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“Mención Internacional”

UNIVERSIDAD DE GRANADA

Exposición a bisfenol A: Efectos sobre la reproducción, neurodesarrollo y obesidad/metabolismo

“Exposure to bisphenol A: Effects on reproduction, neurodevelopment and obesity/metabolism”

UNIVERSIDAD DE GRANADA

FACULTAD DE MEDICINA

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Que la Tesis doctoral titulada ‘’Exposición a bisfenol A: Efectos sobre la reproducción, neurodesarrollo y obesidad/metabolismo” [“Exposure to bisphenol A: Effects on reproduction, neurodevelopment and obesity/metabolism”], de la que es autor Vicente Mustieles Miralles, ha sido realizada bajo mi dirección y asesoramiento y reúne las condiciones y calidad científica deseadas para ser presentada por el interesado para optar al grado de Doctor.

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CERTIFICA

Que la Tesis doctoral titulada ‘’Exposición a bisfenol A: Efectos sobre la reproducción, neurodesarrollo y obesidad/metabolismo” [“Exposure to bisphenol A: Effects on reproduction, neurodevelopment and obesity/metabolism”], de la que es autor Vicente Mustieles Miralles, ha sido realizada en las instalaciones del grupo de investigación Medicina Ambiental, en el Centro de Investigación Biomédica, Universidad de Granada, donde están ubicados los laboratorios del grupo de investigación CTS-206 Oncología Básica y Clínica, perteneciente a este Departamento.

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MEMORIA DE TESIS PRESENTADA POR EL DOCTORANDO

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PARA OPTAR AL GRADO DE

Doctor en Farmacia

BAJO LA DIRECCIÓN DE LA DOCTORA:

Dña. Mariana F. Fernández Cabrera

Profesora Titular

Departamento de Radiología y Medicina Física

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“El viaje hacia un futuro diferente debe comenzar definiendo el problema de un modo distinto a como lo hemos hecho hasta ahora… La tarea no es buscar sustitutos de compuestos químicos que interfieren con las hormonas, atacan la capa de ozono, o causan problemas todavía no conocidos, aunque puedan ser necesarios como una medida temporal. La tarea a hacer frente durante el próximo medio siglo es la de rediseñar.”

“The journey to a different future must begin by defining the problem differently that we have done until now… The task is not to find substitutes for chemicals that disrupt hormones, attack the ozone layer, or cause still undiscovered problems, though it may be necessary to use replacements as a temporary measure. The task that confronts us over the next half century is one of redesign.”

Theo Colborn, Dianne Dumanouski and John Peterson Myers

Our stolen future

FUNDING

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CONTENTS

SUMMARY

INTRODUCTION

Bisphenol A (BPA) is a well-known endocrine disruptor able to interfere with hormonal homeostasis even at low doses. BPA is a high-production man-made chemical used worldwide in the manufacture of polycarbonate plastics, epoxy resin liners of canned food, some dental sealants, medical devices, and thermal receipts, among many other applications. Available data from biomonitoring studies clearly indicate that the general population is ubiquitously exposed to BPA. There is also growing evidence that BPA is harmful in laboratory animals and may adversely affect human health, especially during development. BPA has become a target of current risk assessments and policy regulations, mainly due to concerns about its possible adverse effects on children's reproductive health, neurodevelopment, and obesity/metabolism. Hence, there is an urgent need to advance our knowledge on the potential effects of BPA exposure in human populations, with a special emphasis on different critical periods of development.

OBJECTIVE

The main objective of this thesis project was to investigate whether human BPA exposure is associated with adverse perinatal, childhood, and adult health outcomes, including reproductive, neurodevelopment, and metabolic health effects. For this purpose, three specific objectives were proposed:

Objective 1: Reproduction. To explore whether BPA exposure (before conception, during pregnancy, and in peripuberty) is associated with reproductive outcomes (addressed in papers #1 and #2 of the Results section).

Objective 2: Neurodevelopment. To review all the existing epidemiological literature on the association between BPA exposure and human neurobehavior, and to investigate whether environmental exposure to BPA in childhood is associated with behavior (addressed in papers #3, #4 and #5 of the Results section).

Objective 3: Obesity/Metabolism. To assess the influence of long-term exposure to mixtures of persistent organic pollutants (as well as short-term exposure to BPA) on the risk of obesity and metabolic diseases in children and adults (addressed in paper #6 of the Results section and in the Preliminary results of the Appendix 1).

MATERIALS AND METHODS

In order to achieve the aforementioned objectives, studies were conducted in three established epidemiological cohorts:

The Environment and Reproductive Health Study (EARTH): An ongoing prospective preconception cohort of subfertile couples in Boston (USA).

The Environment and Childhood Study (INMA)-Granada Cohort: A prospective mother–child cohort from Granada province, integrated in the Spanish multicenter INMA Project.

The Granada-Motril (GraMo) Cohort: A prospective cohort based on a hospital-based study of adults from Granada (Southern Spain).

RESULTS & DISCUSSION

Paper #1

Mustieles V, Williams PL, Fernandez MF, Mínguez-Alarcón L, Ford JB, Calafat AM, Hauser R, Messerlian C; Environment and Reproductive Health (EARTH) Study Team. Maternal and paternal preconception exposure to bisphenols and size at birth. *Human Reproduction* 2018; 33(8):1528-37. https://doi.org/10.1093/humrep/dey234

BPA has been classified as an ovarian toxicant based on both experimental and human evidence. We found that maternal preconception urinary BPA concentrations - but not paternal preconception BPA concentrations - were negatively associated with both birth weight and head circumference among singletons conceived by subfertile couples from a large fertility center. Maternal prenatal BPA concentrations also showed suggestive associations with birth size. Although limited by small numbers and low detection frequencies, there was also no evidence of associations with BPS concentrations across all exposure windows. Given that the strongest BPA-associations were found when maternal exposure was assessed before conception, and BPA has been shown to alter the epigenetic programming of human and mammalian oocytes leading to functional impairments, a potential early effect of BPA at the ovary affecting oocyte quality and later resulting in reduced embryo viability/development might be proposed. Although these results were overall consistent with prior studies on prenatal BPA exposure, these findings may not be directly generalizable to women without fertility concerns.

Paper #2

Mustieles V, Ocón-Hernandez O, Mínguez-Alarcón L, Dávila-Arias C, Pérez-Lobato R, Calvente I, Arrebola JP, Vela-Soria F, Rubio S, Hauser R, Olea N, Fernández MF. Bisphenol A and reproductive hormones and cortisol in peripubertal boys: The INMA-Granada cohort. *Science of the Total Environment* 2018; 618: 1046-53.
https://doi.org/10.1016/j.scitotenv.2017.09.093

Higher urinary BPA concentrations in peripubertal boys of 9–11 years of age were cross-sectionally associated with altered hormone levels, including higher serum total testosterone (TT) levels, lower serum cortisol concentrations, and higher serum TT: Luteinizing Hormone (LH) and TT:cortisol ratios. Our results pointed to the adrenal gland as an endocrine organ potentially related to the observed associations, although a possible action at the testis or pituitary cannot be ruled out. Hence, if there were a causal association of urinary BPA concentrations with higher levels of TT and lower levels of cortisol, it could be explained by a differential production of androgens/cortisol triggered by the action of BPA at the adrenal gland.

Paper #3

Mustieles V, Pérez-Lobato R, Olea N, Fernández MF. Bisphenol A: Human exposure and neurobehavior. *Neurotoxicology* 2015; 49:174-84. Review.
https://doi.org/10.1016/j.neuro.2015.06.002

In 2015, we conducted a comprehensive review of the available literature about the relationship between human BPA exposure and neurodevelopment and on proposed mechanisms of action. All scientific publications up to March 2015 were reviewed using the MEDLINE/PubMed database. Although epidemiologic research on this issue was limited at that time, eight out of the twelve available articles reported associations between BPA exposure and altered neurobehavior, including aggressive behavior, attention deficit, hyperactivity disorder, depression, and anxiety, mostly in children exposed *in utero*, indicating disruption of the brain during this critical window of development. Despite the reduced number of studies and their heterogeneity, the results suggested that prenatal BPA exposure

may have a negative impact on neurobehavioral functioning in children and that the effects may be sex-dependent.

Paper #4

Perez-Lobato R, Mustieles V, Calvente I, Jimenez-Diaz I, Ramos R, Caballero-Casero N, López-Jiménez FJ, Rubio S, Olea N, Fernández MF. Exposure to bisphenol A and behavior in school-age children. *Neurotoxicology* 2016; 53:12-9. https://doi.org/10.1016/j.neuro.2015.12.001

In 2016, results from the INMA-Granada cohort showed that higher urinary BPA concentrations at 9-11 years of age were cross-sectionally associated with higher behavior problems among boys, especially internalizing symptoms (somatic complaints, as well as thought and social problems). Our results were in line with both previous epidemiological studies and experimental studies, most of which have supported the ability of BPA to interfere with normal brain development and behavior. Although the effects of BPA on behavior appear to be more pronounced during prenatal exposure, the relevance of postnatal exposure should not be underestimated and warrants further research.

Paper #5

Mustieles V, Messerlian C, Reina I, Rodríguez-Carrillo A, Olea N, Fernández MF. Is Bisphenol A (BPA) a Threat to Children's Behavior? *Journal of Mental Health & Clinical Psychology* 2018;2(1):6-9. ISSN: 2578-2959

In 2018, we conducted a mini-review to assess whether epidemiological studies published since the first review (paper #3) confirmed the direction indicated by previous epidemiological studies. The update showed that, since March 2015, the number of human studies addressing the BPA-neurobehavior hypothesis doubled, mostly reinforcing previous prenatal associations and frequently showing differences between boys and girls. An increasing number of studies also showed an association between postnatal BPA exposure and diverse neurobehavioral impairments, including attention-deficit and hyperactivity disorder (ADHD). Overall, research data on the relationship between human BPA exposure and children's behavior has revealed a relatively consistent pattern that cannot be ignored.

Paper #6

Mustieles V, Fernández MF, Martin-Olmedo P, González-Alzaga B, Fontalba-Navas A, Hauser R, Olea N, Arrebola JP. Human adipose tissue levels of persistent organic pollutants and metabolic syndrome components: Combining a cross-sectional with a 10-year longitudinal study using a multi-pollutant approach. Environment International 2017;104: 48-57. https://doi.org/10.1016/j.envint.2017.04.002

Results from the GraMo cohort confirmed that adult populations continue to be exposed to mixtures of persistent EDCs, including those that were regulated or banned decades ago, due to their accumulation in fatty tissues. According to the results of our study, accumulated levels of some of these compounds [β-Hexachlorocyclohexane (β-HCH) in combination with Hexachlorobenzene (HCB)] were consistently associated with both the prevalence and incidence of being metabolically compromised; that is, of presenting or developing at least one out the four components of the metabolic syndrome considered (hypertension, type 2 diabetes, low HDL-cholesterol or hypertriglyceridemia). To our best knowledge, this investigation is one of the first prospective studies designed to analyze associations between POP exposure and metabolic syndrome components and is the first to combine cross-sectional with longitudinal analyses.

Preliminary results (Appendix 1)

Mustieles V et al. Bisphenol A and adiposity measures among peripubertal boys from Spain. Manuscript in preparation.

Preliminary results showed that urinary BPA concentrations in peripubertal boys at 9-11 years of age from the INMA-Granada cohort were cross-sectionally and positively associated with both BMI z-scores and the prevalence of being overweight/obese. Additionally, BPA was positively associated with the prevalence of abdominal obesity, but not with total body fat mass, suggesting a possible localized effect in visceral adipose tissue. However, these results should be interpreted with caution, because of possible reverse causality and/or confounding by dietary and lifestyle factors.

CONCLUSIONS

Conclusion 1

Our findings highlight the maternal preconception period as a sensitive but largely unexplored critical window for BPA effects on birth size. Given the ubiquity of exposure to BPA, the predictive value of size at birth for future health, and that BPA has been classified as a reproductive toxicant and endocrine disruptor based on experimental and human evidence, we consider these findings to be of public health relevance.

Conclusion 2

BPA exposure during peripuberty could affect hormonal homeostasis in 9- to 11 year-old boys, and our results are compatible with a possible involvement of BPA at the adrenal gland, resulting in a differential production of androgens/cortisol. However, these findings should be interpreted with caution, given the cross-sectional design of the study, the heterogeneous results reported in the literature, and the scant experimental research on BPA effects at the adrenal gland.

Conclusion 3

There is a strong body of experimental evidence to support that BPA, at doses of relevance for human exposure, may affect the developing brain and neurobehavioral functioning of laboratory animals, including non-human primates. Among 9-11 year-old boys from the INMA-Granada cohort, greater exposure to BPA was associated with more behavior problems, in line with a growing number of human studies. Although it may never be possible to establish a causal link between this specific endocrine disruptor and a particular neurobehavioral endpoint, research data on the relationship between human BPA exposure and children's behavior has revealed a relatively consistent pattern that cannot be ignored. The evidence as a whole supports more than ever the need to apply the precautionary principle and undertake preventive measures to reduce inadvertent exposure to BPA, especially during critical periods of development: women of child-bearing age, pregnant women and children.

Conclusion 4

Among adults from the GraMo cohort, the accumulation in adipose tissue of hexachlorobenzene (HCB) and β-hexachlorocyclohexane (β-HCH) was consistently and positively associated with the prevalence and incidence of metabolic disorders. Our results highlight that, although most persistent organic pollutants were regulated decades ago, human exposure remains relevant to public health because current generations might suffer the effects of accumulated exposure. Moreover, part of this body burden may be transferred to the offspring during gestation and breastfeeding, representing a vicious cycle that needs to be ended in order to protect future generations.

Conclusion 5

Our preliminary findings suggest that BPA exposure could exert an obesogenic effect on peripubertal boys from the INMA-Granada cohort, potentially increasing the risk of overweight and obesity. However, given the possibility of reverse causality and/or confounding by dietary habits, these preliminary results must be interpreted with due caution.

RESUMEN

INTRODUCCIÓN

Bisfenol A, (BPA – siglas en inglés –), es un compuesto químico de origen sintético, empleado de forma masiva en todo el mundo en la fabricación del plástico policarbonato, las resinas epoxi, en algunos empastes dentales, equipos médicos, o en el papel térmico, entre otras muchas aplicaciones. BPA es un disruptor endocrino ampliamente estudiado que puede interferir con la homeostasis hormonal incluso a bajas dosis. Los datos disponibles procedentes de estudios de biomonitorización indican que la exposición a BPA es ubicua y generalizada en la población. La evidencia también sugiere que esta exposición puede tener efectos adversos, tanto en animales de laboratorio como en el hombre, especialmente cuando la exposición ocurre durante el desarrollo. BPA está en el punto de mira de las evaluaciones de riesgo y se debate la regulación de su uso, principalmente debido a su posible relación con la salud reproductiva, el neurodesarrollo y la obesidad/metabolismo infantil. Es necesario, por tanto, profundizar en el conocimiento sobre los efectos potenciales de la exposición a BPA en población humana, con especial énfasis cuando su exposición ocurre en los períodos más críticos del desarrollo.

OBJETIVO

El objetivo principal de este proyecto de tesis es investigar si la exposición humana a BPA se asocia con efectos adversos al nacer, durante la infancia y en la adultez, analizando específicamente efectos sobre la reproducción, el neurodesarrollo y la obesidad/metabolismo. Para este propósito, se plantean tres objetivos específicos:

Objetivo 1: Reproducción. Explorar si la exposición a BPA (antes de la concepción, durante el embarazo y en la peri-pubertad) se relaciona con efectos sobre la salud reproductiva (artículos #1 y #2 de la sección de Resultados).

Objetivo 2: Neurodesarrollo. Revisar toda la literatura científica disponible sobre la asociación entre exposición a BPA y neurodesarrollo infantil, e investigar si la exposición a BPA durante la niñez está asociada con el comportamiento de los niños (artículos #3, #4 y #5 de la sección de Resultados).

Objetivo 3: Obesidad/Metabolismo. Evaluar la influencia de la exposición a largo plazo de mezclas de contaminantes orgánicos persistentes (así como la exposición a corto plazo a BPA),

sobre el riesgo de obesidad y enfermedades metabólicas tanto en niños como en adultos (artículo #6 de la sección de Resultados, y resultados preliminares del Apéndice 1).

MATERIAL Y MÉTODOS

A fin de abordar los objetivos planteados, se llevaron a cabo estudios en tres cohortes epidemiológicas establecidas:

El estudio Medio Ambiente y Salud Reproductiva (EARTH Study): Una cohorte prospectiva pre-concepcional de parejas subfértiles en Boston (USA).

La cohorte de Infancia y Medio Ambiente (INMA) de Granada: Una cohorte prospectiva de parejas madre-hijo de la provincia de Granada, integrada en la cohorte prospectiva española multicéntrica INMA.

La cohorte Granada-Motril (GraMo): Una cohorte prospectiva de adultos reclutados en dos hospitales de la provincia de Granada.

RESULTADOS Y DISCUSSION

Artículo #1

Mustieles V, Williams PL, Fernandez MF, Mínguez-Alarcón L, Ford JB, Calafat AM, Hauser R, Messerlian C; Environment and Reproductive Health (EARTH) Study Team. Maternal and paternal preconception exposure to bisphenols and size at birth. *Human Reproduction* 2018; 33(8):1528-37. https://doi.org/10.1093/humrep/dey234

BPA está clasificado como un tóxico ovárico en base tanto a la evidencia experimental como observacional. Nuestros resultados mostraron que, mayores concentraciones de BPA en la orina de las madres antes de la concepción, pero no la de los padres, se asociaban negativamente con el peso al nacer y el perímetro cefálico de los recién nacidos concebidos por parejas subfértiles. Las concentraciones maternas de BPA durante el embarazo también tendieron a asociarse con estos mismos efectos. Aunque limitados por el reducido tamaño de la muestra y por la baja frecuencia de detección, no se observaron asociaciones entre la exposición a bisfenol S (BPS –siglas en inglés–) y el tamaño al nacer de los hijos. Dado que las asociaciones más fuertes se encontraron cuando se evaluó la exposición materna antes de la concepción, y que BPA es capaz de alterar la programación epigenética de ovocitos humanos produciendo alteraciones funcionales, nuestros resultados avalan un posible efecto

temprano de BPA en el ovario que afectaría a la calidad de los ovocitos, y que más tarde podría resultar en una reducción de la viabilidad o desarrollo del embrión. Aunque estos hallazgos son consistentes con estudios previos sobre la exposición prenatal a BPA, podrían no ser generalizables a mujeres sin problemas de fertilidad.

Artículo #2

Mustieles V, Ocón-Hernandez O, Mínguez-Alarcón L, Dávila-Arias C, Pérez-Lobato R, Calvente I, Arrebola JP, Vela-Soria F, Rubio S, Hauser R, Olea N, Fernández MF. Bisphenol A and reproductive hormones and cortisol in peripubertal boys: The INMA-Granada cohort. *Science of the Total Environment* 2018; 618: 1046-53.
https://doi.org/10.1016/j.scitotenv.2017.09.093

Mayores concentraciones urinarias de BPA en niños peripuberales de 9-11 años se asociaron de manera transversal con niveles hormonales alterados, incluyendo mayores niveles séricos de testosterona total y menores concentraciones de cortisol, así como con mayores ratios de testosterona/hormona luteinizante y testosterona/cortisol. Estos resultados apuntan a la glándula adrenal como un órgano endocrino potencialmente relacionado con las asociaciones observadas, aunque no es posible descartar una acción de BPA en el testículo o en la glándula pituitaria. Así, si hubiera una asociación causal entre BPA y mayores niveles de testosterona y menores de cortisol, podría explicarse por una producción diferencial de andrógenos/cortisol desencadenada por la glándula adrenal en respuesta a la exposición a BPA.

Artículo #3

Mustieles V, Pérez-Lobato R, Olea N, Fernández MF. Bisphenol A: Human exposure and neurobehavior. *Neurotoxicology* 2015; 49:174-84. Review.
https://doi.org/10.1016/j.neuro.2015.06.002

En 2015 llevamos a cabo una revisión exhaustiva de la literatura disponible sobre la relación entre la exposición humana a BPA y el neurodesarrollo, así como de los mecanismos de acción propuestos. Utilizando la base de datos MEDLINE/PubMed, se revisaron todas las publicaciones científicas disponibles hasta Marzo de 2015. Aunque la investigación

epidemiológica sobre este tema era limitada en aquel momento, ocho de los doce artículos disponibles mostraban asociaciones entre la exposición a BPA y alteraciones en el comportamiento infantil, incluyendo déficit de atención, síntomas de hiperactividad, agresividad, depresión y ansiedad, principalmente en niños expuestos durante la gestación, indicando un posible efecto adverso sobre la función cerebral durante este período crítico del desarrollo. A pesar del reducido número de estudios y su heterogeneidad, los resultados sugirieron que la exposición prenatal a BPA podía tener un efecto negativo sobre el comportamiento de los niños, y que los efectos podían variar en función del sexo.

Artículo #4

Perez-Lobato R, Mustieles V, Calvente I, Jimenez-Diaz I, Ramos R, Caballero-Casero N, López-Jiménez FJ, Rubio S, Olea N, Fernández MF. Exposure to bisphenol A and behavior in school-age children. *Neurotoxicology* 2016; 53:12-9. https://doi.org/10.1016/j.neuro.2015.12.001

Los resultados del estudio de los niños de la cohorte INMA-Granada, a los 9-11 años de edad, mostraron que mayores concentraciones urinarias de BPA se asociaban transversalmente con mayores problemas de comportamiento, especialmente síntomas internalizantes (quejas somáticas, aunque también problemas sociales y de pensamiento). Estos resultados son similares a los descritos en estudios epidemiológicos y experimentales previos, la mayoría de los cuales respaldan la capacidad de BPA para interferir con un correcto desarrollo cerebral y comportamiento. Aunque los efectos de BPA sobre el comportamiento parecen ser más evidentes cuando la exposición tiene lugar durante la gestación, la relevancia de la exposición postnatal no debe subestimarse y requiere más investigación, especialmente durante el período peripuberal.

Artículo #5

Mustieles V, Messerlian C, Reina I, Rodríguez-Carrillo A, Olea N, Fernández MF. Is Bisphenol A (BPA) a Threat to Children's Behavior? *Journal of Mental Health & Clinical Psychology* 2018;2(1):6-9. ISSN: 2578-2959

En 2018 llevamos a cabo una actualización de la literatura científica (mini-revisión) con objeto de revisar si los estudios epidemiológicos publicados después de la primera revisión (artículo

#3), confirmaban o no, la dirección señalada por los estudios previos. La revisión mostró que, desde marzo de 2015, el número de estudios epidemiológicos se había duplicado, en su mayoría reforzando las asociaciones prenatales previamente descritas y mostrando frecuentemente diferencias entre niños y niñas. También reveló que un número cada vez mayor de estudios encuentra asociaciones entre la exposición posnatal a BPA y diversas alteraciones comportamentales, incluyendo el trastorno por déficit de atención e hiperactividad (TDAH). En general, los datos de investigación disponibles sobre la relación entre la exposición humana a BPA y el comportamiento de los niños revela un patrón relativamente consistente que no puede ser ignorado.

Artículo #6

Mustieles V, Fernández MF, Martin-Olmedo P, González-Alzaga B, Fontalba-Navas A, Hauser R, Olea N, Arrebola JP. Human adipose tissue levels of persistent organic pollutants and metabolic syndrome components: Combining a cross-sectional with a 10-year longitudinal study using a multi-pollutant approach. *Environment International* 2017;104: 48-57. https://doi.org/10.1016/j.envint.2017.04.002

El estudio llevado a cabo en la cohorte GraMo confirmó que los adultos continúan expuestos a mezclas de disruptores endocrinos persistentes, incluso a aquéllos que fueron regulados o prohibidos hace varias décadas, fundamentalmente debido a su capacidad de acumulación en los tejidos grasos. En base a los resultados de nuestro estudio, las concentraciones en tejido adiposo de algunos de los compuestos orgánicos persistentes (COPs) analizados, [β-Hexaclorociclohexano (β-HCH y Hexaclorobenzeno (HCB)], se asociaron tanto con la prevalencia como con la incidencia de estar comprometido a nivel metabólico; es decir, de presentar o desarrollar al menos uno de los cuatro componentes del síndrome metabólico considerados (hipertensión, diabetes tipo 2, colesterol HDL bajo, o hipertrigliceridemia). Este estudio es uno de los primeros en evaluar la relación entre la exposición acumulada a COPs y los componentes del síndrome metabólico, así como en combinar un análisis transversal con otro de tipo longitudinal en la misma población de estudio.

Resultados preliminares (Apéndice 1)

Mustieles V et al. Bisphenol A and adiposity measures among peripubertal boys from Spain. Manuscrito en preparación.

Resultados preliminares de los niños de la cohorte INMA-Granada, durante la peripubertad, muestran que mayores concentraciones de BPA en orina se asocian transversalmente tanto con un mayor IMC (z-scores/puntuaciones z), como con una mayor prevalencia de sobrepeso/obesidad. Además, BPA se asocia de manera positiva con la prevalencia de obesidad abdominal, pero no con el porcentaje de grasa corporal, sugiriendo un posible efecto obesogénico de BPA en el tejido adiposo visceral. Sin embargo, estos resultados deben ser interpretados con precaución debido a la posibilidad de causalidad reversa y/o confusión por factores dietéticos y de estilo de vida.

CONCLUSIONES

Conclusión 1

Nuestros hallazgos destacan la necesidad de estudiar el inexplorado período de la preconcepción como una ventana crítica adicional en relación a la exposición a BPA y sus efectos adversos en el nacimiento. Dada la ubicuidad de la exposición a BPA, y el valor predictivo del tamaño al nacer para la salud humana, consideramos que estos resultados son de relevancia para la salud pública.

Conclusión 2

Los resultados de los niños de la cohorte INMA-Granada, durante la peri-pubertad, son compatibles con un posible efecto de BPA a nivel de la glándula adrenal, que tiene como consecuencia una producción hormonal anómala de andrógenos y cortisol. Sin embargo, dado el diseño transversal de nuestro estudio, la heterogeneidad de los resultados publicados en la literatura, y la poca información experimental disponible sobre los efectos de BPA en la glándula adrenal, los resultados deben ser interpretados con precaución.

Conclusión 3

Los datos que avalan la relación entre exposición a BPA y problemas de comportamiento infantil son cada vez más abundantes y consistentes, y nuestros resultados en los niños de la

cohorte INMA-Granada, a los 9-11 años de edad, apoyan también esta asociación. Aunque puede que nunca sea posible establecer una relación causal entre este disruptor endocrino y comportamientos específicos, la información científica disponible hasta el momento no puede ser ignorada y exige, más que nunca, la necesidad de aplicar el principio de precaución y tomar medidas preventivas para reducir la exposición inadvertida a BPA, especialmente durante períodos críticos del desarrollo: mujeres en edad fértil, mujeres embarazadas y niños.

Conclusión 4

En población adulta de la cohorte GraMo, mayores niveles de hexaclorobenzeno (HCB) y β-hexaclorociclcohexano (β-HCH) en tejido adiposo, se asociaron con la prevalencia e incidencia de enfermedades metabólicas. Estos resultados también indican que, aunque la mayoría de compuestos orgánicos persistentes fueron prohibidos hace décadas, su exposición sigue siendo relevante para la salud humana ya que las generaciones actuales pueden sufrir las consecuencias de su bioacumulación. Además, parte de esta carga corporal puede ser transferida a la descendencia durante la gestación y la lactancia, representando un círculo vicioso que necesita ser prevenido con el objetivo de proteger a las futuras generaciones.

Conclusión 5

Resultados preliminares en niños peripuberales de la cohorte INMA-Granada sugieren que la exposición a BPA podría ejercer un efecto obesogénico, al incrementar el riesgo de sobrepeso y obesidad en la población de estudio. Sin embargo, no se puede descartar la posibilidad de causalidad reversa y/o la confusión de estos resultados por factores dietéticos y de estilo de vida.

INTRODUCTION

The growing use of chemicals in modern society has resulted in their increasing production and use, reaching global dimensions. These chemicals include combustion products of fossil fuels, chemicals released into the environment as a result of industrial activities, pesticides used in agriculture, plastic-derived compounds, substances used in personal care products and even pharmaceuticals of human and veterinary use that finally end up in the environment among many other chemical families of anthropogenic origin. Over recent decades, observations of the effects of exposure to some of these chemicals, mainly on wildlife, have increased public awareness of the risks they pose (Reinen et al., 2010). Analogously, the increase in developmental and non-communicable diseases (NCD) in humans over the past 40 years indicates an important role of the environment in disease etiology. In this line, endocrine disrupting chemicals (EDCs) are considered an important component of environmental influences on disease along nutrition and other factors (Bergman et al., 2013).

Nowadays there are more than 1000 chemicals reported to have endocrine effects (TEDX, 2018). According to the World Health Organization (WHO), EDCs are “exogenous substances, or mixtures, that alter function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations” (Bergman et al., 2013). EDCs are highly diverse, with different origins, structures, and functions (Fernández and Olea, 2014). They can interfere with the endocrine system by mimicking the action of naturally produced hormones; by antagonizing the action of endogenous hormones; by altering the synthesis and function of hormone receptors; or by modifying the synthesis, transport, metabolism, or excretion of hormones (Ropero et al., 2006). Consequently, EDCs have also been defined as “exogenous chemicals, or mixtures of chemicals, that can interfere with any aspect of hormone action” (Zoeller et al., 2012). Notably, increasing evidence suggests that EDCs can affect not only the exposed organism but also its offspring and future generations, through epigenetic programming of the germ line that could lead to multigenerational and transgenerational effects (Anway and Skinner, 2008; Xin et al., 2015).

EDCs can be classified in two wide groups based on their persistence and bioaccumulation potential: a) **persistent EDCs**, which are lipophilic substances that resist environmental

degradation and metabolism in biological organisms leading to a significant bioaccumulation throughout lifespan. This group includes the so-called persistent organic pollutants (POPs), including: organochlorine pesticides; polychlorinated biphenyls (PCBs) used in industrial applications; and polybrominated diphenyl ethers (PBDEs) used as flame retardants, among many others; b) **non-persistent EDCs**, which are rapidly metabolized and excreted in urine. Although they are less likely to be accumulated in biological tissues, a continuous and daily exposure has been confirmed in biomonitoring studies. This group includes bisphenol A (polycarbonate plastic and epoxy resins), phthalates (plasticizers commonly used in polyvinyl chloride, [PVC]), parabens and triclosan (antimicrobials in personal care products), as well as some organophosphate pesticides, among many other chemical families.

Bisphenol A (BPA) is a paradigmatic non-persistent endocrine disruptor that challenges science and risk assessment because of its ability to act at low doses through multiple mechanisms of action, and the difficulty to reliably assess its exposure in humans. BPA was initially synthesized around 1900 and studied in the 1930s as a synthetic estrogen (Dodds and Lawson, 1938). However, it was not until the 1950s when its use was rediscovered due to its technological application in the fabrication of polycarbonate plastic. Since then to nowadays, BPA has become a high-production man-made chemical used worldwide in the manufacture of polycarbonate plastics, epoxy resin liners of canned food, some dental sealants, medical devices, and thermal receipts, among many other applications (Vandenberg et al., 2007). Diet is considered the predominant source of BPA exposure in the general population due to the leaching of BPA from packaging materials and can liners into food and beverages (Vandenberg et al., 2010). In addition, other sources and routes such as inhalation and dermal absorption may also contribute to total human exposure (Ehrlich et al., 2014; Michałowicz, 2014). Although BPA is rapidly metabolized and excreted in urine, chronic daily human exposure has led to the detection of BPA concentrations in the urine of around 90% of the general population in industrialized countries (Becker et al., 2009; Calafat et al., 2008; Casas et al., 2011; Vandenberg et al., 2010). Internal exposure to BPA has also been confirmed in other biological matrices such as maternal blood, amniotic fluid, cord and fetal serum, placenta, and maternal breast milk (Vandenberg et al., 2010).

Canned food constitutes one of the most important dietary sources of BPA
(Kawamura et al., 2014; von Goetz et al., 2017)

BPA is a well-known EDC that can interfere with hormonal balance, even at low doses, *via* multiple steroid hormone receptors that mediate a myriad of cellular effects (Nesan et al., 2018; Vandenberg et al., 2012). The mechanistic understanding of its effects is particularly complex: BPA can bind not only to nuclear and membrane estrogen receptors but also to thyroid, glucocorticoid, and peroxisome proliferator-activated receptors, and it can also interact with steroidogenic enzymes, among other molecular targets (Mustieles et al., 2015; Wolstenholme et al., 2011). This biological promiscuity might explain the pleiotropic effects exerted by BPA on reproduction, behavior and metabolism (Rochester, 2013; vom Saal et al., 2007).

BPA has recently been classified as a substance of very high concern and as an endocrine disruptor by the European Chemicals Agency (ECHA, 2017), in addition to its previous recognition as a reproductive toxicant (ECHA, 2016). Although some governments have implemented preventive measures, such as the banning of BPA in baby bottles by Canada and subsequently by the European Union (Canada Consumer Product Safety Act, 2017; European Commission, 2011), or the total prohibition of BPA in food containers in France (Audran and Salmon, 2012), the issue of BPA continues to generate discussion and is in the spotlight. Regulatory organizations such as the European Food Safety Authority (EFSA) have been progressively reducing their estimation of the tolerable daily intake (TDI) in

subsequent risk assessments. Thus, the TDI for BPA was lowered from 50 µg/kg bw/day to 4 µg/kg bw/day in 2015 (EFSA, 2015). Nevertheless, it has been suggested that EFSA's temporary tolerable daily intake of 4 µg/kg bw/day may not be "sufficiently protective" for humans in the general population (Hass et al., 2016). The greater public awareness around BPA has been reflected by the increased popularity of "BPA-free" products (Vandenberg and Prins, 2016). However, many of these products are manufactured using bisphenol analogues, including bisphenol S and F (BPS and BPF), which have been shown to be at least as hormonally active as BPA (Rochester and Bolden, 2015).

On the other hand, POPs are highly lipophilic compounds that resist metabolism and biodegradation and therefore tend to have a relatively long half-life in the environment and to bioaccumulate and biomagnify in the food chain (Mrema et al., 2013). The result is the virtually universal exposure of living organisms, including humans. These chemicals include organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), which have been used in a variety of commercial products, e.g., insecticides (dichlorodiphenyltrichloroethane [DDT], dicofol, lindane), fungicides (hexachlorobenzene [HCB]), and coolant and heating exchange fluids (polychlorinated biphenyls [PCBs]). Although legal restrictions in most countries have caused a worldwide decline in the production and handling of many POPs, human exposure remains relevant to public health due to their ubiquity and because current generations might suffer the effects of accumulated exposure throughout their lives. Most studies have considered diet, especially fatty food, to be the main current source of exposure in the general population (Gasull et al., 2011).

Although the use of most POPs has been regulated and totally or partially banned following the Stockholm Convention (United Nations, 2001), they continue to be detected in the environment, in the food chain, and in human fatty tissues. Adipose tissue constitutes a reservoir for long-term POP accumulation that can act as a source of chronic exposure to POPs through their slow release into the bloodstream, which might have relevant consequences in several chronic diseases (La Merrill et al., 2013). Moreover, accumulated POPs can be partially transferred to subsequent generations during gestation and breastfeeding (Shen et al., 2007). Additionally, accumulated POPs may exert a local effect

in the adipose tissue, interfering with lipid metabolism, insulin sensitivity, and endocrine function, and could therefore affect all components of the metabolic syndrome. Proposed mechanisms of action include interaction with nuclear receptors such as peroxisome proliferator-activated receptor gamma (PPAR-γ) and aryl hydrocarbon receptor (AhR) (La Merrill et al., 2013), as well as increased adipose tissue oxidative microenvironment and inflammation (Artacho-Cordón et al., 2016).

Since EDCs can interfere with different aspects of hormonal homeostasis, they have been linked to diverse adverse health outcomes including reproduction, neurodevelopment, obesity and metabolic diseases, developmental defects as well as cancer among other health endpoints (Gore et al., 2015). Importantly, the impact of EDC exposure on both animals and humans strongly depends on the period of development at which it occurs. Thus, the effects of *in utero* exposure or during peri-puberty can be expected to differ from those of exposure during adulthood. Embryos, fetuses, neonates and children are highly sensitive to EDC exposure and tend to be more susceptible to adverse effects than adults do (Schug et al., 2011; Vandenberg et al., 2013).

Early developmental exposure to EDCs at low doses may lead to an increased risk of dysfunction and disease later in life, despite the absence of phenotypic changes observable at birth (Anway and Skinner, 2008). Thus, the endocrine disruption hypothesis fits well the paradigm of the “Developmental Origins of Health and Disease (DOHaD)”, which maintains that early life environments permanently influence health outcomes later in life (Wadhwa et al., 2009). Although pregnancy is generally considered as the most critical period of development, increasing evidence also highlights the importance of other critical windows of development such as preconception and peripuberty (Messerlian et al., 2018; Schulz et al., 2009).

Overall, biomonitoring studies have shown that humans are exposed to complex mixtures of EDCs, and the resulting exposure-effect relationships are very difficult to predict. Among EDCs, BPA is in the spotlight of current regulations. Although some prospective birth cohort studies have yielded suggestive results on different health endpoints, there is a need for more observational data. Therefore, the present thesis project has focused on the possible

effects of EDC exposure in human populations, mainly BPA, with a special emphasis on different critical periods of development (preconception, pregnancy and peripuberty), and on the health endpoints of highest concern observed in experimental studies: reproduction, neurodevelopment and obesity/metabolism.

JUSTIFICATION & OBJECTIVE

JUSTIFICATION

Available data from biomonitoring studies clearly indicate that the general population is ubiquitously exposed to BPA. There is also growing evidence that BPA is harmful in laboratory animals and may adversely affect human health, especially during development. BPA has become a target of current risk assessments and policy regulations mainly due to concerns about its possible adverse effects on children's reproductive health, neurodevelopment and obesity/metabolism. Hence, there is a need to advance our knowledge on the potential effects of BPA exposure in human populations, with a special emphasis on different critical periods of development.

OBJECTIVE

The main objective of this thesis project was to investigate whether human BPA exposure is associated with adverse perinatal, childhood, and adult health outcomes, including reproductive, neurodevelopment, and metabolic health effects.

For this purpose, three specific objectives were proposed:

Objective 1: Reproduction. To explore whether BPA exposure (before conception, during pregnancy and in peripuberty) is associated with reproductive outcomes (addressed in articles #1 and #2 of the Results section).

Objective 2: Neurodevelopment. To review all the existing epidemiological literature on the association between BPA exposure and human neurobehavior, and to investigate whether environmental exposure to BPA in childhood is associated with behavior (addressed in papers #3, #4 and #5 of the Results section).

Objective 3: Obesity/Metabolism. To assess the influence of long-term exposure to POP mixtures (as well as short-term exposure to BPA) on the risk of obesity and metabolic diseases in children and adults (addressed in article #6 of the Results section and in the Preliminary results of the Appendix).

MATERIAL & METHODS

In order to achieve all the aforementioned objectives, studies were conducted in three established epidemiological cohorts. The general design and study population of each cohort, as well as the study question of interest for the present thesis are briefly reported in this section to provide a general view. Specific methodological details can be consulted in each of the corresponding published articles, included in the "Results" section.

The Environment and Reproductive Health Study (EARTH): An ongoing prospective preconception cohort of subfertile couples in Boston (USA), designed to study the effects of preconception and prenatal environmental exposures on the reproductive health of couples and the development of their offspring.

The Environment and Childhood Study (INMA)-Granada Cohort: A prospective mother–child cohort from Granada province (Spain), integrated in the Spanish multicenter INMA Project. The INMA Project aims to assess the effect of prenatal environmental exposures and diet on growth, development, and health from early fetal life until adolescence. Since the initial recruitment of the INMA-Granada cohort in 2000-2002, follow-ups of the children have been conducted at the ages of 4-5 and 9-11 years, and a follow-up at 15-16 years is ongoing.

The Granada-Motril (GraMo) Cohort: A prospective cohort based on a hospital-based study of adults from Southern Spain (Granada), primarily centered on the study and identification of environmental factors affecting the risk of chronic diseases. The GraMo cohort has access to adipose tissue samples obtained at recruitment, and one of its main lines of research is the study of historical exposure to mixtures of POPs and its relation to metabolic diseases.

A) The Environment and Reproductive Health Study (EARTH)

Design and study population: The EARTH Study is an ongoing prospective preconception cohort of couples recruited from the Massachusetts General Hospital (MGH) Fertility Center. The study was designed to evaluate the effects of environmental exposures and diet on fertility and pregnancy outcomes. To date, the study has recruited ~800 women ages 18–46 years and 500 men ages 18–55 years. An extensive description of the study is available elsewhere (Messerlian et al., 2018). Briefly, participants enroll independently or as a couple and are followed from study entry and throughout their fertility care, pregnancy and delivery. Participants complete staff-administered baseline questionnaires and provide urine samples at enrollment and again at each fertility treatment cycle. Trained study staff described the study protocol to all participants and answered their questions, before these provided signed informed consent. The study was approved by the Institutional Review Boards of MGH, Harvard T.H. Chan School of Public Health and the Centers for Disease Control and Prevention (CDC).

Study question related to Objective 1 (Reproduction): Are maternal and paternal preconception urinary bisphenol A (BPA) or bisphenol S (BPS) concentrations associated with offspring size at birth?

This question was addressed in **article #1** of the Results section.

Exposure characterization: Men and women provided a single spot urine sample at study entry. Women provided up to two additional urine samples per fertility treatment cycle. During pregnancy, women also provided one spot urine sample per trimester (median: at 6, 21 and 35 weeks of gestation). Men provided one additional spot urine sample per treatment cycle at the time as their female partner underwent oocyte retrieval or intrauterine insemination. Multiple urine samples collected by each participant were used to examine BPA and BPS in three separate windows of exposure—paternal preconception, maternal preconception and maternal prenatal. Urinary BPA and BPS concentrations were quantified at the CDC in Atlanta (USA) using online solid phase extraction coupled with high performance liquid chromatography– isotope dilution tandem mass spectrometry (Silva et

al., 2007). The specific gravity of each urine sample was assessed with a handheld refractometer (National Instrument Company, Inc., Baltimore, MD, USA), and used to account for urinary dilution.

Outcome assessment: Infant birth weight (in grams) and head circumference (in cm) were abstracted from delivery records.

Final sample size analyzed: 346 singletons corresponding to 346 mothers and 190 fathers with available BPA exposure data. Additionally, 107 singletons corresponding to 107 mothers and 37 fathers also had BPS exposure data.

Covariate selection: The selection of covariates as potential confounders was based on substantive knowledge using a directed acyclic graph, and both unadjusted and covariate-adjusted results were examined. The main Maternal preconception-prenatal window covariate-adjusted models included: maternal age and BMI (continuous); maternal education (<college, college, graduate degree); smoking status (never smoked versus ever smoked, defined as a current or former smoker); and Assisted Reproductive Technology (ART) versus non-ART-based treatment. Paternal preconception window covariate-adjusted models included paternal and maternal age and BMI (continuous); paternal and maternal smoking (ever/never); maternal education (<college, college, graduate degree); ART versus non-ART-based treatment. Because gestational age may be a causal intermediate between bisphenol exposure and birth outcomes (Wilcox et al., 2011), it was additionally included in a separate model to assess the potential change in estimates (Ananth and Schisterman, 2017).

Statistical analysis: Urinary BPA and BPS concentrations were adjusted for urine dilution by multiplying each concentration by [(SGp − 1)/(SGi − 1)], where SGi is the specific gravity of the participant's sample and SGp is the mean specific gravity for all male or all female participants included in the study sample (Pearson et al., 2009). The specific gravity-adjusted bisphenol concentrations were natural-log transformed to standardize the distribution and reduce the influence of extreme values. Mean preconception and prenatal exposures were estimated by averaging urinary ln-BPA and ln-BPS concentrations in

multiple maternal and paternal urine samples collected before pregnancy, and maternal pregnancy samples collected in each trimester. The association of maternal and paternal preconception and maternal prenatal urinary BPA and BPS concentrations with birth weight and head circumference was assessed using multivariable linear regression models. Beta coefficients and 95% Confidence Intervals (CIs) represent the mean difference in birth weight (g) and head circumference (cm) for each natural log-unit increase in urinary bisphenol concentration. Natural-log urinary BPA and BPS concentrations were used as the independent variable. Additionally, in order to assess potential non-linear associations, we fitted multivariable linear models to evaluate change in birth weight and head circumference across tertiles of urinary BPA concentrations using the lowest tertile as the reference.

***A priori* study strengths:** The EARTH Study is one of the few existing preconception cohorts for the study of exposure to environmental chemicals. Additionally, its strong design and comprehensive follow-up of the participants allows a good characterization of exposure to non-persistent chemicals due to the collection of multiple urine samples.

***A priori* study limitations:** Because the study population comprises subfertile couples and EDC exposure is associated with fertility problems, the results may not be directly generalizable to couples without fertility concerns.

B) The Environment and Childhood (INMA)-Granada Cohort

The INMA (Infancia y Medio Ambiente – Environment and Childhood) cohort is a population-based study in seven regions of Spain, designed to explore the effects of environmental pollutants during pregnancy and early childhood on child growth and development. An extensive description of the study is available elsewhere (Guxens et al., 2012). The INMA-Granada sub-cohort includes 668 mother-son pairs recruited at delivery (2000−2002) in Granada province (Spain) (Fernandez et al., 2007). When the children reached the age of 9–11 years, all families in the cohort were invited to participate in the 2010–2012 follow-up. Three hundred families (44.9% participation rate) gave their written informed consent. These families completed staff-administered questionnaires to collect

sociodemographic, dietary and clinical information. Out of 298 boys who provided a single spot urine sample, 187 (63%) also agreed to donate a blood sample. The study followed the principles of the declaration of Helsinki and was approved by the Ethics Committee of San Cecilio University Hospital (Granada, Spain).

Study question related to Objective 1 (Reproduction): Are urinary BPA concentrations cross-sectionally associated with reproductive hormones and cortisol in peripubertal boys?

This question was addressed in **article #2** of the Results section.

Study question related to Objective 2 (Neurodevelopment): Are urinary BPA concentrations cross-sectionally associated with behavior in boys at 9-11 years of age?

Before conducting any original research to address this study question, a comprehensive review of the scientific literature was carried out to investigate existing knowledge on the relationship between human BPA exposure and children's behavior. The first scientific review was published in 2015 and constitutes **article #3** of the Results section.

The study question was addressed in **article #4** of the Results section.

In 2018, a new review was conducted to include the epidemiological studies published after the initial 2015 review, assessing whether these studies confirm or not the direction indicated by previous epidemiological studies. This opinionated mini-review constitutes **article #5** of the Results section.

Study question related to Objective 3 (Obesity/metabolism): Are urinary BPA concentrations cross-sectionally associated with adiposity measures among peripubertal boys?

Although not completely finished, preliminary results are shown in the Appendix 1.

Exposure characterization: All children provided a single non-fasting spot urine sample at follow-up visit, always between 5 pm and 8 pm. Total BPA (free plus conjugated) was

determined by liquid chromatography-mass spectrometry in the Department of Analytical Chemistry of the University of Cordoba (Spain). BPA background contamination from the presence of polymers in components of the urine collection containers and/or of the LC equipment or labware was avoided by filtering LiChrosol water through a 47 mm Styrene DVB (SDB-XC) disk from Empore (3 M, St Paul, Minnesota, USA) and rinsing the glassware and Eppendorf microtubes with methanol several times before their use. Moreover, an inter-laboratory comparison was performed with the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA)—Institute of the Ruhr-University Bochum (Germany), with considerable experience in BPA determination. Urinary creatinine (mg/dL) was determined at the Public Health Laboratory of the Basque Country (Spain), and was used to account for urinary dilution.

Outcome assessment:

Reproduction (article #2): Serum total testosterone (TT), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and cortisol. Within 1–3 days of the 2010–2012 follow-up visit, fasting morning blood samples were also collected from 187 out 300 boys at San Cecilio University Hospital in accordance with recommendations of the hospital staff. Serum reproductive hormones and cortisol levels were measured by electrochemiluminescence immunoassay (Elecsys System, Roche Diagnostics) at the Analytical Unit of the University Hospital.

Neurodevelopment (article #3): Behavioral function was assessed at the 2010–2012 follow-up visit using the Child Behavior Checklist (CBCL/6-18), a standardized parent report questionnaire (Achenbach Rescorla, 2001). The assessment was supervised by a psychologist and was completed by 294 parents. The CBCL includes 118 items that parents rate on a three-point scale (Not True, Somewhat True, Very/Often True). The CBCL provides eight syndrome scales [anxious/depressed, withdrawn/depressed, somatic complaints, social problems, thought problems, attention problems, rule-breaking behavior, and aggressive behavior, which are grouped by three composite scales: 1. Internalizing-sum of scores on the anxious/depressed, withdrawn/depressed, and somatic complaints scales; 2. Externalizing-sum of scores on the rule-breaking behavior and aggressive behavior scales;

and 3. Total Problems], reported as both raw scores and sex- and age-normalized T-scores. Children with CBCL/6–18 T-scores >60 on internalizing or externalizing problem scales and T-scores >65 on diagnostic/syndrome scales were classified as borderline/clinical cases (Achenbach Rescorla, 2001).

Obesity/Metabolism (Manuscript in preparation – Appendix 1): BMI was calculated (Kg/m^2) using child's weight (kg) and height (cm), assessed without shoes and in light clothing, and using an electronic scale (TANITA model 354, Seca Corporation, Hamburg, Germany). Body fat mass was also assessed with the TANITA device. Waist circumference (WC) was measured at a level midway between the lower rib margin and iliac crest with a non-stretchable tape. Duplicate measures were taken and values were averaged. BMI z-scores were calculated using the 2007 World Health Organization (WHO) growth reference standards (de Onis, 2007). Overweight and obese children were categorized based on BMI z-scores percentiles (≥85th percentile for age and sex). Waist-to-height ratio (WHtR) was calculated dividing WC by height in the same units. Central obesity was defined as a ratio higher than 0.5 based on the literature for both children and adults (Browning et al., 2010). In the absence of reference cut-offs, body fat mass was not categorized and studied only as a continuous variable.

Final sample size analyzed:

Reproduction (article #2): 172 boys for which urinary BPA data, serum reproductive hormones and cortisol, as well as relevant covariates were available.

Neurodevelopment (article #3): 269 boys for which urinary BPA data, behavioral assessment and relevant covariates were available.

Obesity/metabolism (Manuscript in preparation – Appendix 1): 210 boys for which urinary BPA data, adiposity measures (including body fat mass), dietary data, and relevant covariates were available.

Covariate selection:

Reproduction (article #2): Known or suspected confounders of the association between the exposure and outcome were selected as covariates based on the literature (Scinicariello and Buser, 2016), and/or a ≥10% change in beta coefficients of the independent variable in the statistical models. Final models were adjusted for: maternal education (up to primary, secondary school or university), as an indicator of socio-economic status (SES); BMI (kg/m^2), because body composition is associated with steroid hormone levels; total serum cholesterol levels (mg/dL), because cholesterol is the common precursor of steroid hormones; and urinary cotinine (ng/mL), as a measure of exposure to second-hand smoke (Fernández et al., 2015), because tobacco has been related to BPA exposure (Casas et al., 2011; Covaci et al., 2015) and nicotine can inhibit the activity of the crucial steroid enzyme aromatase. In order to improve the comparability of the results, final models for all hormones were adjusted for the same set of covariates. Models were further adjusted for Tanner stage (I vs. II) in order to account for possible differences in the development stage and/or endogenous hormonal status.

Neurodevelopment (article #4): Known or suspected risk factors for the exposure or outcome were selected as covariates, based on the literature (LaKind and Naiman, 2011; Mustieles et al., 2015). Adjusted models included the children's age, intelligence quotient (IQ) score, body mass index (BMI kg/m^2), exposure to environmental tobacco smoke (any/none) at home, mother's intelligence score and age, parental education level (university/secondary school/up to primary), marital status (married/not married), maternal smoking during pregnancy (yes/no), and breastfeeding (yes/no).

Obesity/metabolism (Manuscript in preparation – Appendix 1): Known or suspected confounders of the association between the exposure and the outcome were considered as covariates, based on the literature (Trasande et al., 2012). Covariates associated with exposure, outcome or both at $p<0.2$ were further explored by entering them in the multivariate models. These covariates were conserved in the models if they produced a ≥10% change in beta coefficients of the independent variable. Because not all selected covariates were available for all the children, a progressive adjustment was conducted. Consequently, final models were progressively adjusted for: urinary creatinine levels (mg/dl), maternal education (up to primary/secondary school/university), calorie intake

(Kcal), total carbohydrates (g) and total fat intake (g), and Tanner stage (I vs. II), followed by maternal BMI (Kg/m^2) and physical activity (sedentary/low activity vs. physically active).

Statistical analysis: Urinary BPA concentrations were used both in crude and adjusted by urinary creatinine levels (mg/dL) as has been proposed (LaKind and Naiman, 2011). Urinary BPA and creatinine-adjusted BPA concentrations were natural-log transformed in order to standardize the distribution and reduce the influence of extreme values. The association between children's urinary BPA concentrations and continuous outcomes was assessed using multivariable linear regression models, while binary outcomes were assessed using logistic regression models. Beta coefficients and 95% CIs were calculated for the multivariable linear regression models, and multivariable-adjusted odds ratios (ORs) with 95% CIs for the logistic regression models. Natural log-transformed BPA concentrations were used as the independent variable, both as a continuous variable and categorized in tertiles or quartiles, depending on the specific outcome studied and the available sample size.

***A priori* study strengths:** Although the peripubertal period is increasingly recognized as another window of heightened vulnerability to environmental exposures, it has been little studied. This investigation, based on the 2010-2012 follow-up of the INMA-Granada cohort, contributes novel data on the potential effects of BPA on health endpoints of concern during this specific developmental period.

***A priori* study limitations:** The cross-sectional design of the children's follow-up at 9-11 years prevents the inference of causal relationships, and reverse causality issues cannot be ruled out. Additionally, the use of a single spot urine sample to estimate BPA exposure increases the chances of exposure misclassification, due to its non-persistent nature and short-term variability. However, this is more likely to produce an underestimation rather than an overestimation of its effects (attenuation bias).

C) The Granada-Motril (GraMo) Cohort

The Granada-Motril (GraMo) study is a hospital-based cohort that initially aimed to characterize long-term exposure to POPs in the adipose tissue of adults from Southern

Spain and to assess its potential health effects, with a particular emphasis on metabolic diseases. The study design, recruitment, and methods have been extensively described elsewhere (Arrebola et al., 2010, 2009). In brief, study subjects were recruited in two public hospitals from Granada province: San Cecilio University Hospital in the city of Granada (240.000 inhabitants, urban area), and Santa Ana Hospital in the town of Motril (50.000 inhabitants, semi-rural area). Participants were recruited between July 2003 and June 2004 from among patients undergoing non-cancer-related surgery. Following the standard surgery protocols of the hospitals, all participants were under 12-h fasting conditions at sample collection. Inclusion criteria were: age over 16 years, absence of cancer, non-prescription of hormonal therapy, and residence in one of the study areas for at least 10 years. Reasons for surgery included a total of 70 different health issues. Given this heterogeneity, they were grouped into four categories: hernias (41%), gallbladder diseases (21%), varicose veins (12%), and other conditions (26%). Out of the 409 individuals who were contacted, 387 agreed to participate and were included in the initial cohort. Data on socio-demographic characteristics, lifestyle, and health status were collected in face-to-face interviews conducted by trained personnel at the time of recruitment during the hospital stay. All participants were users of the public health system. All adipose tissue biopsies were collected at recruitment (n =387) and all participants signed their informed consent to participate in the study, which was approved by the ethics committees of both hospitals.

Study question related to Objective 3 (Obesity/metabolism): Are human adipose tissue concentrations of POPs cross-sectionally and longitudinally associated with the risk of metabolic syndrome components?

This question is addressed in **article #6** of the Results section.

Exposure characterization: Eight POPs were quantified in adipose tissue samples by high-resolution gas chromatography coupled with a mass spectrometry detector in tandem mode. Residues of *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*- DDE, the main metabolite of the pesticide DDT), HCB, dicofol, α- and β-hexachlorocyclohexane (α-and β-HCH, respectively), and PCB congeners−138, −153 and −180 were quantified. Lipid content in

adipose tissue samples was quantified gravimetrically as previously reported (Rivas et al., 2001), and lipid-basis POP concentrations were calculated and expressed in nanograms per gram of lipid (ng/g lipid).

Outcome assessment: The outcome "metabolically compromised" was defined as having ≥1 diagnosis of the following components: type 2 diabetes (fasting glucose ≥126 mg/dL and/or prescription of anti-diabetic therapy); hypertension (systolic blood pressure > 140 mm Hg and/or diastolic blood pressure > 90 mm Hg and/or prescription of antihypertensive therapy); hypertriglyceridemia (serum triglycerides (TG) ≥ 150 mg/dL and/or prescription of lipid-lowering treatment); or low high-density lipoprotein cholesterol (HDL-C), defined by serum level < 50 mg/dL in females or < 40 mg/dL in males (Alberti et al., 2009). Thus, we compared metabolically compromised individuals (≥1 Metabolic Syndrome (MetS) component) with those metabolically healthy (free of any MetS component). This was the final outcome analyzed in both the cross-sectional and longitudinal analyses. For the longitudinal analyses, the follow-up period was from the date of recruitment to the first diagnosis of a MetS component, death of the participant, or December 31, 2013 (the cohort remains under study).

The prevalence of MetS components was gathered using both self-reported information and the clinical record (DIRAYA database), finding no relevant discrepancies between these sources, while the incidence was gathered by using the DIRAYA database. DIRAYA integrates all clinical information for each public health system user, facilitating clinical procedures and epidemiological research.

Final sample size analyzed: 387 adults in the cross-sectional analysis, and 154 participants in the longitudinal analysis, which were free of any MetS component at recruitment and were followed for 10-years.

Covariate selection: Selected covariates included variables whose inclusion in any model produced changes> 10% in beta coefficients and/or those reported as relevant confounders in previous studies: BMI (kg/m^2), age (years), sex (male/female), residence (urban/semirural), education (primary schooling not completed/primary or higher),

alcohol consumption (consumer/non-consumer), and smoking habit (smoker/non-smoker).

Statistical analysis: Lipid-basis adipose tissue POP concentrations (ng/g lipid) were natural-log transformed in order to reduce the influence of extreme values. For the overall cohort (n = 387), the magnitude of associations between POPs and the outcome was analyzed by binomial unconditional logistic regression, calculating multivariable-adjusted ORs with their corresponding 95% CIs. Participants free of any MetS component at recruitment (n = 154) were followed-up, and the magnitude of associations between POPs and the 10-year incidence of MetS components was evaluated using Cox-regression models with time-to-events as the time variable, calculating hazard ratios (HRs) with their corresponding 95% CIs. Both the logistic- and Cox-regression models were adjusted for the same group of covariates in order to enhance the comparability of results. In addition to single-pollutant analyses, elastic-net regression analyses were conducted to select the most relevant predictors, accounting for simultaneous co-exposures.

***A priori* study strengths:** The main strength of the GraMo cohort is the availability of adipose tissue samples, which is considered the most suitable matrix for the assessment of long-term accumulated concentrations of POPs. Another major strength is the combined cross-sectional and longitudinal design, which can reinforce causal inference and minimize potential reverse causality issues. A further strength is the utilization of a complementary statistical multi-pollutant model that assesses several co-exposures simultaneously, which represents a more realistic exposure scenario.

***A priori* study limitations:** The modest sample size, particularly in the longitudinal analyses. It is not possible to rule out the potential influence of other groups of unmeasured environmental pollutants that may have partially contributed to the associations observed.

RESULTS

Paper #1

Mustieles V, Williams PL, Fernandez MF, Mínguez-Alarcón L, Ford JB, Calafat AM, Hauser R, Messerlian C; Environment and Reproductive Health (EARTH) Study Team. (2018). Maternal and paternal preconception exposure to bisphenols and size at birth. *Human Reproduction*, 33(8), 1528-1537. https://doi.org/10.1093/humrep/dey234

Impact factor: 4,990; Q1; Category: OBSTETRICS & GYNECOLOGY; Ranking: 4/82

Paper #2

Mustieles V, Ocón-Hernandez O, Mínguez-Alarcón L, Dávila-Arias C, Pérez-Lobato R, Calvente I, Arrebola JP, Vela-Soria F, Rubio S, Hauser R, Olea N, Fernández MF. (2018). Bisphenol A and reproductive hormones and cortisol in peripubertal boys: The INMA-Granada cohort. *Science of the Total Environment*, 618, 1046-1053. https://doi.org/10.1016/j.scitotenv.2017.09.093

Impact factor: 4,610; Q1; Category: ENVIRONMENTAL SCIENCES; Ranking: 27/241

Cites: 1

Paper #3

Mustieles V, Pérez-Lobato R, Olea N, Fernández MF. (2015). Bisphenol A: Human exposure and neurobehavior. *Neurotoxicology*, 49, 174-184. Review. https://doi.org/10.1016/j.neuro.2015.06.002

Impact Factor: 3,076; Q2; Category: TOXICOLOGY; Ranking: 34/94

Cites: 67

Paper #4

Perez-Lobato R, Mustieles V, Calvente I, Jimenez-Diaz I, Ramos R, Caballero-Casero N, López-Jiménez FJ, Rubio S, Olea N, Fernández MF. (2016). Exposure to bisphenol A and behavior in school-age children. *Neurotoxicology*, 53, 12-19. https://doi.org/10.1016/j.neuro.2015.12.001

Impact factor: 3,076; Q2; Category: TOXICOLOGY; Ranking: 34/94

Cites: 18

Paper #5

Mustieles V, Messerlian C, Reina I, Rodríguez-Carrillo A, Olea N, Fernández MF. (2018). Is Bisphenol A (BPA) a Threat to Children's Behavior? *Journal of Mental Health & Clinical Psychology,* 2(1), 6-9. ISSN: 2578-2959

Paper #6

Mustieles V, Fernández MF, Martin-Olmedo P, González-Alzaga B, Fontalba-Navas A, Hauser R, Olea N, Arrebola JP. (2017). Human adipose tissue levels of persistent organic pollutants and metabolic syndrome components: Combining a cross-sectional with a 10-year longitudinal study using a multi-pollutant approach. *Environment International*, 104, 48-57. https://doi.org/10.1016/j.envint.2017.04.002

Impact factor: 7,297; Q1; Category: ENVIRONMENTAL SCIENCES; Ranking: 7/241

Cites: 7

Preliminary Results (Appendix Section)

Mustieles V et al. Bisphenol A and adiposity measures among peripubertal boys from Spain. *Unsubmitted, manuscript in preparation.*

PAPER 1

Mustieles V, Williams PL, Fernandez MF, Mínguez-Alarcón L, Ford JB, Calafat AM, Hauser R, Messerlian C; Environment and Reproductive Health (EARTH) Study Team. (2018). Maternal and paternal preconception exposure to bisphenols and size at birth. *Human Reproduction*, 33(8), 1528-1537.

Paper #1

Maternal and paternal preconception exposure to bisphenols and size at birth

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Abstract

Study question: Are maternal and paternal preconception urinary bisphenol A (BPA) or bisphenol S (BPS) concentrations associated with offspring birth size?

Summary answer: Maternal—but not paternal—preconception urinary BPA concentrations were associated with lower birth size among couples seeking fertility evaluation.

What is known already: Prenatal BPA exposure has been previously associated with reduced birth size in some but not all epidemiologic studies. However, the potential effect of BPA exposure before conception in either parent is unknown. Data on BPS is practically absent.

Study design, Size, duration: Ongoing prospective preconception cohort of women and men seeking fertility evaluation between 2005 and 2016 in a large fertility center in an academic hospital in Boston, MA, USA.

Participants/materials, setting, methods: We examined the association between maternal and paternal preconception, as well as maternal prenatal urinary BPA and BPS concentrations, and size at birth among 346 singletons from couples recruited in the Environment and Reproductive Health (EARTH) Study using multivariable linear regression. Infant birth weight and head circumference were abstracted from delivery records. Mean preconception and prenatal exposures were estimated by averaging urinary ln-BPA and ln-BPS concentrations in multiple maternal and paternal urine samples collected before pregnancy, and maternal pregnancy samples collected in each trimester.

Main results and the role of chance: Maternal preconception urinary BPA concentrations were inversely associated with birth weight and head circumference in adjusted models: each ln-unit increase was associated with a decrease in birth weight of 119 g (95%CI: −212, −27), and a head circumference decrease of 0.72 cm (95% CI: −1.3, −0.1). Additional adjustment by gestational age or prenatal BPA exposure modestly attenuated results. Women with higher prenatal BPA concentrations had infants with lower mean birth weight (−75 g, 95% CI: −153, 2) although this did not achieve statistical significance. Paternal preconception urinary BPA concentrations were not associated with either birth weight or head circumference. No consistent patterns emerged for BPS concentrations measured in either parent.

Limitations, reasons for caution: We observed a strong negative association between maternal—but not paternal—pre-conception BPA concentrations and offspring birth size among a subfertile population.

Although these results are overall consistent with prior studies on prenatal BPA exposure, these findings may not be generalizable to women without fertility concerns.

Wider implications of the findings: This study suggests that the unexplored maternal preconception period may be a sensitive window for BPA effects on birth outcomes.

Study funding/competing interest(s): Work supported by Grants (ES R01 009718, ES 022955 and ES 000002) from the National Institute of Environmental Health Sciences (NIEHS). C.M. was supported by a post-doctoral fellowship award from the Canadian Institutes of Health Research. There are no competing interests to declare.

Introduction

The Developmental Origins of Health and Disease paradigm maintains that early life environments influence health outcomes later in life (Wadhwa et al., 2009). Birth weight and other measures of infant size at birth are considered important markers of the intrauterine environment, with potential long-term consequences for adult health (Barker, 2007; Basso, 2008; Visentin et al., 2014). There is accumulating epidemiologic evidence associating exposure to non-persistent endocrine disrupting chemicals (EDC), such as bisphenol A (BPA), to adverse reproductive outcomes, including reduced fetal and infant birth weight, with some studies showing differences by infant sex (Veiga-Lopez et al., 2015; Tomza-Marciniak et al., 2018). Moreover, there is a growing body of experimental research showing that EDC exposure before conception may affect offspring health, potentially leading to multi-generational effects (Fan et al., 2013; Xin et al., 2015; Chen et al., 2016). Germ cells are hypothesized to mediate part or the totality of these effects, possibly through epigenetic modifications of oocytes and spermatozoa, which can be inherited by offspring (Fan et al., 2013; Xin et al., 2015; Chen et al., 2016). BPA has been shown to exert epigenetic modifications in mammalian and human sperm (Manikkam et al., 2013; Zheng et al., 2017), oocytes (Trapphoff et al., 2013; Machtinger and Orvieto, 2014) and the placenta (Susiarjo et al., 2013; De Felice et al., 2015). Some of these modifications have been shown to affect the expression of genes and transcription factors related to fetal growth and nutrition (Susiarjo et al., 2013).

BPA is a synthetic high production volume chemical used in the manufacturing of polycarbonate plastics and epoxy resins food can liners, among other consumer products (Vandenberg et al., 2007). Consequently, human exposure is widespread as suggested by the fact that more than 90% of the US population has detectable BPA concentrations in their urine (Calafat et al., 2008). Well-conducted experimental and a small but growing number of epidemiologic studies show that BPA can interfere with several aspects of hormone action, and may produce pleiotropic effects on reproduction, behavior and metabolism (Peretz et al., 2014; Giulivo et al., 2016; Mustieles et al., 2015, 2018). Increasing concern over BPA has prompted its substitution in some consumer products often labeled as 'BPA-free'. However, some replacements are structural analogs such as bisphenol S (BPS), which are also hormonally active (Rochester and Bolden, 2015) and increasingly detected in human urine (Yang et al., 2014; Ye et al., 2015).

Emerging research suggests that the preconception period may be highly sensitive to environmental perturbations, highlighting the importance of considering paternal in addition to maternal exposures (Rando, 2012; Braun et al., 2017). While BPA exposure during pregnancy has been inversely associated with infant size at birth in some epidemiologic studies, the effect of preconception exposure is unknown. Furthermore, epidemiologic data on BPS are practically absent. Therefore, our study aimed to examine whether paternal and maternal preconception, as well as maternal prenatal urinary BPA and BPS concentrations were associated with infant birth weight and head circumference in a prospective preconception cohort of couples attending a large fertility clinic.

Materials and Methods

Study cohort

The Environment and Reproductive Health (EARTH) Study is an ongoing prospective preconception cohort of couples recruited from the Massachusetts General Hospital (MGH) Fertility Center. The study was designed to evaluate the effects of environmental exposures and diet on fertility and pregnancy outcomes. To date, the study has recruited ~800 women ages 18–46 years and 500 men ages 18–55 years. Details of the study have been described elsewhere (Messerlian et al., 2018). Briefly, participants enroll independently or as a couple and are followed from study entry and throughout their fertility care, pregnancy and delivery. Participants complete staff-administered baseline questionnaires and provide urine samples at enrollment and then again at each fertility treatment cycle.

The present study included male and female participants from the EARTH Study with a singleton infant born between 2005 and 2016 (N = 385 singletons). Out of these 385 singletons infants, BPA concentration measurements were available in at least one urine sample before conception of the index pregnancy for 346 mother–child pairs (Fig. 1). As measurement of BPS began in 2015, urinary concentrations were available for only 107 mother–child pairs and 37 father–child pairs. Trained study staff described the study protocol to all participants and answered questions, before participants provided signed informed consent. The study was approved by the Institutional Review Boards of MGH, Harvard T.H. Chan School of Public Health and the Centers for Disease Control and Prevention (CDC).

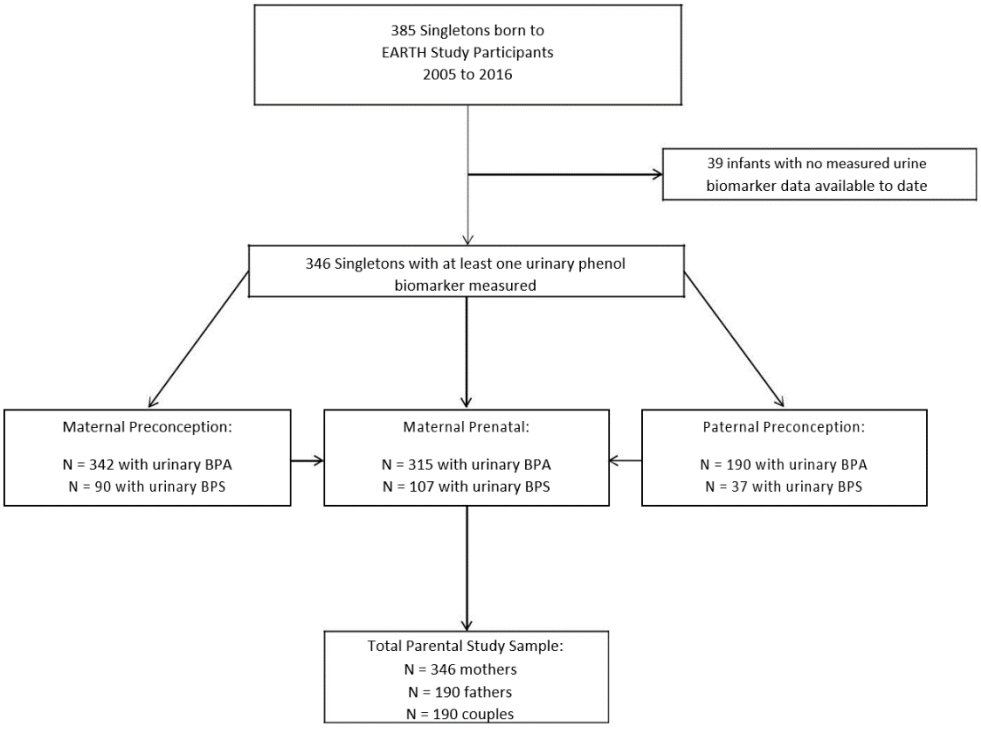

Figure 1. Participant flow chart and bisphenol biomarker data available in the Environment and Reproductive Health (EARTH) study.

Bisphenol exposure assessment

Men and women provided a single spot urine sample at study entry. Women provided up to two additional urine samples per fertility treatment cycle. During pregnancy, women also provided one spot urine sample per trimester (median: 6, 21 and 35 weeks gestation). Men provided one additional spot urine sample per treatment cycle at the time when their female partner underwent oocyte retrieval or IUI. We used multiple urine samples collected by each participant to examine BPA and BPS in three separate windows of exposure—paternal preconception, maternal preconception and maternal prenatal (Fig. 2).

Urine was collected in a polypropylene specimen cup and analyzed for specific gravity with a handheld refractometer (National Instrument Company, Inc., Baltimore, MD, USA), divided into aliquots, and frozen for long-term storage at −80°C. Samples were shipped on dry ice overnight to the CDC (Atlanta, GA, USA) for quantification of urinary BPA and BPS concentrations using online solid phase extraction coupled with high performance liquid chromatography–isotope dilution tandem mass spectrometry (Silva et al., 2007). The limits of detection (LOD) were 0.4 and 0.1 ng/ml for BPA and BPS, respectively. Concentrations below the LOD were assigned the LOD divided by the square root of two (Hornung, 1990). We excluded paternal BPS from analyses given the small number of male participants with available BPS data (N = 37).

Birth weight and head circumference outcome assessment

Birth weight (g) and head circumference (cm) were abstracted from hospital delivery records by trained study staff. Gestational age in days was also abstracted from delivery records and validated using the American College of Obstetricians and Gynecologists (ACOG) guidelines to estimate gestational age for births following medically assisted reproduction (ACOG, 2014). For IVF based conceptions, we estimated gestational age as: (outcome date − date of transfer) + 14 + cycle day of transfer. For IUI and non-medically assisted/naturally conceived pregnancies, we used birth date minus cycle start date. Gestational age was corrected if delivery record estimates (gold standard) differed by over 6 days from the clinically estimated age (corrected for three infants through additional chart verification). Implausible birth weight values were examined through additional verification of delivery record by study nurse (corrected for two infants).

Covariates

Race, age and education of study participants were obtained from the enrollment questionnaire. A study nurse measured the height and weight of the parents at study entry, and BMI (kg/m2) was calculated. Smoking status was self-reported at baseline. Infant sex and mode of delivery (vaginal versus caesarian) was abstracted by study staff from maternal delivery records. The treating infertility physician diagnosed the underlying cause of infertility using the Society for Assisted Reproductive Technology (ART) definitions. Type of medically assisted reproduction used in the conception cycle of the index birth was abstracted from the electronic medical records by trained study staff and dichotomized: ART procedures (e.g. fresh or frozen IVF protocols, including ICSI) versus non-ART protocols (e.g. IUI with or without ovulation induction/stimulation; ovulation induction/stimulation with timed intercourse, or non-medically assisted/naturally conceived).

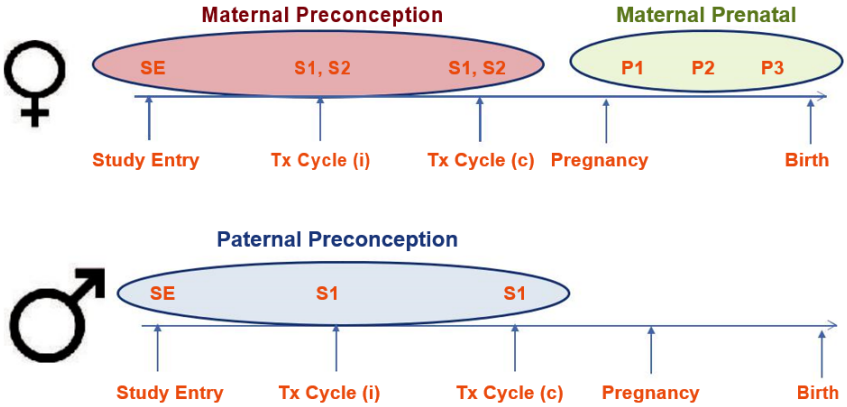

Figure 2. Maternal and paternal assessment in the Environment and Reproductive Health (EARTH) study. Female participants: Study Entry (SE) Assessment includes: baseline urine and completion of the Baseline and Full Questionnaires. Treatment (Tx) Cycle (i) connotes any number of followed cycles including those treated with IVF based technologies or non-IVF based procedures. Assessment occurs at two points in time during each Treatment (Tx) Cycle: S1—includes the first spot urine sample during the follicular phase of the cycle (Days 3–9). S2—includes the second spot urine sample collected at the time of scheduled treatment procedure (oocyte retrieval, embryo transfer or intrauterine insemination). All SE, S1 and S2 samples represent the maternal preconception BPA/BPS exposure period. Treatment (Tx) Cycle (c) connotes the index cycle of conception. Clinical information about the mode of conception (IVF-based, non-IVF based, or non-medically assisted) is abstracted from electronic medical records by trained study staff. Assessment in pregnancy: P1, P2 and P3—includes a single urine sample collected in the first, second and third trimesters of pregnancy, respectively. P1, P2 and P3 samples collected following the index conception represent the maternal prenatal BPA/BPS exposure period. Male participants: Study Entry (SE) Assessment includes: baseline urine and completion of the Baseline and Full Questionnaires. Assessment at Treatment (Tx) cycle: S1 includes a spot urine sample collected on the day their female partner undergoes their scheduled fertility treatment procedure. All SE and S1 samples collected up to and including Tx Cycle (c)—the index cycle of conception—represent the paternal preconception BPA/BPS exposure period. Republished from Messerlian et al. (2018).

Statistical analysis

Urinary BPA and BPS concentrations were adjusted for urine dilution by multiplying each concentration by [(SGp − 1)/(SGi − 1)], where SGi is the specific gravity of the participant's sample and SGp is the mean specific gravity for all male or all female participants included in the study samples (Pearson et al., 2009). The specific gravity-adjusted bisphenol concentrations were natural log-transformed to standardize the distribution and reduce the influence of extreme values. We estimated mean paternal and maternal preconception BPA and BPS concentrations by averaging each participant's natural log-bisphenol A or S concentration obtained from study entry and at each treatment cycle up to and including the cycle of the index conception of the singleton (Fig. 2). We estimated mean maternal prenatal bisphenol concentration by averaging all trimester-specific natural log-bisphenol A or S concentrations obtained from women during the index pregnancy. When only one urine sample was available the bisphenol concentration for that single sample was used. We calculated descriptive statistics for BPA and BPS concentrations for the three exposure windows as well as the percentage of values below the LOD. We also calculated Pearson correlation coefficients for each natural log-bisphenol concentration between couples (paternal versus maternal) and within women across exposure windows (maternal preconception versus maternal prenatal).

We estimated associations of paternal and maternal preconception and maternal prenatal natural log-bisphenol A or S concentrations and birth weight and head circumference using multivariable linear regression. Beta coefficients and 95% CIs represent the mean difference in birth weight (g) and head circumference (cm) for each natural log-unit increase in urinary bisphenol concentration. In order to assess potential non-linear associations, we fit multivariable linear models to evaluate change in birth weight and head circumference across tertiles of urinary BPA concentrations using the lowest tertile as the reference. We conducted statistical tests for trend across tertiles using the urinary bisphenol concentration as an ordinal level indicator variable (1, 2, 3) of each tertile in the regression model.

We selected a priori covariates as potential confounders based on substantive knowledge using a directed acyclic graph and examined unadjusted and covariate-adjusted results (Supplementary Fig. S1). Maternal preconception/prenatal window covariate-adjusted models included: maternal age and BMI (continuous); maternal education (<college, college, graduate degree); smoking status (never smoked versus ever smoked, defined as a current or former smoker); and ART versus non-ART-based treatment. Paternal preconception window covariate-adjusted models included paternal and maternal age and BMI (continuous); paternal and maternal smoking (ever/never); maternal education (<college, college, graduate degree); ART versus non-ART-based treatment. As gestational age may be a causal intermediate between bisphenol exposure and birth outcomes (Wilcox et al., 2011), we initially did not include this in our main covariate-adjusted model. However, we additionally adjusted for gestational age in separate models to assess the potential change in estimates (Ananth and Schisterman, 2017). We also adjusted for bisphenol co-exposures by partner or prenatal window by adding the specific bisphenol concentration into each individual multivariable model. That is, concentrations of BPA (or BPS) from two different

windows were entered into the same model in order to account for possible differences in effects by exposure window (Messerlian et al., 2017). In models examining head circumference, we also controlled for mode of delivery (vaginal versus caesarian birth).

Given previously reported sex-specific differences between BPA exposure and size at birth (Veiga-Lopez et al., 2015; Tomza-Marciniak et al., 2018), we conducted a stratified sensitivity analysis by adding a cross-product term for interaction (bisphenol concentration * sex), with a P-value for the interaction term <0.20 indicating possible effect measure modification by infant sex on the multiplicative scale. We also conducted post-hoc analyses to assess the relationship between urinary BPA concentrations and gestational age (days, continuous) using multivariable linear regression. All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, USA). The significance level was set at $P \leq 0.05$, and all tests were two-tailed.

Results

Study cohort

The study cohort included 346 mothers and 190 fathers (190 couples) with an average age of 34.8 and 35.8 years, respectively, at the time of enrollment. Table I shows parental characteristics of study participants. Among the 346 singletons, the mean (SD) for birth weight and head circumference was 3373 (534) g and 34.3 (2.5) cm respectively; 7.5% were born preterm (<37 weeks gestation) and 3.5% at low birth weight (<2.5 kg) (see Supplementary Table SI).

Table I. Parental characteristics from 346 mothers and 190 fathers participating in the Environment and Reproductive Health (EARTH) Study.

Parental characteristic	Mothers N = 346	Fathers N = 190
Age (years)		
Mean (SD)	34.8 (3.9)	35.8 (4.4)
Age > 35, n (%)	145 (42)	104 (55)
Race, n (%)		
White	298 (86)	167 (88)
Black	7 (2)	3 (2)
Asian	28 (8)	13 (7)
Other	13 (4)	7 (4)
BMI (kg/m^2)		
Mean (SD)	24.1 (4.2)	27.1 (4.3)
BMI > 25, n (%)	107 (31)	130 (68)
Education, n (%)		
<College	20 (6)	26 (14)
College graduate	112 (32)	50 (26)
Graduate degree	189 (55)	76 (40)
Missing	25 (7)	38 (20)
Smoking status, n (%)		
Never	256 (74)	132 (69)
Ever (former or current)	90 (26)	58 (31)
Infertility diagnosis, n (%)		
Female factor	87 (25)	58 (31)
Male factor	114 (33)	56 (29)
Unexplained	145 (42)	76 (40)
Nulliparous, n (%)	287 (83)	

Urinary bisphenol concentrations

Geometric means of the specific gravity-adjusted urinary BPA concentrations were 1.6, 1.5 and 1.2 ng/ml for paternal preconception, maternal preconception, and maternal prenatal BPA, respectively (Supplementary Table SII). The specific gravity-adjusted geometric means for BPS were: 0.51, 0.45 and 0.33 ng/ml for the corresponding exposure windows. BPA detection frequencies ranged between 61 and 81% and between 53 and 68% for BPS. These values were within ranges reported for US adults (Calafat et al., 2008; Ye et al., 2015). Bisphenol concentrations were weakly correlated between couples and within subjects across exposure windows, with Pearson correlation coefficients ranging from 0.10 to 0.26 (Supplementary Table SIII).

Maternal preconception window

Maternal preconception urinary BPA concentrations were inversely associated with birth weight (Table II). After adjusting for covariates in the main model, each log-unit increase in urinary BPA concentration was associated with a 119 g (95% CI: −212, −27) decrease in birth weight. Further adjustment for gestational age attenuated the magnitude of the association (β = −79; 95% CI: −153, −5) (Table II). Models that additionally adjusted for maternal prenatal BPA concentrations did not substantially change the results (Table II). When maternal preconception urinary BPA concentrations were categorized in tertiles, a significant negative trend in birth weight over increasing BPA tertiles was observed (test for P-trend, 0.03) (Table III). Additionally, maternal urinary preconception BPA concentrations were inversely associated with head circumference in adjusted models (Table IV). Each log-unit increase in BPA concentration was associated with a 0.72 cm (95% CI: −1.3, −0.1) decrease in head circumference (Table IV). Although BPA tertiles were not significantly associated with head circumference in adjusted models, a suggestive trend was observed (data not shown). No sex-specific associations or interaction by sex were observed between maternal preconception urinary BPA concentrations and birth weight (Supplementary Table SIV). Maternal preconception urinary BPS concentrations were not associated with birth weight or head circumference (Tables II and IV).

Maternal prenatal window

Maternal prenatal BPA concentrations were associated with non-significant birth weight decrement. After adjustment for covariates, each log-unit increase in urinary BPA concentration during pregnancy was associated with a 75 g (95% CI: −153, 2) decrease in birth weight (Table II). This association did not differ by newborn sex (Supplemental Table IVA). Models that additionally adjusted for maternal preconception BPA concentrations substantially attenuated this association (Table II). Maternal prenatal BPS concentrations were not associated with birth weight (Tables II). However, there was some evidence of effect measure modification by infant sex (P-interaction, 0.10) with boys exhibiting non-significant decreased birth weight compared with increased birth weight in girls in relation to higher BPS concentrations (Supplementary Table SIV). Neither maternal prenatal BPA nor pre-natal BPS concentrations were associated with head circumference (Table IV).

Table II. Association of natural log-unit increase in paternal preconception, maternal preconception, and maternal prenatal urinary bisphenol A (BPA) and bisphenol S (BPS) concentrations and birth weight (g) among all singletons.

Model 1[a]	Paternal preconception			Maternal preconception			Maternal prenatal		
	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value	N
BPA	−58 (−158, 43)	0.26	190	−113 (−203, −24)	0.01	342	−78 (−155, −2)	0.05	315
BPS[f]	–	–	–	−8 (−139, 124)	0.91	90	22 (−82, 126)	0.68	107
Model 2	Paternal preconception[b]			Maternal preconception[c]			Maternal prenatal[c]		
	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value	N
BPA	−45 (−146, 55)	0.38	178	−119 (−212, −27)	0.01	318	−75 (−153, 2)	0.06	292
BPS[f]	–	–	–	9 (−119, 138)	0.89	83	24 (−78, 127)	0.64	100
Model 3	Paternal preconception[d]			Maternal preconception[d]			Maternal prenatal[d]		
	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value	N
BPA	−54 (−143, 35)	0.23	178	−79 (−153, −5)	0.04	318	−38 (−101, 25)	0.24	292
BPS[f]	–	–	–	23 (−85, 131)	0.68	83	13 (−83, 109)	0.79	100
Model 4	Paternal preconception[e]			Maternal preconception[e]			Maternal prenatal[e]		
	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value	N
BPA	−48 (−151, 55)	0.36	166	−89 (−186, 8)	0.07	289	−46 (−127, 36)	0.27	289
BPS[f]	–	–	–	144 (2, 286)	0.05	73	−67 (−210, 76)	0.36	73

[a]Model 1: Unadjusted model.
[b]Model 2: Covariate-Adjusted Paternal Preconception Model: adjusted for maternal and paternal age (continuous); maternal and paternal BMI (continuous); maternal education (<college, college, graduate degree); maternal and paternal smoking (ever/never); and ART (yes/no).
[c]Model 2: Covariate-Adjusted Maternal Preconception and Prenatal Models: adjusted for maternal age (continuous); maternal BMI (continuous); maternal education (<college, college, graduate degree); maternal smoking (ever/never); and ART (yes/no).
[d]Model 3: Covariate Adjusted + Gestational Age Model: covariates from Model 2 plus additional adjustment for gestational age (continuous, days).
[e]Model 4: Co-Adjusted Model: covariates from Model 2 plus additional co-adjustment for:
Paternal Preconception Model includes respective maternal prenatal bisphenol concentration. Maternal Preconception Model includes respective maternal prenatal bisphenol concentration. Maternal Prenatal Model includes respective maternal preconception bisphenol concentration.
[f]Paternal BPS concentrations not analyzed due to the small number of male participants with available BPS data (N = 37).

Paternal preconception window

No associations were observed between paternal preconception urinary BPA concentrations and birth weight or head circumference: β = −45 g; 95% CI: −146, 55 and β = −0.15 cm; 95% CI: −0.68, 0.37, respectively (Tables II and IV).

Discussion

Maternal preconception BPA concentrations—but not paternal pre-conception BPA concentrations—were negatively associated with both birth weight and head circumference among singletons born to subfertile couples from a large fertility center. Maternal prenatal BPA concentrations also showed suggestive associations with birth size. No sex-specific differences were evident. There was also no evidence of associations with BPS concentrations across all exposure windows. However, given the limited detection frequencies (53–68%), the smaller sample size with BPS measured in our cohort, and the fact that this is the first epidemiologic study on BPS exposure during the preconception period, more research is warranted.

Our main results showed a robust inverse association between maternal preconception urinary BPA concentrations and birth size, while associations in relation to maternal prenatal BPA concentrations were more tenuous.

In support of a maternal preconception effect, the observed negative association with birth weight was maintained even after additional adjustment for maternal prenatal BPA concentrations, whereas the converse did not occur in the prenatal BPA exposure models. Furthermore, a dose–response trend was observed across increasing BPA tertiles for the maternal preconception—but not maternal prenatal—window. Because additional adjustment for gestational age attenuated both preconception and prenatal BPA associations, we conducted a post-hoc analysis to assess the relationship between urinary BPA concentrations and gestational age (Supplementary Table SV). Negative trends were observed between both maternal pre-conception and maternal prenatal BPA concentrations and gestational age (β = −1.5 days; 95% CI: −3.6, 0.55 and β = −1.5 days; 95% CI: −3.4, 0.34, respectively), suggesting the possibility of a partial mediating effect by gestational age (Supplementary Table SV). Although maternal preconception BPA associations were attenuated after adjusting for covariates, gestational age, as well as maternal prenatal BPA co-exposure, associations resisted all these adjustments and were particularly robust.

BPA has been classified as an ovarian toxicant based on both experimental and human evidence (Souter et al., 2013;

Table III. Change in birth weight (g) by tertile (T) of urinary bisphenol A (BPA) concentrations in paternal preconception, maternal preconception and maternal prenatal windows of exposure among all singletons.

Model 2	Paternal preconception[a]			Maternal preconception[b]			Maternal prenatal[b]		
	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value	N
BPA									
T1	Ref.		60	Ref.		106	Ref.		98
T2	−117(−303, 70)	0.22	59	−53(−196, 89)	0.46	106	−58(−86, 201)	0.43	97
T3	−40(−219, 140)	0.67	59	−157(−300, −13)	0.03	106	−70(−215, 75)	0.34	97
P-trend		0.68			0.03			0.30	
Model 3	Paternal preconception[c]			Maternal preconception[c]			Maternal prenatal[c]		
	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value	N	Beta(95% CI)	P-value	N
BPA									
T1	Ref.		60	Ref.		106	Ref.		98
T2	−95(−262, 72)	0.26	59	−66(−180, 47)	0.25	106	32(−84, 148)	0.59	97
T3	−53(−213, 107)	0.52	59	−130(−245, −15)	0.03	106	−16(−133, 102)	0.79	97
P-trend		0.53			0.03			0.80	

[a]Model 2: Covariate-Adjusted Paternal Preconception Model: adjusted for maternal and paternal age (continuous); maternal and paternal BMI (continuous); maternal education (<college, college, graduate degree); maternal and paternal smoking (ever/never); and ART (yes/no).

[b]Model 2: Covariate-Adjusted Maternal Model: adjusted for maternal age (continuous); maternal BMI (continuous); maternal education (<college, college, graduate degree); maternal smoking (ever/never); and ART (yes/no).

[c] Model 3: Covariate Adjusted + Gestational Age Model: covariates from Model 2 plus additional adjustment for gestational age (continuous, days).

Table IV. Association of natural log-unit increase in paternal preconception, maternal preconception, and maternal prenatal urinary bisphenol A (BPA) and bisphenol S (BPS) concentrations and head circumference (cm) among all singletons.

Model 1[a]	Paternal preconception			Maternal preconception			Maternal prenatal		
	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value	N
BPA	−0.01 (−0.55, 0.53)	0.97	133	−0.47 (−1.04, 0.10)	0.10	210	−0.25 (−0.70, 0.19)	0.26	196
BPS[e]	–	–	–	−0.09 (−1.10, 0.91)	0.86	62	0.27 (−0.56, 1.1)	0.53	77
Model 2	Paternal preconception[b]			Maternal preconception[c]			Maternal prenatal[c]		
	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value	N
BPA	−0.15 (−0.68, 0.37)	0.57	126	−0.72 (−1.3, −0.16)	0.01	210	−0.33 (−0.77, 0.11)	0.14	196
BPS[e]	–	–	–	0.11 (−1.2, 0.95)	0.84	62	−0.09 (−1.05, 0.87)	0.85	77
Model 3	Paternal preconception[d]			Maternal preconception[d]			Maternal prenatal[d]		
	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value	N	Beta (95% CI)	P-value	N
BPA	−0.14 (−0.66, 0.37)	0.59	126	−0.63 (−1.2, −0.09)	0.02	210	−0.31 (−0.74, 0.11)	0.14	196
BPS[e]	–	–	–	−0.11 (−1.2, 0.96)	0.84	62	−0.02 (−0.99, 0.94)	0.96	77

[a]Model 1: Unadjusted model.

[b]Model 2: Covariate-Adjusted Paternal Preconception Model: adjusted for maternal and paternal age (continuous); maternal and paternal BMI (continuous); maternal education (<college, college, graduate degree); maternal and paternal smoking (ever/never); ART (yes/no); and mode of delivery (vaginal versus c-section).

[c]Model 2: Covariate-Adjusted Maternal Preconception and Prenatal Models: adjusted for maternal age (continuous); maternal BMI (continuous); maternal education (<college, college, graduate degree); maternal smoking (ever/never); ART (yes/no); and mode of delivery (vaginal versus c-section).

[d]Model 3: Covariate Adjusted + Gestational Age Model: covariates from Model 2 plus additional adjustment for gestational age (continuous, days).

[e]Paternal BPS concentrations not analyzed due to the small number of male participants with available BPS data (N = 37).

Peretz et al., 2014), and there is sufficient experimental evidence to support adverse effects on female reproductive physiology (Santangeli et al., 2017). BPA has been shown to affect early oogenesis and follicle formation, female steroidogenesis, oocyte quantity, quality and fertilization, uterine receptivity and implantation, embryo development and the placenta in experimental and some epidemiologic studies (Susiarjo et al., 2013; Peretz et al., 2014). Increasing evidence also highlights the potential of BPA to interfere with epigenetic mechanisms, which may mediate part of its effects on female reproduction (Santangeli et al., 2017). As we found the strongest BPA-associations when maternal exposure was assessed before conception, and BPA has

shown to alter the epigenetic programming of human and mammalian oocytes leading to functional impairments (Eichenlaub-Ritter and Pacchierotti, 2015), a potential early effect of BPA at the ovary (Ikezuki et al., 2002) affecting oocyte quality and later resulting in reduced embryo viability/ development might be proposed (Yuan et al. 2018).

Epidemiologic studies have provided inconsistent results regarding size at birth. Some studies reported lower birth size in response to higher urinary BPA concentrations during pregnancy, in line with our maternal prenatal results (Chou et al., 2011; Snijder et al., 2013; Troisi et al., 2014; Huo et al., 2015; Veiga-Lopez et al., 2015), whereas others found no association (Wolff et al., 2008; Philippat et al., 2011, 2014; Casas et al., 2015) or even higher birth size (Lee et al., 2014; Ding et al., 2017). Most studies used a single spot urine sample for exposure characterization, which might partially explain discrepancies (Snijder et al., 2013; Perrier et al., 2016). Conversely, only one study has evaluated associations between paternal and maternal preconception urinary BPA concentrations and birth outcomes (Smarr et al., 2015). This study also relied on a single spot urine sample for BPA exposure characterization. Although the authors observed some trends between maternal preconception quartiles of BPA concentrations and smaller size at birth, no obvious associations were found (Smarr et al., 2015). Our results are overall consistent with existing research and expand these findings by reporting clear evidence of the maternal preconception period as a potentially critical window for BPA effects on perinatal outcomes.

Only one previous study analyzed the relationship between urinary BPS concentrations during pregnancy and birth weight, reporting that mothers with a detectable concentration of BPS at any of the study visits had lower weight females (Ferguson et al., 2018). Although we did not observe associations between BPS concentrations and birth size— probably influenced by the small subsample and low detection frequencies—increasing experimental research points to a similar or even worse reprotoxic and/or embryofetotoxic potential of BPS compared to BPA (Žalmanová et al. 2017; Campen et al. 2018; Gingrich et al. 2018). Additional epidemiologic research is needed, especially in new cohorts with more recent recruitments, since the substitution of BPA with BPS is already taking place in consumer products, and this process seems to be occurring relatively faster in the US than other countries (Wu et al. 2018). Future follow-up of the EARTH Study will lead to more detailed analyses of the relationship between BPS exposure and reproductive health.

A major strength of our study is the prospective preconception design of the EARTH cohort. Studying subfertile couples from a large fertility center allowed us to assess three critical windows of exposure, including mother's and father's exposure before conception. Although the generalizability of our findings to non-subfertile couples is uncertain, a previous analysis in the EARTH cohort studying paternal pre-conception exposure to phthalates and birth size (Messerlian et al., 2017) was in line with results from a non-subfertile preconception cohort (Smarr et al., 2015). Because we were limited by a lower number of fathers compared to mothers, future analyses with a higher number of male participants will further address whether paternal pre-conception exposure to bisphenols is associated with perinatal outcomes. Another major strength is that most participants provided multiple urine samples for each critical window of exposure, allowing us to better characterize exposure to bisphenols, and thus reduce the chances of exposure misclassification and its expected attenuation bias (Perrier et al., 2016). Even so, some level of exposure misclassification cannot be disregarded given the short biological half-lives of these non-persistent chemicals and episodic nature of the exposures.

Conclusions

Our main results show a clear and robust inverse association between maternal preconception urinary BPA concentrations and infant birth weight and head circumference. Although maternal prenatal BPA concentrations also showed a suggestive association towards decreased birth weight, maternal preconception associations tended to remain after additional adjustment for maternal prenatal BPA exposure, whereas the opposite was not observed. No associations were found for paternal preconception BPA exposure. Although limited by small numbers, and low detection frequencies, no associations were observed for BPS exposure across exposure windows. Taken together, our findings highlight the maternal preconception period as a sensitive, yet largely unexplored critical window for BPA effects on birth size. Given the ubiquity of bisphenol exposures (Calafat et al., 2008), the predictive value of size at birth for future health (Basso, 2008), and that BPA has been classified as a reproductive toxicant and endocrine disruptor based on both experimental and human evidence (ECHA, 2016 and 2017; Peretz et al., 2014), we consider these findings to be of public health importance.

Supplementary data

Supplementary data are available at Human Reproduction online.

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Authors' role

C.M. conceived and designed the study and analysis plan. A.M.C. con-ducted the chemical analysis of urine samples and produced the chem-ical database. J.B.F contributed to the acquisition of data and follow-up of the participants. All statistical analyses were conducted by C.M. in consultation with P.W., V.M. and L.M. V.M. and C.M. prepared the first draft of the article and all authors critically revised the article for important intellectual content, approving the final version.

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Conflict of interest

The authors declare no actual or potential competing financial conflicts of interest. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC). Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the US Department of Health and Human Services.

References

ACOG. Committee opinion no 611: method for estimating due date. Obstet Gynecol 2014;124:863–866.

Ananth CV, Schisterman EF. Confounding, causality, and confusion: the role of intermediate variables in interpreting observational studies in obstetrics. Am J Obstet Gynecol 2017;217:167–175.

Barker DJP. The origins of the developmental origins theory. J Intern Med 2007;261:412–417.

Basso O. Birth weight is forever. Epidemiology 2008;19:204–205.

Braun JM, Messerlian C, Hauser R. Fathers matter: why it's time to con-sider the impact of paternal environmental exposures on children's health. Curr Epidemiol Rep 2017;4:46–55.

Calafat AM, Ye X, Wong L-Y, Reidy JA, Needham LL. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003–2004.Environ Health Perspect 2008;116:39–44.

Campen KA, Kucharczyk KM, Bogin B, Ehrlich JM, Combelles CMH. Spindle abnormalities and chromosome misalignment in bovine oocytes after exposure to low doses of bisphenol A or bisphenol S. Hum Reprod 2018;33:895–904.

Casas M, Valvi D, Ballesteros-Gomez A, Gascon M, Fernández MF, Garcia-Esteban R, Iñiguez C, Martínez D, Murcia M, Monfort N et al. Exposure to bisphenol A and phthalates during pregnancy and ultrasound mea-sures of fetal growth in the INMA-Sabadell cohort. Environ Health Perspect 2015;124:521–528.

Chen Q, Yan W, Duan E. Epigenetic inheritance of acquired traits through sperm RNAs and sperm RNA modifications. Nat Rev Genet 2016;17: 733–743.

Chou W-C, Chen J-L, Lin C-F, Chen Y-C, Shih F-C, Chuang C-Y. Biomonitoring of bisphenol A concentrations in maternal and umbilical cord blood in regard to birth outcomes and adipokine expression: a birth cohort study in Taiwan. Environ Health 2011;10:94.

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

Ding G, Wang C, Vinturache A, Zhao S, Pan R, Han W, Chen L, Wang W, Yuan T, Gao Y et al. Prenatal low-level phenol exposures and birth out-comes in China. Sci Total Environ 2017;607–608:1400–1407.

ECHA (European Chemicals Agency). Member State Committee Support Document for Identification of 4,4'-Isopropylidenediphenol (Bisphenol A) as a Substance of Very High Concern Because of its Toxic For Reproduction (Article 57 C), EC 201-245-8, CAS 80-05-7. 2016. https://echa.europa.eu/documents/10162/b10d6a00-8e47-9b14-4f61-c779a8dc8450.

ECHA (European Chemicals Agency). Member State Committee Unanimously Agrees That Bisphenol A is an Endocrine Disruptor. 2017. https://echa.europa.eu/es/-/msc-unanimously-agrees-that-bisphenol-a-is-an-endocrine-disruptor.

Eichenlaub-Ritter U, Pacchierotti F. Bisphenol A effects on mammalian oogenesis and epigenetic integrity of oocytes: a case study exploring risks of endocrine disrupting chemicals. Biomed Res Int 2015;2015: 698795.

Fan Y, Ding S, Ye X, Manyande A, He D, Zhao N, Yang H, Jin X, Liu J, Tian C et al. Does preconception paternal exposure to a physiologically rele-vant level of bisphenol A alter spatial memory in an adult rat? Horm Behav 2013;64:598–604.

Ferguson KK, Meeker JD, Cantonwine DE, Mukherjee B, Pace GG, Weller D, McElrath TF. Environmental phenol associations with ultrasound and delivery measures of fetal growth. Environ Int 2018;112:243–250.

Gingrich J, Pu Y, Roberts J, Karthikraj R, Kannan K, Ehrhardt R, Veiga-Lopez A. Gestational bisphenol S impairs placental endocrine function and the fusogenic trophoblast signaling pathway. Arch Toxicol 2018;92: 1861–1876.

Giulivo M, Lopez de Alda M, Capri E, Barceló D. Human exposure to endocrine disrupting compounds: their role in reproductive systems, metabolic syndrome and breast cancer. A review. Environ Res 2016;151: 251–264.

Hornung RWRL. Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg 1990;5:46–51.

Huo W, Xia W, Wan Y, Zhang B, Zhou A, Zhang Y, Huang K, Zhu Y, Wu C, Peng Y et al. Maternal urinary bisphenol A levels and infant low birth weight: a nested case–control study of the Health Baby Cohort in China. Environ Int 2015;85:96–103.

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

Lee B-E, Park H, Hong Y-C, Ha M, Kim Y, Chang N, Kim B-N, Kim YJ, Yu S-D, Ha E-H. Prenatal bisphenol A and birth outcomes: MOCEH (Mothers and Children's Environmental Health) study. Int J Hyg Environ Health 2014;217:328–334.

Machtinger R, Orvieto R. Bisphenol A, oocyte maturation, implantation, and IVF outcome: review of animal and human data. Reprod Biomed Online 2014;29:404–410.

Manikkam M, Tracey R, Guerrero-Bosagna C, Skinner MK. Plastics derived endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transge-nerational inheritance of obesity, reproductive disease and sperm epi-mutations. PLoS One 2013;8:e55387.

Messerlian C, Braun JM, Mínguez-Alarcón L, Williams PL, Ford JB, Mustieles V, Calafat AM, Souter I, Toth T, Hauser R. Paternal and maternal urinary phthalate metabolite concentrations and birth weight of singletons con-ceived by subfertile couples. Environ Int 2017;107:55–64.

Messerlian C, Williams PL, Ford JB, Chavarro JE, Mínguez-Alarcón L, Dadd R, Braun JM, Gaskins AJ, Meeker JD, James-Todd T et al. The Environment and Reproductive Health (EARTH) Study: a prospective preconception cohort. Hum Reprod Open 2018:1–11. doi:10.1093/hro-pen/hoy001.

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

Mustieles V, Pérez-Lobato R, Olea N, Fernández MF. Bisphenol A: human exposure and neurobehavior. Neurotoxicology 2015;49:174–184.

Pearson MA, Lu C, Schmotzer BJ, Waller LA, Riederer AM. Evaluation of physiological measures for correcting variation in urinary output: implica-tions for assessing environmental chemical exposure in children. J Expo Sci Environ Epidemiol 2009;19:336–342.

Peretz J, Vrooman L, Ricke WA, Hunt PA, Ehrlich S, Hauser R, Padmanabhan V, Taylor HS, Swan SH, VandeVoort CA et al. Bisphenol a and reproductive health: update of experimental and human evidence, 2007–2013. Environ Health Perspect 2014;122:775–786.

Perrier F, Giorgis-Allemand L, Slama R, Philippat C. Within-subject pooling of biological samples to reduce exposure misclassification in biomarker-based studies. Epidemiology 2016;27:378–388.

Philippat C, Botton J, Calafat AM, Ye X, Charles M-A, Slama R, EDEN Study Group. Prenatal exposure to phenols and growth in Boys. Epidemiology 2014;25:625–635.

Philippat C, Mortamais M, Chevrier C, Petit C, Calafat AM, Ye X, Silva MJ, Brambilla C, Pin I, Charles M-A et al. Exposure to phthalates and phe-nols during pregnancy and offspring size at birth. Environ Health Perspect 2011;120:464–470.

Rando OJ. Daddy issues: paternal effects on phenotype. Cell 2012;151: 702–708.

Rochester JR, Bolden AL. Bisphenol S and F: a systematic review and com-parison of the hormonal activity of bisphenol A substitutes. Environ Health Perspect 2015;123:643–650.

Santangeli S, Maradonna F, Olivotto I, Piccinetti CC, Gioacchini G, Carnevali O. Effects of BPA on female reproductive function: the involvement of epigenetic mechanism. Gen Comp Endocrinol 2017;245: 122–126.

Silva M, Samandar E, Preaujr J, Reidy J, Needham L, Calafat AM. Quantification of 22 phthalate metabolites in human urine. J Chromatogr B Analyt Technol Biomed Life Sci 2007;860:106–112.

Smarr MM, Grantz KL, Sundaram R, Maisog JM, Kannan K, Louis GMB. Parental urinary biomarkers of preconception exposure to bisphenol A and phthalates in relation to birth outcomes. Environ Health 2015;14:73.

Snijder CA, Heederik D, Pierik FH, Hofman A, Jaddoe VW, Koch HM, Longnecker MP, Burdorf A. Fetal growth and prenatal exposure to bis-phenol A: the generation R study. Environ Health Perspect 2013;121: 393–398.

Souter I, Smith KW, Dimitriadis I, Ehrlich S, Williams PL, Calafat AM, Hauser R. The association of bisphenol-A urinary concentrations with antral follicle counts and other measures of ovarian reserve in women undergoing infertility treatments. Reprod Toxicol 2013;42:224–231.

Susiarjo M, Sasson I, Mesaros C, Bartolomei MS. Bisphenol a exposure dis-rupts genomic imprinting in the mouse. PLoS Genet 2013;9:e1003401.

Tomza-Marciniak A, Stępkowska P, Kuba J, Pilarczyk B. Effect of bisphenol A on reproductive processes: a review of in vitro, in vivo and epidemio-logical studies. J Appl Toxicol 2018;38:51–80.

Trapphoff T, Heiligentag M, El Hajj N, Haaf T, Eichenlaub-Ritter U. Chronic exposure to a low concentration of bisphenol A during follicle culture affects the epigenetic status of germinal vesicles and metaphase II oocytes. Fertil Steril 2013;100:1758–1767.e1.

Troisi J, Mikelson C, Richards S, Symes S, Adair D, Zullo F, Guida M. Placental concentrations of bisphenol A and birth weight from births in the Southeastern U.S. Placenta 2014;35:947–952.

Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). Reprod Toxicol 2007;24:139–177.

Veiga-Lopez A, Kannan K, Liao C, Ye W, Domino SE, Padmanabhan V. Gender-specific effects on gestational length and birth weight by early pregnancy BPA exposure. J Clin Endocrinol Metab 2015;100:E1394– E1403.

Visentin S, Grumolato F, Nardelli GB, Di Camillo B, Grisan E, Cosmi E. Early origins of adult disease: low birth weight and vascular remodeling. Atherosclerosis 2014;237:391–399.

Wadhwa PD, Buss C, Entringer S, Swanson JM. Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. Semin Reprod Med 2009;27:358–368.

Wilcox AJ, Weinberg CR, Basso O. On the pitfalls of adjusting for gesta-tional age at birth. Am J Epidemiol 2011;174:1062–1068.

Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, Zhu C, Wetmur J, Calafat AM. Prenatal phenol and phthalate exposures and birth out-comes. Environ Health Perspect 2008;116:1092–1097.

Wu LH, Zhang XM, Wang F, Gao CJ, Chen D, Palumbo JR, Guo Y, Zeng EY. Occurrence of bisphenol S in the environment and implications for human exposure: a short review. Sci Total Environ 2018;615:87–98.

Xin F, Susiarjo M, Bartolomei MS. Multigenerational and transgenerational effects of endocrine disrupting chemicals: a role for altered epigenetic regulation? Semin Cell Dev Biol 2015;43:66–75.

Yang Y, Guan J, Yin J, Shao B, Li H. Urinary levels of bisphenol analogues in residents living near a manufacturing plant in south China. Chemosphere 2014;112:481–486.

Ye X, Wong L-Y, Kramer J, Zhou X, Jia T, Calafat AM. Urinary concentra-tions of bisphenol A and three other bisphenols in convenience samples of U.S. adults during 2000–2014. Environ Sci Technol 2015;49:11834– 11839.

Yuan M, Hu M, Lou Y, Wang Q, Mao L, Zhan Q, Jin F. Environmentally relevant levels of bisphenol A affect uterine decidualization and embryo implantation through the estrogen receptor/serum and glucocorticoid-regulated kinase 1/epithelial sodium ion channel α-subunit pathway in a mouse model. Fertil Steril 2018;109:735–744.

Žalmanová T, Hošková K, Nevoral J, Adámková K, Kott T, Šulc M, Kotíková Z, Prokešová Š, Jílek F, Králíčková M et al. Bisphenol S nega-tively affects the meotic maturation of pig oocytes. Sci Rep 2017;7:485.

Zheng H, Zhou X, Li D-K, Yang F, Pan H, Li T, Miao M, Li R, Yuan W. Genome-wide alteration in DNA hydroxymethylation in the sperm from bisphenol A-exposed men. In Rosenfeld CS, editor. PLoS One 2017;12: e0178535.

Supplementary Material

Maternal and paternal preconception exposure to bisphenols and size at birth

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Supplementary Table I. Birth Characteristics of 346 Singletons from the Environment and Reproductive Health (EARTH) Study.

Child Characteristics	All Children N=346	Boys n=180	Girls n=166
Birth Weight (grams)			
Mean (SD)	3373 (534)	3448 (532)	3292 (526)
min-max	1090-5040	1310-5040	1090-4650
Birth Weight Z-Score			
Mean (SD)	0.05 (1.0)	0.08 (1.04)	0.03 (0.96)
Head Circumference (cm)			
Mean (SD)	34.3 (2.5)	35.0 (2.4)	33.7 (2.4)
Low Birth Weight			
<2500 grams, n (%)	12 (3.5)	4 (2)	8 (5)
Gestational Age at Birth			
Mean weeks (min-max)	39.4 (29-42)	39.4 (32-42)	39.3 (29-42)
Mean days (min-max)	276 (205-294)	276 (224-294)	276 (205-294)
Preterm Birth			
<37 weeks, n (%)	26 (7.5)	12 (6.7)	14 (8)
Mode of Conception[1]			
ART	195 (56)	103 (57)	92 (55)
Non-ART	151 (44)	77 (43)	74 (45)

[1]Mode of conception: ART index infant conceived through fresh or frozen IVF protocols including intracytoplasmic sperm injection; Non-ART, index infant conceived through intrauterine insemination (IUI) with or without ovulation induction/ovarian stimulation; ovulation induction/ovarian stimulation with timed intercourse, or non-medically assisted/naturally conceived.

Supplementary Table II. Distribution of specific gravity normalized urinary bisphenol A (BPA) or bisphenol S (BPS) concentrations in 477 paternal preconception, 1370 maternal preconception, and 796 maternal prenatal urine samples from 346 mothers and 190 fathers from the Environment and Reproductive Health (EARTH) study participants.

Bisphenol	Sample Size (N)	LOD (ng/ml)	% > LOD	SG-Adjusted GM (GSD)	SG-Adjusted Median (ng/ml)	IQR 25^{th} - 75^{th} (ng/ml)
Paternal Preconception						
BPA	190	0.4	81	1.6 (0.09)	1.5	0.98 – 2.4
BPS	37	0.1	68	0.51 (0.06)	0.57	0.30 – 0.71
Maternal Preconception						
BPA	342	0.4	61	1.2 (0.04)	1.1	0.80 – 1.7
BPS	90	0.1	53	0.45 (0.03)	0.46	0.29 – 0.83
Maternal Prenatal						
BPA	315	0.4	68	1.2 (0.05)	1.2	0.78 – 1.73
BPS	107	0.1	55	0.33 (0.03)	0.31	0.19 – 0.55

Abbreviations: number of urinary samples (N); Limit of Detection (LOD); specific gravity (SG); geometric mean (GM); geometric standard deviation (GSD); interquartile range (IQR); 25^{th} percentile (25^{th}); 75^{th} percentile (75^{th}).

Supplementary Table III. Pearson correlations coefficients of bisphenol A (BPA) and bisphenol S (BPS) concentrations across windows of exposure.

Bisphenol	Windows of Exposure		
	Paternal Preconception x Maternal Preconception Pearson's *r*	Maternal Preconception x Maternal Prenatal Pearson's *r*	Paternal Preconception x Maternal Prenatal Pearson's *r*
BPA	0.16	0.26	0.25
BPS	0.25	0.24	0.10

Paternal and maternal preconception: N = 190 for BPA; N = 37 for BPS.
Maternal preconception and prenatal: N = 315 for BPA; N = 90 for BPS.
Paternal preconception and maternal prenatal: N = 190 for BPA; N = 37 for BPS

Supplementary Table IV. Sex-specific associations of natural log-unit increase in paternal preconception, maternal preconception, and maternal prenatal bisphenol A (BPA) and bisphenol S (BPS) concentrations and birth weight (g) among all singletons.

Window/ Bisphenol	All Infants			Boys		Girls		Sex x Bisphenol EMM* p-value
	Beta (95% CI)	p-value	N	Beta (95% CI)	N	Beta (95% CI)	N	
Paternal Preconception [a]								
BPA	-45 (-146, 55)	0.38	178	-54 (-195, 87)	93	-59 (-199, 80)	85	0.96
BPS[c]	-	-	-	-	-	-	-	-
Maternal Preconception [b]								
BPA	-119 (-212, -27)	0.01	318	-100 (-265, 65)	165	-93 (-254, 67)	153	0.96
BPS[c]	9 (-119, 138)	0.89	83	-145 (-459, 169)	45	210 (-73, 494)	38	0.10
Maternal Prenatal [b]								
BPA	-75 (-153, 2)	0.06	292	-62 (-229, 105)	152	-66 (-203, 71)	140	0.97
BPS[c]	24 (-78, 127)	0.64	100	-23 (-191, 145)	53	-5 (-234, 224)	47	0.90

[a] Paternal Preconception Model 2: adjusted for maternal and paternal age (continuous), maternal and paternal Body Mass Index (continuous), maternal education (<college, college, graduate degree), maternal and paternal smoking (ever/never); assisted reproductive technology (yes/no).

[b] Maternal Preconception and Prenatal Model 2: adjusted for maternal age (continuous), maternal Body Mass Index (continuous), maternal education (<college, college, graduate degree), maternal smoking (ever/never); and assisted reproductive technology (yes/no).

[c] Paternal BPS concentrations not analyzed due to the small number of male participants with available BPS data (N=37).

* EMM: effect measure modification.

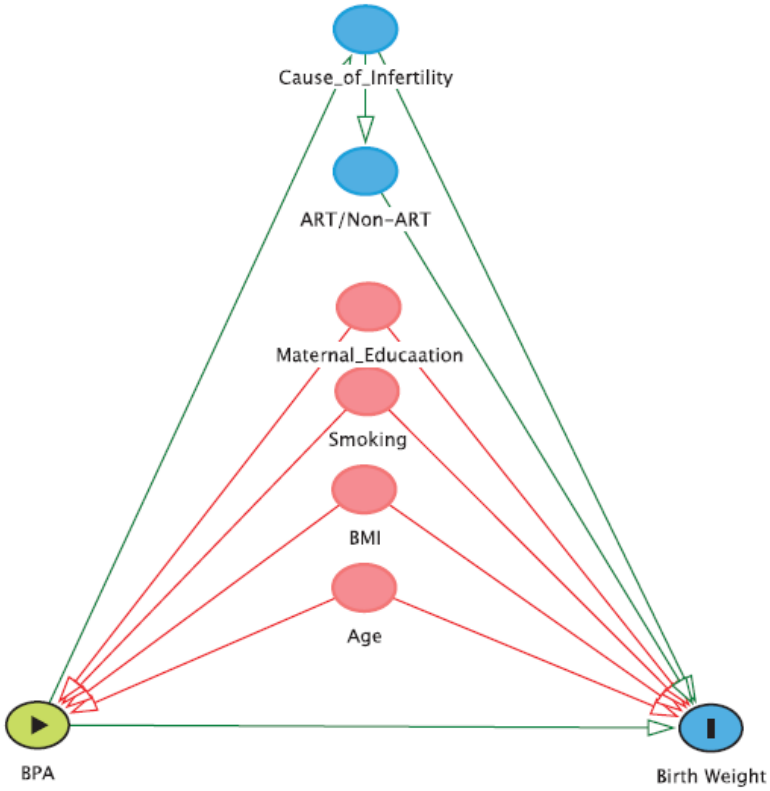
Supplementary Table V. Post-hoc analysis: Association of natural log-unit increase in paternal preconception, maternal preconception, and maternal prenatal bisphenol A (BPA) concentrations and gestational age (days) among all singletons.

Model 2	Paternal Preconception[1]			Maternal Preconception[2]			Maternal Prenatal[2]		
	Beta (95% CI)	p-value	N	Beta (95% CI)	p-value	N	Beta (95% CI)	p-value	N
BPA	0.43 (-1.8, 2.7)	0.70	178	-1.5 (-3.6, 0.55)	0.15	318	-1.5 (-3.4, 0.34)	0.11	292

[1]Paternal Preconception Model 2: adjusted for maternal and paternal age (continuous), maternal and paternal Body Mass Index (continuous), maternal education (<college, college, graduate degree), maternal and paternal smoking (ever/never); assisted reproductive technology (yes/no).

[2]Maternal Preconception and Prenatal Model 2: adjusted for maternal age (continuous), maternal Body Mass Index (continuous), maternal education (<college, college, graduate degree), maternal smoking (ever/never), and assisted reproductive technology (yes/no).

Figure III. Hypothesized directed acyclic graph (DAG) of bisphenol A (BPA) or bisphenol S (BPS) and birth weight.



PAPER 2

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

Paper #2

Bisphenol A and reproductive hormones and cortisol in peripubertal boys: The INMA-Granada cohort

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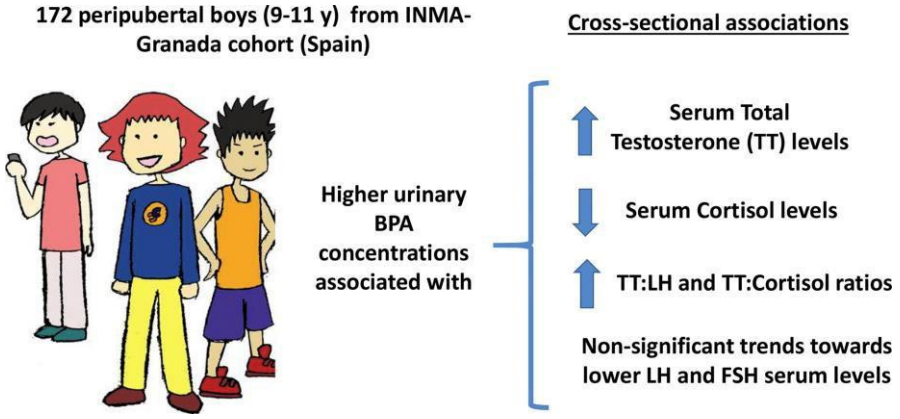
Graphical Abstract



Abstract

Introduction: Bisphenol A (BPA) is a well-known endocrine disrupting compound. Although several studies have investigated the effect of BPA exposure and reproductive hormones in humans, results have been inconsistent.

Objective: To explore the cross-sectional relationship between bisphenol A (BPA) exposure and reproductive hormones/cortisol among peripubertal boys.

Material and methods: Urinary BPA and serum hormones were assessed in 172 boys belonging to the INMA "Environment and Childhood" Granada birth cohort in their follow-up at 9–11 years of age. BPA concentrations were quantified by liquid chromatography-mass spectrometry, and levels of serum total testosterone (TT), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and cortisol were measured by electrochemiluminescence immunoassay.

Result(s): After adjustment for confounders, linear regression models showed that each natural-log unit increase in urinary BPA concentrations was associated with a 19% increase in geometric mean (GM) serum TT levels, and a 16% decrease in GM serum cortisol levels. When urinary BPA concentrations were categorized in tertiles, boys in the 3rd tertile showed 49% higher TT levels and 23% lower cortisol concentrations compared to boys in the 1st tertile. Additionally, urinary BPA concentrations were also significantly associated with higher TT:LH and TT:cortisol ratios, but not with serum LH or FSH levels.

Conclusion(s): Our results suggest the possible endocrine disrupting potential of BPA during this important period of development. Although action at the testis or pituitary cannot be ruled out, our findings are compatible with a possible involvement of BPA at the adrenal gland, resulting in a differential production of androgens/cortisol. However, given the cross-sectional design of our study, the heterogeneous results reported in the literature, and the scant experimental research on BPA effects at the adrenal gland, the present findings should be interpreted with caution.

1. Introduction

Bisphenol A (BPA) is a high-production man-made chemical widely used in the manufacture of polycarbonate plastics, epoxy resin liners of canned food, some dental sealants, medical devices, and thermal receipts, among other applications (Vandenberg et al., 2007). Diet is considered the predominant source of BPA exposure in the general population due to the leaching of BPA from packaging materials and can liners into food and beverages (Vandenberg et al., 2010). In addition, other sources and routes such as inhalation and dermal absorption may also contribute to total human exposure (Ehrlich et al., 2014; Michałowicz, 2014). Biomonitoring studies have confirmed widespread and continuous daily exposure to BPA, finding detectable levels in the urine of around 90% of the general population in industrialized countries (Becker et al., 2009; Calafat et al., 2008; Casas et al., 2011; Vandenberg et al., 2010). BPA has also been detected in other biological matrices such as maternal blood, amniotic fluid, cord and fetal serum, placenta, and maternal breast milk (Vandenberg et al., 2010).

BPA is a well-known endocrine-disrupting compound (EDC) with the potential to disturb the endocrine system and hormonal homeostasis even at environmentally relevant doses (Vandenberg et al., 2012). In vitro studies have shown that BPA can exert estrogenic actions through its binding to nuclear estrogen receptors (ERs) (Wetherill et al., 2007) and other membrane estrogen receptor families (Alonso-Magdalena et al., 2012). In addition, BPA has been found to display antagonistic activity at the androgen receptor (AR) (Molina-Molina et al., 2013), to alter the expression of steroidogenic enzymes, and to interfere with thyroid, PPAR-γ, and glucocorticoid signaling pathways (Mustieles et al., 2015). BPA can also modify the epigenetic programming of several endocrine-related pathways in different tissues, and these modifications can be transferred to offspring through either the maternal or paternal lineage (Manikkam et al., 2013).

Among other developmental effects, BPA has been shown to alter the reproductive system of experimental animals (vom Saal et al., 2007) and has been classified as an ovarian toxicant, based on both experimental and epidemiological data (Peretz et al., 2014). Although there is also evidence that BPA may be a testicular toxicant in experimental animals, data in humans are still equivocal (Peretz et al., 2014). Thus, epidemiologic studies have reported associations between BPA exposure and serum levels of total testosterone (TT), follicle- stimulating hormone (FSH), and luteinizing hormone (LH), among others, but results have been inconsistent (Mínguez-Alarcón et al., 2016). In addition, although BPA has been reported to interfere with the hypothalamic-pituitary-adrenal axis (HPA) in experimental animals and human in vitro models (Panagiotidou et al., 2014; Zhang et al., 2011), there is remarkably little epidemiological information on adrenal corticosteroids, including cortisol (Giesbrecht et al., 2016).

EDCs are thought to exert a stronger effect during specific development stages, and peripuberty may represent a critical window of exposure that has been less well explored (Zawatski and Lee, 2013; Sergeyev et al., 2017). Adrenarche constitutes the earlier phase of pubertal development during which adrenal glands mature, leading to a shift in the balance of adrenal steroidogenesis and resulting in increased androgen production in comparison to cortisol (McKenna et al., 1997). This process is independent of puberty onset and manifests clinically as the appearance of axillary and pubic hair (pubarche), usually around age 8 (Auchus, 2011). In contrast, puberty onset is characterized by maturation of the hypothalamic-pituitary-gonadal (HPG) axis, which triggers nocturnal releases of LH in boys, subsequently stimulating testicular Leydig cells to produce testosterone. Together with other stimuli, this then guides the development of functional gonads (gonadarche), secondary sexual characteristics, and reproductive capacity (Forest et al., 1976). During childhood and the prepubertal period, boys and girls have similar mean serum concentrations of testosterone (≈6 ng/dL), thought to be predominantly of adrenocortical origin and not requiring functional gonads (Forest et al., 1976). Testosterone levels begin to rise at approximately 10 years of age, peaking in adolescence at around the age of 17 years (Elmlinger et al., 2005). Cortisol levels remain quite constant during childhood, while increase throughout puberty (Soldin et al., 2005).

Because puberty is increasingly recognized as another crucial period which can be affected by environmental exposures (Zawatski and Lee, 2013), we aimed to assess the effect of peripubertal urinary BPA concentrations on the levels of several reproductive hormones (TT, FSH and LH) and cortisol in a group of Spanish boys.

2. Materials and methods

2.1. Study population

The INMA (Infancia y Medio Ambiente – Environment and Childhood) cohort is a population-based study in seven regions of Spain designed to explore the effects of environmental pollutants during pregnancy and early childhood on child growth and development (Guxens et al., 2012). The INMA-Granada subcohort includes 668 mother-son pairs recruited at delivery (2000−2002) in Granada province (Spain) (Fernandez et al., 2007). When the children reached the age of 9–11 years, all families in the cohort were invited to participate in the 2010–2012 follow-up (Perez-Lobato et al., 2016). Three hundred families (44.9% participation rate) gave their written informed consent. The study followed the principles of the declaration of Helsinki and was approved by the Ethics Committee of San Cecilio University Hospital. Out of 298 boys who provided a urine sample, 187 (63%) also agreed to donate a blood sample. The final sample included in the present study comprised 172 peripubertal boys for whom urinary BPA concentrations, reproductive hormone levels, and covariate data were available. No significant differences in socio-demographic or clinical characteristics were observed between the children in the present subsample (n = 172) and those in the rest of the follow-up sample (n = 126) (Supplemental Table 1).

2.2. BPA exposure assessment

All children provided a single non-fasting spot urine sample at 9–11 years of age; always between 5 pm and 8 pm. Urine was collected in 10-mL polypropylene tubes and immediately stored at −20 °C.

Total BPA (free plus conjugated) was determined by liquid chromatography-mass spectrometry in the laboratory of the Department of Analytical Chemistry of the University of Cordoba (Spain) as previously described (Perez-Lobato et al., 2016). The limits of detection (LOD) and quantification (LOQ) were 0.1 μg/L and 0.2 μg/L, respectively. All BPA concentrations were divided by urinary creatinine (Cr) concentrations (mg/dL). Urinary creatinine (mg/dL) was determined at the Public Health Laboratory of the Basque Country (Spain). For further in-formation on the analytical procedures used, see Perez-Lobato et al. (2016).

2.3. Reproductive hormones and cortisol levels

Within 1–3 days of the follow-up visit, fasting morning blood samples were collected from 187 boys at San Cecilio University Hospital, in accordance with recommendations of the hospital staff. Aliquots of serum were subsequently prepared and stored in polypropylene tubes at −80 °C for additional analyses. Serum TT, FSH, LH, and cortisol levels were measured by electrochemiluminescence immunoassay (Elecsys System, Roche Diagnostics) at the Analytical Unit of the University Hospital. The limit of detection (LOD) was 0.3 IU/L for FSH, 0.1 IU/L for LH, 0.025 ng/mL for TT, and 0.04 μg/dL for cortisol. Only 3–6% of hormone values was below the LOD and were treated as LOD/√2.

2.4. Physical examination

The weight and height of the children were measured, without shoes and in light clothing, using an electronic scale (TANITA model 354, Seca Corporation, Hamburg, Germany), and Body Mass Index (BMI) calculated (weight/height^2). Pubertal assessment was performed by visual inspection using established Tanner stage criteria for genitalia and pubic hair (Tanner and Whitehouse, 1976). Genital development was used for Tanner stage classification, ranging from 1 (undeveloped) to 5 (fully developed). Genital stage 1, in which penis, testes, and scrotum are unchanged compared to early childhood, represents non-puberty. Stage N 1, when gonads are observed to enlarge and change in texture and color, indicates puberty (Ferguson et al., 2014). Two trained physicians (OOH and CDA) conducted all pubertal assessments and anthropometric measurements.

2.5. Covariates

Known or suspected confounders of the association between the exposure and outcome were selected as covariates based on previous literature (Scinicariello and Buser, 2016), and/or a ≥10% change in beta coefficients of the independent variable. Thus, the final models were adjusted for maternal education (up to primary, secondary school or university), as an indicator of socio-economic status (SES); BMI (kg/m^2), because body composition is associated with steroid hormone levels; total serum cholesterol levels (mg/dL), because cholesterol is the common precursor of steroid hormones; and urinary cotinine (ng/mL), as a measure of exposure to second-hand smoke (Fernández et al., 2015), because tobacco has been related to BPA exposure (Geens et al., 2014; Casas et al., 2011) and nicotine can inhibit the activity of the crucial steroid enzyme aromatase. In order to improve the comparability of the results, final models for all outcomes were adjusted for the same set of covariates. Models were further adjusted for Tanner stage (I vs. II) in order to account for possible differences in the development stage and/or endogenous hormonal status. Only two children were in Tanner stage III (b1.5%), and were therefore included in the stage II group.

Total cholesterol serum levels, measured in the same serum samples in which hormones were assessed, and urinary cotinine levels, deter-mined by competitive enzyme immunoassay (EIA), were analyzed at the Analytical Unit of the San Cecilio University Hospital (Granada). Information on maternal education (up to primary, secondary school or university) was gathered from questionnaires completed by the mothers.

2.6. Statistical analysis

We performed a descriptive analysis of children characteristics using measures of central tendency and dispersion for numerical variables and frequencies for categorical variables. Cr-adjusted urinary BPA concentrations (μg/g) were employed in the analyses as a less biased method in comparison to other creatinine adjustments (O'Brien et al., 2016). Continuous natural log-transformed Cr-adjusted urinary BPA concentrations and natural log-transformed hormone levels were used in the analyses in order to reduce the skewness of distributions, as previously reported (Scinicariello and Buser, 2016). The association of BPA concentrations with reproductive hormone levels and cortisol was studied using multivariate linear regression models. Natural log-transformed Cr-adjusted BPA concentrations were used as the independent variable, both as continuous as well as categorized in tertiles. Because hormone levels were log-transformed, results were back-transformed by exponentiation of the β coefficients and their corresponding 95% CIs, i.e., the percentage change in geometric mean hormone levels associated with a natural log-unit increase in Cr-adjusted BPA concentrations, or as the percentage change for the second and third tertiles in comparison to the first tertile of BPA exposure. The mean percentage change between the first tertile of Cr-adjusted BPA concentrations and the other two tertiles were graphically depicted in figures. Hormone ratios were calculated by simple division as previously reported and natural-log transformed (Chang et al., 2015). The significance level was set at p b 0.05. Data analyses were performed using SPSS v20.0 (IBM, Chicago, IL).

3. Results

3.1. Characteristics of the study population

The median and 25th/75th percentiles (P25, P75) of the children's age and BMI was 9.8 y (9.7, 10.0), and 18.3 (16.1, 21.3) kg/m^2, respectively (Table 1). Peripubertal urinary BPA concentrations were detected in all urine samples (100%) at a wide range of concentrations, with a geometric mean and standard deviation (GM ± GSD) concentration of 5.1 (±1.07 μg/L) and 5.8 (±1.09 μg/g of creatinine). Both demographic and clinical characteristics were similar across tertiles of peripubertal Cr-adjusted urinary BPA concentrations (Table 1). Median (P25, P75) serum concentrations of reproductive hormones were 5 (3, 11) ng/dL for TT, 1.3 (0.8, 1.9) IU/L for FSH, 0.1 (0.1, 0.5) IU/L for LH, and 18.1 (13.4, 21.4) μg/dL for cortisol. These values are within reference ranges (Soldin et al., 2005; Zec et al., 2012).

3.2. Cross-sectional associations of BPA with reproductive hormones and cortisol

When peripubertal Cr-adjusted urinary BPA concentrations were analyzed as continuous independent variable in the adjusted main models, a significant association was observed between BPA and higher TT levels [Exp (β) = 1.19, 95% CI = 1.03, 1.44] (Table 2). Likewise, the positive association between Cr-adjusted urinary BPA concentrations and TT levels remained when BPA was categorized in tertiles. Boys in the 3rd tertile of BPA exposure showed significantly higher mean TT levels [8.66 ng/dL (6.71, 11.2)] compared to boys in the 1st tertile [5.81 ng/dL (4.48, 7.55)] (p-trend = 0.03). Peripubertal Cr-adjusted urinary BPA concentrations were not associated with serum LH or FSH levels, but were negatively and significantly associated with cortisol concentrations, both when BPA concentrations were treated as continuous variable [Exp (β) = 0.84, 95% CI = 0.79, 0.98] and across tertiles of peripubertal urinary BPA exposure (p-trend = 0.04). When the 1st tertile of urinary BPA concentrations was taken as the reference, boys in the 3rd

Table 1. Distribution of peripubertal creatinine (Cr)-adjusted urinary bisphenol A (BPA) concentrations (µg/g) among boys from the INMA-Granada cohort according to demographic and reproductive characteristics.

Percentile										
	GM (±GSD)	Min	5th	10th	25th	50th	75th	90th	95th	Max
BPA (µg/L)[a]	5.1 (±1.07)	0.25	0.99	1.55	3.06	5.03	9.57	13.9	23.2	44.4
Creatinine-adjusted BPA (µg/g)[b]	5.8 (±1.09)	0.46	1.31	1.85	3.20	5.37	11.0	17.0	29.9	63.7

Baseline characteristics	Total sample (n = 172)	Peripubertal Cr-adjusted urinary BPA concentrations (µg/g)			P-value[c]
		T1 (n = 57)	T2 (n = 57)	T3 (n = 58)	
Age (years)	9.8 (9.7, 10.0)	9.9 (9.7, 10.2)	9.8 (9.7, 9.9)	9.7(9.6, 10.0)	0.09
Body Mass Index (kg/m^2)	18.3 (16.1, 21.3)	17.8 (15.8, 21.0)	18.6 (16, 22.3)	18.6(16.5, 20.7)	0.53
Maternal education, n (%)					
Up to primary	80 (47)	30(53)	23 (40)	29(50)	0.63
Secondary school	59 (34)	17(30)	22 (39)	19(33)	
University	33 (19)	10(17)	12 (21)	10(17)	
Tanner stage, n (%)					
1	138 (80)	49(86)	43 (75)	46(79)	0.36
2	34 (20)	8 (14)	14 (25)	12(21)	
Total cholesterol, n (%)	167 (147, 182)	171 (154, 187)	160 (145, 180)	167 (146, 182)	0.31
Urinary cotinine (ng/mL)	6.7 (2, 18.5)	7.3 (2, 18.9)	6.7 (2, 15.5)	6.7 (2, 19.4)	0.76
Cryptorchidism, n (%)	5 (3)	1(2)	2(3)	2(3)	0.82
TT (ng/dL)	5 (3, 11)	5 (3, 9)	5 (3, 11)	8 (3, 12)	0.07
FSH (IU/L)	1.3 (0.8, 1.9)	1.2 (0.8, 2.0)	1.4 (0.8, 1.9)	1.3 (0.7, 1.8)	0.84
LH (IU/L)	0.1 (0.1, 0.5)	0.2 (0.1, 0.5)	0.1 (0.1, 0.5)	0.1 (0.1, 0.5)	0.46
Cortisol (µg/dL)	18.1 (13.4, 21.4)	19.4 (16.2, 21.5)	16.9 (12.9, 21.5)	18 (12.6, 20.7)	0.10
Ratio TT:LH	30 (15, 60)	30 (15, 40)	30 (15, 70)	37 (14, 90)	0.10
Ratio TT:cortisol	0.33 (0.19, 0.65)	0.29 (0.19, 0.49)	0.32 (0.17, 0.71)	0.44(0.23, 0.74)	0.03

Data are presented as medians (IQRs) or n (%).
BPA Limit of Detection (LOD) = 0.1 µg/L; BPA Limit of Quantification (LOQ) = 0.2 µg/L.
Creatinine-adjusted BPA concentrations (µg/g) were calculated by dividing BPA concentrations (µg/L) for urinary creatinine levels (mg/dL).
Value of hypothesis testing is reported for the comparison of covariates across tertiles of BPA exposure. The Kruskal-Wallis test was used for continuous variables and the Chi-square test for categorical variables. T: Tertile, TT: Total testosterone, FSH: follicle-stimulating hormone, LH: luteinizing hormone. Hormone ratios were calculated by simple division.

tertile presented 49% higher TT levels, and 23% lower cortisol concentrations (Fig. 1). In addition, Cr-adjusted urinary BPA concentrations were positively and significantly associated with TT:LH and TT:cortisol ratios [Exp (β) = 1.27, 95% CI = 1.14, 1.69) and Exp (β) = 1.24, 95% CI = 1.10, 1.68), respectively], both when BPA concentrations were treated as continuous variables and categorized in tertiles (Table 3).

3.3. Sensitivity analyses

Given that cryptorchidism might imply changes in reproductive hormone levels, the main models (Table 2) were analyzed after excluding five boys with a diagnosis of cryptorchidism. Results remained unchanged (data not shown).

Given that 20% of the boys in this study were in Tanner stage II, which might have a distinct developmental and/or hormonal profile, the main models were stratified by Tanner stages (I vs. II), finding the same significant associations with TT and cortisol among those in Tanner stage I (n = 1 3 8) [(E x p (β) = 1.26, 95% CI = 1.07, 1.56) for TT and (Exp (β) = 0.81, 95% CI = 0.74, 0.97) for cortisol], but not among those in stage II. Although the sample size of boys in stage II was small (n = 34) and possibly underpowered, the direction of associations was the opposite of that in boys in stage I, observing a negative although non-significant association between

Table 2. Serum total testosterone, FSH, LH and cortisol levels by peripubertal creatinine-adjusted urinary bisphenol A (BPA) concentrations among boys from the INMA-Granada cohort (n = 172).

Creatinine-adjusted urinary	**Testosterone (ng/dL)**		**FSH (IU/L)**		**LH (IU/L)**		**Cortisol (µg/dL)**	
BPA concentrations (µg/g)	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
Tertiles	Mean (95% CI)		Mean (95% CI)		Mean (95% CI)		Mean (95% CI)	
T1 [0.46 –3.83]	5.85 (4.48, 7.65)	5.81 (4.48, 7.55)	1.25 (1.06, 1.47)	1.25 (1.07, 1.46)	0.25 (0.19, 0.33)	0.25 (0.19, 0.33)	17.7 (15.0, 20.9)	17.7 (14.9, 20.9)
T2 [3.88–8.52]	6.47 (4.95, 8.45)	6.50 (5.01, 8.44)	1.23 (1.04, 1.45)	1.23 (1.05, 1.44)	0.21 (0.16, 0.28)	0.21 (0.16, 0.27)	14.6 (12.3, 17.2)	14.7 (12.4, 17.4)
T3 [8.82–63.7]	8.65 (6.64, 11.3)	8.66 (6.71, 11.2)	1.16 (0.98, 1.36)	1.16 (0.99, 1.35)	0.21 (0.16, 0.28)	0.21 (0.16, 0.28)	13.8 (11.6, 16.3)	13.7 (11.6, 16.2)
	Exp (β) (95% CI)		Exp (β) (95% CI)		Exp (β) (95% CI)		Exp (β) (95% CI)	
T1 [0.46–3.83]	Ref.	(1) Ref. (1)	Ref.	(1) Ref. (1)	Ref.	(1) Ref. (1)	Ref.	(1) Ref. (1)
T2 [3.88–8.52]	1.11 (0.76, 1.61)	1.12 (0.77, 1.62)	0.99 (0.78, 1.24)	0.98 (0.79, 1.23)	0.86 (0.58, 1.27)	0.84 (0.57, 1.22)	0.82 (0.65, 1.05)	0.83 (0.65, 1.06)
T3 [8.82–63.7]	1.48 (1.01, 2.15)	1.49 (1.03, 2.15)	0.93 (0.74, 1.17)	0.93 (0.74, 1.16)	0.87 (0.59, 1.27)	0.85 (0.58, 1.24)	0.78 (0.61, 0.99)	0.77 (0.61, 0.98)
p-Trend	0.04	0.03	0.53	0.49	0.46	0.40	0.04	0.04
	Exp (β) (95% CI)		Exp (β) (95% CI)		Exp (β) (95% CI)		Exp (β) (95% CI)	
Continuous	1.17 (1.01, 1.43)	1.19 (1.03, 1.44)	0.88 (0.83, 1.02)	0.89 (0.84, 1.02)	0.87 (0.72, 1.02)	0.89 (0.73, 1.03)	0.84 (0.79, 0.98)	0.84 (0.79, 0.98)
p- value	0.04	0.02	0.09	0.12	0.08	0.10	0.02	0.02

Data are presented as both means (95% CIs) and exponentiation of beta estimates [Exp (β) (95% CIs)] when creatinine-adjusted BPA was categorized in tertiles, and as Exp (β) (95% CIs) when natural log-creatinine-adjusted BPA was treated as continuous variable. Because hormone levels were log-transformed, model coefficients are reported as exponentiated beta es-timates [Exp (β) (95% CIs)], i.e., the percentage change in geometric mean hormone levels associated with a natural log-unit increase in Cr-adjusted BPA concentrations, or as the percent-age change for the second and third tertiles in comparison to the first tertile of BPA exposure.
Models were adjusted for body mass index (kg/m^2), maternal education (up to primary/secondary school/university), total cholesterol (mg/dL), urinary cotinine (ng/mL) and Tanner stage (I vs. II).

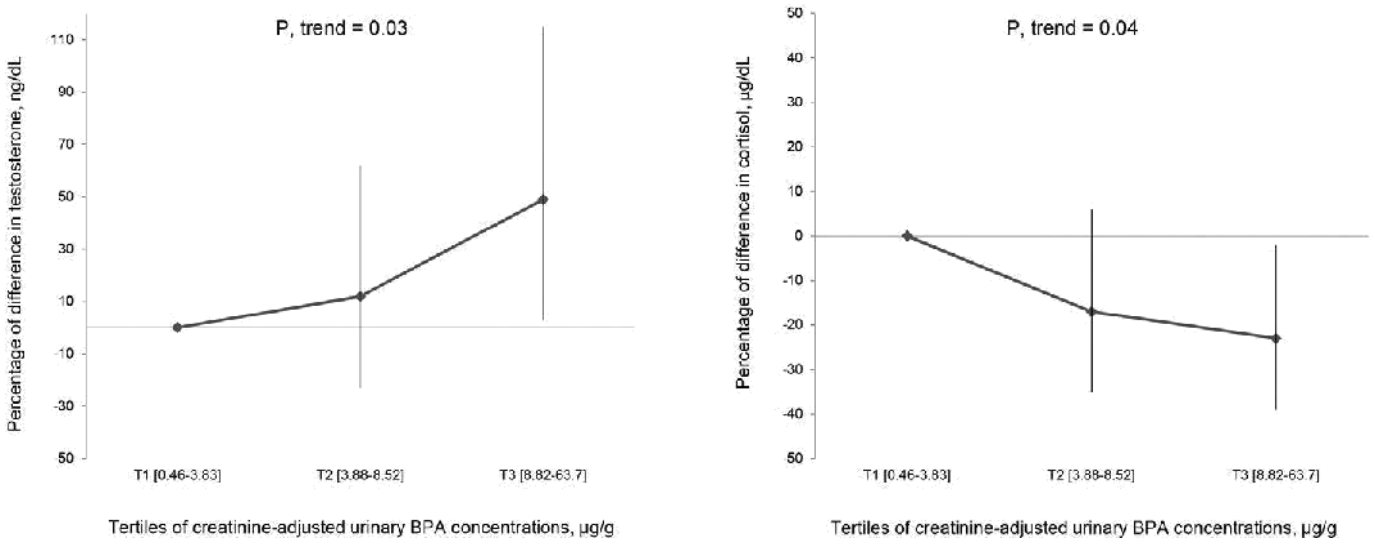


Fig. 1. Percentage of change in total testosterone (ng/dL) and cortisol concentrations (µg/dL) by tertiles of peripubertal creatinine-adjusted urinary bisphenol A (BPA) concentrations among peripubetal boys (n = 172). Models were adjusted body mass index (kg/m^2), maternal education (up to primary/secondary school/university), total cholesterol (mg/dL), urinary cotinine (ng/mL) and Tanner stage (I vs. II). T: Tertile. Reference category = 1st Tertile.

Cr-adjusted urinary BPA concentrations and TT levels [Exp (β) = 0.84, 95% CI 0.52, 1.21) and a positive although non-significant association with cortisol levels [Exp (β) = 1.02, 95% CI = 0.88, 1.15) (Supplemental Table 2).

Although our study sample size is likely underpowered to study interactions, the potential modifying effect of Tanner stage, BMI, cholesterol, cotinine and maternal education on the associations found was studied by entering the interaction terms (BPA concentrations ∗ each potential modifier) in the linear regression models. No significant interactions were found (data not shown), although a possible interaction cannot be ruled out for the product term BPA ∗ Tanner stage in the main model for TT (p-value = 0.13).

4. Discussion

Peripubertal Cr-adjusted urinary BPA concentrations at 9–11 years of age were significantly associated with higher serum TT levels, lower serum cortisol concentrations, and higher serum TT:LH and TT:cortisol ratios, whether the concentrations were treated as a continuous variable or categorized in tertiles. The same associations remained significant and even strengthened when results were separately analyzed for the boys in Tanner stage I (n = 138) but not for those in Tanner stage II, whose results suggested opposite associations; however, only a small number of boys were in this stage (n = 34). Additionally, although a suggestive trend towards lower LH and FSH levels was also observed in the main models, which might be consistent with a negative feedback exerted by increased serum TT levels, statistical significance was not reached for gonadotrophins in this population of Spanish boys.

To our best knowledge, only two epidemiological studies have investigated BPA exposure in relation to cortisol levels. Giesbrecht et al. (2016) found an association between higher urinary BPA concentrations during pregnancy and a flattening of the maternal daytime cortisol pattern, measured in saliva (Giesbrecht et al., 2016). In the same cohort, higher urinary BPA concentrations during pregnancy were also associated with reduced salivary cortisol levels in male infants, whereas these concentrations increased in females (Giesbrecht et al., 2017).

Table 3. Hormone ratios (TT:LH and TT:cortisol) by peripubertal creatinine-adjusted urinary bisphenol A (BPA) concentrations among boys from the INMA-Granada cohort (n = 172).

Creatinine-adjusted urinary BPA	**TT:LH ratio**		**TT:cortisol ratio**	
concentrations (µg/g)	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
Tertiles	Mean (95% CI)		Mean (95% CI)	
T1 [0.46 –3.83]	23.7 (17.3, 32.5)	23.6 (17.3, 32.3)	0.33 (0.24, 0.46)	0.34 (0.24, 0.47)
T2 [3.69–8.52]	30.5 (22.2, 41.8)	30.9 (22.6, 42.2)	0.44 (0.32, 0.62)	0.44 (0.32, 0.61)
T3 [8.82–63.7]	40.5 (29.6, 55.4)	40.1 (29.5, 54.4)	0.63 (0.46, 0.87)	0.62 (0.45, 0.86)
	Exp (β) (95% CI)		Exp (β) (95% CI)	
T1 [0.46–3.83]	Ref. (1)	Ref. (1)	Ref. (1)	Ref. (1)
T2 [3.69–8.52]	1.31 (0.84, 2.04)	1.30 (0.83, 2.03)	1.34 (0.85, 2.13)	1.31 (0.82, 2.11)
T3 [8.82–63.7]	1.70 (1.10, 2.62)	1.69 (1.09, 2.62)	1.90 (1.20, 3.01)	1.86 (1.17, 2.95)
p-Trend	0.02	<0.01	<0.01	<0.01
	Exp (β) (95% CI)		Exp (β) (95% CI)	
Continuous	1.29 (1.15, 1.72)	1.27 (1.14, 1.69)	1.25 (1.11, 1.69)	1.24 (1.10, 1.68)
p-Value	<0.01	<0.01	<0.01	<0.01

Data are presented as both means (95% CIs) and exponentiation of beta estimates [Exp (β) (95% CIs)] when creatinine-adjusted BPA was categorized in tertiles, and as Exp (β) (95% CIs) when natural log-creatinine-adjusted BPA was treated as continuous variable. Because hormone levels were log-transformed, model coefficients are reported as exponentiated beta es-timates [Exp (β) (95% CIs)], i.e., the percentage change in geometric mean hormone levels associated with a natural log-unit increase in Cr-adjusted BPA concentrations, or as the percent-age change for the second and third tertiles in comparison to the first tertile of BPA exposure. T: Tertile, TT: total testosterone; LH: luteinizing hormone. Hormone ratios were calculated by simple division.

Models were adjusted for body mass index (kg/m^2), maternal education (up to primary/secondary school/university), total cholesterol (mg/dL), urinary cotinine (ng/mL) and Tanner stage (I vs. II).

Interestingly, reduced cortisol and a higher TT:cortisol ratio has been associated with behavior problems in humans (Terburg et al., 2009). Giesbrecht et al., have proposed that some of the BPA sexually dimorphic changes in behavior reported in the scientific literature might be mediated by sexually dimorphic changes in HPA axis function (Giesbrecht et al., 2017). Our present results might be in line since we have previously reported associations between BPA exposure and poorer behavior in boys from the same cohort (Perez-Lobato et al., 2016). More experimental and epidemiological researches are warranted to elucidate the effects of BPA on the HPA axis.

Several epidemiologic studies, mostly with a cross-sectional design, have also assessed associations between BPA exposure and male reproductive hormones, yielding heterogeneous results (Mínguez-Alarcón et al., 2016). Thus, a study using data from the National Health and Nutrition Examination Survey (NHANES 2011–2012) by Scinicariello and Buser (2016) found significant associations between urinary BPA concentrations and lower serum TT levels among male adolescents around 15 years of age (n = 161) but not among boys aged around 8 years of age (n = 134). Additionally, Ferguson et al. (2014) reported a non-significant relationship between BPA concentrations and lower TT levels among 8–14 year old boys (n = 113). In contrast, Galloway et al. (2010) and Lassen et al. (2014) observed a positive relationship between urinary BPA concentrations and serum TT levels in male adults, while others found no association with adult TT levels (Meeker et al., 2010; Mendiola et al., 2010). The apparent discrepancy between the present results and the observations of Scinicariello and Buser (2016) may be explained by the completely different development stages investigated. Thus, they examined BPA exposure in relation to serum TT levels in adolescent males with "peaking" TT values (geometric mean TT = 276 ng/dL), whereas we studied peripubertal 9–11 year-old boys with median serum TT levels of 5 ng/dL. Differences in exposure levels could also explain discrepancies, since opposite effects at low and high doses have been documented for EDCs in general and BPA in particular (Vandenberg, 2013). Thus, Scinicariello and Buser (2016) reported a geometric mean of 1.94 μg/L for urinary BPA concentrations in male adolescents, in contrast to the 5.1 μg/L presented by the peripubertal boys. In addition, we cannot rule out the possibility of differences in the metabolism and tissue distribution of BPA between puberty and adolescence.

In the present study, results also suggest some differences in the BPA-hormone associations according to puberty status. Thus, in stratified analyses (Supplemental Table 2), strengthened associations towards higher serum TT and lower serum cortisol levels were found in the prepubertal boys but the opposite was observed for both TT and cortisol levels in the pubertal boys. In addition, a possible interaction cannot be ruled out for the product term BPA ∗ Tanner stage in the main model for TT (p-value = 0.13) given that our analysis was likely underpowered due to the sample size. Therefore, although our results are not sufficient to fully elucidate whether puberty status is a modifier or a confounder of the associations found, they might help to explain differences according to developmental stage in other studies. In this context, it was recently reported that urinary BPA concentrations were associated not only with earlier pubertal onset, but also with delayed progression of puberty in boys aged 9–18 years (Wang et al., 2017). These apparently counterintuitive relationships of BPA with pubertal onset and progression might be consistent with our observations of a BPA-related increase in serum TT levels in prepubertal boys but reduction in adolescent males (Scinicariello and Buser, 2016). Future research is warranted to clarify and integrate these findings.

Both experimental and epidemiologic studies have shown that the effect of BPA appears to depend on the developmental stage of the ex-posed individual, dose, duration of exposure, sex and target tissue (Barrett, 2014; Bourguignon et al., 2016; Brouard et al., 2016; Wetherill et al., 2007). Thus, although BPA exposure has been shown to reduce testosterone levels in adolescent rodents (Peretz et al., 2014), other studies conducted in prepubertal animals have observed increased testosterone levels after BPA exposure (Ramos et al., 2003; Song et al., 2002). However, an accurate explanation for this difference in effects is still lacking, probably because BPA mechanisms of action are more varied and complex than initially thought (Lassen et al., 2014; Mustieles et al., 2015). Hence, although the estrogenic properties of BPA are well documented (Wetherill et al., 2007), Song et al. found that BPA strongly induces the gene expression of an orphan nuclear receptor (Nur77) that plays an important role in LH-mediated steroidogenesis in testicular Leydig cells (Song et al., 2002). Thus, when pre-pubertal mice were locally treated with BPA (intratesticular injection), the induction of Nur77 was followed by an increase in total testicular testosterone in vivo (Song et al., 2002). Interestingly, Nur77 also exerts important functions in the human adrenal gland (Kelly et al., 2004). Therefore, if BPA is able to induce Nur77 expression, it could potentially interfere with androgen/cortisol balance in the human adrenal gland by preferentially regulating steroid enzyme genes relevant for cortisol release (Kelly et al., 2004).

The adrenal gland produces androgens and cortisol in a coordinated manner, and its activity increases with its maturation at around 6– 8 years of age (Kelly et al., 2004). Therefore, an increased production of adrenal androgens such as dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) is triggered during adrenarche (Nakamura et al., 2009). Some of these adrenal androgens are converted to testosterone in peripheral tissues (Zouboulis et al., 2007). The increase in adrenal androgen secretion during adrenarche is not associated with increased levels of gonadotrophins (LH/FSH) or adrenocorticothropic hormone (ACTH) (McKenna et al., 1997; Nakamura et al., 2009). Cortisol levels are centrally regulated by ACTH, and although ACTH normally triggers an increased production of both cortisol and androgens in the human adrenal gland, there are situations in which this production becomes divergent (Kelly et al., 2005). This is the case in adrenarche, when the balance of adrenal steroidogenesis shifts towards increased androgen production (McKenna et al., 1997).

Our results point to the adrenal gland as an endocrine organ potentially related to the observed associations. Hence, if there is a causal association of urinary BPA concentrations with higher levels of TT and lower levels of cortisol in serum, it could be explained by a differential production of androgens/cortisol triggered by action of BPA at the adrenal gland. In this regard, BPA has been shown to increase adrenal weight in both male and female mice (Medwid et al., 2016) and to alter steroidogenesis in the adrenal gland both in vivo (Medwid et al., 2016) and in vitro (Lan et al., 2015). Although there have been scant experimental studies on the role of BPA, increasing data are available on the known EDC di(2-ethylhexyl) phthalate (DEHP) (Martinez-Arguelles and Papadopoulos, 2015). Despite belonging to different chemical families, BPA and DEHP share some mechanisms of action, including binding to estrogen and androgen nuclear receptors and peroxisome proliferator-activated receptors (PPARs). DEHP was recently found to affect adrenal gland development and function in vivo by interacting with PPAR and cholesterol biosynthesis pathways (Lee et al., 2017). Therefore, the relationships of PPARs with other steroidogenic targets are plausible mechanistic pathways by which both EDCs could interact and affect adrenal function.

Median serum TT (5 ng/dL), LH (0.1 IU/L), FSH (1.3 IU/L) and cortisol levels (18.1 μg/dL) were similar to those previously reported for children and prepubertal boys (Forest et al., 1976; Soldin et al., 2005; Zec et al., 2012). All children in this subsample of the INMA-Granada birth cohort had detectable urinary BPA concentrations at this follow-up, with a median of 5.0 μg/L, similar to reports in younger children (Braun et al., 2011; Casas et al., 2011) and those of the same age (Calafat et al., 2008). However, BPA concentrations were higher than those observed in children aged 6–11 years (2.7 μg/L) in the 2005–2006 NHANES study (LaKind and Naiman, 2011) or in those aged 9– 11 years (2.13 μg/L) in the German Environmental Survey on Children (GerES) (Becker et al., 2009). These higher values may be attributable to the timing of urine collection (non-fasting samples collected between 17:00 and 20:00 h) and/or the characteristics of the children and their families (food intake, lifestyle, socio-economic status, etc.).

This study presents some limitations. First, the cross-sectional design of the study prevents the inference of causal relationships, and reverse causality issues cannot be ruled out. Second, we acknowledge the limitation of using a single urine sample to estimate BPA exposure, although the non-persistent nature and short-term variability of BPA is more likely to produce an underestimation rather than an overestimation of its effects (Betts, 2014). Third, our study was limited by a relatively small sample size, which reduces statistical power and might explain some of the non-significant associations observed. Fourth, because we did not measure additional hormones such as ACTH, among others (DHEA and DHEAS), it was not possible to evaluate whether the relationship between BPA and cortisol was driven at central or peripheral level. Free testosterone (FT) values would also be of major interest because they represent the biologically active hormone. However, because we did not have information on serum hormone-binding globulin (SHBG) levels, we could not estimate FT or the free androgen index (Mendiola et al., 2010; Vermeulen et al., 1999). In addition, because other non-persistent EDCs have been associated with serum hormone levels in boys (Ferguson et al., 2014; Wu et al., 2017), the possibility of partial confounding by unmeasured co-exposures cannot be discarded. However, study strengths include the focus on the peripubertal period, an important and less explored critical window of development in relation to BPA exposure, the study sample provides the opportunity to examine the transition from adrenarche to puberty, and the large number of variables collected at the follow-up from questionnaires, biological samples and medical records, allowing us to control for potential confounders.

5. Conclusions

Peripubertal BPA exposure was associated with increased total testosterone and decreased cortisol levels in serum, and with higher ratios of total testosterone to cortisol and LH, raising concerns about the endocrine disrupting potential of BPA during this important period of development. Although action at the testis or pituitary cannot be ruled out, our results are compatible with a possible involvement of BPA at the adrenal gland, resulting in a differential production of androgens/cortisol. However, given the cross-sectional design of our study, the heterogeneous results reported in the literature, and the scant experimental research on BPA effects at the adrenal gland, the present findings should be interpreted with caution.

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Competing financial interest declaration

The authors declare no actual or potential competing financial conflict of interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.09.093.

References

Alonso-Magdalena, P., Ropero, A.B., Soriano, S., García-Arévalo, M., Ripoll, C., Fuentes, E., et al., 2012. Bisphenol-A acts as a potent estrogen via non-classical estrogen triggered pathways. Mol. Cell. Endocrinol. 355 (2), 201–207 (PubMed: 22227557).

Auchus, R.J., 2011. The physiology and biochemistry of adrenarche. Endocr. Dev. 20, 20–27 (PubMed: 21164255).

Barrett, J.R., 2014. BPA and reproductive health reviewing the current state of the science.

Environ. Health Perspect. 122 (8), A223 (PubMed: 25229086).

Becker, K., Göen, T., Seiwert, M., Conrad, A., Pick-Fuss, H., Müller, J., et al., 2009. GerES IV: phthalate metabolites and bisphenol A in urine of German children. Int. J. Hyg. Envi-ron. Health 212 (6), 685–692 (PubMed: 19729343).

Betts, K.S., 2014. Clues to autistic behaviors: exploring the role of endocrine disruptors.

Environ. Health Perspect. 122 (5), A137 (PubMed: 24784413).

Bourguignon, J.-P., Juul, A., Franssen, D., Fudvoye, J., Pinson, A., Parent, A.-S., 2016. Contri-bution of the endocrine perspective in the evaluation of endocrine disrupting chem-ical effects: the case study of pubertal timing. Horm. Res. Paediatr. 86 (4), 221–232 (PubMed: 26799415).

Braun, J.M., Kalkbrenner, A.E., Calafat, A.M., Yolton, K., Ye, X., Dietrich, K.N., et al., 2011. Im-pact of early-life bisphenol A exposure on behavior and executive function in chil-dren. Pediatrics 128 (5), 873–882 (PubMed: 22025598).

Brouard, V., Guénon, I., Bouraima-Lelong, H., Delalande, C., 2016. Differential effects of bisphenol A and estradiol on rat spermatogenesis' establishment. Reprod. Toxicol. 63, 49– 61 (PubMed: 27174447).

Calafat, A.M., Ye, X., Wong, L.-Y., Reidy, J.A., Needham, L.L., 2008. Exposure of the U.S. pop-ulation to bisphenol A and 4-tertiary-octylphenol: 2003–2004. Environ. Health Perspect. 116 (1), 39–44 (PubMed: 2199288).

Casas, L., Fernández, M.F., Llop, S., Guxens, M., Ballester, F., Olea, N., et al., 2011. Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. Environ. Int. 37 (5), 858–866 (PubMed: 21440302).

Chang, W.-H., Li, S.-S., Wu, M.-H., Pan, H.-A., Lee, C.-C., 2015. Phthalates might interfere with testicular function by reducing testosterone and insulin-like factor 3 levels. Hum. Reprod. 30 (11), 2658–2670 (PubMed: 26385792).

Ehrlich, S., Calafat, A.M., Humblet, O., Smith, T., Hauser, R., 2014. Handling of thermal re-ceipts as a source of exposure to bisphenol A. JAMA 311 (8), 859–860 (PubMed: 24570250).

Elmlinger, M.W., Kühnel, W., Wormstall, H., Döller, P.C., 2005. Reference intervals for tes-tosterone, androstenedione and SHBG levels in healthy females and males from birth until old age. Clin. Lab. 51 (11–12), 625–632 (PubMed: 16329620).

Ferguson, K.K., Peterson, K.E., Lee, J.M., Mercado-García, A., Blank-Goldenberg, C., Téllez-Rojo, M.M., et al., 2014. Prenatal and peripubertal phthalates and bisphenol A in rela-tion to sex hormones and puberty in boys. Reprod. Toxicol. 47, 70–76 (PubMed: 24945889).

Fernandez, M.F., Olmos, B., Granada, A., López-Espinosa, M.J., Molina-Molina, J.-M., Fernandez, J.M., et al., 2007. Human exposure to endocrine-disrupting chemicals and prenatal risk factors for cryptorchidism and hypospadias: a nested case–control study. Environ. Health Perspect. 115 (S-1), 8–14 (PubMed: 18174944).

Fernández, M.F., Artacho-Cordón, F., Freire, C., Pérez-Lobato, R., Calvente, I., Ramos, R., et al.,

2015. Trends in children's exposure to second-hand smoke in the INMA-Granada cohort: an evaluation of the Spanish anti-smoking law. Environ. Res. 138, 461–468 (PubMed: 25794848).

Forest, M.G., De Peretti, E., Bertrand, J., 1976. Hypothalamic-pituitary-gonadal relation-ships in man from birth to puberty. Clin. Endocrinol. 5 (5), 551–569 (PubMed: 791540).

Galloway, T., Cipelli, R., Guralnik, J., Ferrucci, L., Bandinelli, S., Corsi, A.M., et al., 2010. Daily bisphenol A excretion and associations with sex hormone concentrations: results from the InCHIANTI adult population study. Environ. Health Perspect. 118 (11), 1603–1608 (PubMed: 20797929).

Geens, T., Bruckers, L., Covaci, A., Schoeters, G., Fierens, T., Sioen, I., et al., 2014. Determi-nants of bisphenol A and phthalate metabolites in urine of Flemish adolescents. Envi-ron. Res. 134, 110–117 (PubMed: 25127521).

Giesbrecht, G.F., Liu, J., Ejaredar, M., Dewey, D., Letourneau, N., Campbell, T., et al., 2016. Urinary bisphenol A is associated with dysregulation of HPA-axis function in preg-nant women: findings from the APrON cohort study. Environ. Res. 151, 689–697 (PubMed: 27640068).

Giesbrecht, G.F., Ejaredar, M., Liu, J., Thomas, J., Letourneau, N., Campbell, T., et al., APrON Study Team, 2017. Prenatal bisphenol A exposure and dysregulation of infant hypothalamic-pituitary-adrenal axis function: findings from the APrON cohort study. Environ. Health 19 (16(1)), 47 (PubMed: 28526030).

Guxens, M., Ballester, F., Espada, M., Fernández, M.F., Grimalt, J.O., Ibarluzea, J., et al., 2012. Co-hort profile: the INMA—INfancia y Medio Ambiente—(environment and childhood) pro-ject. Int. J. Epidemiol. 41 (4), 930–940 (PubMed: 21471022).

Kelly, S.N., McKenna, T.J., Young, L.S., 2004. Modulation of steroidogenic enzymes by or-phan nuclear transcriptional regulation may control diverse production of cortisol and androgens in the human adrenal. J. Endocrinol. 181 (2), 355–365 (PubMed: 15128284).

Kelly, S.N., McKenna, T.J., Young, L.S., 2005. Coregulatory protein-orphan nuclear receptor interactions in the human adrenal cortex. J. Endocrinol. 186 (1), 33–42 (PubMed: 16002533).

LaKind, J.S., Naiman, D.Q., 2011. Daily intake of bisphenol A and potential sources of expo-sure: 2005–2006 National Health and Nutrition Examination Survey. J. Expo. Sci. En-viron. Epidemiol. 21 (3), 272–279 (PubMed: 20237498).

Lan, H.-C., Lin, I.-W., Yang, Z.-J., Lin, J.-H., 2015. Low-dose bisphenol A activates cyp11a1 gene expression and corticosterone secretion in adrenal gland via the JNK signaling pathway. Toxicol. Sci. 148 (1), 26–34 (PubMed: 26209791).

Lassen, T.H., Frederiksen, H., Jensen, T.K., Petersen, J.H., Joensen, U.N., Main, K.M., et al., 2014. Urinary bisphenol A levels in young men: association with reproductive hor-mones and semen quality. Environ. Health Perspect. 122 (5), 478–484 (PubMed: 24786630).

Lee, S., Martinez-Arguelles, D.B., Campioli, E., Papadopoulos, V., 2017. Fetal exposure to low levels of the plasticizer DEHP predisposes the adult male adrenal gland to endo-crine disruption. Endocrinology 158 (2), 304–318 (PubMed: 27849367).

Manikkam, M., Tracey, R., Guerrero-Bosagna, C., Skinner, M.K., 2013. Plastics derived en-docrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inher-itance of obesity, reproductive disease and sperm epimutations. PLoS One 8 (1), e55387 (PubMed: 23359474).

Martinez-Arguelles, D.B., Papadopoulos, V., 2015. Mechanisms mediating environmental chemical-induced endocrine disruption in the adrenal gland. Front. Endocrinol. 6, 29 (PubMed: 25788893).

McKenna, T.J., Fearon, U., Clarke, D., Cunningham, S.K., 1997. A critical review of the origin and control of adrenal androgens. Baillieres Clin. Obstet. Gynaecol. 11 (2), 229–248 (PubMed: 9536209).

Medwid, S., Guan, H., Yang, K., 2016. Prenatal exposure to bisphenol A disrupts adrenal steroidogenesis in adult mouse offspring. Environ. Toxicol. Pharmacol. 43, 203–208 (PubMed: 27017381).

Meeker, J.D., Calafat, A.M., Hauser, R., 2010. Urinary bisphenol A concentrations in relation to serum thyroid and reproductive hormone levels in men from an infertility clinic. Environ. Sci. Technol. 44 (4), 1458–1463 (PubMed: 20030380).

Mendiola, J., Jørgensen, N., Andersson, A.-M., Calafat, A.M., Ye, X., Redmon, J.B., et al., 2010. Are environmental levels of bisphenol A associated with reproductive function in fer-tile men? Environ. Health Perspect. 118 (9), 1286–1291 (PubMed: 20494855).

Michałowicz, J., 2014. Bisphenol A—sources, toxicity and biotransformation. Environ.

Toxicol. Pharmacol. 37 (2), 738–758 (PubMed: 24632011).

Mínguez-Alarcón, L., Hauser, R., Gaskins, A.J., Ehrlich, S., Calafat, A.M., Humblet, O., et al., 2016. Effects of bisphenol A on male and couple reproductive health: a review. Fertil. Steril. 106 (4), 864–870 (PubMed: 27498136).

Molina-Molina, J.-M., Amaya, E., Grimaldi, M., Sáenz, J.-M., Real, M., Fernández, M.F., et al., 2013. In vitro study on the agonistic and antagonistic activities of bisphenol-S and other bisphenol-A congeners and derivatives via nuclear receptors. Toxicol. Appl. Pharmacol. 272 (1), 127–136 (PubMed: 23714657).

Mustieles, V., Pérez-Lobato, R., Olea, N., Fernández, M.F., 2015. Bisphenol A: human expo-

sure and neurobehavior. Neurotoxicology 49, 174–184 (PubMed: 26121921).

Nakamura, Y., Gang, H.X., Suzuki, T., Sasano, H., Rainey, W.E., 2009. Adrenal changes asso-ciated with adrenarche. Rev. Endocr. Metab. Disord. 10 (1), 19–26 (PubMed: 18821019).

O'Brien, K.M., Upson, K., Cook, N.R., Weinberg, C.R., 2016. Environmental chemicals in urine and blood: improving methods for creatinine and lipid adjustment. Environ. Health Perspect. 124 (2), 220–227 (PubMed: 26219104).

Panagiotidou, E., Zerva, S., Mitsiou, D.J., Alexis, M.N., Kitraki, E., 2014. Perinatal exposure to low-dose bisphenol A affects the neuroendocrine stress response in rats. J. Endocrinol. 220

(3), 207–218 (PubMed: 24323913).

Peretz, J., Vrooman, L., Ricke, W.A., Hunt, P.A., Ehrlich, S., Hauser, R., et al., 2014. Bisphenol A and reproductive health: update of experimental and human evidence, 2007–2013. Environ. Health Perspect. 122 (8), 775–786 (PubMed: 24896072).

Perez-Lobato, R., Mustieles, V., Calvente, I., Jimenez-Diaz, I., Ramos, R., Caballero-Casero, N., et al., 2016. Exposure to bisphenol A and behavior in school-age children. Neurotoxicology 53, 12–19 (PubMed: 26654821).

Ramos, J.G., Varayoud, J., Kass, L., Rodríguez, H., Costabel, L., Muñoz-de-Toro, M., et al., 2003. Bisphenol A induces both transient and permanent histofunctional alterations of the hypothalamic-pituitary-gonadal axis in prenatally exposed male rats. Endocri-nology 144

(7), 3206–3215 (PubMed: 12810577).

Scinicariello, F., Buser, M.C., 2016. Serum testosterone concentrations and urinary bisphenol A, benzophenone-3, triclosan, and paraben levels in male and female chil-dren and adolescents: NHANES 2011–2012. Environ. Health Perspect. 124 (12), 1898–1904 (PubMed: 27383665).

Sergeyev, O., Burns, J.S., Williams, P.L., Korrick, S.A., Lee, M.M., Revich, B., et al., 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 (PubMed: 28231067).

Soldin, O.P., Hoffman, E.G., Waring, M.A., Soldin, S.J., 2005. Pediatric reference intervals for FSH, LH, estradiol, T3, free T3, cortisol, and growth hormone on the DPC IMMULITE 1000. Clin. Chim. Acta 355 (1–2), 205–210 (PubMed: 15820497).

Song, K.-H., Lee, K., Choi, H.-S., 2002. Endocrine disrupter bisphenol A induces orphan nu-clear receptor Nur77 gene expression and steroidogenesis in mouse testicular Leydig cells. Endocrinology 143 (6), 2208–2215 (PubMed: 12021184).

Tanner, J.M., Whitehouse, R.H., 1976. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch. Dis. Child. 51 (3), 170–179 (PubMed: 952550).

Terburg, D., Morgan, B., van Honk, J., 2009. The testosterone-cortisol ratio: a hormonal marker for proneness to social aggression. Int. J. Law Psychiatry 32 (4), 216–223 (PubMed: 19446881).

Vandenberg, L.N., 2013. Non-monotonic dose responses in studies of endocrine disrupting chemicals: bisphenol a as a case study. Dose-Response 12 (2), 259–276 (PubMed: 24910584).

Vandenberg, L.N., Hauser, R., Marcus, M., Olea, N., Welshons, W.V., 2007. Human exposure to bisphenol A (BPA). Reprod. Toxicol. 24 (2), 139–177 (PubMed: 17825522).

Vandenberg, L.N., Chahoud, I., Heindel, J.J., Padmanabhan, V., Paumgartten, F.J.R., Schoenfelder, G., 2010. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ. Health Perspect. 118 (8), 1055–1070 (PubMed: 17825522).

Vandenberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs, D.R., Lee, D.-H., et al., 2012. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr. Rev. 33 (3), 378–455 (PubMed: 22419778).

Vermeulen, A., Verdonck, L., Kaufman, J.M., 1999. A critical evaluation of simple methods for the estimation of free testosterone in serum. J. Clin. Endocrinol. Metab. 84 (10), 3666– 3672 (PubMed: 10523012).

vom Saal, F.S., Akingbemi, B.T., Belcher, S.M., Birnbaum, L.S., Crain, D.A., Eriksen, M., et al., 2007. Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. Reprod. Toxicol. 24 (2), 131–138 (PubMed: 17768031).

Wang, Z., Li, D., Miao, M., Liang, H., Chen, J., Zhou, Z., et al., 2017. Urine bisphenol A and pubertal development in boys. Int. J. Hyg. Environ. Health 220 (1), 43–50 (PubMed: 27769633).

Wetherill, Y.B., Akingbemi, B.T., Kanno, J., McLachlan, J.A., Nadal, A., Sonnenschein, C., et al., 2007. In vitro molecular mechanisms of bisphenol A action. Reprod. Toxicol. 24

(2), 178–198 (PubMed: 17628395).

Wu, W., Zhou, F., Wang, Y., Ning, Y., Yang, J.Y., Zhou, Y.K., 2017. Exposure to phthalates in children aged 5–7 years: associations with thyroid function and insulin-like growth factors. Sci. Total Environ. 1 (579), 950–956 (PubMed: 27884522).

Zawatski, W., Lee, M.M., 2013. Male pubertal development: are endocrine-disrupting compounds shifting the norms? J. Endocrinol. 218 (2), R1-12 (PubMed: 23709001).

Zec, I., Kučak, I., Begčević, I., Šimundić, A.-M., Tišlarić-Medenjak, D., Megla, Ž.B., et al., 2012.

Reference intervals for reproductive hormones in prepubertal children on the auto-mated

Roche cobas e 411 analyzer. Clin. Biochem. 45 (15), 1206–1212 (PubMed: 22634601).

Zhang, X., Chang, H., Wiseman, S., He, Y., Higley, E., Jones, P., et al., 2011. Bisphenol A dis-rupts steroidogenesis in human H295R cells. Toxicol. Sci. 121 (2), 320–327 (PubMed: 21427057).

Zouboulis, C., Chen, W.-C., Thornton, M., Qin, K., Rosenfield, R., 2007. Sexual hormones in human skin. Horm. Metab. Res. 39 (2), 85–95 (PubMed: 17326004).

Supplemental Material

Bisphenol A and reproductive hormones and cortisol in peripubertal boys: The INMA-Granada cohort

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Supplemental Table 1. Socio-demographic and clinical characteristics of participating boys for whom reproductive hormones/cortisol data were available in comparison to the rest of the follow-up sample.

Baseline Characteristics	Present subsample with hormone data (n=172)	Rest of the follow-up sample (n=126)	P-value[a]
Age (years)	9.8 (9.7, 10.0)	9.9 (9.7, 10.1)	0.11
Body Mass Index (Kg/m^2)	18.3 (16.1, 21.3)	18.4 (16.5, 21.1)	0.65
Maternal Education, n (%) Up to primary Secondary school University	 80(47) 59(34) 33(19)	 56(44) 37(30) 33(26)	0.31
Tanner stage, n (%) 1 2	 138 (80) 34(20)	 97(77) 29(23)	0.27
Total Cholesterol, n (%)	167 (147, 182)	-	
Urinary Cotinine (ng/mL)	6.7 (2, 18.5)	9.5 (2, 31.6)	0.14
Cryptorchidism, n (%)	5(3)	4(3)	0.88

Data are presented as medians (IQRs) or n (%). [a] Value of hypothesis testing was calculated using the Kruskal-Wallis test for continuous variables and the Chi-square test for categorical variables.

Supplemental Table 2. Stratification by Tanner stage on the relation between peripubertal Cr-adjusted urinary bisphenol A (BPA) concentrations and serum Total Testosterone and Cortisol levels among boys from the INMA-Granada cohort (n=172).

Creatinine-adjusted urinary BPA concentrations (µg/g)	Tanner stage I (n=138)				Tanner stage II (n=34)			
	Testosterone (ng/dL)		Cortisol (µg/dL)		Testosterone (ng/dL)		Cortisol (µg/dL)	
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
Tertiles	Mean (95% CI)		Mean (95%CI)		Mean (95% I)		Mean (95% CI)	
T1 [0.46-3.66]	5.07 (3.81, 6.76)	5.00 (3.75, 6.67)	19.0 (15.5, 23.2)	18.8 (15.3, 23.0)	9.56 (4.78, 19.1)	10.4 (5.47, 19.9)	16.9 (14.1, 20.4)	16.8 (13.9, 20.2)
T2 [3.69-8.52]	5.97 (4.48, 7.96)	6.02 (4.52, 8.03)	13.1 (10.7, 16.1)	13.2 (10.8, 16.1)	10.1 (5.04, 20.1)	10.6 (5.43, 20.5)	18.4 (15.3, 22.2)	19.4 (16.0, 23.6)
T3 [8.83-63.7]	8.32 (6.24, 11.1)	8.37 (6.28, 11.1)	13.0 (10.6, 15.9)	13.0 (10.6, 16.0)	10.1 (5.18, 19.5)	8.09 (4.78, 16.5)	16.5 (13.8, 19.7)	15.8 (13.2, 18.9)
	Exp (β) (95% CI)		Exp (β) (95% CI)		Exp (β) (95% CI)		Exp (β) (95% CI)	
T1 [0.46-3.66]	Ref. (1)	Ref. (1)	Ref. (1)	Ref. (1)	Ref. (1)	Ref. (1)	Ref. (1)	Ref. (1)
T2 [3.69-8.52]	1.18 (0.78, 1.77)	1.20 (0.80, 1.81)	0.69 (0.51, 0.91)	0.70 (0.52, 0.93)	1.05 (0.40, 2.80)	1.01 (0.39, 2.61)	1.09 (0.83, 1.41)	1.16 (0.88, 1.53)
T3 [8.83-63.7]	1.64 (1.09, 2.46)	1.67 (1.11, 2.51)	0.68 (0.51, 0.90)	0.69 (0.51, 0.93)	1.05 (0.40, 2.80)	0.85 (0.35, 2.09)	0.97 (0.75, 1.26)	0.94 (0.73, 1.23)
p-trend	**0.02**	**0.01**	**0.01**	**0.02**	0.91	0.71	0.80	0.64
	Exp (β) (95% CI)		Exp (β) (95% CI)		Exp (β) (95% CI)		Exp (β) (95% CI)	
Continuous	1.27 (1.08, 1.56)	1.26 (1.07, 1.56)	0.82 (0.75, 0.97)	0.81 (0.74, 0.97)	0.89(0.54, 1.37)	0.84 (0.52, 1.21)	1.05 (0.90, 1.16)	1.02 (0.88, 1.15)
p-value	**<0.01**	**<0.01**	**0.02**	**0.02**	0.52	0.27	0.78	0.92

Data are presented as both means (95% CIs) and exponentiation of beta estimates [Exp(β) (95% CIs)] when creatinine-adjusted BPA was categorized in tertiles, and as Exp (β) (95% CIs) when natural log-creatinine-adjusted BPA was treated as continuous variable. Because hormone levels were log-transformed, model coefficients are reported as exponentiated beta estimates [Exp (β) (95% CIs)], i.e., the percentage change in geometric mean hormone levels associated with a natural log-unit increase in Cr-adjusted BPA concentrations, or as the percentage change for the second and third tertiles in comparison to the first tertile of BPA exposure

Models were adjusted for body mass index (Kg/m^2), maternal education (up to primary/secondary school/university), total cholesterol (mg/dL) and urinary cotinine (ng/mL).

T: Tertile.

PAPER 3

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Paper #3

Bisphenol A: Human exposure and neurobehavior

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Abstract

The effect of bisphenol A (BPA) exposure on human brain and behavior is a relatively new issue, and particular concerns have been raised about its potential impact on children. The primary objective of this review was to analyze the current state of knowledge on the association of environmental BPA exposure during pregnancy and/or childhood with child cognitive and/or behavior outcomes. All scientific publications until March 2015 that include examination of this relationship have been reviewed using the MEDLINE/PubMed database. Although research on this issue has not been abundant, an association with altered neurobehavior was reported by eight out of the twelve available articles, including aggressive behavior, attention deficit, hyperactivity disorder, depression and anxiety impairments, mostly in children exposed in utero, indicating disruption of the brain during this critical window of development. Despite the reduced number of studies and their heterogeneity, the results suggest that prenatal BPA exposure may have a negative impact on neurobehavioral functioning in children and that the effects may be sex-dependent. It is therefore necessary to be vigilant towards the potential adverse effects of ubiquitous low-level BPA exposure, although more studies in humans are required to convincingly confirm or rule out the association between BPA exposure and health. Meanwhile, it is desirable to inform women planning or undergoing pregnancy about measures to reduce or avoid exposure to BPA. We discuss some key aspects of the relationship between exposure and neurobehavioral outcomes.

Keywords: Bisphenol A (BPA), Child, Behavior, Neurodevelopment, Endocrine disruptors (EDCs), Human exposure

1. Introduction

Dodds and Lawson (1938) discovered the estrogenic properties of bisphenol A (BPA) while seeking estrogenic compounds without the phenanthrene nucleus. More than 50 years later, Krishnan et al. (1993) reported that leaching of this endocrine disrupting chemical (EDC) from polycarbonate flasks during autoclaving was responsible for the estrogenicity found in yeast culture medium. Over the subsequent two decades, there has been considerable research on the actions of BPA and its possible adverse effects on human health.

BPA is a monomer widely used in the production of epoxy resins and polycarbonate plastics, and several million tons are produced worldwide every year (Vogel, 2009). It is present in the epoxy resin that coats canned food and beverages (Bemrah et al., 2014; Brotons et al., 1995; Cao et al., 2009; Carwile et al., 2009; Vandenberg et al., 2007) and in polycarbonate plastic bottles, food containers, plastic plates and cups, dental sealants, water supply pipes, toys, thermal receipts, cigarette filters, and even medical equipment, including medical tubing and implant devices (Calafat et al., 2009; Duty et al., 2013; Ehrlich et al., 2014; Geens et al., 2012; Vandenberg et al., 2007; Welshons et al., 2006).

Biomonitoring studies indicate that human exposure to BPA is ubiquitous, with more than 90% of the population evidencing detectable levels of BPA in different biological matrices (usually measured in urine in the concentration range of nanograms per milliliter) (Becker et al., 2009; Calafat et al., 2008; Casas et al., 2013; Vandenberg et al., 2010). Food and beverages are thought to be among the main sources of exposure in the general population (Vandenberg et al., 2010; von Goetz et al., 2010), although aquatic, air, soil and dermal routes may also contribute to total human exposure (Michałowicz, 2014).

BPA is one of the most widely studied EDCs (Vandenberg et al., 2010), with varied and well-documented effects in animals and humans (Chapin et al., 2008; Rochester, 2013; vom Saal et al., 2007). BPA has been consistently detected in maternal blood, amniotic fluid, and fetal serum (Vandenberg et al., 2010). It has been found that BPA can cross the placenta and enter the fetus (Corbel et al., 2014; Edlow et al., 2012; Jiménez-Díaz et al., 2010; Tsutsumi, 2005). Neonates can also be exposed to BPA through maternal breast milk (Mendonca et al., 2014; Vandenberg et al., 2010). The effects of BPA in humans depend on the dose and timing, with the prenatal/neonatal period representing the most vulnerable window of exposure (Barker, 2007; Capra et al., 2013; Fernández et al., 2014; Vandenberg et al., 2009).

The human brain is a sexually dimorphic organ (MacLusky and Naftolin, 1981; Yang and Shah, 2014), and major morphological differences are shaped during prenatal development under the regulation of gonadal steroid hormones, especially estrogen and aromatizable androgens (Bao and Swaab, 2011; Berenbaum and Beltz, 2011; Cohen-Bendahan et al., 2005; Manson, 2008; Swaab, 2007). Therefore, the effects on human brain and behavior of EDCs in general and BPA in particular are of special interest. Animal studies have shown that exposure to low (environmentally relevant) doses of BPA during critical periods alter sex-specific structural and behavioral patterns, increasing, decreasing, or eliminating sex differences and thereby affecting the sexually dimorphic development of the brain (Bowman et al., 2014; Chen et al., 2014; Jasarevic et al., 2011; Kubo et al., 2003; Tando et al., 2014) and altering steroid receptor levels (Cao et al., 2013; Rebuli et al., 2014). Experimental animals exposed to low BPA doses have also been found to display behavioral changes, including: hyperactivity (Anderson et al., 2013; Komada et al., 2014; Zhou et al., 2011), increased aggressiveness (Kawai et al., 2003; Patisaul and Bateman, 2008), greater anxiety (Luo et al., 2014; Tian et al., 2010; Xu et al., 2012), and modified socio-sexual behavior (Farabollini et al., 2002; Porrini et al., 2005). Low BPA doses can also alter the development of play behavior (Dessı-Fulgheri et al., 2002), spatial learning, and memory function (Carr et al., 2003; Eilam-Stock et al., 2012; Kuwahara et al., 2013; Wang et al., 2014) in animals. The finding that BPA exposure changes socio-sexual interactions in infant and juvenile nonhuman primates is of special interest (Nakagami et al., 2009; Negishi et al., 2014).

The effect of BPA exposure on human brain and behavior is a relatively new issue, and there is particular concern about the potential impact of BPA exposure on children (Colborn, 2004). The purpose of this study was to review available data on child BPA exposure and its relationship to neurodevelopment and behavioral outcomes.

2. Mechanisms of action and targets in the brain

BPA has varied and complex mechanisms of action that may interfere with normal brain and behavior development, evidencing a plausible causal link (Wolstenholme et al., 2011a). The main mechanisms that may be related to brain development are summarized below.

2.1. Endocrine-related BPA mechanisms

BPA binds to classical nuclear estrogen receptors (ERs) and exerts a mix of agonist and/or antagonist actions depending on the target tissues, cell types, ER subtypes, and differential cofactors recruited by ER-ligand complexes (Welshons et al., 2006). For example, depending on the tissue involved and the ER subtype, BPA acts as an agonist of ERb receptors and as an agonist/antagonist of ERa (Kurosawa et al., 2002), with lower affinity for ER-a and ER-b in comparison to estradiol (Andersen et al., 1999; Perez et al., 1998). BPA has been reported to have an 80-fold greater affinity for ERg, which is highly expressed in placenta and mammalian fetal brain, than for ERa, both in vitro (Matsushima et al., 2007; Takayanagi et al., 2006; Takeda et al., 2009) and in vivo (Tohmé et al., 2014). BPA can also bind to estrogen membrane receptors such as GPR30 (Thomas and Dong, 2006) and elicit nongenomic estrogenic actions in experimental models (Alonso-Magdalena et al., 2012; Watson et al., 2007), producing rapid responses to very low BPA concentrations from 10 fM to 10 nM (Wetherill et al., 2007; Zsarnovszky et al., 2005). Interestingly, a sexually differentiated pattern of GPR30 expression has been reported in some brain areas of hamsters (Canonaco et al., 2008).

BPA is also an antagonist of the androgen receptor (AR) (Molina-Molina et al., 2013; Wetherill et al., 2007). Its anti-androgenic activity has been described in several studies, with varying half maximal inhibitory concentration (IC_{50}) values (Bonefeld-Jørgen-sen et al., 2007; Roy et al., 2004; Xu et al., 2005). Whereas BPA has a half maximal effective concentration (EC_{50}) of 10–100 nM for the ERa, it has an IC_{50} of 1–2 mM against the AR (Teng et al., 2013). Unlike other known AR antagonists, BPA inhibits efficient nuclear translocation of the AR and interferes with its function via multiple mechanisms (Teng et al., 2013).

BPA can also bind to sex hormone-binding globulin (SHBG) (Déchaud et al., 1999), which may alter the androgen–estrogen balance (Takeuchi and Tsutsumi, 2002) and interfere with neuroendocrine regulation of the hypothalamus–pituitary-axis (Gore, 2010; Chen et al., 2014).

Endocrine-related BPA action mechanisms also involve the aryl hydrocarbon receptor (AhR), decreasing its activity in vitro (Bonefeld-Jørgensen et al., 2007) and, at extremely low doses (0.02 mg/kg/d), upregulating brain mRNA expression of this receptor in vivo (Nishizawa et al., 2005). BPA can also reduce aromatase activity in vitro (Bonefeld-Jørgensen et al., 2007) and the synthesis of testosterone and estradiol in vivo (Akingbemi et al., 2004).

BPA binds to thyroid receptors (TRs), acting as an antagonist (Moriyama et al., 2002). In vitro studies have shown that BPA binds to both TR α and β subtypes, although with a relatively low affinity (Delfosse et al., 2014; Kitamura et al., 2005). Moreover, BPA can inhibit thyroid hormone sulfotransferase activity (Butt and Stapleton, 2013) and change the transcription and gene expression of TRs, both in vitro and in experimental models (Gentilcore et al., 2013; Sheng et al., 2012).

BPA has been found to alter glucocorticoid-regulated responses, affecting the sexual differentiation of brain and behavior in rodents (Poimenova et al., 2010), and it has also been proposed as a corticoid agonist (Prasanth et al., 2010). Finally, a recent animal study indicated that chronic perinatal exposure to low BPA concentrations can alter the basal and stress-induced activity of the hypothalamic–pituitary–adrenal axis in a sexually dimorphic manner, increasing susceptibility to stress- and anxiety-related disorders in later life (Panagiotidou et al., 2014).

2.2. Epigenetic effects of BPA

Epigenetic mechanisms of action of BPA include the alteration of some DNA methylation patterns (Dolinoy et al., 2007; Susiarjo et al., 2013). Studies in rodents found that prenatal BPA exposure alters the expression of genes encoding estrogen receptor subtypes (ERα, ERβ and ERγ) in a sex- and brain region-specific manner (Kundakovic et al., 2013) and disrupts normal placental development (Susiarjo et al., 2013). Hence, BPA may predetermine the response of certain brain areas to steroid hormones from a very early stage of development (Wilson and Sengoku, 2013). BPA was reported to impair gene expression of regulatory factors that provide stability and flexibility to epigenetic regulation, adversely affecting the normal development of hypothalamic functions (Warita et al., 2013). Although these changes affect gene expression without altering the underlying DNA, they are heritable and can exert trans-generational effects (Manikkam et al., 2013).

2.3. Synaptic effects

BPA exposure largely abolishes the synaptogenic response to estradiol in hippocampal and prefrontal spine synapses in both rat and non-human primate models (Leranth et al., 2008; MacLusky et al., 2005). This inhibitory effect, at environmentally relevant doses, appears to be mediated via membrane receptors in which rapid responses are elicited (Hajszan and Leranth, 2010). Thus, low BPA doses (40–400 mg/kg/d) altered synaptogenic remodeling in rats (MacLusky et al., 2005) and impacted on dopamine neurons and spine synapses in the hippocampus of in utero exposed non-human primates (Elsworth et al., 2013). As a consequence of this research, concerns have arisen about the effect of BPA exposure on infant human brain functioning and its cognitive and behavioral repercussions.

3. Methods

We reviewed all scientific publications up to March 2015 that addressed the association of human BPA exposure during pregnancy and/or childhood with infant cognitive and/or behavior outcomes. The MEDLINE/PubMed database was searched for publications written in English, using the key words ''BPA'', ''Child'' ''Neurodevelopment'', and ''Behavior/Behaviour''. References cited in the retrieved papers were also examined. We found twelve articles that met the search criteria and gathered the following data: study design and population; (2) type and timing of exposure, (3) exposure assessment (direct assessment/ proxy, spot, or repeated measurements), (4) evaluation of neurodevelopment evaluation (tests); and (5) statistical methods and adjustment for confounders.

4. Results

Up to March 2015, only twelve epidemiological studies explored the relationship between perinatal BPA exposure and neurobehavioral outcomes in childhood (Braun et al., 2009, 2011, 2014; Evans et al., 2014; Harley et al., 2013; Hong et al., 2013; Maserejian et al., 2012a, 2012b; Miodovnik et al., 2011; Perera et al., 2012; Roen et al., 2015; Yolton et al., 2011). Eight of these studies reported a significant association between behavior and BPA exposure during childhood (Harley et al., 2013; Hong et al., 2013; Maserejian et al., 2012a; Roen et al., 2015) and, especially, in utero (Braun et al., 2009, 2011; Evans et al., 2014; Harley et al., 2013; Perera et al., 2012; Roen et al., 2015). In the other four studies, no significant relationship was found between BPA exposure and neurodevelopment or behavioral outcomes (Braun et al., 2014; Maserejian et al., 2012b; Miodovnik et al., 2011; Yolton et al., 2011) (Table 1).

Within the Health Outcomes and Measures of the Environment (HOME) study, a prospective birth cohort in Cincinnati, Ohio (USA), Braun et al. (2009) recruited 249 mother–child pairs between 2003 and 2006, collecting maternal spot urine samples during pregnancy, at 16 and 26 wk of gestation, and at birth. The median concentration of total (free plus conjugated) BPA was 1.8 ng/ml (16 wk), 1.7 ng/ml (26 wk), and 1.3 ng/ml (at birth). The behavior of these infants was assessed at 2 yr of age using the Behavioral Assessment System for Children (BASC-2) completed by parents. After adjustment for confounders, linear regression models showed no association between prenatal BPA exposure and externalizing, internalizing, or Behavior Symptom Index (BSI) scores in the global sample. However, sex-stratified analyses revealed a positive and significant association in the girls, but not in the boys, between BPA levels (at 16 and 26 wk of gestation) and worse externalizing behavior ($\beta = 6.0$; 95%CI, 0.1–12.0; $p < 0.05$) and BSI scores ($\beta = 5.5$; 95% CI, 0.3–10.7). A stronger association was found with BPA levels at 16 wk than with those at 17–21 weeks.

The follow up of this birth cohort (Braun et al., 2011) continued with the collection of new spot urine samples from the children at 1, 2, and 3 yr of age between 2004 and 2009 (median value 4.1 ng/ ml). At the age of 3 yr, the BASC-2 test was once more used to evaluate the children's behavior, and the Behavior Rating Inventory of Executive Function—Preschool (BRIEF—P) test was completed by the parents to assess executive function. Maternal total BPA concentrations during pregnancy (median 2 ng/ml) were again positively associated in the girls with higher BASC-2 scores for hyperactivity ($\beta = 9.1$; 95%CI, 3.1–15; $p < 0.05$), anxiety ($\beta = 12$; 95%CI, 4.7–20, $p < 0.05$), and depression ($\beta = 11$; 95%CI, 3.6–18; $p < 0.05$) and with lower BRIEF—P emotional control ($\beta = 9.1$; 95%CI, 2.8–15; $p < 0.05$) and inhibition ($\beta = 9.3$; 95%CI, 1.8–17) scores. In contrast, the BASC-2 hyperactivity score was negatively associated with maternal BPA in the boys ($\beta = -6.3$; 95%CI, -12 to -0.6; $p < 0.05$). No association was found between childhood total BPA exposure and neurobehavior.

Yolton et al. (2011) studied 350 mother–child pairs enrolled in the HOME cohort, evaluating the neurobehavior of the neonates at 5 wk of age using the Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS). Logistic regression models showed no significant associations between maternal BPA concentrations during pregnancy and neonate neurobehavior, although a trend was observed towards greater hypotonia with higher BPA concentrations at 16 wk of gestation (1.8 ng/ml of total BPA concentration).

Table 1. Human epidemiological studies linking bisphenol A (BPA) and children neurobehavior.

Authorship—Study design	Study population	Urine BPA concentration	Behavior evaluation	Neurobehavior effect
Braun et al. (2009) Prospective Birth Cohort (USA)	HOME study: 249 mother–child pairs Ethnicity: White (62%), Nonwhite (38%) Recruitment: March 2003–January 2006	Maternal gestational Total BPA: 16 wk: 1.8 ng/ml 26 wk: 1.7 ng/ml Birth*: 1.3 ng/ml (*samples collected within 24 h of parturition)	BASC-2 (at 2 years) By mothers	No association between BPA exposure and behavior in the all sample. After sex-stratified analyses, associations were found with externalizing behavior [β = 6.0; 95%CI, 0.1–12.0; p < 0.05] and BSI scores (β = 5.5; 95%CI, 0.3–10.7; p < 0.05), but only among girls. These associations were stronger when considered the earliest mean prenatal exposure (16 wk)
Braun et al. (2011) Prospective Birth Cohort (USA)	HOME Study (follow up): 244 mother–child pairs	Maternal gestational Total BPA (16 wk, 26 wk and birth): 2 ng/ml Childhood Total BPA (1, 2 and 3 years): 4.1 ng/ml	BASC-2 and BRIEF-P (at 3 years) By mothers	Gestational BPA exposure: -Girls: Positive associations were found: BASC-2: Increased hyperactivity (β = 9.1; 95%CI, 3.1–15; p < 0.05), increased anxiety (β = 12; 95%CI, 4.7–20; p < 0.05) and depression (β = 11; 95%CI, 3.6–18; p < 0.05). Interestingly, at the same time hyperactivity decreased among boys (β = -6.3; 95%CI, -12 to -0.6; p < 0.05). BRIEF-P: Poorer emotional control (β = 9.1; 95%CI, 2.8–15; p < 0.05) and inhibition (β = 9.3; 95%CI, 1.8–17; p < 0.05) among girls. Childhood BPA exposure: No association was found
Braun et al. (2014) Prospective Birth Cohort (USA)	HOME Study (follow up): 175 mother–child pairs Boys (n = 95) Girls (n = 80)	Maternal gestational Total BPA: 2.1 ng/ml	SRS (at 4 years and 5 years) By mothers	No association between BPA and SRS scores was observed. They found a mean of 52 EDCs in the biological samples A semi-Bayesian hierarchical regression model showed a direct association between some EDCs and SRS scores (indicating more social problems), while others were inversely associated
Perera et al. (2012) Prospective Birth Cohort (USA)	CCCEH Cohort 198 mother–child pairs Ethnicity: Afro-American and Latina. Low income. Recruitment: 1998–2003	Maternal gestational Total BPA: (34 wk; range 24–40 wk): 1.96 ng/ml Childhood Total BPA (between 3 and 4 years): 3.94 ng/ml	CBCL (between 3–5 years) By mothers (oversaw by trained research workers)	Gestational BPA exposure: Boys: Increased emotional reactivity (1.62 times greater; 95%CI, 1.13–2.32; p <0.008) and aggressive behavior (1.29 times greater; 95%CI, 1.09–1.53; p <0.003). Girls: Decreased anxious/depressed (0.75 times as high; 95%CI, 0.57–0.99; p < 0.04), less aggressive (0.82 times as high; 95%CI, 0.7–0.97; p <0.017). Childhood BPA exposure: Negative association was found for only the Emotionally Reactive scale within all children sample (0.76 times as high; 95%CI, 0.59–0.97; p < 0.029)
Roen et al. (2015)	CCCEH Cohort (follow up): Maternal gestational 250 mother–child pairs	Total BPA: (34 wk; 1.9 ng/ml) Childhood Total BPA (between 3 and 4 years): 3 ng/ml	CBCL (between 7–9 years) By mothers	Gestational BPA exposure: Boys: Increased internalizing (β = 0.41; CI95%, 0.24–0.58; p <0.0001) and externalizing (β = 0.40; CI95%, 0.24–0.56; p <0.0001) problems. Girls: the opposite trend was observed in internalizing composite score (β = -0.17; 95%CI, -0.33, -0.01; p <0.04). Childhood BPA exposure: Boys: fewer symptoms were seen among boys in both internalizing (β = -0.29; CI95%, -0.47, -0.11; p <0.002) and externalizing (β = -0.37; CI95%, -0.54, -0.20; p < 0.0001) problems. Girls: increased problems in both internalizing (β = 0.30; 95%CI, 0.14–0.45; p < 0.0002) and externalizing (β = 0.33; 95%CI, 0.17–0.5; p < 0.0001) symptoms.
Miodovnik et al. (2011) Prospective Birth Cohort (USA)	Mount Sinai Children's Environmental Health Study 137 mother–child pairs Multiethnic Recruitment: May 1998-July 2002	Maternal gestational Total BPA: (31 wk; range 25–40 wk): 1.2 ng/ml (Lot of participants near to LOD)	SRS By mothers (between 7 and 9 years)	Gestational BPA exposure: No association was found with gestational BPA exposure. However, when several outliers were removed (n = 128), they found statistically significant association with higher total SRS score

Table 1 (Continued)

Authorship—Study design	Study population	Urine BPA concentration	Behavior evaluation	Neurobehavior effect
Harley et al. (2013) Prospective Birth Cohort (USA)	CHAMACOS Study 292 mother–child pairs Ethnicity: Latina (99%) Low income. Recruitment: 1999–2000	Maternal gestational Total BPA: (13, 6 wk and 26, 4 wk): 1.1 ng/ml Childhood Total BPA: (at 5 years): 2.5 ng/ml	BASC-2 and CADS (at 7 years) By mothers and teachers CPT (at 9 years) Direct computerized test	Gestational BPA exposure: Boys: higher BPA concentrations during pregnancy were associated with increased internalizing problems [mother report (β = 1.8; 95%CI, 0.3–3.3; p < 0.05)/teacher report (β = 2.5; 95%CI, 0.7–4.4; p <0.01)], with increased symptoms of anxiety* and depression**, and increased aggressive behavior* in boys at 7 years of age, according to either or both mother and teacher report on BASC-2. Prenatal BPA concentrations were not associated with scores on CADS at 7 years. Girls: No significant association was seen between prenatal BPA concentrations and behavior. However, for almost all scales, the point estimates showed trends towards decreased behavior problems. Childhood BPA: Boys: BPA concentrations at age 5 were associated with increased internalizing scores (β = 1.8; 95%CI, 0.4–3.1; p <0.05) and increased anxiety* on the BASC-2 and attention problems* on the BASC-2 and the CADS, at age 7 according to teacher report. No associations were seen with the maternal report. Girls: associations were also found with both internalizing [teacher report (β = 1.8; 95%CI, 0.1–3.6; p <0.05)] and externalizing (by mother and teacher report) problems, both in BASC-2 and CADS, including hyperactivity* and conduct problems**. All together: All associations persisted and became more statistically significant. CPT scores were not associated with gestational or childhood BPA exposure. * p < 0.05 ** p <0.01
Yolton et al. (2011) Prospective Birth Cohort (USA)	HOME Study 350 mother–child pairs Multiethnic Recruitment: March 2003-January 2006	Maternal gestational Total BPA: 16 wk: 1.8 ng/ml 26 wk: 1.7 ng/ml	NNNS (Neonates at 5 wk)	Gestational BPA exposure: No significant associations were found between BPA and behavior in neonates. Only a non-significant trend related to hypotonia was observed (β = 0.170; p = 0.09).
Hong et al., 2013 Cross-sectional study (Korea)	1008 children Ethnicity: Asiatic Recruitment: missing data	Children Total BPA: 1.32 mg/g of creatinine	CBCL LDES (at 8–11 years) By parents	Childhood BPA: BPA levels were positively associated with CBCL total score (β= 0.85; 95% CI, 0.26–1.44; p = 0.00) and negatively associated with LDES total score (β = -1.90; 95%CI, -3.5 to -0.30; p = 0.02). Further, CBCL anxiety/depression score (β = 1.07; 95%CI, 0.57–1.58; p = 0.00) and LDES listening score (β = -0.81; 95%CI, -1.27 to -0.34; p = 0.00) remained significant after correction for multiple comparisons
Evans et al. (2014) Prospective Birth Cohort (USA)	Study for Future Families II 176 mother–child pairs Multiethnic Recruitment: 2002–2005	Maternal gestational Total BPA: 26.6 wk: 1.1 ng/ml	CBCL (at 6–10.5 years) By parents	Gestational BPA exposure: - Boys: Prenatal BPA exposure was significantly associated with behavior problems in several domains, including externalizing composite scale [β(p) = 0.27(0.006)], anxiety [β(p) = 0.15(0.04)], aggressive behavior [β(p) = 0.23(0.01)], conduct disorder [β(p) = 0.22(0.003)], and oppositional/defiant behaviors [β(p) = 0.20(0.008)]. - Girls: No significant association was seen between prenatal BPA concentrations and any CBCL scores; however, the trend was toward negative BPA associations with behavior problems
Maserejian et al. (2012a) Randomized Clinical Safety Trial (USA) Maserejian et al. (2012b)	NECAT: New England children's amalgam trial 434 children Multiethnic recruitment: 1997–2005	Estimation of cumulative exposure from dental treatment (composite vs. amalgam material) establishing surface-years categories. 5 years follow-up	BASC-SR (self-reported) At 8 years CBCL (by parents) (at 6 to 10 years)	Cumulative BPA exposure: Higher exposure to bisGMA-based composite was associated with worse scores on three of the four BASC-SR scales: Emotional Symptoms (β = 0.8; p = 0.003), Clinical Maladjustment (β = 0.7; p = 0.02) and personal adjustment (β = -0.8; SE = 0.2; p = 0.002). No associations were found to amalgam or urethane dimethacrylate-based composite. No associations were found for CBCL
	Secondary analysis 444 children	Idem	WISC-III WIAT WRAML WRAVMA COWAT	Neuropsychological development: Greater exposure to dental composite materials (containing bisGMA and TEGDMA) was not linked to any statistically significant association with neuropsychological scores through follow up. However, a trend was observed between worse scores and children with greater exposure

HOME (Health Outcomes and Measures of the Environment Study). CCCEH (Columbia Center for Children's Environmental Health). CHAMACOS (Center for the Health Assessment of Mothers and Children of Salinas). NECAT (New England Children's Amalgam Trial). BASC-2 (Behavioral Assessment System for Children). BSI (Behavioral Symptom Index). BRIEF-P (Behavior Rating Inventory of Executive Function Preschool Version). LOD (Limit of Detection). CBCL (Child Behavior Check List). SRS (Social Responsiveness Scale). CADS (Conner's ADHD/DSM-IV Scales). CPT (Connors' Continuous Performance Test). NNNS (Neonatal Intensive Care Unit Network Neurobehavioral Scale). LDES

Recently, within the HOME study, Braun et al. (2014) screened a large number of EDCs (n = 70) in relation to autistic behaviors. Blood and/or urine samples of mothers were analyzed during pregnancy and autistic behaviors were assessed when the children were 4 and 5 years old using the Social Responsiveness Scale (SRS). They found a mean of 52 EDCs in the biological samples. A semi-Bayesian hierarchical regression model showed a direct association between some EDCs and SRS scores (indicating more social problems), while others were inversely associated. BPA was not associated with SRS scores.

The Mount Sinai Children's Environmental Health Study is a prospective multiethnic birth cohort of primiparous women recruited between 1998 and 2002 (Miodovnik et al., 2011). Only one maternal urine sample was collected during pregnancy, between 25 and 40 wk of gestation (mean 31.2 wk), yielding a median total BPA concentration of 1.2 ng/ml. Between 7 and 9 yr of age, the social behavior of the children was reported by their mothers (n = 137) using the Social Responsiveness Scale (SRS), and the SRS scores were higher (worse) with greater BPA exposure in utero. According to multivariable adjusted linear regression models, the association between BPA exposure and SRS scores was not statistically significant (β = 1.18; 95%CI, -0.75, 3.11), although significance was reached after the removal of outliers (β = 1.73; 95%CI, 0.02, 3.45).

The Columbia Center for Children's Environmental Health (CCCEH) is a prospective cohort study of 198 mother–child pairs recruited between 1998 and 2003 (Perera et al., 2012). One spot urine sample was collected between 24 and 40 wk of pregnancy (mean 34 wk) and another from the children between 3 and 4 yr of age, finding geometric mean total BPA concentrations of 1.96 ng/ ml and 3.94 ng/ml, respectively. At the age of 3–5 yr, the behavior of the children was reported by their mothers using the Child Behavior Check List (CBCL). Poisson and linear regression models showed an association between gestational BPA exposure (highest quartile vs. lowest three quartiles) and greater problems among the boys in emotionally reactive behavior (1.62-fold greater; 95%CI, 1.13–2.32; p < 0.001) and aggressive behavior (1.29-fold greater; 95%CI, 1.09–1.53; p < 0.003). Among the girls, higher BPA exposure was related to fewer problems in all areas, finding a statistically significant negative association with anxious/de-pressed behavior (0.75-fold higher; 95%CI, 0.57–0.99; p < 0.04) and aggressive behavior (0.82-fold higher; 95%CI, 0.7–0.97; p < 0.02). No significant relationship was found between child-hood BPA exposure and behavior.

The follow up of this birth cohort (Roen et al., 2015) continued with the re-assessment of children' behavior at 7–9 years of age (n = 250). Among the boys (n = 115), higher gestational BPA concentration (upper tertile vs. lower two tertiles) was associated with increased internalizing (β=0.41; 95%CI, 0.24-0.58; P<0.0001) and externalizing (β=0.40; 95%CI, 0.24-0.56; P<0.0001) problems. Among the girls (n=135), the opposite trend was observed trend was observed in internalizing composite score (β = -0.17; 95%CI, -0.33, -0.01; p = 0.04). Moreover, among the girls, higher postnatal BPA concentrations were associated with increased problems in both internalizing (β=0.30; 95%CI, 0.14-0.45; p < 0.0002) and externalizing (β=0.33; 95%CI, 0.17-0.50; p<0.0001) symptoms; while fewer symptoms were seen among the boys in both internalizing (β= -0.29; 95%CI, -0.47, -0.11; p < 0.002) and externalizing (β= -0.37; 95%CI, -0.54, -0.20; p < 0.0001) problems.

The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS, USA) conducted a prospective cohort study of 292 mother– child pairs recruited between 1999 and 2000 on environmental factors and the growth and development of children (Harley et al., 2013). Two maternal urine samples were taken during pregnancy (mean of 13.6 and 26.4 wk) and one sample during childhood (at 5 yr of age), finding mean total BPA concentrations of 1.1 ng/ml during pregnancy and 2.3 ng/ml at 5 yr. At the age of 7 yr, the children's behavior was assessed with the BASC-2 and Conner's ADHD/DSM-IV Scale (CADS), which were both completed by the mothers and teachers; at the age of 9 yr, they were directly assessed using Conner's Continuous Performance Test (CPT). Multivariable linear regression models for the boys showed that each twofold rise in gestational total BPA concentration was significantly associated with an increase at the age of 7 yr in mother- and teacher-reported internalizing problems [mother's report (β 1.8; 95%CI, 0.3–3.3; p < 0.05), teacher's report (β = 2.5; 95%CI, 0.7–4.4; p < 0.01)], symptoms of anxiety (p < 0.05) and depression (p < 0.01), externalizing problems, and aggressive behavior (p < 0.05). No associations were found between prenatal BPA concentrations and behavior at 7 yr in the girls, although there was a trend towards decreased behavior problems. Among the boys in the CHAMACOS cohort, childhood total BPA exposure was associated, as in the case of in utero exposure, with increases in teacher-reported BASC-2 internalizing (β = 1.8; 95%CI, 0.4–3.1; p < 0.05) and anxiety (p < 0.05) scores and BASC-2 and CADS attention problems (p < 0.05); no significant associations with mother-reported scores were observed. Among the girls, associations were also found with internalizing [teacher-reported (β = 1.8; 95%CI, 0.1–3.6; p < 0.05)] and externalizing (mother- and teacher-reported) problems in both BASC-2 and CADS tests, including hyperactivity (p < 0.05) and conduct problems (p < 0.01). When the entire sample was analyzed, these associations persisted and became more statistically significant. No association was found between in utero or childhood BPA exposure and CPT scores.

In a cross-sectional study of 1008 children aged between 8 and 11 yr old (mean of 9.05 0.70 yr), total urinary BPA levels were measured (mean 1.32 mg/g) and the children's behavior was assessed by using the CBCL and the Learning Disabilities Evaluation Scale (LDES), completed by the parents (Hong et al., 2013). Multivariate linear regression adjusted models showed that urinary BPA concentrations were positively associated with the CBCL total problem score (β = 0.85; 95% CI, 0.26–1.44; p = 0.001; R^2 = 0.20) and negatively associated with the LDES score (β = -1.90; 95%CI, -3.5 to -0.30; p = 0.02; R^2 = 0.20). Linear association of anxiety/depression (β = 1.07; 95%CI, 0.57–1.58; p = 0.001) and LDES listening (β = -0.81; 95%CI, -1.27 to -0.34; p = 0.001) scores with the CBCL score remained significant after correction for multiple comparisons.

Maserejian et al. (2012a) studied 434 children (6 to 10 yr old at baseline) in the New England Children's Amalgam Trial (NECAT) between 1997 and 2005. They estimated cumulative exposure to BPA from bisphenol-A-glycidyl-methacrylate (bis-GMA)-based dental composite resins, measured by surface years (each treated surface weighted by no. yrs present in the mouth). The behavior of the children was evaluated with the BASC-SR (Self-reported) and CBCL (parent-reported) tests. Multivariate linear and logistic regression models evidenced an association between greater cumulative exposure to bisGMA and detrimental effects on psychosocial health. The children evidenced more anxiety, depression, social stress, and interpersonal-relation problems with increasing levels and duration of the exposure and were more likely to have clinically significant scores for total problem behaviors. Associations were stronger with posterior-occlusal chewing surfaces, where the degradation of composite is increased and the transfer of BPA derivatives is greater. In the same study population (NECAT), Maserejian et al. (2012b) also examined whether cumulative exposure to bisGMA composite materials was associated with neuropsychological development, finding small but non-significant adverse effects (e.g., slightly lower Scores in intelligence, achievement, and memory tests). Within the Study for Future Families II (SFII), a prospective cohort study of mother–child pairs recruited between 2002 and 2005, maternal urine samples were collected during pregnancy at a mean of 26.6 wk of gestation (range 10–39 wk), yielding a median total BPA concentration of 1.1 ng/ml. Behavior was evaluated

at 6– 10.5 yr of age in 153 children with (parent-reported) CBCL test (Evans et al., 2014). Prenatal BPA exposure was significantly and positively associated with behavior problems among the boys in several domains, including externalizing composite scale [β(p) = 0.27(0.006)], anxiety [β(p) = 0.15(0.04)], aggressive behavior [β(p) = 0.23(0.01)], conduct disorder [β(p) = 0.22(0.003)], and oppositional/defiant behaviors [β(p) = 0.20(0.008)]; observing a significant interaction between BPA and sex for various behaviors. In contrast, a trend towards fewer problems with greater exposure was observed among the girls.

5. Discussion

Increasing attention has been paid over recent years to the impact of prenatal BPA exposure on child neurodevelopment, due to the ubiquitous presence of this EDC and the suspicion of brain and behavior effects based on results obtained in animal models. Only twelve studies on this issue have been published to date, eight of them reporting altered neurobehavior (e.g., hyperactivity, aggressive behavior, anxiety, depression, attention problems, and/or other cognitive function impairments), especially in children exposed in utero, suggesting disruption of the brain during this critical window of development.

The greatest impediment to the comparative analysis of these studies is their heterogeneity. There are disparities in the study design, assessment of exposure (proxy of exposure vs. direct measurements in one or more biological samples), neurodevelopment evaluation (different tests, evaluation periods, and reporters), sample size, adjustment for potential confounders, and/or socio-demographic characteristics of the populations. These shortcomings limit the conclusions that can be drawn on the epidemiology of the potential health risks of BPA exposure on neurodevelopment. Therefore, it is necessary to critically evaluate the main aspects of the most comparable investigations, i.e., those with direct measurements of BPA exposure, a prospective design, and behavior evaluation at a similar developmental period (Braun et al., 2009, 2011; Evans et al., 2014; Harley et al., 2013; Miodovnik et al., 2011; Perera et al., 2012; Roen et al., 2015). Braun et al. (2014) was also excluded, because the main objective of this study was to identify multiple gestational EDC exposure biomarkers and to develop statistical techniques that account for the complex EDC mixtures present in real life.

Among these seven more comparable investigations, six found significant associations between BPA exposure and adverse behavior effects (Braun et al., 2009, 2011; Evans et al., 2014; Harley et al., 2013; Perera et al., 2012; Roen et al., 2015), while Miodovnik et al. (2011) found no relationship. We examined the results of these seven selected studies and discuss below some key aspects of the relationship between BPA exposure and the neurobehavior of children.

5.1. Timing of exposure

A stronger relationship between prenatal vs. childhood BPA exposure and adverse behavioral effects was observed in six out of the seven aforementioned studies. The fetal period is the most important stage of neurodevelopment, and hormones are key factors in numerous developmental events (Fowden and Forhead, 2009). Thus, sex steroids are crucial in the sexual differentiation of central nervous system structures that control neuroendocrine, behavioral, and cognitive functions (Bao and Swaab, 2011; Peperet al., 2011). Further, the period between weeks 8 and 24 of pregnancy has long been considered as the critical period for sexual differentiation of the human brain, especially at around 16 wk (Auyeung et al., 2009, 2013; Berenbaum and Beltz, 2011; Cohen-Bendahan et al., 2005; Manson, 2008). In this line, Braun et al. (2009), found that externalizing problems and anxious and depressed symptoms in children were more strongly associated with the total BPA concentration at 16 wk of gestation than at 26 wk or at the delivery (<24 h postpartum), indicating a possible critical window of exposure (Braun et al., 2009). Nevertheless, the majority of studies that found a significant association considered the gestational period as a whole, based on one or two urine samples collected during different trimesters.

Only two studies have reported relevant associations with postnatal BPA exposure (Harley et al., 2013; Roen et al., 2015), and further research is required to confirm or rule out its effects on later behavior.

The impact of EDC exposure on human health appears to depend strongly on its timing, and the effects of BPA exposure in utero differ from those of exposure during adulthood in both rats (Gore et al., 2014) and non-human primates (Elsworth et al., 2013). Embryos, fetuses, and neonates are highly sensitive to EDC exposure and suffer more severe adverse effects in comparison to adults (Ferna´ndez et al., 2014).

The endocrine disruption hypothesis fits well the paradigm of the fetal origin of disease, which suggests that interactions between the developing organism and the environment exert a major influence on the risk of disease in adulthood (Barker, 2007; Capra et al., 2013). Thus, developmental exposure to EDCs at low doses can result in functional changes in gene expression that do not produce a phenotypic change observable at birth but may increase the risk of dysfunction and disease later in life. Adverse effects may emerge during childhood and adolescence, given that health-disease represents a continuous spectrum of outcomes in response to risk factors (Vandenberg et al., 2010).

5.2. Neurodevelopment assessment

There is no consensus on the most appropriate and sensitive instruments for evaluating cognitive and behavioral problems in children at different ages (Myers et al., 2010). In the reviewed studies, behavior was assessed with multiple different tests completed by different reporters (parents and/or teachers), hampering comparison of the behavioral results. In addition, most of the scales considered only behavioral variables, and there has been very little evaluation of cognitive variables. Hence, there is a need to include cognitive measures in the assessment of BPA effects upon neurodevelopment, given that neuropsychological functions (working memory, behavioral inhibition, cognitive flexibility, reasoning, problem solving and planning) act together to control cognition and modulate complex behaviors (Diamond, 2013). It is also necessary to conduct longitudinal studies with multiple assessments during different developmental periods in order to further quantify the influence of BPA exposure on the mental and behavioral development of children and its long-term effects on their neurobehavioral functioning.

In the seven selected studies, the most frequently applied behavior tests were BASC-2 and CBCL. These two assessment tools showed a correlation of 0.35 to 0.76 for comparable sub-scales and 0.69 to 0.82 for the composite indices [internalizing, externalizing and total] (Myers et al., 2010). Consequently, differences in the scales applied may produce slight-to-moderate variations in behavioral results.

Among behavioral outcomes related to BPA exposure, externalizing composite scores have been found in three out of the six selected studies

that showed a positive association (Braun et al., 2009; Evans et al., 2014; Roen et al., 2015), including aggressive behavior (Evans et al., 2014; Harley et al., 2013; Perera et al. (2012); Roen et al., 2015), and rule-breaking behavior (Evans et al., 2014; Roen et al., 2015). Internalizing composite scores have been associated in Harley et al. (2013) and Roen et al. (2015), including anxiety/depression symptoms reported in four out of these six studies (Braun et al., 2011; Evans et al., 2014; Harley et al., 2013; Roen et al., 2015), emotional control/reactivity problems (Braun et al., 2011;Perera et al., 2012) and somatization (Braun et al., 2011; Roen et al., 2015).

5.3. Sex-dependent effects

Results of the selected studies also indicated that the impact of BPA exposure on neurobehavioral functioning may differ between boys and girls. Thus, in the cohort studied by Braun et al. (2009, 2011), girls were observed to be more susceptible to the impacts of prenatal BPA exposure than were the boys, with findings of increased hyperactivity, anxiety, depression, and somatization symptoms among girls. In contrast, several studies reported that in utero BPA exposure was associated with increased behavior problems in boys but with a trend towards decreased behavior problems in girls (Evans et al., 2014; Harley et al., 2013; Perera et al., 2012; Roen et al., 2015).

An association with postnatal BPA exposure has been predominantly observed in girls rather than boys, indicating that sex-dependent effects may depend upon the developmental period in which exposure occurs. However, only two studies have addressed this issue (Harley et al., 2013; Roen et al., 2015), which requires further investigation.

The above discrepancies in results have been attributed to differences in the timing of exposure, in the neuropsycological tests used, or in the age of the children at assessment as well as to confounders (Perera et al., 2012; Roen et al., 2015; Rosenfeld and Trainor, 2014). With regard to the timing, Harley et al. (2013) and Braun et al. (2009) measured BPA exposure during a similar period (early to mid-pregnancy and observed different effects in boys and girls, respectively). Moreover, this gender discrepancy cannot be attributed to a difference in behavior assessment tools, because Harley et al. (2013) and Braun et al. (2009) both used the BASC-2 test. The age at assessment differed between the studies by Braun et al. (2009, 2011), 2–3 years of age, and those by Harley et al. (2013), Perera et al. (2012), Evans et al. (2014), and Roen et al.

(2015), 3–10 years of age (Table 1). Various authors have pointed out that externalizing problems become more prevalent in boys at 5–7 years of age (Rosenfeld and Trainor, 2014), which may in part explain why associations in boys have largely been observed at an older age. Furthermore, the gender discrepancies observed do not appear to be related to factors such as ethnicity or socioeconomic status; thus, the populations studied by Evans et al. (2014) and Braun et al. (2009, 2011) shared similar sociodemographic characteristics.

The combined effects of exposure to mixtures of EDCs on neurodevelopment have been demonstrated both in vivo and in vitro (Cory-Slechta, 2005; He et al., 2009; Pellacani et al., 2014; Sobolewski et al., 2014; Tiffany-Castiglioni et al., 2006). In this line, a low-level mixture of EDCs recently demonstrated a sex-specific enhanced behavioral toxicity in rodents (Sobolewski et al., 2014). Humans are exposed to many EDCs from different sources (Michałowicz, 2014). Thus, Braun et al. (2014) reported that most pregnant women in the HOME cohort were exposed to a mean of 52 EDCs. A number of epidemiological studies have found that prenatal exposure to EDCs has a negative impact on neuropsychological development during the first years of life (Bellinger, 2013). However, the short- and long-term risks to humans derived from early exposure to environmentally relevant doses of complex mixtures remain unclear and represent an area of increasing concern (Vilahur et al., 2014). Our research group reported that boys appear to be more vulnerable to prenatal exposure to mixtures of xenoestrogens, while no significant associations were seen in girls, with regression coefficients in the opposite direction (Vilahur et al., 2014). The overall mixture of EDCs may affect the androgen/estrogen balance during a critical and sensitive window of neurodevelopment, thereby altering the dimorphic behaviors that characterize boys and girls, which may in part explain both the sex-discrepancy and the observation of opposite trends in males and females across studies.

Furthermore, mechanisms that underlie the neurodevelopmental toxicity and sex-specific effects of BPA are not fully understood. In experimental studies, Kundakovic et al. (2013) observed that in utero exposure to low BPA doses can induce an enduring epigenetic disruption of the brain that may permanently alter brain function and behavior in offspring, especially in relation to sexually dimorphic behaviors. This disruption is associated with long-term changes in ER-related gene expression and DNA methylation in the brain. Moreover, Miodovnik et al. (2012) found that specific polymorphisms in the maternal steroid pathway may be related to behavior problems in boys.

The biological plausibility of the adverse effects of BPA on the brain has been consistently reported in experimental models (Palanza et al., 2008; Wolstenholme et al., 2011a). Results obtained in animals (mostly rodents) have shown that low doses of BPA can disrupt the development of sexually dimorphic behaviors, including anxiety, social interaction, aggression, and spatial memory, and that this disruption has distinct effects on males and females (Adriani et al., 2003; Gioiosa et al., 2013; Palanza et al., 2008; Rubin et al., 2006; Rosenfeld, 2012; Wolstenholme et al., 2011a, 2011b). Notably, administration of low BPA doses (relevant to human exposure) to nonhuman primates abolished the synaptogenic response to estradiol (Leranth et al., 2008) and impacted on midbrain dopamine neurons and hippocampal spine synapses (Elsworth et al., 2013). Moreover, prenatal exposure to low BPA doses (10 mg/kg/d) altered male infant behavior towards the mother [male infants behaved as females] (Nakagami et al., 2009) and modified socio-sexual interactions in male juvenile primates, demasculinizing key sexually dimorphic behaviors (Negishi et al., 2014).

Research on the causal link between BPA and child neurodevelopment is highly challenging. The continual exposure of humans to low doses of BPA means that a scant number of individuals remain unexposed. Furthermore, some authors have concluded that it may never be possible to associate the exposure of a single EDC with a specific neurobehavioral endpoint, at least by using the conventional epidemiological model, due to the ubiquity of BPA, its varied mechanisms of action, the possibility of complex co-exposure effects in humans and living organisms (Braun et al., 2014; Katchy et al., 2014; Rajapakse et al., 2002; Sárria et al., 2011; Vilahur et al., 2014), the difficult characterization of long-term exposure to BPA, and the existence of critical windows of exposure (Colborn, 2004).

6. Conclusion and perspectives

In summary, the mechanisms of BPA action on the brain have been elucidated in experimental models, and the biological plausibility of its adverse cerebral effects has been demonstrated (Elsworth et al., 2013; Hajszan and Leranth, 2010; Nakagami et al., 2009; Negishi et al., 2014; Palanza et al., 2008; Wolstenholme et al., 2011a). The epidemiologic studies conducted to date point in the same worrying direction, suggesting that prenatal exposure to BPA (and possibly postnatal BPA exposure) may be related to increased neurobehavioral problems in children. Although the very small number of

human studies does not yet allow any conclusive link to be established, the results of our comparative analysis show that aggressive behavior and anxiety/depression symptoms are the problems most consistently associated with BPA exposure.

Understandably, there is special concern about the potential effect on the fetus and neonate brain, given their particular vulnerability to neurotoxicants and generally higher exposure to BPA in comparison to adults (Calafat et al., 2008; Mielke and Gundert-Remy, 2009). Moreover, the almost universal exposure of humans to BPA means that small changes in behavior at the individual level may have major social repercussions (Bellinger, 2004, 2007). The magnitude of the potential impact of BPA exposure represents a valuable research line that might lead to a reassessment of the risks for vulnerable populations (Chapin et al., 2008; vom Saal et al., 2007).

Many more studies in humans are required to clarify the relationship between BPA exposure and health. Nevertheless, there is a present need for vigilance in regard to the potential adverse effects of ubiquitous low-level BPA exposure, and it appears desirable to inform women planning or undergoing pregnancy about measures to reduce or avoid EDC exposure (Groff, 2010; Stotland et al., 2014).

Future actions

More prospective birth cohort studies are needed. Vigilance is now essential in regard to the potential adverse effects of ubiquitous low-level BPA exposure.

Physicians, especially gynecologists and pediatricians, should be aware of the hazards of EDC exposure, allowing them to make lifestyle recommendations for preventing and/or reducing exposure, especially in high-risk populations.

A new risk assessment is required in order to take account of increasing evidence of the deleterious effects of BPA on child behavior and cognition (Chapin et al., 2008). It appears appropriate to follow the precautionary principle until more conclusive data are available on the exposure of fetuses and children to BPA.

Conflict of interest

The authors declare no conflict of interest.

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References

Adriani, W., Seta, D.D., et al., 2003. Altered profiles of spontaneous novelty seeking, impulsive behavior, and response to D-amphetamine in rats perinatally exposed to bisphenol A. Environ. Health Perspect. 111 (4), 395–401.

Akingbemi, B.T., Sottas, C.M., et al., 2004. Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. Endocrinology 145 (2), 592–603.

Alonso-Magdalena, P., Ropero, A.B., et al., 2012. Bisphenol-A acts as a potent estrogen via non-classical estrogen triggered pathways. Mol. Cell. Endocrinol. 355 (2), 201–207.

Andersen, H.R., Andersson, A.M., et al., 1999. Comparison of short-term estrogenicity tests for identification of hormone-disrupting chemicals. Environ. Health Perspect. 107 (Suppl. 1), 89–108.

Anderson, O.S., Peterson, K.E., et al., 2013. Perinatal bisphenol A exposure promotes hyperactivity, lean body composition, and hormonal responses across the murine life course. FASEB J. 27 (4), 1784–1792.

Auyeung, B., Baron-Cohen, S., et al., 2009. Fetal testosterone predicts sexually differentiated childhood behavior in girls and in boys. Psychol. Sci. 20 (2), 144–148.

Auyeung, B., Lombardo, M.V., et al., 2013. Prenatal and postnatal hormone effects on the human brain and cognition. Pflugers Arch. 465 (5), 557–571.

Bao, A.M., Swaab, D.F., 2011. Sexual differentiation of the human brain: relation to gender identity, sexual orientation and neuropsychiatric disorders. Front. Neuroendocrinol. 32 (2), 214–226.

Barker, D.J., 2007. The origins of the developmental origins theory. J. Intern. Med. 261 (5), 412–417.

Becker, K., Goen, T., et al., 2009. GerES IV: phthalate metabolites and bisphenol A in urine of German children. Int. J. Hyg. Environ. Health 212 (6), 685–692.

Bellinger, D.C., 2004. What is an adverse effect? A possible resolution of clinical and epidemiological perspectives on neurobehavioral toxicity. Environ. Res. 95 (3), 394–405.

Bellinger, D.C., 2007. Interpretation of small effect sizes in occupational and environmental neurotoxicology: individual versus population risk. Neurotoxicology 28 (2), 245–251.

Bellinger, D.C., 2013. Prenatal exposures to environmental chemicals and children’s neurodevelopment: an update. Saf. Health Work 4 (1), 1–11.

Bemrah, N., Jean, J., et al., 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.

Berenbaum, S.A., Beltz, A.M., 2011. Sexual differentiation of human behavior: effects of prenatal and pubertal organizational hormones. Front. Neuroendocrinol. 32 (2), 183–200.

Bonefeld-Jørgensen, E.C., Long, M., et al., 2007. Endocrine-disrupting potential of bisphenol A, bisphenol A dimethacrylate, 4-n-nonylphenol, and 4-n-octylphenol in vitro: new data and a brief review. Environ. Health Perspect. 115 (Suppl. 1), 69–76.

Bowman, R.E., Luine, V., et al., 2014. Adolescent Bisphenol-A exposure decreases dendritic spine density: role of sex and age. Synapse 68 (11), 498–507.

Braun, J.M., Yolton, K., et al., 2009. Prenatal bisphenol A exposure and early childhood behavior. Environ. Health Perspect. 117 (12), 1945–1952.

Braun, J.M., Kalkbrenner, A.E., et al., 2011. Impact of early-life bisphenol A exposure on behavior and executive function in children. Pediatrics 128 (5), 873–882.

Braun, J.M., Kalkbrenner, A.E., et al., 2014. Gestational exposure to endocrine-disrupting chemicals and reciprocal social, repetitive, and stereotypic behaviors in 4-and 5-year-old children: the HOME study. Environ. Health Perspect. 122 (5), 513–520.

Brotons, J.A., Olea-Serrano, M.F., et al., 1995. Xenoestrogens released from lacquer coatings in food cans. Environ. Health Perspect. 103 (6), 608–612.

Butt, C.M., Stapleton, H.M., 2013. Inhibition of thyroid hormone sulfotransferase activity by brominated flame retardants and halogenated phenolics. Chem. Res. Toxicol. 26 (11), 1692–1702.

Calafat, A.M., Ye, X., et al., 2008. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003–2004. Environ. Health Perspect. 116 (1), 39–44.

Calafat, A.M., Weuve, J., et al., 2009. Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants. Environ. Health Perspect. 117 (4), 639–644.

Canonaco, M., Giusi, G., et al., 2008. A sexually dimorphic distribution pattern of the novel estrogen receptor G-protein-coupled receptor 30 in some brain areas of the hamster. J. Endocrinol. 196 (1), 131–138.

Cao, X.L., Corriveau, J., et al., 2009. Levels of bisphenol A in canned soft drink products in Canadian markets. J. Agric. Food Chem. 57 (4), 1307–1311.

Cao, J., Rebuli, M.E., et al., 2013. Prenatal bisphenol A exposure alters sex-specific estrogen receptor expression in the neonatal rat hypothalamus and amygdala. Toxicol. Sci. 133 (1), 157–173.

Capra, L., Tezza, G., et al., 2013. The origins of health and disease: the influence of maternal diseases and lifestyle during gestation. Ital. J. Pediatr. 39, 7.

Carr, R., Bertasi, F., et al., 2003. Effect of neonatal rat bisphenol a exposure on performance in the Morris water maze. J. Toxicol. Environ. Health A: Curr. Issues 66 (21), 2077–2088.

Carwile, J.L., Luu, H.T., et al., 2009. Polycarbonate bottle use and urinary bisphenol A concentrations. Environ. Health Perspect. 117 (9), 1368–1372.

Casas, M., Chevrier, C., et al., 2013. Exposure to brominated flame retardants, perfluorinated compounds, phthalates and phenols in European birth cohorts: ENRIECO evaluation, first human biomonitoring results, and recommendations. Int. J. Hyg. Environ. Health 216 (3), 230–242.

Chapin, R.E., Adams, J., et al., 2008. NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A. Birth Defects Res. B: Dev. Reprod. Toxicol. 83 (3), 157–395.

Chen, F., Zhou, L., et al., 2014. Sex differences in the adult HPA axis and affective behaviors are altered by perinatal exposure to a low dose of bisphenol A. Brain Res. 1571, 12–24.

Cohen-Bendahan, C.C., van de Beek, C., et al., 2005. Prenatal sex hormone effects on child and adult sex-typed behavior: methods and findings. Neurosci. Biobehav. Rev. 29 (2), 353–384.

Colborn, T., 2004. Neurodevelopment and endocrine disruption. Environ. Health Perspect. 112 (9), 944–949.

Corbel, T., Gayrard, V., et al., 2014. Bidirectional placental transfer of Bisphenol A and its main metabolite, Bisphenol A-Glucuronide, in the isolated perfused human placenta. Reprod. Toxicol. 47, 51–58.

Cory-Slechta, D.A., 2005. Studying toxicants as single chemicals: does this strategy adequately identify neurotoxic risk? Neurotoxicology 26 (4), 491–510.

De´chaud, H., Ravard, C., et al., 1999. Xenoestrogen interaction with human sex hormone-binding globulin (hSHBG). Steroids 64 (5), 328–334.

Delfosse, V., Grimaldi, M., et al., 2014. Nuclear receptor profiling of bisphenol-A and its halogenated analogues. Vitam. Horm. 94, 229–251.

Dessı`-Fulgheri, F., Porrini, S., Farabollini, F., et al., 2002. Effects of perinatal exposure to bisphenol A on play behavior of female and male juvenile rats. Environ. Health Perspect. 110 (Suppl. 3), 403–407.

Diamond, A., 2013. Executive functions. Annu. Rev. Psychol. 64, 135–168. Dodds, E.C., Lawson, W., 1938. Molecular structure in relation to oestrogenic

activity. Compounds without a phenanthrene nucleus. Proc. R. Soc. London B: Biol. Sci. 125, 222–232.

Dolinoy, D.C., Weidman, J.R., et al., 2007. Epigenetic gene regulation: linking early developmental environment to adult disease. Reprod. Toxicol. 23 (3), 297–307.

Duty, S.M., Mendonca, K., et al., 2013. Potential sources of bisphenol A in the neonatal intensive care unit. Pediatrics 131 (3), 483–489.

Edlow, A.G., Chen, M., et al., 2012. Fetal bisphenol A exposure: concentration of conjugated and unconjugated bisphenol A in amniotic fluid in the second and third trimesters. Reprod. Toxicol. 34 (1), 1–7.

Ehrlich, S., Calafat, A.M., et al., 2014. Handling of thermal receipts as a source of exposure to bisphenol A. JAMA 311 (8), 859–860.

Eilam-Stock, T., Serrano, P., et al., 2012. Bisphenol-A impairs memory and reduces dendritic spine density in adult male rats. Behav. Neurosci. 126 (1), 175–185.

Elsworth, J.D., Jentsch, J.D., et al., 2013. Prenatal exposure to bisphenol A impacts midbrain dopamine neurons and hippocampal spine synapses in non-human primates. Neurotoxicology 35, 113–120.

Evans, S.F., Kobrosly, R.W., et al., 2014. Prenatal Bisphenol A Exposure and maternally reported behavior in boys and girls. Neurotoxicology 45, 91–99.

Farabollini, F., Porrini, S., et al., 2002. Effects of perinatal exposure to bisphenol A on sociosexual behavior of female and male rats. Environ. Health Perspect. 110 (Suppl 3), 409–414.

Ferna´ndez, M.F., Roma´n, M., et al., 2014. Endocrine disruptors: time to act. Curr.

Environ Health Rep. 1 (4), 325–332.

Fowden, A.L., Forhead, A.J., 2009. Hormones as epigenetic signals in developmental programming. Exp. Physiol. 94 (6), 607–625.

Geens, T., Aerts, D., et al., 2012. A review of dietary and non-dietary exposure to bisphenol-A. Food Chem. Toxicol. 50 (10), 3725–3740.

Gentilcore, D., Porreca, I., et al., 2013. Bisphenol A interferes with thyroid specific gene expression. Toxicology 304, 21–31.

Gioiosa, L., Parmigiani, S., et al., 2013. The effects of bisphenol A on emotional behavior depend upon the timing of exposure, age and gender in mice. Horm. Behav. 63 (4), 598–605.

Gore, A.C., 2010. Neuroendocrine targets of endocrine disruptors. Hormones (Athens) 9 (1), 16–27.

Gore, A.C., Martien, K.M., et al., 2014. Implications of prenatal steroid perturbations for neurodevelopment, behavior, and autism. Endocr. Rev. 35

(6), 961–991, http://dx.doi.org/10.1210/er.2013-1122.

Groff, T., 2010. Bisphenol A: invisible pollution. Curr. Opin. Pediatr. 22 (4), 524–529.

Hajszan, T., Leranth, C., 2010. Bisphenol A interferes with synaptic remodeling.

Front. Neuroendocrinol. 31 (4), 519–530.

Harley, K.G., Gunier, R.B., et al., 2013. Prenatal and early childhood bisphenol A concentrations and behavior in school-aged children. Environ. Res. 126, 43–50.

He, P., Wang, A.G., et al., 2009. Mechanism of the neurotoxic effect of PBDE-47 and interaction of PBDE-47 and PCB153 in enhancing toxicity in SH-SY5Y cells. Neurotoxicology 30, 10–15.

Hong, S.B., Hong, Y.C., et al., 2013. Bisphenol A in relation to behavior and learning of school-age children. J. Child. Psychol. Psychiatry 54 (8), 890–899.

Jasˇarevic´, E., Sieli, P.T., et al., 2011. Disruption of adult expression of sexually selected traits by developmental exposure to bisphenol A. Proc. Natl. Acad. Sci. U.S.A. 108 (28), 11715–11720.

Jime´nez-Dı´az, I., Zafra-Go´mez, A., et al., 2010. Determination of Bisphenol A and its chlorinated derivatives in placental tissue samples by liquid chromatography–tandem mass spectrometry. J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 878 (32), 3363–3369.

Katchy, A., Pinto, C., et al., 2014. Coexposure to phytoestrogens and bisphenol a mimics estrogenic effects in an additive manner. Toxicol. Sci. 138 (1), 21–35.

Kawai, K., Nozaki, T., et al., 2003. Aggressive behavior and serum testosterone concentration during the maturation process of male mice: the effects of fetal exposure to bisphenol A. Environ. Health Perspect. 111 (2), 175–178.

Kitamura, S., Suzuki, T., et al., 2005. Comparative study of the endocrine-disrupting activity of bisphenol A and 19 related compounds. Toxicol. Sci. 84

(2), 249–259.

Komada, M., Itoh, S., et al., 2014. Newborn mice exposed prenatally to bisphenol A show hyperactivity and defective neocortical development. Toxicology 323, 51–60.

Krishnan, A.V., Stathis, P., et al., 1993. Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology 132

(6), 2279–2286.

Kubo, K., Arai, O., et al., 2003. Low dose effects of bisphenol A on sexual differentiation of the brain and behavior in rats. Neurosci. Res. 45 (3), 345–356.

Kundakovic, M., Gudsnuk, K., et al., 2013. Sex-specific epigenetic disruption and behavioral changes following low-dose in utero bisphenol A exposure. Proc. Natl. Acad. Sci. U.S.A. 110 (24), 9956–9961.

Kurosawa, T., Hiroi, H., et al., 2002. The activity of bisphenol A depends on both the estrogen receptor subtype and the cell type. Endocr. J. 49 (4), 465–471.

Kuwahara, R., Kawaguchi, S., et al., 2013. Perinatal exposure to low-dose bisphenol A impairs spatial learning and memory in male rats. J. Pharmacol. Sci. 123 (2), 132–139.

Leranth, C., Hajszan, T., et al., 2008. Bisphenol A prevents the synaptogenic response to estradiol in hippocampus and prefrontal cortex of ovariectomized nonhuman primates. Proc. Natl. Acad. Sci. U.S.A. 105 (37), 14187–14191.

Luo, G., Wang, S., et al., 2014. Maternal Bisphenol A diet induces anxiety-like behavior in female juvenile with neuroimmune activation. Toxicol. Sci. 140

(2), 364–373.

MacLusky, N.J., Naftolin, F., 1981. Sexual differentiation of the central nervous system. Science 211 (4488), 1294–1302.

MacLusky, N.J., Hajszan, T., et al., 2005. The environmental estrogen bisphenol A inhibits estradiol-induced hippocampal synaptogenesis. Environ. Health Perspect. 113 (6), 675–679.

Manikkam, M., Tracey, R., et al., 2013. Plastics derived endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. PLoS ONE 8 (1), e55387.

Manson, J.E., 2008. Prenatal exposure to sex steroid hormones and behavioral/ cognitive outcomes. Metabolism 57 (Suppl. 2), S16–S21.

Maserejian, N.N., Trachtenberg, F.L., et al., 2012a. Dental composite restorations and psychosocial function in children. Pediatrics 130 (2), e328–e338.

Maserejian, N.N., Trachtenberg, F.L., et al., 2012b. Dental composite restorations and neuropsychological development in children: treatment level analysis from a randomized clinical trial. Neurotoxicology 33 (5), 1291–1297.

Matsushima, A., Kakuta, Y., et al., 2007. Structural evidence for endocrine disruptor bisphenol A binding to human nuclear receptor ERR gamma. J. Biochem. 142 (4), 517–524.

Mendonca, K., Hauser, R., et al., 2014. Bisphenol A concentrations in maternal breast milk and infant urine. Int. Arch. Occup. Environ. Health 87 (1), 13–20.

Michałowicz, J., 2014. Bisphenol A—sources, toxicity and biotransformation.

Environ. Toxicol. Pharmacol. 37 (2), 738–758.

Mielke, H., Gundert-Remy, U., 2009. Bisphenol A levels in blood depend on age and exposure. Toxicol. Lett. 190 (1), 32–40.

Miodovnik, A., Engel, S.M., et al., 2011. Endocrine disruptors and childhood social impairment. Neurotoxicology 32 (2), 261–267.

Miodovnik, A., Diplas, A.I., et al., 2012. Polymorphisms in the maternal sex steroid pathway are associated with behavior problems in male offspring. Psychiatr. Genet. 22 (3), 115–122.

Molina-Molina, J.M., Amaya, E., et al., 2013. In vitro study on the agonistic and antagonistic activities of bisphenol-S and other bisphenol-A congeners and derivatives via nuclear receptors. Toxicol. Appl. Pharmacol. 272 (1), 127–136.

Moriyama, K., Tagami, T., et al., 2002. Thyroid hormone action is disrupted by bisphenol A as an antagonist. J. Clin. Endocrinol. Metab. 87 (11), 5185–5190.

Myers, C.L., Bour, J.L., et al., 2010. Same constructs, different results: examining the consistency of two behavior-rating scales with referred preschoolers. Psychol. Sch. 47, 205–216.

Nakagami, A., Negishi, T., et al., 2009. Alterations in male infant behaviors towards its mother by prenatal exposure to bisphenol A in cynomolgus monkeys (Macaca fascicularis) during early suckling period.

Psychoneuroendocrinology 34 (8), 1189–1197.

Negishi, T., Nakagami, A., et al., 2014. Altered social interactions in male juvenile cynomolgus monkeys prenatally exposed to bisphenol A. Neurotoxicol. Teratol. 44, 46–52.

Nishizawa, H., Imanishi, S., et al., 2005. Effects of exposure in utero to bisphenol A on the expression of aryl hydrocarbon receptor, related factors, and xenobiotic metabolizing enzymes in murine embryos. J. Reprod. Dev. 51 (5), 593–605.

Palanza, P., Gioiosa, L., et al., 2008. Effects of developmental exposure to bisphenol A on brain and behavior in mice. Environ. Res. 108 (2), 150–157.

Panagiotidou, E., Zerva, S., et al., 2014. Perinatal exposure to low-dose bisphenol A affects the neuroendocrine stress response in rats. J. Endocrinol. 220 (3), 207–218.

Patisaul, H.B., Bateman, H.L., 2008. Neonatal exposure to endocrine active compounds or an ERbeta agonist increases adult anxiety and aggression in gonadally intact male rats. Horm. Behav. 53 (4), 580–588.

Pellacani, C., Tagliaferri, S., et al., 2014. Synergistic interactions between PBDEs and PCBs in human neuroblastoma cells. Environ. Toxicol. 29 (4), 418–427.

Peper, J.S., van den Heuvel, M.P., et al., 2011. Sex steroids and connectivity in the human brain: a review of neuroimaging studies. Psychoneuroendocrinology 36 (8), 1101– 1113.

Perera, F., Vishnevetsky, J., et al., 2012. Prenatal bisphenol A exposure and child behavior in an inner-city cohort. Environ. Health Perspect. 120 (8), 1190–1194.

Perez, P., Pulgar, R., et al., 1998. The estrogenicity of bisphenol A-related diphenylalkanes with various substituents at the central carbon and the hydroxy groups. Environ. Health Perspect. 106 (3), 167–174.

Poimenova, A., Markaki, E., et al., 2010. Corticosterone-regulated actions in the rat brain are affected by perinatal exposure to low dose of bisphenol A. Neuroscience 167 (3), 741–749.

Porrini, S., Belloni, V., et al., 2005. Early exposure to a low dose of bisphenol A affects socio-sexual behavior of juvenile female rats. Brain Res. Bull. 65 (3), 261–266.

Prasanth, G.K., Divya, L.M., et al., 2010. Bisphenol-A can bind to human glucocorticoid receptor as an agonist: an in silico study. J. Appl. Toxicol. 30 (8), 769–774.

Rajapakse, N., Silva, E., et al., 2002. Combining xenoestrogens at levels below individual no-observed-effect concentrations dramatically enhances steroid hormone action. Environ. Health Perspect. 110 (9), 917–921.

Rebuli, M.E., Cao, J., et al., 2014. Investigation of the effects of subchronic low dose oral exposure to Bisphenol A (BPA) and Ethinyl Estradiol (EE) on estrogen receptor expression in the juvenile and adult female rat hypothalamus. Toxicol. Sci. 140 (1), 190–203.

Rochester, J.R., 2013. Bisphenol A and human health: a review of the literature. Reprod. Toxicol. 42, 132–155.

Roen, E.L., Wang, Y., et al., 2015. Bisphenol A exposure and behavioral problems among inner city children at 7–9 years of age. Environ. Res. ([Epub ahead of print]).

Rosenfeld, C.S., 2012. Effects of maternal diet and exposure to bisphenol A on sexually dimorphic responses in conceptuses and offspring. Reprod. Domest. Anim. 47 (Suppl. 4), 23–30.

Rosenfeld, C.S., Trainor, B.C., 2014. Environmental health factors and sexually dimorphic differences in behavioral disruptions. Curr. Environ. Health Rep. 1 (4), 287–301.

Roy, P., Salminen, H., et al., 2004. Screening of some anti-androgenic endocrine disruptors using a recombinant cell-based in vitro bioassay. J. Steroid Biochem. Mol. Biol. 88 (2), 157–166.

Rubin, B.S., Lenkowski, J.R., et al., 2006. Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. Endocrinology 147 (8), 3681–3691.

Sa´rria, M.P., Santos, M.M., et al., 2011. The unpredictable effects of mixtures of androgenic and estrogenic chemicals on fish early life. Environ. Int. 37 (2), 418–424.

Sheng, Z.G., Tang, Y., et al., 2012. Low concentrations of bisphenol A suppress thyroid hormone receptor transcription through a nongenomic mechanism. Toxicol. Appl. Pharmacol. 259 (1), 133–142.

Sobolewski, M., Conrad, K., et al., 2014. Sex-specific enhanced behavioral toxicity induced by maternal exposure to a mixture of low dose endocrine-disrupting chemicals. Neurotoxicology 45, 121–130.

Stotland, N.E., Sutton, P., et al., 2014. Counseling patients on preventing prenatal environmental exposures—a mixed-methods study of obstetricians. PLoS ONE 9 (6), e98771.

Susiarjo, M., Sasson, I., et al., 2013. Bisphenol A exposure disrupts genomic imprinting in the mouse. PLoS Genet. 9 (4), e1003401.

Swaab, D.F., 2007. Sexual differentiation of the brain and behavior. Best Pract. Res. Clin. Endocrinol. Metab. 21 (3), 431–444.

Takayanagi, S., Tokunaga, T., et al., 2006. Endocrine disruptor bisphenol A strongly binds to human estrogen-related receptor gamma (ERRgamma) with high constitutive activity. Toxicol. Lett. 167 (2), 95–105.

Takeda, Y., Liu, X., et al., 2009. Placenta expressing the greatest quantity of bisphenol A receptor ERRgamma among the human reproductive tissues: predominant expression of type-1 ERRgamma isoform. J. Biochem. 146 (1), 113–122.

Takeuchi, T., Tsutsumi, O., 2002. Serum bisphenol A concentrations showed gender differences, possibly linked to androgen levels. Biochem. Biophys. Res. Commun. 291 (1), 76–78.

Tando, S., Itoh, K., et al., 2014. Bisphenol A exposure disrupts the development of the locus coeruleus-noradrenergic system in mice. Neuropathology, http://dx.doi.org/10.1111/neu.12137 ([Epub ahead of print]).

Teng, C., Goodwin, B., et al., 2013. Bisphenol A affects androgen receptor function via multiple mechanisms. Chem. Biol. Interact. 203 (3), 556–564.

Thomas, P., Dong, J., 2006. Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: a potential novel mechanism of endocrine disruption. J. Steroid Biochem. Mol. Biol. 102 (1–5), 175–179.

Tian, Y.H., Baek, J.H., et al., 2010. Prenatal and postnatal exposure to bisphenol A induces anxiolytic behaviors and cognitive deficits in mice. Synapse 64 (6), 432–439.

Tiffany-Castiglioni, E., Hong, S., et al., 2006. In vitro models for assessing neurotoxicity of mixtures. Neurotoxicology 27 (5), 835–839.

Tohme´, M., Prud'homme, S.M., et al., 2014. Estrogen-related receptor g is an in vivo receptor of bisphenol A. FASEB J. 28 (7), 3124–3133.

Tsutsumi, O., 2005. Assessment of human contamination of estrogenic endocrine-disrupting chemicals and their risk for human reproduction. J. Steroid Biochem. Mol. Biol. 93 (2–5), 325–330.

Vandenberg, L.N., Hauser, R., et al., 2007. Human exposure to bisphenol A (BPA). Reprod. Toxicol. 24 (2), 139–177.

Vandenberg, L.N., Maffini, M.V., et al., 2009. Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. Endocr. Rev. 30 (1), 75–95.

Vandenberg, L.N., Chahoud, I., et al., 2010. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ. Health Perspect. 118 (8), 1055–1070.

Vilahur, N., Bustamante, M., et al., 2014. Prenatal exposure to mixtures of xenoestrogens and repetitive element DNA methylation changes in human placenta. Environ. Int. 71, 81–87.

Vogel, S.A., 2009. The politics of plastics: the making and unmaking of bisphenol A ''safety''. Am. J. Public Health 99 (Suppl. 3), S559–S566.

vom Saal, F.S., Akingbemi, B.T., et al., 2007. Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. Reprod. Toxicol. 24 (2), 131–138.

von Goetz, N., Wormuth, M., et al., 2010. Bisphenol A: how the most relevant exposure sources contribute to total consumer exposure. Risk Anal. 30 (3), 473–487.

Wang, C., Niu, R., et al., 2014. Changes in memory and synaptic plasticity induced in male rats after maternal exposure to bisphenol A. Toxicology 322, 51–60.

Warita, K., Mitsuhashi, T., et al., 2013. Gene expression of epigenetic regulatory factors related to primary silencing mechanism is less susceptible to lower doses of bisphenol A in embryonic hypothalamic cells. J. Toxicol. Sci. 38 (2), 285–289.

Watson, C.S., Bulayeva, N.N., et al., 2007. Xenoestrogens are potent activators of nongenomic estrogenic responses. Steroids 72 (2), 124–134.

Welshons, W.V., Nagel, S.C., et al., 2006. Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. Endocrinology 147 (6 Suppl.), S56–S69.

Wetherill, Y.B., Akingbemi, B.T., et al., 2007. In vitro molecular mechanisms of bisphenol A action. Reprod. Toxicol. 24 (2), 178–198.

Wilson, M.E., Sengoku, T., 2013. Developmental regulation of neuronal genes by DNA methylation: environmental influences. Int. J. Dev. Neurosci. 31 (6), 448–451.

Wolstenholme, J.T., Rissman, E.F., et al., 2011a. The role of Bisphenol A in shaping the brain, epigenome and behavior. Horm. Behav. 59 (3), 296–305.

Wolstenholme, J.T., Taylor, J.A., et al., 2011b. Gestational exposure to low dose bisphenol A alters social behavior in juvenile mice. PLoS ONE 6 (9), e25448.

Xu, L.C., Sun, H., et al., 2005. Evaluation of androgen receptor transcriptional activities of bisphenol A, octylphenol and nonylphenol in vitro. Toxicology 216 (2–3), 197–203.

Xu, X., Hong, X., et al., 2012. Gestational and lactational exposure to bisphenol-A affects anxiety- and depression-like behaviors in mice. Horm. Behav. 62 (4), 480–490.

Yang, C.F., Shah, N.M., 2014. Representing sex in the brain, one module at a time. Neuron 82 (2), 261–278.

Yolton, K., Xu, Y., et al., 2011. Prenatal exposure to bisphenol A and phthalates and infant neurobehavior. Neurotoxicol. Teratol. 33 (5), 558–566.

Zhou, R., Bai, Y., et al., 2011. Abnormal synaptic plasticity in basolateral amygdala may account for hyperactivity and attention-deficit in male rat exposed perinatally to low-dose bisphenol-A. Neuropharmacology 60 (5), 789–798.

Zsarnovszky, A., Le, H.H., et al., 2005. Ontogeny of rapid estrogen-mediated extracellular signal-regulated kinase signaling in the rat cerebellar cortex: potent nongenomic agonist and endocrine disrupting activity of the xenoestrogen bisphenol A. Endocrinology 146 (12), 5388–5396.

PAPER 4

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Paper #4

Exposure to bisphenol A and behavior in school-age children

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Abstract

Introduction: Bisphenol A (BPA) exposure has been shown to affect human brain neurodevelopment and behavior.
Objective: We aimed to investigate whether environmental exposure to BPA in children was associated with their childhood behavior.
Methods: Urinary BPA concentrations and behavioral characteristics were assessed in 300 children belonging to the INMA ''Environment and Childhood'' Granada birth cohort in their follow-up at 9–11 years of age. BPA concentrations were quantified in urine using liquid chromatography-tandem mass spectrometry (LC–MS–MS), and child behavior reported by parents using the Child Behavior Checklist (CBCL/6–18) under supervision of a psychologist. The association between BPA concentrations and CBCL standardized scores was analyzed using linear regression models, adjusted for important covariates.
Results: Median (P25, P75) BPA concentration was 4.76 (2.77, 9.03) mg/L. Mean (SD) CBCL externalizing and internalizing scores were 56.35 (8.06) and 51.36 (9.22), respectively. In multivariate regression analyses, adjusted for maternal and child characteristics, higher BPA concentrations were associated with worse behavioral scores on all scales. Children with BPA concentrations in the 4th quartile had more somatic complaints (b = 2.35; 95% CI: 0.25, 4.46) and social (b = 1.71; 95% CI: 0.19, 3.22) and thought problems (b = 2.58; 95% CI: 0.66, 4.51) in comparison to those in the 1st quartile. Children with values in the 3rd quartile of BPA concentrations also showed greater social problems (b = 1.94; 95% CI: 0.43, 3.45).
Conclusions: Our results suggest that exposure to BPA in childhood may affect children's behavior. Although further investigations are required, preventive measures should be undertaken to reduce inadvertent exposure to BPA.

1. Introduction

Bisphenol A (BPA) is a high-production synthetic chemical used in the manufacture of polycarbonate plastics and epoxy resins (e.g., in food and drink containers), flame retardants, dental sealants, and in thermal paper, among other applications (Vandenberg et al., 2007). BPA is commonly found in food and beverage products, which are considered as the predominant source of BPA exposure in the general population (Vandenberg et al., 2010), although aquatic, air, soil, and dermal routes may also contribute to total human exposure (Michałowicz, 2014). Biomonitoring studies indicate that human exposure to BPA is nearly ubiquitous, with 95% of general population having detectable levels of BPA in different biological matrices (usually urine at the concentration range of nanograms per milliliter) (Becker et al., 2009; Calafat et al., 2008; Casas et al., 2011; Covaci et al., 2015; Findlay and Kohen, 2015; Koch et al., 2012; LaKind and Naiman, 2015). BPA has also been consistently detected in maternal blood, amniotic fluid, cord and fetal serum, placenta, and maternal breast milk (Jiménez-Díaz et al., 2010; Vandenberg et al., 2010).

BPA is a known endocrine-disrupting compound (EDC) with the potential to disturb hormonal regulation and the normal endocrine

system, even at low doses (Casals-Casas and Desvergne, 2011). BPA has varied and complex mechanisms of action. Thus, BPA can bind to thyroid receptors (TRs) (Moriyama et al., 2002), inhibit thyroid hormone sulfotransferase activity, and change the transcription and gene expression of TRs, both in vitro and in experimental models (Gentilcore et al., 2013; Sheng et al., 2012). BPA can also interfere with estrogen, androgen, and glucocorticoid hormones and appears to affect certain neural and endocrine circuits, disturbing normal brain development and subsequent behavior patterns related to reproduction and both social and non-social behaviors (Delfosse et al., 2014; Mustieles et al., 2015; Palanza et al., 2008).

The effects of BPA on humans depend on the dose and timing of exposure, with the prenatal/neonatal period representing the most vulnerable stages (Fernández et al., 2014). The main morphological differences in the brain are shaped during prenatal development under the regulation of gonadal steroid hormones, especially estrogen and aromatizable androgens (Bao and Swaab, 2011). Animal studies indicate an association of prenatal and early postnatal BPA exposure with several cognitive deficits, including spatial learning and memory function (Wang et al., 2014), as well as behavioral problems, including: increased anxiety (Tian et al., 2010; Luo et al., 2014), hyperactivity (Komada et al., 2014), aggressiveness (Patisaul and Bateman, 2008), and altered socio-sexual behavior (Farabollini et al., 2002; Porrini et al., 2005). These alterations can persist into adulthood and appear to be permanent (Xu et al., 2010).

Over recent years, several human studies have investigated the role of BPA exposure in child neurodevelopment (Braun et al., 2009, 2011; Casas et al., 2015; Evans et al., 2014; Findlay and Kohen, 2015; Harley et al., 2013; Hong et al., 2013; Miodovnik et al., 2011; Perera et al., 2012; Roen et al., 2015; Yolton et al., 2011) and some of them have reported altered neurobehavior (including hyperactivity, aggressive behavior, anxiety, depression, attention problems, and/or other cognitive function impairments), especially in children exposed in utero (Mustieles et al., 2015). Most of these studies have focused on prenatal exposure to BPA, but less is known about the relationship between postnatal exposure and childhood neurobehavior. Therefore, we aimed to investigate the relationship between current BPA exposure, estimated from BPA urinary concentrations, and cognitive functioning and behavioral problems in children from the Spanish Environment and Childhood ''Infancia y Medio Ambiente (INMA)'' mother– child cohort.

2. Methods

2.1. Study population

The INMA cohort is a population-based study in seven regions of Spain. It aims to explore the effects on child growth and development of environmental pollutants during pregnancy and early childhood. The present study includes the INMA cohort set up in Granada (a province in Southern Spain). From 2000 through 2002, 668 mother–son pairs were recruited at delivery, with the initial aim of investigating chronic exposure to endocrine disrupting chemicals and urogenital malformations in newborn boys. The cohort inclusion and exclusion criteria and the recruitment and characteristics of the study population have been reported elsewhere (Freire et al., 2011). When the children reached the age of 9–10 years (2011–2012), all families in the cohort were contacted and invited to participate in the follow-up (Pérez-Lobato et al., 2015). Three hundred families (44.9% participation rate) gave their written informed consent. The study followed the principles of the Declaration of Helsinki and was approved by the Ethics Committee of San Cecilio University Hospital.

2.2. BPA measurement

Children [296 (99%)] provided a single non-fasting spot urine sample at the follow-up visit, always between 5 p.m. and 8 p.m. Urine was collected in 10 mL polypropylene tubes and immediately stored at 20 ºC.

BPA was determined in the laboratory of the Department of Analytical Chemistry of the University of Cordoba (Spain) as previously described (Casas et al., 2013). Briefly, urine samples (1.25 mL) were fortified with 2.5 mg/L of internal standard ($^{13}C_{12}$-bisphenol A) and 625 mL of b-glucoronidase H1 and sulfatase H1 solution (926 U/mL of each enzyme in ammonium acetate buffer 1 M, pH 5) and were then placed in glass tubes (2 mL) closed with aluminum caps and incubated at 37 8C overnight. Total BPA (free plus conjugated) was quantified by liquid chromatography-mass spectrometry with an AB Sciex 4000 Qtrap[1] mass spectrometer coupled to an Agilent 1200 Series LC system with a negative-ion TurboSpray interface. The limit of detection (LOD) was 0.1 mg/L and the limit of quantification (LOQ) was 0.2 mg/L. BPA background contamination from the presence of polymers in components of the urine collection containers and/or of the LC equipment or labware was avoided by filtering LiChrosol water through a 47 mm Styrene DVB (SDB-XC) disk from Empore (3 M, St Paul, Minnesota, USA) and rinsing the glassware and Eppendorf microtubes with methanol several times before their use.

All BPA concentrations were standardized by urinary creatinine (Cr) concentrations (mg/dL). Cr was determined at the Public Health Laboratory of the Basque Country (Spain) (Fernández et al., 2015). The researcher responsible for the urine analyses was blinded to the characteristics of the study population.

2.2.1. Inter-laboratory comparison

An inter-laboratory comparison was performed with the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA)—Institute of the Ruhr-University Bochum (Germany), with considerable experience in BPA determination.

2.3. Behavioral and emotional assessment

Behavioral function was evaluated by the Child Behavior Checklist (CBCL/6-18), a standardized parent report questionnaire (Achenbach and Rescorla, 2001), which was completed by 294 parents. The CBCL includes 118 items that parents rate on a three-point scale (Not True, Somewhat True, Very/Often True). The CBCL provides eight syndrome scales (anxious/depressed, withdrawn/depressed, somatic complaints, social problems, thought problems, attention problems, rule-breaking behavior, and aggressive behavior) grouped by three composite scales (Internalizing-sum of scores on the anxious/depressed, withdrawn/depressed, and somatic complaints scales; Externalizing-sum of scores on the rule-breaking behavior and aggressive behavior scales; and Total Problems), reported as both raw scores and sex- and age-normalized T scores. Children with CBCL/6–18 T-scores 60 on internalizing or externalizing problem scales and T-scores 65 on diagnostic scales were classified as borderline/clinical cases (Achenbach and Rescorla, 2001). We also assessed the general cognitive intelligence of the children using the Kaufman Brief Intelligence Test (K-BIT) (Kaufman and Kaufman, 1997), in which the composite Intelligence Quotient (IQ) is based on verbal and non-verbal scale scores.

The behavioral functioning was assessed at the Monitoring and Early Stimulation Unit of the Hospital, supervised by a trained psychologist (RPL) blinded to the BPA exposure status of the children.

2.4. Additional data collection

Information was gathered at the follow-up visit on parental age, education level, marital status, maternal smoking during pregnancy, breastfeeding, and child's age, IQ score, body mass index [BMI (kg/m^2)], and tobacco exposure at home with an ad hoc self-reported questionnaire. The child's place of residence was classified as urban (city of Granada, 236,000 inhabitants), suburban (towns of >20,000 inhabitants in the proximity of Granada), or rural (<20,000 inhabitants). We estimated the general cognitive ability of the parents by assessing their verbal reasoning ability using the Similarities subtest of the Wechsler Adult Intelligence-Third Edition (WAIS-III) (Wechsler, 1999).

The presence of a chronic disease related to cognitive development, including diabetes (n = 1), hyperthyroidism (n = 1), attention deficit hyperactivity disorder (ADHD) (n = 7), language disorder (n = 2), Asperger syndrome (n = 2), Noonan syndrome (n = 1), Tourette syndrome with ADHD (n = 1), Charcot–Marie–Toth syndrome with ADHD (n = 1), cerebral palsy (n = 1), and brain tumor surgery (n = 1), was considered as an additional exclusion criterion. Therefore, data on BPA in urine, covariates, and behavioral tests were obtained from 269 children (89.67%).

2.5. Statistical analysis

We performed a descriptive analysis of the characteristics of children and their parents, using measures of central tendency and dispersion for numerical variables and absolute frequencies for categorical variables. Differences between the children included in and excluded from the main analyses were estimated using the Mann–Whitney or Kruskal–Wallis test for continuous variables and Pearson's chi-square test for categorical variables.

BPA levels were expressed as median and interquartile range (IQR) in mg/L. Until a consensus is achieved on the best biomonitoring approaches for assessing exposure to short-lived chemicals using urine samples, research on factors associated with BPA exposure should include assessments using both volume-based urinary BPA and creatinine-adjusted urinary BPA data (Lakind and Naiman, 2015). Continuous urinary BPA concentrations and creatinine-adjusted BPA concentrations were $\log_{10}$-transformed to reduce the influence of outliers and to improve the linearity of the association and were analyzed as continuous and categorical (quartiles) variables.

The association of BPA concentrations with CBCL scores was studied using linear regression models. T-scores for all behavioral outcomes were analyzed as continuous variables, taken from the analysis of standardized scores for the Spanish population. In addition, logistic regression models were constructed to estimate the risk (odds ratio [OR]; 95% CI) of obtaining T-scores 60 on internalizing or externalizing problem scales and T-scores 65 on diagnostic scales. We calculated unadjusted and adjusted coefficients, using different independent variables in the models: first, BPA-$\log_{10}$-transformed concentrations; second, $\log_{10}$-transformed Cr-adjusted BPA concentrations; third, BPA concentrations categorized in quartiles; and fourth, Cr-adjusted BPA concentrations categorized in quartiles. Beta coefficients from the continuous models represent the mean change in CBCL scores for each unit of increase in log-BPA concentrations. We calculated adjusted mean scores for each quartile and the difference between the mean first quartile score and the mean score of the other three quartiles. Logistic regression analyses were also performed with both models in which volume-related and Cr-adjusted BPA concentration were categorized in quartiles.

Known or suspected risk factors for the exposure or outcome were selected as covariates, based on the literature (Calafat et al., 2008; Lakind and Naiman, 2015). The adjusted linear and logistic models included the age, IQ score, body mass index (BMI kg/m^2), exposure to environmental tobacco smoke (any/none) at home of the children, mother's intelligence score and age, parental education level (university/secondary school/up to primary), marital status (married/not married), maternal smoking during pregnancy (yes/no), and breastfeeding (yes/no).

The significance level was set at p 0.05, following the recommendations of Rothman (Rothman, 1990) for the evaluation of exposure-outcome relationships in the public health setting. Data analyses were performed using SPSS v20.0 (IBM, Chicago, IL) and R statistical computing environment v3.0.0 (http://www. r-project.org/).

3. Results

3.1. Descriptive analysis

BPA distribution by characteristics of the study population is shown in Table 1. In brief, the median (25th, 75th) BMI (kg/m^2) of the children was 18.48 (16.38–21.57), within the normal variation of the Spanish population according to the criteria of Sobradillo et al. (2004). Nearly a quarter of participating families lived in urban areas, and 50.8% of children had been exposed to tobacco smoke at home.

BPA was detected in all (100%) urine samples at a wide range of concentrations. The GM GSD BPA concentration was 4.58 0.95 mg/L for non-adjusted BPA and 5.07 0.95 mg/g for Cr-adjusted BPA. Table 2 exhibits the ranges and percentile distribution of current volume-related and Cr-adjusted BPA concentrations. A subset of samples (n = 10) was analyzed for free BPA without enzymatic hydrolyses to rule out external contamination or degradation of the conjugates. Free BPA was represented in less than 10% of these samples, indicating that external contamination was unlikely (data not shown).

Urinary BPA concentrations determined in the two laboratories (inter-comparison) were strongly correlated (Spearman r = 0.95, p < 0.001), and the correlation was similar for both low and high total BPA concentrations (low concentrations: Spearman r = 0.78, p < 0.001; high concentrations: Spearman r = 0.79, p < 0.001). The coefficients of variation for our laboratory (Spain) and the IPA laboratories were 96.7 and 147.2, respectively.

The distribution of CBCL scores of children is shown in Table 3, where higher scores indicate more parent-reported behavior problems. Boys showed more internalizing problems (36.43%) – mainly anxious/depressed, withdrawn/depressed, and somatic complaints – than externalizing problems (17.10%) (Table 3).

For the majority of behavior functions and paternal characteristics, no differences were found between the children in the final subsample and those not included (those with chronic disease related to cognitive development and/or missing data on the relevant covariate measures: 269 vs. 31 subjects) (data not shown). However, differences were found in attention problems [55.46 (6.47) vs. 59.50 (8.37), p = 0.003, respectively] and ADHD symptoms [54.79 (6.16) vs. 57.36 (7.78), p = 0.042], respectively.

3.2. Association with behavioral problems

When BPA urinary concentrations were considered as a continuous variable, higher BPA levels were related to greater behavioral problems scores and lower competence scores, but without reaching statistical significance (data not shown). Log_{10}-transformed-BPA concentrations were positively and significantly associated with somatic complaints (p = 0.009) and thought problems (p = 0.023) in the unadjusted model. In the adjusted multivariate model, these associations remained statistically significant in addition to social problems (p = 0.043) (Table 4). A marginally significant (p= 0.06)

Table 1. Geometric mean and geometric standard deviation of urinary BPA concentrations (mg/L) by demographic characteristics of the study population (n = 269).

	BPA (mg/L)			
	Mean (SD)	GM GSD		p Value[1]
Child variables				
Age (years)	9.90(0.33)	–		0.077
BMI (kg/m^2)	19.23(3.46)	–		0.933
Creatinine (mg/dL)[a]	90.22(0.50)	–		0.065
IQ	108.20(11.80)	–		0.150
Area of residence (%)				
Urban	24.53	4.71	2.72	0.659
Semi- urban	58.00	4.66	2.48	
Rural	17.47	4.18	2.80	
Tobacco exposure (%)				
Yes	51.63	4.66	2.51	0.850
No	48.37	4.35	2.77	
Maternal variables				
Age (years)	39.70(4.94)	–		0.553
Verbal reasoning[2]	14.88(4.58)	–		0.759
Marital status (%)				
Married	90.71	4.57	2.53	0.572
Unmarried	9.29	4.62	4.26	
Education level (%)				
University	23.40	5.21	2.72	0.260
Secondary school	31.60	4.81	2.25	
Up to primary	45.00	4.14	2.75	
Breastfeeding (%)				
Yes	86.99	4.06	2.64	0.878
No	13.01	4.57	3.16	
Smoking during pregnancy[3] (%)				
Yes	21.56	5.05	3.06	0.845
No	78.44	3.94	2.64	
Paternal variables				
Age (years)	42.14(5.37)	–		0.424
Verbal reasoning[2]	15.63(5.36)	–		0.063
Marital status (%)				
Married	90.33	4.18	2.61	0.615
Unmarried	9.67	3.60	3.32	
Education level (%)				
University	21.60	3.25	1.95	0.300
Secondary school	32.00	5.75	2.86	
Up to primary	45.40	3.86	2.61	

GM: geometric mean; GSD: geometric standard deviation; SD = standard deviation; Parent's age at follow-up.
Creatinine (mg/dL) is expressed as GM GSD.

p-Value: Value of statistical significance reached in hypothesis testing (Mann– Whitney test for breastfeeding, smoking during pregnancy, Kruskal– Wallis test for area of residence, maternal and paternal education, and bivariate analysis with

Spearmen correlation test for continuous variables.

Verbal reasoning, measured by Similarities subtest of WAIS-III. [3] Mother's habit.

association with internalizing composite scores also emerged (Table 4). When Cr-adjusted log_{10}-transformed BPA concentration was considered, the adjusted model showed a statistically significant association with somatic complaints (p = 0.005) and thought problems (p = 0.006) (data not shown).

As observed in Table 5, mean scores for somatic, social and thought problems scores were approximately 2 points higher for the children in the top quartile of BPA concentrations than for those in the lowest quartile. Children in the second and third quartiles also showed higher mean scale scores compared with children in the first quartile, although significance was only reached for social problems (third quartile; β = 1.94; 95% CI: 0.423, 3.460; p = 0.012) (Table 5). We found similar significant associations using linear regression analysis with either non-adjusted or Cr-adjusted BPA concentrations, except for social problems (Table 5).

We also explored the association between quartiles of BPA concentrations and behavioral outcomes in multivariate logistic regression models. Children with BPA concentrations in the top quartile had a higher risk of a T-score above 60 in thought problems (OR = 2.93; 95% CI = 1.44, 7.490; p = 0.025), rule-breaking problems (OR = 4.03; 95% CI = 1.266, 12.850; p = 0.018), and total problems (OR = 2.66; 95% CI = 1.105, 6.410; p = 0.029) vs. those with BPA concentrations in the 1st quartile. Multivariable logistic regression analysis of Cr-adjusted BPA concentrations only showed a statistically significant association with thought problems (OR = 2.55; 95% CI = 1.056, 6.151; p = 0.038) (data not shown).

Finally, we explored the robustness of our analyses by excluding extreme creatinine values that ranged between <0.3 g/L and >3.0 g/L (n = 10) to prevent 'dilution effects' in the urine samples (Kasper-Sonnenberg et al., 2014), and by excluding children born small-for-gestational age (SGA) (n = 19). The results showed no substantive differences in the relationship between BPA concentrations and behavioral scores (data not shown).

4. Discussion

BPA exposure at 10 years of age was positively and significantly associated with higher scores in three out of nine behavior scales, i.e., with internalizing symptoms (especially somatic complaints) and with thought and social problems. Our results also showed increased subclinical internalizing symptoms, which may signal greater vulnerability to the subsequent development of a mental disorder in adolescence and adulthood (Paus et al., 2008).

Somatization is described as the tendency to experience and express psychological distress through somatic complaints such as headache, stomach ache, or nausea (Campo and Fritsch, 1994; Shannon et al., 2010). Somatic problems may appear if children have difficulties in communicating their feelings (Friedberg and McClure, 2002). Moreover, a greater tendency to think about oneself and the future can lead to an increase in obsessive thinking, worries, and sleeping disorders, among other problems. This intensified thinking activity may also be related to the perception of greater social interaction difficulties and school stress, as well as to the increase in somatic symptoms (Shannon et al., 2010).

Although the effects of BPA on behavior appear to be more pronounced after prenatal exposure, the relevance of postnatal exposure should not be underestimated and warrants further research (Mustieles et al., 2015). Thus, our study contributes data on BPA exposure during the prepubertal stage of development, the second most critical period for the sexual differentiation of the brain and behavior after gestation (Auyeung et al., 2013). Brain and behavior are modulated by gonadal hormones in a dimorphic manner during development, exerting an organizational effect in utero and permanently changing brain structures and behaviors (Wallen, 2009). Later in life, these

Table 2. Distribution of urinary BPA concentrations in boys from INMA-Granada cohort (n = 269).

		Percentile							
	Min	5th	25th	50th	75th	95th	Max	Mean SD	GM GSD
Unadjusted BPA (mg/L)	0.25	0.82	2.77	4.76	9.03	18.97	62.40	6.92 7.54	4.58 0.95
Creatinine adjusted BPA (mg/g)	0.26	1.06	2.75	4.75	10.23	23.56	76.30	7.97 9.37	5.07 0.95

Limit of detection (LOD) was 0.1 mg/L; GM = geometric mean; GSD = geometric standard deviation. Min = minimum; Max = maximum.

Table 3
Distribution of CBCL typical behavior score outcomes in the INMA-Granada cohort (n = 269 boys).

CBCL	Score range	Mean of scores[a]	SD	Median	n (%) in borderline or clinical range[b]
Syndrome scores					
Anxious depressed	50–78	56.93	6.41	57.00	78(28.99)
Withdrawn	50–79	56.85	6.50	54.00	73(27.14)
Somatic complaints	50–82	56.48	6.23	57.00	79(29.37)
Social problems	50–73	54.12	4.62	53.00	34(12.64)
Thought problems	50–74	54.39	5.75	51.00	50(18.59)
Attention problems	50–83	55.35	6.17	53.00	55(20.45)
Rule-break problems	50–76	53.98	5.39	51.00	40(14.87)
Aggressive behavior	50–75	54.93	5.59	53.00	49(18.21)
ADHD problems (DSM-IV)	50–77	54.67	5.95	51.00	22(8.18)
Composite scores					
Internalizing problems	34–76	56.35	8.06	57.00	98(36.43)
Externalizing problems	33–74	51.36	9.22	51.00	46(17.10)
Total problems	36–73	53.27	8.37	53.00	63(23.42)
Competences					
Social	26–64	45.36	7.72	45.00	8(2.97)
School	24–55	50.36	6.68	50.86	7(2.60)

CBCL: child behabior checklist (6–18). ADHD: attention deficit hyperactivity disorder. Internalizing-sum of scores on the anxious/depressed, withdrawn/depressed, and somatic complaints scales; externalizing-sum of scores on the rule-breaking behavior and aggressive behavior scales; total problems-sum of the eight individual syndrome scores.

We converted the raw scores of the seven syndrome scales to T-scores to compare each child to a normative sample of children.

We grouped the scores of the children into two outcome groups for each syndrome: borderline or clinical (60), or normal (<60) T-score.

hormones have ''activational'' effects, producing temporary alterations to the brain and behavior throughout puberty and adolescence (Berenbaum and Beltz, 2011; Wallen, 2009). Some disorders (e.g., conduct problems) with an early onset have a marked male prevalence, while others (e.g., depression, anxiety) with onset in puberty/adolescence are more frequent among females (Zahn-Waxler et al., 2008). In this context, our results may suggest a BPA-induced reduction in this behavior dimorphism, specifically in the direction of a demasculinization of behavior, as recently reported in juvenile non-human primates (Negishi et al., 2014).

Experimental studies have consistently reported that BPA may interfere with normal brain and behavior development, suggesting a plausible biological causal link (Mustieles et al., 2015). BPA has shown varied and complex mechanisms of action (epigenetic, endocrine, and synaptic). For example, BPA exposure largely abolishes the synaptogenic response to estradiol in hippocampal and prefrontal spine synapses in both rat and non-human primate models (Leranth et al., 2008), and exposure to low environmentally relevant doses of BPA during critical periods has been found to alter sex-specific brain structures and behavioral patterns, increasing, decreasing, or eliminating sex differences (Bowman et al., 2014). Results of epidemiological studies also indicate that the impact of BPA exposure on neurobehavioral functioning may differ between boys and girls (Mustieles et al., 2015), although the very small number of human studies does not allow any conclusive conclusions to be drawn.

Table 4. Distribution of the CBCL behaviour scores by log10 BPA (mg/L) in the INMA-Granada cohort (n = 269 boys).

Behavioral functions	BPA (µg/L)							
	BPA (log10 transformed) unadjusted				BPA (log10 transformed) adjusted			
	β (SE)		p	95% CI	β (SE)		p	95% CI
Syndrome scores								
Anxious depressed	1.14	(0.95)	0.228	-0.720, 3.007	1.24	(0.95)	0.191	-0.622, 3.106
Withdrawn	0.53	(0.96)	0.575	-1.354, 2.432	0.89	(0.96)	0.352	-0.994, 2.784
Somatic complaints	2.38	(0.91)	**0.009***	0.589, 4.171	2.23	(0.91)	**0.015***	0.438, 4.030
Social problems	1.17	(0.68)	0.086†	-0.166, 2.515	1.34	(0.66)	**0.043***	0.041, 2.639
Thought problems	1.93	(0.84)	**0.023***	0.273, 3.592	2.01	(0.83)	**0.017***	0.362, 3.654
Attention problems	-0.94	(0.91)	0.300	-2.742, 0.849	-0.37	(0.88)	0.671	-2.111, 1.362
Rule-breaking problems	0.89	(0.80)	0.266	-0.681, 2.457	1.04	(0.77)	0.177	-0.475, 2.556
Aggressive behavior	0.23	(0.83)	0.780	-1.398, 1.864	0.50	(0.80)	0.531	-1.073, 2.075
ADHD problems (DSM-IV)	-0.33	(0.89)	0.712	-2.074, 1.419	0.06	(0.88)	0.941	-1.673, 1.803
Composite scores								
Internalizing problems	2.10	(1.19)	0.077†	-0.232, 4.442	2.24	(1.18)	0.060†	-0.090, 4.574
Externalizing problems	0.23	(1.36)	0.867	-2.459, 2.917	0.63	(1.33)	0.634	-1.981, 3.246
Total problems	1.20	(1.24)	0.332	-1.223, 3.638	1.60	(1.18)	0.178	-0.731,3.931
Competences								
Social	-0.08	(1.15)	0.945	-2.345, 2.186	-0.30	(1.16)	0.791	-2.582, 1.970
School	0.29	(0.99)	0.769	-1.656, 2.238	-0.32	(0.91)	0.720	-2.119, 1.467

CBCL: child behavior checklist (6–18). ADHD: attention deficit hyperactivity disorder. Internalizing-sum of scores on the anxious/depressed, withdrawn/depressed, and somatic complaints scales; externalizing-sum of scores on the rule-breaking behavior and aggressive behavior scales; total problems-sum of the eight individual syndrome scores. Models were adjusted for child's age, intelligence quotient (IQ) score, BMI (kg/m^2), and exposure to environmental tobacco smoke (any/none) in the home based on questionnaire, mother's intelligence score, mother's age at the time of assessment, education level (university/secondary school/up to primary), marital status (married/not married), maternal smoking during pregnancy (yes/no), breastfeeding (yes/no).

*p ≤ 0.05
† p ≤0.1

Table 5
Distribution of the CBCL scores by quartiles of urinary bisphenol A and creatinine-adjusted bisphenol A concentrations (n = 269).

	BPA quartiles (μg/L)				BPA/creatinine quartiles (mg/g)			
	Reference scores	Coefficients (SE)			Reference scores	Coefficients (SE)		
Behavioural functions	Mean(SD)	2nd	3rd	4th	Mean(SD)	2nd	3rd	4th
	(0.25–2.76)	(2.77–4.75)	(4.76–9.02)	(9.03–62.40)	(0.26–2.74)	(2.75–4.74)	(4.75–10.22)	(10.23–76.30)
Syndrome scores								
Anxious depressed	56.19 (5.65)	1.36 (1.11)	0.92 (1.11)	1.34 (1.11)	57.25 (6.79)	-0.84 (1.11)	0.23 (1.12)	0.18 (1.12)
Withdrawn	56.27 (6.34)	0.90 (1.12)	0.96 (1.12)	1.62 (1.12)	57.21 (7.29)	-0.11 (1.13)	0.21 (1.13)	0.02 (1.14)
Somatic complaints	55.43 (5.58)	1.69 (1.06)	0.33 (1.07)	**2.35 (1.07)***	56.27 (6.76)	-1.02 (1.06)	0.19 (1.07)	**2.15(1.07)***
Social problems	53.07 (3.49)	1.13 (0.77)	**1.94 (0.77)***	**1.71 (0.77)***	53.90 (4.68)	-0.27 (0.77)	0.96 (0.77)	1.17 (0.78)
Thought problems	53.52 (4.56)	1.20 (0.98)	0.46 (0.97)	**2.58 (0.98)***	53.87 (4.95)	0.40 (0.97)	0.24 (0.98)	**2.53(0.98)***
Attention problems	55.67 (5.30)	0.30 (1.03)	0.84 (1.03)	0.79 (1.03)	56.70 (6.51)	-1.78 (1.03)†	1.39 (1.03)	0.38 (1.03)
Rule-breaking problems	53.40 (4.74)	0.58 (0.90)	0.91 (0.90)	1.50 (0.90)†	54.39 (5.53)	-0.67 (0.90)	0.38 (0.90)	0.56 (0.91)
Aggressive behavior	54.84 (5.27)	0.23 (0.94)	0.54 (0.93)	0.70 (0.94)	55.61 (5.86)	-1.10 (0.94)	0.42 (0.94)	0.08 (0.94)
ADHD problems (DSM-IV)	54.49 (5.85)	0.58 (1.03)	0.25 (1.03)	0.99 (1.03)	55.46 (6.62)	-1.55 (1.03)	0.53 (1.03)	0.19 (1.04)
Composite scores								
Internalizing problems	55.22 (7.45)	1.59 (1.39)	1.35 (1.39)	2.44 (1.39)†	56.31 (8.85)	-0.47 (1.39)	0.43 (1.40)	1.17 (1.41)
Externalizing problems	51.37 (8.62)	0.29 (1.55)	0.65 (1.55)	0.68 (1.56)	52.63 (9.07)	-1.40 (1.56)	1.23 (1.56)	0.23 (0.89)
Total problems	52.51 (7.22)	1.40 (1.39)	1.11 (1.38)	2.30 (1.39)	54.10 (8.50)	-1.62 (0.39)	0.48 (1.39)	1.01 (1.39)
Competences								
Social	45.67 (6.84)	0.03 (1.35)	0.87 (1.35)	0.46 (1.35)	44.54 (7.06)	1.65 (1.35)	1.28 (1.35)	0.13 (1.36)
School	50.37 (7.27)	0.96 (1.06)	0.77 (1.06)	0.67 (0.52)	51.27 (6.01)	-0.59 (1.07)	1.35 (1.07)	1.13 (1.08)

Internalizing problems include anxious/depressed, withdrawn/depressed and somatic complains; externalizing problems include rule-breaking and aggressive behavior; total problems include the eight individual syndrome scores. Models were adjusted for child's age, intelligence quotient (IQ) score, BMI (kg/m^2), and exposure to environmental tobacco smoke (yes/no) in the home based on questionnaire, mother's intelligence score, mother's age at the time of assessment, education level (university/ secondary school/up to primary), marital status (married/not married), maternal smoking during pregnancy (yes/no), breastfeeding (yes/no).

*$p \leq 0.05$.
† $p \leq 0.1$.

All children in our study evidenced detectable BPA levels, with a median concentration of 4.76 mg/L (4.75 mg/g Cr), similar to reports in younger children (Braun et al., 2011; Casas et al., 2011; Perera et al., 2012) and in those of the same age (Calafat et al., 2008). The present levels were higher than those reported for children aged from 6 to 11 years (2.7 ng/mL) in the 2005–2006 NHANES study (LaKind and Naiman, 2011), for those aged from 6 to 19 years (1.3 ng/mL) in the 2007–2011 Canadian Health measures survey (Findlay and Kohen, 2015), for those aged from 9 to 11 years (2.13 ng/mL) in the German Environmental Survey on Children (GerES) (Becker et al., 2009), and for children of similar ages in some other studies (Covaci et al., 2015; Hong et al., 2013; Teitelbaum et al., 2008). The slightly higher values in our study may be attributable to the timing of urine collections (non-fasting samples collected between 17:00 and 20:00 h), or the characteristics of the children (e.g., food intake, life-style, paternal education or household income, among other socio-demographical characteristics). Importantly, it has been pointed out that the non-persistent nature and short-term variability of BPA is more likely to produce an underestimation than an overestimation of its effects (Betts, 2014; Perera et al., 2012).

Our results are in line with previous findings. Six studies have explored the association between postnatal BPA exposure, assessed at different time points, and behavior problems in school-aged children (Braun et al., 2011; Findlay and Kohen, 2015; Harley et al., 2013; Hong et al., 2013; Perera et al., 2012; Roen et al., 2015), and four of these found positive associations (Findlay and Kohen, 2015; Harley et al., 2013; Hong et al., 2013; Roen et al., 2015). Harley et al. (2013) reported that total urinary BPA levels were associated with increased teacher-reported internalizing behaviors in boys at the age of 7 years (b = 1.8; 95% CI, 0.4–3.1; $p < 0.05$), specifically anxiety ($p < 0.05$) and inattention and hyperactivity ($p < 0.05$), but no significant associations were observed with mother-reported scores. In addition, a cross-sectional study of 1008 children aged between 8 and 11 years (mean of 9.05 0.70 y), showed that total urinary BPA levels were positively associated with the CBCL total problem score, i.e., the sum of internalizing and externalizing symptoms (β = 0.85; 95% CI, 0.26– 1.44; p = 0.001) (Hong et al., 2013). Further, results from the Canadian Health Measures Survey indicate that BPA concentration was positively associated with lower pro-social behavior (OR = 1.24; 95% CI, 1.06–1.45) among boys aged 6 to 17 years (Findlay and Kohen, 2015). However, in the follow-up of the Columbia Center for Children's Environmental Health cohort, at the age of 7–9 years (Roen et al., 2015), high postnatal BPA exposure was associated with more internalizing (anxious/depressed, withdrawn/depressed and somatic complaints) and externalizing problems among girls but with fewer problems among boys (Roen et al., 2015).

A more consistent relationship has been reported between prenatal vs. childhood BPA exposure and adverse behavioral effects (Braun et al., 2011; Casas et al., 2015; Evans et al., 2014; Harley et al., 2013; Perera et al., 2012; Roen et al., 2015). However, some authors found no significant relationship between BPA prenatal exposure and social behavior symptoms related to autistic spectrum disorders in children (Miodovnik et al., 2011) or neurobehavior scores in neonates (Yolton et al., 2011). The above discrepancies can be attributed to differences in the timing of exposure, neurodevelopment evaluation (different tests, evaluation periods, and reporters), sample size, adjustment for potential confounders, and/or socio-demographic characteristics of the study populations, among others (Mustieles et al., 2015; Rosenfeld, 2015).

Evidence has been published on in-utero and early exposure to BPA in the Granada INMA cohort. Thus, the presence of BPA was confirmed in 10 (20.4%) of 49 placenta specimens from a random sample of newborns from the cohort (2000–2002), at concentrations ranging from 5.7 to 22.2 ng/g) (Jiménez-Díaz et al., 2010). However, only 15 of these 49 children participated in the present follow-up (2011–2012). We also analyzed urinary BPA levels in a subsample of 30 children in their follow-up at 4–5 years of age (2004–2005), with a median concentration of 4.2 ng/mL (Casas et al., 2011).

Our study presents some limitations. First, the study sample only included boys, and it has been suggested that the association between BPA and behavior may be sex-dependent (Braun et al., 2009, 2011; Mustieles et al., 2015; Perera et al., 2012). Second, the cross-sectional design of the study prevents the inference of causal relationships, although experimental findings and the results of epidemiological birth cohort studies indicate a possible causal role for the effects of BPA upon behavior. Third, a single urine sample may not adequately reflect long-term exposure levels and represents only one time point in a continuum of brain development (Miodovnik et al., 2011; Ye et al., 2011). Fourth, some potential confounders were not taken into account, such as the pubertal development or maturity of the child or prenatal exposure to other neurotoxic chemicals. Finally, behavior assessment was based on a parent-reported test. Although some symptoms, such as somatic complaints, are easier to observe and objectively recognized by parents due to their physical manifestations, data from other sources (for example psychologists) should be explored (Findlay and Kohen, 2015). Strengths of this study include the representative nature of the cohort and the large number of variables collected at the follow-up from questionnaires, biological samples, and medical records, which allowed us to control for multiple potential confounders. Further-more, the study focused on children aged between 9 and 11 years, a crucial developmental period that has received inadequate attention to date.

On the basis of accumulating evidence of BPA exposure and its adverse health impact and the need to avoid this exposure during time-specific vulnerable windows of human development (pregnancy, childhood and adolescence), leading reproductive health professional societies have called for timely action to reduce exposure and to address the consequences of this exposure (Bergman et al., 2013; Zoeller et al., 2012). This vulnerable population should be protected by changes in product regulations and educational programs on environment and health (Di Renzo et al., 2015).

5. Conclusions

Our results suggest that childhood BPA exposure may affect the behavior of prepubertal boys during a crucial brain developmental stage. Mental and behavioral problems or disorders in children represent a major and growing public health concern because of their increasing prevalence, early onset, and impact on the child, family, and community (Perou et al., 2013). New epidemiological studies are needed to investigate different critical periods of exposure and development, to clarify the sex-dependent nature of BPA effects and possible long-term consequences on neurobehavioral functioning, and to address the influence of multiple EDC co-exposures (Braun et al., 2014; Mustieles et al., 2015; Vilahur et al., 2014).

Conflict of interest statement

The authors declare no conflict of interest. This paper will form part of the doctoral thesis developed by Vicente Mustieles in the context of the ''Clinical Medicine and Public Health Program'' of the University of Granada.

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References

Achenbach, T.M., Rescorla, L.A., 2001. Manual of the ASEBA School-Age Forms and Profiles. University of Vermont, Research Center for Children, Youth and Families, Burlington, VT.

Auyeung, B., Lombardo, M.V., et al., 2013. Prenatal and postnatal hormone effects on the human brain and cognition. Pflugers Arch. 465 (5), 557–571.

Bao, A.M., Swaab, D.F., 2011. Sexual differentiation of the human brain: relation to gender identity, sexual orientation and neuropsychiatric disorders. Front. Neuroendocrinol. 32 (2), 214–226.

Becker, K., Guen, T., et al., 2009. GerES IV: phthalate metabolites and bisphenol A in urine of German children. Int. J. Hyg. Environ. Health 212 (6), 685–692.

Bergman, A ̈., Heindel, J.J., et al., 2013. The impact of endocrine disruption: a consensus statement about the state of the science. Environ. Health Perspect. 121 (4), A104–A106.

Berenbaum, S.A., Beltz, A.M., 2011. Sexual differentiation of human behavior: effects of prenatal and pubertal organizational hormones. Front. Neuroendocrinol. 32 (2), 183–200.

Betts, K.S., 2014. Clues to autistic behaviors: exploring the role of endocrine disruptors. Environ. Health Perspect. 122 (5), A137.

Bowman, R.E., Luine, V., et al., 2014. Adolescent bisphenol-A exposure decreases dendritic spine density: role of sex and age. Synapse 68 (11), 498–507.

Braun, J.M., Yolton, K., et al., 2009. Prenatal bisphenol A exposure and early childhood behavior. Environ. Health Perspect. 117 (12), 1945–1952.

Braun, J.M., Kalkbrenner, A.E., et al., 2011. Impact of early-life bisphenol A exposure on behavior and executive function in children. Pediatrics 128 (5), 873–882.

Braun, J.M., Kalkbrenner, A.E., et al., 2014. Gestational exposure to endocrine-disrupting chemicals and reciprocal social, repetitive, and stereotypic behaviors in 4- and 5-year-old children: the HOME study. Environ. Health Perspect. 122 (5), 513–520.

Calafat, A., Ye, X., et al., 2008. Exposure of the US population to bisphenol A and 4 tertiary-octylphenol: 2003–2004. Environ. Health Perspect. 116 (1), 39–44.

Campo, J.V., Fritsch, S.L., 1994. Somatization in children and adolescents. J. Am.

Acad. Child Adolesc. Psychiatry 33 (9), 1223–1235.

Casals-Casas, C., Desvergne, B., 2011. Endocrine disruptors: from endocrine to metabolic disruption. Annu. Rev. Physiol. 73, 135–162.

Casas, L., Ferna´ndez, M.F., et al., 2011. Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. Environ. Int. 37 (5), 858–866.

Casas, M., Valvi, D., et al., 2013. Dietary and socio-demographic determinants of bisphenol A urine concentrations in pregnant women and children. Environ. Int. 56, 10–18.

Casas, M., Forns, J., et al., 2015. Exposure to bisphenol A during pregnancy and child neuropsychological development in the INMA-Sabadell cohort. Environ. Res. 142, 671–679.

Covaci, A., Hond, E.D., et al., 2015. Urinary BPA measurements in children and mothers from six European member states: overall results and determinants of exposure. Environ. Res. 141, 77–85.

Delfosse, V., Grimaldi, M., et al., 2014. Nuclear receptor profiling of bisphenol-A and its halogenated analogues. Vitam. Horm. 94, 229–251.

Di Renzo, G.C., Conry, J.A., et al., 2015. International Federation of Gynecology and Obstetrics opinion on reproductive health impacts of exposure to toxic environmental chemicals. Int. J. Gynaecol. Obstet. 131 (3), 219–225.

Evans, S.F., Kobrosly, R.W., et al., 2014. Prenatal bisphenol A exposure and maternally reported behavior in boys and girls. Neurotoxicology 45, 91–99.

Farabollini, F., Porrini, S., et al., 2002. Effects of perinatal exposure to bisphenol A on socio sexual behavior of female and male rats. Environ. Health Perspect. 110 (Suppl. 3), 409– 414.

Fernández, M.F., Artacho-Cordón, F., et al., 2015. Trends in children's exposure to second-hand smoke in the INMA-Granada cohort: an evaluation of the Spanish anti-smoking law. Environ. Res. 138, 461–468.

Fernández, M.F., Román, M., et al., 2014. Endocrine disruptors: time to act. Curr.

Environ. Health Rep. 1 (4), 325–332.

Findlay, L.C., Kohen, D.E., 2015. Bisphenol A and child and youth behaviour:

Canadian Health Measures Survey 2007 to 2011. Health Rep. 26 (8), 3–9.

Freire, C., López-Espinosa, M.J., et al., 2011. Prenatal exposure to organochlorine pesticides and TSH status in newborns from Southern Spain. Sci. Total Environ. 409 (18), 3281– 3287.

Friedberg, R.D., McClure, J.M., 2002. Clinical Practice of Cognitive Therapy with Children and Adolescents: The Nuts and Bolts. Guilford Press, New York, NY.

Gentilcore, D., Porreca, I., et al., 2013. Bisphenol A interferes with thyroid specific gene expression. Toxicology 304, 21–31.

Harley, K.G., Gunier, R.B., et al., 2013. Prenatal and early childhood bisphenol A concentrations and behavior in school-aged children. Environ. Res. 126, 43–50.

Hong, S.B., Hong, Y.C., et al., 2013. Bisphenol-A in relation to behavior and learning of school-age children. J. Child Psychol. Psychiatry 54 (8), 890–899.

Jiménez-Díaz, I., Zafra-Gómez, A., et al., 2010. Determination of bisphenol A and its chlorinated derivatives in placental tissue samples by liquid chromatography–tandem mass spectrometry. J. Chromatogr. B 878 (32), 3363–3369.

Kaufman, A.S., Kaufman, N.L., 1997. Kaufman Brief Intelligence Test (K BIT) Manual. TEA Ediciones, S.A., Madrid, Spain (Spanish Adaptation. Cordero A, Calonge I. 2000).

Kasper-Sonnenberg, M., Koch, H.M., et al., 2014. Phthalate metabolites and bisphenol A in urines from German school-aged children: results of the Duisburg birth cohort and Bochum cohort studies. Int. J. Hyg. Environ.

Health 217 (8), 830–838.

Koch, H.M., Kolossa-Gehring, M., et al., 2012. Bisphenol A in 24 h urine and plasma samples of the German Environmental Specimen Bank from 1995 to 2009: a retrospective exposure evaluation. J. Exposure Sci. Environ. Epidemiol. 22, 610–616.

Komada, M., Itoh, S., et al., 2014. Newborn mice exposed prenatally to bisphenol A show hyperactivity and defective neocortical development. Toxicology 323, 51–60.

LaKind, J.S., Naiman, D.Q., 2011. Daily intake of bisphenol A and potential sources of exposure: 2005–2006 National Health and Nutrition Examination Survey. J. Exposure Sci. Environ. Epidemiol. 21 (3), 272–279.

LaKind, J.S., Naiman, D.Q., 2015. Temporal trends in bisphenol A exposure in the United States from 2003–2012 and factors associated with BPA exposure: spot samples and urine dilution complicate data interpretation. Environ. Res. 142, 84–95.

Leranth, C., Hajszan, T., et al., 2008. Bisphenol A prevents the synaptogenic response to estradiol in hippocampus and prefrontal cortex of ovariectomized nonhuman primates. Proc. Natl. Acad. Sci. 105 (37), 14187–14191.

Luo, G., Wang, S., et al., 2014. Maternal bisphenol-A diet induces anxiety-like behavior in female juvenile with neuroimmune activation. Toxicol. Sci. 140

(2), 364–373.

Michałowicz, J., 2014. Bisphenol A sources, toxicity and biotransformation.

Environ. Toxicol. Pharmacol. 37 (2), 738–758.

Miodovnik, A., Engel, S.M., et al., 2011. Endocrine disruptors and childhood social impairment. Neurotoxicology 32 (2), 261–267.

Moriyama, K., Tagami, T., et al., 2002. Thyroid hormone action is disrupted by bisphenol A as an antagonist. J. Clin. Endocrinol. Metab. 87 (11), 5185–5190.

Mustieles, V., Pe´rez-Lobato, R., et al., 2015. Bisphenol A: human exposure and neurobehavior. Neurotoxicology 49, 174–184.

Negishi, T., Nakagami, A., et al., 2014. Altered social interactions in male juvenile cynomolgus monkeys prenatally exposed to bisphenol A. Neurotoxicol. Teratol. 44, 46–52.

Palanza, P., Gioiosa, L., et al., 2008. Effects of developmental exposure to bisphenol A on brain and behavior in mice. Environ. Res. 2, 150–157.

Patisaul, H.B., Bateman, H.L., 2008. Neonatal exposure to endocrine active compounds or an ERbeta agonist increases adult anxiety and aggression in gonadally intact male rats. Horm. Behav. 53 (4), 580–588.

Paus, T., Keshavan, M., et al., 2008. Why do many psychiatric disorders emerge during adolescence? Nat. Rev. Neurosci. 9 (12), 947–957.

Perera, F., Vishnevetsky, J., et al., 2012. Prenatal bisphenol A exposure and child behavior in an inner-city cohort. Environ. Health Perspect. 120 (8), 1190– 1194.

Pérez-Lobato, R., Ramos, R., et al., 2015. Thyroid status and its association with cognitive functioning in healthy boys at 10 years of age. Eur. J. Endocrinol. 172 (2), 129–139.

Perou, R., Bitsko, R.H., et al., 2013. Mental health surveillance among children— United States, 2005–2011. MMWR Surveill. Summ. 62 (Suppl. 2), 1–35.

Porrini, S., Belloni, V., et al., 2005. Early exposure to a low dose of bisphenol A affects socio-sexual behavior of juvenile female rats. Brain. Res. Bull. 65 (3), 261–266.

Roen, E.L., Wang, Y., et al., 2015. Bisphenol A exposure and behavioral problems among inner city children at 7–9 years of age. Environ. Res. 142, 739–745.

Rosenfeld, C.S., 2015. Bisphenol A and phthalate endocrine disruption of parental and social behaviors. Front. Neurosci. 9, 57.

Rothman, K.J., 1990. No adjustments are needed for multiple comparisons.

Epidemiology 1, 43–46.

Shannon, R.A., Bergren, M.D., Matthews, A., 2010. Frequent visitors: somatization

in school-age children and implications for school nurses. J. Sch. Nurs. 26 (3), 169–182.

Sheng, Z.G., Tang, Y., et al., 2012. Low concentrations of bisphenol A suppress thyroid hormone receptor transcription through a nongenomic mechanism. Toxicol. Appl. Pharmacol. 259 (1), 133–142.

Sobradillo, B., Aguirre, A., Bilbao, A., et al., 2004. Curvas y tablas de crecimiento: estudios longitudinal y transversal. Fundacio´n Faustino Orbegozo Eizaguirre Available in: hwww.aepap.org/crecorbegozo.htmi

Teitelbaum, S.L., Britton, J.A., et al., 2008. Temporal variability in urinary concentrations of phthalate metabolites, phytoestrogens and phenols among minority children in the United States. Environ. Res. 106 (2), 257–269.

Tian, Y., Baek, J., et al., 2010. Prenatal and postnatal exposure to bisphenol A induces anxiolytic behaviors and cognitive deficits in mice. Synapse 6, 432–439.

Vandenberg, L.N., Hauser, R., et al., 2007. Human exposure to bisphenol A (BPA). Reprod. Toxicol. 24 (2), 139–177.

Vandenberg, L.N., Chahoud, I., et al., 2010. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ. Health Perspect. 118 (8), 1055–1070.

Vilahur, N., Bustamante, M., et al., 2014. Prenatal exposure to mixtures of xenoestrogens and repetitive element DNA methylation changes in human placenta. Environ. Int. 71, 81–87.

Wallen, K., 2009. The organizational hypothesis: reflections on the 50th anniversary of the publication of Phoenix, Goy, Gerall, and Young (1959). Horm. Behav. 55 (5), 561–565.

Wang, C., Niu, R., et al., 2014. Changes in memory and synaptic plasticity induced in male rats after maternal exposure to bisphenol A. Toxicology 322, 51–60.

Wechsler, D., 1999. Escala de inteligencia de Wechsler para adultos (WAIS-III).

TEA Ediciones, S.A., Madrid, Spain.

Xu, X., Zhang, J., et al., 2010. Perinatal exposure to bisphenol-A impairs learning-memory by concomitant down-regulation of N-methyl-ᴅ-aspartate receptors of hippocampus in male offspring mice. Horm. Behav. 58 (2), 326–333.

Ye, X., Wong, L.Y., et al., 2011. Variability of urinary concentrations of bisphenol A in spot samples, first morning voids, and 24-hour collections. Environ. Health Perspect. 119 (7), 983–988.

Yolton, K., Xu, Y., et al., 2011. Prenatal exposure to bisphenol A and phthalates and infant neurobehavior. Neurotoxicol. Teratol. 33 (5), 558–566.

Zahn-Waxler, C., Shirtcliff, E.A., et al., 2008. Disorders of childhood and adolescence: gender and psychopathology. Annu. Rev. Clin. Psychol. 4, 275–303.

Zoeller, R.T., Brown, T.R., et al., 2012. Endocrine-disrupting chemicals and public health protection: a statement of principles from The Endocrine Society. Endocrinoly 153 (9), 4097–4110.

PAPER 5

Mustieles V, Messerlian C, Reina I, Rodríguez-Carrillo A, Olea N, Fernández MF. (2018). Is Bisphenol A (BPA) a Threat to Children's Behavior? *Journal of Mental Health & Clinical Psychology,* 2(1), 6-9. ISSN: 2578-2959.

Paper #5

Is Bisphenol A (BPA) a Threat to Children's Behavior?

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Abstract

In 2015 we reviewed the state of knowledge regarding the potential impact of bisphenol A (BPA) exposure on child neurobehavior. At that time, we expressed concern about the effects of BPA on children's behavior, especially when exposure takes place in utero. Since then, the number of human studies addressing the BPA-neurobehavior hypothesis has doubled, most of them reinforcing previous prenatal associations and frequently showing differences between boys and girls. An increasing number of studies have also shown an association between postnatal BPA exposure and diverse neurobehavioral impairments, including attention-deficit and hyperactivity disorder (ADHD). It may never be possible to establish a causal link between this specific endocrine disruptor and a particular neurobehavioral endpoint; however, research data on the relationship between human BPA exposure and children's behavior has revealed a relatively consistent pattern that cannot be ignored. The mounting experimental and epidemiologic evidence on neurobehavioral effects support more than ever the need to apply the precautionary principle during development, especially in relation to pregnant women and children. It seems that the time to act has arrived.

Neurobehavioral disabilities affect millions of children worldwide, and the prevalence of some neurodevelopmental disorders appears to be increasing. Subclinical symptoms are even more frequent and also contribute to a poorer quality of life and lower academic achievement[1]. Epidemiologic and animal studies have demonstrated that exposure to several families of endocrine-disrupting chemicals (EDCs) contribute to the risk of neurodevelopmental impairment[1,2].

Bisphenol A (BPA) is a well-known EDC that can interfere with hormonal balance, even at low doses, *via* multiple steroid hormone receptors that mediate a myriad of cellular effects[3]. The mechanistic understanding of its effects is particularly complex: BPA can bind not only to nuclear and membrane estrogen receptors but also to thyroid, glucocorticoid, and peroxisome proliferator-activated receptors, and it can also interact with steroidogenic enzymes, among other molecular targets[4,5]. This biological promiscuity might explain the pleiotropic effects exerted by BPA on behavior, reproduction, and metabolism[6,7]. The developing brain is a key target for this compound, and pre-, peri- and post-natal BPA exposure has been linked to a variety of altered behaviors, as demonstrated in multiple experimental models[3].

In 2015 we reviewed the state of knowledge on the relationship between human BPA exposure and neurobehavior[5]. We

expressed concern about the effects of BPA on children's behavior, especially when exposure takes place *in utero*. Although only 12 epidemiologic studies were available at that time, their findings pointed in the same direction as experimental studies, suggesting a negative impact of prenatal BPA exposure on children's neurobehavioral functioning in a sex-dependent manner[5]. The results suggested that male fetuses were more frequently affected by prenatal BPA exposure than females, in line with the current hypothesis of environmental intrauterine sex-dependent vulnerability[8].

Since March 2015, the number of human studies addressing the BPA-neurobehavior hypothesis has doubled[9– 19], most of them reinforcing previous associations and frequently showing differences between boys and girls. For example, earlier findings by Perera et al. (2012)[20] and Roen et al. (2015)[21] were confirmed by Perera et al. (2016)[13], showing a consistent longitudinal pattern of internalizing problems, including anxiety and depression symptoms, among boys from childhood to adolescence in a birth cohort from the U.S.. More recently, Casas et al. (2015)[9] in Spain, Philippat et al. (2017)[15] in France, and Braun et al. (2017)[14] in Canada all supported the findings of previous studies by Harley et al. (2013)[22] in California and by Evans et al. (2014)[23] in a multicenter U.S. cohort. These reported more frequent behavior problems related to prenatal BPA exposure in the males than in the females. Notably, Braun and colleagues (2017)[14] studied 812 mother-child pairs belonging to the Maternal–Infant Research on Environmental Chemicals (MIREC) study, finding that higher prenatal urinary BPA concentrations were significantly associated with more frequent internalizing problems among three-year-old boys and with poorer executive function and higher social impairments. In contrast, two birth cohorts observed more behavior problems[24] and social impairments[17] related to prenatal BPA exposure in girls than in boys. Overall, epidemiologic data support a probable negative effect of prenatal BPA exposure on children's behavior in a sex-dependent manner.

An increasing number of studies show that postnatal BPA exposure is also associated with diverse neurobehavioral impairments among both boys and girls[10,11,16,21,22]. For example, some studies, including two representative national surveys, have reported associations with symptoms of attention-deficit and hyperactivity (ADHD) and with the prevalence of ADHD. Findlay and Kohen (2015)[10] observed positive cross-sectional associations between urinary BPA concentrations and ADHD symptoms in 2730 Canadian children/youths aged 6-17, while Tewar et al. (2016)[12] found a positive cross-sectional association between BPA concentrations and the risk of ADHD in 460 North American children/youths aged 8-15 years. A more recent case-control study in China also reported a stepwise increase in the risk of ADHD across increasing quartiles of urinary BPA concentrations[19]. These associations reinforce data from some previous prospective studies that found more ADHD symptoms in response to higher prenatal BPA concentrations[9,15,24]. These associations are also supported by experimental data[25,26].

The human brain is a sexually dimorphic organ, and major morphological differences are permanently shaped during prenatal development under the influence of steroid hormones, especially estrogen and aromatizable androgens[27,28]. BPA has been shown to alter sex-specific structural and behavioral patterns in experimental animals, including non-human primates, increasing, decreasing, and/or eliminating sex differences[29–32]. It is possible that BPA induces these effects through epigenetic modifications related to the estrogen-androgen balance, given evidence that BPA affects the gene expression of several estrogen receptor subtypes (ERα, ERβ and ERγ) in a sex- and brain region-specific manner[29]. Hence, *in utero* BPA exposure could predetermine later responses of certain brain areas to steroid hormones. Moreover, a recent high-quality experimental study by the Consortium Linking Academic and Regulatory Insights on BPA Toxicity (CLARITY-BPA) demonstrated that low BPA doses *in utero* alter the rat brain transcriptome, mainly in the hypothalamus, finding sex-specific effects on hypothalamic ERα and ERβ expression[33].

More than 90% of Europeans and Americans have detectable concentrations of BPA in their urine, and diet is considered the main source of BPA exposure in humans[34–37]. Despite the ubiquity of BPA, its non-persistence and short biological half-life and the episodic nature of its exposure make BPA characterization very difficult, frequently producing an important degree of exposure misclassification. This results in a greater tendency to obtain null results, the so-called attenuation bias[15,38]. Therefore, it is possible that we have been systematically underestimating the effects of BPA, and researchers in the field should consider the potential impact of this attenuation when interpreting epidemiologic findings[11,15,38]. However, despite these methodological limitations, the overall picture of the relationship between human BPA exposure and behavior is relatively consistent across studies and populations. Moreover, the widespread nature of exposure to BPA means that even subtle changes in behavior at the individual level may have relevant effects at the population level, with public health repercussions[39,40].

In 2008, a National Toxicology Program assessment, based on animal data, reported that BPA-related neural and behavioral endpoints were a major concern for fetuses, infants, and children[41]. Since then, some governments have implemented preventive measures, such as the banning of BPA in baby bottles by Canada and subsequently by the European Union[42,43], and the total prohibition of BPA in France[44]. Additionally, regulatory organizations such as the European Food Safety Authority (EFSA) have been progressively reducing their estimation of the tolerable daily intake (TDI)

in subsequent risk assessments. Thus, the TDI for BPA was lowered from 50 µg/kg bw/day to 4 µg/kg bw/day in 2015[45]. Nevertheless, recent well-conducted experimental studies have shown that BPA can impact the brain and behavior of rats at doses near to or even below the current TDI[33,46], and it has been suggested that EFSA's temporary tolerable daily intake of 4µg/kg bw/day may not be "sufficiently protective" for humans in the general population[46]. EFSA's forthcoming assessment will be an opportunity to integrate new experimental and epidemiologic data and provide evidence in support of action to protect children's behavior.

BPA is commonly found in food packaging materials, being used in the manufacture of polycarbonate plastics and in the epoxy resin liners of food cans. The greater public awareness around BPA has been reflected by the increased popularity of "BPA-free" products[47]. However, many of these products are manufactured using bisphenol analogues, including bisphenol S and F (BPS and BPF), which have been shown to be at least as hormonally active as BPA[48]. Furthermore, experimental studies of bisphenol substitutes such as BPS and BPF suggest similar and in some cases greater adverse neurobehavioral effects than those associated with BPA[49].

It may never be possible to establish a causal link between a specific EDC and a particular neurobehavioral endpoint[50]. Nevertheless, it is impossible to ignore the consistent and accumulating human evidence on the effects of BPA on child neurobehavior. While future research will provide greater clarity, the mounting experimental and epidemiologic data on its neurobehavioral effects support more than ever the need to apply the precautionary principle during development, especially in relation to pregnant women and children[5,51]. It seems that the time to act has arrived[52].

Conflict of interest

The authors declare no actual or potential financial conflicts of interest.

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References

1. Grandjean P, Landrigan PJ. Neurobehavioural effects of developmental toxicity. Lancet Neurol. 2014;13(3): 330-338. doi:10.1016/S1474-4422(13) 70278-3.

2. Braun JM. Early-life exposure to EDCs: role in childhood obesity and neurodevelopment. *Nat Rev Endocrinol*. 2017; 13(3): 161-173. doi:10.1038/nrendo.2016.186.

3. Nesan D, Sewell LC, Kurrasch DM. Opening the black box of endocrine disruption of brain development: Lessons from the characterization of Bisphenol A. *Horm Behav*. January 2018. doi:10.1016/j. yhbeh.2017.12.001.

4. Wolstenholme JT, Rissman EF, Connelly JJ. The role of Bisphenol A in shaping the brain, epigenome and behavior. *Horm Behav*. 2011; 59(3): 296-305. doi:10.1016/j.yhbeh.2010.10.001.

5. Mustieles V, Pérez-Lobato R, Olea N, et al. Bisphenol A: Human exposure and neurobehavior. *Neurotoxicology*. 2015. doi:10.1016/j. neuro.2015.06.002.

6. vom Saal FS, Akingbemi BT, Belcher SM, et al. Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. *Reprod Toxicol*. 2007; 24(2): 131-138. doi:10.1016/j. reprotox.2007.07.005.

7. Rochester JR. Bisphenol A and human health: a review of the literature. *Reprod Toxicol*. 2013; 42: 132-155. doi:10.1016/j. reprotox.2013.08.008.

8. DiPietro JA, Voegtline KM. The gestational foundation of sex differences in development and vulnerability. *Neuroscience*. 2017; 342: 4-20. doi:10.1016/j.neuroscience.2015.07.068.

9. Casas M, Forns J, Martínez D, et al. Exposure to bisphenol A during pregnancy and child neuropsychological development in the INMA-Sabadell cohort. *Environ Res*. 2015; 142: 671-679. doi:10.1016/j. envres.2015.07.024.

10. Findlay LC, Kohen DE. Bisphenol A and child and youth behaviour: Canadian Health Measures Survey 2007 to 2011. *Heal reports*. 2015; 26(8): 3-9. http://www.ncbi.nlm.nih.gov/pubmed/26288316. Accessed January 21, 2018.

11. Perez-Lobato R, Mustieles V, Calvente I, et al. Exposure to bisphenol A and behavior in school-age children. *Neurotoxicology*. 2016; 53: 12-19. doi:10.1016/j.neuro.2015.12.001.

12. Tewar S, Auinger P, Braun JM, et al. Association of Bisphenol A exposure and Attention-Deficit/Hyperactivity Disorder in a national sample of U.S. children. *Environ Res*. 2016; 150: 112-118. doi:10.1016/j.envres.2016.05.040.

13. Perera F, Nolte ELR, Wang Y, et al. Bisphenol A exposure and symptoms of anxiety and depression among inner city children at 10–12 years of age. *Environ Res*. 2016; 151: 195-202. doi:10.1016/j. envres.2016.07.028.

14. Braun JM, Muckle G, Arbuckle T, et al. Associations of Prenatal Urinary Bisphenol A Concentrations with Child Behaviors and Cognitive Abilities. *Environ Health Perspect*. 2017; 125(6): 67008. doi:10.1289/EHP984.

15. Philippat C, Nakiwala D, Calafat AM, et al. Prenatal Exposure to Nonpersistent Endocrine Disruptors and Behavior in Boys at 3 and 5 Years. *Environ Health Perspect*. 2017; 125(9): 97014. doi:10.1289/ EHP1314.

16. Stacy SL, Papandonatos GD, Calafat AM, et al. Early life bisphenol A exposure and neurobehavior at 8 years of age: Identifying windows of heightened vulnerability. *Environ Int*. 2017; 107: 258-265. doi:10.1016/j.envint.2017.07.021.

17. Lim YH, Bae S, Kim BN, et al. Prenatal and postnatal bisphenol A exposure and social impairment in 4-year-old children. *Environ Heal*. 2017; 16(1): 79. doi:10.1186/s12940-017-0289-2.

18. Minatoya M, Araki A, Nakajima S, et al. Cord blood BPA level and child neurodevelopment and behavioral problems: The Hokkaido Study on Environment and Children's Health. *Sci Total Environ*. 2017; 607-608: 351-356. doi:10.1016/J.SCITOTENV.2017.06.060.

19. Li Y, Zhang H, Kuang H, et al. Relationship between bisphenol A exposure and attention-deficit/ hyperactivity disorder: A case-control study for primary school children in Guangzhou, China. *Environ Pollut*. 2018; 235: 141-149. doi:10.1016/j.envpol.2017.12.056.

20. Perera F, Vishnevetsky J, Herbstman JB, et al. Prenatal bisphenol a exposure and child behavior in an inner-city cohort. *Environ Health Perspect*. 2012; 120(8): 1190-1194. doi:10.1289/ehp.1104492.

21. Roen EL, Wang Y, Calafat AM, et al. Bisphenol A exposure and behavioral problems among inner city children at 7-9 years of age. *Environ Res*. 2015; 142: 739-745. doi:10.1016/j.envres.2015.01.014.

22. Harley KG, Gunier RB, Kogut K, et al. Prenatal and early childhood bisphenol A concentrations and behavior in school-aged children. *Environ Res*. 2013; 126: 43-50. doi:10.1016/j.envres.2013.06.004.

23. Evans SF, Kobrosly RW, Barrett ES, et al. Prenatal bisphenol A exposure and maternally reported behavior in boys and girls. *Neurotoxicology*. 2014; 45: 91-99. doi:10.1016/j.neuro.2014.10.003.

24. Braun JM, Kalkbrenner AE, Calafat AM, et al. Impact of Early-Life Bisphenol A Exposure on Behavior and Executive Function in Children. *Pediatrics*. 2011; 128(5): 873-882. doi:10.1542/peds.2011-1335.

25. Zhou R, Bai Y, Yang R, et al. Abnormal synaptic plasticity in basolateral amygdala may account for hyperactivity and attention-deficit in male rat exposed perinatally to low-dose bisphenol-A. *Neuropharmacology*. 2011; 60(5): 789-798. doi:10.1016/j.neuropharm.2011.01.031.

26. Ishido M, Masuo Y, Kunimoto M, et al. Bisphenol A causes hyperactivity In the rat concomitantly with impairment of tyrosine hydroxylase immunoreactivity. *J Neurosci Res*. 2004; 76(3): 423-433. doi:10.1002/ jnr.20050.

27. Bao AM, Swaab DF. Sexual differentiation of the human brain: Relation to gender identity, sexual orientation and neuropsychiatric disorders. *Front Neuroendocrinol*. 2011; 32(2): 214-226. doi:10.1016/j. yfrne.2011.02.007.

28. Berenbaum SA, Beltz AM. Sexual differentiation of human behavior: Effects of prenatal and pubertal organizational hormones. *Front Neuroendocrinol*. 2011; 32(2): 183-200. doi:10.1016/j. yfrne.2011.03.001.

29. Kundakovic M, Gudsnuk K, Franks B, et al. Sex-specific epigenetic disruption and behavioral changes following low-dose in utero bisphenol A exposure. *Proc Natl Acad Sci*. 2013; 110(24): 9956-9961. doi:10.1073/pnas.1214056110.

30. Rubin BS, Lenkowski JR, Schaeberle CM, et al. Evidence of Altered Brain Sexual Differentiation in Mice Exposed Perinatally to Low, Environmentally Relevant Levels of Bisphenol A. *Endocrinology*. 2006; 147(8): 3681-3691. doi:10.1210/en.2006-0189.

31. Nakagami A, Negishi T, Kawasaki K, et al. Alterations in male infant behaviors towards its mother by prenatal exposure to bisphenol A in cynomolgus monkeys (Macaca fascicularis) during early suckling period. *Psychoneuroendocrinology*. 2009; 34(8): 1189-1197. doi:10.1016/j.psyneuen.2009.03.005.

32. Negishi T, Nakagami A, Kawasaki K, et al. Altered social interactions in male juvenile cynomolgus monkeys prenatally exposed to bisphenol A. *Neurotoxicol Teratol*. 2014; 44: 46-52. doi:10.1016/j.ntt.2014.05.004.

33. Arambula SE, Belcher SM, Planchart A, et al. Impact of Low Dose Oral Exposure to Bisphenol A (BPA) on the Neonatal Rat Hypothalamic and Hippocampal Transcriptome: A CLARITY-BPA Consortium Study. *Endocrinology*. 2016; 157(10): 3856-3872. doi:10.1210/en.2016-1339.

34. Becker K, Göen T, Seiwert M, et al. GerES IV: phthalate metabolites and bisphenol A in urine of German children. *Int J Hyg Environ Health*. 2009; 212(6): 685-692. doi:10.1016/j.ijheh.2009.08.002.

35. Calafat AM, Ye X, Wong LY, et al. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003-2004. *Environ Health Perspect*. 2008; 116(1): 39-44. doi:10.1289/ehp.10753.

36. Covaci A, Hond E Den, Geens T, et al. Urinary BPA measurements in children and mothers from six European member states: Overall results and determinants of exposure. *Environ Res*. 2015; 141: 77-85. doi:10.1016/j.envres.2014.08.008.

37. Mustieles V, Ocón-Hernandez O, Mínguez-Alarcón L, et al. Bisphenol A and reproductive hormones and cortisol in peripubertal boys: The INMA-Granada cohort. *Sci Total Environ*. 2017. doi:10.1016/j. scitotenv.2017.09.093.

38. Perrier F, Giorgis-Allemand L, Slama R, et al. Within-subject Pooling of Biological Samples to Reduce Exposure Misclassification in Biomarker-based Studies. *Epidemiology*. 2016; 27(3): 378-388. doi:10.1097/EDE.0000000000000460.

39. Bellinger DC. What is an adverse effect? A possible resolution of clinical and epidemiological perspectives on neurobehavioral toxicity. *Environ Res*. 2004; 95(3): 394-405. doi:10.1016/j.envres.2003.07.013.

40. Bellinger DC. Comparing the population neurodevelopmental burdens associated with children's exposures to environmental chemicals and other risk factors. *Neurotoxicology*. 2012; 33(4): 641-643. doi:10.1016/j.neuro.2012.04.003.

41. Chapin RE, Adams J, Boekelheide K, et al. NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A. *Birth Defects Res B Dev Reprod Toxicol*. 2008; 83(3): 157-395. doi:10.1002/bdrb.20147.

42. Canada Consumer Product Safety Act. 2017. http://laws-lois.justice.gc.ca/PDF/C-1.68.pdf. Accessed January 16, 2018.

43. European Commission - Bisphenol A: EU ban on baby bottles to enter into force tomorrow. http://europa.eu/rapid/press-release_IP-11-664_en.htm. Published 2011. Accessed January 16, 2018.

44. Audran X, Salmon DG. French Law Banning Bisphenol A in Food Containers Enacted BPA Ban in Food Containers by 2015. 2012. https://gain.fas.usda.gov/RecentGAINPublications/French Law Banning Bisphenol A in Food Containers Enacted_Paris_ France_2-5-2013.pdf. Accessed January 16, 2018.

45.Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. *EFSA J*. 2015; 13(1): 3978. doi:10.2903/j.efsa.2015.3978.

46. Hass U, Christiansen S, Boberg J, et al. Low-dose effect of developmental bisphenol A exposure on sperm count and behaviour in rats. *Andrology*. 2016; 4(4): 594-607. doi:10.1111/andr.12176.

47. Vandenberg LN, Prins GS. Clarity in the face of confusion: new studies tip the scales on bisphenol A (BPA). *Andrology*. 2016; 4(4): 561-564. doi:10.1111/andr.12219.

48. Rochester JR, Bolden AL. Bisphenol S and F: A Systematic Review and Comparison of the Hormonal Activity of Bisphenol A Substitutes. *Environ Health Perspect*. 2015; 123(7): 643-650. doi:10.1289/ ehp.1408989.

49. Rosenfeld CS. Neuroendocrine disruption in animal models due to exposure to bisphenol A analogues. *Front Neuroendocrinol*. 2017; 47: 123-133. doi:10.1016/j.yfrne.2017.08.001.

50. Colborn T. Neurodevelopment and endocrine disruption. *Environ Health Perspect*. 2004; 112(9): 944-949. http://www.ncbi.nlm.nih.gov/pubmed/15198913. Accessed January 22, 2018.

51. Vandenberg LN. Exposure to bisphenol A in Canada: invoking the precautionary principle. *Can Med Assoc J*. 2011; 183(11): 1265-1270. doi:10.1503/cmaj.101408.

52. Fernández MF, Román M, Arrebola JP, et al. Endocrine Disruptors: Time to Act. *Curr Environ Heal Reports*. 2014; 1(4): 325-332. doi:10.1007/ s40572-014-0025-9.

PAPER 6

Mustieles V, Fernández MF, Martin-Olmedo P, González-Alzaga B, Fontalba-Navas A, Hauser R, Olea N, Arrebola JP. (2017). Human adipose tissue levels of persistent organic pollutants and metabolic syndrome components: Combining a cross-sectional with a 10-year longitudinal study using a multi-pollutant approach. *Environment International*, 104, 48-57.

Paper #6

Human adipose tissue levels of persistent organic pollutants and metabolic syndrome components: Combining a cross-sectional with a 10-year longitudinal study using a multi-pollutant approach

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Abstract

We aimed to assess the influence of long-term exposure to POPs on the risk of metabolic syndrome, combining a cross-sectional with a 10-year longitudinal follow-up design. Residues of eight POPs were quantified in adipose tissue samples from 387 participants recruited between 2003 and 2004 in Granada province (Spain). The outcome ("metabolically compromised") was defined as having ≥1 diagnosis of type 2 diabetes, hypertension, hypertriglyceridemia, and/or low HDL cholesterol. The cross-sectional analysis was conducted in the initial cohort, while the 10-year longitudinal analysis was conducted in those 154 participants free of any of the so-mentioned metabolic diseases and classified as "metabolically healthy" at recruitment. Statistical analyses were performed using single and multi-pollutant approaches through logistic and Cox regression analyses with elastic net penalty. After adjusting for confounders, β-hexachlorocyclohexane (β-HCH) and hexachlorobenzene (HCB) were independently associated with an increased risk of being metabolically compromised (unpenalized ORs = 1.17, 95% CI = 1.01–1.36 and 1.17, 95% CI = 0.99–1.38, respectively). Very similar results were found in the 10-year longitudinal analysis [HRs = 1.28, 95% CI = 1.01–1.61 (β-HCH); 1.26, 95% CI = 1.00–1.59 (HCB)] and were in line with those obtained using elastic net regression. Finally, when the arithmetic sum of both compounds was used as independent variable, risk estimates increased to OR = 1.25, 95% CI = 1.03–1.52 and HR = 1.32, 95% CI = 1.02–1.70. Our results suggest that historical exposure to HCB and β-HCH is consistently associated with the risk of metabolic disorders, and that these POPs might be partly responsible for the morbidity risk traditionally attributed to age and obesity.

1. Introduction

The emergent obesity epidemic is at the center of worldwide public health concerns, along with its implications for chronic diseases (Guh et al., 2009). Although unhealthy dietary patterns and sedentary lifestyles are recognized as the main triggers of this epidemic, mounting evidence is signaling other environmental stressors, such as exposure to endocrine disrupting chemicals (EDCs), as an additional risk factor for obesity and meta-bolic disorders (Dhurandhar and Keith, 2014). Thus, increasing data suggest that long-term exposure to a group of EDCs designated persistent organic pollutants (POPs) may have a relevant impact on a cluster of metabolic conditions (obesity, dyslipidemia, high blood pressure and insulin resistance) known as the metabolic syndrome (MetS) (Alberti et al., 2009).

POPs are highly lipophilic compounds that resist metabolism and biodegradation and therefore tend to have a relatively long half-life in the

environment and to bioaccumulate and biomagnify in the food chain (Mrema et al., 2013). The result is the virtually universal exposure of living organisms, including humans (Jakszyn et al., 2009). These chemicals include organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), which have been used in a variety of commercial products, e.g., insecticides (dichlorodiphenyltrichlor-oethane [DDT], dicofol, lindane), fungicides (hexachlorobenzene [HCB]), and coolant and heating exchange fluids (polychlorinated biphenyls [PCBs]). Although legal restrictions in most countries have caused a worldwide decline in the production and handling of many POPs, human exposure remains relevant to public health due to their ubiquity and because current generations might suffer the effects of accumulated exposure throughout their lives, especially during critical windows of development (Tang-Péronard et al., 2015). Moreover, part of the POP body burden is transferred to subsequent generations during gestation and breastfeeding (Shen et al., 2007), and most studies have considered diet, especially fatty food, to be the main current source of exposure in the general population (Gasull et al., 2011; Arrebola et al., 2012). Other sources, such as indoor inhalation or dermal exposure, might also be important for certain POPs and population groups (Bräuner et al., 2016; Luo et al., 2014).

Although adipose tissue was once considered a simple energy storage depot, it is now known to be a complex endocrine organ with autocrine, paracrine, and neuroendocrine actions that influence appetite, energy regulation, lipid oxidation, immune and vascular functions, and hormonal status (Galic et al., 2010). Adipose tissue also appears to have an important toxicological function by sequestering POPs and other lipophilic contaminants in order to protect other more sensitive lipophilic organs (e.g., the brain) from an overload (La Merrill et al., 2013). Therefore, adipose tissue constitutes a reservoir for long-term POP accumulation and can act as a source of chronic exposure to POPs through their slow release into the bloodstream, which might have relevant consequences in several chronic diseases (La Merrill et al., 2013).

Adipose tissue is itself a target of pollutants, and some authors have suggested that POPs are taken up by adipocytes and accumulate within lipid droplets, where they might exert a major local effect by interfering with lipid metabolism, insulin sensitivity, and endocrine function (Bourez et al., 2013; La Merrill et al., 2013). Given the relative frequency of clinical exceptions to the paradigm “more fat means more metabolic disease” (Muñoz-Garach et al., 2016), lipophilic contaminants are therefore increasingly seen as potentially explaining, at least in part, the link with adipose tissue inflammation and dysfunction, the underlying mechanisms thought to determine whether obese individuals remain metabolically healthy or not (Muñoz-Garach et al., 2016).

The action mechanisms proposed for POPs include interaction with nuclear receptors such as peroxisome proliferator-activated receptor gamma (PPAR-γ) and aryl hydrocarbon receptor (AhR) (La Merrill et al., 2013), and with endogenous endocrine-related enzymes and inflammation pathways, causing oxidative stress and epigenetic modulation (Mrema et al., 2013). The diverse action mechanisms of different POP families and the potential interaction of complex mixtures, with additive, synergistic and/or antagonistic effects, complicate elucidation of the effects of POPs on metabolism (Rajapakse et al., 2002; Biemann et al., 2014). Furthermore, direct metabolic disrupting effects may coexist with long-term obesogenic effects that would lead to increased adiposity and therefore higher metabolic risk (Heindel et al., 2015; Lee et al., 2011).

Further complications are introduced by the simultaneous exposure of humans to complex low-level mixtures of EDCs that can potentially interfere with metabolism, including POPs (CDC, 2015; Braun et al., 2014). The short and long-term health risks posed by these mixtures remain unclear and are causes of increasing concern. However, the vast majority of epidemiological studies have considered exposure to chemicals in a one-compound-at-a-time approach that may not address the true effect of chemical mixtures on human health (Braun et al., 2016). Consequently, one of the goals of current environmental epidemiology is the development of alternative and complementary multi-pollutant approaches to disentangle independent associations among several co-exposures and assess their combined effect (Lenters et al., 2016).

Few epidemiological studies have analyzed the association between POP exposure and MetS (Lee et al., 2007b; Lee et al., 2011; Lee et al., 2014a; Park et al., 2010; Tomar et al., 2013). The present study was prompted by previous reports on associations between exposure to individual POPs and the risk of diabetes, hypertension, and elevated serum lipids in this same population (GraMo cohort) (Arrebola et al., 2013, 2014, 2015a). The study objectives were to assess the relation-ship between long-term exposure to eight POPs and the risk of developing MetS components and to examine whether POP exposure is in part responsible for the metabolic risk traditionally attributed to body mass index (BMI) and age. The causality of these relationships was explored by combining a cross-sectional with a 10-year longitudinal design, using both a single-chemical and a multi-pollutant approach.

2. Methods

2.1. Study cohort

This research is part of a wider hospital-based study that aims to characterize the exposure to POPs of an adult cohort from Southern Spain and to assess its potential health effects (GraMo cohort). The study design, recruitment, and methods are extensively described elsewhere (Arrebola et al., 2009, 2010). In brief, study subjects were recruited in two public hospitals from Granada province: San Cecilio University Hospital in the city of Granada (240.000 inhabitants, urban area) and Santa Ana Hospital in the town of Motril (50.000 inhabitants, semi-rural area). Participants were recruited between July 2003 and June 2004 from patients undergoing non-cancer-related surgery. Following the standard surgery protocols of the hospitals, all participants were under 12-h fasting conditions at sample collection. Inclusion criteria were: age over 16 years, absence of cancer, non-prescription of hormonal therapy, and residence in one of the study areas for at least 10 years. Reasons for surgery included a total of 70 different health issues. Given this heterogeneity, they were grouped into four cate-gories: hernias (41%), gallbladder diseases (21%), varicose veins (12%), and other conditions (26%). All participants signed their informed consent to participate in the study, which was approved by the ethics committees of both hospitals.

Out of the 409 individuals who were contacted, 387 agreed to participate and were included in the initial cohort. All analyzed adipose tissue biopsies were collected at recruitment (n = 387) and were used for the cross-sectional analyses in the present study. Out of this initial cohort, all participants free of any metabolic disease at recruitment (n = 169) were then included in the 10-year follow-up study. Out of these 169 participants, 15 were excluded from the follow-up due to missing information or discrepancies in their clinical records, leaving a final subsample of 154 individuals. All participants were users of the public health system. No statistically significant differences in sex distribution or age were found between participants and non-participants (data not shown in tables). Main characteristics of the study population are summarized in Table 1.

2.2. Exposure assessment

Samples of 5–10 g of adipose tissue were intra-operatively collected and immediately coded and stored at −80 °C until chemical analysis. Sample analysis and purification procedures were conducted as previously described (Rivas et al., 2001; Moreno Frías et al., 2004). In brief, chemical extraction with n-hexane was conducted on 200 mg of adipose tissue, and the solution was then purified through 2 g of alumina in a glass column. All extracts were stored in glass tubes at −80 °C.

Table 1. Characteristics of the study population.

	Initial cohort						10-year follow-up					
	Total (n = 387)		At risk of MetS (n = 218)		Metabolically healthy (N = 169)		Total (n = 154)		At risk of MetS (n = 53)		Metabolically healthy (n = 101)	
	n	%	n	%	n	%	n	%	n	%	n	%
Sex = male	197	50.9	92	42.2	105	62.1	95	61.7	36	67.9	59	58.4
Education = primary or higher	275	71.1	143	65.6	132	78.1	121	78.6	39	73.6	82	81.2
Residence												
Urban (Granada)	186	48.1	110	50.5	76	45.0	68	44.2	21	39.6	47	46.5
Semi-rural (Motril)	201	51.9	108	49.5	93	55.0	86	55.8	32	60.4	54	53.5
Alcohol consumption = yes	200	51.7	89	40.8	111	65.7	102	66. 2	33	62.3	69	68.3
Smoker = yes	126	32.6	59	27.1	67	39.7	60	39	18	44.0	42	41.6

	Initial cohort									10-year follow-up								
	Total (n = 387)			At risk of MetS (n = 218)			Metabolically healthy (N = 169)			Total (n = 154)			At risk of MetS (n = 53)			Metabolically healthy (n = 101)		
	Median	Percentiles		Median	Percentiles		Median	Percentiles		Median	Percentiles		Median	Percentiles		Median	Percentiles	
		25th	75th		25th	75th		25th	75th		25th	75th		25th	75th		25th	75th
Age	52	37	63	58	45	68	42	31	56	42	30	56	56	46	66	34	26	48
BMI (kg/m^2)	26.6	23.9	29.5	27.4	24.2	30.8	25.8	23.5	28.4	26.0	23.5	28.3	27.5	24.6	29.2	25.3	22.8	27.6
PCB 138[a]	82.7	31.1	136.0	89.0	42.6	146.6	65.3	14.4	120.4	62.9	12.3	124.1	84.1	34.4	144.0	50.0	11.1	115.9
PCB 153[a]	223.2	136.0	361.5	262.7	153.9	406.9	183.5	105.0	295.6	180.9	102.2	299.6	204.3	149.2	363.3	165.3	68.5	270.7
PCB 180[a]	179.6	104.0	290.2	203.7	119.5	329.8	161.8	84.1	257.2	158.8	78.5	262.9	169.2	114.9	292.5	153.1	39.9	248.9
p,p′-DDE[a]	93.0	32.9	210.2	117.6	54.6	254.6	53.9	22.7	159.1	51.5	21.3	155.2	121.1	33.1	293.6	41.7	13.74	132.2
β-HCH[a]	10.6	3.7	21.4	14.1	6.5	26.2	6.8	0.7	14.8	6.9	0.6	14.8	11.3	3.5	22.6	5.5	0.3	10.8
HCB[a]	14.5	5.0	39.6	24.7	8.6	50.6	8.2	2.9	21.2	8.1	2.9	22.4	14.7	7.1	43.4	5.8	1.8	16.6
Σβ-HCH + HCB[a]	16.1	6.4	41.2	26.3	10.5	51.6	10.0	3.3	26.0	15.4	3.6	42.5	28.4	11.5	66.3	11.1	2.9	26.9

	Initial cohort						10-year follow-up					
	Total (n = 387)		At risk of MetS (n = 218)		Metabolically healthy (N = 169)		Total (n = 154)		At risk of MetS (n = 53)		Metabolically healthy (n = 101)	
	n	%	n	%	n	%	n	%	n	%	n	%
Dicofol (> limit of detection)	76	19.6	37	17.0	39	23.1	36	23.4	14	26.4	22	21.8
α-HCH (> limit of detection)	84	21.7	60	27.5	24	14.2	22	14.3	13	24.5	9	8.9

BMI (Body Mass Index); PCB 138, 153 and 180 (Polychlorinated Biphenyls 138, 153 and 180); p,p′-DDE (p,p′-Dichlorodiphenyldichloroethylene); β-HCH (β-Hexachlorocyclohexane); HCB (Hexachlorobenzene); α-HCH (α-Hexachlorocyclohexane); MetS (Metabolic Syndrome).

[a]ng/g lipid.

POPs were quantified by high-resolution gas chromatography coupled with a mass spectrometry detector in tandem mode, using a Saturn 2000 ion trap system (Varian, Walnut Creek, CA). We employed a 2 m × 0.25 mm silica capillary column (Bellefonte, PA) coupled with 30 m × 0.25 mm analytical column (Factor FOUR vf-5MS, Varian Inc., Walnut Creek, CA). The limit of detection (LOD) was set at 0.01 μg/L for all POPs under study. Chromatographic concentrations below the limit of detection were assigned a random value between 0 and the LOD. Residues of p,p′-dichlorodiphenyldichloroethylene (p,p′-DDE, the main metabolite of the pesticide DDT), HCB, dicofol, α- and β-hexachlorocyclohexane (α- and β-HCH, respectively), and PCB congeners −138, −153 and −180 were quantified. Recovery of the POPs from adipose tissue was studied to assess the extraction efficiency of the method and ranged from 90 to 98%.

Lipid content in adipose tissue samples was quantified gravimetrically as reported by Rivas et al. (2001), including a homogenization step of 100 mg adipose tissue with 5 mL chloroform:methanol:hydro-chloric acid (20:10:0.1) and acidification with 0.1 N hydrochloric acid before collection and weighing of the organic phase. Lipid-basis POP concentrations were calculated and expressed in nanograms per gram of lipid (ng/g lipid).

2.3. Outcome assessment

The outcome ("metabolically compromised") was defined as having ≥ 1 diagnosis of the following components: type 2 diabetes (fasting glucose ≥126 mg/dL and/or prescription of anti-diabetic therapy); hypertension (systolic blood pressure > 140 mm Hg and/or diastolic blood pressure > 90 mm Hg and/or prescription of antihypertensive therapy); hypertriglyceridemia (serum triglycerides (TG) ≥ 150 mg/dL and/or prescription of lipid-lowering treatment); or low high-density lipoprotein cholesterol (HDL-C), defined by serum level < 50 mg/dL in females or < 40 mg/dL in males (Alberti et al., 2009). Thus, we compared metabolically compromised individuals (≥1 MetS component) with those metabolically healthy (free of any MetS component). This was the final outcome analyzed in both the cross-sectional and longitudinal analyses. For the longitudinal analyses, the follow-up period was from the date of recruitment to diagnosis of the first metabolic condition, death of the participant, or December 31, 2013 (the cohort remains under study).

The classification criteria were based on the harmonized definition of the International Diabetes Federation (IDF) (Alberti et al., 2009), and the same criteria were used in the cross-sectional and the longitudinal analyses. However, we did not include obesity in the outcome classification criteria for two main reasons: i) our secondary aim was to disentangle the metabolic effects of POPs from the metabolic risk traditionally attributed to obesity, as previously suggested (Lee et al., 2007a; Gauthier et al., 2014); and ii) we considered BMI as a confounder of the association between POPs and metabolic diseases because it is strongly and positively correlated with both the exposure and the outcome (Vaclavik et al., 2006; Arrebola et al., 2010).

The prevalence of MetS components was gathered using both self-reported information and the DIRAYA clinical record database, finding no relevant discrepancies between these sources, while the incidence was gathered by using the DIRAYA database. DIRAYA, which was implemented in 2003, integrates all clinical information for each public health system user, including primary and specialized care, storing data on diagnostic tests performed and pharmaceutical treatments received. The aim of this system is to facilitate clinical procedures and epidemiological research.

2.4. Covariates

Data on socio-demographic characteristics, lifestyle, and health status were collected in face-to-face interviews conducted by trained personnel at the time of recruitment during the hospital stay.

Questionnaire and research procedures were standardized and validated in a pilot study with 50 subjects. The questionnaire was designed and validated in a previous investigation (Buckland et al., 2009; Gonzalez and Riboli, 2010).

Participants' weight and height were measured, and the BMI was calculated as weight/height squared (kg/m^2). A subject was considered a smoker or alcohol consumer with any level of daily tobacco (≥1 cig/ day) or weekly alcohol (≥1 drink/week) consumption. Residence in the city of Granada at the time of the surgery was considered "urban" and residence in the area of Motril was considered "semi-rural".

The dietary section was composed of a semi-quantitative questionnaire on food consumption habits which included the following food groups: meat, cold meats, fats, fish, eggs, milk, dairy products, cheese, vegetables, pulses, fruit, bread, and pasta. The frequency of food consumption was gathered in four categories: < 1 portion/week, 1 portion/week, 2–6 portions/week, or > 7 portions/week. We also recorded the type of milk predominantly consumed (skimmed/semi-skimmed/whole) and the types of fish (lean/fatty) and meat (white/ red) consumed.

2.5. Statistical methods

Descriptive analysis included the calculation of medians and 25/ 75th percentiles for the interval variables and percentages for the categorical variables. ∑(HCB + β-HCH) was calculated as the arithmetic sum of the two chemical concentrations. Correlations between pairs of POPs were assessed using Spearman test.

For the overall cohort (n = 387), the magnitude of associations between POPs and the outcome was analyzed by binomial unconditional logistic regression, calculating multivariable-adjusted odds ratios (ORs) with their corresponding 95% confidence intervals (CIs). Then, those participants that were free of any MetS component at recruitment (n = 154) were followed-up, and the magnitude of associations be-tween POPs and the 10-year incidence of MetS components was evaluated using Cox-regression models with time-to-events as the time variable, calculating hazard ratios (HRs) with their corresponding 95% CIs. Estimations of time-to-events were based on the dates of recruitment, diagnosis, and end of follow-up. Data on participants who died before the observation of a study outcome were censored; therefore, only their disease-free time was considered in the analyses.

The shape of the relationships between individual POP concentrations and the outcome was evaluated by using locally weighted scatterplot smoothing (LOWESS) and Generalized Additive Models (GAM). The influence of extreme values was minimized by log-transforming the concentrations of all POPs and treating them as continuous variables (for POPs whose LOD ranged between 86 and 100%), with the exception of dicofol (% > LOD = 20) and α-HCH (% > LOD = 22), which were treated as dichotomous (≥LOD /LOD) due to their low detection level. In addition, the comparability was enhanced by adjusting both the logistic- and Cox-regression models for the same group of covariates, which included variables whose inclusion in any model produced changes > 10% in beta coefficients and/or those reported as relevant confounders in previous studies, i.e., BMI (kg/m^2), age (years), sex (male/female), residence (urban/semi-rural), education (primary schooling not completed/primary or higher), alcohol consumption (consumer/non-consumer), and smoking habit (smoker/non-smoker). Fig. S1 (supplementary material) depicts the hypothesized causal pathway between exposure and outcome and the role of the potential confounders. The potential modifying effect of sex, age, BMI, residence, education, smoking habit, and alcohol consumption on the associations found was studied by entering the interaction terms (POP levels ∗ each potential modifier) in the models. No significant interactions were found. Multivariable models displayed in the manuscript were performed using lipid-basis POP concentrations (ng/g lipid), although they were repeated using wet-basis concentrations (ng/ g adipose tissue), and no relevant changes were observed in the associations found (data not shown in tables).

Most POP concentrations were highly correlated, showing variance inflation factors of 3.2–5.4 when entered simultaneously in a model. Therefore, in addition to single-pollutant analyses, elastic-net regression analyses were conducted to select the most relevant predictors, accounting for simultaneous co-exposures (Friedman et al., 2010; Simon et al., 2011; Tibshirani et al., 2012). The degree of penalization was estimated using cross-validation, and the process was repeated 100 times to calculate the combination of α and γ that produced a cross-validation error that was one standard error of the minimum (error.1se) (Zou and Hastie, 2005).

Data were stored and processed using the R statistical computing environment v3.2.3 (http://www.r-project.org/), and elastic-net models were built with the glmnet package. The significance level was set at $p \leq 0.05$, and all tests were two-tailed.

3. Results

3.1. Characteristics of study population

Table 1 summarizes the characteristics of the study population and the results of the chemical analyses. In comparison to the initially recruited population, the individuals included in the follow-up were considerably younger (median age: 52 vs. 42 years, respectively), had a similar BMI (median BMI: 26.6 vs. 26.0 kg/m^2, respectively), and there was a higher proportion of males (61.7% vs. 50.9%, respectively). There was also a higher proportion of alcohol consumers in the follow-up study population (66.2% vs. 51.7%, respectively). Out of the 387 initially recruited participants, 218 (56.3%) were classified as "metabolically compromised" (≥1 MetS component). Out of the 154 metabolically healthy individuals at recruitment, 53 (34.4%) developed at least one MetS component during the 10-year follow-up and were consequently classified as "metabolically compromised" in the longitudinal analysis. The median follow-up time, including censored and non-censored data, was 117.5 months. Levels of accumulated POPs in the adipose tissue samples from our cohort were comparable to other contemporary European and Asian populations (Malarvannan et al., 2013; Pauwels et al., 2000: Shen et al., 2009) and have been extensively described elsewhere (Arrebola et al., 2009, 2010, 2013). On the other hand, POP concentrations in the subsample included in the follow-up were 12–24% lower for PCBs and 35–45% lower for OCPs in comparison to levels in the initial cohort.

3.2. Prevalence at recruitment and incidence of MetS components

The prevalence and incidence of MetS components are reported as Supplementary material (Table S1). At recruitment, 136 (35.1%) participants presented one metabolic component, 63 (16.3%) two components, 17 (4.4%) three components, and only 2 (0.5%) presented with the four metabolic components considered in our classification criteria. During the 10-year follow-up, one of the four metabolic components was developed by 43 participants (27.9%), two by 9 (5.9%), and three by 1 (0.6%). Among the four components, hypertension and low HDL-C were more prevalent at recruitment, whereas incident hypertension predominated over the other three MetS components at the follow-up.

3.3. Associations between POP exposure and risk of MetS

Fig. 1 a and b depict POP levels according to the number of MetS components at recruitment in the study population. Median β-HCH and HCB concentrations increased in parallel with the number of MetS components (Fig. 1a), but this trend was not so clear for the other POPs (Fig. 1b).

Table 2 displays the results of the logistic regression analyses of the associations between POPs and MetS components. Among the eight POPs

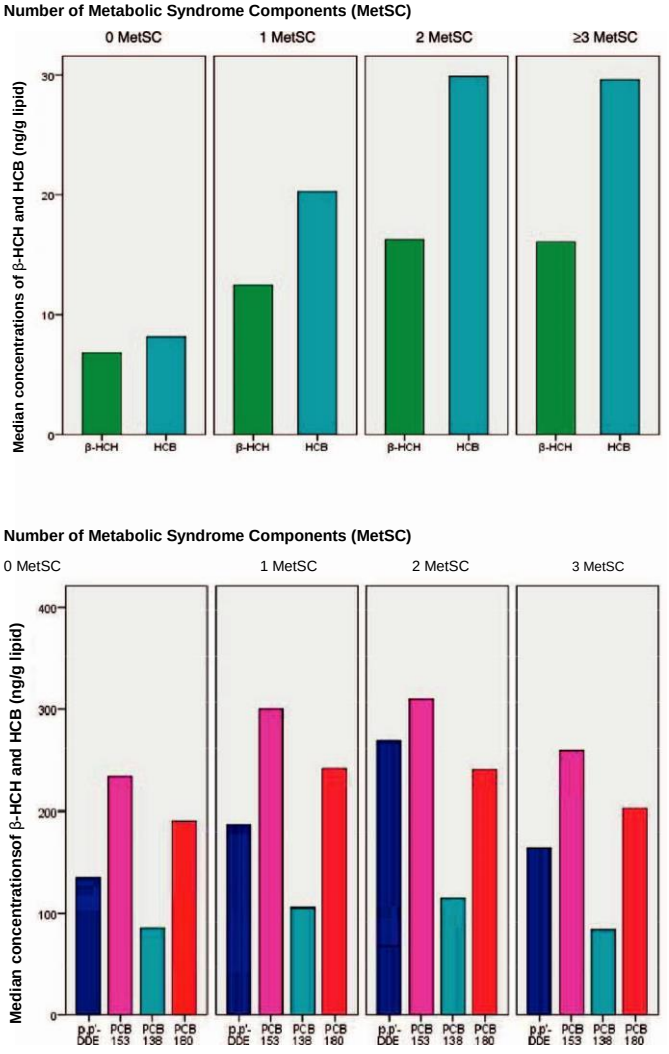

Fig. 1. a and b. POP levels according to the number of MetS components at recruitment in the study population. Median β-HCH and HCB concentrations increased in parallel with the number of MetS components (panel a), but this trend was not so clear for the other POPs (panel b).

measured, individual models using single chemical concentrations as independent variables showed that β-HCH was positively and significantly associated with the outcome (OR = 1.17, 95% CI = 1.01–1.36). Exposure to HCB showed a similar positive association, although the association was only marginally significant (OR = 1.17, 95% CI = 0.99–1.38). When all POPs were included in elastic net regression analyses, only coefficients for β-HCH [exp(beta) = 1.09] and HCB [exp(beta) = 1.03] were non-zero values. Furthermore, when ∑(HCB + β-HCH) was analyzed as an independent variable, we observed a stronger OR than that found for each chemical alone (OR = 1.25, 95% CI = 1.03–1.52).

Table 2 also exhibits the results of Cox regression analyses on the influence of historical exposure to POPs and the 10-year incidence of MetS components in the individuals classified as "metabolically healthy" at recruitment. Multivariable models with single POPs showed that β-HCH and HCB were again positively and significantly associated with the risk of MetS components (HRs = 1.28, 95% CI = 1.01–1.61 and 1.26, 95% CI = 1.00–1.59, respectively). In addition, elastic net regression analyses once more showed positive beta coefficients for both β-HCH [exp(beta) = 1.02] and HCB [exp(beta) = 1.01]. Furthermore, and similar to the cross-sectional analyses, ∑(HCB + β-HCH) was more strongly associated with the outcome in comparison to each individual compound (HR = 1.32, 95% CI = 1.02–1.70).

Table 2. Associations of adipose tissue POP concentrations (ng/g lipid) with prevalence at recruitment (n = 387) and 10-year incidence (n = 154) of MetS components.

	Prevalence at recruitment					10-year incidence				
	Ordinary logistic regression				Elastic-net regression	Ordinary Cox regression				Elastic-net regression
	OR[c]	95% CI[d]		p-Value	exp (beta)	HR[c]	95% CI[d]		p-Value	exp (beta)
		Lower	Upper				Lower	Upper		
Dicofol[a]	0.73	0.40	1.31	0.292	0	0.72	0.34	1.54	0.398	0
α-HCH[a]	1.21	0.61	2.39	0.592	0	1.12	0.50	2.49	0.786	0
PCB 138[b]	1.03	0.91	1.16	0.683	0	1.13	0.92	1.38	0.235	0
PCB 153[b]	1.01	0.85	1.20	0.955	0	1.15	0.85	1.56	0.356	0
PCB 180[b]	0.98	0.86	1.13	0.903	0	1.10	0.87	1.40	0.407	0
p,p′-DDE[b]	1.06	0.85	1.30	0.617	0	1.23	0.89	1.72	0.217	0
β-HCH[b]	1.17	1.01	1.36	0.035*	1.09	1.28	1.01	1.61	0.039*	1.02
HCB[b]	1.17	0.99	1.38	0.069†	1.03	1.26	1.00	1.59	0.048*	1.01
Σβ-HCH + HCB[b]	1.25	1.03	1.52	0.023*	–	1.32	1.02	1.70	0.036*	–

POP (Persistent Organic Pollutants); α-HCH (α-Hexachlorocyclohexane); PCBs-138, 153 and 180 (Polychlorinated Biphenyls 138, 153 and 180); p,p′-DDE (p-p′-Dichlorodiphenyldichloroethylene); β-HCH (β-Hexachlorocyclohexane); HCB (Hexachlorobenzene). Models in both designs were adjusted for age (years), BMI (kg/m^2), sex (male/ female), smoking (yes/no), alcohol (yes/no), place of residence (urban vs. semi-rural) and education (primary or higher vs. less than primary education). Estimations for Cox regression models used time-to-events as the time variable.

Cross-validation penalization in cross-sectional analysis: α = 0.95, λ = 0.07787609, error.1se = 1.246588.

Cross-validation penalization in longitudinal analysis: α = 1.0, λ = 0.2844456, error.1se = 9.993487.

[a] > limit of detection vs. < limit of detection.

[b] ng/g lipid.

[c] Odds Ratio (OR) and Hazard Ratio (HR).

[d] Confidence interval.

4. Discussion

In order to test the potential influence of the reason for surgery on the associations found, all multivariable models were adjusted for this variable (hernias/gallbladder diseases/varicose veins/other conditions), finding no relevant modifications in model coefficients (Supplementary material, Table S2).

In addition, we examined whether POP exposure might account for part of the contribution of age and obesity to the risk of MetS components. Thus, Tables 3a, 3b and Fig. 2 exhibit risk estimates when BMI or age is used as independent variable in relation to the outcome (dependent variable), in both cross-sectional and longitudinal analyses, at different levels of POP adjustment. In summary, β-HCH and HCB concentrations might account for 14–25% of the effect of the associations between BMI or age and the risk of presenting or developing the outcome.

Given that diet, especially animal fatty food, is considered a major contributor to both metabolic health and POP exposure in the general population, we conducted sensitivity analyses of the final models (both logistic and Cox regression), which were sequentially adjusted for selected dietary components potentially associated with both the exposure and the outcome (i.e., milk, fatty fish, cheese, meat, fruit, and vegetable consumption). These adjustments for dietary variables did not substantially affect the initial associations shown in Table 2, with only p-p′-DDE changing to a borderline significant association (Supplementary material, Table S3).

According to the results of this study, accumulated levels of β-HCH in combination with HCB were consistently associated with the risk of being metabolically compromised. Moreover, these pollutants might account for part of the risk of morbidity traditionally attributed to obesity and age. To our best knowledge, this investigation is one of the first prospective studies to analyze associations between POP exposure and MetS components as a whole and the first to combine cross-sectional with longitudinal analyses.

Our findings are in line with those of Lee et al. (2007b), who studied five POP subclasses and observed that serum OCP levels, especially β-HCH levels, showed the strongest and most consistent association with MetS. Park et al. (2010) found that β-HCH and heptachlor were associated with MetS in a case-control study, while Lee et al. (2014a) reported that levels of β-HCH and HCB (as well as oxychlordane and heptachlor) predicted the risk of MetS in a nested case-control study. Moreover, Tomar et al. (2013) found higher serum levels of nine OCPs in MetS patients compared to controls, although only β-HCH and aldrin were significantly associated with the risk of MetS.

There is also increasing evidence on the relationship between POP exposure and individual MetS components. Thus, β-HCH and HCB have been associated with the prevalence of type 2 diabetes (Al-Othman et al., 2014; Dirinck et al., 2011; Evangelou et al., 2016), and both β-HCH and HCB (as well as PCB-138 and PCB-153) were associated with incident hypertension in the GraMo

Table 3a
Cross-sectional analysis. Changes in odds ratios (ORs) of age and body mass index (BMI) for being metabolically compromised in logistic regression models at different levels of POP adjustment.

	Independent variable = age (years)				Independent variable = BMI (kg/m^2)			
	OR	95% CI[a]		p-Value	OR	95% CI[a]		p-Value
		Lower	Upper			Lower	Upper	
Model 1	1.041	1.024	1.058	< 0.001	1.076	1.026	1.129	0.003
Model 2	1.032	1.014	1.051	0.001	1.065	1.014	1.118	0.012
Model 3	1.034	1.016	1.053	< 0.001	1.060	1.009	1.114	0.021
Model 4	1.031	1.013	1.050	0.001	1.057	1.006	1.111	0.027

[a]Confidence interval.

Table 3b
Longitudinal analysis. Changes in hazard ratios (HRs) of age and body mass index (BMI) for being metabolically compromised in logistic regression models at different levels of POP adjustment.

	Independent variable = age (years)				Independent variable = BMI (kg/m^2)			
	HR	95% CI[a]		p-Value	HR	95% CI[a]		p-Value
		Lower	Upper			Lower	Upper	
Model 1	1.069	1.045	1.093	< 0.001	1.029	0.978	1.084	0.274
Model 2	1.059	1.034	1.084	< 0.001	1.007	0.949	1.069	0.809
Model 3	1.057	1.032	1.083	< 0.001	1.007	0.946	1.071	0.830
Model 4	1.057	1.031	1.083	< 0.001	1.005	0.944	1.070	0.873

Model 1: adjusted for [age (years) or BMI (kg/m^2)] + sex (male/female) + smoking (yes/no) + alcohol (yes/no) + place of residence (urban vs. semi-rural) + education (primary or higher vs. less than primary education). Model 2: model 1 + additional adjustment for β-HCH. Model 3: model 1 + additional adjustment for HCB. Model 4: model 1 + additional adjustment for Σ(HCB + β-HCH). Dependent variable: metabolic status (0 vs. ≥1 MetS components).
[a]Confidence interval.

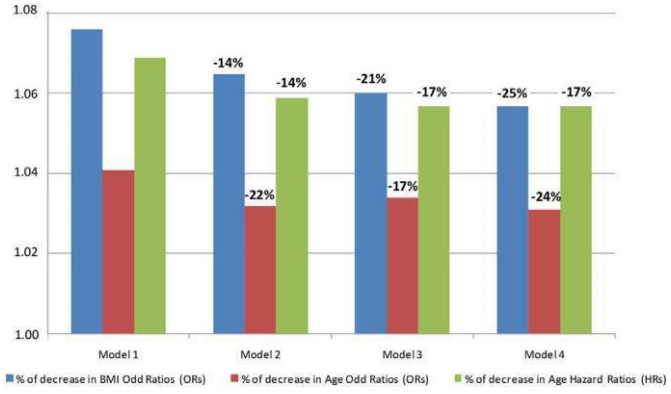

Fig. 2. Changes in risk estimates of BMI and age for MetS components at different levels of POP adjustment. Each column depicts the risk estimates (Odd Ratios [ORs] or Hazard Ratios [HRs]) of age and BMI in the models before and after adjustment for β-HCH and/or HCB concentrations. Percentages above the columns represent the relative decrease in model coefficients after adjustment for POPs. Model 1: adjusted for [age (years) or BMI (kg/m^2)] + sex (male vs. female) + smoking (yes/no) + alcohol (yes/no) + place of residence (urban vs. semi-rural) + education (primary or higher vs. less than primary education). Model 2: model 1 + adjustment for β-HCH. Model 3: model 1 + adjustment for HCB. Model 4: model 1 + adjustment for Σ(HCB + β-HCH).

cohort (Arrebola et al., 2015a). Similar findings have been reported in vulnerable groups, with findings of positive associations between serum POP concentrations and insulin resistance in pregnant women (Arrebola et al., 2015b) and between HCB exposure during pregnancy and higher plasma insulin levels in female offspring at 5 years of age (Tang-Péronard et al., 2015). Furthermore, higher plasma concentrations of HCB (among other POPs) were found to increase the likelihood of insulin resistance in pre-pubertal boys (Burns et al., 2014). In contrast, Lee et al. (2016) reported positive associations between PCB exposure and blood pressure and/or triglyceride levels among children but found no association with OCPs. There has even been a report of an inverse association between β-HCH levels and hypertension in adults (Valera et al., 2013).

In addition, the above epidemiological data on POP exposure and the metabolic syndrome are supported by experimental findings (La Merrill et al., 2013; Lee et al., 2014b). When Ruzzin et al. (2010) exposed rats to an environmentally relevant mixture of POPs from fish oil (farmed Atlantic salmon), animals developed insulin resistance, abdominal obesity, and hepatosteatosis. Moreover, these results were confirmed in differentiated adipocytes. A similar study in exposed mice also found an increased insulin resistance linked to visceral adiposity and inflammatory markers (Ibrahim et al., 2011). More recently, Zhang et al. (2015) reported a POP-induced modification of gut microbiota-host metabolic homeostasis in mice via activation of the AhR. These effects are in agreement with mechanistic data on POPs, suggesting interplay among endocrine-related mechanisms, chronic low-grade inflammation, and mitochondrial dysfunction (Kim et al., 2012; La Merrill et al., 2013; Lim et al., 2010). Although there remains a need for experimental data on the effect of specific POPs (including β-HCH and HCB) on metabolic outcomes, a recent systems biology approach has revealed converging molecular mechanisms that link three different POP families with common metabolic diseases (Ruiz et al., 2016).

Interestingly, the present data indicate that β-HCH and HCB levels might partially account for the effects of age and BMI as risk factors for MetS components (Tables 3a, 3b, Fig. 2). Although this finding needs to be further investigated and confirmed in future studies, it is consistent with the relatively new concept of the metabolically healthy obese phenotype. In this line, Gauthier et al. (2014) found significantly higher levels of 12 out of 18 POPs in metabolically unhealthy versus healthy obese women, despite having a similar age, BMI, and fat mass. Moreover, Lee et al. (2007a) found that, among obese individuals, only those with high levels of OCPs were at increased risk of insulin resistance, and there was no apparent association with the lowest quartile of OCP concentrations.

Although the ability of specific POPs to interfere with lipid and glucose metabolism is well documented (Evangelou et al., 2016; Lee et al., 2014a,b), the isolated interpretation of specific compounds may provide apparently inconsistent results (Lee et al., 2014b). This can be due to the existence of different patterns of POP mixtures between and within populations and/or the possibility that the observed effect of a specific chemical might be a surrogate of a POP combined effect. The influence of isolated individual compounds therefore has limited meaning, because humans are exposed to complex mixtures of POPs and the biological concentrations of many PCBs and OCPs are highly correlated, as observed in the present population (Spearman coefficients ranging from 0.6 to 0.9, Supplementary material, Fig. S2). In this regard, our elastic-net regression models demonstrated that both β-HCH and HCB were independently associated with the outcome. Additionally, the fact that risk estimates for ∑(HCB + β-HCH) were stronger than for the individual chemicals might point to an additive effect. This possibility is supported by the similar chemical structures of these two compounds. Thus, the equatorial position of the chlorine atoms in the molecule of β-HCH causes a more planar conformation that is highly similar to that of HCB (Fig. 3) (Mrema et al., 2013). This structural and conformational analogy might explain their similar half-lives and bioaccumulation patterns as well as their comparable pharmacokinetics and mechanisms of action (Mrema et al., 2013).

The main strength of this study is its combined design, which showed consistent results between the cross-sectional and longitudinal analyses (despite differences in population characteristics), thereby reinforcing causal inference and minimizing potential reverse causality. In this context, some authors have noted that certain inflammatory processes associated with metabolic diseases may lead to an alteration of POP metabolism, the

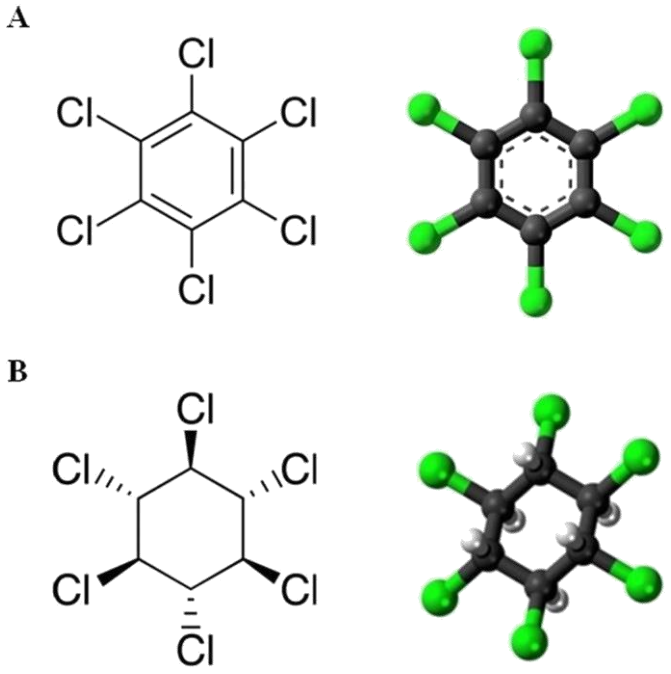

Fig. 3. A: Hexachlorobenzene (HCB); B: β-hexachlorocyclohexane (β-HCH). Interestingly, the equatorial position of the chlorine atoms in the molecule of β-HCH causes a more planar conformation, very similar to that of HCB.

so-called disease progression bias (Porta, 2014). However, the fact that similar findings were observed in the follow-up group (even with lower POP levels, younger age and healthier status) minimizes the aforementioned bias. A further strength is the utilization of a complementary statistical multi-pollutant model that assesses several co-exposures simultaneously, which represents a more realistic exposure scenario. Furthermore, historical exposure to POPs was estimated by using samples of adipose tissue, which is considered the most suitable matrix for the assessment of long-term accumulated concentrations of these compounds, accounting for all routes and sources of exposure (Kohlmeier and Kohlmeier, 1995; Quintana et al., 2004). In this regard, adipose tissue POP concentrations have been reported to show less variability in comparison to serum concentrations, which can be influenced by both point exposures and the mobilization of POPs stored in fatty tissues (Archibeque-Engle et al., 1997; Gaskins and Schisterman, 2009). In the present study, the validity of adipose tissue POP concentrations as a biomarker of long-term exposure is supported by the similar findings in the cross-sectional and longitudinal analyses, which can be of relevance for future epidemiological studies.

In addition, sensitivity analyses (Table S3) showed that adjustment for dietary items did not substantially affect the associations with β-HCH and HCB. These diet-adjusted results are timely, given a recent claim that the BMI might not be sufficient when estimating the confounding effect of diet as a source of POPs and as a predictor of metabolic disease (Tuomisto et al., 2016).

Among the limitations of our study is the relatively modest sample size, particularly in the longitudinal analyses and, therefore, we cannot completely rule out associations in those POPs with no statistically-significant results. However, the sample size was sufficient to yield robust associations and to reaffirm the results found in the cross-sectional analyses. Another limitation is that the final models were not adjusted for physical activity or for diet as a global pattern. As previously mentioned, diet might be a potential confounder of the associations found. However, POP concentrations can be considered as intermediate variables on the causal path from fatty food consumption to metabolic disorders, and the inclusion of dietary variables and POP exposure in the same model might have led to an over-adjustment. Interestingly, sensitivity analyses adjusting for dietary items showed no relevant changes in the previously observed associations. Additionally, all models were adjusted for BMI, which may represent, at least partially, both an excessive caloric intake and a sedentary lifestyle. Therefore, diet does not seem to have been a relevant modifier of the associations found in our study. A further limitation is that we cannot exclude the potential influence of other groups of unmeasured environ-mental pollutants that may have partially contributed to the observed associations. In this regard, further analyses of different groups of persistent and non-persistent contaminants are currently being con-ducted in the GraMo cohort.

5. Conclusions

Our results show that historical exposure to β-HCH and/or HCB is consistently related to the risk of MetS components, and that these pollutants might account for part of the risk of morbidity traditionally attributed to obesity and age. Given the ubiquity of the exposure and the frequency of metabolic diseases, we consider these results to be of major public health importance.

Conflicts of interest

The authors declare no actual or potential competing financial conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx. doi.org/10.1016/j.envint.2017.04.002.

References

Alberti, K.G., Eckel, R.H., Grundy, S.M., Zimmet, P.Z., Cleeman, J.I., Donato, K.A., et al., 2009. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 120 (16), 1640–1645.

Al-Othman, A., Yakout, S., Abd-Alrahman, S.H., Al-Daghri, N.M., 2014. Strong associations between the pesticide hexachlorocyclohexane and type 2 diabetes in Saudi adults. Int. J. Environ. Res. Public Health 11 (9), 8984–8995.

Archibeque-Engle, S.L., Tessari, J.D., Winn, D.T., Keefe, T.J., Nett, T.M., Zheng, T., 1997. Comparison of organochlorine pesticide and polychlorinated biphenyl residues in human breast adipose tissue and serum. J. Toxicol. Environ. Health 52 (4), 285–293.

Arrebola, J.P., Martin-Olmedo, P., Fernandez, M.F., Sanchez-Cantalejo, E., Jimenez-Rios, J.A., Torne, P., et al., 2009. Predictors of concentrations of hexachlorobenzene in human adipose tissue: a multivariate analysis by gender in Southern Spain. Environ. Int. 35 (1), 27–32.

Arrebola, J.P., Fernandez, M.F., Porta, M., Rosell, J., de la Ossa, R.M., Olea, N., et al., 2010. Multivariate models to predict human adipose tissue PCB concentrations in Southern Spain. Environ. Int. 36 (7), 705–713.

Arrebola, J.P., Mutch, E., Cuellar, M., Quevedo, M., Claure, E., Mejía, L.M., et al., 2012. Factors influencing combined exposure to three indicator polychlorinated biphenyls in an adult cohort from Bolivia. Environ. Res. 116, 17–25.

Arrebola, J.P., Fernández, M.F., Olea, N., Ramos, R., Martin-Olmedo, P., 2013. Human exposure to p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) in urban and semi-rural areas in southeast Spain: a gender perspective. Sci. Total Environ. 458-460, 209–216.

Arrebola, J.P., Ocaña-Riola, R., Arrebola-Moreno, A.L., Fernández-Rodríguez, M., Martin-Olmedo, P., Fernández, M.F., et al., 2014. Associations of accumulated exposure to persistent organic pollutants with serum lipids and obesity in an adult cohort from Southern Spain. Environ. Pollut. 195, 9–15.

Arrebola, J.P., Fernández, M.F., Martin-Olmedo, P., Bonde, J.P., Martín-Rodriguez, J.L., Expósito, J., et al., 2015a. Historical exposure to persistent organic pollutants and risk of incident hypertension. Environ. Res. 138, 217–223.

Arrebola, J.P., González-Jiménez, A., Fornieles-González, C., Artacho-Cordón, F., Olea, N., Escobar-Jiménez, F., et al., 2015b. Relationship between serum concentrations of persistent organic pollutants and markers of insulin resistance in a cohort of women with a history of gestational diabetes mellitus. Environ. Res. 136, 435–440.

Biemann, R., Fischer, B., Navarrete, Santos A., 2014. Adipogenic effects of a combination of the endocrine-disrupting compounds bisphenol A, diethylhexylphthalate, and tributyltin. Obes. Facts 7 (1), 48–56.

Bourez, S., Van den Daelen, C., Le Lay, S., Poupaert, J., Larondelle, Y., Thomé, J.P., et al., 2013. The dynamics of accumulation of PCBs in cultured adipocytes vary with the cell lipid content and the lipophilicity of the congener. Toxicol. Lett. 216 (1), 40–46.

Braun, J.M., Kalkbrenner, A.E., Just, A.C., Yolton, K., Calafat, A.M., Sjödin, A., et al., 2014. Gestational exposure to endocrine-disrupting chemicals and reciprocal social, repetitive, and stereotypic behaviors in 4- and 5-year-old children: the HOME study. Environ. Health Perspect. 122 (5), 513–520.

Braun, J.M., Gennings, C., Hauser, R., Webster, T.F., 2016. What can epidemiological studies tell us about the impact of chemical mixtures on human health? Environ. Health Perspect. 124 (1), A6–A9.

Bräuner, E.V., Andersen, Z.J., Frederiksen, M., Specht, I.O., Hougaard, K.S., Ebbehøj, N., et al., 2016. Health effects of PCBs in residences and schools (HESPERUS): PCB - health cohort profile. Sci. Rep. 6, 24571.

Buckland, G., González, C.A., Agudo, A., Vilardell, M., Berenguer, A., Amiano, P., et al., 2009. Adherence to the Mediterranean diet and risk of coronary heart disease in the Spanish EPIC cohort study. Am. J. Epidemiol. 170 (12), 1518–1529.

Burns, J.S., Williams, P.L., Korrick, S.A., Hauser, R., Sergeyev, O., Revich, B., et al., 2014. Association between chlorinated pesticides in the serum of prepubertal Russian boys and longitudinal biomarkers of metabolic function. Am. J. Epidemiol. 180 (9), 909–919.

CDC (Centers for Disease Control and Prevention), 2015. Fourth National Report on Human Exposure to Environmental Chemicals. (Accessed on August, 2016: http:// www.cdc.gov/biomonitoring/pdf/FourthReport_UpdatedTables_Feb2015.pdf).

Dhurandhar, E.J., Keith, S.W., 2014. The aetiology of obesity beyond eating more and exercising less. Best Pract. Res. Clin. Gastroenterol. 28 (4), 533–544.

Dirinck, E., Jorens, P.G., Covaci, A., Geens, T., Roosens, L., Neels, H., et al., 2011. Obesity and persistent organic pollutants: possible obesogenic effect of organochlorine pesticides and polychlorinated biphenyls. Obesity 19 (4), 709–714.

Evangelou, E., Ntritsos, G., Chondrogiorgi, M., Kavvoura, F.K., Hernández, A.F., Ntzani, E.E., et al., 2016. Exposure to pesticides and diabetes: a systematic review and meta-analysis. Environ. Int. 91, 60–68.

Friedman, J., Hastie, T., Tibshirani, R., 2010. Regularization paths for generalized linear models via coordinate descent. J. Stat. Softw. 33 (1), 1–22.

Galic, S., Oakhill, J.S., Steinberg, G.R., 2010. Adipose tissue as an endocrine organ. Mol. Cell. Endocrinol. 316 (2), 129–139.

Gaskins, A.J., Schisterman, E.F., 2009. The effect of lipid adjustment on the analysis of environmental contaminants and the outcome of human health risks. Methods Mol. Biol. 580, 371–381.

Gasull, M., Bosch de Basea, M., Puigdomènech, E., Pumarega, J., Porta, M., 2011. Empirical analyses of the influence of diet on human concentrations of persistent organic pollutants: a systematic review of all studies conducted in Spain. Environ. Int. 37 (7), 1226–1235.

Gauthier, M.S., Rabasa-Lhoret, R., Prud'homme, D., Karelis, A.D., Geng, D., van Bavel, B., et al., 2014. The metabolically healthy but obese phenotype is associated with lower plasma levels of persistent organic pollutants as compared to the metabolically abnormal obese phenotype. J. Clin. Endocrinol. Metab. 99 (6), E1061–E1066. http:// dx.doi.org/10.1210/jc.2013-3935.

Gonzalez, C.A., Riboli, E., 2010. Diet and cancer prevention: contributions from the European prospective investigation into cancer and Nutrition (EPIC) study. Eur. J. Cancer 46 (14), 2555–2562.

Guh, D.P., Zhang, W., Bansback, N., Amarsi, Z., Birmingham, C.L., Anis, A.H., 2009. The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. BMC Public Health 9, 88.

Heindel, J.J., vom Saal FS, Blumberg B, Bovolin P, Calamandrei G, Ceresini G, et al., 2015. Parma consensus statement on metabolic disruptors. Environ. Health 14, 54.

Ibrahim, M.M., Fjære, E., Lock, E.J., Naville, D., Amlund, H., Meugnier, E., et al., 2011. Chronic consumption of farmed salmon containing persistent organic pollutants causes insulin resistance and obesity in mice. PLoS One 6 (9), e25170. http://dx.doi. org/10.1371/journal.pone.0025170.

Jakszyn, P., Goñi, F., Etxeandia, A., Vives, A., Millán, E., López, R., et al., 2009. Serum levels of organochlorine pesticides in healthy adults from five regions of Spain. Chemosphere 76 (11), 1518–1524.

Kim, M.J., Pelloux, V., Guyot, E., Tordjman, J., Bui, L.C., Chevallier, A., et al., 2012. Inflammatory pathway genes belong to major targets of persistent organic pollutants in adipose cells. Environ. Health Perspect. 120 (4), 508–514.

Kohlmeier, L., Kohlmeier, M., 1995. Adipose tissue as a medium for epidemiologic exposure assessment. Environ. Health Perspect. 103 (Suppl. 3), 99–106.

La Merrill, M., Emond, C., Kim, M.J., Antignac, J.P., Le Bizec, B., Clément, K., et al., 2013. Toxicological function of adipose tissue: focus on persistent organic pollutants. Environ. Health Perspect. 121 (2), 162–169.

Lee, D.H., Lee, I.K., Jin, S.H., Steffes, M., Jacobs Jr., D.R., 2007a. Association between serum concentrations of persistent organic pollutants and insulin resistance among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999– 2002. Diabetes Care 30 (3), 622–628.

Lee, D.H., Lee, I.K., Porta, M., Steffes, M., Jacobs Jr., D.R., 2007b. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999–2002. Diabetologia 50 (9), 1841–1851.

Lee, D.H., Steffes, M.W., Sjödin, A., Jones, R.S., Needham, L.L., Jacobs Jr., D.R., 2011. Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes. PLoS One 6 (1), e15977. http://dx.doi.org/10.1371/journal.pone.0015977.

Lee, Y.M., Kim, K.S., Kim, S.A., Hong, N.S., Lee, S.J., Lee, D.H., 2014a. Prospective associations between persistent organic pollutants and metabolic syndrome: a nested case-control study. Sci. Total Environ. 496, 219–225.

Lee, D.H., Porta, M., Jacobs Jr., D.R., Vandenberg, L.N., 2014b. Chlorinated persistent organic pollutants, obesity, and type 2 diabetes. Endocr. Rev. 4, 557–601.

Lee, H.A., Park, S.H., Hong, Y.S., Ha, E.H., Park, H., 2016. The effect of exposure to persistent organic pollutants on metabolic health among Korean children during a 1-year follow-up. Int. J. Environ. Res. Public Health 13 (3).

Lenters, V., Portengen, L., Rignell-Hydbom, A., Jönsson, B.A., Lindh, C.H., Piersma, A.H., et al., 2016. Prenatal phthalate, perfluoroalkyl acid, and organochlorine exposures and term birth weight in three birth cohorts: multi-pollutant models based on elastic net regression. Environ. Health Perspect. 124 (3), 365–372.

Lim, S., Cho, Y.M., Park, K.S., Lee, H.K., 2010. Persistent organic pollutants, mitochondrial dysfunction, and metabolic syndrome. Ann. N. Y. Acad. Sci. 1201, 166–176.

Luo, F., Song, J., Chen, M.F., Wei, J., Pan, Y.Y., Yu, H.B., 2014. Risk assessment of manufacturing equipment surfaces contaminated with DDTs and dicofol. Sci. Total Environ. 468-469, 176–185.

Malarvannan, G., Dirinck, E., Dirtu, A.C., Pereira-Fernandes, A., Neels, H., Jorens, P.G., et al., 2013. Distribution of persistent organic pollutants in two different fat compartments from obese individuals. Environ. Int. 55, 33–42.

Moreno Frías, M., Jiménez Torres, M., GarridoFrenich, A., Martínez Vidal, J.L., Olea-Serrano, F., Olea, N., 2004. Determination of organochlorine compounds in human biological samples by GC-MS/MS. Biomed. Chromatogr. 18 (2), 102–111.

Mrema, E.J., Rubino, F.M., Brambilla, G., Moretto, A., Tsatsakis, A.M., Colosio, C., 2013. Persistent organochlorinated pesticides and mechanisms of their toxicity. Toxicology 307, 74–88.

Muñoz-Garach, A., Cornejo-Pareja, I., Tinahones, F.J., 2016. Does metabolically healthy obesity exist? Nutrients 8 (6). http://dx.doi.org/10.3390/nu8060320. (pii: E320).

Park, S.K., Son, H.K., Lee, S.K., Kang, J.H., Chang, Y.S., Jacobs, D.R., et al., 2010. Relationship between serum concentrations of organochlorine pesticides and metabolic syndrome among non-diabetic adults. J. Prev. Med. Public Health 43 (1), 1–8.

Pauwels, A., Covaci, A., Weyler, J., Delbeke, L., Dhont, M., De Sutter, P., et al., 2000. Comparison of persistent organic pollutant residues in serum and adipose tissue in a female population in Belgium, 1996–1998. Arch. Environ. Contam. Toxicol. 39 (2), 265–270.

Porta, M., 2014. A Dictionary of Epidemiology, Sixth edition. Oxford University Press. Quintana, P.J., Delfino, R.J., Korrick, S., Ziogas, A., Kutz, F.W., Jones, E.L., et al., 2004. Adipose tissue levels of organochlorine pesticides and polychlorinated biphenyls and risk of non-Hodgkin's lymphoma. Environ. Health Perspect. 112 (8), 854–861.

Rajapakse, N., Silva, E., Kortenkamp, A., 2002. Combining xenoestrogens at levels below individual no-observed-effect concentrations dramatically enhances steroid hormone action. Environ. Health Perspect. 110 (9), 917–921.

Rivas, A., Fernandez, M.F., Cerrillo, I., Ibarluzea, J., Olea-Serrano, M.F., Pedraza, V., et al., 2001. Human exposure to endocrine disrupters: standardization of a marker of estrogenic exposure in adipose tissue. APMIS 109 (3), 185–197.

Ruiz, P., Perlina, A., Mumtaz, M., Fowler, B.A., 2016. A systems biology approach reveals converging molecular mechanisms that link different POPs to common metabolic diseases. Environ. Health Perspect. 124 (7), 1034–1041.

Ruzzin, J., Petersen, R., Meugnier, E., Madsen, L., Lock, E.J., Lillefosse, H., et al., 2010. Persistent organic pollutant exposure leads to insulin resistance syndrome. Environ. Health Perspect. 118 (4), 465–471.

Shen, H., Main, K.M., Virtanen, H.E., Damggard, I.N., Haavisto, A.M., Kaleva, M., et al., 2007. From mother to child: investigation of prenatal and postnatal exposure to persistent bioaccumulating toxicants using breast milk and placenta biomonitoring. Chemosphere 67 (9), S256–S262.

Shen, H., Han, J., Tie, X., Xu, W., Ren, Y., Ye, C., 2009. Polychlorinated dibenzo-p-dioxins/furans and polychlorinated biphenyls in human adipose tissue from Zhejiang Province, China. Chemosphere 74 (3), 384–388.

Simon, N., Friedman, J., Hastie, T., Tibshirani, R., 2011. Regularization paths for cox's proportional hazards model via coordinate descent. J. Stat. Softw. 39 (5), 1–13.

Tang-Péronard, J.L., Heitmann, B.L., Jensen, T.K., Vinggaard, A.M., Madsbad, S., Steuerwald, U., et al., 2015. Prenatal exposure to persistent organochlorine pollutants is associated with high insulin levels in 5-year-old girls. Environ. Res. 142, 407–413.

Tibshirani, R., Bien, J., Friedman, J., Hastie, T., Simon, N., Taylor, J., et al., 2012. Strong rules for discarding predictors in lasso-type problems. J. R. Stat. Soc. Ser. B Stat Methodol. 74 (2), 245–266.

Tomar, L.R., Agarwal, M.P., Avasthi, R., Tyagi, V., Mustafa, M., Banerjee, B.D., 2013. Serum organochlorine pesticide levels in patients with metabolic syndrome. Indian J. Endocrinol. Metab. 17 (Suppl. 1), S342–S344.

Tuomisto, J., Airaksinen, R., Kiviranta, H., Tukiainen, E., Pekkanen, J., Tuomisto, J.T., 2016. A pharmacokinetic analysis and dietary information are necessary to confirm or reject the hypothesis on persistent organic pollutants causing type 2 diabetes. Toxicol. Lett. 261, 41–48.

Vaclavik, E., Tjonneland, A., Stripp, C., Overvad, K., Philippe Weber, J., Raaschou-Nielsen, O., 2006. Organochlorines in Danish women: predictors of adipose tissue concentrations. Environ. Res. 100 (3), 362–370.

Valera, B., Ayotte, P., Poirier, P., Dewailly, E., 2013. Associations between plasma persistent organic pollutant levels and blood pressure in Inuit adults from Nunavik. Environ. Int. 59, 282–289.

Zhang, L., Nichols, R.G., Correll, J., Murray, I.A., Tanaka, N., Smith, P.B., et al., 2015. Persistent organic pollutants modify gut microbiota-host metabolic homeostasis in mice through aryl hydrocarbon receptor activation. Environ. Health Perspect. 123 (7), 679–688.

Zou, H., Hastie, T., 2005. Regularization and variable selection via the elastic net. J. R. Stat. Soc. 67 (2), 301–320.

Supplemental Material

Human adipose tissue levels of persistent organic pollutants and metabolic syndrome components: Combining a cross-sectional with a 10-year longitudinal study using a multi-pollutant approach.

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Table S1. Prevalence and 10-year incidence of MetS components in GraMo cohort

	Prevalence (N=387)		10-year Incidence (N=154)	
	Metabolically compromised (N=218)		Metabolically compromised (N=53)	
	N	%	n	%
Type 2 diabetes	39	10.1	11	7.1
Hypertension	101	26.1	34	22.1
↑ Triglycerides	77	19.9	10	6.5
↓ HDL-C	104	26.9	9	5.8
Number of MetS components	n	%	n	%
0	169	43.7	101	65.6
1	136	35.1	43	27.9
2	63	16.3	9	5.9
3	17	4.4	1	0.6
4	2	0.5	0	0.0

MetS (Metabolic Syndrome).↑ Triglycerides (serum levels ≥150 mg/dl). ↓ HDL-C (serum levels of HDL-cholesterol <50mg/dl in women and <40 mg/dl in men).

Table S2. Associations of adipose tissue POP concentrations (ng/g lipid) with prevalence and 10-year incidence of MetS components after adjustment for the reason for surgery.

	Prevalence at recruitment				10-yearIncidence			
	OR[c]	95% CI[d]		p-value	HR[e]	95% CI[d]		p-value
		Lower	Upper			Lower	Upper	
Dicofol[a]	0.68	0.38	1.24	0.211	0.77	0.36	1.65	0.499
α-HCH[a]	1.20	0.60	2.38	0.610	1.11	0.50	2.50	0.793
PCB 138[b]	1.05	0.92	1.18	0.484	1.12	0.91	1.37	0.282
PCB 153[b]	1.02	0.86	1.22	0.826	1.13	0.83	1.52	0.439
PCB 180[b]	1.00	0.87	1.15	0.987	1.09	0.86	1.37	0.472
***p,p´*-DDE[b]**	1.10	0.88	1.36	0.395	1.22	0.87	1.69	0.248
β-HCH[b]	1.20	1.03	1.39	0.020*	1.27	1.01	1.61	0.044*
HCB[b]	1.20	1.01	1.42	0.041*	1.27	1.01	1.60	0.043*
Σβ-HCH + HCB[b]	1.29	1.06	1.57	0.011*	1.32	1.02	1.70	0.038*

a: >Limit of Detection vs.< Limit of Detection; b: ng/g lipid; c: Odds Ratio; d: Confidence Interval. POP (Persistent Organic Pollutants); α-HCH (α-Hexachlorocyclohexane); PCBs-138, 153 and 180 (Polychlorinated Biphenyls 138, 153 and 180); p,p'-DDE (p-p'-Dichlorodiphenyldichloroethylene); β-HCH (β-Hexachlorocyclohexane); HCB (Hexachlorobenzene). Models in both designs were adjusted for age (years), BMI (Kg/m^2), sex (male/ female), smoking (yes/no), alcohol (yes/no), place of residence (urban vs. semi-rural) and education (primary or higher vs. less than primary education). In addition, models have been further adjusted for the reason for surgery (hernias / gallbladder diseases / varicose veins / other conditions). Estimations for Cox regression models used time-to-events as the time variable.

Table S3. Associations of adipose tissue POP concentrations (ng/g lipid) with prevalence and 10-year incidence of MetS components after adjustment for selected dietary components.

	Prevalence at recruitment				10-yearIncidence			
	OR[c]	95% CI[d]		p-value	HR[e]	95% CI[d]		p-value
		Lower	Upper			Lower	Upper	
Dicofol[a]	0.75	0.40	1.41	0.367	0.75	0.34	1.69	0.492
α-HCH[a]	1.21	0.57	2.55	0.619	1.46	0.59	3.61	0.410
PCB 138[b]	1.03	0.91	1.18	0.628	1.20	0.94	1.52	0.137
PCB 153[b]	1.01	0.84	1.22	0.903	1.21	0.86	1.70	0.271
PCB 180[b]	0.99	0.85	1.15	0.874	1.13	0.88	1.47	0.337
***p,p´*-DDE[b]**	1.01	0.81	1.27	0.915	1.39	0.97	1.99	0.076†
β-HCH[b]	1.17	0.99	1.38	0.057†	1.44	1.10	1.90	0.009*
HCB[b]	1.21	1.00	1.46	0.049*	1.52	1.14	2.03	0.004*
Σβ-HCH + HCB[b]	1.27	1.03	1.56	0.027*	1.59	1.17	2.18	0.004*

a: >Limit of Detection vs.< Limit of Detection; b: ng/g lipid; c: Odds Ratio; d: Confidence Interval. POP (Persistent Organic Pollutants); α-HCH (α-Hexachlorocyclohexane); PCBs-138, 153 and 180 (Polychlorinated Biphenyls 138, 153 and 180); p,p'-DDE (p-p'-Dichlorodiphenyldichloroethylene); β-HCH (β-Hexachlorocyclohexane); HCB (Hexachlorobenzene). Models in both designs were adjusted for age (years), BMI (Kg/m^2), sex (male/ female), smoking (yes/no), alcohol (yes/no), place of residence (urban vs. semi-rural) and education (primary or higher vs. less than primary education). In addition, models have been further adjusted for selected dietary components: fish, milk, meat, dairy products, fruit, and vegetables. Estimations for Cox regression models used time-to-events as the time variable.

Figure S1. Conceptual framework of the study

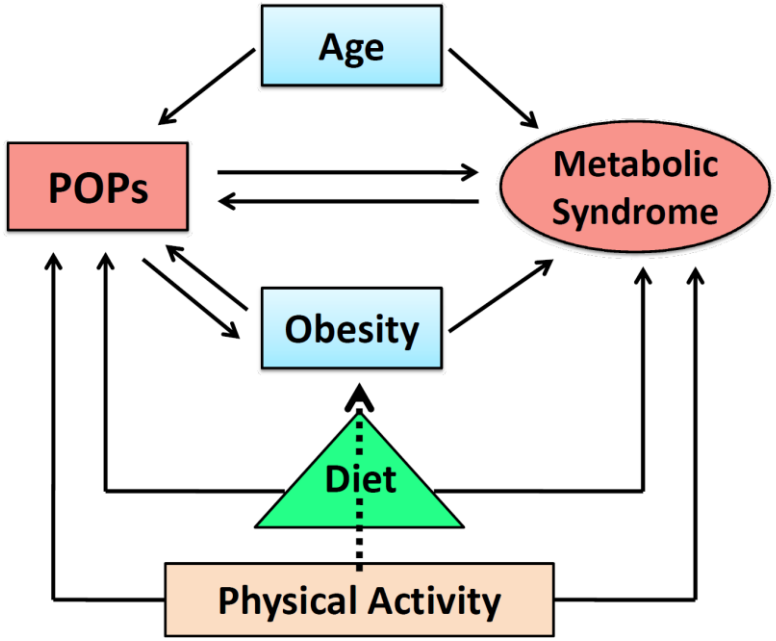
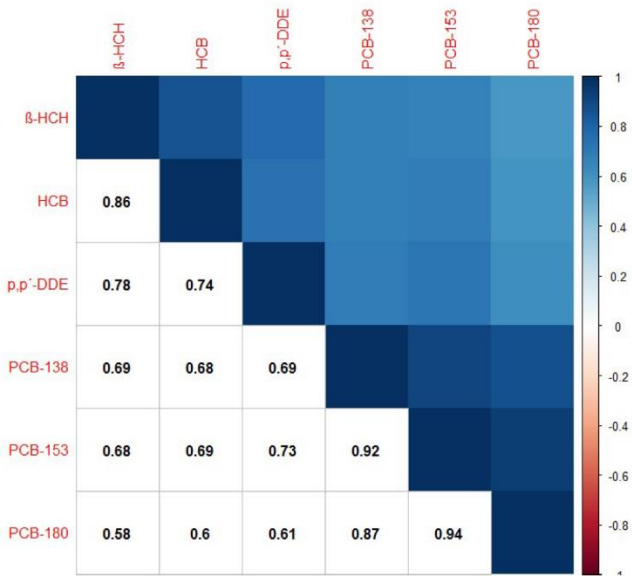


Figure S2. Spearman correlations among adipose tissue POP concentrations in the study population



DISCUSSION

One of the major challenges for environmental health and environmental epidemiology is the evaluation of possible effects of human exposure to non-persistent EDCs such as BPA, in order to facilitate risk assessment procedures and help to protect public health. Exposure during critical periods of development is especially important, from as early as the preconception period and throughout pregnancy up to infancy, puberty and adolescence. Although several birth cohorts have studied the effects of prenatal exposure to BPA, few studies have investigated the possible consequences of BPA exposure during preconception or peripuberty.

Based on previous experimental and observational data, our hypothesis was that developmental exposure to BPA could be associated with several human adverse health effects. To test this hypothesis, we conducted several studies in three different epidemiological cohorts, focusing on reproductive, neurodevelopmental, and metabolic outcomes.

Reproductive outcomes

Results from the EARTH Study showed that maternal preconception BPA concentrations—but not paternal preconception BPA concentrations—were negatively associated with both birth weight and head circumference in singletons born to subfertile couples from a large fertility center [paper #1 (Mustieles et al., 2018c)]. Maternal prenatal BPA concentrations also showed suggestive associations with birth size. No sex-specific differences were evident. There was also no evidence of associations with BPS concentrations across all exposure windows. This is the first epidemiological study on BPS exposure during the preconception period and further research is warranted, given the limited detection frequencies (53–68%) and the small sample size with available BPS data in the cohort [paper #1 (Mustieles et al., 2018c)].

While a robust inverse association between maternal preconception urinary BPA concentrations and birth size was observed, associations in relation to maternal prenatal BPA concentrations were more tenuous. In support of a maternal preconception effect,

the observed negative association with birth weight was maintained even after additional adjustment for maternal prenatal BPA concentrations, whereas the converse did not occur in the prenatal BPA exposure models. Furthermore, a dose–response trend was observed across increasing BPA tertiles for the maternal preconception but not maternal prenatal window.

BPA has been classified as an ovarian toxicant based on both experimental and human evidence (Peretz et al., 2014; Souter et al., 2013), and there is sufficient experimental evidence to support adverse effects on female reproductive physiology (Santangeli et al., 2017). BPA has been shown to affect early oogenesis and follicle formation, female steroidogenesis, oocyte quantity, quality and fertilization, uterine receptivity and implantation, embryo development and the placenta in experimental and some epidemiologic studies (Peretz et al., 2014; Susiarjo et al., 2013). Increasing evidence also highlights the potential of BPA to interfere with epigenetic mechanisms, which may mediate part of its effects on female reproduction (Santangeli et al., 2017). Given our findings of stronger associations with BPA when maternal exposure was assessed before conception and the capacity of BPA to alter the epigenetic programming of human and mammalian oocytes leading to functional impairments (Eichenlaub-Ritter and Pacchierotti, 2015), a potential early effect of BPA at the ovary (Ikezuki et al., 2002) affecting oocyte quality and later resulting in reduced embryo viability/development might be proposed (Yuan et al., 2018).

Epidemiological studies have provided inconsistent results on birth size. Some studies reported lower birth size in response to higher urinary BPA concentrations during pregnancy, in line with our maternal prenatal results (Chou et al., 2011; Huo et al., 2015; Snijder et al., 2013; Troisi et al., 2014; Veiga-Lopez et al., 2015), whereas others found no association (Casas et al., 2015; Philippat et al., 2011; Wolff et al., 2008) or even reported higher birth size (Ding et al., 2017; Lee et al., 2014). Most studies used a single spot urine sample for exposure characterization, which might partially explain discrepancies (Perrier et al., 2016; Snijder et al., 2013). Conversely, only one study has evaluated associations between paternal and maternal preconception urinary BPA

concentrations and birth outcomes and also relied on a single spot urine sample for BPA exposure characterization (Smarr et al., 2015). Although the authors observed some trends between maternal preconception quartiles of BPA concentrations and smaller size at birth, no obvious associations were found (Smarr et al., 2015). Our results are overall consistent with existing research and take them further by contributing clear evidence of the maternal preconception period as a potentially critical window for BPA effects on perinatal outcomes.

A major strength of our study was the prospective preconception design of the EARTH cohort. Investigation of subfertile couples from a large fertility center allowed us to assess three critical windows of exposure, including mother's and father's exposure before conception. Although the generalizability of our findings to non-subfertile couples is uncertain, a previous analysis in the EARTH cohort studying paternal preconception exposure to phthalates and birth size (Messerlian et al., 2017) was in line with results from a non-subfertile preconception cohort (Smarr et al., 2015). Because there was a lower number of fathers than mothers, future analyses with a higher number of male participants will further address whether paternal preconception exposure to bisphenols is associated with perinatal outcomes. Another major strength was that most participants provided multiple urine samples for each critical window of exposure, allowing us to better characterize exposure to bisphenols, and thereby reduce the chances of exposure misclassification and its expected attenuation bias (Perrier et al., 2016). Even so, some level of exposure misclassification cannot be ruled out, given the short biological half-lives of these non-persistent chemicals and episodic nature of exposures.

Additionally, results from the INMA-Granada cohort showed that higher peripubertal urinary BPA concentrations at 9–11 years of age were cross-sectionally associated with higher serum total testosterone (TT) levels, lower serum cortisol concentrations, and higher serum TT: Luteinizing Hormone (LH) and TT:cortisol ratios in 172 boys, whether the concentrations were treated as a continuous variable or categorized in tertiles [paper #2: (Mustieles et al., 2018b)]. The same associations were found, and even

strengthened, when only boys in Tanner stage I (n = 138) were separately analyzed, whereas opposite associations were observed for those in Tanner stage II (n = 34). Additionally, although a suggestive trend towards lower LH and FSH levels was also observed in the main models, which might be consistent with a negative feedback due to increased serum TT levels, statistical significance was not reached for gonadotrophins in this population of boys from Southern Spain [paper #2: (Mustieles et al., 2018b)].

Several epidemiological studies, mostly with a cross-sectional design, have also assessed associations between BPA exposure and male reproductive hormones, yielding heterogeneous results (Mínguez-Alarcón et al., 2016). Thus, a study using data from the National Health and Nutrition Examination Survey (NHANES 2011–2012) by Scinicariello and Buser (2016) found significant associations between urinary BPA concentrations and lower serum TT levels among male adolescents around 15 years of age (n = 161) but not among boys aged around 8 years of age (n = 134). Additionally, Ferguson et al. (2014) reported a non-significant relationship between BPA concentrations and lower TT levels among 8–14 year old boys (n=113) (Ferguson et al., 2014). In contrast, Galloway et al. (2010) and Lassen et al. (2014) observed a positive relationship between urinary BPA concentrations and serum TT levels in male adults (Galloway et al., 2010; Lassen et al., 2014), while others found no association with adult TT levels (Meeker et al., 2010; Mendiola et al., 2010). The apparent discrepancy between the results of the present project and the observations of Scinicariello and Buser (2016) may be explained by the completely different development stages investigated. Thus, they examined BPA exposure in relation to serum TT levels in adolescent males with “peaking” TT values (geometric mean TT = 276 ng/dL), whereas we studied peripubertal 9–11 year-old boys with median serum TT levels of 5 ng/dL, similar to the levels normally found during childhood in both boys and girls.

The present results also suggest some differences in BPA-hormone associations according to puberty status. Thus, in stratified analyses (paper #2: Supplemental Table 2), strengthened associations towards higher serum TT and lower serum cortisol levels were found in the prepubertal boys but the opposite was observed for both TT and

cortisol levels in the pubertal boys. In addition, a possible interaction cannot be ruled out for the product term BPA ∗ Tanner stage in the main statistical model for TT (p-value=0.13), given that our analysis was likely underpowered due to the modest sample size. Therefore, although our results are not sufficient to fully elucidate whether puberty status is a modifier or a confounder of the associations found, they might help to explain differences according to developmental stage in other studies. In this context, it was recently reported that urinary BPA concentrations were associated not only with earlier pubertal onset, but also with delayed progression of puberty in boys aged 9–18 years (Wang et al., 2017). These apparently counterintuitive relationships of BPA with pubertal onset and progression might be consistent with our observations of a BPA related increase in serum TT levels in prepubertal boys but reduction in adolescent males (Scinicariello and Buser, 2016). Future research is warranted to clarify and integrate these findings.

Both experimental and epidemiologic studies have shown that the effect of BPA appears to depend on the developmental stage of the exposed individual, the dose, the duration of exposure, the sex and the target tissue (Barrett, 2014; Bourguignon et al., 2016; Brouard et al., 2016; Wetherill et al., 2007). Thus, although BPA exposure has been shown to reduce testosterone levels in adolescent rodents (Peretz et al., 2014), other studies conducted in prepubertal animals have observed increased testosterone levels after BPA exposure (Ramos et al., 2003; Song et al., 2002). However, there is still no convincing explanation for this difference in effects, probably because BPA mechanisms of action are more varied and complex than initially thought (Lassen et al., 2014; Mustieles et al., 2015). Although the estrogenic properties of BPA are well-documented (Wetherill et al., 2007), Song et al. found that BPA strongly induces the gene expression of an orphan nuclear receptor (Nur77) that plays an important role in LH-mediated steroidogenesis in testicular Leydig cells (Song et al., 2002). Hence, when pre-pubertal mice were locally treated with BPA (intratesticular injection), the induction of Nur77 was followed by an increase in total testicular testosterone *in vivo* (Song et al., 2002). Interestingly, Nur77 also exerts important functions in the human adrenal gland (Kelly et al., 2004).

Our results point to the adrenal gland as an endocrine organ potentially related to the observed associations. Thus, if there is a causal association of urinary BPA concentrations with higher levels of TT and lower levels of cortisol in serum, it could be explained by a differential production of androgens/cortisol triggered by action of BPA at the adrenal gland. In this regard, BPA has been shown to increase adrenal weight in both male and female mice (Medwid et al., 2016) and to alter steroidogenesis in the adrenal gland both *in vivo* (Medwid et al., 2016) and *in vitro* (Lan et al., 2015).

The focus on the peripubertal period, an important and less-explored critical window of development in relation to BPA exposure was one of our study strengths. However, the study also presents some limitations. First, the cross-sectional design of the study prevents the inference of causal relationships, and reverse causality issues cannot be ruled out. Second, we acknowledge the limitation of using a single urine sample to estimate BPA exposure, although the non-persistent nature and short-term variability of BPA is more likely to produce an underestimation rather than an overestimation of its effects (Betts, 2014). Third, our study was limited by a relatively small sample size, which reduces the statistical power and might explain some of the non-significant associations observed. Fourth, because we did not measure additional hormones such as ACTH, among others (DHEA and DHEAS), it was not possible to evaluate whether the relationship between BPA and cortisol was driven at central or peripheral level. Free testosterone (FT) values would also be of major interest because they represent the biologically active hormone. However, because we did not have information on serum hormone binding globulin (SHBG) levels, we could not estimate FT or the free androgen index (Mendiola et al., 2010). Finally, because other non-persistent EDCs have been associated with serum hormone levels in boys (Ferguson et al., 2014; Wu et al., 2017)(Ferguson et al., 2014), the possibility of partial confounding by unmeasured co-exposures cannot be discarded.

Neurodevelopment

One of the greatest concerns about human exposure to low doses of BPA is neurodevelopmental toxicity (Chapin et al., 2008; vom Saal et al., 2007b). There is special concern about the potential effect on the fetus and neonate brain, given their particular vulnerability to neurotoxicants and generally higher exposure to BPA in comparison to adults (Calafat et al., 2008). Moreover, the almost universal exposure of humans to BPA means that small changes in behavior or cognition at the individual level may have major social repercussions (Bellinger, 2004).

In 2015, we conducted a comprehensive review of the available literature on the relationship between human BPA exposure and neurodevelopment and on proposed mechanisms of action [paper #3: (Mustieles et al., 2015)]. All scientific publications up to March 2015 were reviewed using the MEDLINE/PubMed database. Although epidemiological research on this issue was limited at that time, eight out of the twelve available articles retrieved described associations between BPA exposure and altered neurobehavior, including aggressive behavior, attention deficit, hyperactivity disorder, depression, and anxiety, mostly among children exposed *in utero*, indicating disruption of the brain during this critical window of development. Despite the reduced number of studies and their heterogeneity, the results suggested that prenatal BPA exposure may have a negative impact on neurobehavioral functioning in children and that the effects may be sex-dependent.

In 2016, results from the INMA-Granada cohort showed that BPA exposure at 9-11 years of age was positively and significantly associated with higher internalizing symptoms, especially somatic complaints, as well as thought and social problems [paper #4: (Pérez Lobato et al., 2016)]. Our results also showed increased total subclinical internalizing symptoms, which may signal greater vulnerability to the subsequent development of a mental disorder in adolescence and adulthood. Although the effects of BPA on behavior appear to be more pronounced during prenatal exposure, the relevance of postnatal exposure should not be underestimated and warrants further research. In this regard,

our study contributes data on BPA exposure during peripuberty [paper #4: (Pérez Lobato et al., 2016)], the second most critical period for the sexual differentiation of the brain and behavior after gestation (Auyeung et al., 2013).

Brain and behavior are modulated by gonadal hormones in a dimorphic manner during development, exerting an organizational effect *in utero* and permanently changing brain structures and behaviors (Wallen, 2009). Later in life, these hormones have ‘‘activational’’ effects, producing transient changes in brain and behavior throughout puberty and adolescence (Berenbaum and Beltz, 2011; Wallen, 2009). Experimental studies have consistently reported that BPA may interfere with normal brain and behavior development (Nesan et al., 2018). Exposure to low, environmentally relevant doses of BPA during critical periods of development has been found to alter sex-specific brain structures and behavioral patterns, increasing, decreasing, or eliminating sex differences in experimental animals (Bowman et al., 2014). Results of epidemiological studies also indicate that the impact of BPA exposure on neurobehavioral functioning may differ between boys and girls (Mustieles et al., 2015).

Our results are in line with previous findings. Several studies have explored the association between postnatal BPA exposure, assessed at different time points, and behavior problems in school-aged children (Braun et al., 2011; Findlay and Kohen, 2015; Harley et al., 2013; Hong et al., 2013; Perera et al., 2012; Roen et al., 2015). Harley et al. (2013) reported that total urinary BPA levels were associated with increased teacher-reported internalizing behaviors in boys at the age of 7 years ($\beta = 1.8$; 95% CI, 0.4–3.1; $p < 0.05$), specifically anxiety ($p < 0.05$) and inattention and hyperactivity ($p < 0.05$), but no significant associations were observed with mother-reported scores. In addition, a cross-sectional study of 1008 children aged between 8 and 11 years showed that total urinary BPA levels were positively associated with the CBCL total problem score, i.e., the sum of internalizing and externalizing symptoms ($\beta = 0.85$; 95% CI, 0.26– 1.44; $p = 0.001$) (Hong et al., 2013). Furthermore, results from the Canadian Health Measures Survey indicated that BPA concentration was positively associated with lower pro-social

behavior (OR = 1.24; 95% CI, 1.06–1.45) among boys aged 6 to 17 years (Findlay and Kohen, 2015).

Our study presents some limitations. First, the study sample only included boys, and it has been suggested that the association between BPA and behavior may be sex-dependent (Braun et al., 2011; Mustieles et al., 2015; Perera et al., 2012). Second, the cross-sectional design of the study prevents the inference of causal relationships, although experimental findings and the results of epidemiological birth-cohort studies indicate a possible causal role for the effects of BPA on behavior. Third, a single urine sample may not adequately reflect long-term exposure levels and represents only one time point in a continuum of brain development (Miodovnik et al., 2011; Ye et al., 2011). Strengths of this study include the representative nature of the cohort and the large number of variables collected at the follow-up from questionnaires, biological samples, and medical records, which allowed us to control for multiple potential confounders. Furthermore, the study focused on children aged between 9 and 11 years, a crucial developmental period that has received inadequate attention to date.

Finally, in 2018, we conducted an update mini-review [paper #5: (Mustieles et al., 2018a)] to assess whether epidemiological studies published since the first review [paper #3: (Mustieles et al., 2015)] confirmed the direction indicated by previous epidemiological studies. This update revealed that the number of human studies addressing the BPA-neurobehavior hypothesis had doubled since March 2015, mostly supporting previous prenatal associations and frequently showing differences between boys and girls. An increasing number of studies also reported an association between postnatal BPA exposure and diverse neurobehavioral impairments, including attention-deficit and hyperactivity disorder (ADHD). Overall, although it may never be possible to establish a causal link between this specific endocrine disruptor and a particular neurobehavioral endpoint, research data on the relationship between human BPA exposure and children's behavior reveal a relatively consistent pattern that cannot be ignored. The mounting experimental and epidemiological evidence on its neurobehavioral effects emphasizes more than ever the need to apply the precautionary

principle during development, especially in relation to pregnant women and children. It appears that the time for action t has arrived [paper #5: (Mustieles et al., 2018a)].

Obesity/Metabolism

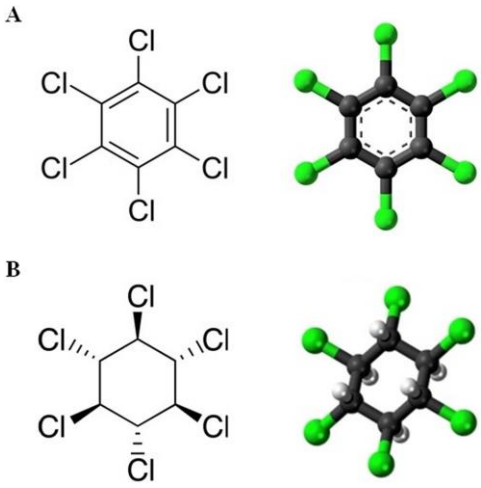
Results obtained by this thesis project in the Spanish GraMo cohort showed that besides continuous exposure to non-persistent EDCs like BPA, human populations continue to be exposed to mixtures of persistent EDCs, including those regulated or banned decades ago, because of their accumulation in fatty tissues. According to our results, accumulated levels of some of these compounds (β-HCH in combination with HCB) were consistently associated with the risk of being metabolically compromised; that is, of presenting or developing at least one out the four components of the metabolic syndrome (MetS) considered (hypertension, type 2 diabetes, low HDL-cholesterol or hypertriglyceridemia). To our best knowledge, this investigation is one of the first prospective studies aimed to analyze associations between POP exposure and MetS components and the first to combine cross-sectional with longitudinal analyses [paper #6: (Mustieles et al., 2017)].

Our findings are in line with those of Lee et al. (2007b), who studied five POP subclasses and observed that serum OCP levels, especially β-HCH levels, showed the strongest and most consistent association with the MetS (Lee et al., 2007). Park et al. (2010) found that β-HCH and heptachlor were associated with MetS in a case-control study, while Lee et al. (2014a) reported that levels of β-HCH and HCB (as well as oxychlordane and heptachlor) predicted the risk of MetS in a nested case-control study (Park et al., 2010). Moreover, Tomar et al. (2013) found higher serum levels of nine OCPs in MetS patients compared to controls, although only two compounds, β-HCH and aldrin, were significantly associated with the risk of MetS (Tomar et al., 2013).

The above epidemiological data on POP exposure and the metabolic syndrome are supported by experimental findings (La Merrill et al., 2013). Thus, when Ruzzin et al. (2010) exposed rats to an environmentally relevant mixture of POPs from fish oil

(farmed Atlantic salmon), animals developed insulin resistance, abdominal obesity, and hepatosteatosis. Moreover, these results were confirmed in differentiated adipocytes (Ruzzin et al., 2010). A similar study in exposed mice also found increased insulin resistance linked to visceral adiposity and inflammatory markers (Ibrahim et al., 2011). These effects are in agreement with mechanistic data on POPs, suggesting interplay among endocrine-related mechanisms, chronic low-grade inflammation, and mitochondrial dysfunction (La Merrill et al., 2013). Although there remains a need for experimental data on the effect of specific POPs (including β-HCH and HCB) on metabolic outcomes, a recent systems biology approach has revealed converging molecular mechanisms that link three different POP families to common metabolic diseases (Ruiz et al., 2015).

It has been suggested that the influence of isolated individual EDCs has limited meaning. This is because humans are exposed to complex mixtures of chemicals and POPs, and the biological concentrations of many PCBs and OCPs are highly correlated, as observed in the present population (Spearman coefficients ranging from 0.6 to 0.9, Supplementary material, Fig. S2). In this regard, the elastic-net regression models showed that both β-HCH and HCB were independently associated with the studied outcome. Moreover, the fact that risk estimates for Σ(HCB + β-HCH) were stronger than for the individual chemicals might point to an additive effect. This possibility is supported by the similar chemical structures of these two compounds. Thus, the equatorial position of the chlorine atoms in the molecule of β-HCH causes a more planar conformation that is highly similar to that of HCB. This structural and conformational analogy might explain their similar half-lives and bioaccumulation patterns as well as their comparable pharmacokinetics and mechanisms of action (Mrema et al., 2013).



A: Hexachlorobenzene (HCB); B: β-hexachlorocyclohexane (β-HCH). The equatorial position of the chlorine atoms in the molecule of β-HCH causes a more planar conformation, very similar to that of HCB.

The main strength of this study was its combined design, which showed consistent results between the cross-sectional and longitudinal analyses (despite differences in population characteristics), thereby reinforcing causal inference and minimizing potential reverse causality. Regarding reverse causality, some authors have noted that certain inflammatory processes associated with metabolic diseases may lead to an alteration of POP metabolism and toxicokinetics, the so-called disease progression bias (Porta, 2014). However, the fact that similar findings were observed in the follow-up group (even with lower POP levels, younger age and healthier status) minimizes the aforementioned bias. A further strength was the utilization of a complementary statistical multi-pollutant model that assesses several co-exposures simultaneously, which represents a more realistic exposure scenario. Furthermore, historical exposure to POPs was estimated by using samples of adipose tissue, which is considered the most suitable matrix for the assessment of long-term accumulated concentrations of these compounds (Kohlmeier and Kohlmeier, 1995). In this regard, adipose tissue POP concentrations have been reported to show less variability in comparison to serum concentrations, which can be influenced both by point exposures and by the mobilization of POPs stored in fatty tissues (Archibeque-Engle et al., 1997). In addition, sensitivity analyses (Table S3) showed that adjustment for dietary items did not substantially affect the associations with β-HCH and HCB. These diet-adjusted results

are timely, given a recent claim that the BMI might not be sufficient when estimating the confounding effect of diet as a source of POPs and as a predictor of metabolic disease (Tuomisto et al., 2016).

Among the limitations of our study was the relatively modest sample size, particularly in the longitudinal analyses and, therefore, we cannot completely rule out associations for the POPs without statistically significant results. However, the sample size was sufficient to yield robust associations and to reaffirm the results found in the cross-sectional analyses. Another limitation was that the final models were not adjusted for physical activity or for diet as a global pattern. However, sensitivity analyses adjusting for dietary items showed no relevant changes in the previously observed associations. Additionally, all models were adjusted for BMI, which may represent, at least partially, both an excessive caloric intake and a sedentary lifestyle. Therefore, diet does not seem to have been a relevant modifier of the associations found in our study. Finally, we cannot exclude the potential influence of other groups of unmeasured environmental pollutants that might have partially contributed to the associations observed [paper #6: (Mustieles et al., 2017)].

Preliminary findings on childhood BPA exposure and adiposity measures in the INMA-Granada cohort are displayed in the Appendix. Overall, urinary BPA concentrations were cross-sectionally and positively associated with BMI z-scores and the prevalence of overweight/obese status in peripubertal boys from Southern Spain. Additionally, BPA was positively associated with the prevalence of abdominal obesity but was not related to total body fat mass, suggesting a possible localized effect on visceral adipose tissue. Diet constitutes the main source of exposure due to the leaching of BPA from packaging materials and can liners into food and beverages (Vandenberg et al., 2010). Therefore, although our models were adjusted for total calorie intake among other relevant confounders, further in-depth research into possible specific dietary predictors of urinary BPA concentrations in order to complete the analysis.

BPA is a suspected obesogen able to increase adiposity and alter metabolism as shown in experimental models (Valentino et al., 2016; Wassenaar et al., 2017). Obesogens are thought to promote obesity in three ways: by increasing the number of adipocytes and/or the fat storage/content in existing fat cells; by altering the energy balance towards storage of calories (i.e., decreasing thermogenesis); or by disrupting signals that regulate appetite and satiety mechanisms are regulated (Janesick and Blumberg, 2016). In this line, BPA has been shown to promote adipogenesis in human stem cells *in vitro* (Boucher et al., 2014; Ohlstein et al., 2014) mainly *via* estrogenic pathways, and to affect the expression of crucial genes in abdominal adipose tissue explants from children (Menale et al., 2015; Wang et al., 2013). In fact, our own laboratory has tested the *in vitro* effects of BPA on adipocyte differentiation by differentiating human adipose-derived mesenchymal stem cells into adipocytes and using the Oil Red O assay to assess lipid accumulation. BPA was found to promote adipogenesis at 10 nM, 100 nM, 1 µM, and 10 µM (day 21 of adipogenic differentiation) with a maximal response at 10 µM (1.31 ± 0.07–fold increase), concentrations which might be relevant to humans and the environment (Figure 1, below) [Mustieles et al. unpublished results].

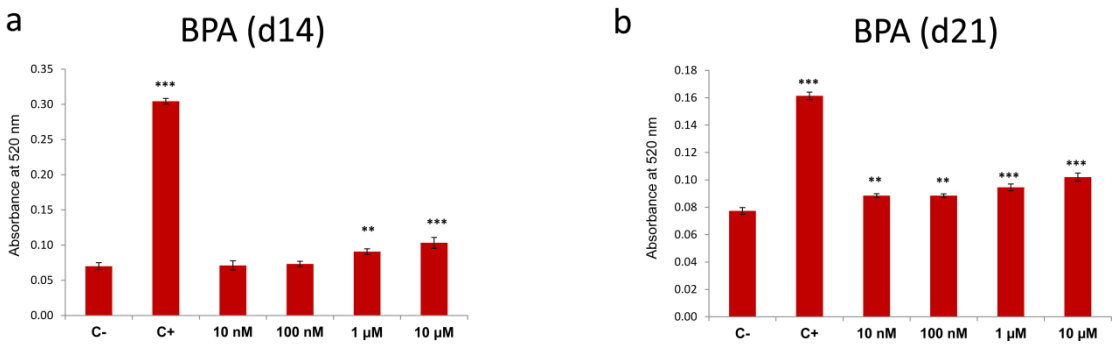


Figure 1: **Effect of bisphenol A (BPA) on intracellular lipid accumulation in human adipose-derived stem cells (hADSCs) at days 14 (a) and 21 (b) of differentiation.** Lipid accumulation was quantified by Oil Red O staining assay (absorbance at 520 nm). All values are the means ± s.e.m. of three independent experiments. Significant differences were analyzed using the Mann-Whitney U test; **P*<0.05, ***P*<0.01, and ****P*<0.001.

Action of BPA in the brain can also stimulate the appetite of mice, increasing their food intake (Mackay et al., 2013), and a recent systematic review of rodent studies concluded

that early-life exposure to BPA might increase adiposity and metabolic disease (Wassenaar et al., 2017).

Although the experimental literature is compelling, the epidemiological evidence has been less consistent and remains limited. While some reviews of the human literature support the role of BPA as an obesogen (Rancière et al., 2015)(Rancière et al., 2015), other works have been more critical and cautious in the light of inherent methodological limitations (Oppeneer and Robien, 2015). Among the most important limitations are exposure assessment issues, confounding by dietary habits and lifestyle, the critical window of development assessed, the use of only indirect measures of adiposity, and study design (Liu and Peterson, 2015; Oppeneer and Robien, 2015; Romano et al., 2014; Sharpe and Drake, 2013). Therefore, although our preliminary findings point towards an association between increasing childhood urinary BPA concentrations and increases in BMI z-scores and in the prevalence of overweight/obesity and of abdominal obesity among boys from Granada, a cautious interpretation is warranted since reverse causality and confounding by dietary and lifestyle factors is more probable than for the other health endpoints examined.

Taken as a whole, the combined results of this thesis project show that BPA exposure can affect human health as early as before conception. Notably, size at birth is highly important, because a reduced birth weight and head circumference can influence reproductive, neurodevelopmental, and metabolic health later in life. In addition, exposure to BPA during peripuberty, another sensitive and understudied period of development, was associated with altered serum hormone levels, more behavior problems and, possibly, with a higher prevalence of overweight/obesity and abdominal obesity in 9-11 year-old boys. Besides continuous exposure to mixtures of non-persistent EDCs, humans are internally exposed to mixtures of persistent and lipophilic EDCs, even when many have been banned for decades, mainly due to their accumulation in fatty tissues. Moreover, these accumulated POPs seem to play a relevant role in the etiology of MetS components and other chronic diseases. Importantly, adult men and women with accumulated POPs in their bodies and who are continuously exposed to

ubiquitous non-persistent EDCs may plan to have a baby at some point. However, since POPs have also been related to fertility issues and preconception health (Robledo et al., 2015), society faces a highly interconnected cycle of exposure to mixtures of environmental chemicals with hormonal activity that cannot only affect the exposed organism but also their offspring. This can be either a vicious or virtuous cycle (Mori and Todaka, 2017), and the difference, at least for the general population, depends mostly on dietary habits and lifestyle choices. A diet primarily based on plants, with a minimal intake of ultra-processed foods, both key dietary recommendations for the prevention of chronic diseases (Katz and Meller, 2014), can also help to reduce both the exposure to POPs (predominantly accumulated in fatty foods of animal origin) [Arrebola et al., 2018], and the exposure to those non-persistent EDCs used in cans and food packaging materials (e.g., BPA and phthalates). Even in the absence of totally conclusive evidence - which is not realistic – the precautionary principle should prevail to reduce possible risks when a sufficient amount of data is available and cannot be ignored. The Royal College of Obstetricians and Gynecologists supports this approach, declaring: “Despite uncertainty surrounding the effects of common environmental chemicals, mothers should be made aware of the sources and routes of exposure, the potential risks to the fetus/baby, and the important role that the mother can play in minimizing her baby’s chemical exposure” (Stotland et al., 2014). In the light of new data (Messerlian et al., 2017), we would add that not only maternal but also paternal exposure to EDCs could influence perinatal outcomes (Braun et al., 2017). Therefore, it appears desirable to inform couples planning or undergoing pregnancy about measures to reduce or avoid exposure to EDCs, including both BPA and POPs (Stotland et al., 2014). Strategies to prevent exposure and reduce the body burden of accumulated EDCs are urgently needed to protect public health and future generations.

CONCLUSIONS

Conclusion 1

Among subfertile couples from the EARTH Study, higher maternal urinary BPA concentrations measured before conception were strongly associated with a reduction in offspring birth weight and head circumference. No associations were found for paternal preconception exposure to BPA. Although these results are overall consistent with prior studies on prenatal BPA exposure, they might not be directly generalizable to women without fertility concerns. Nevertheless, these results highlight the need to study the unexplored maternal preconception period as a further critical window of exposure to BPA and its adverse reproductive birth outcomes.

En parejas sub-fértiles del estudio EARTH, mayores concentraciones de BPA en la orina de las madres, medidas antes de la concepción, fueron asociadas con un menor peso al nacer y perímetro craneal en sus descendientes. No se encontraron asociaciones en relación con las concentraciones de BPA de los padres. Aunque estos resultados son consistentes con estudios previos en los que se ha evaluado el efecto de la exposición prenatal a BPA, los hallazgos podrían no ser directamente generalizables a mujeres sin problemas de fertilidad. No obstante, estos resultados destacan la necesidad de estudiar el inexplorado período de la preconcepción como una ventana crítica adicional en relación a la exposición a BPA y sus efectos adversos en el nacimiento.

Conclusion 2

Among peripubertal boys from the INMA-Granada cohort, aged 9-11 years, higher BPA exposure was associated with increased total testosterone and decreased cortisol levels in serum. Although action at the testis or pituitary gland cannot be ruled out, our results are compatible with a possible involvement of BPA at the adrenal gland, resulting in a differential production of androgens/cortisol. However, our findings should be interpreted with caution, given the cross-sectional study design, the heterogeneous

results reported in the literature, and the scant experimental research on BPA effects at the adrenal gland.

En varones peri-puberales de la cohorte INMA-Granada, con edades comprendidas entre los 9 y 11 años, mayores concentraciones urinarias de BPA se asociaron con mayores niveles de testosterona y menores niveles de cortisol en suero. Estos resultados sugieren una posible acción de BPA en la glándula adrenal, que provocaría una producción alterada de andrógenos/cortisol; sin embargo, no se puede descartar una posible acción a nivel testicular o hipofisario. Estos hallazgos deben ser interpretados con precaución, dado el diseño transversal del estudio desarrollado, los resultados heterogéneos encontrados en la literatura, y la poca información experimental sobre los efectos de BPA en la glándula adrenal.

Conclusion 3

A strong body of experimental evidence supports the impact of BPA on the developing brain and neurobehavioral functioning of laboratory animals, including non-human primates, at doses of relevance for human exposure. Among 9-11 year-old boys from the INMA-Granada cohort, greater exposure to BPA was associated with more behavior problems, in line with an increasing number of human studies. Although it may never be possible to establish a causal link between this specific endocrine disruptor and a particular neurobehavioral endpoint, research data on the relationship between human BPA exposure and children's behavior has revealed a relatively consistent pattern that cannot be ignored. Taken together, the evidence supports more than ever the need to apply the precautionary principle and undertake preventive measures to reduce inadvertent exposure to BPA, especially during critical periods of development: women of child-bearing age, pregnant women and children.

Existe un importante cuerpo de evidencia científica que apoya que la exposición a BPA, a dosis similares a las que la población humana está generalmente expuesta, afecta el desarrollo cerebral y comportamiento de animales de experimentación, incluyendo a los

primates no humanos. En los niños de 9-11 años de la cohorte INMA-Granada, mayores concentraciones urinarias de BPA se asociaron con mayores problemas de comportamiento, en línea con los ya no tan escasos estudios epidemiológicos publicados. Aunque puede que nunca sea posible establecer una relación causal entre la exposición a este disruptor endocrino y un efecto adverso sobre el comportamiento, la investigación realizada en los últimos años sobre la relación entre la exposición humana a BPA y el comportamiento infantil revela un patrón relativamente consistente que no puede ser ignorado. La evidencia en su conjunto apoya más que nunca la necesidad de aplicar el principio de precaución y tomar medidas preventivas para reducir la exposición inadvertida a BPA, especialmente durante períodos críticos del desarrollo: mujeres en edad fértil, embarazadas y niños.

Conclusion 4

Among adults from the GraMo cohort, the accumulation in adipose tissue of hexachlorobenzene and β-hexachlorocyclohexane was consistently and positively associated with the prevalence and incidence of metabolic disorders. Our results highlight that, although these compounds were regulated decades ago, human exposure remains a public health concern, because current generations may suffer from the effects of accumulated exposure. Moreover, part of this body burden is transferred to subsequent generations during gestation and breastfeeding, representing a vicious cycle that needs to ended.

En población adulta de la cohorte GraMo, mayores niveles en tejido adiposo de los compuestos organoclorados hexaclorobenzeno (HCB) y β-hexaclorociclohexano (β-HCH), se asociaron de manera consistente con una mayor prevalencia e incidencia de enfermedades metabólicas. Aunque estos compuestos fueron regulados en España hace más de tres décadas, nuestros resultados indican que la población sigue expuesta y que esta exposición es relevante para la salud pública ya que las generaciones actuales pueden sufrir los efectos de su acumulación en el tejido graso. Además, parte de esta

carga corporal puede ser transferida a las generaciones subsiguientes durante la gestación y lactancia, constituyendo un círculo vicioso que es necesario evitar.

Conclusion 5

Our preliminary findings about peripubertal BPA exposure and adiposity measures in boys from the INMA-Granada cohort suggest that BPA could exert an obesogenic effect during this period of development, potentially increasing the risk of overweight and obesity. However, given the possibility of reverse causality and/or confounding by dietary habits, these preliminary results must be interpreted with due caution.

Nuestros resultados preliminares indican que la exposición a BPA en los niños de la cohorte INMA-Granada durante la peri-pubertad podría tener un efecto pro-obesogénico, al asociarse con la prevalencia de sobrepeso y obesidad infantil. No obstante, el diseño transversal del estudio planteado, que impide descartar la posibilidad de causalidad reversa, y/o la posibilidad de confusión por variables dietéticas, aconsejan precaución en la interpretación de estos resultados preliminares.

REFERENCES

Achenbach Rescorla, 2001. Manual of the ASEBA School-Age Forms and Profiles. University of Vermont, Research Center for Children, Youth and Families, Burlington, VT.

Alberti, K.G.M.M., Eckel, R.H., Grundy, S.M., Zimmet, P.Z., Cleeman, J.I., Donato, K.A., Fruchart, J.-C., James, W.P.T., Loria, C.M., Smith, S.C., International Diabetes Federation Task Force on Epidemiology and Prevention, Hational Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society, International Association for the Study of Obesity, 2009. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 120, 1640–5. https://doi.org/10.1161/CIRCULATIONAHA.109.192644

Ananth, C. V, Schisterman, E.F., 2017. Confounding, causality, and confusion: the role of intermediate variables in interpreting observational studies in obstetrics. Am. J. Obstet. Gynecol. 217, 167–175. https://doi.org/10.1016/j.ajog.2017.04.016

Anway, M.D., Skinner, M.K., 2008. Epigenetic programming of the germ line: effects of endocrine disruptors on the development of transgenerational disease. Reprod. Biomed. Online 16, 23–5.

Archibeque-Engle, S.L., Tessari, J.D., Winn, D.T., Keefe, T.J., Nett, T.M., Zheng, T., 1997. Comparison of organochlorine pesticide and polychlorinated biphenyl residues in human breast adipose tissue and serum. J. Toxicol. Environ. Health 52, 285–93. https://doi.org/10.1080/00984109708984065

Arrebola JP, Castaño A, Esteban M, Bartolomé M, Pérez-Gómez B, Ramos JJ; BIOAMBIENT.ES, 2018. Differential contribution of animal and vegetable food items on persistent organic pollutant serum concentrations in Spanish adults. Sci Total Environ. 634:235-242. https://doi: 10.1016/j.scitotenv.2018.03.283.

Arrebola, J.P., Fernandez, M.F., Porta, M., Rosell, J., de la Ossa, R.M., Olea, N., Martin-Olmedo, P., 2010. Multivariate models to predict human adipose tissue PCB concentrations in Southern Spain. Environ. Int. 36, 705–713.

https://doi.org/10.1016/j.envint.2010.05.004

Arrebola, J.P., Martin-Olmedo, P., Fernandez, M.F., Sanchez-Cantalejo, E., Jimenez-Rios, J.A., Torne, P., Porta, M., Olea, N., 2009. Predictors of concentrations of hexachlorobenzene in human adipose tissue: A multivariate analysis by gender in Southern Spain. Environ. Int. 35, 27–32. https://doi.org/10.1016/J.ENVINT.2008.05.009

Artacho-Cordón, F., León, J., Sáenz, J.M., Fernández, M.F., Martin-Olmedo, P., Olea, N., Arrebola, J.P., 2016. Contribution of Persistent Organic Pollutant Exposure to the Adipose Tissue Oxidative Microenvironment in an Adult Cohort: A Multipollutant Approach. Environ. Sci. Technol. 50, 13529–13538. https://doi.org/10.1021/acs.est.6b03783

Audran, X., Salmon, D.G., 2012. French Law Banning Bisphenol A in Food Containers Enacted BPA Ban in Food Containers by 2015.

Auyeung, B., Lombardo, M. V., Baron-Cohen, S., 2013. Prenatal and postnatal hormone effects on the human brain and cognition. Pflügers Arch. - Eur. J. Physiol. 465, 557–571. https://doi.org/10.1007/s00424-013-1268-2

Barrett, J.R., 2014. BPA and reproductive health reviewing the current state of the science. Environ. Health Perspect. 122, A223.

Becker, K., Göen, T., Seiwert, M., Conrad, A., Pick-Fuss, H., Müller, J., Wittassek, M., Schulz, C., Kolossa-Gehring, M., 2009. GerES IV: phthalate metabolites and bisphenol A in urine of German children. Int. J. Hyg. Environ. Health 212, 685–92. https://doi.org/10.1016/j.ijheh.2009.08.002

Bellinger, D.C.D.C., 2004. What is an adverse effect? A possible resolution of clinical and epidemiological perspectives on neurobehavioral toxicity. Environ. Res. 95, 394–405. https://doi.org/10.1016/j.envres.2003.07.013

Berenbaum, S.A., Beltz, A.M., 2011. Sexual differentiation of human behavior: Effects of prenatal and pubertal organizational hormones. Front. Neuroendocrinol. 32, 183–200. https://doi.org/10.1016/j.yfrne.2011.03.001

Bergman, A., Heindel, J.J., Jobling, S., Kidd, K.A., Zoeller, R.T., World Health Organization., United Nations Environment Programme., 2013. State of the science of endocrine disrupting chemicals - 2012 : an assessment of the state of

the science of endocrine disruptors prepared by a group of experts for the United Nations Environment Programme (UNEP) and WHO. United National Environment Programme.

Bergman, Å., Heindel, J.J., Kasten, T., Kidd, K.A., Jobling, S., Neira, M., Zoeller, R.T., Becher, G., Bjerregaard, P., Bornman, R., Brandt, I., Kortenkamp, A., Muir, D., Drisse, M.-N.B., Ochieng, R., Skakkebaek, N.E., Byléhn, A.S., Iguchi, T., Toppari, J., Woodruff, T.J., 2013. The Impact of Endocrine Disruption: A Consensus Statement on the State of the Science. Environ. Health Perspect. 121, a104–a106. https://doi.org/10.1289/ehp.1205448

Betts, K.S., 2014. Clues to autistic behaviors: exploring the role of endocrine disruptors. Environ. Health Perspect. 122, A137. https://doi.org/10.1289/ehp.122-A137

Boucher, J.G., Boudreau, A., Atlas, E., 2014. Bisphenol A induces differentiation of human preadipocytes in the absence of glucocorticoid and is inhibited by an estrogen-receptor antagonist. Nutr. Diabetes 4, e102. https://doi.org/10.1038/nutd.2013.43

Bourguignon, J.-P., Juul, A., Franssen, D., Fudvoye, J., Pinson, A., Parent, A.-S., 2016. Contribution of the Endocrine Perspective in the Evaluation of Endocrine Disrupting Chemical Effects: The Case Study of Pubertal Timing. Horm. Res. Paediatr. 86, 221–232. https://doi.org/10.1159/000442748

Bowman, R.E., Luine, V., Khandaker, H., Villafane, J.J., Frankfurt, M., 2014. Adolescent bisphenol-A exposure decreases dendritic spine density: Role of sex and age. Synapse 68, 498–507. https://doi.org/10.1002/syn.21758

Braun, J.M., Kalkbrenner, A.E., Calafat, A.M., Yolton, K., Ye, X., Dietrich, K.N., Lanphear, B.P., 2011. Impact of Early-Life Bisphenol A Exposure on Behavior and Executive Function in Children. Pediatrics 128, 873–882. https://doi.org/10.1542/peds.2011-1335

Braun, J.M., Messerlian, C., Hauser, R., 2017. Fathers Matter: Why It's Time to Consider the Impact of Paternal Environmental Exposures on Children's Health. Curr. Epidemiol. Reports 4, 46–55. https://doi.org/10.1007/s40471-017-0098-8

Brouard, V., Guénon, I., Bouraima-Lelong, H., Delalande, C., 2016. Differential effects of bisphenol A and estradiol on rat spermatogenesis' establishment. Reprod.

Toxicol. 63, 49–61. https://doi.org/10.1016/j.reprotox.2016.05.003

Browning, L.M., Hsieh, S.D., Ashwell, M., 2010. A systematic review of waist-to-height ratio as a screening tool for the prediction of cardiovascular disease and diabetes: 0·5 could be a suitable global boundary value. Nutr. Res. Rev. 23, 247–69. https://doi.org/10.1017/S0954422410000144

Calafat, A.M., Ye, X., Wong, L.-Y., Reidy, J.A., Needham, L.L., 2008. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003-2004. Environ. Health Perspect. 116, 39–44. https://doi.org/10.1289/ehp.10753

Canada Consumer Product Safety Act, 2017. Canada Consumer Product Safety Act.

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., INMA Project, 2011. Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. Environ. Int. 37, 858–66. https://doi.org/10.1016/j.envint.2011.02.012

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., 2015. Exposure to Bisphenol A and Phthalates during Pregnancy and Ultrasound Measures of Fetal Growth in the INMA-Sabadell Cohort. Environ. Health Perspect. 124, 521–8. https://doi.org/10.1289/ehp.1409190

Chapin, R.E., Adams, J., Boekelheide, K., Gray, L.E., Hayward, S.W., Lees, P.S.J., McIntyre, B.S., Portier, K.M., Schnorr, T.M., Selevan, S.G., Vandenbergh, J.G., Woskie, S.R., 2008. NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A. Birth Defects Res. B. Dev. Reprod. Toxicol. 83, 157–395. https://doi.org/10.1002/bdrb.20147

Chou, W.-C., Chen, J.-L., Lin, C.-F., Chen, Y.-C., Shih, F.-C., Chuang, C.-Y., 2011. Biomonitoring of bisphenol A concentrations in maternal and umbilical cord blood in regard to birth outcomes and adipokine expression: a birth cohort study in Taiwan. Environ. Health 10, 94. https://doi.org/10.1186/1476-069X-10-94

Covaci, A., Hond, E. Den, Geens, T., Govarts, E., Koppen, G., Frederiksen, H., Knudsen, L.E., Mørck, T.A., Gutleb, A.C., Guignard, C., Cocco, E., Horvat, M., Heath, E.,

Kosjek, T., Mazej, D., Tratnik, J.S., Castaño, A., Esteban, M., Cutanda, F., Ramos, J.J., Berglund, M., Larsson, K., Jönsson, B.A.G., Biot, P., Casteleyn, L., Joas, R., Joas, A., Bloemen, L., Sepai, O., Exley, K., Schoeters, G., Angerer, J., Kolossa-Gehring, M., Fiddicke, U., Aerts, D., Koch, H.M., 2015. Urinary BPA measurements in children and mothers from six European member states: Overall results and determinants of exposure. Environ. Res. 141, 77–85. https://doi.org/10.1016/j.envres.2014.08.008

de Onis, M., 2007. Development of a WHO growth reference for school-aged children and adolescents. Bull. World Health Organ. 85, 660–667. https://doi.org/10.2471/BLT.07.043497

Ding, G., Wang, C., Vinturache, A., Zhao, S., Pan, R., Han, W., Chen, L., Wang, W., Yuan, T., Gao, Y., Tian, Y., 2017. Prenatal low-level phenol exposures and birth outcomes in China. Sci. Total Environ. 607–608, 1400–1407. https://doi.org/10.1016/j.scitotenv.2017.07.084

Dodds, E.C., Lawson, W., 1938. Molecular Structure in Relation to Oestrogenic Activity. Compounds without a Phenanthrene Nucleus. Biol. Sci. 125, 222–232.

EFSA 2015, 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA J. 13, 3978. https://doi.org/10.2903/j.efsa.2015.3978

Ehrlich, S., Calafat, A.M., Humblet, O., Smith, T., Hauser, R., 2014. Handling of thermal receipts as a source of exposure to bisphenol A. JAMA 311, 859–60. https://doi.org/10.1001/jama.2013.283735

Eichenlaub-Ritter, U., Pacchierotti, F., 2015. Bisphenol A Effects on Mammalian Oogenesis and Epigenetic Integrity of Oocytes: A Case Study Exploring Risks of Endocrine Disrupting Chemicals. Biomed Res. Int. 2015, 698795. https://doi.org/10.1155/2015/698795

European Chemicals Agency (ECHA), 2017. Member State Committee Unanimously Agrees that Bisphenol A is an Endocrine Disruptor. [WWW Document]. URL https://echa.europa.eu/es/-/msc-unanimously-agrees-that-bisphenol-a-is-an-endocrine-disruptor (accessed 12.1.17).

European Chemicals Agency (ECHA), 2016. ECHA. Member State Committee Support

Document for Identification of 4,4'-Isopropylidenediphenol (Bisphenol A) as a Substance of Very High Concern Because of its Toxic For Reproduction (Article 57 C), EC 201-245-8, CAS 80-05-7.

European Commission, 2011. Restriction of use of Bisphenol A in plastic infant feeding bottles.

Ferguson, K.K., Peterson, K.E., Lee, J.M., Mercado-García, A., Blank-Goldenberg, C., Téllez-Rojo, M.M., Meeker, J.D., 2014. Prenatal and peripubertal phthalates and bisphenol A in relation to sex hormones and puberty in boys. Reprod. Toxicol. 47, 70–76. https://doi.org/10.1016/j.reprotox.2014.06.002

Fernández, M.F., Artacho-Cordón, F., Freire, C., Pérez-Lobato, R., Calvente, I., Ramos, R., Castilla, A.M., Ocón, O., Dávila, C., Arrebola, J.P., Olea, N., 2015. Trends in children's exposure to second-hand smoke in the INMA-Granada cohort: An evaluation of the Spanish anti-smoking law. Environ. Res. 138, 461–468. https://doi.org/10.1016/j.envres.2015.03.002

Fernández, M.F., Olea, N., 2014. [Endocrine disruptors. Is there sufficient evidence to act?]. Gac. Sanit. 28, 93–5. https://doi.org/10.1016/j.gaceta.2013.11.005

Fernandez, M.F., Olmos, B., Granada, A., López-Espinosa, M.J., Molina-Molina, J.-M., Fernandez, J.M., Cruz, M., Olea-Serrano, F., Olea, N., 2007. Human Exposure to Endocrine-Disrupting Chemicals and Prenatal Risk Factors for Cryptorchidism and Hypospadias: A Nested Case–Control Study. Environ. Health Perspect. 115, 8–14. https://doi.org/10.1289/ehp.9351

Findlay, L.C., Kohen, D.E., 2015. Bisphenol A and child and youth behaviour: Canadian Health Measures Survey 2007 to 2011. Heal. reports 26, 3–9.

Galloway, T., Cipelli, R., Guralnik, J., Ferrucci, L., Bandinelli, S., Corsi, A.M., Money, C., McCormack, P., Melzer, D., 2010. Daily bisphenol A excretion and associations with sex hormone concentrations: results from the InCHIANTI adult population study. Environ. Health Perspect. 118, 1603–8. https://doi.org/10.1289/ehp.1002367

Gasull, M., Bosch de Basea, M., Puigdomènech, E., Pumarega, J., Porta, M., 2011. Empirical analyses of the influence of diet on human concentrations of persistent organic pollutants: a systematic review of all studies conducted in Spain. Environ.

Int. 37, 1226–35. https://doi.org/10.1016/j.envint.2011.05.008

Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J., Zoeller, R.T., 2015. EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocr. Rev. 36, E1–E150. https://doi.org/10.1210/er.2015-1010

Guxens, M., Ballester, F., Espada, M., Fernández, M.F., Grimalt, J.O., Ibarluzea, J., Olea, N., Rebagliato, M., Tardón, A., Torrent, M., Vioque, J., Vrijheid, M., Sunyer, J., INMA Project, 2012. Cohort Profile: the INMA--INfancia y Medio Ambiente--(Environment and Childhood) Project. Int. J. Epidemiol. 41, 930–40. https://doi.org/10.1093/ije/dyr054

Harley, K.G., Gunier, R.B., Kogut, K., Johnson, C., Bradman, A., Calafat, A.M., Eskenazi, B., 2013. Prenatal and early childhood bisphenol A concentrations and behavior in school-aged children. Environ. Res. 126, 43–50. https://doi.org/10.1016/j.envres.2013.06.004

Hass, U., Christiansen, S., Boberg, J., Rasmussen, M.G., Mandrup, K., Axelstad, M., 2016. Low-dose effect of developmental bisphenol A exposure on sperm count and behaviour in rats. Andrology 4, 594–607. https://doi.org/10.1111/andr.12176

Hong, S.-B., Hong, Y.-C., Kim, J.-W., Park, E.-J., Shin, M.-S., Kim, B.-N., Yoo, H.-J., Cho, I.-H., Bhang, S.-Y., Cho, S.-C., 2013. Bisphenol A in relation to behavior and learning of school-age children. J. Child Psychol. Psychiatry 54, 890–899. https://doi.org/10.1111/jcpp.12050

Huo, W., Xia, W., Wan, Y., Zhang, B., Zhou, A., Zhang, Y., Huang, K., Zhu, Y., Wu, C., Peng, Y., Jiang, M., Hu, J., Chang, H., Xu, B., Li, Y., Xu, S., 2015. Maternal urinary bisphenol A levels and infant low birth weight: A nested case–control study of the Health Baby Cohort in China. Environ. Int. 85, 96–103. https://doi.org/10.1016/j.envint.2015.09.005

Ibrahim, M.M., Fjære, E., Lock, E.-J., Naville, D., Amlund, H., Meugnier, E., Le Magueresse Battistoni, B., Frøyland, L., Madsen, L., Jessen, N., Lund, S., Vidal, H., Ruzzin, J., 2011. Chronic consumption of farmed salmon containing persistent organic pollutants causes insulin resistance and obesity in mice. PLoS One 6, e25170. https://doi.org/10.1371/journal.pone.0025170

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, 2839–41.

Janesick, A.S., Blumberg, B., 2016. Obesogens: an emerging threat to public health. Am. J. Obstet. Gynecol. 214, 559–565. https://doi.org/10.1016/j.ajog.2016.01.182

Katz, D.L., Meller, S., 2014. Can We Say What Diet Is Best for Health? Annu. Rev. Public Health 35, 83–103. https://doi.org/10.1146/annurev-publhealth-032013-182351

Kawamura, Y., Etoh, M., Hirakawa, Y., Abe, Y., Mutsuga, M., 2014. Bisphenol A in domestic and imported canned foods in Japan. Food Addit. Contam. Part A 31, 330–340. https://doi.org/10.1080/19440049.2013.874047

Kelly, S.N., McKenna, T.J., Young, L.S., 2004. Modulation of steroidogenic enzymes by orphan nuclear transcriptional regulation may control diverse production of cortisol and androgens in the human adrenal. J. Endocrinol. 181, 355–65.

Kohlmeier, L., Kohlmeier, M., 1995. Adipose tissue as a medium for epidemiologic exposure assessment. Environ. Health Perspect. 103 Suppl 3, 99–106. https://doi.org/10.1289/ehp.95103s399

La Merrill, M., Emond, C., Kim, M.J., Antignac, J.-P., Le Bizec, B., Clément, K., Birnbaum, L.S., Barouki, R., 2013. Toxicological function of adipose tissue: focus on persistent organic pollutants. Environ. Health Perspect. 121, 162–9. https://doi.org/10.1289/ehp.1205485

LaKind, J.S., Naiman, D.Q., 2011. Daily intake of bisphenol A and potential sources of exposure: 2005–2006 National Health and Nutrition Examination Survey. J. Expo. Sci. Environ. Epidemiol. 21, 272–279. https://doi.org/10.1038/jes.2010.9

Lan, H.-C., Lin, I.-W., Yang, Z.-J., Lin, J.-H., 2015. Low-dose Bisphenol A Activates Cyp11a1 Gene Expression and Corticosterone Secretion in Adrenal Gland via the JNK Signaling Pathway. Toxicol. Sci. 148, 26–34. https://doi.org/10.1093/toxsci/kfv162

Lassen, T.H., Frederiksen, H., Jensen, T.K., Petersen, J.H., Joensen, U.N., Main, K.M., Skakkebaek, N.E., Juul, A., Jørgensen, N., Andersson, A.-M., 2014. Urinary bisphenol A levels in young men: association with reproductive hormones and semen quality. Environ. Health Perspect. 122, 478–84.

https://doi.org/10.1289/ehp.1307309

Lee, B.-E., Park, H., Hong, Y.-C., Ha, M., Kim, Y., Chang, N., Kim, B.-N., Kim, Y.J., Yu, S.-D., Ha, E.-H., 2014. Prenatal bisphenol A and birth outcomes: MOCEH (Mothers and Children's Environmental Health) study. Int. J. Hyg. Environ. Health 217, 328–334. https://doi.org/10.1016/j.ijheh.2013.07.005

Lee, D.-H., Lee, I.-K., Porta, M., Steffes, M., Jacobs, D.R., 2007. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002. Diabetologia 50, 1841–1851. https://doi.org/10.1007/s00125-007-0755-4

Liu, Y., Peterson, K.E., 2015. Maternal Exposure to Synthetic Chemicals and Obesity in the Offspring: Recent Findings. Curr. Environ. Heal. Reports 2, 339–347. https://doi.org/10.1007/s40572-015-0068-6

Mackay, H., Patterson, Z.R., Khazall, R., Patel, S., Tsirlin, D., Abizaid, A., 2013. Organizational effects of perinatal exposure to bisphenol-A and diethylstilbestrol on arcuate nucleus circuitry controlling food intake and energy expenditure in male and female CD-1 mice. Endocrinology 154, 1465–75. https://doi.org/10.1210/en.2012-2044

Medwid, S., Guan, H., Yang, K., 2016. Prenatal exposure to bisphenol A disrupts adrenal steroidogenesis in adult mouse offspring. Environ. Toxicol. Pharmacol. 43, 203–8. https://doi.org/10.1016/j.etap.2016.03.014

Meeker, J.D., Calafat, A.M., Hauser, R., 2010. Urinary Bisphenol A Concentrations in Relation to Serum Thyroid and Reproductive Hormone Levels in Men from an Infertility Clinic. Environ. Sci. Technol. 44, 1458–1463. https://doi.org/10.1021/es9028292

Menale, C., Piccolo, M.T., Cirillo, G., Calogero, R.A., Papparella, A., Mita, L., Giudice, E.M.D., Diano, N., Crispi, S., Mita, D.G., 2015. Bisphenol A effects on gene expression in adipocytes from children: association with metabolic disorders. J. Mol. Endocrinol. 54, 289–303. https://doi.org/10.1530/JME-14-0282

Mendiola, J., Jørgensen, N., Andersson, A.-M., Calafat, A.M., Ye, X., Redmon, J.B., Drobnis, E.Z., Wang, C., Sparks, A., Thurston, S.W., Liu, F., Swan, S.H., 2010. Are

environmental levels of bisphenol a associated with reproductive function in fertile men? Environ. Health Perspect. 118, 1286–91. https://doi.org/10.1289/ehp.1002037

Messerlian, C., Braun, J.M., Mínguez-Alarcón, L., Williams, P.L., Ford, J.B., Mustieles, V., Calafat, A.M., Souter, I., Toth, T., Hauser, R., Environment and Reproductive Health (EARTH) Study Team, 2017. Paternal and maternal urinary phthalate metabolite concentrations and birth weight of singletons conceived by subfertile couples. Environ. Int. 107, 55–64. https://doi.org/10.1016/j.envint.2017.06.015

Messerlian, C., Williams, P.L., Ford, J.B., Chavarro, J.E., Mínguez-Alarcón, L., Dadd, R., Braun, J.M., Gaskins, A.J., Meeker, J.D., James-Todd, T., Chiu, Y.-H., Nassan, F.L., Souter, I., Petrozza, J., Keller, M., Toth, T.L., Calafat, A.M., Hauser, R., 2018. The Environment and Reproductive Health (EARTH) Study: a prospective preconception cohort. Hum. Reprod. Open 1–11. https://doi.org/10.1093/hropen/hoy001

Michałowicz, J., 2014. Bisphenol A--sources, toxicity and biotransformation. Environ. Toxicol. Pharmacol. 37, 738–58. https://doi.org/10.1016/j.etap.2014.02.003

Mínguez-Alarcón L., Hauser R., Gaskins A.J., 2016. Effects of bisphenol A on male and couple reproductive health: a review. Fertil Steril.106(4):864-70. https://doi: 10.1016/j.fertnstert.2016.07.1118.

Miodovnik, A., Engel, S.M., Zhu, C., Ye, X., Soorya, L. V, Silva, M.J., Calafat, A.M., Wolff, M.S., 2011. Endocrine disruptors and childhood social impairment. Neurotoxicology 32, 261–7. https://doi.org/10.1016/j.neuro.2010.12.009

Mori, C., Todaka, E., 2017. For a healthier future: a virtuous cycle for reducing exposure to persistent organic pollutants. J. Epidemiol. Community Health 71, 660–662. https://doi.org/10.1136/jech-2016-208088

Mrema, E.J., Rubino, F.M., Brambilla, G., Moretto, A., Tsatsakis, A.M., Colosio, C., 2013. Persistent organochlorinated pesticides and mechanisms of their toxicity. Toxicology 307, 74–88. https://doi.org/10.1016/j.tox.2012.11.015

Mustieles, V., Fernández, M.F., Martin-Olmedo, P., González-Alzaga, B., Fontalba-Navas, A., Hauser, R., Olea, N., Arrebola, J.P., 2017. Human adipose tissue levels of persistent organic pollutants and metabolic syndrome components: Combining a

cross-sectional with a 10-year longitudinal study using a multi-pollutant approach. Environ. Int. 104. https://doi.org/10.1016/j.envint.2017.04.002

Mustieles, V., Messerlian, C., Reina, I., Rodríguez-Carrillo, A., Olea, N., Fernández F., M., 2018a. Is Bisphenol A (BPA) a Threat to Children's Behavior? J. Ment. Heal. Clin. Psychol. 2, 6–9.

Mustieles, V., Ocón-Hernandez, O., Mínguez-Alarcón, L., Dávila-Arias, C., Pérez-Lobato, R., Calvente, I., Arrebola, J.P., Vela-Soria, F., Rubio, S., Hauser, R., Olea, N., Fernández, M.F., 2018b. Bisphenol A and reproductive hormones and cortisol in peripubertal boys: The INMA-Granada cohort. Sci. Total Environ. 618, 1046–1053. https://doi.org/10.1016/j.scitotenv.2017.09.093

Mustieles, V., Pérez-Lobato, R., Olea, N., Fernández, M.F., 2015. Bisphenol A: Human exposure and neurobehavior. Neurotoxicology. https://doi.org/10.1016/j.neuro.2015.06.002

Mustieles, V., Williams, P.L., Fernandez, M.F., Mínguez-Alarcón, L., Ford, J.B., Calafat, A.M., Hauser, R., Messerlian, C., Environment and Reproductive Health (EARTH) Study Team, 2018c. Maternal and paternal preconception exposure to bisphenols and size at birth. Hum. Reprod. 33, 1528–1537. https://doi.org/10.1093/humrep/dey234

Nesan, D., Sewell, L.C., Kurrasch, D.M., 2018. Opening the black box of endocrine disruption of brain development: Lessons from the characterization of Bisphenol A. Horm. Behav. https://doi.org/10.1016/j.yhbeh.2017.12.001

Ohlstein, J.F., Strong, A.L., McLachlan, J.A., Gimble, J.M., Burow, M.E., Bunnell, B.A., 2014. Bisphenol A enhances adipogenic differentiation of human adipose stromal/stem cells. J. Mol. Endocrinol. 53, 345–53. https://doi.org/10.1530/JME-14-0052

Oppeneer, S.J., Robien, K., 2015. Bisphenol A exposure and associations with obesity among adults: a critical review. Public Health Nutr. 18, 1847–1863. https://doi.org/10.1017/S1368980014002213

Park, S.-K., Son, H.-K., Lee, S.-K., Kang, J.-H., Chang, Y.-S., Jacobs, D.R., Lee, D.-H., 2010. Relationship Between Serum Concentrations of Organochlorine Pesticides and Metabolic Syndrome Among Non-Diabetic Adults. J. Prev. Med. Public Heal. 43, 1.

https://doi.org/10.3961/jpmph.2010.43.1.1

Pearson, M.A., Lu, C., Schmotzer, B.J., Waller, L.A., Riederer, A.M., 2009. Evaluation of physiological measures for correcting variation in urinary output: Implications for assessing environmental chemical exposure in children. J. Expo. Sci. Environ. Epidemiol. 19, 336–42. https://doi.org/10.1038/jes.2008.48

Perera, F., Vishnevetsky, J., Herbstman, J.B., Calafat, A.M., Xiong, W., Rauh, V., Wang, S., 2012. Prenatal bisphenol a exposure and child behavior in an inner-city cohort. Environ. Health Perspect. 120, 1190–4. https://doi.org/10.1289/ehp.1104492

Peretz, J., Vrooman, L., Ricke, W.A., Hunt, P.A., Ehrlich, S., Hauser, R., Padmanabhan, V., Taylor, H.S., Swan, S.H., VandeVoort, C.A., Flaws, J.A., 2014. Bisphenol a and reproductive health: update of experimental and human evidence, 2007-2013. Environ. Health Perspect. 122, 775–86. https://doi.org/10.1289/ehp.1307728

Pérez Lobato, R., Mustieles, V., Calvente, I., Jiménez Díaz, I., Ramos, R., Caballero-Casero, N., López-Jiménez, F.J., Rubio, S., Olea, N., Fernandez, M.F., 2016. Exposure to bisphenol A and behavior in school-age children. Neurotoxicology 53, 12–19. https://doi.org/10.1016/j.neuro.2015.12.001

Perrier, F., Giorgis-Allemand, L., Slama, R., Philippat, C., 2016. Within-subject Pooling of Biological Samples to Reduce Exposure Misclassification in Biomarker-based Studies. Epidemiology 27, 378–88. https://doi.org/10.1097/EDE.0000000000000460

Philippat, C., Mortamais, M., Chevrier, C., Petit, C., Calafat, A.M., Ye, X., Silva, M.J., Brambilla, C., Pin, I., Charles, M.-A., Cordier, S., Slama, R., 2011. Exposure to Phthalates and Phenols during Pregnancy and Offspring Size at Birth. Environ. Health Perspect. 120, 464–470. https://doi.org/10.1289/ehp.1103634

Porta, M., 2014. A Dictionary of Epidemiology, Sixth. ed. Oxford University Press.

Ramos, J.G., Varayoud, J., Kass, L., Rodríguez, H., Costabel, L., Muñoz-de-Toro, M., Luque, E.H., 2003. Bisphenol A Induces Both Transient and Permanent Histofunctional Alterations of the Hypothalamic-Pituitary-Gonadal Axis in Prenatally Exposed Male Rats. Endocrinology 144, 3206–3215. https://doi.org/10.1210/en.2002-0198

Rancière, F., Lyons, J.G., Loh, V.H.Y., Botton, J., Galloway, T., Wang, T., Shaw, J.E.,

Magliano, D.J., 2015. Bisphenol A and the risk of cardiometabolic disorders: a systematic review with meta-analysis of the epidemiological evidence. Environ. Heal. 14, 46. https://doi.org/10.1186/s12940-015-0036-5

Reinen, J., Suter, M.J.-F., Vögeli, A.C., Fernandez, M.F., Kiviranta, H., Eggen, R.I.L., Vermeulen, N.P.E., 2010. Endocrine disrupting chemicals-Linking internal exposure to vitellogenin levels and ovotestis in Abramis brama from Dutch surface waters. Environ. Toxicol. Pharmacol. 30, 209–23. https://doi.org/10.1016/j.etap.2010.07.004

Rivas, A., Fernandez, M.F., Cerrillo, I., Ibarluzea, J., Olea-Serrano, M.F., Pedraza, V., Olea, N., 2001. Human exposure to endocrine disrupters: Standardisation of a marker of estrogenic exposure in adipose tissue. APMIS 109, S189–S202. https://doi.org/10.1111/j.1600-0463.2001.tb05767.x

Robledo, C.A., Yeung, E., Mendola, P., Sundaram, R., Maisog, J., Sweeney, A.M., Barr, D.B., Louis, G.M.B., 2015. Preconception maternal and paternal exposure to persistent organic pollutants and birth size: the LIFE study. Environ. Health Perspect. 123, 88–94. https://doi.org/10.1289/ehp.1308016

Rochester, J.R., 2013. Bisphenol A and human health: a review of the literature. Reprod. Toxicol. 42, 132–55. https://doi.org/10.1016/j.reprotox.2013.08.008

Rochester, J.R., Bolden, A.L., 2015. Bisphenol S and F: A Systematic Review and Comparison of the Hormonal Activity of Bisphenol A Substitutes. Environ. Health Perspect. 123, 643–50. https://doi.org/10.1289/ehp.1408989

Roen, E.L., Wang, Y., Calafat, A.M., Wang, S., Margolis, A., Herbstman, J., Hoepner, L.A., Rauh, V., Perera, F.P., 2015. Bisphenol A exposure and behavioral problems among inner city children at 7-9 years of age. Environ. Res. 142, 739–45. https://doi.org/10.1016/j.envres.2015.01.014

Romano, M.E., Savitz, D.A., Braun, J.M., 2014. Challenges and Future Directions to Evaluating the Association Between Prenatal Exposure to Endocrine-Disrupting Chemicals and Childhood Obesity. Curr. Epidemiol. Reports 1, 57–66. https://doi.org/10.1007/s40471-014-0007-3

Ropero, A.B., Alonso-Magdalena, P., Ripoll, C., Fuentes, E., Nadal, A., 2006. Rapid endocrine disruption: environmental estrogen actions triggered outside the

nucleus. J. Steroid Biochem. Mol. Biol. 102, 163–9. https://doi.org/10.1016/j.jsbmb.2006.09.019

Ruiz, P., Perlina, A., Mumtaz, M., Fowler, B.A., 2015. A Systems Biology Approach Reveals Converging Molecular Mechanisms that Link Different POPs to Common Metabolic Diseases. Environ. Health Perspect. 124, 1034–41. https://doi.org/10.1289/ehp.1510308

Ruzzin, J., Petersen, R., Meugnier, E., Madsen, L., Lock, E.-J., Lillefosse, H., Ma, T., Pesenti, S., Sonne, S.B., Marstrand, T.T., Malde, M.K., Du, Z.-Y., Chavey, C., Fajas, L., Lundebye, A.-K., Brand, C.L., Vidal, H., Kristiansen, K., Frøyland, L., 2010. Persistent organic pollutant exposure leads to insulin resistance syndrome. Environ. Health Perspect. 118, 465–71. https://doi.org/10.1289/ehp.0901321

Santangeli, S., Maradonna, F., Olivotto, I., Piccinetti, C.C., Gioacchini, G., Carnevali, O., 2017. Effects of BPA on female reproductive function: The involvement of epigenetic mechanism. Gen. Comp. Endocrinol. 245, 122–126. https://doi.org/10.1016/j.ygcen.2016.08.010

Schug, T.T., Janesick, A., Blumberg, B., Heindel, J.J., 2011. Endocrine disrupting chemicals and disease susceptibility. J. Steroid Biochem. Mol. Biol. 127, 204–215. https://doi.org/10.1016/j.jsbmb.2011.08.007

Schulz, K.M., Molenda-Figueira, H.A., Sisk, C.L., 2009. Back to the future: The organizational-activational hypothesis adapted to puberty and adolescence. Horm. Behav. 55, 597–604. https://doi.org/10.1016/j.yhbeh.2009.03.010

Scinicariello, F., Buser, M.C., 2016. Serum Testosterone Concentrations and Urinary Bisphenol A, Benzophenone-3, Triclosan, and Paraben Levels in Male and Female Children and Adolescents: NHANES 2011-2012. Environ. Health Perspect. 124, 1898–1904. https://doi.org/10.1289/EHP150

Sharpe, R.M., Drake, A.J., 2013. Obesogens and obesity-An alternative view? Obesity 21, 1081–1083. https://doi.org/10.1002/oby.20373

Shen, H., Main, K.M., Virtanen, H.E., Damggard, I.N., Haavisto, A.-M., Kaleva, M., Boisen, K.A., Schmidt, I.M., Chellakooty, M., Skakkebaek, N.E., Toppari, J., Schramm, K.-W., 2007. From mother to child: Investigation of prenatal and postnatal exposure to persistent bioaccumulating toxicants using breast milk and

placenta biomonitoring. Chemosphere 67, S256–S262. https://doi.org/10.1016/J.CHEMOSPHERE.2006.05.106

Silva, M.J., Samandar, E., Preau, J.L., Reidy, J.A., Needham, L.L., Calafat, A.M., 2007. Quantification of 22 phthalate metabolites in human urine. J. Chromatogr. B 860, 106–112. https://doi.org/10.1016/J.JCHROMB.2007.10.023

Smarr, M.M., Grantz, K.L., Sundaram, R., Maisog, J.M., Kannan, K., Louis, G.M.B., 2015. Parental urinary biomarkers of preconception exposure to bisphenol A and phthalates in relation to birth outcomes. Environ. Heal. 14, 73. https://doi.org/10.1186/s12940-015-0060-5

Snijder, C.A., Heederik, D., Pierik, F.H., Hofman, A., Jaddoe, V.W., Koch, H.M., Longnecker, M.P., Burdorf, A., 2013. Fetal growth and prenatal exposure to bisphenol A: the generation R study. Environ. Health Perspect. 121, 393–8. https://doi.org/10.1289/ehp.1205296

Song, K.-H., Lee, K., Choi, H.-S., 2002. Endocrine disrupter bisphenol a induces orphan nuclear receptor Nur77 gene expression and steroidogenesis in mouse testicular Leydig cells. Endocrinology 143, 2208–15. https://doi.org/10.1210/endo.143.6.8847

Souter, I., Smith, K.W., Dimitriadis, I., Ehrlich, S., Williams, P.L., Calafat, A.M., Hauser, R., 2013. The association of bisphenol-A urinary concentrations with antral follicle counts and other measures of ovarian reserve in women undergoing infertility treatments. Reprod. Toxicol. 42, 224–231. https://doi.org/10.1016/j.reprotox.2013.09.008

Stotland N.E., Sutton P., Trowbridge J., Atchley D.S., Conry J., Trasande L., Gerbert B., Charlesworth A., Woodruff T.J., 2014. Counseling patients on preventing prenatal environmental exposures--a mixed-methods study of obstetricians. PLoS One. 25;9(6):e98771. https://doi: 10.1371/journal.pone.0098771.

Susiarjo, M., Sasson, I., Mesaros, C., Bartolomei, M.S., 2013. Bisphenol a exposure disrupts genomic imprinting in the mouse. PLoS Genet. 9, e1003401. https://doi.org/10.1371/journal.pgen.1003401

TEDX, 2018. The Endocrine Disruptor Exchange. TEDX List of Potential Endocrine Disruptors. https://endocrinedisruption.org/interactive-tools/tedx-list-of-

potential-endocrine-disruptors/search-the-tedx-list#sname=&searchfor=any&sortby=chemname&action=search&searchcats=all&sortby=chemname

Tomar, L.R., Agarwal, M.P., Avasthi, R., Tyagi, V., Mustafa, M., Banerjee, B.D., 2013. Serum organochlorine pesticide levels in patients with metabolic syndrome. Indian J. Endocrinol. Metab. 17, S342-4. https://doi.org/10.4103/2230-8210.119612

Trasande, L., Attina, T.M., Blustein, J., 2012. Association Between Urinary Bisphenol A Concentration and Obesity Prevalence in Children and Adolescents. JAMA 308, 1113. https://doi.org/10.1001/2012.jama.11461

Troisi, J., Mikelson, C., Richards, S., Symes, S., Adair, D., Zullo, F., Guida, M., 2014. Placental concentrations of bisphenol A and birth weight from births in the Southeastern U.S. Placenta 35, 947–52. https://doi.org/10.1016/j.placenta.2014.08.091

Tuomisto, J., Airaksinen, R., Kiviranta, H., Tukiainen, E., Pekkanen, J., Tuomisto, J.T., 2016. A pharmacokinetic analysis and dietary information are necessary to confirm or reject the hypothesis on persistent organic pollutants causing type 2 diabetes. Toxicol. Lett. 261, 41–48. https://doi.org/10.1016/j.toxlet.2016.08.024

United Nations, 2001. Stockholm Convention on Persistent Organic Pollutants [WWW Document]. URL https://treaties.un.org/Pages/ViewDetails.aspx?src=IND&mtdsg_no=XXVII-15&chapter=27&clang=_en (accessed 8.15.18).

Valentino, R., D'Esposito, V., Ariemma, F., Cimmino, I., Beguinot, F., Formisano, P., 2016. Bisphenol A environmental exposure and the detrimental effects on human metabolic health: is it necessary to revise the risk assessment in vulnerable population? J. Endocrinol. Invest. 39, 259–263. https://doi.org/10.1007/s40618-015-0336-1

Vandenberg, L.N., Chahoud, I., Heindel, J.J., Padmanabhan, V., Paumgartten, F.J.R., Schoenfelder, G., 2010. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ. Health Perspect. 118, 1055–70. https://doi.org/10.1289/ehp.0901716

Vandenberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs, D.R., Lee, D.-H., Myers, J.P., Shioda, T., Soto, A.M., vom Saal, F.S., Welshons, W. V, Zoeller, R.T., 2013. Regulatory decisions on endocrine disrupting chemicals should be based on the principles of endocrinology. Reprod. Toxicol. 38, 1–15. https://doi.org/10.1016/j.reprotox.2013.02.002

Vandenberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs, D.R., Lee, D.-H., Shioda, T., Soto, A.M., vom Saal, F.S., Welshons, W. V, Zoeller, R.T., Myers, J.P., 2012. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr. Rev. 33, 378–455. https://doi.org/10.1210/er.2011-1050

Vandenberg, L.N., Hauser, R., Marcus, M., Olea, N., Welshons, W. V, 2007. Human exposure to bisphenol A (BPA). Reprod. Toxicol. 24, 139–77. https://doi.org/10.1016/j.reprotox.2007.07.010

Vandenberg, L.N., Prins, G.S., 2016. Clarity in the face of confusion: new studies tip the scales on bisphenol A (BPA). Andrology 4, 561–4. https://doi.org/10.1111/andr.12219

Veiga-Lopez, A., Kannan, K., Liao, C., Ye, W., Domino, S.E., Padmanabhan, V., 2015. Gender-Specific Effects on Gestational Length and Birth Weight by Early Pregnancy BPA Exposure. J. Clin. Endocrinol. Metab. 100, E1394-403. https://doi.org/10.1210/jc.2015-1724

vom Saal, F.S., Akingbemi, B.T., Belcher, S.M., Birnbaum, L.S., Crain, D.A., Eriksen, M., Farabollini, F., Guillette, L.J., Hauser, R., Heindel, J.J., Ho, S.-M., Hunt, P.A., Iguchi, T., Jobling, S., Kanno, J., Keri, R.A., Knudsen, K.E., Laufer, H., LeBlanc, G.A., Marcus, M., McLachlan, J.A., Myers, J.P., Nadal, A., Newbold, R.R., Olea, N., Prins, G.S., Richter, C.A., Rubin, B.S., Sonnenschein, C., Soto, A.M., Talsness, C.E., Vandenbergh, J.G., Vandenberg, L.N., Walser-Kuntz, D.R., Watson, C.S., Welshons, W. V, Wetherill, Y., Zoeller, R.T., 2007a. Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. Reprod. Toxicol. 24, 131–8. https://doi.org/10.1016/j.reprotox.2007.07.005

vom Saal, F.S., Akingbemi, B.T., Belcher, S.M., Birnbaum, L.S., Crain, D.A., Eriksen, M.,

Farabollini, F., Guillette, L.J., Hauser, R., Heindel, J.J., Ho, S.-M., Hunt, P.A., Iguchi, T., Jobling, S., Kanno, J., Keri, R.A., Knudsen, K.E., Laufer, H., LeBlanc, G.A., Marcus, M., McLachlan, J.A., Myers, J.P., Nadal, A., Newbold, R.R., Olea, N., Prins, G.S., Richter, C.A., Rubin, B.S., Sonnenschein, C., Soto, A.M., Talsness, C.E., Vandenbergh, J.G., Vandenberg, L.N., Walser-Kuntz, D.R., Watson, C.S., Welshons, W. V, Wetherill, Y., Zoeller, R.T., 2007b. Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. Reprod. Toxicol. 24, 131–8. https://doi.org/10.1016/j.reprotox.2007.07.005

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

Wadhwa, P.D., Buss, C., Entringer, S., Swanson, J.M., 2009. Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. Semin. Reprod. Med. 27, 358–68. https://doi.org/10.1055/s-0029-1237424

Wallen, K., 2009. The Organizational Hypothesis: Reflections on the 50th anniversary of the publication of Phoenix, Goy, Gerall, and Young (1959). Horm. Behav. 55, 561–565. https://doi.org/10.1016/j.yhbeh.2009.03.009

Wang, J., Sun, B., Hou, M., Pan, X., Li, X., 2013. The environmental obesogen bisphenol A promotes adipogenesis by increasing the amount of 11β-hydroxysteroid dehydrogenase type 1 in the adipose tissue of children. Int. J. Obes. (Lond). 37, 999–1005. https://doi.org/10.1038/ijo.2012.173

Wang, Z., Li, D., Miao, M., Liang, H., Chen, J., Zhou, Z., Wu, C., Yuan, W., 2017. Urine bisphenol A and pubertal development in boys. Int. J. Hyg. Environ. Health 220, 43–50. https://doi.org/10.1016/j.ijheh.2016.10.004

Wassenaar, P.N.H., Trasande, L., Legler, J., 2017. Systematic Review and Meta-Analysis of Early-Life Exposure to Bisphenol A and Obesity-Related Outcomes in Rodents. Environ. Health Perspect. 125, 106001. https://doi.org/10.1289/EHP1233

Wetherill, Y.B., Akingbemi, B.T., Kanno, J., McLachlan, J.A., Nadal, A., Sonnenschein, C., Watson, C.S., Zoeller, R.T., Belcher, S.M., 2007. In vitro molecular mechanisms of bisphenol A action. Reprod. Toxicol. 24, 178–198. https://doi.org/10.1016/j.reprotox.2007.05.010

Wilcox, A.J., Weinberg, C.R., Basso, O., 2011. On the Pitfalls of Adjusting for Gestational Age at Birth. Am. J. Epidemiol. 174, 1062–1068. https://doi.org/10.1093/aje/kwr230

Wolff, M.S., Engel, S.M., Berkowitz, G.S., Ye, X., Silva, M.J., Zhu, C., Wetmur, J., Calafat, A.M., 2008. Prenatal phenol and phthalate exposures and birth outcomes. Environ. Health Perspect. 116, 1092–7. https://doi.org/10.1289/ehp.11007

Wolstenholme, J.T., Rissman, E.F., Connelly, J.J., 2011. The role of Bisphenol A in shaping the brain, epigenome and behavior. Horm. Behav. 59, 296–305. https://doi.org/10.1016/j.yhbeh.2010.10.001

Wu, H., Estill, M.S., Shershebnev, A., Suvorov, A., Krawetz, S.A., Whitcomb, B.W., Dinnie, H., Rahil, T., Sites, C.K., Pilsner, J.R., 2017. Preconception urinary phthalate concentrations and sperm DNA methylation profiles among men undergoing IVF treatment: a cross-sectional study. Hum. Reprod. 32, 2159–2169. https://doi.org/10.1093/humrep/dex283

Xin, F., Susiarjo, M., Bartolomei, M.S., 2015. Multigenerational and transgenerational effects of endocrine disrupting chemicals: A role for altered epigenetic regulation? Semin. Cell Dev. Biol. 43, 66–75. https://doi.org/10.1016/j.semcdb.2015.05.008

Ye, X., Wong, L.-Y., Bishop, A.M., Calafat, A.M., 2011. Variability of Urinary Concentrations of Bisphenol A in Spot Samples, First Morning Voids, and 24-Hour Collections. Environ. Health Perspect. 119, 983–988. https://doi.org/10.1289/ehp.1002701

Yuan, M., Hu, M., Lou, Y., Wang, Q., Mao, L., Zhan, Q., Jin, F., 2018. Environmentally relevant levels of bisphenol A affect uterine decidualization and embryo implantation through the estrogen receptor/serum and glucocorticoid-regulated kinase 1/epithelial sodium ion channel α-subunit pathway in a mouse model. Fertil. Steril. 109, 735–744.e1. https://doi.org/10.1016/j.fertnstert.2017.12.003

Zoeller, R.T., 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, 4097–110. https://doi.org/10.1210/en.2012-1422

“Life is what happens to you while you are busy making other plans”

“La vida es aquello que te va sucediendo mientras te empeñas en hacer otros planes”

John Lennon

APPENDIX

APPENDIX 1. Preliminary Results

Mustieles V et al. Bisphenol A and adiposity measures among peripubertal boys from Spain.

Unsubmitted, manuscript in preparation.

Table 1. Univariate analysis between urinary natural log-transformed BPA concentrations and sociodemographic characteristics and dietary composition of peripubertal boys (N=210)

Baseline characteristics	N (%)	Urinary BPA			
			Linear regression		
		Median (µg/l)	%	95%CI	p-value
Area of residence (N=210)					
Rural	37 (17.6)	4.39	0	–	–
Semi-Rural	130 (61.9)	4.72	15	−18; 62	0.42
Urban	43 (20.5)	4.91	21	−20; 82	0.37
Maternal education (N=210)					
Primary	101 (48.1)	4.13	0	–	–
Secondary	60 (28.6)	4.89	17	−14; 57	0.32
University	49 (23.3)	5.03	27	−7; 75	0.14
Maternal IMC (N=201)	24.5 (4.57)*	–	1	−2; 4	0.41
Tanner Stage (N=203)					
Stage I	167 (82.3)	4.57	0	–	–
Stage II	36 (17.7)	5.85	13	−19; 59	0.46
Child's Physical activity (N=200)					
Low-moderate	105 (52.5)	4.80	0	–	–
High	95 (47.5)	4.72	−13	−33; 13	0.28
Child's urinary creatinine (mg/dL) (N=210)	95.8 (44.2)*	–	0.5	0.2; 0.8	**0.00**
Child's weight (kg) (N=210)	37.2 (8.38)*	–	1.4	−0.1; 2.9	**0.08**
Child's height (cm) (N=210)	139.1 (5.80)*	–	0.9	−1.3; 3.1	0.43
BMI (kg/m^2) (N=210)	18.4 (3.49)*	–	3.3	−0.3; 7	**0.07**
BMI z-scores (N=210)	0.96 (1.33)*	–	12	2; 24	**0.02**
BMI z-scores categorized (N=210)					
Underweight/eutrophic (<85th)	107 (51.0)	4.44	0	–	–
Overweight & Obese (≥85th)	103 (49.0)	4.96	38	8; 78	**0.01**
Hip circumference (N=209)	77.2 (8.06)*	–	1.4	−0.2; 3	**0.09**
Waist circumference (N=209)	67.1 (8.33)*	–	1.4	−0.2; 3	**0.08**
Waist-to-hip ratio (WHR) (N=209)	0.87 (0.05)*	–	255	−79; 3092	0.46
Waist-to-height ratio (WtHR) (N=209)	0.48 (0.05)*	–	720	−32; 7634	**0.10**
WtHR categorized (N=209)					
Normal (WthR<0.50)	134 (64.1)	4.62	0	–	–
Central obesity (WtHR≥0.50)	75 (35.9)	4.83	32	2; 72	**0.04**
Fat body mass (%) (N=203)	19.3 (6.87)*	–	1.5	−0.4; 3.4	0.13
Child's dietary composition (N=210)					
Total calorie intake (Kcal)	2305 (988)*	–	$4x10^{-5}$	$-1x10^{-5}$; $2x10^{-4}$	0.59
Total protein intake (g)	96.3 (34.6)*	–	$2x10^{-3}$	$-2x10^{-3}$; $5x10^{-3}$	0.35
Total carbohydrate intake (g)	285.5 (159.9)*	–	$2x10^{-4}$	$-4x10^{-4}$; $1x10^{-3}$	0.72
Total fats intake (g)	91.7 (35.5)*	–	$1x10^{-3}$	$-3x10^{-3}$; $5x10^{-3}$	0.58

*Mean (SD)

%: percent change in BPA urinary level

Table 2. Univariate analysis between child's BMI z-scores and sociodemographic characteristics and dietary composition of peripubertal boys (N=210)

Baseline characteristics	N (%)	Linear regression		
		%	95%CI	p-value
Area of residence (N=210)				
Rural	37 (17.6)	0	–	–
Semi-Rural	130 (61.9)	−21	−52; 29	0.34
Urban	43 (20.5)	−26	−59; 33	0.31
Maternal education (N=210)				
Primary	101 (48.1)	0	–	–
Secondary	60 (28.6)	41	−8; 116	0.11
University	49 (23.3)	10	−30; 73	0.69
Maternal IMC (N=201)	24.5 (4.57)*	5	1; 9	**0.02**
Tanner Stage (N=203)				
Stage I	167 (82.3)	0	–	–
Stage II	36 (17.7)	−14	−47; 40	0.54
Child's Physical activity (N=200)				
Low-moderate	105 (52.5)	0	–	–
High	95 (47.5)	−57	−70; −39	**0.00**
Child's urinary creatinine (mg/dL) (N=210)	95.8 (44.2)*	0.3	−0.1; 1	0.18
Total calorie intake (Kcal)	2305 (988)	$-2x10^{-4}$	$-4x10^{-4}$; $-5x10^{-5}$	**0.01**
Total protein intake (g)	96.3 (34.6)	$-4x10^{-3}$	$-9x10^{-3}$; $1x10^{-3}$	0.15
Total carbohydrate intake (g)	285.5 (159.9)	$-1x10^{-3}$	$-2x10^{-3}$; $-1x10^{-5}$	**0.05**
Total fats intake (g)	91.7 (35.5)	$-8x10^{-3}$	$-1x10^{-2}$; $-3x10^{-3}$	**0.00**

*Mean(SD)

%: percent change in BMI z-score

Table 3. Urinary bisphenol A (BPA) concentrations and anthropometric measures among peripubertal boys from the INMA-Granada cohort (n=210).

BPA	BMI z-scores			Overweight/Obesity			Waist-to-Height Ratio (WtHR)			WtHR (0.5 *vs* ≥ 0.5)			Body fat mass (%)		
	β (95% CI)	P-value	N	OR (95% CI)	P-value	N	β (95% CI)	P-value	N	OR (95% CI)	P-value	N	β (95% CI)	P-value	N
Model 1	0.24 (0.05, 0.43)	**0.02**	210	1.48 (1.09, 2.01)	**0.01**	210	0.007 (-0.001, 0.014)	0.10	209	1.40 (1.02, 1.92)	**0.04**	209	0.79 (-0.23, 1.80)	0.13	203
Model 2	0.22 (0.02, 0.42)	**0.03**	210	1.42 (1.04, 1.95)	**0.03**	210	0.007 (-0.002, 0.015)	0.11	209	1.40 (1.01, 1.94)	**0.04**	209	0.68 (-0.38, 1.73)	0.21	203
Model 3	0.22 (0.02, 0.42)	**0.03**	210	1.41 (1.03, 1.94)	**0.03**	210	0.007 (-0.002, 0.015)	0.11	209	1.40 (1.00, 1.95)	**0.05**	209	0.65 (-0.41, 1.70)	0.23	203
Model 4	0.23 (0.03, 0.42)	**0.02**	210	1.46 (1.05, 2.03)	**0.03**	210	0.007 (-0.001, 0.015)	0.09	209	1.46 (1.02, 2.08)	**0.04**	209	0.70 (-0.32, 1.73)	0.18	203
Model 5	0.23 (0.03, 0.43)	**0.02**	203	1.43 (1.02, 2.01)	**0.04**	203	0.008 (0.000, 0.016)	**0.04**	203	1.57 (1.09, 2.27)	**0.02**	203	0.73 (-0.31, 1.76)	0.17	197
Model 6	0.22 (0.02, 0.41)	**0.03**	193	1.39 (0.98, 1.97)	0.07	193	0.008 (0.000, 0.015)	**0.05**	193	1.61 (1.10, 2.36)	**0.02**	193	0.65 (-0.37, 1.66)	0.21	187
Model 7	0.15 (-0.04, 0.34)	0.12	186	1.26 (0.87, 1.83)	0.22	186	0.005 (-0.002, 0.013)	0.17	186	1.54 (1.04, 2.29)	**0.03**	186	0.33 (-0.67, 1.34)	0.51	180

Data are presented Beta estimates and 95% Confidence Intervals [β (95% CIs)] for continuous outcomes or Odds Ratios and 95% Confidence Intervals [OR (95%CIs)] for categorized outcomes.

Model 1: Natural log-transformed BPA concentrations (lnBPA).
Model 2: lnBPA + urinary creatinine levels (mg/dL).
Model 3: lnBPA + urinary creatinine levels (mg/dL) + maternal education (up to primary/secondary school/university).
Model 4: Further adjustment for total calorie intake (Kcal) + carbohydrates (g) + total fats intake (g).
Model 5: Further adjustment for Tanner stage (I *vs.* II).
Model 6: Further adjustment for maternal BMI (Kg/m^2).
Model 7: Further adjustment for physical activity (sedentary/low activity *vs.* physically active).

Supplemental Table 1. Socio-demographic and clinical characteristics of participating boys for whom dietary data were available in comparison to the rest of the sample.

Baseline Characteristics	Present subsample with dietary data in addition to BPA and anthropometric measurements (n=210)	Rest of the sample (n=60)	P-value[a]
BPA concentrations (µg/L)	4.74 (2.86, 8.96)	5.08 (2.03, 9.30)	0.61
Child's urinary creatinine (mg/dL)	95.8 (67.1, 128.2)	96.6 (75.4, 121.7)	0.99
BMI z-scores	1.02 (-0.07, 2.05)	1.09 (0.40, 2.02)	0.26
Overweight/Obesity, n (%)	103 (49)	34 (57)	0.30
Waist-to-height ratio (WtHR)	0.47 (0.44, 0.52)	0.48 (0.44, 0.52)	0.56
Central obesity (WtHR≥0.50), n (%)	75 (36)	22 (37)	0.91
Fat body mass (%)	18.2 (13.8, 24.0)	17.9 (13.8, 24.6)	0.83
Tanner Stage, n (%) (n=259)			
1	167 (82)	40 (71)	0.07
2	36 (18)	16 (29)	
Physical Activity, n (%) (n=220)			
Low-moderate	105 (52)	7 (35)	0.14
High	95 (48)	13 (65)	
Maternal IMC (Kg/m^2)	23.6 (21.6, 26.4)	23.5 (21.1, 27.9)	0.80
Maternal education, n (%) (n=270)			
Primary	101 (48)	22 (37)	0.24
Secondary	60 (29)	23 (38)	
University	49 (23)	15 (25)	

Data are presented as medians (IQRs)* in the case of continuous variables or as n (%) for categorical variables. **a** Value of hypothesis testing was calculated using the Kruskal-Wallis test for continuous variables and the Chi-square test for categorical variables.

APPENDIX 2. Curriculum Vitae

CURRÍCULUM VÍTAE NORMALIZADO

Vicente Mustieles Miralles

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GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA, INDUSTRIA Y COMPETITIVIDAD

FECYT FUNDACIÓN ESPAÑOLA PARA LA CIENCIA Y LA TECNOLOGÍA

Vicente Mustieles Miralles

Surname(s):	**Mustieles Miralles**
Name:	**Vicente**
DNI:	**77720564E**
Date of birth:	**07/06/1990**
Gender:	**Male**
Nationality:	**Spain**
Country of birth:	**Spain**
Aut. region/reg. of birth:	**Region of Murcia**
Contact province:	**Granada**
City of birth:	**Cartagena**
Contact country:	**Spain**
Contact aut. region/reg.:	**Andalusia**
Contact city:	**Granada**
Email:	**vmustieles@ugr.es**

Current professional situation

Employing entity: Univeristy of Granada **Type of entity:** University
Department: Department of Radiology and Physical Medicine
Professional category: Research Contract associated to the Human Biomonitoring for Europe project (HBM4EU)
Start date: 01/03/2017
Type of contract: Temporary employment contract **Dedication regime:** Full time

Previous positions and activities

	Employing entity	Professional category	Start date
	FIBAO - Instituto de Investigación Biosanitaria ibs. GRANADA	Contract for Research Support	05/10/2015

Employing entity: FIBAO - Instituto de Investigación Biosanitaria ibs. GRANADA **Type of entity:** University Research Institute

Professional category: Contract for Research Support
Start-End date: 05/10/2015 - 05/07/2016 **Duration:** 9 months

Education

University education

1st and 2nd cycle studies and pre-Bologna degrees

1 **University degree:** Higher degree
Name of qualification: Master's Degree in Human Nutrition
Degree awarding entity: University of Granada **Type of entity:** University
Date of qualification: 2014

2 **University degree:** Higher degree
Name of qualification: Bachelor of Pharmacy
Degree awarding entity: University of Granada **Type of entity:** University
Date of qualification: 30/09/2013

Doctorates

Doctorate programme: Clinical Medicine and Public Health Program
Degree awarding entity: Universidad de Granada **Type of entity:** University
Date of degree: 01/10/2014

Language skills

Language	Listening skills	Reading skills	Spoken interaction	Speaking skills	Writing skills
English	C1	C1	C1	C1	C1

Teaching experience

General teaching experience

1 **Name of the course:** Cáncer y Alimentación / Seminario Dieta vegetariana "Pros y Contras"
University degree: Nutrición Humana y Dietética
Start date: 23/05/2014 **End date:** 23/05/2014
End date: 23/05/2014
Entity: Universidad de Granada **Type of entity:** University

2 **Name of the course:** Seminario "Nutriéndonos con Psicología"
University degree: Licenciado en Psicología Especialidad Psicología Clínica
Start date: 2014 **End date:** 2014
End date: 2014
Entity: Colegio Mayor Santa Fé (GRANADA) **Type of entity:** Colegio Mayor

Faculty, institute or centre: Colegio Mayor Santa Fé (GRANADA)

3 Name of the course: Formación Nutricional de Voluntarios de Cruz Roja
University degree: Variadas
Start date: 2014
Entity: Cruz Roja Española Granada

Experience supervising doctoral thesis and/or final yearprojects

1 Project title: Influence of a lifestyle intervention (diet, physical activity and mindfulness) on quality of life of breast cancer patients (Final year project)
Entity: University of Granada **Type of entity:** University
Student: Miriam López
Date of reading: 24/06/2016

2 Project title: Vegetarian dietary patterns and cancer (Final year project)
Entity: University of Granada
Student: Mariana Fernández Rodríguez
Date of reading: 24/06/2016

Other activities/achievements not includedabove

1 Description of the activity: Charla Alimentación Saludable **Organising entity:** Ayuntamiento de Huétor Vega (GRANADA) **End date:** 2014

2 Description of the activity: Charla Alimentación Saludable
Organising entity: Ayuntamiento de Cájar (GRANADA) **Type of entity:** State agency
End date: 2014

3 Description of the activity: Taller de Alimentación y Memoria
Organising entity: Ayuntamiento de La Zubia (Granada)
End date: 2014

Healthcare experience

Courses and seminars given to healthcare professionals focused on improving medical care

1 Name of the course: IV Jornadas sobre Envejecimiento Activo, Dependencia y Protección de las Personas Mayores
City organizing entity: La Zubia-Granada, Andalusia, Spain
"The equation of health: the art of aging".
Start-End date: 30/10/2014 - 30/10/2014

2 Name of the course: III Jornadas sobre Envejecimiento Activo, Dependencia y Protección de las Personas Mayores- "Beneficios de la dieta vegetariana en las personas mayores"
City organizing entity: Granada, Andalusia, Spain

Entity where project took place: Ayuntamiento deLa Zubia (Granada)

Start-End date: 2013 - 2013**ype of entity:** State agency

City of entity: Granada, Andalusia, Spain

"Benefits of vegetarian diets in aging".

Scientific and technological experience

Scientific or technological activities

R&D projects funded through competitive calls of public or private entities

1 Name of the project: European Human Biomonitoring Initiative (HBM4EU).

Funding entity or bodies:

Comisión Europea. Horizonte 20-20 (H-2020). (Instituto de Salud Carlos III).

Start-End date: 01/01/2017 - 31/12/2021

Total amount: 74.059.590 €

2 Name of the project: Exposición a pesticidas no persistentes, tiempo de la pubertad y susceptibilidad genética en niños y adolescentes: Proyecto INMA-Ado-PubPest

Entity where project took place: University of Granada

Type of entity: University

City of entity: Granada, Andalusia, Spain

Name principal investigator (PI, Co-PI.....): Carmen Freire

Nº of researchers: 6

Funding entity or bodies:

Ministerio de economía, industria y competitividad

Type of entity: Administrative Body of the National Health System

City funding entity: Andalusia, Spain

Start-End date: 01/01/2018 - 01/01/2021

Total ammount: 144.667, 6€

3 Name of the project: Carga obesogénica total efectiva: nuevo biomarcador de efecto combinado a "obesógenos" como factor de riesgo en obesidad

Entity where project took place: Instituto de Investigación Biosanitaria de Granada, ibs. GRANADA

Type of entity: University Research Institute

City of entity: Granada, Spain

Name principal investigator (PI, Co-PI.....): Mariana F. Fernández Cabrera

Nº of researchers: 13

Start-End date: 01/01/2017 - 31/12/2019

Total amount: 74.415 €

4 Name of the project: Exposición del recién nacido de muy bajo peso a disruptores endocrinos (DEs) en la Unidad de Cuidados Neonatales (UCIN) y evaluación de las consecuencias sobre el desarrollo. PI16/01820.

Entity where project took place: (Instituto de Investigación Biosanitaria de Granada (ibs.GRANADA)).

Type of entity: University Research Institute

City of entity: Granada, Spain

Funding entity or bodies:
Instituto de Salud Carlos III.

Type of entity: Administrative Body of the National Health System

City funding entity: Madrid, Spain

Start-End date: 01/01/2017 - 31/12/2019
Total amount: 92.565 €

Scientific and technological activities

Scientific production

Publications, scientific and technical documents

1 Vicente Mustieles; Paige L. Williams; Mariana Fernandez; Lidia Minguez Alarcón; Jennifer B Ford; Antonia M Calafat; Russ Hauser; Carmen Messerlian. Maternal and paternal preconception exposure to bisphenols and size at birth.Human Reproduction. 33, pp. 1528 - 1537. Oxford Academic, 04/07/2018.
Type of production: Scientific paper **Format:** Journal
Corresponding author: No

2 Fernando Vela-Soria; Luz María Iribarne-Durán; Vicente Mustieles; Inmaculada Jiménez-Díaz; Mariana F. Fernández; Nicolás Olea. QuEChERS and ultra-high performance liquid chromatography-tandem mass spectrometry method for the determination of parabens and ultraviolet filters in human milk samples.Journal of Chromatography A. 1546, pp. 1 - 9. Elsevier, 06/03/2018.
Type of production: Scientific paper **Format:** Journal

3 Vicente Mustieles; Carmen Messerlian; Iris Reina; Andrea Rodríguez-Carrillo; Nicolás Olea; Mariana F. Fernández. Is Bisphenol A (BPA) a threat for children's behavior?. Journal of Mental Health and Clinical Psychology. 2 - 1, pp. 6 - 9. 05/03/2018.
Type of production: Scientific paper **Format:** Journal

4 Carmen Messerlian; Vicente Mustieles; Lidia Mínguez-Alarcón; Jennifer B. Ford; Ana María Calafat; Irene Souter; Paige L Williams; Russ Hauser. Preconception and prenatal urinary concentrations of phenols and birth size of singleton infants born to mothers and fathers from the Environment and Reproductive Health (EARTH) study.Environment International. 114, pp. 60 - 68. Elsevier, 22/02/2018.
Type of production: Scientific paper **Format:** Journal

5 Vicente Mustieles; Olga Ocón-Hernández; Lidia Mínguez-Alarcón; Cristina Dávila-Arias; Rocío Pérez-Lobato; Irene Calvente; Juan P. Arrebola; Fernando Vela-Soria; Soledad Rubio; Russ Hauser; Nicolás Olea; Mariana F. Fernández. Bisphenol A and reproductive hormones and cortisol in peripubertal boys: The INMA-Granada cohort.Science of the Total Environment. 15;618:1046-1053, Elsevier, 31/10/2017.
Type of production: Scientific paper **Format:** Journal
Corresponding author: No

6 Mariana Fernandez; José Antonio López Medina; Vicente Mustieles; Nicolas Olea. Obesógenos¿ Una nueva amenaza para la salud pública?. Revista de Salud Ambiental. 17 - 1, pp. 93 - 99. 15/10/2017.
Type of production: Scientific paper **Format:** Journal
Corresponding author: No

7 Carmen Messerlian; Vicente Mustieles; Blair J. Wiley; Jennifer B. Ford; Myra Keller; Ye Xiaoyun; Antonia M. Calafat; Paige L. Williams; Russ Hauser. Ultrasound gel as an unrecognized source of exposure to phthalates and phenols among pregnant women undergoing routine scan.International Journal of Hygiene and Environmental Health. Elsevier, 14/08/2017.

Type of production: Scientific paper **Format:** Journal
Corresponding author: No

8 Carmen Messerlian; Joseph M. Braun; Lidia Mínguez-Alarcón; Paige L. Williams; Jennifer B. Ford; Vicente Mustieles; Antonia M. Calafat; Irene Souter; Thomas Toth; Russ Hauser. Paternal and maternal urinary phthalate metabolite concentrations and birth weight of singletons conceived by subfertile couples.Environment International. 107, pp. 55 - 64. Elsevier, 27/06/2017.

Type of production: Scientific paper **Format:** Journal
Corresponding author: No

9 Vicente Mustieles; Mariana F. Fernández; Piedad Martín-Olmedo; Beatriz González-Alzaga; Andrés Fontalba-Navas; Russ Hauser; Nicolás Olea; Juan P. Arrebola. Human adipose tissue levels of persistent organic pollutants and metabolic syndrome components: Combining a cross-sectional with a 10-year longitudinal study using a multi-pollutant approach. Environment International. 104, pp. 48 - 57. Elsevier, 14/04/2017.

Type of production: Scientific paper **Format:** Journal

10 Rocío Pérez-Lobato; Vicente Mustieles; Irene Calvente; Inmaculada Jiménez-Díaz; Soledad Rubio; Nicolás Olea; Mariana F. Fernández. Exposure to bisphenol A and behavior in school-age children.Neurotoxicoloy. 53, pp. 12 - 19. 04/12/2015.

Type of production: Scientific paper **Format:** Journal

11 Vicente Mustieles; Nicolás Olea; Maria José Sánchez; Mariana F. Fernández. Controversial messages on cancer. Asian Pacific Journal of Cancer Prevention. 16 - 14, pp. 6171 - 6172. Asian Pacific Journal of Cancer Prevention, 01/09/2015.

Type of production: Scientific paper **Format:** Journal

12 Vicente Mustieles; Rocío Pérez-Lobato; Nicolás Olea; Mariana F. Fernández. Bisphenol A: Human exposure and neurobehavior. Neurotoxicology. 49, pp. 174 - 184. Neurotoxicology, 27/06/2015.

Type of production: Scientific paper **Format:** Journal

Works submitted to national or international conferences

1 **Title of the work:** Preconception urinary bisphenol A (BPA) and bisphenol S (BPS) concentrations among subfertile couples in relation to offspring birth size

Name of the conference: Gordon Research Conference on Environmental Endocrine Disruptors
Corresponding author: No
Date of event: 03/06/2018
End date: 08/06/2018
Organising entity: Les Diablerets Conference Center
City organizing entity: Les Diablerets, Switzerland
Vicente Mustieles. "Preconception urinary bisphenol A (BPA) and bisphenol S (BPS) concentrations among subfertile couples in relation to offspring birth size".

2 **Title of the work:** Effect Biomarkers in The Human Biomonitoring for Europe Project (HBM4EU)

Name of the conference: Work Package 15 Lisbon Workshop
Corresponding author: Yes
City of event: Lisbon, Lisboa, Portugal
Date of event: 09/05/2018

End date: 10/05/2018
Organising entity: INSA **Type of entity:** R&D Centre
City organizing entity: Lisbon, Lisboa, Portugal
Vicente Mustieles; Andrea Rodríguez Carrillo; Mariana Fernandez; Nicolas Olea. "Effect Biomarkers in Human Biomonitoring for Europe Project (HBM4EU)".

3 Title of the work: Infant birth weight in relation to paternal and maternal exposure to phenols among subfertile couples
Name of the conference: 9th Copenhagen Workshop on Endocrine Disrupters
Corresponding author: Yes
City of event: Copenhagen, Denmark
Date of event: 02/05/2017
End date: 05/05/2017
Organising entity: Rigshospitalet University Hospital
City organizing entity: Copenhagen, Denmark
Carmen Messerlian; Vicente Mustieles; Rémy Slama; Lidia Mínguez-Alarcón; Jennifer B. Ford; Paige Williams; Russ Hauser. "Infant birth weight in relation to paternal and maternal exposure to phenols among subfertile couples".

4 Title of the work: Paternal and maternal preconception and prenatal urinary concentrations of bisphenol A and size at birth
Name of the conference: 9th Copenhagen Workshop on Endocrine Disrupters
Corresponding author: Yes
City of event: Copenhagen, Denmark
Date of event: 02/05/2017
End date: 05/05/2017
Organising entity: Rigshospitalet University Hospital
City organizing entity: Copenhagen, Denmark
Vicente Mustieles; Carmen Messerlian; Lidia Mínguez-Alarcón; Mariana F. Fernández; Jennifer B. Ford; Paige Williams; Russ Hauser. "Paternal and maternal preconception and prenatal urinary concentrations of bisphenol A and size at birth".

5 Title of the work: Urinary bisphenol A concentrations and levels of reproductive hormones and cortisol in peripubertal boys: the INMA-Granada cohort
Name of the conference: 9th Copenhagen Workshop on Endocrine Disrupters
Corresponding author: Yes
City of event: Copenhagen, Denmark
Date of event: 02/05/2017
End date: 05/05/2017
Organising entity: Righospitalet University Hospital **Type of entity:** Healthcare Institutions
City organizing entity: Copenhagen, Denmark
Vicente Mustieles; Olga Ocón-Hernández; Lidia Mínguez-Alarcón; Cristina Dávila-Arias; Rocío Pérez-Lobato; Irene Calvente; Juan P. Arrebola; Fernando Vela-Soria; Soledad Rubio; Russ Hauser; Nicolás Olea; Mariana F. Fernández. "Urinary bisphenol A concentrations and levels of reproductive hormones and cortisol in peripubertal boys: the INMA-Granada cohort".

6 Title of the work: Bisphenol-A and other bisphenol-A congeners promotes adipogenic > differentiation of human adipose stromal/stem cells.
Name of the conference: 2nd Paris Workshop on Endocrine Disruptors Effects on > Wildlife and Human Health.
City of event: París, France
Date of event: 21/01/2016

End date: 22/01/2016

Vicente Mustieles; Francisco Ruiz-Ojeda; Jose Manuel Molina-Molina; Jose María Saenz; Juan Pedro Arrebola; Nicolás Olea; Mariana F. Fernandez.

7 **Title of the work:** Bisphenol A: Human exposure and neurobehavior

Name of the conference: XIII Congreso Español de Salud Ambiental y IX Conferencia Nacional de Disruptores Endocrinos

City of event: Cartagena, Region of Murcia, Spain

Date of event: 24/06/2015

End date: 26/06/2015

Organising entity: SESA (Sociedad Española de Sanidad Ambiental)

Mustieles V; Pérez-Lobato R; Calvente I; Molina JM; Olea N; Fernández MF.

8 **Title of the work:** Exposición a bisfenol A y comportamiento en escoalres

Name of the conference: XIII Congreso Español de Salud Ambiental y IX Conferencia Nacional de Disruptores Endocrinos

City of event: Cartagena, Region of Murcia, Spain

Date of event: 24/06/2015

End date: 26/06/2015

Organising entity: SESA (Sociedad Española de Sanidad Ambiental)

Pérez-Lobato R; Mustieles V; Ocón O; Dávila C; Olea N; Mariana F. Fernández.

9 **Title of the work:** Niveles de exposición a bisfenol A en niños de la cohorte INMA-Granada.

Name of the conference: XIII Congreso Español de Salud Ambiental y IX Conferencia Nacional de Disruptores Endocrinos

City of event: Cartagena, Region of Murcia, Spain

Date of event: 24/06/2015

End date: 26/06/2015

Organising entity: SESA (Sociedad Española de Sanidad Ambiental)

Mustieles V; Calvente I; Pérez-Lobato R; Vela-Soria F; Olea N; Fernández MF.

10 **Title of the work:** Exposure to Bisphenol A and behavior in school-age children **Name of the conference:** 8th Copenhagen workshop on Endocrine Disrupters **City of event:** Copenhage, Denmark

Date of event: 27/04/2015

End date: 30/04/2015

Rocío Pérez-Lobato; Vicente Mustieles; José Manuel Molina-Molina; Fernando Vela-Soria; Mariana F. Fernandez; Nicolás Olea.

11 **Title of the work:** Estudio piloto de las concentraciones de bisfenol-A y 4-nonilfenol en tejido adiposo humano

Name of the conference: XII Congreso Español de Salud Ambiental

City of event: GRANADA, Andalusia, Spain

Date of event: 12/06/2013

End date: 14/06/2013

Organising entity: Sociedad Española de Salud Ambiental (SESA) **Type of entity:** State agency

City organizing entity: Spain

Juan Pedro Arrebola; Laura Mercado; Esperanza Amaya; Vicente Mustieles Miralles; Francisco Artacho; Nicolás Olea. "Estudio piloto de las concentraciones de bisfenol-A y 4-nonilfenol en tejido adiposo humano".

12 **Title of the work:** Exposición a contaminantes orgánicos persistentes como factor de riesgo de patologías asociadas a la obesidad
Name of the conference: XXXIV REUNIÓN CIENTÍFICA DE LA SEE
City of event: Sevilla, Spain
Organising entity: Sociedad Española de Epidemiología (SEE)
Vicente Mustieles Miralles; Elena Salamanca Fernández; Mariana Fátima Fernández Cabrera; Nicolás Olea Serrano; Juan Pedro Arrebola.

Other achievements:

Stays in public or private R&D Centres

1 **Entity:** ISGlobal **Type of entity:** Healthcare Institutions
Faculty, institute or centre: Barcelona Institute for Global Health
City of entity:
Barcelona,
Catalonia, Spain
Start-End date:
22/02/2018 -
02/03/2018
Goals of the stay: Guest
Provable tasks: Certificate

2 **Entity:** Harvard University **Type of entity:** University
Faculty, institute or centre: Harvard School of Public Health
City of entity: Boston - Massachusetts, United States of America
Start-End date: 30/08/2016 - 30/11/2016 **Duration:** 3 months
Goals of the stay: Guest
Provable tasks: Four collaborative scientific papers published

Obtained grants and scholarships

1 **Name of the grant:** Mobility Scholarships for Doctoral Students
Aims: Pre-doctoral
Awarding entity: University of Granada **Type of entity:** University
Conferral date: 31/08/2016 **Duration:** 3 months
End date: 30/11/2016
Entity where activity was carried out: Harvard T.H. Chan School of Public Health

2 **Name of the grant:** Colaboration Scholarship
Aims: Introduction to Research
Awarding entity: Spanish Ministry of Education
Conferral date: 2012 **Duration:** 9 months
End date: 2013