.

Lai, D. Y., Kacew, S., Dekant, W., 2015. Tetrabromobisphenol A (TBBPA): Possible modes of action of toxicity and carcinogenicity in rodents. *Food and Chemical Toxicology*, *80*, 206–214. https://doi.org/10.1016/j.fct.2015.03.023.

Lankova, D., Lacina, O., Pulkrabova, J., Hajslova, J., 2013. The determination of perfluoroalkyl substances, brominated flame retardants and their metabolites in human breast milk and infant formula. Talanta 117, 318–325. https://doi.org/10.1016/j.talanta.2013.08.040.

Latini, G., De Felice, C., Presta, G., Del Vecchio, A., Paris, I., Ruggieri, F., Mazzeo, P., 2003. Exposure

to Di(2-ethylhexyl)phthalate in humans during pregnancy: A preliminary report. Biol. Neonate 83, 22–24. https://doi.org/10.1159/000067012.

Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jørgensen, E.H., Sonne, C., Verreault, J., Vijayan, M.M., Gabrielsen, G.W., 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. Sci. Total Environ. 408, 2995-3043. https://doi.org/10.1016/j.scitotenv.2009.10.038.

Li, L., Chen, L., Meng, X., Chen, B., Chen, S., Zhao, Y., Zhao, L., 2013. Exposure Levels of Environmental Endocrine Disruptors in Mother-Newborn Pairs in China and Their Placental Transfer Characteristics 8, 1–9. https://doi.org/10.1371/journal.pone.0062526.

Lindstrom, A. B., Strynar, M. J., Libelo, E. L., 2011. Polyfluorinated Compounds: Past, Present, and Future. *Environmental Science & Technology*, *45*(19), 7954–7961. https://doi.org/10.1021/es2011622.

Liu, A., Zhao, Z., Qu, G., Shen, Z., Shi, J., Jiang, G., 2018. Transformation/degradation of tetrabromobisphenol A and its derivatives: A review of the metabolism and metabolites. Environ Pollut 243, 1141–1153. https://doi.org/10.1016/j.envpol.2018.09.068.

Liu, B., Lehmler, H. J., Sun, Y., Xu, G., Sun, Q., Snetselaar, L. G., Bao, W., 2019. Association of bisphenol A and its substitutes, bisphenol F and bisphenol S, with obesity in United States children and adolescents. *Diabetes and Metabolism Journal*, *43*(1), 59–75. https://doi.org/10.4093/dmj.2018.0045.

Liu, J., Li, J., Liu, Y., Chan, H.M., Zhao, Y., Cai, Z., Wu, Y., 2011. Comparison on gestation and lactation exposure of perfluorinated compounds for newborns. Environ. Int. 37, 1206-1212. https://doi.org/10.1016/j.envint.2011.05.001.

Lu, S., Yu, Y., Ren, L., Zhang, X., Liu, G., Yu, Y., 2017. Estimation of intake and uptake of bisphenols and triclosan from personal care products by dermal contact. Sci. Total Environ. 1–8. https://doi.org/10.1016/j.scitotenv.2017.10.088.

Lyngsø, J., Ramlau-Hansen, C.H., Høyer, B.B., Støvring, H., Bonde, J.P., Jönsson, B.A.G., Lindh, C.H., Pedersen, H.S., Ludwicki, J.K., Zviezdai, V., Toft, G., 2014. Menstrual cycle characteristics in fertile women from Greenland, Poland and Ukraine exposed to perfluorinated chemicals: A cross-sectional study. Hum. Reprod. 29, 359-367. https://doi.org/10.1093/humrep/det390.

Machtinger, R., Berman, T., Adir, M., Mansur, A., Baccarelli, A.A., Racowsky, C., Calafat, A.M., Hauser, R., Nahum, R., 2018. Urinary concentrations of phthalate metabolites, bisphenols and personal care product chemical biomarkers in pregnant women in Israel. Environ. Int. 116, 319–325.

https://doi.org/10.1016/j.envint.2018.04.022.

Mallozzi, M., Bordi, G., Garo, C., Caserta, D., 2016. Review The Effect of Maternal Exposure to Endocrine Disrupting Chemicals on Fetal and Neonatal Development: A Review on the Major Concerns. *Wiley Periodicals, Inc.*, 19. https://doi.org/10.1002/bdrc.21137.

Mancini, F. R., Cano-Sancho, G., Gambaretti, J., Marchand, P., Boutron-Ruault, M., Severi, G., Arveux, P., Antignac, J.P., Kvaskoff, M., 2019. Perfluorinated alkylated substances serum concentration and breast cancer risk: Evidence from a nested case-control study in the French E3N cohort. *International Journal of Cancer*, 917-928. https://doi.org/10.1002/ijc.32357.

Manzano-Salgado, C.B., Casas, M., Lopez-Espinosa, M.J., Ballester, F., Martinez, D., Ibarluzea, J., Santa-Marina, L., Vioque, J., Sunyer, J., Vrijheid, M., 2016. Variability of perfluoroalkyl substance concentrations in pregnant women by socio-demographic and dietary factors in a Spanish birth cohort. Environ. Int. 92-93, 357-365. https://doi.org/10.1016/j.envint.2016.04.004.

Martínez, M. A., Rovira, J., Sharma, R. P., Nadal, M., Schuhmacher, M., Kumar, V., 2018. Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure : A Catalonia (Spain) case study. Environmental Research, 166, 25–34. https://doi.org/10.1016/j.envres.2018.05.008.

Meng, Q., Inoue, K., Ritz, B., Olsen, J., Liew, Z. 2018. Prenatal exposure to perfluoroalkyl substances and birth outcomes; An updated analysis from the Danish National Birth Cohort. Int. J. Environ. Res. Public Health, 9, 1-15. https://doi.org/10.3390/ijerph15091832.

Mitro, S.D., Johnson, T., Zota, A.R. 2015. Cumulative Chemical Exposures During Pregnancy and Early Development. Curr. Environ. Health Rep. 2, 367-378. https://doi.org/10.1007/s40572-015-0064-x.

Moon, M. K., 2019. Concern about the safety of bisphenol a substitutes. *Diabetes and Metabolism Journal*, *43* (1), 46–48. https://doi.org/10.4093/dmj.2019.0027.

Morales, L., Martrat, M.G., Olmos, J., Parera, J., Vicente, J., Bertolero, A., Ábalos, M., Lacorte, S., Santos, F.J., Abad, E., 2012. Persistent Organic Pollutants in gull eggs of two species (Larus michahellis and Larus audouinii) from the Ebro delta Natural Park. Chemosphere 88, 1306-1316. https://doi.org/10.1016/j.chemosphere.2012.03.106.

Motas Guzmàn, M., Clementini, C., Pérez-Cárceles, M.D., Jiménez Rejón, S., Cascone, A., Martellini, T., Guerranti, C., Cincinelli, A., 2016. Perfluorinated carboxylic acids in human breast milk from Spain and estimation of infant's daily intake. Sci. Total Environ. 544, 595-600.

https://doi.org/10.1016/j.scitotenv.2015.11.059.

Muncke, J., 2011. Endocrine disrupting chemicals and other substances of concern in food contact materials: an updated review of exposure, effect and risk assessment. J Steroid Biochem Mol Biol, 127 (1-2), 118-27. https://doi.org/10.1016/j.jsbmb.2010.10.004.

Myridakis, A., Chalkiadaki, G., Fotou, M., Kogevinas, M., Chatzi, L., Stephanou, E. G. 2016. Exposure of Preschool-Age Greek Children (RHEA Cohort) to Bisphenol A, Parabens, Phthalates, and Organophosphates. *Environmental Science and Technology*, *50* (2), 932–941. https://doi.org/10.1021/acs.est.5b03736.

Nagorka, R., Conrad, A., Scheller, C., Susenbach, B., Moriske, H.J., 2011. Diiononyl 1,2-cyclohexanedicarboxylic acid (DINCH) and di(2-ethylhexyl) terephthalate (DEHT) in indoor dust samples: concentration and analytical problems. Int J Hyg Environ Health, 214, 26-35. https://doi.org/10.1016/j.ijheh.2010.08.005.

Pirkle, J.L., Sampson, E.J., Needham, L.L., Patterson, D.G., Ashley, D.L., 1995. Using biological monitoring to assess human exposure to priority toxicants. Environ Health Perspect. 103, 45–8. https://doi.org/10.1289/ehp.95103s345.

Olsen, G.W., Burris, J.M., Ehresman, D.J., Froehlich, J.W., Seacat, A.M., Butenhoff, J.L., Zobel L.R., 2007. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ Health Perspect, 115 (9), 1298-1305. https://doi.org/10.1289/ehp.10009.

Prevedouros, K., Cousins, I.T., Buck, R.C., Korzeniowski, S.H., 2006. Sources, fate and transport of perfluorocarboxylates. Environ Sci Technol, 40(1),32-44. https://doi.org/10.1021/es0512475.

Rezg, R., El-Fazaa, S., Gharbi, N., Mornagui, B., 2014. Bisphenol A and human chronic diseases: Current evidences, possible mechanisms, and future perspectives. *Environment International*, *64*, 83–90. https://doi.org/10.1016/j.envint.2013.12.007.

Ribeiro, E., Ladeira, C., Viegas, S., 2017. EDCs Mixtures: A Stealthy Hazard for Human Health? Toxics 5, (5) 2-17. https://doi.org/10.3390/toxics5010005.

Rochester, J. R., 2013. Bisphenol A and human health: A review of the literature. *Reproductive Toxicology*, *42*, 132–155. https://doi.org/10.1016/j.reprotox.2013.08.008.

Rudel, R. A., Perovich, L. J., 2009. *Endocrine disrupting chemicals in indoor and outdoor air*. Atmospheric Environment, 170–181. https://doi.org/10.1016/j.atmosenv.2008.09.025.

Sarigiannis, D.A., Karakitsios, S.P., Handakas, E., Simou, K., Solomou, E., Gotti, A., 2016. Integrated exposure and risk characterization of bisphenol-A in Europe. Food Chem. Toxicol. 98, 134–147.

doi:10.1016/j.fct.2016.10.017.

Sergeyev, O., Burns, J., Williams, P., Korrick, S., Lee, M., Revich, B., Hauser R., 2017. The association of peripubertal serum concentrations of organochlorine chemicals and blood lead with growth and pubertal development in a longitudinal cohort of boys: a review of published results from the Russian Children's Study. Rev Environ Health. 32 (1-2) 83-92. https://doi.org/10.1515/reveh-2016-0052.

Silva, M. J., Furr, J., Preau, J. L., Samandar, E., Gray, L. E., Calafat, A. M., 2012. Identification of potential biomarkers of exposure to di(isononyl)cyclohexane-1,2-dicarboxylate (DINCH), an alternative for phthalate plasticizers. *Journal of Exposure Science & Environmental Epidemiology*, *22* (2), 204–211. https://doi.org/10.1038/jes.2011.43.

Song, Q., Li, R., Zhao, Y., Zhu, Q., Xia, B., Chen, S., Zhang, Y., 2018. Evaluating effects of prenatal exposure to phthalates on neonatal birth weight: Structural equation model approaches. Chemosphere 205, 674–681. https://doi.org/10.1016/j.chemosphere.2018.04.063

Sungur, Ş., 2018. Dietary exposure to perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). Review of recent literature. Toxin Rev. 37, 106-116. https://doi.org/10.1080/15569543.2017.1346685.

Suja, F., Pramanik, B.K., Zain, S.Md., 2009. Contamination, bioaccumulation and toxic effects of perfluorinated chemicals (PFCs) in the water environment: A review paper. Water Sci. Technol. 60, 1533-1554. https://doi.org/10.2166/wst.2009.504.

Tsai, M.S., Lin, C.Y., Lin, C.C., Chen, M.H., Hsu, S.H.J., Chien, K.L., Sung, F.C., Chen, P.C., Su, T.C., 2015. Association between perfluoroalkyl substances and reproductive hormones in adolescents and young adults. Int. J. Hyg. Environ. Health, 218, 437-443. https://doi.org/10.1016/j.ijheh.2015.03.008.

Tewar, S., Auinger, P., Braun, J. M., Lanphear, B., Yolton, K., Epstein, J. N., Ehrlich S., Froehlich, T. E., 2016. Association of Bisphenol A exposure and Attention-De fi cit / Hyperactivity Disorder in a national sample of U. S. children. *Environmental Research*, 150, 112–118. https://doi.org/10.1016/j.envres.2016.05.040

Tittlemier, S.A., Pepper, K., Seymour, C., Moisey, J., Bronson, R., Cao, X.L. Dabeca R.W., 2007. Dietary exposure of Canadians to perfluorinated carboxylates and perfluorooctane sulfonate via consumption of meat, fish, fast foods, and food items prepared in their packaging. J Ag Food Chem, 55,3203-3210. https://doi.org/10.1021/jf0634045.

Vandenberg, L. N., Chahoud, I., Heindel, J. J., 2012. Urinary, circulating, and tissue biomonitoring

studies indicate widespread exposure to BPA. Cien Saude Colet, 17, 407–434. https://doi.org/10.1590/s1413-81232012000200015.

Vandermeersch, G., Lourenço, H. M., Alvarez-Muñoz, D., Cunha, S., Diogène, J., Cano-Sancho, G., Sloth, J.J., Kwadijk, C., Barcelo, D., Allegaert, W., Bekaert, K., Oliveira Fernandes, J., Marques, A., Robbens, J., 2015. Environmental contaminants of emerging concern in seafood - European database on contaminant levels. https://doi.org/10.1016/j.envres.2015.06.011.

Van Leeuwen, S.P., Bovee, T.F., Awchi, M., Klijnstra, M.D., Hamers, A.R., Hoogenboom, R.L., Portier, L., Gerssen, A., 2019. BPA, BADGE and analogues: A new multi-analyte LC-ESI-MS/MS method for their determination and their in vitro (anti)estrogenic and (anti)androgenic properties. Chemosphere 221, 246–253. https://doi.org/10.1016/j.chemosphere.2018.12.189.

Verbanck, M., Canouil, M., Leloire, A., Dhennin, V., Coumoul, X., Yengo, L., Froguel, P., Poulain-Godefroy, O., 2017. Low-dose exposure to bisphenols A, F and S of human primary adipocyte impacts coding and non-coding RNA profiles. PLoS One 12, 1–20. https://doi.org/10.1371/journal.pone.0179583.

Van 't Erve, T.J., Rosen, E.M., Barrett, E.S., Nguyen, R.H.N., Sathyanarayana, S., Milne, G.L., Calafat, A.M., Swan, S.H., Ferguson, K.K., 2019. Phthalates and Phthalate Alternatives Have Diverse Associations with Oxidative Stress and Inflammation in Pregnant Women. Environ. Sci. Technol. 53, 3258–3267. https://doi.org/10.1021/acs.est.8b05729.

Vom Saal, F. S.,Welshons, W. V., 2014. Evidence that bisphenol A (BPA) can be accurately measured without contamination in human serum and urine, and that BPA causes numerous hazards from multiple routes of exposure. *Molecular and Cellular Endocrinology*, *398* (1–2), 101–113. https://doi.org/10.1016/j.mce.2014.09.028.

Von Goetz, N., Pirow, R., Hart, A., Bradley, E., Poças, F., Arcella, D., Lillegard, I.T.L., Simoneau, C., van Engelen, J., Husoy, T., Theobald, A., Leclercq, C., 2017. Including non-dietary sources into an exposure assessment of the European Food Safety Authority: The challenge of multi-sector chemicals such as Bisphenol A. Regul. Toxicol. Pharmacol. 85, 70–78. https://doi.org/10.1016/j.yrtph.2017.02.004

Volkel, W., Colnot, T., Csanady, G.A., Filser, J.G., Dekant, W., 2002. Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. Chem Res Toxicol, 15, 1281-1287. https://doi.org/10.1021/tx025548t.

Weihrauch-Blüher, S., Wiegand, S. 2018. Risk Factors and Implications of Childhood Obesity. *Current Obesity Reports*, *7*(4), 254–259. https://doi.org/10.1007/s13679-018-0320-0.

WHO, 2018. Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Available from: http://apps.who.int/food-additives-contaminants-jecfa-database/search.aspx Last accessed: June 8th, 2018.

WHO, 2008. Interim Summary of Conclusions and Dietary Recommendations on Total Fat & Fatty Acids. From the Joint FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition, 10-14 November, 2008, WHO, Geneva. Available from: http://www.who.int/nutrition/topics/FFA_summary_rec_conclusion.pdf. Last accesed: 08-06-2018.

WHO, 2012. State of the Science: Endocrine Disrupting Chemicals*.,* ISBN: 978-92-807-3274-0 (UNEP) and 978 92 4 150503 1 (WHO) (NLM classification: *WK 102).*

WHO, 2013. State of the Science of Endocrine Disrupting Chemicals. WHO Library Cataloguing-in-Publication Data. ISBN: 978-92-807-3274-0.

Wilson, N.K., Chuang, J.C., Morgan, M.K., Lordo, R.A., Sheldon, L.S., 2007. An observational study of the potential exposures of preschool children to pentachlorophenol, bisphenol-A, and nonylphenol at home and daycare. Environ Res, 103(1), 9-20. https://doi.org/10.1016/j.envres.2006.04.006.

Wormuth, M., Scheringer, M., Vollenweider, M., & Hungerbühler, K., 2006. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? *Risk Analysis*, *26* (3), 803–824. https://doi.org/10.1111/j.1539-6924.2006.00770.x.

Zhao, Y., Chen, L., Li, L.X., Xie, C.M., Li, D., Shi, H.J., Zhang, Y.H., 2014. Gender-specific relationship between prenatal exposure to phthalates and intrauterine growth restriction. Pediatr. Res. 76, 401–408. https://doi.org/10.1038/pr.2014.103.

Zhang, H., Yolton, K., Webster, G.M., Ye, X., Calafat, A.M., Dietrich, K.N., Xu, Y., Xie, C., Braun, J.M., Lanphear, B.P., Chen, A., Chen, A., 2018. Prenatal and childhood perfluoroalkyl substances exposures and children's reading skills at ages 5 and 8 years. Environ. Int. 111, 224–231. https://doi.org/10.1016/j.envint.2017.11.031.

Zoeller, T., Brown, T. R., Doan, L. L., Gore, A. C., Skakkebaek, N. E., Soto, A. M., Woodruff, T. J., Vom F. S., 2012. Endocrine-Disrupting Chemicals and Public Health Protection: A Statement of Principles from The Endocrine Society. Endocrinology, 153, 9, 4097–4110, https://doi.org/10.1210/en.2012-1422.

ANNEX

List of publications and congress obtained during the period of the doctoral thesis:

Publicacions

Authors (signature): **M.Á. Martínez**; J. Rovira; R. Prasad Sharma; M. Nadal; M. Schuhmacher; V. Kumar.
Title: Prenatal exposure estimation of BPA and DEHP using integrated external and internal dosimetry: A case study
Journal: Environmental Research. Quartile 1. Impact Factor: 5.026, SJR: 1.567
Number or authors: 6 Position in authors' list: 01
Volume: 158 Number: --- Pages, Initial: 566 final: 575 Year: **2017**

Authors (signature): **M.Á. Martínez**; J. Rovira; R. Prasad Sharma; M. Nadal; M. Schuhmacher; V. Kumar
Title: Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure: A Catalonia (Spain) case study.
Journal: Environmental Research. Quartile 1. Impact Factor: 5.026, SJR: 1.567
Number or authors: 6 Position in authors' list: 01
Volume: 166 Number: --- Pages, Initial: 25 final: 34 Year: **2018**

Authors (signature): **M.Á. Martínez**; I. Castro; J. Rovira; S. Ares; J.M. Rodríguez; S.C. Cunha; S. Casal; J. Olivera-Fernandes; M. Schuhmacher; M. Nadal.
Title: Early-life intake of major trace elements, bisphenol A, tetrabromobisphenol A and fatty acids: Comparing human milk and commercial infant formulas.
Journal: Environmental Research. Quartile 1. Impact Factor: 5.026, SJR: 1.567
Number or authors: 10 Position in authors' list: 01
Volume: 169 Number: --- Pages, Initial: 246 final: 255 Year: **2018**

Authors (signature): J. Rovira; **M.Á. Martínez**; T. espuis; M. Nadal.; V. kumar; D. Costopoulou; I. Vassiliadou; L. Leondiadis; J.L. Domingo; M. Schuhmacher
Title: Prenatal exposure to PFOS and PFOA in a Catalan pregnant women cohort.
Journal: Environmental Research. Quartile 1. Impact Factor: 5.026, SJR: 1.567
Number or authors: 10 Position in authors' list: 02
Volume: 175 Number: --- Pages, Initial: 384 final: 392 Year: **2019**

Authors (signature): N. Bravo; S. Peralta; J. O. Grimalt; **M.Á. Martínez**; J. Rovira; M. Schuhmacher
Title: Organophosphate metabolite concentrations in maternal urine during pregnancy
Journal: Environmental Research. Quartile 1. Impact Factor: 5.026, SJR: 1.567
Number or authors: 6 Position in authors' list: 04
Volume: 182 Number: -- Pages, Initial: 1 final: 6 Year: **2019**

Authors (signature): B. Bocca; F. Ruggieri; A. Pino; J. Rovira; G. Calamandrei; F. Mirabella; **M.Á. Martínez**; J. L. Domingo; A. Alimonti; M. Schuhmacher
Title: Human biomonitoring to evaluate exposure to toxic and essential trace elements during pregnancy. Part A. concentrations in maternal blood, urine and cord blood.
Journal: Environmental Research. Quartile 1. Impact Factor: 5.026, SJR: 1.567
Number or authors: 9 Position in authors' list: 06
Volume: 177 Number: -- Pages, Initial: 1 final: 13 Year: **2019**

Authors (signature): E. Junqué; S. Garcia; **M.Á. Martínez**; J. Rovira; M. Schuhmacher, J. O. Grimalt
Title: Changes of organochlorine compound concentrations in maternal serum during pregnancy and comparison to serum cord blood composition
Journal: Environmental Research. Quartile 1. Impact Factor: 5.026, SJR: 1.567
Number or authors: 6 Position in authors' list: 03
Volume: 182 Number:-- Pages, Initial: 1 final: 9 Year: **2019**

Authors (signature): B. Bocca; F. Ruggieri; A. Pino; J. Rovira; G. Calamandrei; F. Mirabella; **M.Á. Martínez**; J. L. Domingo; A. Alimonti; M. Schuhmacher
Title: Human biomonitoring to evaluate exposure to toxic and essential trace elements during pregnancy. Part B: Predictors of exposure.
Journal: Environmental Research. Quartile 1. Impact Factor: 5.026, SJR: 1.567
Number or authors: 9 Position in authors' list: 06
Volume: 182 Number: -- Pages, Initial: 1 final: 36 Year: **2020**

Authors (signature): **M.Á. Martínez**; J. Blanco; J. Rovira; V. Kumar; J.L. Domingo; M. Schuhmacher
Title: Bisphenol A analogues (BPS and BPF) present a greater obesogenic capacity in 3T3-L1 cell line
Journal: Food and Chemical Toxicology. *In press*
Number or authors: 6 Position in authors' list: 01
Volume: Number: -- Pages, Initial: -- final: -- Year: **2020**

Authors (signature): **M.Á. Martínez;** J. Rovira; R.P.Sharma; V. Kumar; M. Schuhmacher
Title: Reconstruction of phthalate exposure and DINCH metabolites from biomonitoring data from the EXHES cohort of Tarragona, Spain: a case study on Estimated vs Reconstructed DEHP using the PBPK model
Journal: Environmental Research, In press.
Number or authors: 5 Position in authors' list: 01
Volume: Number: -- Pages, Initial: -- final: -- Year: **2020**

Authors (signature): T. Husøy; **M. Á. Martínez;** R.P. Sharma; V. Kumar; M. Andreassen; A.K. Sakhi; C.Thomsen; H. Dirven
Title: Comparison of aggregated exposure using a PBPK model to di(2-ethylhexyl) phthalate from diet and personal care products with urinary concentrations of metabolites – Results from the Norwegian biomonitoring study in EuroMix.
Journal: Food and Chemical Toxicology. Submitted
Number or authors: 8 Position in authors' list: 02
Volume: Number: -- Pages, Initial: -- final: -- Year: **2020**

Congress

Authors: **M.Á. Martínez**, J. Rovira, R.P. Sharma, V. Kumar, M. Nadal, J. L. Domingo, M. Schuhmacher.
Title: Prenatal exposure to the environmental endocrine disruptors.
Type of participation: **Poster
Conference: **8th International Conference on Children's Health and the Environment.**
Number or authors: 7 National / International: International
Organizer Entity:--
City: Barcelona Country: Spain Year: **2017**

Authors: **M.Á. Martínez**, J. Rovira, R.P. Sharma, M. Nadal, M. Schuhmacher, V. Kumar
Title: Estimación de la exposición prenatal dietética a dos disruptores endocrinos: BPA y DEHP.
Type of participation: ** Platform
Conference: **XIV Congreso Español, IV Congreso Iberoamericano de Salud Ambiental**
Number or authors: 6 National / International: National
Organizer Entity: SESA
City: Zaragoza Country: Spain Year: **2017**

Authors: **M.Á. Martínez,** J. Rovira, R. P. Sharma, M. Nadal, M. Schuhmacher, V. Kumar
Title: prenatal exposure to environmental endocrine disruptors (BPA and DEHP) through diet.
Type of participation: **Poster
Conference: **14th Doctoral Day**
Number or authors: 6 National / International: International
Organizer Entity: Rovira I Virgily University
City: Tarragona Country: Spain Year: **2017**

Authors: **M.Á. Martínez,** J. Rovira, R. P. Sharma, M. Nadal, M. Schuhmacher, V. Kumar
Title How much do dietary and non-dietary sources contribute to prenatal exposure to BPA and DEHP? A Catalonia (Spain) case study.

Type of participation: ** Platform
Conference: **15th Doctoral Day**
Number or authors: 6 National / International: International
Organizer Entity: Rovira I Virgily University
City: Tarragona Country: Spain Year: **2018**

Authors: **M.Á. Martínez**, J. Rovira, R. P. Sharma, M. Nadal, M. Schuhmacher, V. Kumar
Title: Dietary and non-dietary prenatal exposure to endocrine disruptors (BPA and DEHP). Spanish case study.
Type of participation: **Poster
Conference: **SETAC Europe 28th Annual Meeting**
Number or authors: 6 National / International: International
Organizer Entity: SETAC
City: Roma Country: Italy Year: **2018**

Authors: **M.Á. Martínez**, J. Rovira, R. P. Sharma, M. Nadal, M. Schuhmacher, V. Kumar
Title: Prediction and evaluation of the internal dosimetry of two widespread endocrine disruptors in fetus using a physiologically based pharmacokinetic model.
Type of participation: **Poster
Conference: **EUROTOX- 54th Congress of the European Societies of Toxicology**
Number or authors: 6 National / International: International
Organizer Entity: EUROTOX
City: Brussels Country: Belgium Year: **2018**

Authors: **M.Á. Martínez**, J. Rovira, V. Kumar, M. Schuhmacher
Title: Tarragona mother-child risk exposure assessment to widespread Endocrine disruptors (EDs) and in Vitro EDs exposure effects on adipogenesis.
Type of participation: **Poster corner
Conference: **8th Young Environmental Scientists Meeting**
Number or authors: 4 National / International: International
Organizer Entity: SETAC
City: Ghent Country: Belgium Year: **2019**

Authors: **M.Á. Martínez**, J. Blanco, J. Rovira, V. Kumar, M. Schuhmacher
Title: Do the principals analogues of bisphenol A have endocrine activity? In vitro case study.
Type of participation: ** Poster
Conference: **SETAC Helsinki 29th Annual Meeting**
Number or authors: 5 National / International: International
Organizer Entity: SETAC
City: Helsinki Country: Finland Year: **2019**

Authors: **M.Á. Martínez**; J. Rovira, R. P. Sharma, M. Nadal, M. Schuhmacher, V. Kumar

Title: How much do dietary and non-dietary sources contribute to prenatal exposure to BPA and DEHP? A Catalonia (Spain) case study.
Type of participation: ** Platform
Conference: **SETAC Helsinki 29th Annual Meeting**
Number or authors: 6 National / International: International
Organizer Entity: SETAC
City: Helsinki Country: Finland Year: **2019**

Authors: T. Husøy, **M.Á. Martínez**, V. Kumar, R. P. Sharma, M. Andreassen, F. Sonnet, A. KAUR SAKHI and H. Dirven
Title: Comparison of modeled internal exposures to Di(2-ethylhexyl)phthalate from foods and cosmetics with urinary metabolite concentrations – results from the EuroMix biomonitoring study.
Type of participation: ** Poster
Conference: **IUTOX 15th International Congress of Toxicology**
Number or authors: 7 National / International: International
Organizer Entity: INCHES
City: Honolulu Country: Hawaii, USA Year: **2019**

Authors: E. Junqué, S. Garcia, **M.Á. Martínez**, J. Rovira, M. Schuhmacher, J. Grimalt
Title: Changes of organochlorine compound concentrations in maternal serum during pregnancy and comparison to serum cord blood composition.
Type of participation: ** Platform
Conference: **10th International Conference on Children's Health and the Environment**
Number or authors: 6 National / International: International
Organizer Entity: INCHES
City: Barcelona Country: Spain Year: **2020**

Authors: **M.Á. Martínez**, J. Rovira, V. Kumar, J.L. Domingo, M. Schuhmacher
Title: Biomonitoring of phthalate and DINCH metabolites in a Spanish pregnant women cohort. Estimated vs Reconstructed exposure of DEHP.
Type of participation: **
Conference: **SETAC Europe 30th Annual Meeting**
Number or authors: 5 National / International: International
Organizer Entity: SETAC
City: Dublin Country: Ireland Year: **2020. Submitted**

Authors: **M.Á. Martínez**, J. Rovira, V. Kumar, M. Schuhmacher
Title: Early-life exposure to major and trace elements trough breastfeeding.
Type of participation: Poster**
Conference: **SETAC Europe 30th Annual Meeting**
Number or authors: 4 National / International: International
Organizer Entity: SETAC

City: Dublin Country: Ireland Year: **2020. Submitted**

Project(s) involved:

-Title of the project / contract: Health and Environment-wide Associations based on Large population Surveys
Type of contract/Program:--
Financing Firm/administration: UNEU – European Union
Institutions participating: ---
Number of the project / contract: 603946
Amount: 31.289.300,00
Duration, since: 2013 Until: 2018
Researcher/s in charge: SCHUHMACHER ANSUATEGUI, MARTA
Number of researchers participating: ---
Keywords:--
Code of the project / contract: 024763 Order: 001

-Title of the project / contract: HDHL-INTIMIC Call for Joint Transnational Research Proposals on "Interrelation of the Intestinal Microbiome, Diet and Health"
Type of contract/Program:--
Financing Firm/administration: 4842 - MINECO
Institutions participating: ---
Number of the project / contract: ---
Amount: 149.780,00 Duration, since: 2017 Until: ---
Researcher/s in charge: SCHUHMACHER ANSUATEGUI, MARTA
Number of researchers participating: ---
Keywords:--
Code of the project / contract: 313071 Order: 002

Grants/Awards received:

-CORBEL funding (28/9/2018): The CORBEL project support your project by giving you access to high-end facilities and service providers free of charge. Furthermore, offers financial support for your travel and accommodation costs. This travel grant is limited to 5000 Euros per project.

-EuroMix human biomonitoring - Norway (September 2018): The ongoing collaboration of UGR and NIPH on the H2020 project EuroMix included all travel expenses, accommodation and diets.

-YES travel grant (5/2/2019): The travel grant application for the 8th Young Environmental Scientists (YES) meeting at Ghent University, Belgium. This travel grant is 100€ to offset your travel costs to the meeting.

-2nd Award for the best poster in the 14th Doctoral Day (May 24, 2017) Nanoscience, Materials and Chemicals Engineering. Rovira i Virgili University.

UNIVERSITAT ROVIRA I VIRGILI
ENDOCRINE DISRUPTION ASSESSMENT: EXPOSURE, BIOMONITORING AND POTENTIAL ACTIVITY OF WIDESPREAD ENDOCRINE DISRUPTORS DURING
PREGNANCY AND EARLY STAGES OF LIFE
María Ángeles Martínez Rodríguez

UNIVERSITAT ROVIRA I VIRGILI
ENDOCRINE DISRUPTION ASSESSMENT: EXPOSURE, BIOMONITORING AND POTENTIAL ACTIVITY OF WIDESPREAD ENDOCRINE DISRUPTORS DURING
PREGNANCY AND EARLY STAGES OF LIFE
María Ángeles Martínez Rodríguez

UNIVERSITAT ROVIRA I VIRGILI
ENDOCRINE DISRUPTION ASSESSMENT: EXPOSURE, BIOMONITORING AND POTENTIAL ACTIVITY OF WIDESPREAD ENDOCRINE DISRUPTORS DURING
PREGNANCY AND EARLY STAGES OF LIFE
María Ángeles Martínez Rodríguez

UNIVERSITAT
ROVIRA i VIRGILI