
# EXPOSICIÓN HISTÓRICA A DISRUPTORES ENDOCRINOS NO PERSISTENTES Y MARCADORES DE SÍNDROME METABÓLICO, EN LA COHORTE EPIC-GRANADA.

**Programa de Doctorado de Medicina Clínica y Salud Pública**

**Tesis Doctoral**

**Elena Salamanca Fernández**

**Granada 2020**

**Editor:** Universidad de Granada. Tesis Doctorales
**Autor:** Elena Salamanca Fernández
**ISBN:** 978-84-1306-736-0
**URI:** http://hdl.handle.net/10481/65409

**Memoria que presenta para optar al grado de Doctora dentro del Programa de Doctorado de Medicina Clínica y Salud Pública de la Universidad de Granada, la Licenciada en Ciencias Ambientales**

**ELENA SALAMANCA FERNÁNDEZ**

*El ser humano es parte de la naturaleza y su guerra contra ella es, inevitablemente, una guerra contra sí mismo.*

Rachel Carson

# Agradecimientos

Cualquier trabajo de esta índole no es obra de un solo individuo, sino que lleva detrás un gran conjunto de personas que han colaborado de una u otra forma a lo largo del proceso. Es por ello que me siento profundamente agradecida a todas ellas y quiero reflejarlo lo mejor que pueda.

Quiero agradecer al Dr. Juan Pedro Arrebola, mi director de tesis, su generosidad, entusiasmo y paciencia, así como la confianza que tuvo siempre en mí y en este trabajo. Él me propuso este tema de tesis que me atrapó desde un principio y que seguía mis inquietudes: la combinación de contaminación ambiental y salud humana. Juan Pedro me ha proporcionado no solo conocimiento sino apoyo moral incondicional que me ha acompañado constantemente durante estos años. Su actitud positiva y su energía en los momentos más difíciles han hecho esta etapa más llevadera. Estas palabras no logran reflejar toda la gratitud que siento hacia él.

A la Dra. María José Sánchez, mi directora de tesis, por su exigencia y sus aportaciones, que han mejorado este trabajo. Por su admirable capacidad multi-tarea y por la enorme oportunidad de dejarme participar en el estudio EPIC, de tanto prestigio y que me ha dado la oportunidad de conocer a grandes investigadores en el mundo de la epidemiología y la salud pública. Sin ella esta tesis no hubiera sido posible.

A mi tutora, la Dra. Marieta Fernández, que inició conmigo este camino y me ha guiado no solo en la investigación, sino en todo el proceso burocrático del doctorado. Quiero agradecerle su enorme paciencia, buen talante, mano izquierda y cariño que siempre hacen todo más fácil. Por abrirme las puertas del mundo de la investigación.

Al excelente grupo del Registro de Cáncer de Granada: A Miguel, por su ayuda y enseñanzas con la estadística. A Victoria, por su apoyo incondicional, su afecto, su vitalidad, alegría y sus ganas contagiosas de trabajar. A Yoe Ling, Carmela, Maribel y Carmen, que me han cuidado como una hija tantos años. Al grupo de investigación del Registro de Cáncer: Ana, Daniel, David y Dafina, por compartir tantos proyectos y congresos juntos. A Miguel Ángel Luque, por su sabiduría y valiosas enseñanzas, académicas y de la vida. Es un verdadero placer poder trabajar con todos ellos.

A mis compañeros y amigos de la Escuela Andaluza de Salud Pública, por sus saberes, consejos y buenos ratos. Por interesarse siempre por mi tesis y darme aliento: Ainhoa, Maribel, Marita, Lupe, Antonio, Clara, Jaime, Pablo, Bea, Helena, Manuela, Eva, Angel, Leticia, y tantos otros.

Al personal de la unidad de apoyo a la investigación del Hospital Clínico San Cecilio de Granada, y el Departamento de Radiología, donde empecé todo este camino: Nicolás Olea, Rocío Pérez, Inma Jiménez, Fran Artacho, Irene Calvente… Gracias por enseñarme los primeros pasos.

A mis amig@s Elena, Victoria, Juani, Alex, Cintia… incondicionales para todo en la vida. Por interesarse siempre por este trabajo, por sus preguntas, que me hacen intentar llevar la ciencia a todos los ámbitos, y por sus ánimos infinitos.

A Manu, por soportar la etapa más difícil de este largo proceso. Por escucharme siempre, consolarme y sacarme una sonrisa. Gracias por ayudarme a combatir mis miedos y acompañarme entre tantas incertidumbres.

Y gracias, sobre todo, a mi familia. Muchas gracias a mi hermano Alberto, por hacerme reír siempre, por su complicidad y por su experiencia vital, que me abre los ojos y me enseña que no solo hay un camino. A mis padres Pura y Alberto, no hay palabras para explicar tanto apoyo, amor y atención recibidos por su parte. Por aguantar los altibajos propios de este camino y hacerme ver siempre el lado bueno. Gracias por saber transmitir esa calma y ayudarme a poner el foco en lo importante. Papá, gracias por inculcarme parte de tu enorme sabiduría científica y médica y el amor por lo bien hecho, con tanta paciencia. Mamá, gracias por darme el mejor ejemplo de mujer luchadora e incansable y por darme perspectiva, para que el frenesí del mundo de la investigación no me arrolle. Vosotros equilibráis mi vida y la llenáis de luz.

A mis abuelos, siempre.

Endelea…

# Índice

# 1. Resumen

Actualmente existen cientos de miles de sustancias químicas registradas en Europa para su uso comercial, y cada año se registran cientos de sustancias nuevas. La exposición humana a determinados contaminantes ambientales puede jugar un papel importante en la etiología de enfermedades cada vez más frecuentes en nuestra sociedad. Además, la exposición a estos compuestos se produce, tanto de forma conocida y programada, como no intencionada, accidental o simplemente inadvertida. Algunas de estas sustancias se comportan como disruptores endocrinos (DE), que se definen como una sustancia o mezcla exógena que altera las funciones del sistema endocrino y, en consecuencia, causa efectos adversos para la salud en un organismo intacto, o su progenie, o poblaciones.

Esta tesis se centra en el estudio de los contaminantes no persistentes (siglas en inglés npEPs: *non-persistent environmental pollutants*) que actúan como disruptores endocrinos. Los npEPs, son un grupo de productos químicos ampliamente utilizados, que no se bioacumulan en el organismo, sino que tienden a ser metabolizados y excretados a través de la orina en lugar de acumularse en el tejido adiposo. El grupo npEPs incluye al bisfenol A (BPA), parabenos (BP) y benzofenonas (BP). Sin embargo, la preocupación de estos contaminantes recae en que su exposición es prolongada y prácticamente constante en la rutina diaria de millones de personas en el mundo y su actuación como DE.

Por otro lado, en las últimas décadas, la prevalencia de varias enfermedades crónicas, no transmisibles, junto con sus factores de riesgo, se ha incrementado dramáticamente, convirtiéndose en un problema importante en todo el mundo. Así se ha observado un aumento de las patologías relacionadas con el síndrome metabólico: obesidad, hipertensión, diabetes tipo 2 y enfermedad cardiovascular, entre otras.

El Estudio Prospectivo Europeo sobre Nutrición y Cáncer (EPIC) es uno de los estudios de cohorte más grandes del mundo, con más de medio millón (521.000) de participantes reclutados, que han sido seguidos hasta la actualidad. El estudio EPIC se inició en 1992 e incluye 23 centros de 10 países europeos. Este estudio constituye un referente internacional y una excelente oportunidad para la investigación de la etiología de las enfermedades crónicas.

Esta tesis se basa en la hipótesis de que la exposición prolongada a dosis bajas de BPA, PB, BP, o su combinación, podría estar asociado con un incremento del riesgo de enfermedades relacionadas con el síndrome metabólico. Es por ello que el objetivo del presente trabajo es estudiar los niveles de exposición histórica a 7 productos químicos, Bisfenol A, 4 Parabenos (Metilparabeno, Etilparabeno, propilparabeno, butilparabeno) y 2 benzofenonas (Benzophenone-1 y Benzophenone-3) en la cohorte EPIC-España y EPIC-Granada y evaluar su relación con la incidencia de enfermedades relacionadas con el síndrome metabólico (Enfermedad coronaria isquémica, diabetes tipo 2 e hipertensión arterial), medido 20 años después del reclutamiento.

El estudio EPIC-España, consta de un total de 41.446 participantes de entre 29 y 69 años que se reclutaron entre 1992 y 1996 en cinco áreas geográficas de España. Dentro de esta cohorte, una de las provincias colaboradoras: EPIC-Granada reclutó 7,879 participantes aparentemente sanos, de los que el 77% eran mujeres.

Para esta tesis se seleccionó una sub-cohorte de 1.000 participantes de 4 centros EPIC-España (Guipuzkoa, Navarra, Murcia, Granada) mediante un muestreo aleatorio estratificado por sexo y edad, excluyendo a las personas con enfermedades crónicas. También se seleccionaron 946 casos de enfermedad coronaria isquémica, infarto agudo de miocardio o angina de pecho (259 de Gipuzkoa, 158 de Granada, 204 de Murcia y 325 de Navarra) para el estudio de caso-cohorte sobre enfermedad coronaria isquémica. Para los estudios sobre hipertensión arterial, diabetes tipo 2 y npEPs, se utilizó una sub-cohorte de la población de EPIC-Granada de 670 participantes.

Nuestros resultados mostraron que el 70% de la población del estudio tenía niveles detectables de BPA. Según los modelos de regresión ajustados, un aumento de 50 g / día en el consumo de grasas y aceites añadidos se asoció con niveles séricos de BPA un 43% más bajos, mientras que el azúcar y los productos de confitería se asociaron con niveles un 25% más altos de BPA sérico. Evidenciamos diferentes niveles de exposición por provincia, sexo y edad, pero no por características antropométricas o de estilo de vida. Además, no observamos asociaciones significativas entre las concentraciones séricas de BPA y el riesgo de enfermedad coronaria isquémica, infarto agudo de miocardio o angina de pecho. Aquellos individuos dentro del cuarto cuartil de propilparabeno (0.53-9.24 ng / ml) mostraron un aumento estadísticamente significativo del riesgo de padecer diabetes tipo 2 (HR = 1.668 p = 0.012), así como un aumento

del riesgo de hipertensión arterial estadísticamente significativo (HR = 1,40, p = 0,015). Las concentraciones de BP1 mostraron una tendencia inversa no significativa con el riesgo de DT2.

A pesar de las limitaciones metodológicas inherentes al diseño epidemiológico, y en vista de la plausibilidad biológica de las asociaciones encontradas, la alta prevalencia de la exposición, así como la carga epidémica de la enfermedad en la sociedad actual, consideramos que nuestros resultados son relevantes para la salud pública, aunque requieren confirmación en futuros estudios científicos de carácter multidisciplinar.

# 2. Abstract

There are currently hundreds of miles of chemicals registered in Europe for commercial use, and hundreds of new substances are registered each year. Human exposure to certain environmental pollutants can play an important role in the etiology of diseases that are increasingly common in our society. Furthermore, exposure to these compounds occurs, both in a known and programmed way, as well as unintentional, accidental or simply inadvertent. Some of these substances behave as endocrine disruptors (EDs), which are defined as an exogenous substance or mixture that disrupts the functions of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or populations.

This thesis focuses on the study of non-persistent pollutants (npEPs) that act as endocrine disruptors. NpEPs are a group of widely used chemicals that do not bioaccumulate in the body, but tend to be metabolized and excreted through urine instead of accumulating in adipose tissue. The group npEPs includes bisphenol A (BPA), parabens (BP) and benzophenones (BP). However, the concern of these pollutants lies in the fact that their exposure is prolonged and practically constant in the daily routine of millions of people in the world and their performance as EDs.

On the other hand, in recent decades, the prevalence of various chronic, non-communicable diseases, together with their risk factors, has increased dramatically, becoming a major problem worldwide. Thus, an increase in pathologies related to metabolic syndrome has been observed: obesity, hypertension, type 2 diabetes and cardiovascular disease, among others.

The European Prospective Study on Nutrition and Cancer (EPIC) is one of the largest cohort studies in the world, with more than half a million (521,000) recruited participants, who have been followed to date. The EPIC study began in 1992 and includes 23 centers from 10 European countries. This study constitutes an international benchmark and an excellent opportunity to research the etiology of chronic diseases.

This thesis is based on the hypothesis that prolonged exposure to low doses of BPA, PB, BP, or their combination, could be associated with an increased risk of diseases related to metabolic

syndrome. That is why the objective of the present work is to study the levels of historical exposure to 7 chemical products, Bisphenol A, 4 Parabens (Methylparaben, Ethylparaben, propylparaben, butylparaben) and 2 benzophenones (Benzophenone-1 and Benzophenone-3) in the cohort EPIC-Spain and EPIC-Granada and to evaluate their relationship with the incidence of diseases related to metabolic syndrome (ischemic coronary artery disease, type 2 diabetes and arterial hypertension), measured 20 years after recruitment.

The EPIC-Spain study consists of a total of 41,446 participants between 29 and 69 years old who were recruited between 1992 and 1996 in five geographical areas of Spain. Within this cohort, one of the collaborating provinces: EPIC-Granada recruited 7,879 apparently healthy participants, of which 77% were women.

For this thesis, a sub-cohort of 1,000 participants from 4 EPIC-Spain centers (Guipuzkoa, Navarra, Murcia, Granada) was selected through a random sampling stratified by sex and age, excluding people with chronic diseases. 946 cases of ischemic coronary artery disease, acute myocardial infarction or angina pectoris (259 from Gipuzkoa, 158 from Granada, 204 from Murcia, and 325 from Navarra) were also selected for the case-cohort study on ischemic coronary artery disease. For the studies on hypertension, type 2 diabetes, and npEPs, a sub-cohort of the EPIC-Granada population of 670 participants was used.

Our results showed that 70% of the study population had detectable levels of BPA. According to the adjusted regression models, a 50 g / day increase in the consumption of added fats and oils was associated with 43% lower serum levels of BPA, while sugar and confectionery products were associated with levels 25 % higher serum BPA. We evidenced different levels of exposure by province, sex, and age, but not by anthropometric or lifestyle characteristics. Furthermore, we did not observe significant associations between serum BPA concentrations and the risk of ischemic coronary artery disease, acute myocardial infarction, or angina pectoris. Those individuals within the fourth quartile of propylparaben (0.53-9.24 ng / ml) showed a statistically significant increase in the risk of suffering from type 2 diabetes (HR = 1.668 p = 0.012), as well as a statistically significant increased risk of arterial hypertension (HR = 1.40, p = 0.015). BP1 concentrations showed a non-significant inverse trend with the risk of T2D.

Despite the methodological limitations inherent to the epidemiological design, and in view of the biological plausibility of the associations found, the high prevalence of exposure, as well as

the epidemic burden of the disease in today's society, we consider that our results are relevant for public health, although they require confirmation in future multidisciplinary scientific studies.

# 3. Introducción

## 3.1. Situación actual del conocimiento

### 3.1.1 El problema de los contaminantes químicos y los plásticos

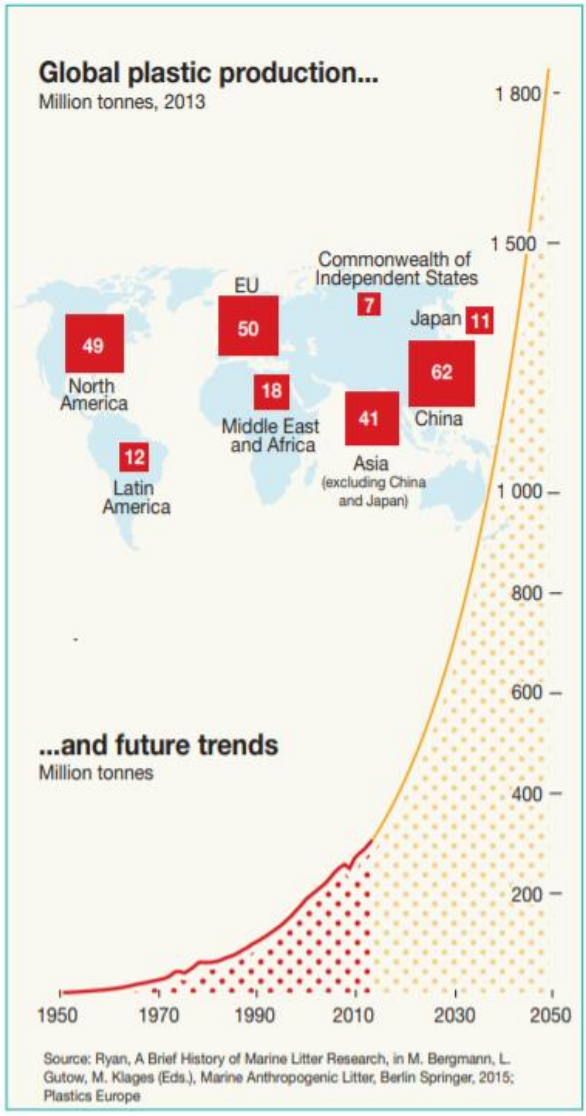
En la actualidad se estiman en más de 150.000 las sustancias químicas registradas en Europa para su uso comercial, y cada año se registran cientos de sustancias nuevas [1]. 10 Mt de sustancias químicas son liberadas cada año al aire, al agua y en vertederos [2,3], lo que conlleva una gran capacidad de diseminación en el medio ambiente.

Por otro lado, algunos de estos contaminantes son inherentes a los plásticos (por ejemplo, el Bisfenol A), lo que resulta preocupante ya que la producción mundial de plástico ha aumentado exponencialmente desde que la fabricación a gran escala comenzó en la década de 1950 (Figura 1). Esta producción mundial aumentó de 322 millones de toneladas (Mt) en 2015 a 348 Mt en 2017 [4]. Teniendo en cuenta la tasa estimada de crecimiento de la población mundial y los hábitos actuales de consumo y desperdicio, se prevé que la producción de plástico se duplique para 2025 y sea más del triple para 2050 [5].

Del total de la producción de plástico, el 36% es Polietileno (PE), el 21% es Polipropileno (PPP) el 12% es cloruro de polivinilo (PVC) y menos del 10% son tereftalato de polietileno (PET), poliuretano (PUR) y poliestireno (PS). La producción de poliamidas (PA) de poliéster y fibra acrílica es el siguiente grupo más grande, gran parte del cual es PET. Juntos, estos siete grupos representan el 92% de todos los plásticos fabricados [6].

Nuestra capacidad para hacer frente a los desechos plásticos está sobrepasada. Solo el nueve por ciento de los nueve mil millones de toneladas de plástico que el mundo ha producido ha sido reciclado [7]. El mayor problema es la eliminación de estos plásticos que se producen sin parar (muchos son de un solo uso). Los vertidos a los océanos y vertederos en países del tercer mundo se han convertido en un problema mundial tanto para los ecosistemas marinos y terrestres como de salud pública. En respuesta a las preocupaciones sobre el impacto de la contaminación plástica y microplástica [8], ha aumentado la participación pública y el compromiso político. Más de 60 países ya están restringiendo o prohibiendo los plásticos de un solo uso, principalmente las bolsas de plástico [7].

**Figura 1.** Producción global de plástico desde 1950




Fuente: Maphoto/Riccardo Pravettoni (http://www.grida.no/resources/6923).

Los plásticos persistentes, con una vida útil estimada para la degradación de cientos de años en condiciones marinas, pueden dividirse en micro y nanoplásticos en escalas de tiempo más cortas, lo que facilita su absorción por la biota marina en toda la cadena alimentaria [9]. Estos polímeros pueden contener aditivos químicos y contaminantes, incluidos algunos disruptores endocrinos conocidos que pueden ser dañinos a concentraciones extremadamente bajas para la biota marina, lo que plantea riesgos potenciales para los ecosistemas marinos, la biodiversidad y la disponibilidad de alimentos. Aunque todavía es necesario llevar a cabo una investigación científica enfocada para cubrir las lagunas de conocimiento sobre los impactos de la basura

plástica en el medio marino, la cadena alimentaria y la salud humana [10]. La evidencia científica existente y las preocupaciones ya son suficientes para apoyar las acciones de las comunidades científica, industrial, política y de la sociedad civil para frenar el flujo continuo de plásticos y los productos químicos tóxicos que contienen en el medio marino. Sin medidas preventivas fuertes e inmediatas, los impactos ambientales y los costes económicos solo empeorarán, incluso a corto plazo. Los continuos aumentos en la producción y el consumo de plástico, combinados con usos derrochadores, infraestructuras ineficientes de recolección de residuos e instalaciones de gestión de residuos insuficientes, especialmente en los países en desarrollo, significan que incluso alcanzar objetivos ya establecidos para la reducción de la basura marina sigue siendo un gran desafío.

### 3.1.2 Contaminantes ambientales y efectos en la salud humana

La aparición de estas nuevas sustancias, exógenas al organismo humano, ha originado nuevos riesgos, pudiendo suponer una nueva amenaza para la salud de las personas. Además, la exposición a estos compuestos se produce, tanto de forma conocida y programada, como no intencionada, accidental o simplemente inadvertida [11].

El aire, el agua, el suelo y por tanto los alimentos, y, en definitiva, las personas y su entorno, quedan expuestos a estas sustancias, desde el momento de su fabricación hasta los procesos de distribución, utilización y degradación final.

Según investigaciones recientes, la exposición humana a determinados contaminantes ambientales puede jugar un papel importante en la etiología de enfermedades cada vez más frecuentes en nuestra sociedad, cuya tendencia al alza no puede explicarse únicamente por la mejora en el diagnóstico o por el envejecimiento de la población. Entre estas enfermedades se encuentra la obesidad, la diabetes, las enfermedades cardiovasculares y el cáncer. Al mismo tiempo, diversos estudios en animales han confirmado el efecto que numerosas sustancias químicas antropogénicas pueden ocasionar en el desarrollo, y en los sistemas reproductivos y endocrinos [12–14].

### 3.1.3 Disruptores endocrinos

Desde los años 60 se empezó a advertir sobre la difusión a todo el planeta de algunos compuestos químicos de síntesis, utilizados fundamentalmente como plaguicidas, y del gran impacto de esa exposición sobre el equilibrio de las especies [15]. Las alteraciones sobre la salud de distintas especies animales (peces, reptiles, pájaros, mamíferos) e incluso de las personas, que han sido evidenciadas tras la exposición a cierto grupo de contaminantes ambientales, incluyen, junto a otras, enfermedades hormono-dependientes entre las que se encuentran: disfunciones tiroideas, alteraciones en el crecimiento, aumento en la incidencia de problemas relacionados con el tracto reproductor masculino, disminución de la fertilidad, pérdida en la eficacia del apareamiento, anomalías del comportamiento, alteraciones metabólicas evidentes desde el nacimiento, desmasculinización, feminización y alteraciones del sistema inmune, e incluso aumento en la incidencia de diferentes tipos de cáncer [16].

Surgió entonces la hipótesis de que algunas de estas sustancias químicas se comportan como hormonas, alterando la homeostasis normal del sistema endocrino, o lo que es lo mismo, produciendo un desequilibrio en el balance de estrógenos, andrógenos, progestágenos y hormonas tiroideas, a través de mecanismos de acción diversos [17]. Los primeros compuestos químicos exógenos o xenobióticos identificados se comportaban como estrógenos, es decir, interferían con la hormona femenina estradiol, imitando o bloqueando su acción natural.

El término disruptor endocrino (Endocrine Disrupting Chemicals EDCs) se define hoy día como el concepto adoptado por el Programa Internacional para la Seguridad Química (que involucra a la Organización Mundial de la salud y el Programa de las Naciones Unidas para el Medio Ambiente), junto con expertos japoneses, estadounidenses, canadienses, de la Organización para la Cooperación y el Desarrollo Económico (OCDE) y de la Unión Europea:

*"Un disruptor endocrino es una sustancia o mezcla exógena que altera las funciones del sistema endocrino y, en consecuencia, causa efectos adversos para la salud en un organismo intacto, o su progenie, o (sub) poblaciones".*

Por lo tanto, los EDCs son un grupo sustancias químicas de muy diferente origen, estructura y uso. Se trata de sustancias exógenas al organismo, naturales o sintéticas, que interfieren con la producción, liberación, transporte, metabolismo, unión, acción biológica o eliminación de las

hormonas responsables del mantenimiento de la homeostasis y regulación del desarrollo [18,19]. Muchos de ellos presentan gran estabilidad e inercia para reaccionar químicamente, por lo que reúnen las características óptimas para haber sido, y ser, empleados en grandes cantidades y con gran libertad, sin especial protección medioambiental.

Algunos de los compuestos disruptores endocrinos son bien conocidos por su capacidad para acumularse y persistir en las cadenas tróficas, como es el caso de los contaminantes orgánicos persistentes, sobre los que sí se han establecido medidas de control adecuadas. Sin embargo, otros parecen no bioacumularse, es decir, son no persistentes en el organismo, pero su presencia como contaminantes en el entorno (agua, aire, alimentos, utensilios) es tan frecuente que la exposición diaria es inevitable.

Esta tesis se centra en el estudio de los contaminantes no persistentes que actúan como disruptores endocrinos. Estos EDCs alcanzan el organismo humano fundamentalmente a través de una exposición ambiental «de fondo», y posiblemente en dosis bajas en la mayor parte de los casos [20]. La principal vía de entrada de los EDCs no persistentes es la dieta, ya que multitud de embalajes alimentarios se encuentran recubiertos de ciertos plásticos y de las sustancias químicas que los forman. Además, existen otras fuentes que incluyen la cosmética, la farmacología, los plásticos y las resinas sintéticas. Esta diversidad de fuentes, junto a la gran variedad de compuestos químicos, dificulta extraordinariamente la aplicación de medidas de prevención.

Es por ello que la comunidad científica defiende la aplicación del *Principio de precaución*, basado en la idea de que las políticas y las decisiones en materia de medio ambiente y salud humana, como medida preventiva, deben estar orientadas a evitar o reducir tanto como sea razonablemente alcanzable la exposición a agentes potencialmente nocivos para la salud, mientras se llevan a cabo estudios que demuestren la seguridad en el uso del compuesto de que se trate. Se trata de prevenir la exposición antes de necesitar paliar el daño producido en salud. Para poder hacer una prevención primaria de la exposición es necesario comprender los mecanismos de acción y efectos biológicos de los compuestos químicos, establecer curvas dosis y determinar la dosis de referencia (concentración desprovista de riesgo) para compuestos particulares. Como complemento imprescindible son necesarios los estudios epidemiológicos que establezcan relaciones entre la exposición y efectos en la salud de una población, sumando así a la evidencia de los efectos tóxicos de determinadas sustancias químicas.

Basado en este principio, se han realizado restricciones de BPA en varios países como en Canadá en 2010 [21,22] donde los recién nacidos y los bebés están protegidos contra la exposición al BPA según la Ley de seguridad de productos de consumo de Canadá [23]. Esta ley prohíbe la fabricación, importación, publicidad o venta de biberones de policarbonato que contienen BPA. Además, se realiza monitorización de la exposición de los canadienses al BPA.

Posteriormente a finales de diciembre de 2012, Francia aprobó oficialmente una ley por la que se suspendía la producción, el comercio y la comercialización de envases de alimentos que contienen bisfenol A [24]. Dichos envases quedaron prohibidos desde enero de 2013 para productos alimenticios destinados a bebés y desde enero de 2015 para todos los demás productos. Francia lo notificó a la Unión Europea y a la Organización Mundial del Comercio. Mientras tanto, los recipientes de comida que contengan BPA llevan una etiqueta de advertencia para mujeres embarazadas y bebés.

Por su parte, la Unión Europea (UE) puso en marcha el programa REACH [25,26] que es el Reglamento de registro, evaluación, autorización y restricción de sustancias químicas. A través de este programa, las empresas fabricantes y proveedoras deben proporcionar información sobre los riesgos que presentan las sustancias y cómo deben manipularse en toda la cadena de suministro. Además, REACH también exige a las empresas o particulares que utilizan una sustancia química sola o en una mezcla, en el curso de sus actividades industriales o profesionales, que transmitan información a las empresas fabricantes y proveedoras de productos químicos o a la Agencia Europea de Sustancias y Preparados Químicos. Así pues, el BPA está permitido para su uso en materiales en contacto con alimentos en la Unión Europea según el Reglamento 10/2011 / UE [27], relacionado con materiales y artículos plásticos que tengan la intención de entrar en contacto con productos alimenticios. En enero de 2011, la Comisión Europea prohibió el uso de BPA en la fabricación de biberones de policarbonato para bebés [28]. En febrero de 2018, la UE introdujo límites más estrictos de BPA en materiales en contacto con alimentos, derivados de la ingesta diaria tolerable temporal establecida por la EFSA en 2015[29].

Sin embargo, a excepción de los alimentos para bebés, la industria y la legislación a nivel mundial está lejos de estar lista, ya que el BPA también está permitido para uso en contacto con

alimentos en otros países, como Estados Unidos y Japón y esto afecta a las importaciones en Europa de alimentos enlatados de estos países.

En cuanto a la legislación de otros disruptores endocrinos, el control se limita a unas pocas leyes nacionales en la mayoría de los países desarrollados debido al hecho de que los efectos adversos clínicos del grupo de contaminantes no persistentes han sido menos estudiados. Por ejemplo, el 21 de marzo de 2011, Dinamarca notificó a la Comisión Europea la prohibición del propilparabeno, butilparabeno, sus isoformas y sales en cosméticos para niños como medida de precaución [30,31]. En 2014, la UE bajó la concentración máxima en el producto preparado para el uso de: butylparaben, propylparaben, entre otros [31].

### 3.1.4 Exposición humana: Biomonitorización

Como se ha mencionado, en los compuestos químicos no persistentes, resulta especialmente importante la exposición diaria a componentes de productos cosméticos, que pueden acceder al organismo por vía dérmica, así como los que forman parte del procesamiento o están contenidos en envases de uso alimentario, que pueden liberarse al producto de consumo y llegar al organismo a través de la dieta, o componentes de los productos textiles que pueden ser liberados al no estar estructuralmente incorporados.

Esta exposición constante unida a los riesgos para la salud anteriormente mencionados, así como el principio de precaución hace necesario estimar la exposición humana a los contaminantes no persistentes. Para ello se realiza la biomonitorización, que mide la concentración de los compuestos (tanto persistentes como no persistentes) en diferentes matrices biológicas en un momento determinado, o a lo largo del tiempo. La información que proporciona la biomonitorización sirve para estimar la cantidad total absorbida del compuesto por el organismo, incluyendo todas las posibles fuentes de exposición, sean éstas conocidas o no. Se podrían determinar las cantidades de la exposición un contaminante a través de sus fuentes conocidas (por ejemplo, para Bisfenol A: comida, aire, agua, polvo, etc.) y sumarlas para estimar la cantidad diaria absorbida. Sin embargo, la estimación basada en la biomonitorización para calcular la cantidad total absorbida integra todas las fuentes de exposición posibles, aunque estas no estén identificadas. Es por ello que los estudios de biomonitorización presentan ciertas ventajas frente a otros métodos de estimación de la exposición a contaminantes ya que se analizan los niveles de los contaminantes directamente

sobre los participantes en el estudio. Esto permite que la exposición evaluada sea mucho más fiel a la realidad. Aun así, la biomonitorización tiene también inconvenientes, ya que obtener muestras biológicas resulta más difícil de cara a los participantes y más cara para los investigadores [32].

En cuanto a las matrices biológicas utilizadas en la biomonitorización de los contaminantes no persistentes pueden ser muy variadas (sangre [33–40], orina [41–43], pelo [44,45], suero de cordón umbilical [39,46], leche materna [39,47,48], placenta [49,50], tejido adiposo [51] y sangre menstrual [52]). Éstas van a depender del compuesto a estudiar, ya que sus características físico-químicas harán que estas sustancias puedan ser acumuladas o no en el organismo, o bien ser metabolizadas y excretadas con mayor o menor facilidad. Los compuestos químicos fácilmente metabolizables, generalmente sustancias muy solubles en agua, podrán encontrarse como la molécula que es absorbida por el organismo o como sus metabolitos excretados por el organismo.

Todas las matrices mencionadas anteriormente tienen su propia variabilidad y características que pueden interferir en la estabilidad de la medición de la exposición y, en consecuencia, sus concentraciones pueden tener significados biológicos disímiles, que es necesario aclarar. Así pues, el suero presenta mayor variabilidad que la orina en cuanto a la exposición a contaminantes no persistentes.

Por otro lado, existen programas de biomonitorización en algunos países que están permitiendo conocer las concentraciones de compuestos químicos que podrían estar interfiriendo con la salud de sus ciudadanos. A esta información obtenida hasta ahora sobre niveles de contaminantes ambientales en población humana puede accederse a través de las bases de datos de publicaciones científicas, así como de organismos como la *Agencia Europea del Medioambiente* (*European Environmental Agency, EEA*) y el *Centro para el Control y la Prevención de Enfermedades (Center for Disease Control and Prevention, CDC)* de Estados Unidos y sus informes periódicos en materia de salud y medioambiente [53,54]. Además de estas bases de datos, existen diversos proyectos de biomonitorizacion de la población que aportan información útil para las políticas de las administraciones, para los profesionales sanitarios, del medio ambiente, de la agricultura y la ganadería, y para otros profesionales, investigadores y organizaciones ciudadanas interesados en las relaciones entre contaminación química, salud y medio ambiente [55]. Algunos de los más relevantes son:

- HBM4EU [56] pertenece a la Agencia Europea de Medio Ambiente y la Comisión Europea, cofinanciado por Horizonte 2020, en el que participan 30 países europeos. La iniciativa está coordinando y promoviendo la biomonitorización humana en Europa. Su objetivo es generar evidencia de la exposición real de los ciudadanos a los productos químicos y los posibles efectos en la salud para apoyar la formulación de políticas.

- DEMOCOPHES [57], se basa en encuestas de biomonitorización humanas en 17 países europeos. Se recogen datos la distribución de biomarcadores específicos y datos relacionados con el estilo de vida entre las poblaciones de estudio definidas que, por primera vez, son comparables a escala europea. Se estudia la exposición al mercurio, cadmio, humo de tabaco y algunos ftalatos y posibles relaciones con el estilo de vida, utilizando biomarcadores y datos de cuestionarios. Además, se añadió bisfenol A como sustancia adicional para un grupo de 6 países.

- Bioambient.es, [58] es un estudio realizado con población trabajadora que el Centro Nacional de Sanidad Ambiental, responsable de DEMOCOPHES en España, viene desarrollando desde el año 2007, con el fin de iniciar el proceso de instauración en nuestro país de un sistema de biomonitorización de la salud. El proyecto es un primer paso para la instauración de una red de biomonitorización a nivel estatal que permita establecer interrelaciones entre las concentraciones de compuestos orgánicos persistentes (COPs) y posibles patologías, y que tengan representación en la misma los diferentes sectores de la población. El estudio, pionero en España, ha recogido muestras de sangre, orina y cabello de una muestra de población trabajadora, consiguiéndose un tamaño muestral no alcanzado hasta ahora en nuestro país. Se miden los niveles de mercurio y otros metales de interés, los niveles de determinados compuestos orgánicos, sus metabolitos, etc. El objetivo final es describir los niveles promedio de los contaminantes estudiados en la población participante según las diferentes variables socio-demográficas (sexo, edad, ocupación y dieta) que han sido recogidas mediante un cuestionario.

- La Agencia Federal Alemana de Medio Ambiente [59], a través de la Encuesta Ambiental Alemana (GerES), monitoriza regularmente la exposición ambiental de la población general a contaminantes químicos y otras fuentes de exposición ambiental, agua de consumo, contaminación del aire interior y exterior, mohos y ruido, a los que la población

está expuesta en su vida diaria. Es el sistema de biomonitorización más exhaustivo de Europa; lleva 30 años realizándose, con independencia de quien gobierne. Su propósito es contribuir a la protección de la población y del medio ambiente.

- El proyecto INMA (Infancia y Medio Ambiente) [60] se constituyó en el año 2003 con el objetivo de estudiar los efectos del medio ambiente y la dieta en el desarrollo fetal e infantil en diversas zonas geográficas en España. El estudio comenzó con el seguimiento de aproximadamente 4000 mujeres embarazadas y sus hijos/as, desde el inicio del embarazo, en siete cohortes españolas (Ribera d'Ebre, Menorca, Granada, Valencia, Sabadell, Asturias y País Vasco).

- NHANES [61], se trata de la encuesta de nutrición y salud americana que incluye la biomonitorización de la exposición a un gran número de contaminantes medioambientales.

Además de la identificación de enfermedades humanas relacionadas con la exposición a EDC, en los últimos años se ha hecho un gran esfuerzo para comprender los mecanismos de acción de estos compuestos, así como para comprender cómo diferentes compuestos sin estructuras químicas similares pueden ejercer efectos fisiológicos similares.

En este sentido, algunas de las propiedades de los EDCs pueden hacer que el estudio de los mecanismos de acción sea más difícil [62]. Estas características de los EDC incluyen:

- Los EDC tienen un potencial de acción muy bajo en comparación con las hormonas naturales.
- La variedad en la naturaleza y estructura química dificulta la identificación de nuevos EDCs usando modelos QSAR tradicionales.
- La incertidumbre sobre el efecto específico que cada EDC puede ejercer sobre cada órgano objetivo, el punto de tiempo de exposición o los niveles de hormonas endógenas que existen simultáneamente.
- La posibilidad de que el efecto combinado de varios EDC pueda ser crucial para ejercer un efecto hormonal. En este aspecto, la OCDE tiene un programa de trabajo para desarrollar métodos de identificación de algunos EDC y ha elaborado un marco conceptual y varias guías [63,64]. Además, la Unión Europea está preparando una guía específica para nuevos marcadores.

Los EDC pueden afectar todas las vías hormonales posibles pero sus interacciones con los receptores de hormonas nucleares, especialmente ERα, ERβ, que son, con mucho, las más estudiadas dentro del mecanismo de disrupción endocrina [65]. Los receptores de hormonas nucleares pueden unirse al ADN y modificar la expresión de los genes y, por lo tanto, pueden actuar como orquestadores en el desarrollo, fisiología y enfermedad. Sin embargo, los EDC también actúan fuera del núcleo celular interactuando con los receptores unidos a la membrana celular, lo que afecta a una variedad de señales conocidas de proteínas en cascada [66]. Por otro lado, hay pruebas sólidas que demuestran que los EDC también pueden alterar el sistema hormonal de manera indirecta, mediante la modificación de la biosíntesis, el metabolismo y / o la excreción hormonal [65]. Además, hay algunos estudios que otorgan a los EDC un papel supuesto en otros mecanismos fisiológicos, como interferir con la remodelación epigenética del ADN [67,68] o modificar la actividad de los receptores hormonales [69].

### 3.1.5 Evaluación de la exposición: Contaminantes objeto de estudio

#### *Contaminantes no persistentes*

Los contaminantes ambientales no persistentes (siglas en inglés npEPs: *non-persistent environmental pollutants*), son un grupo de productos químicos ampliamente utilizados, que no se bioacumulan en el organismo, sino que tienden a ser metabolizados y excretados a través de la orina [70,71], en lugar de acumularse en el tejido adiposo. El grupo npEPs incluye al bisfenol A, parabenos, ftalatos o filtros UV. Sin embargo, la preocupación de estos contaminantes recae en que su exposición es prolongada y prácticamente constante en la rutina diaria de millones de personas en el mundo y su actuación como DE. Todos estos contaminantes se asocian a un ritmo de vida más actual, debido al uso exponencial de comidas envasadas, precocinadas, latas, así como cosméticos.

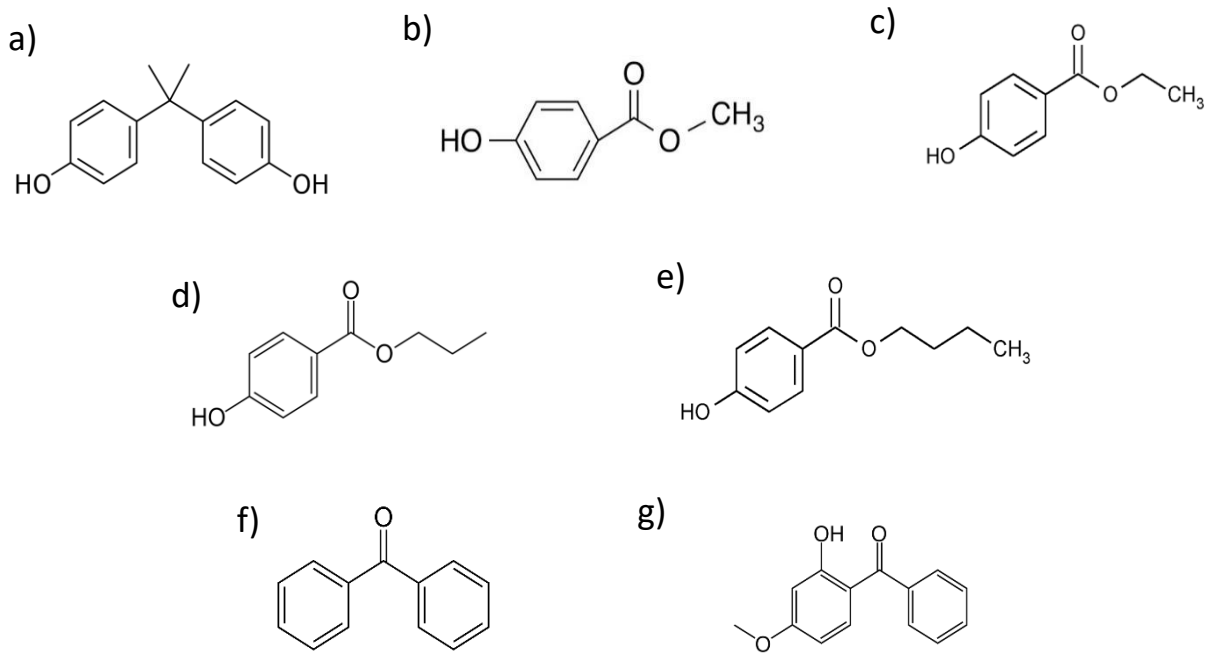
A continuación, se detallan los contaminantes no persistentes que se incluyen en esta tesis:

#### *Bisfenol A*

El bisfenol A (BPA) es un químico industrial desarrollado originalmente como un estrógeno sintético, y uno de los químicos de mayor volumen producido en todo el mundo [72]. Es ampliamente utilizado en la fabricación de polímeros y resinas epoxídicas, policarbonatos y

polisulfones plásticos. En 2015 se fabricaron más de 4 millones de toneladas de este compuesto, que se emplea como aditivo en el PVC, ABS y poliestireno. Por tanto, se encuentra en una gran variedad de productos cotidianos como envases alimentarios, dispositivos médicos y dentales, CD y DVD, tintas y toners, etc. Esto implica que la población está expuesta con frecuencia e inadvertidamente a este compuesto [73,74]. De hecho, se estima que más del 90% de la población en EEUU, Europa y Asia está expuesta a BPA, presentando niveles detectables de BPA (> 0,4 ng/ml) [75–78].

**Figura 2.** Estructura química de los contaminantes estudiados en esta tesis.




a) Bisfenol A, b) metilparaben, c) etilparaben, d) propilparaben, e) butilparaben, f) benzofenona 1, g) benzofenona 3

Las fuentes principales de exposición a BPA son la alimentaria (vía oral), la ocupación (vía inhalatoria) y el contacto (vía dérmica) con materiales, tipo plásticos y dispositivos médicos. La dieta es la principal fuente de exposición a BPA. Los alimentos enlatados presentan la concentración de BPA más elevada (65-842 ng/g), seguido de las bebidas enlatadas (0,61-8,1 ng/ml) y los alimentos envasados en materiales que contienen resinas epoxi y policarbonatos. Otras vías de exposición al BPA son la inhalación de polvo y la absorción dérmica. Estos

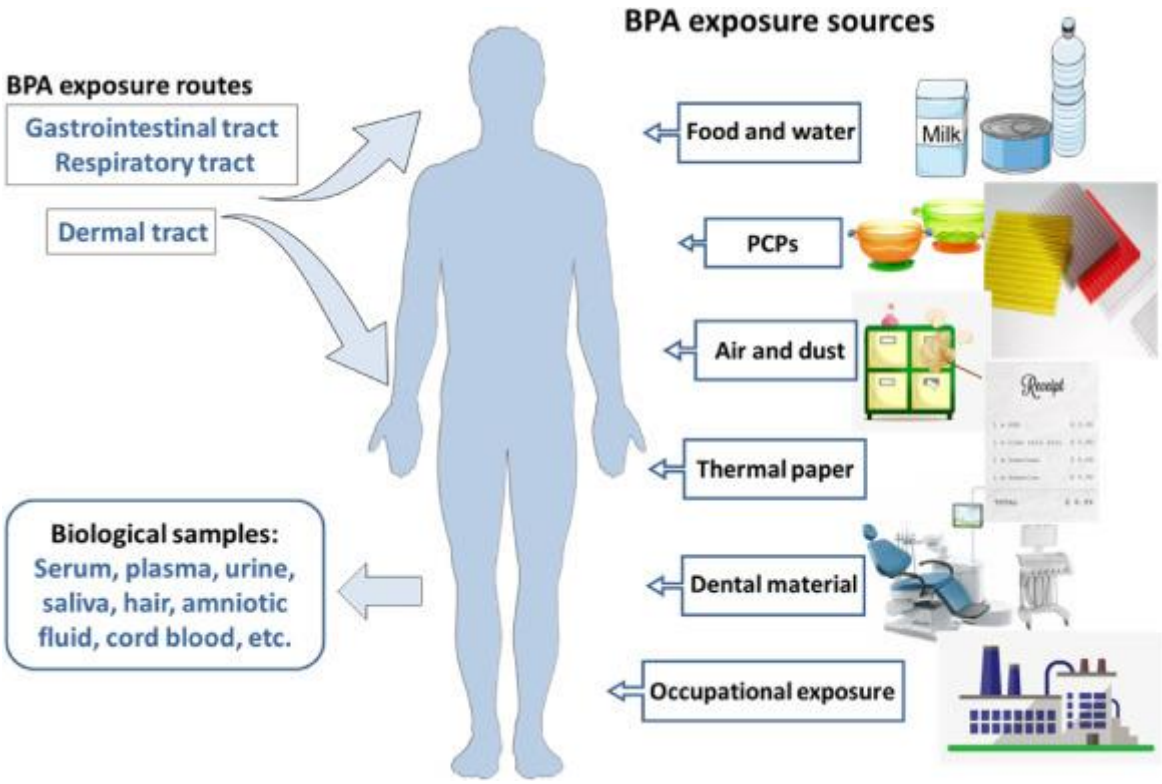
pueden ser una fuente dominante de exposición ocupacional en plantas de reciclaje de desechos electrónicos e instalaciones de producción de materiales que contienen BPA. [79,80].

Estudios recientes de laboratorio han informado sobre el potencial estrogénico de BPA en animales de experimentación [81–85], pero también puede actuar como antiestrógeno al competir con la hormona endógena 17-beta estradiol [86]. Se estima que más del 90% de la población en los EE. UU., Europa y Asia está expuesta al BPA, presentando niveles detectables en la orina (> 0.4 ng / ml) [75–78]. Se considera que la población general está expuesta al BPA principalmente a través de la dieta, ya que con frecuencia está presente en los envases de alimentos, por ejemplo, latas, o cajas de plástico, desde las cuales el BPA puede migrar a los alimentos bajo ciertas condiciones ambientales [73,87–91]. Las crecientes preocupaciones sobre los posibles efectos sobre la salud de la exposición al BPA indujeron a la Autoridad Europea de Seguridad Alimentaria (EFSA) a reducir la ingesta diaria tolerable de BPA en 2015: de 50 μg / kg por día a 4 μg / kg por día; y se está preparando una reevaluación para 2020 [90]. Recientemente, el Tribunal General de la Unión Europea publicó una confirmación de la inclusión del bisfenol A como una sustancia muy preocupante [92].

Sus efectos sobre la salud son controvertidos debido a la escasez de estudios epidemiológicos prospectivos. El BPA tiene una alta capacidad para interaccionar con receptores estrogénicos ERα, ERß y ER, androgénicos, tiroideos, y receptores PPAR y GPR30, involucrados en el metabolismo de la glucosa y en el almacenamiento de los ácidos grasos. BPA ejerce, por tanto, su acción a través de varios receptores hormonales y mecanismos, implicando posiblemente también el estrés oxidativo, la disfunción mitocondrial y la señalización celular. Tiene un potencial efecto tóxico y carcinógeno en base a lo observado en estudios experimentales *in vivo* e *in vitro*.

Es importante señalar también que los seres humanos estamos expuestos a mezclas complejas de contaminantes ambientales, y que esta exposición combinada puede resultar en interacciones de tipo aditivo, sinérgico y/o antagónico, lo que dificulta en gran manera el estudio de los posibles efectos sobre la salud humana derivados de la exposición a compuestos de manera individual. En este sentido, se ha evidenciado actividad disruptora endocrina inducida por mezclas de contaminantes a dosis tan bajas que la exposición individual a cada uno de los componentes de la mezcla no resultaría en ningún efecto.

**Figura 3.** Fuentes de exposición al BPA.




Fuente: Ma, Y., et al. 2019. The adverse health effects of bisphenol A and related toxicity mechanisms. Environ. Res. https://doi.org/10.1016/j.envres.2019.108575 [93]

### *Parabenos*

Los parabenos (PB) pertenecen a la familia de alquil-ésteres del ácido parahidroxibenzoico, donde el grupo éster se localiza en la posición C-4 del anillo benzoico. Los más utilizados son metil-, etil-, propil-, butil- y bencil-paraben. Se trata de moléculas inodoras, incoloras, no volátiles, económicas y eficaces en un amplio rango de pH. Estas características permiten que los parabenos se empleen como conservantes (antimicrobianos) para prolongar la vida de los productos a los que se añaden [94]. Además, se utilizan en una amplia gama de productos cosméticos, artículos de aseo, fármacos, productos para niños, e incluso algunos están permitidos, en cantidades limitadas en alimentos, como por ejemplo metil- y etil-paraben. El hecho de que estén presentes en productos de uso cotidiano hace que los seres humanos estén expuestos de forma continua a estos contaminantes.

Los PB se metabolizan rápidamente y se excretan del cuerpo [95]. Sin embargo, han mostrado propiedades de alteración endocrina con actividades estrogénicas y antiandrogénicas en estudios in vitro e in vivo [96–98]. Es por lo que, actualmente existe una gran preocupación debido a los altos niveles de exposición a estos compuestos en la población general, lo que podría producir efectos indeseables en la salud de los sujetos expuestos. Darbre et al., han descrito la presencia de parabenos en tejido mamario procedente de 20 pacientes con cáncer de mama, encontrando una concentración media de 26,6 ± 4,2 ng/g de tejido, y siendo el metil-paraben el éster cuantificado en mayor concentración [99]. La detección de pequeñas cantidades de parabenos, no metabolizados, en el tumor de mama humano puede sugerir que estas sustancias podrían acumularse y, con el tiempo, provocar efectos tóxicos.

Algunos parabenos actúan como disruptores endocrinos, presentando una moderada actividad estrogénica y anti-androgénica y pudiendo alterar las concentraciones de hormonas sexuales. La actividad estrogénica de los parabenos se relaciona con la longitud del grupo éster, siendo mayor para aquellos con una mayor longitud de la cadena. Así, estudios *in vitro* con líneas celulares que contienen el receptor de estrogénos (por ejemplo, células MCF-7) han demostrado que la exposición a estos compuestos incrementa la proliferación celular. En cuanto a la actividad anti-androgénica, estudios experimentales con ratas macho jóvenes, han observado efectos adversos en los niveles de producción de esperma y de testosterona después de la exposición oral a parabenos de cadenas laterales más largas, como por ejemplo butil-propil-paraben.

### *Benzofenonas*

Las benzofenonas (BP) son compuestos sintéticos utilizados como ingrediente de sabor, potenciador de fragancia, perfume fijador y aditivo para plásticos, recubrimientos y formulaciones adhesivas. También se utilizan en productos de lavandería y limpieza del hogar y en la fabricación de insecticidas, productos químicos agrícolas, medicamentos hipnóticos, antihistamínicos y otros productos farmacéuticos. Los BP se utilizan como agentes de protección ultravioleta (UV) en gafas de sol y para evitar que la luz UV dañe los olores y colores en productos como perfumes y jabones[100], por lo que su uso más extendido es en productos de cuidado personal [101].

Debido a su uso como aditivo en fragancias, cosméticos, productos farmacéuticos, insecticidas y productos de limpieza para el hogar, la exposición a BP a través del contacto dérmico puede ser significativa [102]. Las fuentes dietéticas de exposición incluyen alimentos y agua potable, donde las BP pueden estar presentes debido a la adición como saborizante o la migración del envase [103]. Las BP están clasificadas como sustancias del grupo 2B, "posibles carcinógenos para los humanos" [103,104].

Se han detectado BP en la orina [101] y algunas otras matrices biológicas, como la placenta [50], la leche materna humana [47] y la sangre menstrual [52]. Los efectos disruptores endocrinos (estrogenicidad, problemas de fecundidad, maduración de gónadas, etc.) de las BPs se han demostrado in vitro y en estudios con animales [105–107]. Sin embargo, sigue habiendo una falta de evidencia científica acerca de los posibles efectos adversos de las BPs en la salud humana.

### 3.1.6 Mecanismos de acción y toxicocinética

En ese sentido, resulta necesario estudiar los mecanismos de acción y efectos biológicos de los compuestos químicos, para comprender cómo el sistema biológico responde a una sustancia o conjunto de sustancias químicas, y determinar la dosis de referencia, o concentración sin riesgo.

Para conocer los mecanismos de acción de los contaminantes no persistentes en el organismo humano se habla de metabolismo y toxicocinética de los mismos. Así, algunos estudios han determinado que el hígado juega un papel esencial en la metabolización de Bisfenol A (BPA). Los monómeros de BPA liberados al medio, a partir de los materiales que lo contienen, son absorbidos por el organismo y pasan a través de la circulación enterohepática al hígado, donde se metabolizan mediante glucorono o sulfoconjugación, siendo eliminados vía renal. La glucuronización es una ruta metabólica hepática utilizada para eliminar distintos compuestos tanto endógenos como exógenos.

Algunos estudios toxicocinéticos han puesto de manifiesto que no todo el BPA es conjugado en el hígado; ya que varios estudios han examinado la absorción y el metabolismo del BPA en el intestino y en el colon. La existencia de glucuronidasas en el tracto digestivo ha sido descrita por varios autores (que además ponen de manifiesto el incremento de los niveles desde la infancia a la edad adulta) [108] por lo que el BPA-conjugado podría ser desconjugado y

activado en el tracto digestivo de la población infantil durante el proceso digestivo, dando lugar a BPA nuevamente libre que puede ser absorbido por el colon [109]. Además, se ha comprobado en un estudio sobre personas voluntarias que, en hombres, el 85% de la dosis aplicada de BPA se recuperó en orina después de 5 horas, la mayoría en forma de BPA-glucurónido. En cuanto a las mujeres, se recuperó el 75% del BPA, igualmente como BPA-glucurónido y en el mismo período de tiempo [110]. Esto parece indicar potenciales diferencias de género en el metabolismo del BPA, sugeridas por otros estudios previos [111,112]. La eliminación de BPA se suele dar a las 24 horas posteriores a la exposición [113]. Así pues, el BPA es absorbido rápidamente desde el tracto gastrointestinal, conjugado con ácido glucurónico a BPA-glucurnónido en el hígado, y rápidamente filtrado desde la sangre por el riñón para excretarse por la orina.

En cuanto a la toxicocinética de parabenos y benzofenonas es memos estudiada, pero se conoce que, en los seres humanos, los parabenos son principalmente ácido hidroxibenzoico superior hidrolizado y luego se excretan en la orina como conjugados de glicina, glucurónido y sulfato, pero también los parabenos inalterados pueden excretarse en diversas formas, como conjugados de glucurónido y sulfato. Se ha descubierto que el perfil metabólico de los parabenos depende de la ruta de exposición [95,114]. En un estudio con personas voluntarias, la fracción de la dosis administrada excretada en la orina fue de 0,05% para propilparaben libre, 8,6% para propilparaben total (libre + conjugados) [115]. A pesar del uso generalizado de cosméticos y productos para el cuidado de la piel con ftalatos y parabensina, se carece de información sobre la absorción sistémica humana de estos compuestos a través de la piel.

## 3.2 Síndrome Metabólico

En las últimas décadas, la prevalencia de varias enfermedades crónicas, no transmisibles, junto con sus factores de riesgo, se ha incrementado dramáticamente, convirtiéndose en un problema importante en todo el mundo [116]. Así, se ha observado un aumento de la obesidad, la hipertensión, la diabetes tipo 2, y la aterosclerosis. Este conjunto de condiciones muy relacionadas entre sí y que suponen factores de riesgo para la enfermedad cardiovascular se conoce actualmente como “síndrome metabólico”, que se estima afecta a un 20-30% de la población europea [117]. De hecho, las últimas estimaciones indican que la diabetes tipo 2 afecta, al menos, a 366 millones de personas y que alrededor de 500 millones de adultos son

obesos [118]. Por tanto, las personas con síndrome metabólico experimentan una alta incidencia de la diabetes tipo 2 y de enfermedades cardiovasculares [119]. Por otra parte, las enfermedades cardiovasculares (ECV) y la hipertensión son algunas de las causas más frecuentes de mortalidad adulta en los países desarrollados [120].

Los factores de riesgo metabólico más importantes incluyen:

- El perímetro de la cintura u obesidad abdominal. El exceso de grasa en el área del estómago es un factor de riesgo mayor de enfermedad cardíaca que el exceso de grasa en otras partes del cuerpo, como en las caderas.
- Un nivel alto de triglicéridos (o estar tomando medicamentos para tratar los triglicéridos altos).
- Un nivel bajo de colesterol HDL (o estar tomando medicamentos para tratar el colesterol HDL bajo). El HDL a veces se denomina colesterol "bueno" porque ayuda a eliminar el colesterol de las arterias. Un nivel bajo de colesterol HDL aumenta el riesgo de enfermedad cardíaca.
- Presión arterial alta (o estar tomando medicamentos para tratar la presión arterial alta). La presión arterial es la fuerza que ejerce la sangre contra las paredes de las arterias cuando el corazón bombea sangre. Si esta presión aumenta y se mantiene alta con el tiempo, puede dañar el corazón y provocar la acumulación de placas en las arterias.
- Nivel alto de azúcar en sangre en ayunas (o estar tomando medicamentos para tratar el nivel alto de azúcar en sangre). Un nivel levemente alto de azúcar en sangre puede ser un signo temprano de diabetes.

Diabetes

La diabetes tipo 2 se caracteriza por la resistencia a la insulina y la deficiencia relativa de insulina. La resistencia a la insulina se ve agravada por el envejecimiento, la inactividad física y el sobrepeso (índice de masa corporal [IMC] 25-29.9 kg / $m^2$) u obesidad (IMC> 30 kg / $m^2$) [121]. Entre los pacientes obesos, la pérdida de peso a menudo reduce el grado de resistencia a la insulina y puede retrasar la aparición de diabetes o mejorar la gravedad de la diabetes y, por lo tanto, reducir el riesgo de complicaciones a largo plazo. La resistencia a la insulina afecta principalmente al hígado, los músculos y los adipocitos, y se caracteriza por alteraciones complejas en los receptores celulares, la función intracelular de la glucosa quinasa y otros procesos metabólicos intracelulares [122]. La complejidad y variedad de estos trastornos

intracelulares sugieren que lo que ahora se clasifica como diabetes tipo 2 puede ser, de hecho, un grupo más amplio de afecciones que esperan una definición futura.

La prevalencia global de diabetes mellitus tipo 2 (DM2) entre adultos es de aproximadamente 415 millones, con proyecciones de 642 millones para 2040 (1). La creciente prevalencia de DM2 tiene un impacto importante en la salud mundial, ya que los datos más recientes de la Federación Internacional de Diabetes sugieren que cada año 5 millones de muertes son directamente atribuibles a la diabetes [123].

Entre los factores de riesgo externos que pueden afectar el desarrollo de DM2, existe una preocupación creciente sobre el papel de la exposición crónica a bajas dosis de contaminantes ambientales [124–134]. Los contaminantes químicos incluyen los contaminantes ambientales no persistentes ya que estos compuestos pueden actuar como obesógenos, que se definen funcionalmente como sustancias químicas que promueven la obesidad en humanos o animales [135]. En este sentido, existe evidencia acumulada que vincula estos químicos con la obesidad y las enfermedades crónicas relacionadas con la obesidad y el síndrome metabólico [133,136]. Sin embargo, las implicaciones de la exposición a npEP y T2DM en la población general aún son discutibles debido a informes controvertidos en la literatura científica [133,137,138].

Enfermedad coronaria isquémica

La enfermedad cardiovascular (ECV), principalmente la enfermedad coronaria isquémica (ECI), es la principal causa de mortalidad y morbilidad en todo el mundo [139] con 17,8 millones de muertes en 2017 [139,140]. Además, la incidencia de ECV excede la incidencia conjunta de cáncer de próstata y de mama en todo el mundo [141–143]. En España, la ECV fue la principal causa de muerte con 120.859 muertes en 2018, seguida del cáncer con 112.714 muertes [144].

La ECI se debe a la acumulación de placa en las paredes de las arterias que suministran sangre al corazón (llamadas arterias coronarias) y otras partes del cuerpo [145]. Esa placa está formada por depósitos de colesterol y otras sustancias en la arteria. La acumulación de esta placa hace que el interior de las arterias se estreche con el tiempo, lo que puede bloquear parcial o totalmente el flujo sanguíneo. Este proceso es la aterosclerosis.

Como enfermedad no transmisible, algunos de los factores de riesgo asociados con la ECV y la ECI están relacionados con el estilo de vida: dieta, hábito tabáquico, consumo de alcohol y actividad física; siendo la obesidad uno de los principales factores de riesgo potencialmente modificables [139]. Un historial familiar de enfermedad cardíaca también aumenta su riesgo de CAD, especialmente un historial familiar de tener una enfermedad cardíaca a una edad temprana (50 años o menos). Además, Existe una creciente evidencia de que algunos contaminantes ambientales tienen el potencial de actuar como obesógenos [146] y, por lo tanto, podrían desempeñar un papel importante en el desarrollo de ECI.

Hipertensión arterial

La hipertensión arterial (HTA) es un importante problema de salud pública, y también se sabe que es un factor de riesgo fuerte e independiente para la enfermedad cardiovascular [147]. La presión arterial es la fuerza necesaria para que la sangre circule a través de los vasos arteriales. Cuando esta fuerza ejercida por el corazón a las arterias de forma sostenida es excesiva o más alta de lo recomendable, se habla de hipertensión arterial (HTA). Actualmente existe consenso entre los expertos para definir la hipertensión como aquellas cifras de tensión arterial por encima de 140/90, si bien lo deseable sería estar en 130/80 como límite máximo [148]. Entre los factores de riesgo modificables de hipertensión alta se incluyen las dietas poco saludables (consumo excesivo de sal, dieta alta en grasas saturadas y grasas trans, baja ingesta de frutas y verduras), inactividad física, consumo de tabaco y alcohol, y sobrepeso u obesidad. Los factores de riesgo no modificables incluyen antecedentes familiares de hipertensión, edad mayor de 65 años y enfermedades coexistentes como diabetes o enfermedad renal.

La prevalencia global de HTA entre adultos mayores de 25 años en 2010 fue de aproximadamente el 40% [148,149] y en 2015, 1 de cada 4 hombres y 1 de cada 5 mujeres tenían hipertensión [150]. Esta creciente prevalencia de hipertensión se atribuye al crecimiento de la población, el envejecimiento y los factores de riesgo conductuales, como la dieta poco saludable, el consumo de alcohol, la falta de actividad física, la obesidad y la exposición al estrés persistente [148].

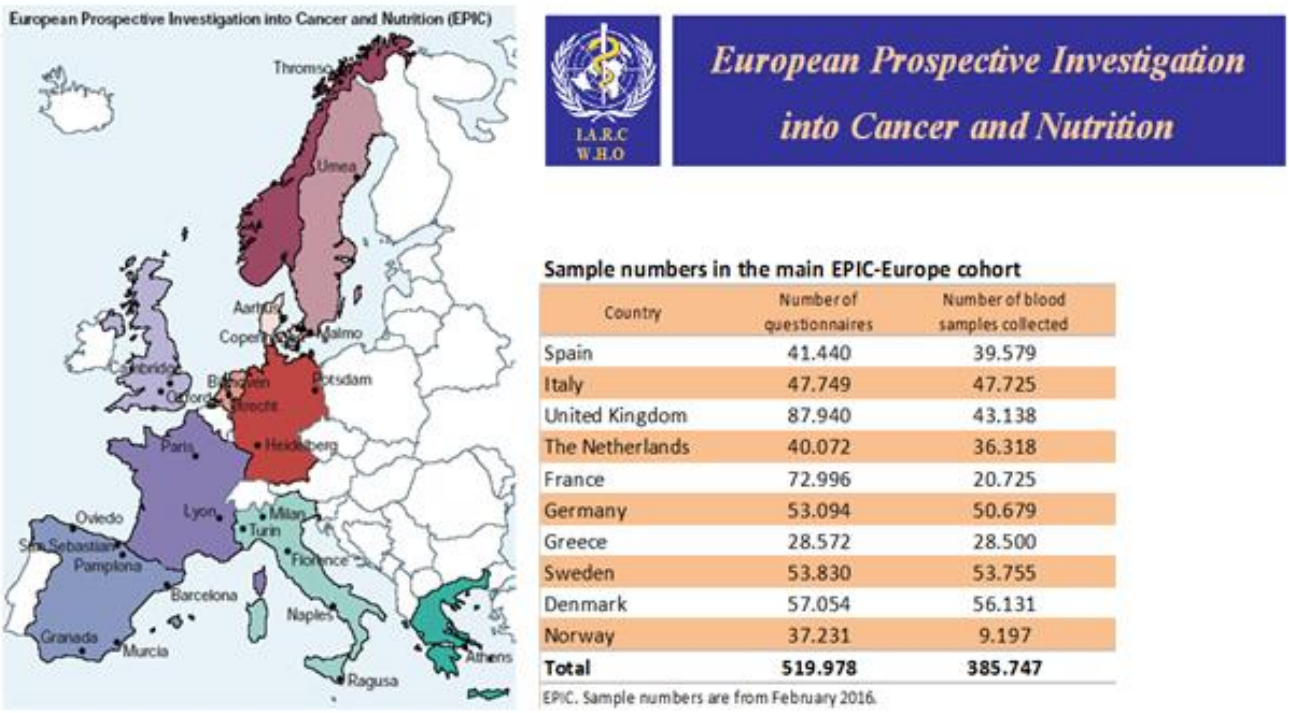
Varios de los mecanismos biológicos que se sabe que están asociados con un mayor riesgo de hipertensión incluyen disfunción tiroidea [151], aumento de peso [152], resistencia a la insulina [153], hiperlipidemia [154], estrés oxidativo [155], e inflamación sistémica más alta [156]. Sin embargo, a pesar de que existe una creciente preocupación sobre el papel de la exposición

humana a bajas dosis de contaminantes ambientales que se sospecha que inducen posibles efectos adversos en los humanos [51], el papel de las exposición a npEP y riesgo de hipertensión arterial en la población general resulta controvertido debido a la falta de resultados homogéneos [157–163].

## 3.3 Cohorte EPIC

El Estudio Prospectivo Europeo sobre Nutrición y Cáncer (EPIC) es uno de los estudios de cohorte prospectivo más grandes del mundo, con más de medio millón (521.000) de participantes reclutados que han sido seguidos hasta la actualidad [164]. El estudio EPIC se inició en 1992 e incluye 23 centros de 10 países europeos (Alemania, Dinamarca, España, Francia, Grecia, Holanda, Italia, Noruega, Reino Unido, Suecia).

**Figura 4.** Cohorte EPIC




Sample numbers in the main EPIC-Europe cohort

| Country | Number of questionnaires | Number of blood samples collected |
|---|---|---|
| Spain | 41.440 | 39.579 |
| Italy | 47.749 | 47.725 |
| United Kingdom | 87.940 | 43.138 |
| The Netherlands | 40.072 | 36.318 |
| France | 72.996 | 20.725 |
| Germany | 53.094 | 50.679 |
| Greece | 28.572 | 28.500 |
| Sweden | 53.830 | 53.755 |
| Denmark | 57.054 | 56.131 |
| Norway | 37.231 | 9.197 |
| **Total** | **519.978** | **385.747** |

EPIC. Sample numbers are from February 2016.

Fuente: modificado de https://epic.iarc.fr/

EPIC fue diseñado para investigar las relaciones entre la dieta, el estado nutricional, el estilo de vida y los factores ambientales, y la incidencia de cáncer y otras enfermedades crónicas. Debido al amplio rango de distribución geográfica proporciona una población con una gran variabilidad de consumo y de hábitos alimentarios [165]. Dadas las características particulares del estudio

(es decir, la población sana en el reclutamiento, más de 20 años de seguimiento y la disponibilidad de muestras biológicas), EPIC constituye un referente internacional y una excelente oportunidad para la investigación de la etiología de las enfermedades crónicas.

En la fase de reclutamiento, llevada a cabo entre 1992 y 1998, se englobaban a mujeres y hombres voluntarios con edades comprendidas entre los 35 y 70 años. Se recogió información común de todos los individuos: se recogió una amplia información a través de un cuestionario sobre factores de riesgo no alimentarios, que incluía historia de consumo de tabaco, actividad física ocupacional, deportes y actividad en el tiempo libre, consumo de anticonceptivos y uso de terapia hormonal sustitutoria, historia de la actividad reproductiva, exposición a ocupaciones de riesgo y antecedentes médicos y quirúrgicos. En algunos centros, como en España, esta información se obtuvo mediante entrevista personal. Además, se recogió una muestra de sangre, que se encuentra almacenada en contenedores de nitrógeno líquido para posteriores análisis.

También se recogió información detallada de la dieta [166] a través de una entrevista personal, utilizándose métodos de medición de la dieta adaptados a la realidad de cada país. Es España se utilizaron cuestionarios de historia de dieta (con más de 600 ítems alimentarios), administrados mediante entrevistas, introducidos directamente en formato informatizado. Además, se implementó en EPIC una nueva aproximación metodológica destinada a calibrar los instrumentos de medición de la dieta de los diferentes países, con el objetivo de corregir los errores sistemáticos de sobre o subestimación de la ingesta. Para ellos se obtuvo una segunda medición dietética en una muestra aleatoria de aproximadamente el 8-10% de cada cohorte, utilizando en todos los centros un mismo método estandarizado: el método del recuerdo de la dieta de 24 horas, mediante un programa informático específicamente desarrollado para este propósito (EPIC-SOFT)[167].

Los estudios de EPIC generalmente se llevan a cabo dentro del contexto de grupos de trabajo específicos de enfermedades que cubren las principales localizaciones anatómicas del cáncer, las enfermedades cardiovasculares, la diabetes tipo 2, la mortalidad general y el envejecimiento saludable. En este contexto, además de los numerosos estudios relevantes en el área de cáncer, en EPIC, la diabetes es tanto una exposición importante como un resultado de interés en sí mismo. Existe un grupo de trabajo de EPIC para diabetes (InterAct) [168] que ha realizado análisis que involucran los casos con diabetes prevalente al inicio del estudio y la investigación de la asociación de los factores iniciales con la diabetes incidente. Además de analizar la

asociación de los factores del estilo de vida y la diabetes incidente, EPIC-InterAct ha completado las medidas bioquímicas básicas, incluidos los ácidos grasos fosfolípidos, y tiene información del genotipo de todo el genoma de todos los individuos. Este estudio ha producido gran cantidad de resultados [169,170] como la evidencia de que la actividad física reduce el riesgo de incidencia de diabetes tipo 2 en general y en personas delgadas y obesas [171].

Por otro lado, el estudio EPIC tiene también un grupo de trabajo de enfermedad coronaria EPIC-Heart, cuyo objetivo es evaluar los efectos separados y combinados de los factores de riesgo genéticos, bioquímicos y del estilo de vida en las enfermedades del corazón. EPIC-Heart utiliza un diseño de estudio de casos y cohortes que incluye > 15.000 casos incidentes confirmados de cardiopatía coronaria y un número similar de participantes seleccionados al azar para actuar como controles (la "subcohorte")[172]. Esta subcohorte se comparte con el proyecto EPIC-InterAct. Dentro de EPIC-Heart, existe el grupo EPIC-CVD que incluye análisis de aproximadamente 10.000 casos de accidentes cerebrovasculares incidentes. EPIC-CVD tiene como objetivo desarrollar y validar puntajes de riesgo innovadores y estrategias de detección eficaces mediante el estudio de 75 biomarcadores y variantes genéticas seleccionadas en este estudio prospectivo poblacional.

En España, el estudio EPIC-España, consta de un total de 41.446 participantes de entre 29 y 69 años que se reclutaron entre 1992 y 1996 en cinco provincias de España (Asturias, Guipuzkoa, Navarra, Murcia, Granada) (Figura 5). La mayoría (60%) de estos participantes fueron reclutados de donantes de sangre y la población de estudio incluyó una amplia gama de niveles socioeconómicos y educativos [173]. En el momento del reclutamiento, se obtuvo una muestra de sangre en ayunas de cada participante. En todos los casos se obtuvo el consentimiento informado firmado y el estudio fue aprobado por el Comité de Ética del Hospital de Bellvitge (Barcelona).

## Cohorte EPIC - Granada

La cohorte EPIC en España cuenta con 5 centros (Guipuzkoa, Murcia, Navarra, Granada y Asturias), siendo Granada el centro más al sur de Europa. En Granada, el proyecto se coordina

desde la Escuela Andaluza de Salud Pública, organismo de la Consejería de Salud y Familias de la Junta de Andalucía.

La fase de reclutamiento de los individuos del estudio se inició en octubre de 1992 y finalizó en junio de 1996. La cohorte EPIC en Granada está formada por 7.879 personas de Granada capital (26,5%) y de distintos municipios de la provincia (73,5%). Los participantes son en su mayoría mujeres (77%) y en menor proporción hombres (23%). La edad media en el momento de su inclusión en la cohorte fue de 49 y 51 años respectivamente.

El primer colectivo de personas al que se solicitó su participación voluntaria fue el de donantes de sangre de la provincia, que constituyen un 45% del total de la cohorte. La elección de este colectivo estuvo motivada por su presumible mayor predisposición a colaborar en un estudio de este tipo, en el que se precisaba la obtención de una muestra de sangre. Además, se valoró la previsible mayor facilidad para su posterior seguimiento a largo plazo. También se recurrió a otros colectivos tales como participantes en programas de educación para adultos, asociaciones de amas de casa, trabajadores de instituciones públicas y grandes empresas y, finalmente, población general de distintos municipios de la provincia.

El contacto inicial con los individuos se realizó mediante el envío de una carta informativa, tras la cual, pasados unos días, se procedía al contacto telefónico para concertar una cita individual con cada posible participante. Como norma general, en una primera cita se realizaba la entrevista personal de dieta, el cuestionario de otros factores y las medidas antropométricas. Posteriormente, se concertaba una segunda cita para la obtención de una muestra de sangre.

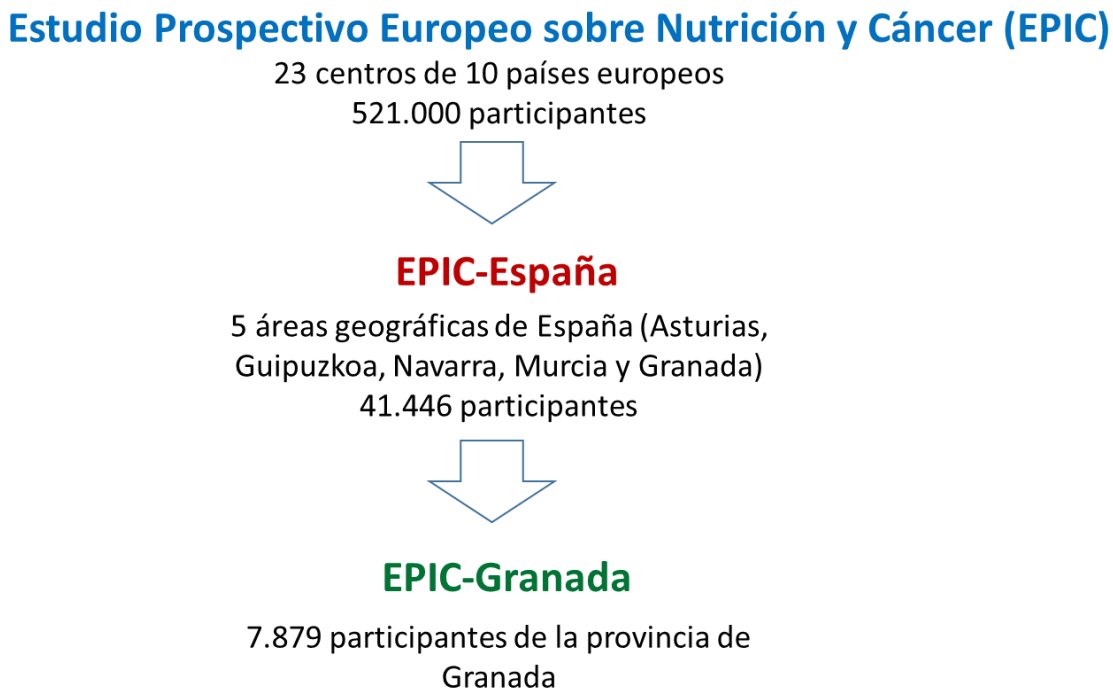
En una posterior fase de seguimiento, a los 3 años de la inclusión en el estudio, se contactó telefónicamente con los participantes con objeto de recabar información sobre cambios en el estado de salud, así como, sobre hábitos y estilos de vida asociados a un mayor riesgo de cáncer. En Granada, esta fase comenzó en marzo de 1996 y concluyó a finales de 1998.

El seguimiento de los sujetos participantes en un estudio de cohorte como es EPIC es un elemento esencial para su desarrollo. Por este motivo, con objeto de reducir al mínimo las pérdidas de seguimiento, el contacto telefónico con los sujetos se intentó repetidamente en distintos momentos del día y, en caso de no conseguirse, el cuestionario se enviaba por correo acompañado de una carta informativa. Finalmente, se realizaron 7.061 entrevistas (96,5%). En

un 2,5% de los sujetos no se logró contactar con ellos por diferentes motivos, un 0,5% rehusó seguir participando en el estudio y en un 0,5% los familiares o amigos con los que se contactó comunicaron su fallecimiento.

Desde su inclusión en el estudio y hasta la actualidad, además de la citada entrevista telefónica, el seguimiento de los participantes se realiza también mediante el enlace de la base de datos de EPIC-Granada con el Registro de Cáncer de Granada, el Registro de Mortalidad de Andalucía y el Registro de Mortalidad del Instituto Nacional de Estadística, lo que ha permitido identificar, hasta el año 2015, aproximadamente 1.459 casos de cáncer y hasta el año 2018, 1.292 defunciones por todas las causas.

**Figura 5.** Estudio EPIC, EPIC-España y EPIC-Granada



# 4. Hipótesis y justificación.

Ante la enorme controversia científica existente en relación a los posibles efectos nocivos del BPA, los parabenos y benzofenonas, y dada la ubicuidad de la exposición, se hacen necesarios estudios epidemiológicos prospectivos que profundicen en esta compleja relación, tal y como indica la OMS en su último informe [174]. El estudio EPIC ofrece la oportunidad única de evaluar la asociación a contaminantes no persistentes y salud prospectivamente, proporcionando información exhaustiva y de calidad sobre las fuentes de exposición a BPA (dieta y otras), y permitiendo comparar la exposición en el reclutamiento y en la actualidad, tras más de 20 años de seguimiento.

La hipótesis que supuso el punto de partida del presente trabajo es que exposición prolongada a dosis bajas de BPA, PB, filtros UV, o su combinación, podría estar relacionado con un incremento del riesgo de enfermedades relacionadas con el síndrome metabólico: resistencia a la insulina, obesidad, hipertensión arterial y/o enfermedad cardiovascular.

# 5. Objetivos

El objetivo general del presente trabajo es estudiar los niveles de exposición a contaminantes no persistentes en la cohorte EPIC en el momento del reclutamiento, así como evaluar su relación con la incidencia de componentes específicos del síndrome metabólico (resistencia a la insulina, hipertensión arterial y riesgo de enfermedad cardiovascular), medido más de 20 años después del reclutamiento.

Objetivos específicos:

1. Estimar la exposición histórica en la cohorte EPIC mediante la cuantificación de los niveles séricos de BPA y sus factores predictores en muestras de suero recogidas en el reclutamiento de una sub-cohorte de EPIC-España.

2. Estudiar la relación entre los niveles séricos de BPA en el reclutamiento y la incidencia de enfermedad coronaria isquémica de una sub-cohorte de EPIC-España durante seguimiento.

3. Estudiar la relación entre los niveles séricos de BPA, parabenos y benzofenonas y la incidencia de diabetes tipo 2 en la sub-cohorte de EPIC-Granada durante el seguimiento.

4. Estudiar la relación entre los niveles séricos de contaminantes y la incidencia de hipertensión arterial en la sub-cohorte de EPIC-Granada durante el seguimiento.

# 6. Metodología

Esta tesis se encuentra en el marco de trabajo de dos proyectos de investigación financiados por el Instituto de Salud Carlos III. Los estudios con la cohorte EPIC-España reciben la financiación del Instituto de Salud Carlos III (ISCIII) (Exps: PI14/00067, PI14/01716, PI14/01880, PI14/00556) y los estudios con la sub-cohorte EPIC Granada reciben la financiación también del ISCIII, a través de un Contrato Miguel Servet (CP15/00193).

Para alcanzar los objetivos de esta tesis se han elaborado 4 artículos científicos basados en las cohortes EPIC-España y EPIC-Granada. En cada una de las publicaciones se detalla la metodología utilizada, que se resume en esta sección.

El estudio de los determinantes de la exposición a BPA, así como el estudio de los niveles de contaminantes no persistentes y su relación con la incidencia de enfermedad coronaria isquémica se llevó a cabo en una sub-cohorte de EPIC-España, compuesta de una muestra aleatoria de los participantes en el estudio EPIC reclutados en los centros de Guipuzkoa, Navarra, Murcia y Granada. Se estudió en el marco de los proyectos ISCIII (Exps: PI14/00067, PI14/01716, PI14/01880, PI14/00556).

La relación entre los niveles de exposición a BPA, parabenos y BPs con la incidencia de hipertensión arterial y diabetes tipo 2 se investigaron en el marco del proyecto Miguel Servet (CP15/00193), enfocado al análisis longitudinal de una muestra de los participantes reclutados en la cohorte EPIC-Granada.

## 6.1 Diseño y población de estudio: EPIC-España y la subcohorte EPIC-Granada

El estudio EPIC-España, consta de un total de 41.446 participantes de entre 29 y 69 años que se reclutaron entre 1992 y 1996 en cinco provincias de España (Asturias, Guipuzkoa, Navarra, Murcia, Granada). La mayoría (60%) de estos participantes fueron reclutados de donantes de sangre y la población de estudio incluyó una amplia gama de niveles socioeconómicos y educativos [173]. En el momento del reclutamiento, se obtuvo una muestra de sangre en ayunas de cada participante. En todos los casos se obtuvo el consentimiento informado firmado y el estudio fue aprobado por el Comité de Ética del Hospital de Bellvitge (Barcelona). Parte de la

cohorte EPIC-España (de 4 centros: Guipuzkoa, Navarra, Murcia, Granada) se ha utilizado para la realización de dos artículos de esta tesis.

Dentro de EPIC-España, la población de uno de sus centros (EPIC-Granada) se ha utilizado para realizar los otros dos artículos de esta tesis. En EPIC-Granada el reclutamiento de los 7,879 participantes aparentemente sanos, tuvo lugar entre 1992-1996. En Granada el 77% de los participantes eran mujeres con una edad media de 51 años, mientras que el 23% restante de hombres, tenía una edad media de 49 años. La mayoría (60%) de estos participantes fueron reclutados de donantes de sangre y la población de estudio incluyó una amplia gama de niveles socioeconómicos y educativos [173]. En total, se realizaron 7.879 entrevistas de historia de dieta y 7.879 sobre otros factores de interés, 7.813 medidas antropométricas y se obtuvieron 6.892 muestras de sangre. Aproximadamente el 90% de los sujetos de la cohorte poseen información completa sobre las cuatro medidas de exposición mencionadas previamente.

Para esta tesis se han realizado cuatro estudios epidemiológicos sobre la exposición a contaminantes persistentes y riesgo para la salud: dos artículos con la cohorte EPIC-España y dos artículos con la sub-cohorte EPIC-Granada (Figura 6). Para cumplir el objetivo de cada uno se planteó una metodología concreta que a continuación se detalla junto con la población escogida para los artículos:

**Figura 6.** Objetivo, diseño y población de los estudios incluidos en esta tesis.

**Estudio de los determinantes de los niveles de exposición a Bisfenol A** (**objetivo 1).** Se seleccionó una sub-cohorte de 1.000 participantes de 4 centros EPIC-España (Guipuzkoa, Navarra, Murcia, Granada) mediante un muestreo aleatorio estratificado por sexo y edad, excluyendo a las personas con enfermedades crónicas (cáncer, enfermedad cardiovascular). En esta sub-cohorte, el 90% de los participantes proporcionaron una muestra de sangre en ayunas en el momento del reclutamiento, extraída entre las 6 am y las 11 am. Tras excluir las muestras consideradas inadecuadas (volumen de suero insuficiente o muestra de suero descompuesta durante el análisis químico), la población final del estudio analizada estuvo formada por una sub-cohorte de 3.553 participantes de los cuatro centros EPIC-España (807 de Granada, 934 de Murcia, 903 de Navarra y 909 de Gipuzkoa). Las características de nuestra muestra con respecto al resto de la cohorte EPIC-España fueron similares para las variables incluidas en el estudio, salvo en la distribución por sexo y edad que fue deliberadamente diferente debido al diseño muestral estratificado utilizado para extraer la muestra (Tabla 1). La estimación de la exposición histórica en la cohorte EPIC-España y el análisis de sus factores predictores se llevó a cabo mediante un estudio transversal, en el que se describieron los niveles séricos de BPA y se investigaron las variables sociodemográficas, dietéticas y de estilo de vida asociadas, siendo BPA la variable dependiente.

**Tabla 1. Comparación entre participantes seleccionados y no seleccionados de toda la cohorte EPIC-España**

| | **Seleccionados** | **No seleccionados** |
|---|---|---|
| **N (%)** | 3553 (100) | 29342 (100) |
| **Centro** | | |
| Gipuzkoa | 909 (25,6) | 7508 (25,6) |
| Granada | 807 (22,7) | 7072 (24,1) |
| Murcia | 934 (26,3) | 7581 (25,8) |
| Navarra | 903 (25,4) | 7181 (24,5) |
| **Sexo** | | |
| Masculino | 1728 (48,6) | 10818 (36,9) |
| Femenino | 1825 (51,4) | 18524 (63,1) |
| **Edad** | | |
| <45 | 679 (19,1) | 10298 (35,1) |
| 45-49 | 572 (16,1) | 6380 (21,7) |
| 50-54 | 757 (21,3) | 5097 (17,4) |
| 55-59 | 687 (19,3) | 4213 (14,4) |

| | | |
|---|---|---|
| 60+ | 858 (24,1) | 3354 (11,4) |
| **Nivel educativo** | | |
| None | 1405 (39,8) | 10683 (36,6) |
| Primary school | 1242 (35,2) | 11010 (37,8) |
| Technical school | 281 (7,9) | 2365 (8,1) |
| Secondary school | 206 (5,8) | 1728 (5,9) |
| University | 392 (11,1) | 3376 (11,6) |
| **IMC** | | |
| <25 kg/m2 | 650 (18,3) | 6654 (22,7) |
| 25-<30 kg/m2 | 1789 (50,3) | 13768 (46,9) |
| ≥30 kg/m2 | 1114 (31,3) | 8920 (30,4) |
| **Estado fumador** | | |
| Nunca | 2114 (59,5) | 16292 (55,5) |
| Antiguo | 669 (18,8) | 5029 (17,1) |
| Fumador actual | 767 (21,59) | 8008 (27,3) |
| **Actividad física** | | |
| Inactivo | 528 (14,9) | 3904 (13,3) |
| Moderadamente inactivo | 807 (22,7) | 6059 (20,6) |
| Moderadamente activo | 1909 (53,7) | 17024 (58,0) |
| Activo | 309 (8,7) | 2355 (8,0) |
| **Consumo de energía** | | |
| Media (SD) – kcal/día | 2211 (704,4) | 2158 (680,9) |

IMC: índice de masa corporal

Para cubrir el **objetivo 2**, **estudio la relación entre los niveles de BPA y la incidencia de enfermedad coronaria isquémica de una sub-cohorte de EPIC-España al seguimiento,** se seleccionaron 3.690 participantes sanos de 4 centros EPIC-España (Guipuzkoa, Navarra, Murcia, Granada) y 946 casos de ECI (259 de Gipuzkoa, 158 de Granada, 204 de Murcia y 325 de Navarra). Se diseñó un estudio de caso-cohorte dentro de la cohorte EPIC-España en el que se describieron los niveles de BPA entre los casos y la sub-cohorte así como las posibles asociaciones observadas con ECI, tomando los niveles séricos de BPA como variable independiente.

Para los **objetivos 3 y 4**, **estudio la relación entre los niveles de contaminantes no persistentes y la incidencia de diabetes tipo 2 e hipertensión en la sub-cohorte de EPIC-Granada al seguimiento,** se diseñaron dos estudios longitudinales en la sub-cohorte EPIC-Granada en los que se usaron los niveles séricos de npEPs como variable independiente y la incidencia de cada patología de interés como variable dependiente. Se utilizó una subcohorte

seleccionada de 670 participantes reclutados en el centro EPIC-Granada, sin casos prevalentes de DM2 o HTA. Esta población fue seleccionada mediante muestreo aleatorio estratificado por sexo y edad con el fin de mantener la representatividad de la cohorte EPIC-Granada.

## 6.2 Aspectos éticos

Los participantes de la cohorte EPIC fueron informados en todo momento del procedimiento y tenían derecho a retractarse de su participación, que fue voluntaria. Durante todo este proceso se respetaron los principios de la declaración de Helsinki y los protocolos fueron aprobados por el Comité de Ética del Hospital de Bellvitge (Barcelona).

Ambos proyectos que enmarcan esta tesis (financiados por del Instituto de Salud Carlos III (Exps: PI14/00067, PI14/01716, PI14/01880, PI14/00556) y Contrato Miguel Servet (CP15/00193)) gozan de un dictamen favorable del Comité de Ética de la Investigación provincial de Granada.

## 6.3 Recogida de muestras biológicas y análisis químico

Durante el proceso de reclutamiento se recogieron 10 ml de sangre a los participantes, con 12-h de ayuno. Las muestras se han mantenido congeladas a -70ºC usando un software para la gestión de muestras y trazabilidad desarrollado previamente en el proyecto EPIC. Las muestras fueron almacenadas en nitrógeno líquido a -180ºC. Las muestras de suero recogidas en el reclutamiento fueron procesadas en se analizaron en la Plataforma de Cromatografía del Instituto de Investigación Biosanitaria de Granada (ibs. GRANADA).

Los niveles de BPA se cuantificaron en muestras de suero utilizando dos pajuelas de 0,5 ml después de la adaptación de una metodología previamente validada [175]. En resumen, el análisis de BPA se realizó mediante microextracción líquido-líquido dispersiva (DLLME) y cromatografía líquida de ultra alto rendimiento con detección de espectrometría de masas en tándem (UHPLC-MS / MS). Las muestras se descongelaron completamente a temperatura ambiente, se centrifugaron a 2600 g durante 10 minutos y se tomaron 0,75 ml para llevar a cabo el análisis. Para determinar la cantidad total de BPA (libre más conjugada) en suero, se añadió a cada muestra 50 µL de solución enzimática (β-glucuronidasa / sulfatasa) y se incubó a 37 ° C durante 24 h. El suero tratado se colocó en un tubo de vidrio con tapón de rosca de 15 ml y se

añadió 30 µL de la solución estándar sustituta (1.25 mg / L de BPA-d16). El suero se diluyó a 10,0 ml con solución acuosa de NaCl al 5% (p / v) y el pH se ajustó a 2,0. A continuación, se mezclaron 0,75 ml de acetona y 0,75 ml de triclorometano y se inyectaron rápidamente en la muestra acuosa con una jeringa. Después de agitación manual, centrifugación y evaporación del extracto, el residuo se disolvió con 100 µl de una mezcla que consiste en agua (0.1% de amoníaco) / acetonitrilo (0.1% de amoníaco), 70:30 (v / v), y finalmente se inyectaron 10 µl. en el sistema LC. El límite de detección (LOD) fue de 0.2 ng / ml. A los valores por debajo de LOD se les asignó el LOD dividido por la raíz cuadrada de 2.

Se han analizado un total de 7 productos químicos, Bisfenol A, 4 Parabenos (Metilparabeno, Etilparabeno, propilparabeno, butilparabeno) y 2 benzofenonas (Benzophenone-1 y Benzophenone-3).

## 6.4 Evaluación de variables de dieta y estilos de vida

La información sobre el estilo de vida y otros factores relacionados con la salud se obtuvo mediante un cuestionario administrado por el entrevistador al inicio del estudio con personal entrenado para tal efecto.

La información sobre la dieta habitual durante los últimos 12 meses se recopiló mediante una versión computarizada administrada por entrevista de un cuestionario de historia alimentaria que había sido validado previamente en España [176]. El cuestionario fue estructurado por comidas e incluyó una lista de 662 comidas y recetas comunes de cada región. Las recetas se desglosaron en alimentos simples, y la frecuencia de consumo de alimentos y recetas consumidas al menos dos veces al mes se registró y clasificó en 16 grupos de alimentos.

Las medidas de altura, peso y circunferencia de cadera y cintura se tomaron en el reclutamiento utilizando procedimientos estandarizados [177]. Incluyó preguntas sobre el nivel educativo, antecedentes de enfermedades previas, antecedentes de consumo de tabaco, actividad física, ocupación e historial reproductivo (Riboli et al., 2003). El IMC (kg / m²) se utilizó para clasificar a los sujetos en tres categorías: <25 kg / m2, 25- <30 kg / m2, ≥30 kg / m2. El nivel educativo se clasificó según cinco categorías: ninguna, primaria, secundaria, formación técnica o profesional y título universitario. El tabaquismo se resumió en tres categorías: nunca fumador,

exfumador y fumador actual. Se reunieron diferentes dominios de actividad física teniendo en cuenta la variación estacional. Un índice simple de actividad física de cuatro niveles (inactivo, moderadamente inactivo, moderadamente activo y activo) fue derivado y validado combinando actividad ocupacional y recreativa [178]. La ingesta total de energía se analizó como variable continua en kcal / día.

La variable "consumo de alimentos ultraprocesados" se definió como el porcentaje de calorías proporcionadas por los alimentos ultraprocesados a la ingesta diaria de energía. Cada elemento de la base de datos de alimentos se clasificó independientemente en una de las cuatro categorías de la clasificación NOVA (material complementario): alimentos no procesados o mínimamente procesados (grupo 1, por ejemplo, alimentos naturales como frutas), ingredientes culinarios (grupo 2, por ejemplo, aceite), alimentos procesados (grupo 3, p. ej. pescado enlatado) y alimentos ultraprocesados (grupo 4, p. ej. refrescos) (Material complementario 3). Las discrepancias sobre la clasificación se resolvieron mediante la investigación bibliográfica y la consulta de expertos. La variable se calculó utilizando la contribución total de energía a la ingesta diaria de energía de alimentos clasificados como ultraprocesados y divididos en cuartiles de consumo [179,180].

Todas estas variables se usaron como variables independientes para el objetivo específico 1 y como covariables en los demás objetivos específicos.

## 6.5 Variables clínicas

En los objetivos 2, 3 y 4, las variables objetivo de estudio fueron: la incidencia de enfermedad coronaria isquémica, la incidencia de diabetes tipo 2 y la incidencia de hipertensión en las poblaciones de estudio.

**Estudio de la relación entre los niveles séricos de BPA en el reclutamiento y la incidencia de enfermedad coronaria isquémica de una sub-cohorte de EPIC-España (objetivo 2).** Los casos de ECI se definieron como participantes con un diagnóstico de cardiopatía isquémica (es decir, códigos ICD10 I20-I25) durante el período de estudio. Los casos de ECI incluyeron casos de infarto agudo de miocardio (IAM), angina de pecho (PA) y paro cardíaco. Los casos de incidentes fueron identificados por personal capacitado a través del enlace de la base de datos

de EPIC con el Conjunto Mínimo Básico de Datos al Alta (CMBD), atención primaria, registros de ECV y registros de mortalidad de cada región. Posteriormente, los casos se confirmaron mediante la revisión de historias clínicas y la aplicación de los criterios diagnósticos de MONICA (255). Las fechas de incidencia se determinaron a partir del primer evento cardíaco diagnosticado en la historia clínica del participante y se excluyeron los casos prevalentes (participantes con diagnóstico de ECI antes del reclutamiento). Las fechas de finalización del seguimiento para la identificación de casos fueron: 31/12/2008 para Granada, 31/12/2012 para Murcia, 31/12/2011 para Navarra y 30/12/2013 para Guipuzkoa. La historia previa de hiperlipidemia e hipertensión fue autoinformada. La subcohorte y los casos se seleccionaron mediante un muestreo aleatorio estratificado por sexo y edad, excluyendo a las personas con enfermedades crónicas. En nuestra subcohorte, el 80% de los participantes proporcionaron una muestra de sangre en ayunas en el momento del reclutamiento, extraída entre las 6 am y las 11 am. El 20% restante de las muestras no se encontraba en ayunas. Sin embargo, podemos generalizar y asumir que la mayoría de nuestras muestras se tomaron durante las mañanas y en ayunas. Las características de nuestra muestra respecto al resto de la cohorte EPIC-España fueron similares en las variables incluidas en el estudio, salvo en la distribución por sexo y edad que fue deliberadamente diferente debido a la selección muestral estratificada. Se eliminaron del análisis los casos prevalentes y los casos en los que no se pudo determinar una fecha de diagnóstico.

**Estudio de la relación entre los niveles séricos de contaminantes no persistentes y la incidencia de diabetes tipo 2 e hipertensión arterial en la sub-cohorte de EPIC-Granada durante el seguimiento (objetivos 3 y 4).** La determinación de los casos incidentes de diabetes tipo 2 e hipertensión arterial se realizó de forma retrospectiva mediante la revisión de las historias clínicas de los pacientes en las bases de datos disponibles en el Sistema Sanitario Público de Andalucía. Se consideró diabético tipo 2 a un participante cuando se le había diagnosticado diabetes tipo 2 durante el tiempo de seguimiento y / o cuando se había registrado prescripción continua de medicación antidiabética en la historia clínica. También, se consideró hipertenso a un participante cuando había sido diagnosticado de HTA durante el tiempo de seguimiento y / o cuando se había registrado prescripción continua de antihipertensivos en la historia clínica. Se excluyeron los casos prevalentes de DM2 y HTA de los análisis estadísticos, que se identificaron mediante el autoinforme inicial de antecedentes de diabetes, el diagnóstico previo de un médico y / o la prescripción crónica de medicamentos para la diabetes. El tiempo de seguimiento comenzó en la fecha de reclutamiento de EPIC y continuó hasta el diagnóstico

de DM2 o HTA o la muerte del paciente. Si el participante no experimentó ninguno de estos eventos, se consideró el 31 de julio de 2017 como fecha final del seguimiento.

## 6.6 Análisis estadístico

El análisis estadístico fue diferente en cada uno de los artículos realizados para la consecución de los objetivos, si bien el descriptivo de las características de la población son similares. Se utilizaron los siguientes programas de análisis: Stata v14 (Stata Statistical Software: Release 14. College Station, TX: StataCorp LP), SPSS Statistics 22.0 (IBM, Chicago, IL) y R 3.0 (R Core team 2018) [181], utilizando los paquetes *pspline* [182] y *survival* [183,184].

Se resume a continuación específicamente las técnicas estadísticas usadas para cada objetivo:

### *Objetivo específico 1: Estudio de los determinantes de los niveles de exposición a Bisfenol A*

En el estudio de los determinantes de exposición a BPA, los niveles de BPA se transformaron usando un logaritmo natural para suavizar su fuerte distribución asimétrica y para fijar la suposición de normalidad de los residuos en los análisis de regresión. Las medias geométricas y los intervalos de confianza del 95% de los niveles de BPA (en ng / ml) se calcularon en general y según el centro, el sexo, el grupo de edad, el nivel educativo, el índice de masa corporal, la actividad física, el estado de fumador y la ingesta total de energía. Igualmente, las concentraciones séricas de npEP se expresaron como media geométrica (GM), mediana, intervalo de confianza del 95% (IC del 95%) y percentiles 25 y 75. A las concentraciones séricas de npEP por debajo del LOD se les asignó un valor de LOD / $\sqrt{2}$. Las diferencias entre variables categóricas se calcularon mediante la prueba de Chi-cuadrado. También se realizó un análisis de la correlación entre contaminantes mediante la prueba de correlación de Spearman.

Para el estudio de los determinantes de la exposición a BPA, las diferencias en los niveles de BPA entre las categorías de las posibles variables asociadas se evaluaron mediante la regresión de Tobit de efecto mixto. Se realizaron modelos univariados con cada variable como predictor, y finalmente se ajustó un modelo multivariado con todas esas variables para evaluar el efecto

independiente de cada variable. El exponencial del coeficiente de regresión menos uno multiplicado por 100 corresponde al porcentaje de cambio en los niveles de BPA para una categoría dada de una variable en relación con la categoría de referencia [185]. La variable que identifica el centro se incluyó en el modelo de regresión utilizando el enfoque de codificación de efectos [186], de modo que el coeficiente de cada centro compara su nivel promedio de BPA con la gran media. Este enfoque evita elegir una categoría de referencia, lo que no tiene sentido en este caso y obliga a omitir el resultado para uno de los centros.

Sin embargo, para el análisis de la dieta, 63 sujetos que tenían datos muy extremos de dieta (consumo extremadamente bajo o extremadamente alto de energía) fueron descartados y finalmente se analizaron 3.490 sujetos. Se utilizaron modelos de regresión de Tobit de efecto mixto para evaluar la asociación de la ingesta de cada grupo de alimentos con los niveles de BPA. El centro se incluyó como un efecto aleatorio en esos modelos. Por lo tanto, controlamos la correlación entre los participantes de la misma área geográfica. La ingesta de grupos de alimentos individuales se ajustó previamente para la ingesta total de energía mediante el método de regresión residual [187].

El ajuste para estos modelos se abordó utilizando 3 estrategias: A) sin ajustar con un efecto aleatorio a nivel central; B) ajustado por sexo y edad con un efecto aleatorio a nivel central; C) ajustado por sexo, edad e ingesta de todos los grupos de alimentos con un efecto aleatorio a nivel central.

### *Objetivo específico 2: Estudio de la relación entre los niveles séricos de BPA en el reclutamiento y la incidencia de enfermedad coronaria isquémica de una sub-cohorte de EPIC-España*

Para el estudio de la posible asociación de BPA con ECI, el tiempo de entrada se definió como la fecha de reclutamiento y el tiempo de salida como la fecha del diagnóstico de ECI, la muerte, la pérdida de seguimiento o el final del seguimiento específico del centro, lo que ocurra primero. Los tiempos hasta el evento de ECI se modelaron mediante modelos de riesgo proporcional de Cox ponderados por Borgan II (256), estratificados por centro. Se utilizaron errores estándar robustos como se recomienda en este diseño de cohortes de casos (257). Las razones de riesgo y los intervalos de confianza del 95% se derivaron de los modelos de Cox. El nivel de BPA, la principal variable independiente, se trató como variable continua y se transformó mediante un

logaritmo en base 2 para suavizar su distribución fuertemente asimétrica. Se utilizaron tres modelos de regresión de Cox: A) BPA lineal estratificado por centro; B) BPA transformado log2 estratificado por centro; C) BPA transformado log2 estratificado por centro y ajustado por sexo, edad, nivel educativo, IMC, actividad física, tabaquismo, consumo de alcohol, hiperlipidemia e hipertensión.

*Objetivos específicos 3 y 4: Estudio de la relación entre los niveles séricos de contaminantes no persistentes y la incidencia de diabetes tipo 2 e hipertensión arterial en la sub-cohorte de EPIC-Granada durante el seguimiento*

Todas las concentraciones séricas de npEP se transformaron logarítmicamente para minimizar la influencia de valores extremos; por lo tanto, los coeficientes β también se presentan como exp (β). BPA, MP, EP y BP3 también se categorizaron en quintiles y PP, BP y BP1 en cuartiles debido al mayor número de valores por debajo del LOD. Las posibles asociaciones de las concentraciones séricas de npEP y DM2 / HTA se evaluaron mediante el modelo de riesgos proporcionales de Cox multivariantes. La forma de las asociaciones se evaluó con modelos aditivos generalizados (GAM). Se comprobó el supuesto de riesgos proporcionales como parte del diagnóstico de los modelos, utilizando la función cox.zph implementada en el paquete de supervivencia R. Esta función correlaciona los residuos de Schoenfeld escalados con la estimación de Kaplan-Meier de la función de supervivencia. La edad, el sexo, el IMC y el nivel educativo se mantuvieron siempre en los modelos, independientemente de su significación estadística, dada la evidencia publicada de su posible asociación. Se realizaron análisis adicionales mediante estratificación por sexo e IMC. El nivel de significancia se fijó en $p = 0,05$.

# 7 Resultados

A continuación, se presentan cuatro artículos científicos publicados en revistas internacionales que responden a los objetivos del presente trabajo. Para su lectura se han ordenado por su posición en el proceso de evaluación del riesgo de la exposición a disruptores endocrinos.

Por esta razón, se presenta en primer lugar el trabajo de caracterización de los niveles de exposición y factores asociados a la misma de Bisfenol A en una sub-cohorte de EPIC España. Posteriormente, se describen los trabajos que presentan las posibles asociaciones con factores de síndrome metabólico de la cohorte EPIC Granada (enfermedad coronaria isquémica, diabetes e hipertensión). Se ha añadido la carátula de cada artículo en pdf pero no el artículo completo para evitar posibles conflictos en relación a los derechos de autor. Es por eso que se incluyen los manuscritos aceptados por la revista en cada caso.

La intención de esta tesis es que cada artículo pueda leerse de forma independiente, por lo que parte de la información presentada en cada artículo, especialmente aquella concerniente a los apartados de introducción y métodos, puede ser parcialmente repetitiva. La intención de esta repetición es proporcionar al lector toda la información necesaria para leer cada artículo de forma independiente.

## 7.1 Objetivo específico 1

1. Cuantificar los niveles de BPA en muestras de suero recogidas en el reclutamiento de una sub-cohorte de EPIC-España.

### 7.1.1 Determinantes de la exposición a Bisfenol A

**Bisphenol-A in the European Prospective Investigation into Cancer and Nutrition cohort in Spain: levels at recruitment and associated dietary factors**

Elena Salamanca-Fernández, Miguel Rodríguez-Barranco, Juan Pedro Arrebola, Fernando Vela, Caridad Díaz, María Dolores Chirlaque, Sandra Colorado-Yohar, Ana Jiménez-Zabala, Amaia Irizar, Marcela Guevara, Eva Ardanaz, Luz María Iribarne-Durán, José Pérez del Palacio, Nicolás Olea, Antonio Agudo, Maria-José Sánchez.

Publicado en Environmental Research [188]. https://doi.org/10.1016/j.envres.2019.109012.
Cuartil 1, Decil 1, Factor de impacto: 5.715

Environmental Research 182 (2020) 109012

Contents lists available at ScienceDirect

Environmental Research

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

# Bisphenol-A in the European Prospective Investigation into Cancer and Nutrition cohort in Spain: Levels at recruitment and associated dietary factors

Elena Salamanca-Fernández[a,b], Miguel Rodríguez-Barranco[a,b,c,*], Juan Pedro Arrebola[b,c,d], Fernando Vela[b], Caridad Díaz[e], María Dolores Chirlaque[c,f,g], Sandra Colorado-Yohar[c,f,h], Ana Jiménez-Zabala[c,i,j], Amaia Irizar[j], Marcela Guevara[c,k,l], Eva Ardanaz[c,k,l], Luz María Iribarne-Durán[b,m], José Pérez del Palacio[e], Nicolás Olea[b,c,m], Antonio Agudo[n], Maria-José Sánchez[a,b,c,o]

[a] Andalusian School of Public Health (EASP). Granada, Spain
[b] Instituto de Investigación Biosanitaria ibs.GRANADA. Granada, Spain
[c] CIBER de Epidemiología y Salud Pública (CIBERESP). Madrid, Spain
[d] Department of Public Health, School of Medicine, University of Granada. Granada, Spain
[e] MEDINA Foundation, Center of Excellence in Research into Innovative Medicines in Andalusia, Technology Park of Health Sciences, Granada, Spain
[f] Department of Epidemiology, Murcia Regional Health Council, IMIB-Arrixaca, Murcia, Spain
[g] Department of Health and Social Sciences, University of Murcia, Spain
[h] Research Group on Demography and Health, National Faculty of Public Health, University of Antioquia, Medellín, Colombia
[i] Public Health Division of Gipuzkoa, Basque Government, Avenida Navarra No 4, 20013, San Sebastián, Gipuzkoa, Spain
[j] Health Research Institute, Biodonostia, San Sebastián, Spain
[k] Navarra Public Health Institute, Pamplona, Spain
[l] IdiSNA, Navarra Institute for Health Research, Pamplona, Spain
[m] Department of Radiology, School of Medicine, University of Granada. Granada, Spain
[n] Unit of Nutrition and Cancer, Catalan Institute of Oncology - ICO, Nutrition and Cancer Group, Bellvitge Biomedical Research Institute - IDIBELL, L'Hospitalet de Llobregat, Barcelona 08908, Spain
[o] Universidad de Granada. Granada, Spain

ARTICLE INFO

*Keywords:*
Exposure factors
Biomonitoring
Bisphenol a
Endocrine disruptor
Diet

ABSTRACT

Bisphenol A (BPA) is considered an endocrine disruptor and it is present in numerous products of daily use. The aim of this study was to analyze serum BPA concentrations in a subcohort of the Spanish European Prospective Investigation into Cancer and Nutrition (EPIC), as well as to identify potential predictors of the exposure. The population consisted on 3553 subjects from 4 EPIC-Spain centres and BPA levels were measured in serum samples by UHPLC-MS/MS. Almost 70% of the participants showed detectable BPA values (> 0.2 ng/ml), with a geometric mean of 1.19 ng/ml (95% CI: 1.12–1.25). By sex, detectable percentages were similar ($p = 0.56$) but with higher serum levels in men (1.27 vs 1.11 ng/ml, $p = 0.01$). Based on the adjusted regression models, a 50 g/day increase in the consumption of added fats and oils were associated with 43% lower BPA serum levels, while sugar and confectionary was associated with 25% higher levels of serum BPA. We evidenced differential exposure levels by province, sex and age, but not by anthropometric or lifestyle characteristics. Further investigation is needed to understand the influence of diet in BPA exposure.

## 1. Introduction

Bisphenol A (BPA) is an industrial chemical that was first developed in the 1890s and is now one of the highest-volume chemicals produced worldwide, with an output of 372,000 tons in 2012 (Mcgroup, 2013). BPA is a synthetic oestrogen that is widely used in the manufacture of polymers and epoxy resins, polycarbonates and polysulphones plastics. It is also used as an additive in polyvinyl chloride (PVC), acrylonitrile butadiene styrene (ABS), and polystyrene (Hahladakis et al., 2018; Rezg et al., 2014) and is part of a great variety of everyday products such as

* Corresponding author. Andalusian School of Public Health (EASP), Campus Universitario de Cartuja, C/Cuesta del Observatorio 4, 18080, Granada, Spain.
*E-mail address:* miguel.rodriguez.barranco.easp@juntadeandalucia.es (M. Rodríguez-Barranco).

https://doi.org/10.1016/j.envres.2019.109012
Received 24 September 2019; Received in revised form 26 November 2019; Accepted 5 December 2019
Available online 07 December 2019
0013-9351/ © 2019 Elsevier Inc. All rights reserved.

## ABSTRACT

Bisphenol A (BPA) is considered an endocrine disruptor and it is present in numerous products of daily use. The aim of this study was to analyze serum BPA concentrations in a subcohort of the Spanish European Prospective Investigation into Cancer and Nutrition (EPIC), as well as to identify potential predictors of the exposure. The population consisted on 3,553 subjects from 4 EPIC-Spain centers and BPA levels were measured in serum samples by UHPLC-MS / MS. Almost 70% of the participants showed detectable BPA values (>0.2 ng/ml), with a geometric mean of 1.19 ng / ml (95% CI: 1.12-1.25). By sex, detectable percentages were similar (p = 0.56) but with higher serum levels in men (1.27 vs 1.11 ng / ml, p = 0.01). Based on the adjusted regression models, a 50 g/day increase in the consumption of added fats and oils were associated with 43% lower BPA serum levels, while sugar and confectionary was associated with 25% higher levels of serum BPA. We evidenced differential exposure levels by province, sex and age, but not by anthropometric or lifestyle characteristics. Further investigation is needed to understand the influence of diet in BPA exposure.


***Keywords**:* Exposure factors; biomonitoring; Bisphenol A; endocrine disruptor; diet

## 1. Introduction

Bisphenol A (BPA) is an industrial chemical that was first developed in the 1890s and is now one of the highest-volume chemicals produced worldwide, with an output of 372,000 tons in 2012 [72]. BPA is a synthetic oestrogen that is widely used in the manufacture of polymers and epoxy resins, polycarbonates and polysulphones plastics. It is also used as an additive in polyvinyl chloride (PVC), acrylonitrile butadiene styrene (ABS), and polystyrene [87,189] and is part of a great variety of everyday products such as food packaging, medical and dental devices, CDs and DVDs, inks and toners. Its ubiquity means that the general population is frequently and inadvertently exposed to this compound [73,78].

BPA is considered a non-persistent chemical, i.e., it is eliminated from the organism (half-life in humans: 7-8 hours), ), despite the constant level of human exposure [74]. It is estimated that over 90% of the population in the US, Europe and Asia is exposed to BPA, with detectable levels in urine (>0.4 ng/ml) [75–78]. BPA has also been detected in the serum of the general population and in pregnant women in the placenta, breast milk and amniotic fluid [190–194]. Humans are exposed to BPA through several routes: food (orally), occupation (inhalation) and contact (dermal) via plastic-type materials and medical devices [73,195]. However, the main exposure route is through diet, as many forms of food packaging such as tins and plastic wrap contain BPA, which migrates towards the food consumed, especially with heat [73,87–91].

Once absorbed in the intestine, BPA is readily glucuro-conjugated or sulpho-conjugated in the liver, until it is finally excreted in urine [196]. BPA concentrations in biological matrices are commonly expressed as the sum of conjugated and unconjugated BPA (total BPA), but also as free BPA, which is considered the biologically active form [197].

BPA is known to be an endocrine disruptor, which means it has the ability to interfere with the production, secretion, transport, action, function and elimination of natural hormones, even at very low doses [198]. Recent laboratory studies have reported an oestrogenic potential of BPA in experimental animals [81–85]. However, it can also act as an anti-oestrogen by competing with the endogenous hormone 17-beta oestradiol [86]. Studies have shown that environmental exposure to BPA could play a role in cancer, insulin resistance, and obesity [51,189,199]. However, its potential effects on human health remain controversial due to the lack of large prospective studies in this respect.

In 2015, increasing concerns about the potential health effects of BPA exposure led the European Food Safety Authority (EFSA) to reduce the tolerable daily intake of BPA from 50 to 4 μg/kg per day and this recommendation will be reviewed in 2020 [90]. Moreover, the General Court of the European Union recently confirmed the inclusion of Bisphenol A as a substance of very high concern [92].

In Spain, some studies have assessed BPA exposure in different populations and biological tissues (Supplementary Material Table 1). There are several studies assessing BPA in urine samples in children and their mothers, , in human milk or in hospitalised patients [51,200–203], as well as in adult population [204]. To our knowledge, therefore, this could one of the first studies assessing exposure to BPA among the adult general population and its possible determinants in Spain.

The aim of the present study is to characterise the exposure to BPA in a sub-cohort of the Spanish European Prospective Investigation into Cancer and Nutrition (EPIC) cohort by an analysis of serum BPA concentrations as well as to identify the potential dietary determinants of exposure.

## 2. Methods

*Study design*

This cross-sectional study was conducted of a sub-cohort of EPIC-Spain. EPIC is a prospective multi-centre cohort study undertaken to investigate the relationship between diet, lifestyles and cancer. It involves 23 research centres in ten European countries, with five centres in Spain: Asturias, Granada, Murcia, Navarra and Gipuzkoa [165]. The study participants reported information about their dietary, lifestyle, reproductive and anthropometric factors at baseline.

*Study population*

In the EPIC-Spain study, a total of 41,446 participants aged 29-69 years were enrolled between 1992 and 1996 in five provinces of Spain. The majority (60%) of these participants were recruited from blood donors and the study population included a broad range of socioeconomic and educational levels. At recruitment, a fasting blood sample was obtained from each participant. Signed informed consent was obtained in every case and the study was approved by the Ethics Committee of the Bellvitge Hospital (Barcelona).

A sub-cohort of 1,000 participants from each centre was selected using stratified random sampling by sex and age, excluding persons with chronic disease. In our sub-cohort, 90% of the participants provided a fasting blood sample at recruitment, extracted between 6 am and 11 am. The remaining 10% of the samples did not have the fasting status. However, we can generalize and assume most of our samples were taken during the mornings and in fasting conditions. After excluding the samples considered inadequate (insufficient serum volume, or decayed serum sample during chemical analysis), the final study population analysed consisted of a sub-cohort of 3,553 participants from the four EPIC-Spain centres (807 from Granada, 934 from Murcia, 903 from Navarra and 909 from Gipuzkoa). The characteristics of our sample regarding the rest of the EPIC-Spain cohort were similar for the variables included in the study, except for the distribution by sex and age that was deliberately different due to the stratified sample design used to extract the sample (Supplementary Material Table 2).

*Assessment of diet and lifestyle variables*

Information on lifestyle and other health-related factors was obtained by an interviewer-administered questionnaire at baseline. All interviewers had received appropriate training for this task.

Information on the usual diet over the last twelve months was collected by means of an interviewer-administered computerised version of a dietary history questionnaire that had been previously validated in Spain [173,176]. The questionnaire was structured by meals and included a list of 662 common foods and recipes from each region. Recipes were broken down into simple foods, and the frequency of consumption of foods and recipes consumed at least twice a month was recorded and classified into 16 food groups.

Measurements of height, weight, and hip and waist circumferences were taken at recruitment using standardised procedures [177]. The questionnaire included items on educational level, history of previous illnesses, history of tobacco use, physical activity, occupation and reproductive history [177]. The participants were classified into three categories by body mass index (BMI): $<25$ kg/m$^2$, 25-$<30$ kg/m$^2$, $\geq 30$ kg/m$^2$. Educational level was classified according to five categories: none, primary school, secondary school, technical or vocational training and university degree. Smoking status was summarised in three categories: never smoked, former smoker and current smoker. Information on the domains of physical activity was compiled

taking seasonal variation into account. A simple four-level physical activity index (inactive, moderately inactive, moderately active and active) was derived and validated by combining occupational and recreational activity [178]. Total energy intake was analysed as a continuous variable in kcal/day.

The variable 'consumption of ultra-processed foods' was defined as the percentage of calories provided by ultra-processed foods to the daily energy intake. Each item on the food database was independently classified in one of the four categories of the NOVA classification (supplementary material): unprocessed or minimally processed foods (group 1, e.g. natural foods like fruits), culinary ingredients (group 2, e.g. oil), processed foods (group 3, e.g. canned fish) and ultra-processed foods (group 4, e.g. soft drinks) (Supplementary Material 3). Discrepancies over classification were resolved through literature research and expert consultation. The variable was calculated using the total energy contribution to daily energy intake from foods classified as ultra-processed, and divided into quartiles of consumption [179,180].

*Sample collection and chemical analyses*

Blood samples were drawn from each participant at recruitment. The samples were then centrifuged, and aliquots of plasma, serum, red blood cells and buffy coat in 0.5 mL straws were stored in liquid nitrogen (-196ºC).

BPA levels were quantified in serum samples using two of 0.5 mL straws, in an adaptation of a previously-validated methodology [175]. In brief, BPA was analysed by dispersive liquid–liquid micro-extraction (DLLME) and ultra-high performance liquid chromatography with tandem mass spectrometry detection (UHPLC-MS/MS). Samples were thawed completely at room temperature, centrifuged at 2600g for 10 min and 0.75 mL was extracted for analysis. In order to determine total BPA (free plus conjugated) in serum, each sample was spiked with 50µL of enzyme solution (β-glucuronidase/sulphatase) and incubated at 37 °C for 24 h. The treated serum was placed in a 15 mL screw-cap glass tube and spiked with 30µL of the surrogate standard solution (1.25 mg/L of BPA-d16). The serum was then diluted to 10.0 mL with 5% NaCl aqueous solution (w/v) and the pH was adjusted to 2.0. Next, 0.75 mL of acetone and 0.75 mL of trichloromethane were mixed and injected rapidly into the aqueous sample with a syringe. After manual shaking, centrifugation and evaporation of the extract, the residue was dissolved with 100µL of a mixture consisting of water (0.1% ammonia)/acetonitrile (0.1%

ammonia), 70:30 (v/v), and finally 10µL was injected into the LC system. Limit of detection (LOD) was 0.2 ng/ml. Values below LOD were assigned the LOD divided by the square root of 2.

*Statistical analysis:*

The BPA levels were transformed using natural logarithms to smooth their strong asymmetric distribution and to assure compliance with the normality assumption of the residuals in the regression analyses. Geometric means and 95% confidence intervals of the BPA levels (in ng/ml) were calculated overall and according to centre, sex, age group, educational level, body mass index, physical activity, smoker status and total energy intake. The differences in the BPA levels across the categories of the potential associated variables were assessed by mixed-effect Tobit regression. Tobit regression is suitable to estimate linear relationships between variables when there is either left- or right-censoring in the dependent variable, as happens in the case of BPA levels with a minimum detection limit. On the other hand, mixed-effect models, including a random effect at center level, allow to model the non-independence in data for the clustering of individuals in the same province of residence. Univariate models were generated with each variable as a predictor, and finally a multivariate model including all the study variables was adjusted to identify the independent effect of each variable. The exponential of the regression coefficient minus one multiplied by 100 corresponded to the percentage of change in BPA levels for a given category of a variable relative to the reference category [185].

The variable identifying the centre was included in the regression model using the effect coding approach [186], so that the coefficient for each centre compared its BPA average level with the overall mean. This approach avoids the need to choose a reference category, which would not be meaningful in this case and would force us to omit the result for one of the centres.

For the diet analysis, 63 subjects who presented extreme data in this respect (extremely low or extremely high consumption of energy) were discarded. Thus, 3,490 participants were finally analysed. Mixed-effect Tobit regression models were used to assess the association of each food group intake with BPA levels, and in these models the centre was included as a random effect, thus controlling for the correlation between participants from the same geographic area. The intake of individual food groups was previously adjusted for total energy intake by the residual regression method [187].

The models were adjusted using three strategies: A) unadjusted with a random effect for the centre; B) adjusted for sex and age with a random effect for the centre; C) adjusted for sex, age, and intake of all foods groups with a random effect for the centre.

Statistical analysis was conducted with Stata v14 (Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

## 3. Results

In our study population, the average age of the participants was 53 years (range 30-69), and 49% were men. At baseline, 50.4% of them had overweight and almost 60% had never smoked (Table 1). Differences between centres were observed in all the participants' characteristics, including the percentage of energy intake obtained from ultra-processed foods (highest in Murcia and Navarra) and the mean energy intake (Kcal/ day) (Table 1).

Of the 3,553 samples analysed, 2,476 (69.7%) had detectable BPA values, with a geometric mean of 1.19 ng/ml (95% CI: 1.12-1.25) (Table 2). There were significant differences in BPA concentrations according to the centre, with the highest concentrations in Granada (1.83 ng/ml; 95% CI: 1.64 to 2.04) and the lowest in Gipuzkoa (0.67 ng/ml; 95% CI: 0.61 to 0.75). The adjusted regression analysis revealed that the participants from Granada and Navarra had, respectively, 84% and 34% significantly higher levels of BPA in serum than the overall sample, while the levels of those from Gipuzkoa were 56% lower than the mean. The levels recorded in the participants from Murcia did not vary significantly from the overall average (Table 2).

By sex, the detectable BPA values were similar (about 70%), although they were lower in women (unadjusted geometric mean 1.11 vs 1.27 ng/ml, respectively), and 32% lower according to the adjusted models (Table 2). Comparison of age groups revealed an inverted V shape, with higher values in the central age group (1.31 ng/dl for the participants aged 45-54 years). Those aged 45-49 and 50-54 years had 35% and 34% significantly higher levels of BPA, respectively, than the younger age-group (<45), but those older than 55 years had similar levels to those of the participants who were younger than 45 years (Table 2). No significant associations were observed between BPA levels and BMI, educational level, physical activity or smoking status. However, the former smokers had 19% lower levels than the never-smokers, and the participants with a university education had 24% lower levels than those with no formal education. Regarding the energy obtained from ultra-processed food, the participants in the

second and third tertiles presented 9% and 20% higher levels of BPA, respectively, than those in the first tertile. A borderline significant negative association was observed between total energy intake and BPA levels (Table 2).

Based on the adjusted Tobit regression models for diet, a 50 g/day increase in the intake of added fats and oils (fats used for seasoning or cooking, such as olive oil, sunflower oil or butter) was associated with a 43% lower level of serum BPA (95% CI: 0.36 – 0.89) (Table 3). This association was consistent in the three adjusted models. A positive association, borderline significant, was also observed between sugar and confectionery (honey, jam, chocolate, sweet bars, ice cream, etc.) and 25% higher levels of serum BPA (95% CI: 1.00 – 1.56), which was detected in adjusted model C (Table 3).

Analysis of individual oils showed that the strongest association of lower serum BPA levels was for mixed oils (51% lower levels (95% CI: 0.19 – 1.25) followed by sunflower oil (49% lower levels (95% CI: 0.28 – 0.96)) (Table 4). In our cohort, however, olive oil was the most commonly consumed source of fats, by 86.8% of participants, and was associated with a 35% lower serum BPA level (95% CI: 0.43 – 0.97) (Table 4).

## 4. Discussion

BPA was frequently detected in our study population. The highest levels were found in men recruited in Southern Spain (Granada). Serum concentrations in our participants (GM= 1.19 ng/mL) were somewhat lower (although of a similar order of magnitude) than those observed in other countries, such as (date of the collection of the serum sample): Japan 1998 (GM= 2.24 ng/ml), Japan 2004 (GM= 2.5 ng/ml), USA 2008 (GM= 5.9 ng/ml), Italy 2009 (GM= 2.91 ng/ml), China 2015 (GM= 9.73 ng/ml in working time ≤ 5 years and 27.18 ng/ml in working time > 5 years), China 2014 (GM= 1.50 ng/ml), Korea 2012 (GM= 1.56 ng/ml) and China 2015 (GM= 3.2 ng/ml) [33–40]. However, several previous studies have reported lower concentrations than those found in our study population, including Thailand 2009 (GM= 0.34 ng/ml), Japan 2003 (GM= 0.46 ng/ml), Japan 2004 (GM= 1.17 ng/ml) [205–207] and Spain (GM= 0.58 ng/ml) [204]. In order to compare different studies, the date of sampling should be taken into account, since most previous research has been performed on populations recruited years or decades after the study period corresponding to our cohort (1992-1996). Date of sampling is relevant because during the 90s plastic containers or canned food were less usual as they were during the following years up to date. Being food the main rout of BPA exposure,

dietary habits have also been gradually changed to use pre-cooked meals more frequently. However, due to the increasing concern of BPA, some analogues have raised their use in substitution of BPA in the recent years and therefore it would be expected that in new exposure measures, BPA biological levels may be lower [208].

In our population, the main determinants of BPA serum levels were sex, recruitment centre, and diet. Regarding dietary predictors, sugar and confectionery consumption was positively associated with serum BPA levels and added fats and oils was negatively associated with BPA.

In our sub-cohort, the consumption of added fats and oils was mainly that of vegetable oils, particularly olive oil, which accounted for 70.4% of total added fats and oils consumed, and sunflower oil with 12.2%. These items, together with mixed and unspecified oils, constituted 93% of the consumption in this main group. In our opinion, it is difficult to assess the negative association between BPA levels and the consumption of olive oil, as information on olive oil packaging was lacking at the time of recruitment. Regarding packaging, a study of BPA in Mediterranean olive oil revealed higher BPA levels in samples stored in plastic vs. non-plastic packaging (B = 121.56, 95% CI 53.44-194.39, p value = 0.009) [209]. Similar findings were obtained in a study of canned tuna, where the levels of bisphenols detected were higher than the mean values for oil [210]. Some studies show that BPA is slightly lipophilic [211,212]. and one reported finding detectable BPA concentrations in 86.8% of the adipose tissue samples from an adult cohort (GraMo cohort) [51]. Some chemical substances similar to BPA present a log of the octanol-water partition coefficient (Kow) for phenols and parabens ranging from 1 to 5; in consequence, they should be considered at least partially lipophilic compounds that would potentially be distributed in adipose tissues [213]. Therefore, because of their presence in food packaging, it seems reasonable to conclude that when there is an environmental release, such as the migration of BPA from different types of plastic packaging [73,86,88], (Geens et al., 2012a; López-Cervantes and Paseiro-Losada, 2003; Rochester, 2013), foods cooked with fats (which, in our country, generally means olive oil) would have lower levels of BPA. Our results show that a 50 g/day increase in the consumption of added fats and oils is associated with 43% lower levels of BPA in serum. However, account should be taken of the lack of information about olive oil packaging in this respect when the study participants were recruited. Furthermore, the negative association observed between olive oil consumption and BPA serum levels might be due to biological mechanisms and/or hepatic metabolism. Thus, olive oil is expected to exert a protective effect related to liver metabolism, as shown by studies according

to which olive oil phenols inhibit human hepatic microsomal activity [214]. Therefore, it could be hypothesized that olive oil could be playing a role in BPA metabolism and elimination. Also, it could be assumed that olive oil consumption and its protective effect against BPA could be reflecting a higher adherence to a Mediterranean diet. Mediterranean diet is inversely associated with BPA levels [215] as it appear to represent less BPA migrating from food packaging and microwave containers [215].

In our cohort, the intake of sugar and confectionery was positively associated with serum BPA levels. In this regard, Larsson et al. found higher levels of BPA in children who often ate chocolate and suggested this might reflect a more frequent consumption of foods contaminated from food wrapping materials [216]. In line with this view, as said before, Rivas *et al*. [215] concluded in their dietary study that adherence to a Mediterranean diet (with very low or zero consumption of confectionery) was inversely associated with BPA levels in human matrices. However, evidence shows that BPA dietary exposure is more determined by food packaging than the food by itself. Therefore, this association could the reflecting some dietary habits more related to BPA exposure as people that consume more confectionary products may be consuming more canned foods, precooked meals or soft drinks, which are products related to BPA exposure [217–219].

Our results also highlighted the existence of a positive association with ultra-processed food consumption. This relationship corroborates that found for sugar intake, as ultra-processed foods usually include high levels of sugars [220–222]. However, although sugar and confectionery products are usually wrapped in plastic, canned foods have also been shown to make a major dietary contribution to BPA levels [223].

Overall our results suggest that dietary exposure to BPA goes beyond the individual food items, and might be affected by the different methods of cooking, packing or preparing food – which, in fact, could be the key route of exposure to BPA.

We found significant gender-related differences in exposure, with men having significantly higher levels of BPA in serum than the women in our study. Previous studies, too, have reported significantly higher BPA concentrations in male plasma than in that of females [224] [225]. On the other hand, González *et al.* [204] found BPA levels 2-fold higher in female workers (0.68 and 1.20 μg/L in men and women, respectively), but this difference did not reach the level of

statistical significance ($p < 0.05$) and may be related to the different work place among gender in their study. However, other studies have found no significant differences in this respect [40,226]. Gender differences in BPA levels could be explained through the mechanisms by which BPA is metabolised in the liver. Biologically, hepatic physiology differs by sex; men have more liver enzymes than women, [227], and so they should eliminate BPA faster. In fact, however, the men in our cohort had higher levels of BPA than the women. Accordingly, unmeasured variables, such as life habits, occupation or even biological reasons must be assumed to play a role in this context. The smoking habit, high androgen levels in blood and dietary habits are other possible explanations of the higher BPA concentrations observed in our male participants. In a related study, Takeuchi *et al.* [228] analysed the serum concentrations and the metabolism of BPA in rats, finding significantly higher concentrations in the males than in the females. These authors commented that the gender difference in serum BPA concentrations might be explained by differences in clearance, according to the resultant enzyme activities. In our own analysis, male participants have significantly higher levels of BPA in serum than female. In this regard, we constructed gender-adjusted models to study the association with added fats and oils and we observed that consumption of added fats and oils was inversely associated with serum BPA levels in women. However, when the study group was stratified by sex, this association was not observed in men. The protective effect of added fats and oils against BPA levels that we observed only in women could be interpreted as reflecting differences in vegetable oil consumption among the participants in our cohort, in which olive oil was consumed by more women than men (52.3% vs 47.7%, respectively; data not shown). Moreover, as stated above, olive oil could play a role in the hepatic metabolism and exert a protective effect [214]. Moreover, this metabolism differs between the sexes [227]. On the other hand, and despite the differences observed, stratifying the participants by sex reduced the statistical power of our analysis and therefore we decided to combine both sexes in the dietary models constructed.

Age and BPA were positively associated in our cohort. However, an earlier study observed no significant age-related differences in blood BPA concentration in people living in Shanghai, China [225]. Some publications have reported higher urinary levels of BPA in younger adults [51,229], probably reflecting their greater consumption of bottled water [230,231] and food packaged in plastic containers [232]. Moreover, other studies have suggested that non-persistent pollutants such as BPA may not be completely excreted, and that a proportion may be stored in body compartments [233,234]. In this respect, a pharmacokinetic study revealed that

unconjugated BPA levels remained for up to 20 h in the adipose tissue, whereas serum concentrations were rapidly converted (< 5 h) into the non-oestrogenic BPA monoglucuronide-iso-form [234].

Our study has some limitations, being one the design of our study, as being a cross-sectional study we may be overestimating or underestimating BPA exposure through time. Another limitation would be the type of dietary information compiled at recruitment, since no questions referred to the use or otherwise of precooked containers or meals, which at the time (the 1990s) were less common than today. It could be hypothesised that the packaging of food, together with its processing, preparation and cooking mechanism, could be even more important than the frequency of consumption alone. This would be especially true of the use of canned food and beverages, as BPA is part of the material from which these containers are manufactured. Moreover, the migration of BPA from canned foods and beverages has been established [235] and its migration from canned foods has been recorded in infant products, canned fish and meat products, canned vegetables, canned soft drinks, coffee and sauces [89,217,236–240]. Another limitation of the present study might be the biological matrix where BPA was measured, as serum presents greater variability than urine as regards BPA exposure. Serum BPA concentrations can be relatively unstable, representing recent exposures [197,241]. Its representativeness of long-term exposures can only be assumed when external, lifestyle and biological determinants of serum concentrations remain constant at a certain degree, which is unlikely in a cohort study with a long follow-up time. Some studies point out that, among the biomonitoring matrices, urine contains the highest BPA concentrations, followed by serum [39] which implies a greater capacity to detect levels of exposure and also an improved estimator of medium-term exposure. In this regard, Spanish cohort included members of local blood donor associations. Blood samples were taken in fasting conditions and they were aliquoted into blood plasma, blood serum, white blood cells and erythrocytes. In this study, we only had the one single sample per participant taken at recruitment, and therefore we assumed BPA exposure to be similar through the follow-up period. The estimated exposure of the general population to BPA from canned food may reach a body burden of 9 µg/kg/day (Commission, 2002). However, BPA levels in the organism are not stable: levels are highest immediately following exposure and are not metabolised and excreted until seven or eight hours after incorporation into the body. Fortunately, BPA is rapidly conjugated and excreted by humans, due to its efficient glucuronidation [242]. Other pathways of BPA exposure include dust inhalation and dermal absorption, which can be a predominant source of occupational exposure in electronic

waste recycling plants and production facilities of BPA-containing materials [79,80]. Finally in this respect, our study does not take occupational exposure into account, as the production facilities of BPA were not included in the EPIC questionnaire at recruitment.

On the other hand, our study also has a number of strengths. The population considered is large and well representative of exposure to BPA in the 1990s. This characteristic is valuable, enabling us to study possible associations between BPA exposure at recruitment and certain chronic illnesses currently present in the participants. In this respect, it is of notable importance that almost 70% of the study population had detectable levels of BPA. In addition, we identify certain determinants of exposure. Although the cohort was recruited during the 1990s, analysis of our study results reveals the temporal evolution of BPA levels, both by comparing our levels with those obtained for more recent populations and also by comparing them with the current levels in our cohort. Our research group is currently working to determine the possible long-term implications of the levels found and their temporal evolution, by following up the EPIC subcohort. We expect to find that in the updated measures of BPA exposure among our cohort, serum levels will be lower due to the extended use of BPA substitutes BPS and BPF [208]. Moreover, we accomplished the objectives of this study as we measured BPA serum concentrations in our population at recruitment as well as we identified potential dietary determinants of the exposure. In this regard, food assessment of the diet of this study and its statistical management are some of the novelties of this study.

In conclusion, almost 70% of the study population had detectable levels of BPA, in a range similar to that found for other populations. We identify certain predictors of exposure, although this finding needs to be confirmed in further research. The historical characterisation of BPA exposure obtained in the present study represents a first step towards an assessment of the long-term implications of BPA exposure in the EPIC cohort. Further investigation is needed to understand the influence of diet on BPA exposure.

## Conflict of interest

None declared.

## Acknowledgements

The authors thank Pilar Guallar-Castillón from Department of Preventive Medicine and Public Health, School of Medicine, Universidad Autónoma de Madrid and Catalina Bonet from the Catalan Institute of Oncology-Bellvitge Biomedical Research Institute, Barcelona, for their contributions to the manuscript. This paper will form part of the doctoral thesis developed by Elena Salamanca-Fernández in the context of the "Clinical Medicine and Public Health Program" of the University of Granada (Spain).

## Funding

This work was supported by the Ministry of economy and competitiveness and the National Institute of Health: Instituto de Salud Carlos III (ISCIII). Exps: PI14/00067, PI14/01716, PI14/01880, PI14/00556, BA15/00093 (Co-funded by European Regional Development Fund. ERDF, a way to build Europe). AECC Junta Provincial de Murcia, Exp.: FFIS-CC 2016-06. Dr. J.P. Arrebola is under contract within Ramon y Cajal program (RYC-2016-20155, Ministerio de Economía, Industria y Competitividad, Spain).

## Ethics

This paper includes human samples for the investigation. All participants were informed at recruitment and they signed an informed consent. This study was approved by Ethics Committee of the Bellvitge Hospital (Barcelona).

**Table 1. Characteristics at recruitment of EPIC sub-cohort participants by center.**

| | Total | Gipuzkoa | Granada | Murcia | Navarra | p |
|---|---|---|---|---|---|---|
| **N (%)** | 3553 (100) | 909 (25.6) | 807 (22.7) | 934 (26.3) | 903 (25.4) | |
| **Sex** | | | | | | 0.65 |
| Male | 1728 (48.6) | 437 (48.1) | 407 (50.4) | 455 (48.7) | 429 (47.5) | |
| Female | 1825 (51.4) | 472 (51.9) | 400 (49.6) | 479 (51.3) | 474 (52.5) | |
| **Age** | | | | | | 0.02 |
| <45 | 679 (19.1) | 163 (17.9) | 178 (22.1) | 187 (20.0) | 151 (16.7) | |
| 45-49 | 572 (16.1) | 149 (16.4) | 109 (13.5) | 141 (15.1) | 173 (19.2) | |
| 50-54 | 757 (21.3) | 198 (21.8) | 182 (22.6) | 206 (22.1) | 171 (18.9) | |
| 55-59 | 687 (19.3) | 170 (18.7) | 147 (18.2) | 176 (18.8) | 194 (21.5) | |
| 60+ | 858 (24.1) | 229 (25.2) | 191 (23.7) | 224 (24.0) | 214 (23.7) | |
| **Education level** | | | | | | <0.01 |
| None | 1405 (39.8) | 266 (29.4) | 376 (47.3) | 479 (51.4) | 284 (31.7) | |
| Primary school | 1242 (35.2) | 390 (43.1) | 201 (25.3) | 215 (23.1) | 436 (48.7) | |
| Technical school | 281 (7.9) | 143 (15.8) | 29 (3.6) | 36 (3.9) | 73 (8.2) | |
| Secondary school | 206 (5.8) | 51 (5.6) | 54 (6.8) | 47 (5.09) | 54 (6.0) | |
| University | 392 (11.1) | 54 (6.0) | 135 (17.0) | 155 (16.6) | 48 (5.4) | |
| **BMI** | | | | | | <0.01 |
| <25 kg/m2 | 650 (18.3) | 236 (26.0) | 110 (13.6) | 147 (15.7) | 157 (17.4) | |
| 25-<30 kg/m2 | 1789 (50.3) | 488 (53.7) | 398 (49.3) | 462 (49.5) | 441 (48.8) | |
| ≥30 kg/m2 | 1114 (31.3) | 185 (20.4) | 299 (37.1) | 325 (34.8) | 305 (33.8) | |
| **Smoke status** | | | | | | <0.01 |
| Never | 2114 (59.5) | 538 (59.2) | 485 (60.1) | 537 (57.5) | 554 (61.4) | |
| Former | 669 (18.8) | 157 (17.3) | 184 (22.8) | 201 (21.5) | 127 (14.1) | |
| Smoker | 767 (21.59) | 214 (23.5) | 138 (17.1) | 193 (20.7) | 222 (24.6) | |
| **Physical activity** | | | | | | <0.01 |
| Inactive | 528 (14.9) | 155 (17.1) | 129 (16.0) | 135 (14.5) | 109 (12.1) | |
| Moderately inactive | 807 (22.7) | 181 (19.9) | 186 (23.0) | 240 (25.7) | 200 (22.1) | |
| Moderately active | 1909 (53.7) | 462 (50.8) | 447 (55.4) | 481 (51.5) | 519 (57.5) | |
| Active | 309 (8.7) | 111 (12.2) | 45 (5.6) | 78 (8.4) | 75 (8.3) | |
| **% energy from ultra-processed food*** | | | | | | 0.03 |
| Tertile 1 (0-16%) | 1164 (33.4) | 306 (34.3) | 273 (34.1) | 301 (32.9) | 284 (32.2) | |
| Tertile 2 (16%-24.5%) | 1163 (33.3) | 318 (35.7) | 280 (35.0) | 288 (31.4) | 277 (31.4) | |
| Tertile 3 (24.5%-79.6%) | 1163 (33.3) | 267 (30.0) | 247 (30.9) | 327 (35.7) | 322 (36.5) | |
| **Energy intake*** | | | | | | <0.01 |
| Mean (SD) – kcal/day | 2211 (704.4) | 2265 (671.0) | 1979 (639.4) | 2259 (728.4) | 2314 (722.6) | |

(*) on 3490 subjects with reliable dietary information

**Table 2. Blood BPA levels (ng/ml) (percentage above the limit of detection (LOD) and geometric mean (GM) with 95% confidence interval) and exponentiated coefficients from Tobit regression models by participants' sociodemographic and life style characteristics.**

| | > LOD (%) | GM (ng/ml) | 95% CI | Tobit univariate models | | Tobit multivariate model | |
|---|---|---|---|---|---|---|---|
| **Total** | 69.7 | 1.19 | 1.12-1.25 | $e^{\beta}$ | p | $e^{\beta}$ | p |
| **Centre** | | | | | | | |
| Gipuzkoa | 57.7 | 0.67 | 0.61-0.75 | 0.44 | <0.01 | 0.44 | <0.01 |
| Granada | 82.0 | 1.83 | 1.64-2.04 | 1.85 | <0.01 | 1.84 | <0.01 |
| Murcia | 69.7 | 1.10 | 0.99-1.22 | 0.91 | 0.19 | 0.92 | 0.22 |
| Navarra | 70.8 | 1.54 | 1.36-1.73 | 1.33 | <0.01 | 1.34 | <0.01 |
| **Sex** | | | | | | | |
| Male | 70.2 | 1.27 | 1.17-1.38 | Ref. | - | Ref. | - |
| Female | 69.2 | 1.11 | 1.03-1.20 | 0.85 | 0.05 | 0.68 | 0.00 |
| **Age group** | | | | | | | |
| <45 | 68.0 | 1.04 | 0.92-1.18 | Ref. | - | Ref. | - |
| 45-49 | 70.1 | 1.31 | 1.13-1.52 | 1.32 | 0.05 | 1.35 | 0.03 |
| 50-54 | 70.0 | 1.31 | 1.15-1.48 | 1.31 | 0.03 | 1.34 | 0.03 |
| 55-59 | 70.5 | 1.23 | 1.08-1.39 | 1.23 | 0.11 | 1.23 | 0.13 |
| 60+ | 69.8 | 1.10 | 0.98-1.23 | 1.08 | 0.52 | 1.09 | 0.52 |
| **Educational level** | | | | | | | |
| None | 71.1 | 1.24 | 1.14-1.36 | Ref. | - | Ref. | - |
| Primary school | 68.8 | 1.19 | 1.08-1.31 | 0.92 | 0.38 | 1.00 | 0.97 |
| Technical school | 65.8 | 0.98 | 0.80-1.20 | 0.72 | 0.04 | 0.96 | 0.79 |
| Secondary school | 72.3 | 1.26 | 1.00-1.59 | 1.03 | 0.86 | 1.02 | 0.92 |
| University | 69.1 | 1.09 | 0.92-1.29 | 0.84 | 0.22 | 0.76 | 0.07 |
| **BMI** | | | | | | | |
| <25 kg/m2 | 66.8 | 1.02 | 0.90- 1.16 | Ref. | - | Ref. | - |
| 25-<30 kg/m2 | 70.3 | 1.21 | 1.12-1.31 | 1.25 | 0.04 | 1.04 | 0.74 |
| ≥30 kg/m2 | 70.4 | 1.25 | 1.13- 1.38 | 1.30 | 0.03 | 0.93 | 0.57 |
| **Physical activity** | | | | | | | |
| Inactive | 68.9 | 1.21 | 1.04-1.40 | Ref. | - | Ref. | - |
| Moderately inactive | 69.0 | 1.18 | 1.04-1.33 | 0.97 | 0.84 | 0.91 | 0.51 |
| Moderately active | 70.7 | 1.21 | 1.12-1.31 | 1.03 | 0.81 | 1.05 | 0.74 |
| Active | 66.7 | 1.03 | 0.85-1.25 | 0.81 | 0.24 | 0.98 | 0.91 |
| **Smoke status** | | | | | | | |
| Never | 70.7 | 1.23 | 1.14-1.32 | Ref. | - | Ref. | - |
| Former | 69.1 | 1.12 | 0.99-1.28 | 0.89 | 0.27 | 0.81 | 0.08 |
| Smoker | 67.4 | 1.12 | 0.99-1.27 | 0.87 | 0.16 | 0.84 | 0.13 |
| **% energy from ultra-processed food** | | | | | | | |
| Tertile 1 (0-16%) | 69.0 | 1.13 | 1.02-1.25 | Ref. | - | Ref. | - |
| Tertile 2 (16%-24.5%) | 69.9 | 1.18 | 1.07-1.31 | 1.06 | 0.54 | 1.09 | 0.41 |
| Tertile 3 (24.5%-79.6%) | 70.3 | 1.27 | 1.15-1.40 | 1.15 | 0.16 | 1.20 | 0.07 |
| **Energy intake (**per an increment of 1000 kcal/day**)** | | | | 0.92 | 0.15 | 0.87 | 0.07 |

**Table 3. Exponentiated coefficients from mixed-effects Tobit regression models and 95% confidence intervals (9**

| **Food groups** (per 50 g/day energy-adjusted increase) | **Model A** | | | **Model B** | | | |
|---|---|---|---|---|---|---|---|
| | $e^{\beta}$ | 95% CI | p | $e^{\beta}$ | 95% CI | p | |
| Potatoes and other tubers | 1.03 | (0.95-1.12) | 0.48 | 1.04 | (0.96-1.13) | 0.34 | 1 |
| Vegetables | 0.99 | (0.96-1.02) | 0.52 | 0.99 | (0.96-1.02) | 0.49 | 1 |
| Legumes | 1.00 | (0.88-1.13) | 0.99 | 0.98 | (0.87-1.11) | 0.75 | 0 |
| Fruits, nuts and seeds | 1.01 | (0.99-1.03) | 0.27 | 1.01 | (0.99-1.03) | 0.38 | 1 |
| Dairy products | 0.99 | (0.97-1.01) | 0.38 | 0.99 | (0.97-1.02) | 0.66 | 0 |
| Cereals and derivatives | 0.98 | (0.92-1.03) | 0.43 | 0.98 | (0.92-1.03) | 0.44 | 0 |
| Meat and derivatives | 0.97 | (0.89-1.05) | 0.44 | 0.97 | (0.89-1.05) | 0.43 | 0 |
| Fish and seafood | 1.09 | (0.98-1.2) | 0.10 | 1.07 | (0.97-1.18) | 0.18 | 1 |
| Eggs and derivatives | 0.84 | (0.67-1.06) | 0.14 | 0.83 | (0.66-1.05) | 0.12 | 0 |
| Added fats and oils | 0.65 | (0.45-0.95) | 0.02 | 0.65 | (0.45-0.95) | 0.03 | 0 |
| Sugar and confectionery | 1.17 | (0.95-1.45) | 0.15 | 1.20 | (0.97-1.48) | 0.10 | 1 |
| Cakes and cookies | 0.97 | (0.88-1.07) | 0.54 | 0.99 | (0.89-1.09) | 0.79 | 0 |
| Non-alcoholic drinks | 0.98 | (0.96-1.00) | 0.09 | 0.98 | (0.96-1.01) | 0.15 | 0 |
| Alcoholic drinks | 1.02 | (1.00-1.04) | 0.11 | 1.01 | (0.99-1.04) | 0.29 | 1 |
| Condiments and sauces | 1.20 | (0.75-1.92) | 0.45 | 1.22 | (0.76-1.95) | 0.41 | 1 |
| Soups and broths | 0.99 | (0.94-1.04) | 0.67 | 0.98 | (0.93-1.04) | 0.48 | 0 |

Model A: unadjusted, random effect at centre level

Model B: adjusted for sex and age, random effect at centre level

Model C: adjusted for sex, age, random effect at centre level, and all food groups

**Table 4. Exponentiated coefficients from mixed-effects Tobit regression models and 95% confidence intervals (95% CI) for components of the main food group "Added fats and oils" ***

| **Food groups** (per 50 g/day energy-adjusted increase) | **Consumers** (%) | **Model C** | | |
|---|---|---|---|---|
| | | $e^{\beta}$ | 95% CI | p |
| Oil not specified | 18.4 | 0.80 | (0.40-1.60) | 0.53 |
| Olive oil | 86.8 | 0.65 | (0.43-0.97) | 0.04 |
| Sunflower oil | 23.6 | 0.51 | (0.28-0.96) | 0.04 |
| Mixed oil | 5.8 | 0.49 | (0.19-1.25) | 0.13 |
| Butter | 6.7 | 0.54 | (0.07-4.04) | 0.55 |
| Margarine | 22.7 | 0.85 | (0.37-1.97) | 0.70 |

(*) Food items with consumption in less than 5% of the sample have been omitted (soya oil, peanut oil, corn/maize oil, grape oil, rapeseed oil, safflower oil, walnut oil, other oils, deep frying fats, marine oils, other animal fats)

**Supplementary Material**

**Supplementary Material, Table 1. Comparison to other studies assessing BPA in human matrices from Spain**

| | GM | Mean age | Date of sample | Matrix |
|---|---|---|---|---|
| **EPIC-Spain** | 1.19 ng/ml | 53 | 1992-1996 | Serum |
| Gonzalez (2019)[204] | 0.58 µg/L | - | 2019 | Blood |
| Gonzalez (2019)[204] | 0.86 µg/L | - | 2019 | Urine |
| Sanchis (2019)[243] | 0.2-12 ng/ml | 32-39 | 2015 | Urine |
| Dualde (2019)[201] | 0.29 ng/ml | 33 | 2015 | Human milk |
| Artacho-Cordon (2018)[51] | 0.54 ng/g | 52.3 | 2003-2004 | Adipose tissue |
| Adoamnei (2018)[229] | 2.3 ng/ml | 20.4 | 2011 | Urine |
| Mustieles (2018)[203] | 5.1 µg/L | 9.8 | 2000-2002 | Urine |
| Artacho-Cordon (2017) [244] | 1.14 ng/ml | 47.2 | 2015 | Urine |
| Artacho-Cordon (2017)[244] | 0.60 ng/g | 47.2 | 2015 | Adipose tissue |
| Perez-Lobato (2016)[245] | 4.76 µg/L | 9.9 | 2000-2002 | Urine |
| Fernández (2016)[246] | 2.59 ng/g | 30.0 | 2000-2002 | Placenta |
| Casas (2013)[247] | 2.3 µg/L | 30.6 | 2004-2006 | Urine |
| Casas (2016)[248] | 2.6 µg/g | 30.6 | 2004-2006 | Urine |
| Gascon (2015)[249] | 2.4 µg/g | 30.6 | 2004-2008 | Urine |
| Covaci (2015)[250] | 1.83 µg/L | Children and mothers | 2011-2012 | Urine |

**Supplementary Material, Table 2. Comparison between selected and non-selected participants from the entire cohort.**

| | Selected | Non-selected |
|---|---|---|
| **N (%)** | 3553 (100) | 29342 (100) |
| **Centre** | | |
| Gipuzkoa | 909 (25.6) | 7508 (25.6) |
| Granada | 807 (22.7) | 7072 (24.1) |
| Murcia | 934 (26.3) | 7581 (25.8) |
| Navarra | 903 (25.4) | 7181 (24.5) |
| **Sex** | | |
| Male | 1728 (48.6) | 10818 (36.9) |
| Female | 1825 (51.4) | 18524 (63.1) |
| **Age** | | |
| <45 | 679 (19.1) | 10298 (35.1) |
| 45-49 | 572 (16.1) | 6380 (21.7) |
| 50-54 | 757 (21.3) | 5097 (17.4) |
| 55-59 | 687 (19.3) | 4213 (14.4) |
| 60+ | 858 (24.1) | 3354 (11.4) |
| **Education level** | | |
| None | 1405 (39.8) | 10683 (36.6) |
| Primary school | 1242 (35.2) | 11010 (37.8) |
| Technical school | 281 (7.9) | 2365 (8.1) |
| Secondary school | 206 (5.8) | 1728 (5.9) |
| University | 392 (11.1) | 3376 (11.6) |
| **BMI** | | |
| <25 kg/m2 | 650 (18.3) | 6654 (22.7) |
| 25-<30 kg/m2 | 1789 (50.3) | 13768 (46.9) |
| ≥30 kg/m2 | 1114 (31.3) | 8920 (30.4) |
| **Smoke status** | | |
| Never | 2114 (59.5) | 16292 (55.5) |
| Former | 669 (18.8) | 5029 (17.1) |
| Smoker | 767 (21.59) | 8008 (27.3) |
| **Physical activity** | | |
| Inactive | 528 (14.9) | 3904 (13.3) |
| Moderately inactive | 807 (22.7) | 6059 (20.6) |
| Moderately active | 1909 (53.7) | 17024 (58.0) |
| Active | 309 (8.7) | 2355 (8.0) |
| **Energy intake** | | |
| Mean (SD) – kcal/day | 2211 (704.4) | 2158 (680.9) |

**Supplementary Material 3**

**NOVA classification:**

https://world.openfoodfacts.org/nova

The NOVA classification assigns a group to food products based on how much processing they have been through:


Group 1 - Unprocessed or minimally processed foods

Group 2 - Processed culinary ingredients

Group 3 - Processed foods

Group 4 - Ultra-processed food and drink products

Group 1. Unprocessed or minimally processed foods

Unprocessed (or natural) foods are edible parts of plants (seeds, fruits, leaves, stems, roots) or of animals (muscle, offal, eggs, milk), and also fungi, algae and water, after separation from nature.

Minimally processed foods are natural foods altered by processes that include removal of inedible or unwanted parts, and drying, crushing, grinding, fractioning, filtering, roasting, boiling, non-alcoholic fermentation, pasteurization, refrigeration, chilling, freezing, placing in containers and vacuum-packaging. These processes are designed to preserve natural foods, to make them suitable for storage, or to make them safe or edible or more pleasant to consume. Many unprocessed or minimally processed foods are prepared and cooked at home or in restaurant kitchens in combination with processed culinary ingredients as dishes or meals.

Group 2. Processed culinary ingredients

Processed culinary ingredients, such as oils, butter, sugar and salt, are substances derived from Group 1 foods or from nature by processes that include pressing, refining, grinding, milling and drying. The purpose of such processes is to make durable products that are suitable for use in home and restaurant kitchens to prepare, season and cook Group 1 foods and to make with them varied and enjoyable hand-made dishes and meals, such as stews, soups and broths, salads, breads, preserves, drinks and desserts. They are not meant to be consumed by themselves, and are normally used in combination with Group 1 foods to make freshly prepared drinks, dishes and meals.

Group 3. Processed foods

Processed foods, such as bottled vegetables, canned fish, fruits in syrup, cheeses and freshly made breads, are made essentially by adding salt, oil, sugar or other substances from Group 2 to Group 1 foods.

Processes include various preservation or cooking methods, and, in the case of breads and cheese, non-alcoholic fermentation. Most processed foods have two or three ingredients, and are recognizable as modified versions of Group 1 foods. They are edible by themselves or, more usually, in combination with other foods. The purpose of processing here is to increase the durability of Group 1 foods, or to modify or enhance their sensory qualities.

Group 4. Ultra-processed foods

Ultra-processed foods, such as soft drinks, sweet or savoury packaged snacks, reconstituted meat products and pre-prepared frozen dishes, are not modified foods but formulations made mostly or entirely from substances derived from foods and additives, with little if any intact Group 1 food.

Ingredients of these formulations usually include those also used in processed foods, such as sugars, oils, fats or salt. But ultra-processed products also include other sources of energy and nutrients not normally used in culinary preparations. Some of these are directly extracted from foods, such as casein, lactose, whey and gluten.

Many are derived from further processing of food constituents, such as hydrogenated or interesterified oils, UN, NOVA and the trouble with ultra-processing 9 hydrolysed proteins, soya protein isolate, maltodextrin, invert sugar and high-fructose corn syrup.

Additives in ultra-processed foods include some also used in processed foods, such as preservatives, antioxidants and stabilizers. Classes of additives found only in ultra-processed products include those used to imitate or enhance the sensory qualities of foods or to disguise unpalatable aspects of the final product. These additives include dyes and other colours, colour stabilizers; flavours, flavour enhancers, non-sugar sweeteners; and processing aids such as carbonating, firming, bulking and anti-bulking, de-foaming, anti-caking and glazing agents, emulsifiers, sequestrants and humectants.

A multitude of sequences of processes is used to combine the usually many ingredients and to create the final product (hence 'ultra-processed'). The processes include several with no domestic equivalents, such as hydrogenation and hydrolysation, extrusion and moulding, and pre-processing for frying.

The overall purpose of ultra-processing is to create branded, convenient (durable, ready to consume), attractive (hyper-palatable) and highly profitable (low-cost ingredients) food products designed to displace all other food groups. Ultra-processed food products are usually packaged attractively and marketed intensively.

More NOVA information in:

1. Monteiro CA et al. NOVA The Food System. World Nutr. 2016;7(7):1–3.

2. Carlos A. Monteiro, Geoffrey Cannon, Renata Levy J-CM et al. Food classification. Public health NOVA. 2016.

3. Open Food Facts. Nova groups for food processing [Internet]. [cited November 8, 2019]. Available at: https://world.openfoodfacts.org/nova

## 7.2 Objetivo específico 2

3. Evaluar la exposición a contaminantes no persistentes y riesgo de enfermedad coronaria isquémica de una sub-cohorte de EPIC-España al seguimiento.

### 7.2.1 Exposición a contaminantes no persistentes y riesgo de enfermedad coronaria isquémica

**Bisphenol A exposure and risk of ischemic heart disease in the Spanish European Prospective Investigation into Cancer and Nutrition study**

Elena Salamanca-Fernández, Miguel Rodríguez-Barranco, Dafina Petrova, Nerea Larrañaga, Marcela Guevara, Conchi Moreno-Iribas, Maria Dolores Chirlaque, Sandra Colorado Yohar, Juan Pedro Arrebola, Fernando Vela, Nicolás Olea, Antonio Agudo, Maria-José Sánchez.

Publicado en Chemosphere [251] https://doi.org/10.1016/j.chemosphere.2020.127697C
Cuartil 1, Factor de Impacto: 5.778

Chemosphere 261 (2020) 127697

Contents lists available at ScienceDirect

Chemosphere

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

# Bisphenol A exposure and risk of ischemic heart disease in the Spanish European Prospective Investigation into cancer and nutrition study

Elena Salamanca-Fernández [a, b], Miguel Rodríguez-Barranco [a, b, c, *], Dafina Petrova [a, b, c], Nerea Larrañaga [c, d], Marcela Guevara [c, e, f], Conchi Moreno-Iribas [e, f], Maria Dolores Chirlaque [c, g, h], Sandra Colorado-Yohar [c, g, i], Juan Pedro Arrebola [b, c, j], Fernando Vela [b], Nicolás Olea [b, c, k], Antonio Agudo [l], Maria-José Sánchez [a, b, c, j]

[a] Andalusian School of Public Health (EASP), Granada, Spain
[b] Instituto de Investigación Biosanitaria ibs.GRANADA, Granada, Spain
[c] CIBER de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain
[d] Public Health Department of Gipuzkoa, Donostia, Spain
[e] Navarra Public Health Institute, Pamplona, Spain
[f] IdiSNA, Navarra Institute for Health Research, Pamplona, Spain
[g] Department of Epidemiology, Murcia Regional Health Council, IMIB-Arrixaca, Murcia, Spain
[h] Department of Health and Sciences, University of Murcia, Spain
[i] Research Group on Demography and Health, National Faculty of Public Health, University of Antioquia, Medellín, Colombia
[j] Department of Preventive Medicine and Public Health, University of Granada, Granada, Spain
[k] Department of Radiology, School of Medicine, University of Granada, Granada, Spain
[l] Unit of Nutrition and Cancer, Catalan Institute of Oncology - ICO, Nutrition and Cancer Group, Bellvitge Biomedical Research Institute - IDIBELL, L'Hospitalet de Llobregat, Barcelona, 08908, Spain

## HIGHLIGHTS

- BPA concentrations were quantified in serum from 946 adults with ischemic heart disease.
- There was a higher proportion of overweight and obesity in cases than in the sub-cohort.
- BPA serum concentration was higher in cases than in participants of the sub-cohort.
- We observed no increased risk on IHD with BPA exposure.

## ARTICLE INFO

*Article history:*
Received 1 April 2020
Received in revised form
26 June 2020
Accepted 12 July 2020
Available online 20 July 2020

Handling Editor: A. Gies

*Keywords:*
Cardiovascular disease
Ischemic heart disease
Case-cohort
Bisphenol A
Environmental exposure

## ABSTRACT

*Background:* Cardiovascular disease, particularly ischemic heart disease (IHD), is the leading cause of mortality worldwide. Bisphenol A (BPA) is considered an endocrine disruptor and obesogen, present in numerous products of daily use. The aim of this study was to assess the potential association of serum BPA concentrations and the risk of incident IHD in a sub-cohort of the Spanish European Prospective Investigation into Cancer and Nutrition (EPIC).
*Methods:* We designed a case-cohort study within the EPIC-Spain cohort. The population consisted of 4636 participants from 4 EPIC-Spain centers (946 IHD cases and 3690 sub-cohort participants). BPA exposure was assessed by means of chemical analyses of serum samples collected at recruitment. Follow-up was performed by linking with national and regional databases and reviewing patients' clinical records. Cox Proportional Hazards Models were used for the statistical analyses.
*Results:* Median follow-up time was 16 years and 70% of the participants showed detectable BPA values (>0.2 ng/ml). Geometric mean (GM) values of cases and sub-cohort were 1.22 ng/ml vs 1.19 ng/ml respectively (p = 0.90). Cox regression models showed no significant association of BPA serum levels and IHD, acute myocardial infarction or angina pectoris risk.

* Corresponding author. Andalusian School of Public Health (EASP), Campus Universitario de Cartuja, C/Cuesta del Observatorio 4, 18080, Granada, Spain.
*E-mail address:* miguel.rodriguez.barranco.easp@juntadeandalucia.es (M. Rodríguez-Barranco).

https://doi.org/10.1016/j.chemosphere.2020.127697
0045-6535/© 2020 Elsevier Ltd. All rights reserved.

## ABSTRACT

*Background:* Cardiovascular disease, particularly ischemic heart disease (IHD), is the leading cause of mortality worldwide. Bisphenol A (BPA) is considered an endocrine disruptor and obesogen, present in numerous products of daily use. The aim of this study was to assess the potential association of serum BPA concentrations and the risk of incident IHD in a sub-cohort of the Spanish European Prospective Investigation into Cancer and Nutrition (EPIC).

*Methods:* We designed a case-cohort study within the EPIC-Spain cohort. The population consisted of 4,636 participants from 4 EPIC-Spain centers (946 IHD cases and 3,690 sub-cohort participants). BPA exposure was assessed by means of chemical analyses of serum samples collected at recruitment. Follow-up was performed by linking with national and regional databases and reviewing patients' clinical records. Cox Proportional Hazards Models were used for the statistical analyses.

*Results:* Median follow-up time was 16 years and 70% of the participants showed detectable BPA values (>0.2 ng/ml). Geometric mean (GM) values of cases and sub-cohort were 1.22 ng/ml vs 1.19 ng/ml respectively (p=0.90). Cox regression models showed no significant association of BPA serum levels and IHD, acute myocardial infarction or angina pectoris risk.

*Conclusions:* We evidenced a similar percentage of detection of BPA among cases and sub-cohort participants from our population, and no clear association with IHD risk was observed. However, further investigation is needed to understand the influence of BPA on IHD risk.


*Keywords:* Cardiovascular disease; ischemic heart disease; case-cohort; Bisphenol A; environmental exposure.

## Introduction

Cardiovascular disease (CVD), mainly ischemic heart disease (IHD), is the leading cause of mortality and morbidity worldwide [139] with 17.8 million deaths in 2017 [139,140]. In Spain, CVD was the main mortality cause with 122,465 deaths in 2017 followed by cancer with 113,266 deaths [252]. Being non-communicable diseases, some of the causes of CVD and IHD are related to lifestyle, and hence are modifiable risk factors [139]: smoking, alcohol consumption, diet physical activity, and obesity .

However, there is a growing evidence that some environmental pollutants have the potential to act as obesogens [146] and could therefore play an important role in the development of CVD. Bisphenol A (BPA), considered an endocrine disruptor (ED), was first developed in the 1890s. BPA is widely produced for the manufacture of polysulfones and polycarbonate plastic, polymers and epoxy resin, and thermal paper, and is one of the highest volume chemicals produced worldwide with 372,000 tonnes produced in 2012 [72]. Its presence is considered to be ubiquitous in the environment and human exposure is continuous [73,74]. BPA has been detected in the urine (>0.4 ng/ml) [75–78] of nearly 90% of adults and children as well as in the serum of general population, pregnant women, placenta, breast milk and amniotic fluid [188,190–194]. Humans are exposed to BPA through several routes: food (oral), occupation (inhalation) and contact materials, plastic type and medical devices (dermal)[73,195]. However, the main exposure route of BPA is through diet, as many types of food packaging such as tins, cans, plastic boxing etc. have BPA in their composition, allowing it to migrate to the food [73,87–91].

Although BPA is considered a non-persistent chemical, i.e., it is degraded in the organism, there is evidence from animal models that BPA induces arterial hypertension, endothelial dysfunction, and atherosclerosis by increasing susceptibility to endothelial damage, arrhythmias, dyslipidemia, and hyperinsulinemia [253] as well as carcinogenesis in mammary glands [81,84,85]. BPA also activates ion channels Maxi-K (KCa1.1) of arterial muscle cells [254]. Therefore, BPA exposure could play an important role in the development of CVD and IHD [255–259]. However, the harmful effect of BPA in human health remains controversial, although some studies show that BPA could play a role in cancer, insulin resistance, and obesity [189,199,260].

The European Food Safety Authority (EFSA) reduced the tolerable daily intake of BPA in 2015 from 50 μg / kg per day to 4 μg / kg per day; and a re-evaluation is being prepared for 2020 [90]. Recently, the General Court of the European Union published a confirmation of the inclusion of BPA as a substance of very high concern [92]. Moreover, the French Senate adopted the legal ban of bisphenol A (BPA) for food contact materials intended for children below 3 by early 2013, and for all food contact materials by January 2015 [261].

Given the increasing concerns regarding the harmful effects of BPA on human health, the present study aims to assess the potential association of serum BPA concentrations and the risk of incident IHD in a sub-cohort of the Spanish European Prospective Investigation into Cancer and Nutrition (EPIC).

**Methods**

*Study design*

We designed a case-cohort study within the EPIC-Spain cohort. EPIC is a prospective multi-centric cohort study planned to investigate the relationship between diet, lifestyle, and cancer. It involves 23 research centers in 10 European countries, including five Spanish centers: Asturias, Granada, Murcia, Navarra, and Gipuzkoa [165].

*Study population*

In the EPIC-Spain study, a total of 41,446 participants aged 29-69 years were enrolled between 1992 and 1996 in five provinces of Spain. Participants were recruited mostly among blood donors (about 60%) and the study population covered a broad range of socioeconomic and educational levels. At recruitment, study participants reported information about dietary, lifestyle, reproductive, and anthropometric factors, and a fasting blood sample was collected from most of them (79.73%). Furthermore, participants signed an informed consent and the study was approved by the Ethics Committee of the Bellvitge Hospital (Barcelona). Further details regarding the EPIC study populations and data collection are explained elsewhere [177].

The study population in the present study consisted of 3,690 participants selected for the sub-cohort and 946 IHD cases from the four EPIC-Spain centres (259 from Gipuzkoa,

158 from Granada, 204 from Murcia and 325 from Navarra). Participants selected for the sub-cohort included, by design, an overlap of 139 IHD cases.

Follow-up time began at EPIC recruitment and IHD cases were defined as participants with a diagnosis of ischemic heart disease (i.e. ICD10 codes I20-I25) during the study period. IHD cases included acute myocardial infarction (AMI), angina pectoris (AP), and cardiac arrest cases. Incident cases were identified by trained staff using a linkage to the datasets of the hospital discharges, primary care, CVD registries, and mortality registries of each region. Then, cases were confirmed by reviewing medical records and applying MONICA diagnostic criteria [262]. Incidence dates were determined from the first cardiac event diagnosed in the participant's clinical record and prevalent cases were excluded (participants with IHD diagnosis prior to recruitment). Dates of end of follow-up for cases identification were: 31/12/2008 for Granada, 31/12/2012 for Murcia, 31/12/2011 for Navarra and 30/12/2013 for Guipuzkoa. Previous history of hyperlipidemia and hypertension was self-reported.

The sub-cohort and cases were selected using stratified random sampling by sex and age, excluding persons with chronic diseases. In our sub-cohort, 80% of the participants provided a fasting blood sample at recruitment, extracted between 6 am and 11 am. The remaining 20% of the samples did not have the fasting status. However, we can generalize and assume most of our samples were taken during the mornings and in fasting conditions. The characteristics of our sample in comparison to the rest of the EPIC-Spain cohort were similar on the variables included in the study, except for the distribution by sex and age that was deliberately different due to the stratified sample selection (Appendices Table A1). Prevalent cases and cases in which a diagnosis date could not be determined were eliminated from the analysis.

*Assessment of lifestyle variables*

Information on lifestyle and other health-related factors was obtained by an interviewer-administered questionnaire at baseline. All interviewers received appropriate training for this task.

Measurements of height, weight, and hip and waist circumferences were taken at recruitment using standardised procedures [177]. The participants were classified into

three categories as a function of their body mass index (BMI): <25 kg/m$^2$, 25-<30 kg/m$^2$, ≥30 kg/m$^2$. The questionnaire included items on educational level, history of previous illnesses, history of tobacco use, physical activity, occupation, and reproductive history [177]. Educational level was classified according to five categories: none, primary school, secondary school, technical or vocational training, and university degree. Smoking status was summarised in three categories: never smoked, former smoker, and current smoker. Information on the domains of physical activity was compiled taking seasonal variation into account. A simple four-level physical activity index (inactive, moderately inactive, moderately active, and active) was derived and validated by combining occupational and recreational activity [178].

*Sample collection and chemical analyses*

Blood samples were drawn from each participant at recruitment, and they were subsequently centrifuged, and aliquots of plasma, serum, red blood cells, and buffy coat in 0.5 mL straws were stored in liquid nitrogen (-196ºC).

BPA levels were quantified in serum samples using two of 0.5 mL straws, in an adaptation of a previously-validated methodology [175]. In brief, BPA was analysed by dispersive liquid–liquid micro-extraction (DLLME) and ultra-high performance liquid chromatography with tandem mass spectrometry detection (UHPLC-MS/MS). Samples were thawed completely at room temperature, centrifuged at 2600g for 10 min and 0.75 mL was extracted for analysis. In order to determine total BPA (free plus conjugated) in serum, each sample was spiked with 50µL of enzyme solution (β-glucuronidase/sulphatase) and incubated at 37 °C for 24 h. The treated serum was placed in a 15 mL screw-cap glass tube and spiked with 30µL of the surrogate standard solution (1.25 mg/L of BPA-d16). The serum was then diluted to 10.0 mL with 5% NaCl aqueous solution (w/v) and the pH was adjusted to 2.0. Next, 0.75 mL of acetone and 0.75 mL of trichloromethane were mixed and injected rapidly into the aqueous sample with a syringe. After manual shaking, centrifugation and evaporation of the extract, the residue was dissolved with 100µL of a mixture consisting of water (0.1% ammonia)/acetonitrile (0.1% ammonia), 70:30 (v/v), and finally 10µL was injected into the LC system. Limit of detection (LOD) was 0.2 ng/ml. Values below LOD were assigned the LOD divided by the square root of 2.

*Statistical analysis:*

Geometric means and 95% confidence intervals of the BPA levels (in ng/ml) were calculated for cases and sub-cohort and according to center, sex, age group, educational level, body mass index, physical activity, alcohol consumption, smoker status, and previous history of hyperlipidemia and hypertension. Statistical differences between means were assessed through the Mann-Whitney or Kruskall-Wallis tests.

Entry time was defined as the date of recruitment and exit time as the date of IHD diagnosis, death, loss of follow-up, or the center-specific end-of-follow-up, whichever happened first. Times to IHD event were modelled by means of Borgan II weighted Cox proportional hazard models [263], stratified by centre. Robust standard errors were used as recommended in such case-cohort design [264]. Hazard ratios and 95% confidence intervals were derived from Cox models. BPA level, the principal independent variable, was treated as continuous variable and transformed by base 2 logarithm to smooth its strongly asymmetric distribution. Three Cox-regression models were used: A) linear BPA stratified by centre; B) log2-tranformed BPA stratified by centre; C) log2-tranformed BPA stratified by centre and adjusted for sex, age, education level, BMI, physical activity, smoking status, alcohol consumption, hyperlipidemia, and hypertension.

Statistical analysis was conducted with Stata v14 (Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

**Results**

Median follow-up time was 15.92 years. Among cases, 74% were men in contrast to 48% of the sub-cohort ($p < 0.001$) and the mean age of the cases was 54.17 years compared to 52.75 years of the sub-cohort ($p < 0.001$). There was a higher proportion of overweight or obesity in the cases than in the sub-cohort (89% versus 82%; $p < 0.001$). 43% of the cases were current smokers, while in the sub-cohort this percentage was 22% ($p < 0.001$). The prevalence of hypertension and hyperlipidemia was significantly higher in cases compared to the sub-cohort by 10-12 percentage points (Table 1).

Percentage of detection was similar between cases and sub-cohort, with around 70% of the samples showing levels above LOD ($p=0.45$). BPA geometric mean was higher in cases than in participants of the sub-cohort (1.22 ng/ml vs 1.19 ng/ml) but with no

statistical significance (p=0.90). The analysis stratified by other variables did not reveal any subgroup that had different concentrations between the cases and the sub-cohort (Table 2).

In the Cox regression analyses, no statistically significant association was found between BPA levels as continuous variable and IHD incidence (HR=0.998; p=0.749). Similarly, no significant effect was observed when the exposure variable was included with log2 transformation, neither in the univariate model nor in the multivariate model adjusted for potential confounders. The results were similar in the analogous analyses of the sub-types of IHD: AMI and AP (Table 3).

Male sex, age, obesity, smoking habit (current or past), hyperlipidemia, and hypertension were risk factors for incidence of IHD, AMI, and AP in the multivariate model (data not shown).

## Discussion

Our study presents novel longitudinal results of the potential contribution of long-term human BPA exposure to the development of IHD, over a relatively large follow-up period in Spain. Although BPA is considered an ED with obesogenic potential [257,258,265], no evident associations were found between BPA serum concentrations and risk of incident IHD.

In this regard, our results are in accord with some previous epidemiological studies that observed no association between urinary BPA exposure and CVD, heart attack, or coronary artery disease [266,267]. Specifically, LaKind et al. examined NHANES data from 2003–04 to 2009–10 in adults aged ≥20 years, and found no significant associations between self-reported coronary heart disease, heart attack, and urinary BPA as a continuous variable in either separate or pooled populations [267]. Moreover, a systematic review conducted by Lakind et al. also concluded that due to the epidemiological design of the studies, the understanding of health effects associated with BPA exposure is limited [266].

However, other epidemiological studies reported an increased risk of coronary artery disease in apparently healthy population exposed to BPA [189,268–270]. The study of

Shankar et al. showed that urinary BPA levels were significantly associated with peripheral arterial disease, independently of traditional CVD risk factors [157]. A meta-analysis concluded that urinary BPA at levels found in the general population are associated with increased prevalence of diabetes, general and abdominal obesity, and hypertension [271]. In NHANES 2003–04 [268], authors found that greater concentration of urinary BPA was associated with an increased risk of self-reported CVD (myocardial infarction, angina, or coronary heart disease) (OR = 1.39, 95 % CI: 1.18–1.63), but not of stroke (OR = 0.97, 95 % CI: 0.74–1.27). Melzer et al. reported similar associations with NHANES 2005–06 data [270]. Casey & Neidell reported significant positive associations between urinary BPA and coronary heart disease in NHANES 2003–04 but results were not consistent in the subsequent 2005/06 and 2007/08 cycles [272]. In fact, Melzer et al. showed that urinary BPA concentrations were significantly associated with severe coronary artery disease (OR = 1.43, 95 % CI: 1.03–1.98; n = 385), and near significantly associated with intermediate coronary artery disease (OR = 1.69, 95 % CI: 0.98–2.94; n = 86) [273]. The prospective nested case–control study within the EPIC-Norfolk cohort, reported a positive association between urinary BPA concentrations and incidence of coronary artery disease: OR = 1.11, 95 % CI = 1.00-1.23 [269]. Thus, several cross-sectional epidemiological studies have reported positive relationships between levels of BPA (urine) with risk factors for CVD, such as blood pressure, cholesterolemia, etc. [86].

These mixed results may be due to several factors. These include the heterogenous populations of the studies, the different ways of disease measurement, and the different assessment of BPA exposure and the biological matrix that can have substantial influence on exposure levels. Moreover, human exposure to many potential endocrine disruptors (ED) can be confounded because most existing cohorts and epidemiological studies were designed to measure the impact of a single chemical without accounting for the effects of mixtures [274]. All these factors must be taken into account when considering study results because different methodologies can result in inconsistent outcomes. Specifically, Lakind et al. suggested that conclusions should be made with caution regarding the NHANES surveys [267].

Some possible mechanisms of action of BPA on IHD risk could be the following: genetic damages, epigenetic effects, endocrine disruption, oxidative stress, and mitochondrial

dysfunction and cell signaling [146,189,275]. However, the more extensively explored mechanism is the BPA potential to act as an obesogen. Obesity has genetic, epigenetic, and environmental components, including environmental chemicals [146]. The recent scientific literature points out that BPA can have an obesogenic potential in humans because of its ED potential [255–258,265,276,277]. It has been suggested that exposure to BPA can be a promoter for IHD risk factors like obesity by distressing neural circuits that regulate feeding behavior or altering differentiation of adipocytes [259]. In this regard, studies carried out in the United States found a positive association between urinary BPA and obesity in the general adult population [259], as well as in children and adolescents [136]. The study of Amin et al. showed significant associations of BPA exposure with obesity and some cardio-metabolic risk factors in children and adolescents [125]. Moreover, BPA exposure was associated with decreased heart rate variability (which is known to increase the risk of cardiac events) and increased blood pressure [161], which are other IHD risk factors. Another suspected mechanism of action of BPA is by disrupting epigenetic signalling (e.g. DNA methylation, histone marks, chromatin remodelling and noncoding RNAs) [275], which might affect normal tissue development, eventually leading to obesity and related conditions [278]. Another potential mechanism is oxidative stress, which has been implicated in aging and many pathological disorders such as ischemic diseases, neurodegenerative diseases, diabetes, and cancer, although the underlying mechanisms are not completely understood [279]. Growing evidence suggests that BPA-induced damage is associated with oxidative stress [189,280,281] as BPA can disturb oxidative homeostasis through direct or indirect pathways, including mitochondrial function [282] and modulation of antioxidant enzymes of mice exposed throughout the embryonic/fetal route [283].

However, experimental studies have overall yielded conflicting results, most likely due to different experimental designs, timing of exposure, and uncontrolled or residual confounding factors, such as the route of administration of these pollutants, the degradation time of BPA or low exposure doses [132,137,146]. Further mechanistic studies in conjunction with epidemiologic approaches should test if the abovementioned mechanisms are sufficient to produce a relevant metabolic effect in the general populations at current exposure levels.

A limitation of our study is that exposure to BPA was estimated using serum concentrations at recruitment, and we do not have information on changes in BPA concentrations and covariates during the follow-up period. The same refers to changes in possible confounding variables during follow-up such as BMI, which we were not able to assess. In addition, the use of one BPA point measurement might not take into account intra-day fluctuations, which might be relevant in certain populations [284,285]. However, we assumed baseline concentrations since 80% of the participants were in fasting conditions.

The biological matrix used for biomonitoring also plays an important role. Serum BPA concentrations can be relatively unstable, representing recent exposures [197,241]. Its representativeness of long-term exposures can only be assumed when external, lifestyle, and biological determinants of serum concentrations remain constant to a certain degree, which is unlikely in a cohort study with a long follow-up time. Some studies point out that, among the biomonitoring matrices, urine contains the highest BPA concentrations, followed by serum [39], which implies that urine has a greater capacity to detect levels of exposure and estimate medium-term exposure. In this regard, the EPIC-Spain cohort included members of local blood donor associations. Blood samples were taken in fasting conditions and were aliquoted into blood plasma, blood serum, white blood cells, and erythrocytes. In this study, we only had one sample per participant taken at recruitment, and therefore we assumed BPA exposure to be similar through the follow-up period. Baseline levels of BPA can be expected to be more or less constant as long as individuals do not significantly change their diet patterns, since diet is considered the main BPA source of exposure in the general population [286]. Whereas we used serum for BPA assessment, other studies used urine [41,42], hair [44,45], umbilical cord serum [39,46], breast milk [39], and placenta [49]. In this regard, the biological matrix most commonly used to determine BPA is urine [287]. Indeed, some authors acknowledge urine concentrations as the best biomarker of BPA exposure, since metabolites in blood can be several orders of magnitude lower than in urine and it can be indicative of a relatively longer exposure period in comparison to other matrices [197]. Additionally, on the basis of the moderate lipophilicity of this compound (log octanol–water partition coefficients (Kow) ranging from 1 to 5), some authors have hypothesized that it might also reach fatty tissues [51,213,288]. In this regard, previous research in the same study area found detectable BPA concentrations in 86.8% of the adipose tissue samples from GraMo adult

cohort [51]. All the above mentioned matrices have their own variability and characteristics that can interfere in the stability of the exposure measurement and, consequently, their concentrations might have dissimilar biological meanings, which need to be clarified. In this study, exposure measure acts as a non-differential error. However, BPA levels in the organism are not stable, as levels are higher the moment the person has been exposed and until BPA is metabolized and excreted 7 or 8 hours after its incorporation into the body. BPA is rapidly conjugated and excreted by humans due to the efficient glucuronidation of BPA [242].

Other pathways of BPA exposure are dust inhalation and dermal absorption. These can be a dominant source for occupational exposure in electronic waste recycling plants and production facilities of BPA-containing materials [79,80]. However, we have not taken into account the occupational exposure, as production facilities of BPA were not included in the EPIC questionnaire at recruitment. Moreover, we have to consider that our population has been exposed to other pollutants. Therefore, the associations that we found from one single contaminant may be due to other highly correlated (and unmeasured) co-exposures, potentially including both persistent and non-persistent pollutants, or could be due to interactions among different co-exposures [289,290]. Finally, causality of the observed associations should be reinforced by addressing the mechanisms of action (e.g., measuring subclinical disease markers).

Our study has several important strengths. The population considered is large and well representative of exposure to BPA in the 1990s (>20 years of follow-up). This characteristic is valuable, enabling us to study possible associations between BPA exposure at recruitment and certain chronic illnesses currently present in the participants. In this respect, it is of notable importance that almost 70% of the study population had detectable levels of BPA. We used previously validated questionnaires which allow to have a precise characterization of the covariates. Besides, BPA was screened using validated analytical methodologies [175]. BPA serum concentrations were measured at recruitment and potential associations were assessed with IHD, AMI and AP. Finally, this study adds valuable information about serum BPA concentrations in the general population in Spain, which is another strength of this research.

## 5. Conclusions

We observed a similar percentage of BPA detection among cases and sub-cohort participants, and evedenced no significant associations between serum BPA concentrations and risk of IHD, AMI, or AP. However, the methodological approach used warrants further investigation of the influence of BPA exposure on IHD risk.

## Conflict of interest

Authors declare they have no conflict of interest.

## Funding

This work was supported by the Ministry of economy and competitiveness and the National Institute of Health: Instituto de Salud Carlos III (ISCIII). Exps: PI14/00067, PI14/01716, PI14/01880, PI14/00556. BA15/00093. FEDER and AECC Junta Provincial de Murcia, Exp.: FFIS-CC 2016-06. Dr. J.P. Arrebola is under contract within Ramon y Cajal program (RYC-2016-20155, Ministerio de Economía, Industria y Competitividad, Spain). The EPIC-Spain cohort was supported by the Health Research Fund (FIS) - Instituto de Salud Carlos III (ISCIII), the Regional Governments of Andalucía, Asturias, Basque Country, Murcia and Navarra, and the Catalan Institute of Oncology – ICO.

## Acknowledgments

This paper will be part of the doctoral thesis developed by Elena Salamanca-Fernández in the context of the “Clinical Medicine and Public Health Program” of the University of Granada (Spain).

**Table 1. Characteristics at recruitment of the EPIC-Spain IHD cases and sub-cohort participants.**

| | Cases | Sub-cohort | p* |
|---|---|---|---|
| **N (%)** | 946 (20.42) | 3,690 (79.58) | |
| **Sex** | | | <0.001 |
| Male | 703 (74.31) | 1,772 (48.02) | |
| Female | 243 (25.69) | 1,918 (51.98) | |
| **Age** | | | <0.001 |
| <45 | 117 (12.37) | 728 (19.73) | |
| 45-49 | 168 (17.76) | 598 (16.21) | |
| 50-54 | 191 (20.19) | 778 (21.08) | |
| 55-59 | 210 (22.20) | 698 (18.92) | |
| 60+ | 260 (27.48) | 888 (24.07) | |
| **Education level** | | | 0.386 |
| None | 383 (40.70) | 1,489 (40.66) | |
| Primary school | 345 (36.66) | 1,268 (34.63) | |
| Technical school | 70 (7.44) | 284 (7.76) | |
| Secondary school | 58 (6.16) | 214 (5.84) | |
| University | 85 (9.03) | 407 (11.11) | |
| **BMI** | | | <0.001 |
| Normal weight | 106 (11.26) | 665 (18.02) | |
| Overweight | 472 (49.89) | 1,846 (50.03) | |
| Obesity | 368 (38.90) | 1,179 (31.95) | |
| **Smoking status** | | | <0.001 |
| Never | 342 (36.19) | 2,193 (59.48) | |
| Former | 195 (20.63) | 696 (18.88) | |
| Smoker | 408 (43.17) | 798 (21.64) | |
| **Physical activity** | | | <0.001 |
| Inactive | 213 (22.52) | 541 (14.66) | |
| Moderately inactive | 267 (28.22) | 845 (22.90) | |
| Moderately active | 400 (42.28) | 1,990 (53.93) | |
| Active | 66 (6.98) | 314 (8.51) | |
| **Alcohol consumption** | | | <0.001 |
| None | 280 (29.60) | 1,195 (32.38) | |
| Drinker <25 g/d | 365 (38.58) | 1,702 (46.13) | |
| Heavy drinker ≥25 g/d | 301 (31.82) | 793 (21.49) | |
| **Hyperlipidemia** | 334 (35.46) | 838 (22.85) | <0.001 |
| **Hypertension** | 325 (34.43) | 902 (24.48) | <0.001 |

*Chi-square

**Table 2. Serum BPA levels (ng/ml) (percentage above the limit of detection (LOD) and geometric mean (GM) with 95% confidence intervals) as a function of sociodemographic and life style characteristics in IHD cases and sub-cohort.**

| | Cases | | | Sub-cohort | | | p* |
|---|---|---|---|---|---|---|---|
| | > LOD (%) | GM (ng/ml) | 95% CI | > LOD (%) | GM (ng/ml) | 95% CI | |
| **Total** | 71.38 | 1.22 | 1.09-1.36 | 70.08 | 1.19 | 1.13-1.26 | 0.900 |
| **Centre** | | | | | | | |
| Gipuzkoa | 59.85 | 0.72 | 0.59-0.88 | 57.46 | 0.67 | 0.61-0.75 | 0.424 |
| Granada | 74.05 | 1.30 | 0.98-1.73 | 81.12 | 1.68 | 1.52-1.86 | 0.083 |
| Murcia | 72.55 | 1.23 | 0.98-1.53 | 69.72 | 1.11 | 1.00-1.24 | 0.451 |
| Navarra | 78.53 | 1.79 | 1.49-2.15 | 70.92 | 1.54 | 1.37-1.74 | 0.267 |
| **Sex** | | | | | | | |
| Male | 72.40 | 1.30 | 1.15-1.48 | 70.94 | 1.29 | 1.19-1.40 | 0.899 |
| Female | 68.31 | 1.01 | 0.82-1.26 | 69.29 | 1.10 | 1.02-1.19 | 0.557 |
| **Age group** | | | | | | | |
| <45 | 71.79 | 1.10 | 0.82-1.47 | 71.32 | 1.06 | 0.94-1.19 | 0.682 |
| 45-49 | 71.43 | 1.46 | 1.11-1.93 | 69.48 | 1.25 | 1.09-1.45 | 0.269 |
| 50-54 | 70.68 | 1.28 | 1.00-1.64 | 65.49 | 1.31 | 1.16-1.48 | 0.884 |
| 55-59 | 76.78 | 1.52 | 1.21-1.90 | 72.43 | 1.24 | 1.09-1.41 | 0.129 |
| 60+ | 67.31 | 0.92 | 0.75-1.13 | 69.53 | 1.13 | 1.01-1.26 | 0.095 |
| **Educational level** | | | | | | | |
| None | 70.31 | 1.18 | 0.99-1.41 | 71.32 | 1.24 | 1.13-1.35 | 0.746 |
| Primary school | 72.17 | 1.23 | 1.03-1.46 | 69.48 | 1.22 | 1.10-1.34 | 0.838 |
| Technical school | 71.43 | 1.18 | 0.79-1.76 | 65.49 | 0.96 | 0.79-1.17 | 0.342 |
| Secondary school | 68.97 | 1.32 | 0.82-2.11 | 72.43 | 1.26 | 1.01-1.58 | 0.903 |
| University | 74.12 | 1.38 | 0.97-1.95 | 69.53 | 1.08 | 0.92-1.28 | 0.206 |
| **BMI** | | | | | | | |
| Normal weight | 72.64 | 1.37 | 0.98-1.92 | 68.27 | 1.06 | 0.94-1.21 | 0.150 |
| Overweight | 70.82 | 1.20 | 1.03-1.40 | 70.37 | 1.21 | 1.12-1.31 | 0.954 |
| Obese | 71.74 | 1.20 | 1.01-1.44 | 70.65 | 1.24 | 1.12-1.37 | 0.870 |
| **Physical activity** | | | | | | | |
| Inactive | 73.24 | 1.41 | 1.13-1.77 | 69.69 | 1.21 | 1.05-1.40 | 0.265 |
| Moderately inactive | 70.04 | 1.12 | 0.91-1.37 | 69.82 | 1.21 | 1.08-1.37 | 0.533 |
| Moderately active | 71.82 | 1.21 | 1.02-1.44 | 70.80 | 1.20 | 1.12-1.30 | 0.768 |
| Active | 68.18 | 1.14 | 0.75-1.73 | 66.88 | 1.04 | 0.86-1.26 | 0.740 |
| **Smoke status** | | | | | | | |
| Never | 70.47 | 1.19 | 0.99-1.43 | 70.73 | 1.21 | 1.13-1.30 | 0.999 |
| Former | 70.26 | 1.15 | 0.90-1.47 | 70.55 | 1.16 | 1.02-1.31 | 0.954 |
| Smoker | 72.62 | 1.28 | 1.09-1.51 | 67.79 | 1.15 | 1.02-1.30 | 0.292 |
| **Alcohol consumption** | | | | | | | |
| None | 69.29 | 1.11 | 0.90-1.36 | 71.05 | 1.20 | 1.09-1.33 | 0.531 |
| Drinker <25 g/d | 71.51 | 1.20 | 1.01-1.42 | 70.39 | 1.19 | 1.09-1.28 | 0.906 |
| Heavy drinker ≥25 g/d | 73.09 | 1.36 | 1.12-1.66 | 67.97 | 1.18 | 1.04-1.33 | 0.172 |
| **Hyperlipidemia** | 70.36 | 1.14 | 0.96-1.37 | 72.20 | 1.25 | 1.12-1.40 | 0.438 |
| **Hypertension** | 72.00 | 1.24 | 1.03-1.50 | 70.18 | 1.24 | 1.11-1.39 | 0.998 |

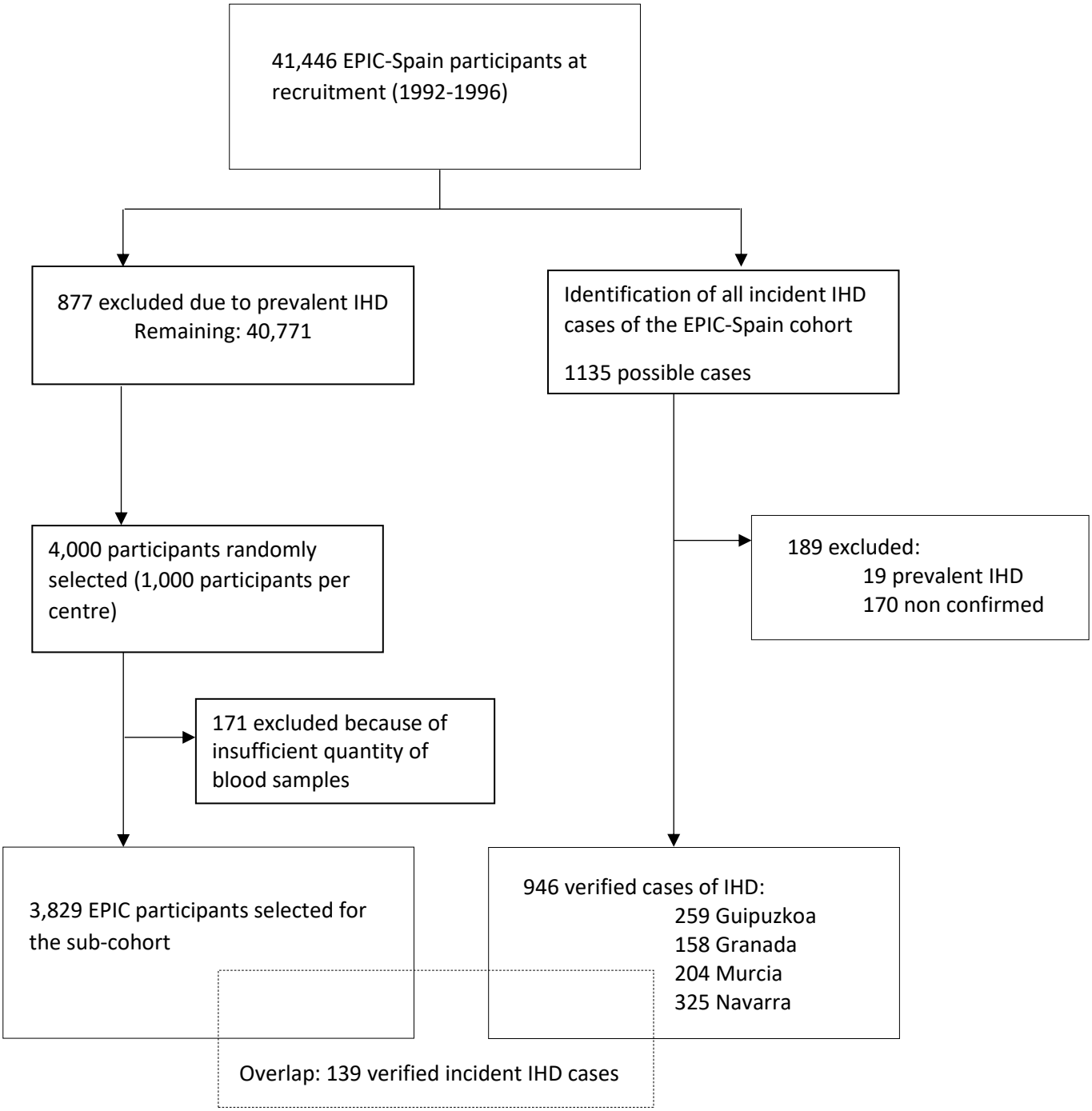
*p-value from Mann-Whitney test

**Table 3. Cox regression and risk of IHD, AMI and AP.**

| | | IHD | | | | AMI | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **HR** | **SE** | **p** | **95% CI** | **HR** | **SE** | **p** | **95% CI** | **HR** |
| **Model A** | **BPA levels (for 1 ng/ml increase)** | 0.998 | 0.005 | 0.749 | 0.99-1.01 | 1.001 | 0.005 | 0.767 | 0.99-1.01 | 0.99 |
| **Model B** | **$log_2$(BPA)** | 1.009 | 0.015 | 0.516 | 0.98-1.03 | 1.019 | 0.017 | 0.269 | 0.98-1.05 | 0.98 |
| **Model C** | **$log_2$(BPA)** | 1.002 | 0.016 | 0.895 | 0.97-1.03 | 1.009 | 0.18 | 0.602 | 0.97-1.04 | 0.97 |

Model A: linear BPA stratified by centre
Model B: log2BPA stratified by centre
Model C: log2BPA stratified by centre adjusted by sex, age, education level, BMI, physical activity, smoking status, alcohol consu

**Figure 1.** Flow chart: Case-cohort design of the study and the number of participants included in the analysis.



## Appendix

**Table A1. Comparison between selected and non-selected participants from the entire cohort.**

| | Selected sub-cohort | Non-selected |
|---|---|---|
| **N (%)** | 3,690 (79.58) | 29342 (100) |
| **Centre** | | |
| Gipuzkoa | 891 (24.15) | 7508 (25.6) |
| Granada | 980 (26.56) | 7072 (24.1) |
| Murcia | 918 (24.88) | 7581 (25.8) |
| Navarra | 901 (24.42) | 7181 (24.5) |
| **Sex** | | |
| Male | 1,772 (48.02) | 10818 (36.9) |
| Female | 1,918 (51.98) | 18524 (63.1) |
| **Age** | | |
| <45 | 728 (19.73) | 10298 (35.1) |
| 45-49 | 598 (16.21) | 6380 (21.7) |
| 50-54 | 778 (21.08) | 5097 (17.4) |
| 55-59 | 698 (18.92) | 4213 (14.4) |
| 60+ | 888 (24.07) | 3354 (11.4) |
| **Education level** | | |
| None | 1,489 (40.66) | 10683 (36.6) |
| Primary school | 1,268 (34.63) | 11010 (37.8) |
| Technical school | 284 (7.76) | 2365 (8.1) |
| Secondary school | 214 (5.84) | 1728 (5.9) |
| University | 407 (11.11) | 3376 (11.6) |
| **BMI** | | |
| <25 kg/m2 | 665 (18.02) | 6654 (22.7) |
| 25-<30 kg/m2 | 1,846 (50.03) | 13768 (46.9) |
| ≥30 kg/m2 | 1,179 (31.95) | 8920 (30.4) |
| **Smoke status** | | |
| Never | 2,193 (59.48) | 16292 (55.5) |
| Former | 696 (18.88) | 5029 (17.1) |
| Smoker | 798 (21.64) | 8008 (27.3) |
| **Physical activity** | | |
| Inactive | 541 (14.66) | 3904 (13.3) |
| Moderately inactive | 845 (22.90) | 6059 (20.6) |
| Moderately active | 1,990 (53.93) | 17024 (58.0) |
| Active | 314 (8.51) | 2355 (8.0) |

## 7.3 Objetivo específico 3

2. Estudiar la exposición a contaminantes no persistentes y riesgo de diabetes tipo 2 en la sub-cohorte de EPIC-Granada al seguimiento.

### 7.3.1 Exposición a contaminantes no persistentes riesgo de diabetes tipo 2

**Historical exposure to non-persistent environmental pollutants and risk of type 2 diabetes in a Spanish sub-cohort from the European Prospective Investigation into Cancer and Nutrition study**

Elena Salamanca-Fernández, Luz María Iribarne-Durán, Miguel Rodríguez-Barranco, Fernando Vela, Nicolás Olea, Maria-José Sánchez*, Juan Pedro Arrebola*

Publicado en Environmental Research [291] https://doi.org/10.1016/j.envres.2020.109383
Cuartil 1, Decil 1, Factor de impacto: 5.715

Environmental Research 185 (2020) 109383

Contents lists available at ScienceDirect

Environmental Research

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

# Historical exposure to non-persistent environmental pollutants and risk of type 2 diabetes in a Spanish sub-cohort from the European Prospective Investigation into Cancer and Nutrition study

E. Salamanca-Fernández[a,b], L.M. Iribarne-Durán[b], M. Rodríguez-Barranco[a,b,c], F. Vela-Soria[b], N. Olea[b,c,d], M.J. Sánchez-Pérez[a,b,c,1], J.P. Arrebola[b,c,e,1,*]

[a] Andalusian School of Public Health (EASP), Granada, Spain
[b] Instituto de Investigación Biosanitaria Ibs.GRANADA, Granada, Spain
[c] CIBER of Epidemiology and Public Health (CIBERESP), Madrid, Spain
[d] Department of Radiology, School of Medicine, University of Granada, Granada, Spain
[e] Department of Preventive Medicine and Public Health, University of Granada, Granada, Spain

ARTICLE INFO

*Keywords:*
Non-persistent environmental pollutants
Parabens
Benzophenones
Bisphenol A
Diabetes

ABSTRACT

*Background:* Environmental factors are believed to account for a substantial burden of type 2 diabetes mellitus (T2DM). Non-persistent environmental pollutants (npEPs) are a group of widely-used chemicals identified as endocrine/metabolic disrupting chemicals and obesogens. The aim of this study was to analyse the potential associations of serum levels of three groups of npEPs with the risk of incident T2DM.

*Methods:* This is a longitudinal study within a sub-sample of Granada EPIC-Spain cohort (n = 670). We quantified serum concentrations of 7 npEPs: four parabens (Methylparaben (MP) ethylparaben (EP), propylparaben (PP) and butilparaben (BP); two benzophenones: Benzophenone 1 (BP1), Benzophenone 3 (BP3); and Bisphenol A (BPA). Exposure was assessed by means of chemical analyses of serum samples collected at recruitment, and information on potential confounders was gathered by using validated questionnaires at baseline. Follow-up was performed by review of patients' clinical records. Cox Proportional Hazards Models were used for the statistical analyses.

*Results:* Median follow-up time was 23 years. There were 182 (27%) incident T2DM diagnoses in our sub-cohort. MP was the most frequently detected npEP, 88.42% samples above the limit of detection, and BP showed the lowest percentage of detection (19.21%). Those individuals within the fourth PP quartile (0.53–9.24 ng/ml) showed a statistically significant increased risk of T2DM (HR = 1.668 p = 0.012), while BP1 concentrations showed an inverse non-significant trend with the risk.

*Conclusions:* We evidenced a potential contribution of npEP exposure on T2DM, but no clear trend was observed. However, limitations in relation to exposure estimation might influence our findings and further research is warranted to confirm our results.

## 1. Introduction

Worldwide prevalence of type 2 diabetes mellitus (T2DM) among adults is nearly 415 million, with projections of 642 million for 2040 (Ogurtsova et al., 2017). This increasing prevalence of T2DM is causing an important impact on global health, since recent data of the International Diabetes Federation suggest that each year 5 million deaths are directly attributable to diabetes (Ogurtsova et al., 2017).

Among external risk factors potentially affecting the development of T2DM, there is an increasing concern on the role of chronic exposure to low doses of environmental pollutants (Ahmadkhaniha et al., 2014; Amin et al., 2019; Duan et al., 2018; Gong et al., 2013; Grice et al., 2017; Hwang et al., 2018; Ngwa et al., 2015; Provvisiero et al., 2016; Ruzzin et al., 2010; Silver et al., 2011; Yang et al., 2017). Chemical pollutants include non-persistent environmental pollutants (npEPs), are a group of widely-used chemicals including substances with very heterogonous chemical structures (Artacho-Cordón et al., 2018).

Bisphenol A (BPA) is an industrial chemical originally developed as

* Corresponding author. University of Granada, Avenida de la Investigación no 11, C.P. 18071, Granada, Spain.
*E-mail address:* jparrebola@ugr.es (J.P. Arrebola).
[1] These authors equally contributed to this work.

https://doi.org/10.1016/j.envres.2020.109383
Received 12 December 2019; Received in revised form 3 March 2020; Accepted 10 March 2020
Available online 12 March 2020
0013-9351/ © 2020 Elsevier Inc. All rights reserved.

**ABSTRACT**

*Background:* Environmental factors are believed to account for a substantial burden of type 2 diabetes mellitus (T2DM). Non-persistent environmental pollutants (npEPs) are a group of widely-used chemicals identified as endocrine/metabolic disrupting chemicals and obesogens. The aim of this study was to analyse the potential associations of serum levels of three groups of npEPs with the risk of incident T2DM.

*Methods:* This is a retrospective study within a sub-sample of Granada EPIC-Spain cohort (n=670). We quantified serum concentrations of 7 npEPs: four parabens (Methylparaben (MP) ethylparaben (EP), propylparaben (PP) and butilparaben (BP); two benzophenones: Benzophenone 1 (BP1), Benzophenone 3 (BP3); and Bisphenol A (BPA). Exposure was assessed by means of chemical analyses of serum samples collected at recruitment, and information on potential confounders was gathered by using validated questionnaires at baseline. Follow-up was performed by review of patients' clinical records. Cox Proportional Hazards Models were used for the statistical analyses.

*Results:* Median follow-up time was 23 years. There were 182 (27%) incident T2DM diagnoses in our sub-cohort. MP was the highest detected compound since 88.42% of the population presented levels above LOD and BP the less detected (19.21% of the population). Those individuals within the fourth PP quartile (0.53-9.24 ng/ml) showed a statistically significant increased risk of T2DM (HR=1.668 p=0.012), while BP1 concentrations showed an inverse non-significant trend with the risk.

*Conclusions:* We evidenced a potential contribution of npEP exposure on T2DM, but no clear trend was observed. However, limitations in relation to exposure estimation might influence our findings and further research is warranted to confirm our results.

*Keywords:* non-persistent Environmental Pollutants; Parabens; Benzophenones; Bisphenol A, Diabetes.

**Funding sources**

This research was supported in part by research grants from the ISCIII (PI14/00067). The authors are grateful to Instituto de Salud Carlos III (Miguel Servet Type I Program CP15/00193) for the research contract. Dr. J.P. Arrebola is under contract within Ramon y Cajal program (RYC-2016-20155, Ministerio de Economía, Industria y Competitividad, Spain). Funding sources had no involvement in the conduct of the research.

**Ethics statement**

All participants were informed at recruitment and they signed an informed consent. This study was approved by Ethics Committee of Granada (Comité de Ética de la Investigación Biomédica de Granada).

## 1. Introduction

Worldwide prevalence of type 2 diabetes mellitus (T2DM) among adults is nearly 415 million, with projections of 642 million for 2040 [123]. This increasing prevalence of T2DM is causing an important impact on global health, since recent data of the International Diabetes Federation suggest that each year 5 million deaths are directly attributable to diabetes [123].

Among external risk factors potentially affecting the development of T2DM, there is an increasing concern on the role of chronic exposure to low doses of environmental pollutants [124–134]. Chemical pollutants include non-persistent environmental pollutants (npEPs), are a group of widely-used chemicals including substances with very heterogonous chemical structures [51].

Bisphenol A (BPA) is an industrial chemical originally developed as a synthetic estrogen, and one of the highest volume chemicals produced worldwide [72]. It is widely used in the manufacture of polymers and epoxy resins, polycarbonates and polysulfones plastics. This implies that the population is exposed frequently and inadvertently to this compound [73,74]. Recent experimental animal studies have reported estrogenic potential of BPA [81–85], but it can also act as an antiestrogen by competing with the endogenous hormone 17-beta estradiol [86]. It is estimated that over 90% of the population in the US, Europe and Asia is exposed to BPA, presenting detectable levels in urine (>0.4 ng/ml) [75–78]. General population is considered to be exposed to BPA mainly through diet, since it is frequently present in food packaging, e.g., tins, cans, or plastic packaging, from which BPA can migrate to the food under certain environmental conditions [73,87–91]. Once absorbed in the intestine, BPA is readily glucuro-conjugated or sulfo-conjugated in the liver, and finally excreted in urine (half-life in humans: 7-8 hours) [74]. The increasing concerns on the potential health effects of BPA exposure induced the European Food Safety Authority (EFSA) to reduce the tolerable daily intake of BPA in 2015: from 50 μg / kg per day to 4 μg / kg per day; and a re-evaluation is being prepared for 2020 [90]. Recently, the General Court of the European Union published a confirmation of the inclusion of BPA as a substance of very high concern [92]. Moreover, French Senate definitively adopted the legal ban of bisphenol A (BPA) for food contact materials intended for children below 3 by early 2013, and for all food contact materials by January 2015 [292].

Parabens (e.g., methylparaben [MP], ethylparaben [EP], n-propyl and isopropylparaben ([n-PrP and i-PrP], n-butyl and isobutylparaben [n- BuP and i-BuP]) and benzylparaben [BzP]) are widely used as antimicrobial preservatives in cosmetics, pharmaceuticals, food and beverages [94]. PBs are rapidly metabolized and excreted from the body, mainly during the first 24h [95,293]. They have shown endocrine disruption properties with estrogenic and anti-androgenic activities in vitro and in vivo studies [96–98].

Benzophenones (BPs) are synthetic compounds used in personal care products due to their protection against UV radiation [101]. BPs have been detected in urine and some other biological matrices, such as placenta [50], human breast milk [47] and menstrual blood [52]. BPs endocrine disrupting effects have mainly been observed *in vitro* and in animals studies, including effects on reproduction and ontogeny [105–107].

The abovementioned npEPs are considered potential metabolic and endocrine disruptors, because they can alter the programming or sensitivity for developing obesity/diabetes or aspects of metabolic syndrome [294–297]. Moreover, these compounds can act as obesogens, which are functionally defined as chemicals that promote obesity in humans or animals [135]. In this regard, there is accumulated evidence linking these chemicals to obesity and obesity-related chronic diseases, including hormone-dependent cancers and metabolic syndrome [133,136]. However, the implications of the exposure to npEPs and T2DM in the general population are still arguable due to controversial reports in the literature [133,137,138].

The present study, which is framed in the European Prospective Investigation into Cancer and Nutrition (EPIC) study, aims to assess associations of serum concentrations of a selection of npEPs with the risk of incident T2DM.

## 2. Methods

### *2.1 Study design and study population: The EPIC cohort*

This research is a longitudinal study within Granada EPIC-Spain cohort (N = 7,879). The European Prospective Investigation into Cancer and Nutrition (EPIC) is a prospective multi-centric cohort study designed to investigate the relationship between diet, lifestyles and cancer. It recruited 41,000 subjects in Spain between 1992 and 1996 and involves 23 research centres in 10 European countries, including 5 Spanish centres: Asturias, Granada, Murcia, Navarra and

Gipuzkoa [165]. Subjects reported information about dietary, lifestyle, reproductive and anthropometric factors at baseline. EPIC study populations and data collection were explained elsewhere [177].

In Granada, EPIC recruitment took place between 1992-1996 with 7,879 apparently healthy subjects (23% men and 77% women) mean aged 49 and 51 years respectively. Study population consisted on a sub-cohort of 807 participants (10,24%) with no prevalent T2DM cases. Our population was selected using stratified random sampling by sex and age in order to maintain the representativeness of EPIC-Granada cohort. A total of 137 individuals were excluded, 125 individuals because of not providing enough sample volume and 12 because of missing data on socio-economic, educational or lifestyle information variables (Supplementary material, Table 1). Therefore, final study population consisted on a sub-cohort of 670 participants.

*2.2 Covariate assessment*

Covariates were gathered during a personal interview at recruitment, when measurements of height, weight, and hip and waist circumferences were performed, using standardized procedures [177]. Information on educational level, history of previous illnesses, history of tobacco use, physical activity, occupation, and reproductive history [177] was also included.

BMI (kg/m²) was considered both as continuous variables as well as in three categories: <25 (normal weight), 25-<30 (overweight), ≥30 (obesity) [298]. Educational level was classified according to five categories: none, primary school, secondary school, technical or professional training and university degree. Smoking status was summarized in three categories: never smoker, former smoker and current smoker. Different domains of physical activity were gathered taking into account seasonal variation. A simple four-level physical activity index (inactive, moderately inactive, moderately active and active) was derived and validated by combining occupational and recreational activity [178].

Dietary information over the last 12 months was collected at recruitment by means of an interview-administered computerized version of a dietary history questionnaire of 662 food items, that had been previously validated in Spain [176,177].

*2.3 Outcome assessment*

All the study participants were residents in Granada province and users of the Andalusian Public Health System. Ascertainment of incident T2DM cases was performed retrospectively by reviewing the datasets of Andalusian CMBD (minimum basic set of health data), and then the review of patient´s clinical records at the databases available in the Andalusian Public Health System. A participant was considered as type 2 diabetic when he/she had been diagnosed of T2DM during follow-up time and/or when continuous prescription of anti-diabetic medication had been registered in the clinical records. We excluded prevalent T2DM cases from the statistical analyses, that were identified by baseline self-report of a history of diabetes, previous diagnosis by a clinician, and/or chronic prescription of diabetes medication. The follow-up time began at EPIC recruitment date and continued until T2DM diagnosis or patient's death. If the participant did not experience any of these events, the follow-up period ended at 31st July 2017.

*2.4 Chemical analysis*

One spot blood sample was drawn from each participant at recruitment (1992-1996). Blood samples were subsequently centrifuged, and 0.5 mL serum aliquots were stored in liquid nitrogen (-196ºC).

Serum concentrations of three groups of npEPs (Bisphenol A (BPA), four parabens (Methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butilparaben (BP)), and two benzophenones (Benzophenone 1 (BP1), Benzophenone 3 (BP3)), were quantified in 2018-2019 in serum samples extracted at recruitment. An adaptation of a previously-validated methodology [175] was used. In brief, analysis of npEPs was carried out by dispersive liquid–liquid micro-extraction (DLLME) and ultra-high performance liquid chromatography with tandem mass spectrometry detection (UHPLC-MS/MS: Agilent Series 1290 LC system (Agilent Technologies, Santa Clara, CA) and API 4000 (triple quadrupole) mass spectrometer (AB SCIEX). Samples were thawed completely at room temperature, centrifuged at 2600g for 10 min and 0.75 mL were taken to carry out the analysis. In order to determine total BPA amount (free plus conjugated) in serum, each sample was spiked with 50µL of enzyme solution (β-glucuronidase/sulfatase) and incubated at 37 °C for 24 h. The treated serum was placed in a 15 mL screw-cap glass tube and spiked with 30µL of the surrogate standard solution (1.25 mg/L of BPA-d16 and EP-6C13). Serum was diluted to 10.0 mL with 5% NaCl aqueous solution (w/v) and the pH was adjusted to 2.0. Next, 0.75 mL acetone and 0.75 mL trichloromethane

were mixed and injected rapidly into the aqueous sample with a syringe. After manual shaking, centrifugation and evaporation of the extract, the residue was dissolved with 100µL of a mixture consisting of water (0.1% ammonia)/acetonitrile (0.1% ammonia), 70:30 (v/v), and finally 10µL was injected in the LC system. Limit of detection (LOD) was 0.14 ng/ml.

*2.5 Statistical analysis*

In the descriptive analysis, npEP concentrations ((ng/mL) serum sample) were expressed as geometric mean (GM), median, 95% Confidence Interval (95% CI) and 25$^{th}$ and 75th percentiles. Serum concentrations of npEP below the LOD were assigned a value of LOD/√2. Differences between categorical variables were calculated using the Chi-square test. An analysis of the correlation between pollutants was performed by Spearman correlation test (Supplementary Material, Table 2).

All npEP serum concentrations were log-transformed to minimise the influence of extreme values; therefore, β coefficients are also presented as exp(β). BPA, MP, EP, and BP3 were also categorized in quintiles and PP, BP and BP1 in quartiles due to the higher number of values below LOD. Potential associations of serum concentrations of npEPs and T2DM were assessed by multivariable Cox Proportional Hazards Model. The shape of the associations was evaluated with generalized additive models (GAM) (Figure 1). A test for the proportional hazards assumption was performed with satisfactory results as part of the diagnostics of the models, using the cox.zph function implemented in the R survival package. This function correlates the scaled Schoenfeld residuals with the Kaplan–Meier estimate of the survival function. Age, sex, BMI and educational level were always retained in the models, regardless of their statistical significance, given published evidence of their potential association. Additional analyses were performed by stratification for sex and BMI (Supplementary Material, Tables 3-7). The significance level was set at p=0.05.

Statistical analyses were conducted with SPSS Statistics 22.0 (IBM, Chicago, IL) and R statistical computing environment 3.0 (R Core team 2018) [181], using *pspline* [182] and *survival* [183,184] libraries.

## 3. Results

In our study population, the average participants´ age was 53.04 years (range 35.19-67.58), and 52.1% were women. At recruitment, 46.5% of them had overweight and 60.7% had never smoked (Table 1). Regarding physical activity, 58.7% of participants were considered to be moderately active. Median follow-up time was 22.8 years. During the follow-up, there were 182 (27.2%) incident T2DM diagnoses in our sub-cohort (Table 1).

Levels of npEPs in serum are described in Table 2 and in supplementary Figure 1. BPA and MP were found in 81.8% and 88.5% of the study population respectively. More than a half of the population showed detectable levels of EP and BP3 (56.4% and 68.6%, respectively). The highest GM concentrations were found in MP, BPA and BP3, with 2.03 ng/ml, 1.76 ng/ml and 0.51 ng/ml respectively. PP was detected in 38.06% of the population with a GM of 0.22 ng/ml (Table 2). Pollutant distributions were highly skewed, with a number of potentially influential outliers. Log-transformation normalized the distributions for chemicals with the highest detection rates (BPA and MP) (Supplementary Material, Figure 1). Male showed higher percentage of detection and GM concentrations than female for BPA and, on the contrary, female showed higher percentage of detection and GM concentrations than men for MP (Supplementary Material, Table 8).

Those individuals within the fourth PP percentile (P4: 0.53-9.24 ng/ml) showed a statistically significant increased risk of T2DM (HR=1.67 p=0.012) (Table 3), which was consistent with the observed trend in GAM models (Figure 1). No relevant differential associations were observed after stratification by sex categories (Supplementary Material, Tables 3-4).

Regarding BP1, we found a negative trend with T2DM risk in GAM models (Figure 1), although not significant in the analyses using percentiles and log-transformed concentrations. Interestingly, this association was only suggestive in females (HR for log[BP1]= 0.77, p=0.09) (Supplementary Material, Tables 3 and 4). Notably, females showed a higher percentage of BP1 detection than males (29.4% *vs* 13.1% respectively) as well as a higher GM concentrations (0.10 vs 0.08 ng/ml respectively) (Supplementary Material, Table 8). No trends nor significant associations were found between the rest of npEPs and T2DM risk (Figure 1 and Table 3).

When stratifying by BMI, we observed a positive association of BP1 concentrations with T2DM risk in normal weight individuals, that was less noticeable in overweight and obese participants. Similarly, in normal weight individuals, those within the 5th percentile of BPA concentrations showed an increased T2DM risk (Supplementary Material, Tables 5-7).

Due to the low detection rate of some npEPs and the skewed distributions (Supplementary Figure 2), we also performed Cox-regression analyses considering dichotomized independent variables (> LOD / <LOD). The results were consistent with those from models using log-transformed concentrations and percentiles (Table 3 and Supplementary Tables 3 to 7).

## 4. Discussion

To the best of our knowledge, our study is one of the very first attempts to longitudinally explore the potential contribution to T2DM of historical human exposure to parabens, benzophenones, and BPA, over a relatively large follow-up time. Despite all npEPs included in our study are considered endocrine disruptors (EDs) with diabetogenic potential [133,138,266,287,299,300], no evident associations were found for most of the pollutants, although those individuals with the highest PP serum concentrations showed an increased risk of incident T2DM. Unexpectedly, a negative trend was found between BP1 and T2DM risk, which was only evident in females.

As abovementioned, the relatively scarce scientific literature on epidemiologic studies provides highly conflicting evidences on the metabolic disrupting potential of these npEPs. A prospective nested case–control study did not find an association of serum BPA exposure with 5-year T2D incidence among Chinese participants: OR 0.93 (95% CI 0.41, 2.13) [301] which is in consonance with our results. On the contrary, a cross-sectional study in a Thai population found positive associations between serum BPA concentrations and diabetes risk (ORs for 3$^{rd}$ and 4$^{th}$ BPA quartiles= 1.88 [95% CI= 1.18, 2.99] and 1.83 [95% CI= 1.12, 2.95], respectively) [205]. Regarding epidemiologic studies using urine as the matrix for exposure assessment, Li et al. observed a 6-fold increase in the odds of diabetes in those individuals with the highest urinary MP and EP concentrations, compared to those with the lowest concentrations [302]. Likewise, Kim et al. observed a significant association between BPA levels and T2DM risk in a case-control study (OR=1.71; 95% CI: 0.89-3.26; p= 0.374) [303]. Surprisingly, the study of Wang et al. found a protective effect of BPA on GDM risk (OR = 0.73; 95% CI = 0.56, 0.97)

[304]. On the other hand, a prospective study on 200 pregnant women concluded that the use of UV-filters (including BPs) could disrupt the endocrine homeostasis and adversely affect the development of foetuses and children [305]. Overall, there are a number of epidemiologic studies [301,303] and reviews [266,287] that did not find a clear association between npEPs and T2DM. In light of the above, there is currently sparse evidence about the potential diabesogenic effects for most npEPs.

Interestingly, parabens and benzophenones are frequently used in cosmetics (apart from food items) [94,306,307], which likely account for a relevant percentage of our sex-related differences in the effects npEPs exposure. In our cohort, serum concentrations of most npEPs were higher in females than in males, with the exception of BPA (Supplementary Material, Table 3-4). Despite the use of cosmetics was not addressed in our questionnaires, the abovementioned findings suggest marked sex-related differences in their use. Moreover, in females we found a higher number of extreme values and, thus, this increased variability might make their potential effect more evident in females. Further research is warranted on this aspect.

Some researchers have argued the role of npEPs on the aetiology of T2DM, GDM or obesity, on the bases of the relatively low exposure levels as well as their rapid metabolism and excretion [137]. Indeed, recent scientific literature suggests that BPA and parabens have an obesogenic potential in humans [255–258,265,276,277]. However, Lakind *et al.* [266] suggested that the design of epidemiological studies as well as the different methodological approaches could be limiting the understanding this possible association of npEPs exposure and T2DM risk. A relevant aspect to be considered is the method used for the assessment of the exposure. In our study, we analysed npEP concentrations in serum samples, which can be relatively unstable, being more representative of recent exposures [197,241]. Some studies point out that, among the biomonitoring matrices, urine contains the highest BPA concentrations, followed by serum [39] which implies a superior capacity to detect low exposure levels, being also a more adequate estimator of medium-term exposure. Another important limitation of current epidemiologic studies is the lack of exposure assessment during critical windows of development [308].

Potential mechanisms of action of the selected npEPs still remain unclear [146,275]. Liu et al. [309] hypothesized that parabens exposure during pregnancy could cause islet beta-cell dysfunction by inducing oxygen free radicals and, thus, increase the risk of GDM. In this regard, a cross-sectional study on a Korean mother-child cohort found positive associations of maternal

urinary EP levels (but not MP or PP) with urinary malondialdehyde levels [310], which reinforces the abovementioned hypothesis of oxidative stress induction. In addition, experimental studies showed that animal beta islets have relatively low concentrations of anti-oxidative hydroxide and oxygen free radicals [311] and, therefore, can be relatively sensitive to oxidative stress [312]. Certain npEPs may also disrupt epigenetic signaling (e.g. DNA methylation, histone marks, chromatin remodeling and noncoding RNAs) [275], which might affect normal tissue development, eventually leading to obesity and related conditions [278]. There is also experimental and in vivo evidence supporting that BPA and BPs exposure can produce an overstimulation of the estrogen receptor α (ERα) in pancreatic β-cells, eventually leading to alteration in the biosynthesis and secretion of insulin [107,313–315]. Other studies suggested that BPA may contribute to insulin resistance by suppressing adiponectin [316,317], a hormone involved in the maintenance of insulin sensitivity [318] and, thus, inducing the impairment of insulin receptors signaling in skeletal muscle and liver [319]. However, experimental studies have overall yielded to conflicting results, most likely due to different experimental designs, timing of exposure, and uncontrolled or residual confounding factors, such as the route of the administration of these pollutants, the degradation time of npEPs or low exposure doses [132,137,146]. It should be considered that the abovementioned mechanisms of action may not be applicable for all the selected npEPs, since there is still scant research in this field, particularly for some of the chemicals (e.g., parabens and benzophenones). Further research should combine mechanistic and epidemiologic approaches to assess whether the abovementioned mechanisms are sufficient to produce relevant metabolic effects in the general populations at current exposure levels.

Strengths of our study include our longitudinal design and relatively large number of participants, with >20 years of follow-up. We used previously-validated questionnaires which allow to have a precise characterization of the covariates. Another strength is that our cohort was covered by the public health system of Andalusia and, thus, accessible by our clinical record review. Besides, the included chemicals were screened using validated analytical methodologies [175]. However, our study has also certain limitations, since the exposure to npEPs was estimated by using serum concentrations at recruitment, and we do not have information on changes in npEPs concentrations and covariates during the follow-up time. In addition, the use of one npEP point measurement might not take into account intra-day fluctuations, which might be relevant in certain populations [284,285]. This increased variability would not likely bias our results towards false positive associations but would

probably cause a non-differential error and, therefore, hamper the identification of certain real associations. On the other hand, the biological matrix used for biomonitoring plays an important role as we used serum for npEPs assessment, other studies used urine [41–43], hair [44,45], umbilical cord serum [39,46], breast milk [39,47,48], placenta [49,50], and even menstrual blood [52]. In this regard, the biological matrix most commonly used to determine BPA is urine [287]. Indeed, some authors acknowledge urine concentrations as the best biomarker of BPA exposure, since metabolites in blood can be several orders of magnitude lower than in urine and it can be indicative of a relatively longer exposure period in comparison to other matrices [197]. Additionally, on the bases of the moderate lipophilicity of these chemicals (log octanol–water partition coefficients (Kow) ranging from 1 to 5), some authors have hypothesized that they might also reach fatty tissues [51,213,288]. In this regard, previous research in the same study area found detectable BPA concentrations in 86.8% of the adipose tissue samples from an adult cohort (GraMo cohort) [51]. All the abovementioned matrices have their own variability and characteristics that can interfere in the stability of the exposure measurement and, consequently, their concentrations might have dissimilar biological meanings, which need to be clarified. Moreover, we have to take into account that our population have been exposed to other pollutants. Therefore, the associations that we found from one single contaminant may be due to other highly correlated (and unmeasured) co-exposures, potentially including both persistent and non-persistent pollutants, or even a result of interactions among different co-exposures [289,290]. Finally, causality of the observed associations should be reinforced by addressing mechanisms of action (e.g., measuring subclinical disease markers).

## 5. Conclusions

We evidenced a potential contribution of npEPs exposure on T2DM, specifically for PP, although no clear relationship was observed. No significant associations were observed for parabens, BPA and benzophenones. We consider that these results are relevant for public health, in view of the high prevalence of the exposure as well as the epidemic burden of the disease in current society. Further research on the potential health implications of endocrine disruptors and T2DM risk is needed.

## Conflict of interest

The authors disclose any financial or personal conflict of interest that could inappropriately influence the work.

## Acknowledgments

This work would not have been possible without the generous collaboration of the EPIC participants. This paper will be part of the doctoral thesis developed by Elena Salamanca-Fernández in the context of the "Clinical Medicine and Public Health Program" of the University of Granada (Spain).

**Figure 1. npEPs exposure and risk of type 2 diabetes. Generalized Additive Model plots.**

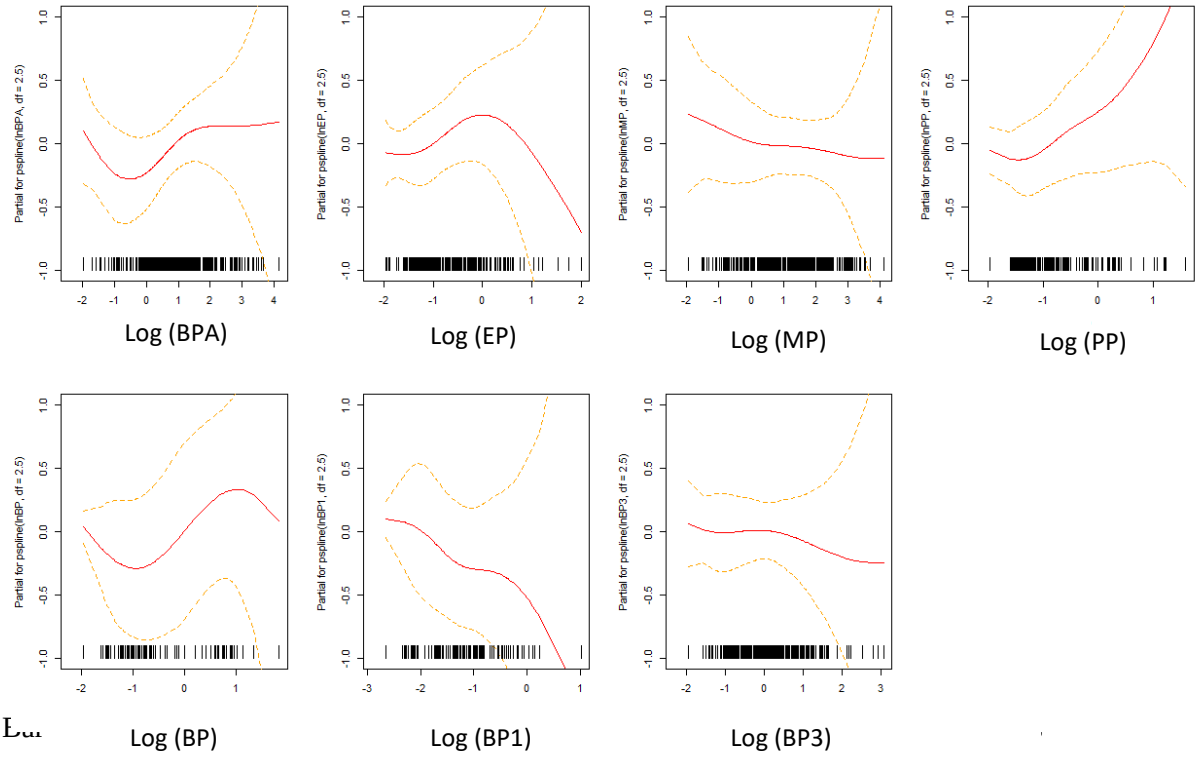


**Table 1. Baseline characteristics of the study population**

| | N (%) | T2DM | p value* |
|---|---|---|---|
| | 670 (100) | 182 (27.2) | |
| **Sex** | | | 0.219 |
| Male | 320 (47.8) | 94 (29.4) | |
| Female | 350 (52.2) | 88 (25.1) | |
| **Age** | | | 0.002 |
| <45 years | 142 (21.2) | 23 (16.2) | |
| 45-54 years | 233 (34.8) | 61(26.2) | |
| 55-59 years | 126 (18.8) | 39 (31.0) | |
| ≥60 years | 169 (25.2) | 59 (34.9) | |
| **Education level** | | | 0.028 |
| None | 352 (52.5) | 109 (31.0) | |
| Primary school | 172 (25.7) | 45 (26.2) | |
| Technical school | 25 (3.7) | 1 (4.0) | |
| Secondary school | 43 (6.4) | 10 (23.3) | |
| University | 78 (11.6) | 17 (21.8) | |
| **BMI** | | | <0.001 |
| Normal weight (20 – 25 kg/m$^2$) | 93 (13.9) | 12 (12.9) | |
| Overweight (25 – 30 kg/m$^2$) | 311 (46.4) | 63 (20.3) | |
| Obese (≥ 30 kg/m$^2$) | 266 (39.7) | 107 (40.2) | |
| **Smoke status** | | | 0.548 |
| Never | 410 (61.2) | 106 (25.9) | |
| Former smoker | 144 (21.5) | 44 (30.6) | |
| Current smoker | 116 (17.3) | 32 (27.6) | |
| **Physical activity** | | | 0.964 |
| Inactive | 93 (13.9) | 25 (26.9) | |
| Moderately inactive | 143 (21.3) | 41 (28.7) | |
| Moderately active | 394 (58.8) | 106 (26.9) | |
| Active | 40 (6.0) | 10 (25.0) | |
| 12-hours fasting conditions (yes) | 342 (51.0) | 82 (45.1) | 0.058 |
| Energy intake Mean (SD) – kcal/day | 1954.20 (646.73) | 1937.91 (715.91) | 0.482 |

* Pearson chi-square

Physical activity measures were obtain from [178]

**Table 2. Serum npEP concentrations (ng/mL) in the study population.**

| | > LOD (%) | GM (ng/ ml) | 95% CI (ng/ ml) | Median (ng/ ml) | Maximum (ng/ml) | Percentile 25 (ng/ ml) | Percentile 75 (ng/ ml) |
|---|---|---|---|---|---|---|---|
| BPA | 81.64 | 1.76 | (1.56-1.99) | 2.14 | 62.95 | 0.58 | 5.80 |
| MP | 88.51 | 2.03 | (1.82-2.28) | 2.27 | 75.55 | 0.73 | 6.52 |
| EP | 56.42 | 0.29 | (0.27-0.31) | 0.24 | 16.91 | < LOD | 0.50 |
| PP | 38.06 | 0.22 | (0.21-0.24) | < LOD | 9.24 | < LOD | 0.32 |
| BP | 19.55 | 0.18 | (0.17-0.19) | < LOD | 6.21 | < LOD | < LOD |
| BP1 | 21.64 | < LOD | (0.09-0.10) | < LOD | 4.21 | < LOD | < LOD |
| BP3 | 68.66 | 0.51 | (0.47-0.56) | 0.55 | 21.20 | < LOD | 1.10 |

LOD: Limit of detection; GM: Geometric mean; 95% CI: Confidence Interval
Bisphenol A (BPA), Methylparaben (MP), Ethylparaben (EP), Propylparaben (PP), Butilparaben (BP), Benzophenone 1 (BP1), Benzophenone 3 (BP3)

## Table 3. Serum npEP concentrations and risk of incident type 2 diabetes. Cox Proportional Hazard Models

| | | | | Parabens | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BPA | | | MP | | | EP | | | PP | | | BP | | |
| | HR | SE | p-value | HR | SE | p-value | HR | SE | p-value | HR | SE | p-value | HR | SE | p-value |
| **Percentiles of npEPs** | | | *0.998 | | | *0.605 | | | *0.730 | | | *0.017 | | | *0.797 |
| **P2**** | 1.055 | 1.262 | 0.818 | 0.675 | 1.279 | 0.110 | 1.122 | 1.266 | 0.627 | 0.694 | 1.313 | 0.179 | 0.771 | 1.449 | 0.483 |
| **P3**** | 0.976 | 1.280 | 0.922 | 0.903 | 1.260 | 0.658 | 0.800 | 1.296 | 0.390 | 0.889 | 1.290 | 0.647 | 0.796 | 1.416 | 0.511 |
| **P4**** | 1.008 | 1.275 | 0.975 | 0.858 | 1.275 | 0.527 | 1.104 | 1.255 | 0.663 | 1.668 | 1.225 | 0.012 | 1.083 | 1.330 | 0.780 |
| **P5**** | 1.027 | 1.278 | 0.914 | 0.826 | 1.275 | 0.432 | 1.174 | 1.259 | 0.487 | - | - | - | - | - | - |
| **>LOD *vs* <LOD** | 0.991 | 1.215 | 0.962 | 0.828 | 1.247 | 0.394 | 1.050 | 1.171 | 0.758 | 1.071 | 1.171 | 0.667 | 0.896 | 1.223 | 0.584 |
| **Log-transformed concentrations (ng/ml)** | 1.008 | 1.049 | 0.876 | 0.988 | 1.054 | 0.821 | 1.063 | 1.100 | 0.518 | 1.027 | 1.114 | 0.802 | 1.167 | 1.101 | 0.109 |
| **Unadjusted model** | 0.985 | 1.048 | 0.740 | 0.969 | 1.051 | 0.534 | 1.036 | 1.095 | 0.693 | 1.190 | 1.097 | 0.062 | 1.113 | 1.113 | 0.318 |

BPA: Bisphenol A; MP: Methylparaben; EP: Ethylparaben; PP: Propylparaben; BP: Butilparaben; BP1: Benzophenone 1; BP3:Benzophenone 3; HR: Hazard ratio; Detection

Adjusted by sex, age, BMI, fasting, energy intake, physical activity, educational level, extraction time and smoking status

*p trend

**: reference category: P1

Ranges of concentrations (ng/mL) in each percentile:

BPA: P1 (<LOD - 0.31), P2 (0.32- 1.47), P3 (1.49 - 3.34), P4 (3.38- 7.42), P5 (7.51- 62.95)

MP: P1 (<LOD - 0.48), P2 (0.49- 1.54), P3 (1.55- 3.42), P4 (3.44- 8.05), P5 (8.16-75.55)

EP: P1 (<LOD - 0.18), P2 (0.20- 0.29), P3 (0.30-0.45), P4 (0.45-0.75), P5 (0.76-16.91)

PP: P1 (<LOD - 0.20), P2 (0.20-0.31), P3 (0.31-0.52), P4 (0.53-9.24)

BP: P1 (<LOD - 0.20), P2 (0.20-0.36), P3 (0.37-0.57), P4 (0.61-6.21)

BP1: P1 (<LOD - 0.10), P2 (0.10-0.20), P3 (0.21-0.37), P4 (0.37-4.46)

BP3: P1 (<LOD - 0.14), P2 (0.21-0.48), P3 (0.49-0.85), P4 (0.86-1.43), P5 (1.46-21.20)

## Supplementary Material

**Supplementary Table 1. Comparison between selected and non-selected participants from the entire cohort.**

| | Selected n (%) | Non-selected n (%) |
|---|---|---|
| **Total** | 670 (83.02) | 125 (16.98) |
| **Age** | | |
| <45 | 142 (21.2) | 34 (27.2) |
| 45-54 | 233 (34.8) | 55 (44.0) |
| 55-59 | 126 (18.8) | 17 (13.6) |
| 60+ | 169 (25.2) | 19 (15.2) |
| **Education level** | | |
| None | 352 (52.5) | 24 (19.2) |
| Primary school | 172 (25.7) | 29 (23.2) |
| Technical school | 25 (3.7) | 4 (3.2) |
| Secondary school | 43 (6.4) | 11 (8.8) |
| University | 78 (11.6) | 57 (45.6) |
| **BMI** | | |
| <25 kg/m$^2$ | 93 (13.9) | 17 (13.6) |
| 25-<30 kg/m$^2$ | 311 (46.4) | 81 (64.8) |
| ≥30 kg/m$^2$ | 266 (39.7) | 27 (21.6) |
| **Smoke status** | | |
| Never | 410 (61.2) | 71 (56.8) |
| Former | 144 (21.5) | 36 (28.8) |
| Smoker | 116 (17.3) | 18 (14.4) |
| **Physical activity** | | |
| Inactive | 93 (13.9) | 33 (26.4) |
| Moderately inactive | 143 (21.3) | 40 (32.0) |
| Moderately active | 394 (58.8) | 47 (37.6) |
| Active | 40 (6.0) | 5 (4.0) |

**Supplementary Table 2. Spearman correlation among npEPs included in the study**

| | | BPA | MP | EP | PP | BP | BP1 | BP3 |
|---|---|---|---|---|---|---|---|---|
| BPA | Correlation coefficient | - | -0.130 | -0.131 | -0.115 | -00.178 | -0.127 | -0.078 |
| | p-value | | 0.001 | 0.001 | 0.003 | <0.001 | 0.001 | 0.044 |
| MP | Correlation coefficient | | - | 0.436 | 0.315 | 0.370 | 00.116 | 0.268 |
| | p-value | | | <0.001 | <0.001 | <0.001 | 0.003 | <0.001 |
| EP | Correlation coefficient | | | - | 0.448 | 0.244 | 0.068 | 0.200 |
| | p-value | | | | <0.001 | <0.001 | 0.078 | <0.001 |
| PP | Correlation coefficient | | | | - | 0.382 | 0.253 | 0.193 |
| | p-value | | | | | <0.001 | <0.001 | <0.001 |
| BP | Correlation coefficient | | | | | - | 0.165 | 0.107 |
| | p-value | | | | | | <0.001 | 0.006 |
| BP1 | Correlation coefficient | | | | | | - | 0.271 |
| | p-value | | | | | | | <0.001 |
| BP3 | Correlation coefficient | | | | | | | - |
| | p-value | | | | | | | |

## Supplementary Table 3. Serum npEPs exposure and risk of incident type 2 diabetes. Cox Proportional Hazard M

| | | | | Parabens | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **BPA** | | | **MP** | | | **EP** | | | **PP** | | | **BP** | | |
| | HR | SE | p | HR | SE | p | HR | SE | p | HR | SE | p | HR | SE | p |
| **Percentiles of npEPs** | | | *0.835 | | | *0.833 | | | *0.621 | | | *0.050 | | | *0.7 |
| **P2** | 0.690 | 1.402 | 0.273 | 0.728 | 1.522 | 0.45 | 0.929 | 1.465 | 0.847 | 0.812 | 1.430 | 0.560 | 0.595 | 1.700 | 0.3 |
| **P3** | 0.816 | 1.391 | 0.537 | 0.656 | 1.456 | 0.263 | 0.840 | 1.446 | 0.636 | 0.732 | 1.430 | 0.383 | 0.800 | 1.607 | 0.6 |
| **P4** | 0.921 | 1.399 | 0.806 | 0.699 | 1.456 | 0.341 | 1.408 | 1.372 | 0.280 | 1.883 | 1.329 | 0.026 | 1.170 | 1.427 | 0.6 |
| **P5** | 0.949 | 1.473 | 0.893 | 0.694 | 1.433 | 0.31 | 1.326 | 1.385 | 0.386 | - | - | - | - | - | - |
| **>LOD *vs* <LOD** | 0.740 | 1.298 | 0.268 | 0.754 | 1.474 | 0.468 | 1.125 | 1.261 | 0.612 | 1.097 | 1.249 | 0.676 | 0.882 | 1.301 | 0.6 |
| **Log-transformed concentrations (ng/ml)** | 1.023 | 1.079 | 0.764 | 0.957 | 1.083 | 0.58 | 1.136 | 1.142 | 0.34 | 1.309 | 1.160 | 0.070 | 1.046 | 1.140 | 0.7 |
| **Unadjusted model** | 1.018 | 1.075 | 0.801 | 0.971 | 1.079 | 0.701 | 1.055 | 1.130 | 0.660 | 1.258 | 1.171 | 0.075 | 1.147 | 1.138 | 0.3 |

Bisphenol A (BPA), Methylparabel (MP) Ethylparaben (EP), Propylparaben (PP) and Butilparaben (BP), Benzophenone 1 (BP1) and Benzophenone 3 (BP3), HR: H
Limit of Detection
Adjusted by age, BMI, fasting, energy intake, physical activity, educational level, extraction time and smoking status
*p trend
Ranges of concentrations (ng/mL) in each percentile:
BPA: P1 (<LOD - 0.31), P2 (0.32- 1.47), P3 (1.49 - 3.34), P4 (3.38- 7.42), P5 (7.51- 62.95)
MP: P1 (<LOD - 0.48), P2 (0.49- 1.54), P3 (1.55- 3.42), P4 (3.44- 8.05), P5 (8.16-75.55)
EP: P1 (<LOD - 0.18), P2 (0.20- 0.29), P3 (0.30-0.45), P4 (0.45-0.75), P5 (0.76-16.91)
PP: P1 (<LOD - 0.20), P2 (0.20-0.31), P3 (0.31-0.52), P4 (0.53-9.24)
BP: P1 (<LOD - 0.20), P2 (0.20-0.36), P3 (0.37-0.57), P4 (0.61-6.21)
BP1: P1 (<LOD - 0.10), P2 (0.10-0.20), P3 (0.21-0.37), P4 (0.37-4.46)
BP3: P1 (<LOD - 0.14), P2 (0.21-0.48), P3 (0.49-0.85), P4 (0.86-1.43), P5 (1.46-21.20)

## Supplementary Table 4. Serum npEPs exposure and risk of incident type 2 diabetes. Cox Proportional Hazard M

| | | | | Parabens | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **BPA** | | | **MP** | | | **EP** | | | **PP** | | | **BP** | | |
| | HR | SE | p | HR | SE | p | HR | SE | p | HR | SE | p | HR | SE | p |
| **Percentiles of npEPs** | | | *0.51 | | | *0.658 | | | *0.684 | | | *0.393 | | | *0.977 |
| **P2** | 1.533 | 1.404 | 0.207 | 0.669 | 1.362 | 0.195 | 1.457 | 1.368 | 0.229 | 0.732 | 1.543 | 0.471 | 1.149 | 1.688 | 0.791 |
| **P3** | 0.880 | 1.499 | 0.753 | 1.060 | 1.031 | 0.850 | 0.872 | 1.456 | 0.715 | 1.340 | 1.454 | 0.434 | 1.027 | 1.695 | 0.960 |
| **P4** | 1.050 | 1.429 | 0.892 | 0.995 | 1.394 | 0.987 | 0.903 | 1.408 | 0.765 | 1.507 | 1.364 | 0.187 | 0.833 | 1.685 | 0.727 |
| **P5** | 1.017 | 1.408 | 0.961 | 0.877 | 1.438 | 0.718 | 1.125 | 1.418 | 0.736 | - | - | - | - | - | - |
| **>LOD *vs* <LOD** | 1.128 | 1.344 | 0.684 | 0.846 | 1.317 | 0.544 | 1.080 | 1.255 | 0.735 | 1.190 | 1.262 | 0.455 | 0.985 | 1.372 | 0.963 |
| **Log-transformed variable (ng/ml)** | 0.965 | 1.066 | 0.580 | 1.010 | 1.074 | 0.886 | 1.048 | 1.153 | 0.741 | 1.141 | 1.146 | 0.329 | 0.986 | 1.208 | 0.940 |
| **Unadjusted model** | 0.946 | 1.065 | 0.377 | 0.987 | 1.073 | 0.852 | 1.028 | 1.147 | 0.843 | 1.146 | 1.138 | 0.314 | 1.099 | 1.203 | 0.608 |

Bisphenol A (BPA), Methylparabel (MP) Ethylparaben (EP), Propylparaben (PP) and Butilparaben (BP), Benzophenone 1 (BP1) and Benzophenone 3 (BP3) HR: H
Limit of Detection
Adjusted by age, BMI, fasting, energy intake, physical activity, educational level, extraction time and smoking status
*p trend
Ranges of concentrations (ng/mL) in each percentile:
BPA: P1 (<LOD - 0.31), P2 (0.32- 1.47), P3 (1.49 - 3.34), P4 (3.38- 7.42), P5 (7.51- 62.95)
MP: P1 (<LOD - 0.48), P2 (0.49- 1.54), P3 (1.55- 3.42), P4 (3.44- 8.05), P5 (8.16-75.55)
EP: P1 (<LOD - 0.18), P2 (0.20- 0.29), P3 (0.30-0.45), P4 (0.45-0.75), P5 (0.76-16.91)
PP: P1 (<LOD - 0.20), P2 (0.20-0.31), P3 (0.31-0.52), P4 (0.53-9.24)
BP: P1 (<LOD - 0.20), P2 (0.20-0.36), P3 (0.37-0.57), P4 (0.61-6.21)
BP1: P1 (<LOD - 0.10), P2 (0.10-0.20), P3 (0.21-0.37), P4 (0.37-4.46)
BP3: P1 (<LOD - 0.14), P2 (0.21-0.48), P3 (0.49-0.85), P4 (0.86-1.43), P5 (1.46-21.20)

**Supplementary Table 5. Serum npEPs exposure and risk of incident type 2 diabetes. Cox Proportional Hazar**

**weight**

| | | | | Parabens | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **BPA** | | | **MP** | | | **EP** | | | **PP** | | | **BP** | | |
| | HR | SE | p | HR | SE | p | HR | SE | p | HR | SE | p | HR | SE | p |
| **Percentiles of npEPs** | | | *0.307 | | | *0.665 | | | *0.747 | | | *0.953 | | | *0.72 |
| **P2** | 1.979 | 4.671 | 0.658 | 0.240 | 4.756 | 0.360 | 0.000 | e | 0.978 | 0.000 | e | 0.981 | 0.156 | 5.294 | 0.26 |
| **P3** | 1.865 | 5.624 | 0.718 | 0.124 | 4.130 | 0.141 | 2.155 | 4.728 | 0.621 | 1.250 | 3.844 | 0.868 | 0.386 | 3.967 | 0.49 |
| **P4** | 3.984 | 6.606 | 0.464 | 0.468 | 2.821 | 0.463 | 0.821 | 3.976 | 0.886 | 0.633 | 2.463 | 0.612 | 0.000 | e | 0.99 |
| **P5** | 15.620 | 4.624 | 0.073 | 0.000 | e | 0.965 | 0.285 | 3.023 | 0.257 | - | - | - | - | - | - |
| **>LOD *vs* <LOD** | 3.105 | 3.330 | 0.346 | 0.210 | 2.875 | 0.140 | 0.435 | 2.195 | 0.290 | 0.592 | 2.179 | 0.501 | 0.239 | 3.456 | 0.24 |
| **Log-transformed variable (ng/ml)** | 1.712 | 1.357 | 0.078 | 0.705 | 1.305 | 0.189 | 0.578 | 1.611 | 0.250 | 0.559 | 1.677 | 0.260 | 0.264 | 3.684 | 0.30 |
| **Unadjusted model** | 1.315 | 1.240 | 0.203 | 0.908 | 1.217 | 0.621 | 0.868 | 1.401 | 0.674 | 0.733 | 1.559 | 0.484 | 0.548 | 2.175 | 0.43 |

e: not calculated because of low sample sixe

Bisphenol A (BPA), Methylparabel (MP) Ethylparaben (EP), Propylparaben (PP) and Butilparaben (BP), Benzophenone 1 (BP1) and Benzophenone 3 (BP3) HR: H
Limit of Detection
Adjusted by sex, age, fasting, energy intake, physical activity, educational level, extraction time and smoking status
*p trend
Ranges of concentrations (ng/mL) in each percentile:
BPA: P1 (<LOD - 0.31), P2 (0.32- 1.47), P3 (1.49 - 3.34), P4 (3.38- 7.42), P5 (7.51- 62.95)
MP: P1 (<LOD - 0.48), P2 (0.49- 1.54), P3 (1.55- 3.42), P4 (3.44- 8.05), P5 (8.16-75.55)
EP: P1 (<LOD - 0.18), P2 (0.20- 0.29), P3 (0.30-0.45), P4 (0.45-0.75), P5 (0.76-16.91)
PP: P1 (<LOD - 0.20), P2 (0.20-0.31), P3 (0.31-0.52), P4 (0.53-9.24)
BP: P1 (<LOD - 0.20), P2 (0.20-0.36), P3 (0.37-0.57), P4 (0.61-6.21)
BP1: P1 (<LOD - 0.10), P2 (0.10-0.20), P3 (0.21-0.37), P4 (0.37-4.46)
BP3: P1 (<LOD - 0.14), P2 (0.21-0.48), P3 (0.49-0.85), P4 (0.86-1.43), P5 (1.46-21.20)

## Supplementary Table 6. Serum npEPs exposure and risk of incident type 2 diabetes. Cox Proportional Hazard M

| | | | | Parabens | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **BPA** | | | **MP** | | | **EP** | | | **PP** | | | **BP** | | |
| | HR | SE | p | HR | SE | p | HR | SE | p | HR | SE | p | HR | SE | p |
| **Percentiles of npEPs** | | | *0.996 | | | *0.053 | | | *0.604 | | | *0.688 | | | *0.295 |
| **P2** | 0.912 | 1.548 | 0.833 | 0.583 | 1.494 | 0.179 | 1.457 | 1.422 | 0.285 | 0.868 | 1.637 | 0.773 | 0.203 | 2.768 | 0.118 |
| **P3** | 1.003 | 1.546 | 0.994 | 1.254 | 1.453 | 0.545 | 0.779 | 1.586 | 0.589 | 0.699 | 1.543 | 0.408 | 0.312 | 2.792 | 0.257 |
| **P4** | 1.026 | 1.524 | 0.952 | 0.450 | 1.598 | 0.088 | 0.939 | 1.521 | 0.881 | 1.302 | 1.469 | 0.493 | 1.093 | 1.638 | 0.857 |
| **P5** | 1.094 | 1.526 | 0.832 | 0.514 | 1.557 | 0.132 | 0.757 | 1.567 | 0.536 | - | - | - | - | - | - |
| **>LOD *vs* <LOD** | 0.928 | 1.426 | 0.834 | 0.665 | 1.438 | 0.260 | 1.007 | 1.320 | 0.979 | 0.940 | 1.324 | 0.825 | 0.553 | 1.508 | 0.150 |
| **Log-transformed variable (ng/ml)** | 1.034 | 1.089 | 0.691 | 0.880 | 1.094 | 0.153 | 0.864 | 1.188 | 0.398 | 1.118 | 1.201 | 0.544 | 0.939 | 1.242 | 0.773 |
| **Unadjusted model** | 1.011 | 1.083 | 0.887 | 0.903 | 1.089 | 0.227 | 0.930 | 1.178 | 0.659 | 1.220 | 1.176 | 0.221 | 1.063 | 1.133 | 0.436 |

Bisphenol A (BPA), Methylparabel (MP) Ethylparaben (EP), Propylparaben (PP) and Butilparaben (BP), Benzophenone 1 (BP1) and Benzophenone 3 (BP3) HR: H
Limit of Detection
Adjusted by sex, age, fasting, energy intake, physical activity, educational level, extraction time and smoking status
*p trend
Ranges of concentrations (ng/mL) in each percentile:
BPA: P1 (<LOD - 0.31), P2 (0.32- 1.47), P3 (1.49 - 3.34), P4 (3.38- 7.42), P5 (7.51- 62.95)
MP: P1 (<LOD - 0.48), P2 (0.49- 1.54), P3 (1.55- 3.42), P4 (3.44- 8.05), P5 (8.16-75.55)
EP: P1 (<LOD - 0.18), P2 (0.20- 0.29), P3 (0.30-0.45), P4 (0.45-0.75), P5 (0.76-16.91)
PP: P1 (<LOD - 0.20), P2 (0.20-0.31), P3 (0.31-0.52), P4 (0.53-9.24)
BP: P1 (<LOD - 0.20), P2 (0.20-0.36), P3 (0.37-0.57), P4 (0.61-6.21)
BP1: P1 (<LOD - 0.10), P2 (0.10-0.20), P3 (0.21-0.37), P4 (0.37-4.46)
BP3: P1 (<LOD - 0.14), P2 (0.21-0.48), P3 (0.49-0.85), P4 (0.86-1.43), P5 (1.46-21.20)

## Supplementary Table 7. Serum npEPs exposure and risk of incident type 2 diabetes. Cox Proportional Hazard M

| | | | | Parabens | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **BPA** | | | **MP** | | | **EP** | | | **PP** | | | **BP** | | |
| | HR | SE | p | HR | SE | p | HR | SE | p | HR | SE | p | HR | SE | p |
| **Percentiles of npEPs** | | | *0.791 | | | *0.757 | | | *0.317 | | | *0.019 | | | *0.993 |
| **P2** | 1.161 | 1.337 | 0.606 | 0.834 | 1.394 | 0.585 | 1.136 | 1.416 | 0.715 | 0.739 | 1.403 | 0.372 | 0.982 | 1.580 | 0.969 |
| **P3** | 0.908 | 1.382 | 0.764 | 0.844 | 1.390 | 0.607 | 0.708 | 1.438 | 0.342 | 0.861 | 1.411 | 0.663 | 1.050 | 1.497 | 0.903 |
| **P4** | 1.120 | 1.372 | 0.720 | 0.983 | 1.397 | 0.958 | 1.330 | 1.341 | 0.331 | 2.043 | 1.309 | 0.008 | 1.104 | 1.436 | 0.784 |
| **P5** | 0.800 | 1.412 | 0.518 | 1.226 | 1.378 | 0.525 | 1.574 | 1.351 | 0.132 | - | - | - | - | - | - |
| **>LOD *vs* <LOD** | 1.013 | 1.280 | 0.958 | 1.121 | 1.361 | 0.710 | 1.173 | 1.237 | 0.454 | 1.151 | 1.232 | 0.502 | 1.052 | 1.280 | 0.837 |
| **Log-transformed variable (ng/ml)** | 0.979 | 1.065 | 0.731 | 1.075 | 1.076 | 0.319 | 1.244 | 1.133 | 0.082 | 1.244 | 1.138 | 0.091 | 1.068 | 1.137 | 0.607 |
| **Unadjusted model** | 0.978 | 1.063 | 0.719 | 1.036 | 1.068 | 0.587 | 1.123 | 1.126 | 0.328 | 1.186 | 1.130 | 0.161 | 1.102 | 1.133 | 0.436 |

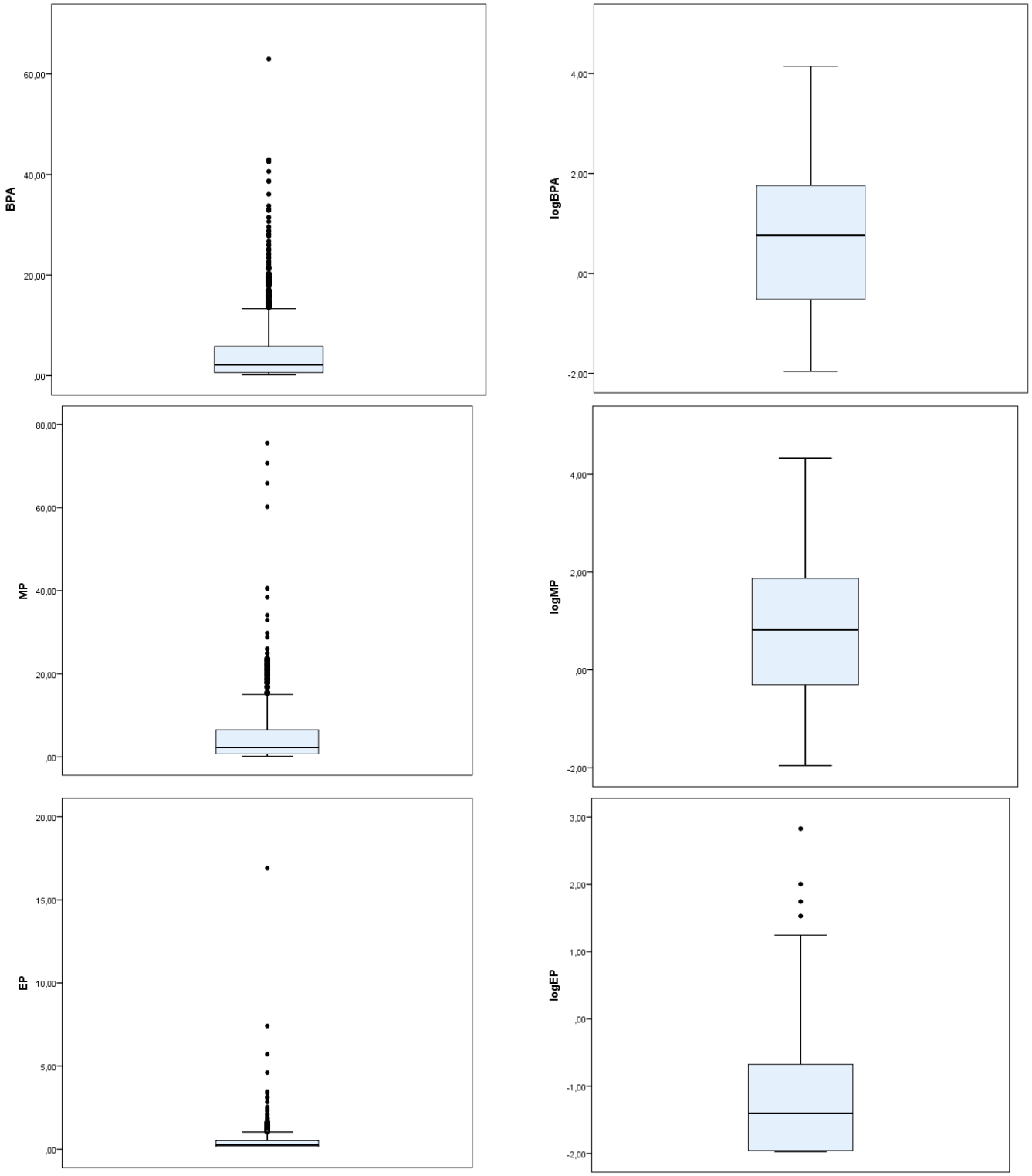
Bisphenol A (BPA), Methylparabel (MP) Ethylparaben (EP), Propylparaben (PP) and Butilparaben (BP), Benzophenone 1 (BP1) and Benzophenone 3 (BP3), HR: H
Limit of Detection
Adjusted by sex, age, fasting, energy intake, physical activity, educational level, extraction time and smoking status
*p trend
Ranges of concentrations (ng/mL) in each percentile:
BPA: P1 (<LOD - 0.31), P2 (0.32- 1.47), P3 (1.49 - 3.34), P4 (3.38- 7.42), P5 (7.51- 62.95)
MP: P1 (<LOD - 0.48), P2 (0.49- 1.54), P3 (1.55- 3.42), P4 (3.44- 8.05), P5 (8.16-75.55)
EP: P1 (<LOD - 0.18), P2 (0.20- 0.29), P3 (0.30-0.45), P4 (0.45-0.75), P5 (0.76-16.91)
PP: P1 (<LOD - 0.20), P2 (0.20-0.31), P3 (0.31-0.52), P4 (0.53-9.24)
BP: P1 (<LOD - 0.20), P2 (0.20-0.36), P3 (0.37-0.57), P4 (0.61-6.21)
BP1: P1 (<LOD - 0.10), P2 (0.10-0.20), P3 (0.21-0.37), P4 (0.37-4.46)
BP3: P1 (<LOD - 0.14), P2 (0.21-0.48), P3 (0.49-0.85), P4 (0.86-1.43), P5 (1.46-21.20)

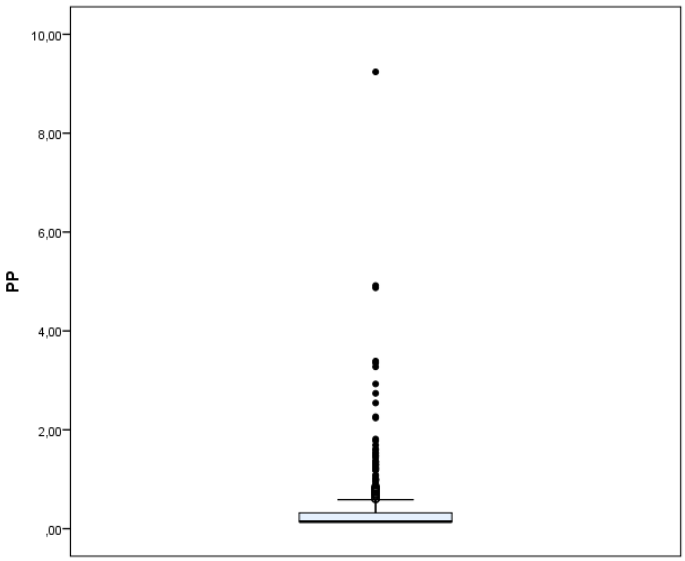
**Supplementary Table 8. Serum npEP concentrations (ng/mL) in women and men.**

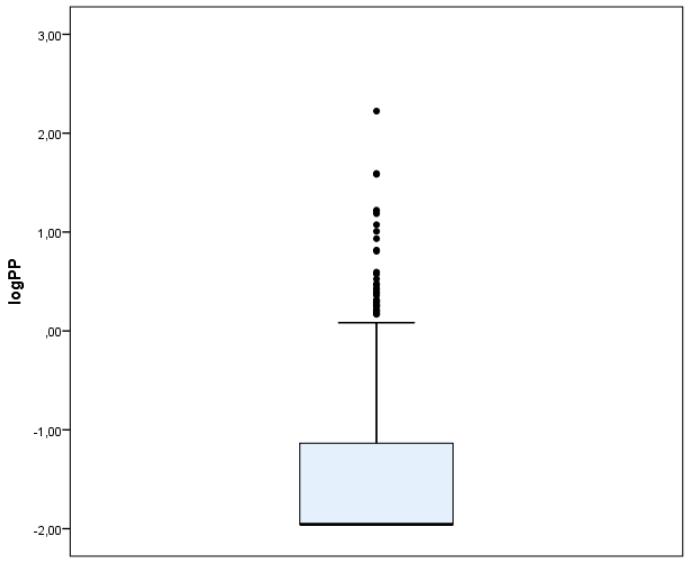
| | Women | | | | | | Me | | |
|---|---|---|---|---|---|---|---|---|---|
| | **> LOD (%)** | **GM (ng/ ml)** | **95% CI** | **Median (ng/ml)** | **25%** | **75%** | **> LOD (%)** | **GM (ng/ ml)** | **95% CI** |
| BPA | 80.0 | 1.43 | (1.22-1.68) | 1.736 | 0.45 | 4.25 | 83.4 | 2.21 | (1.84-2.66) |
| MP | 92.9 | 2.81 | (2.42-3.27) | 3.306 | 1.30 | 8.75 | 83.8 | 1.43 | (1.21-1.69) |
| EP | 58.0 | 0.30 | (0.28-0.33) | 0.249 | < LOD | 0.54 | 54.7 | 0.27 | (0.25-0.30) |
| PP | 46.3 | 0.24 | (0.23-0.26) | < LOD | < LOD | 0.39 | 29.1 | 0.20 | (0.19-0.22) |
| BP | 26.0 | 0.20 | (0.19-0.22) | < LOD | < LOD | 0.21 | 12.5 | 0.16 | (0.16-0.18) |
| BP1 | 29.4 | 0.10 | (0.10-0.11) | 0.070 | < LOD | < LOD | 13.1 | 0.08 | (0.07-0.08) |
| BP3 | 73.1 | 0.63 | (0.55-0.71) | 0.706 | < LOD | 1.37 | 63.7 | 0.41 | (0.37-0.46) |

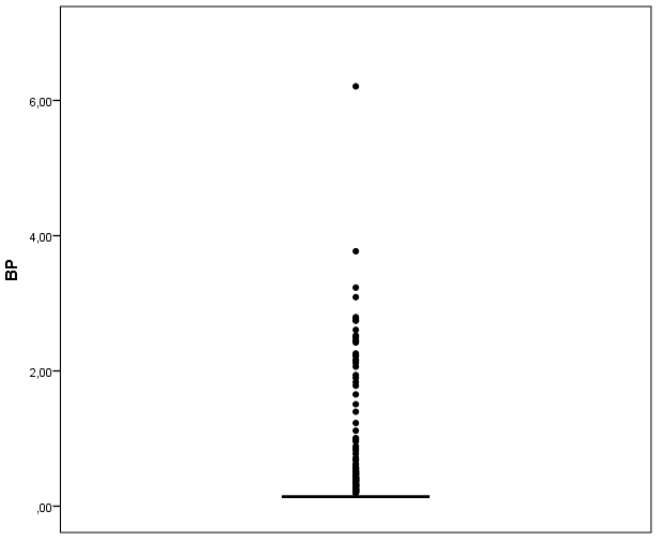
LOD: Limit of detection; GM: Geometric mean; 95% CI: Confidence Interval

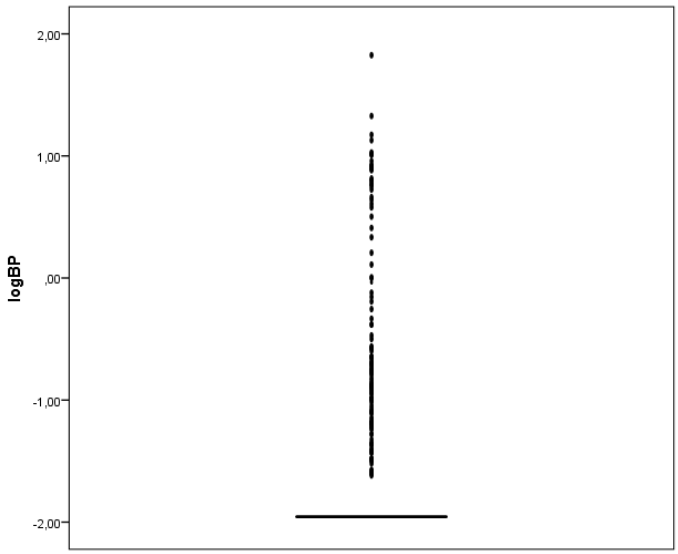
**Supplementary Figure 1. Box diagrams: distribution of npEP concentrations (raw and logarithmic).**

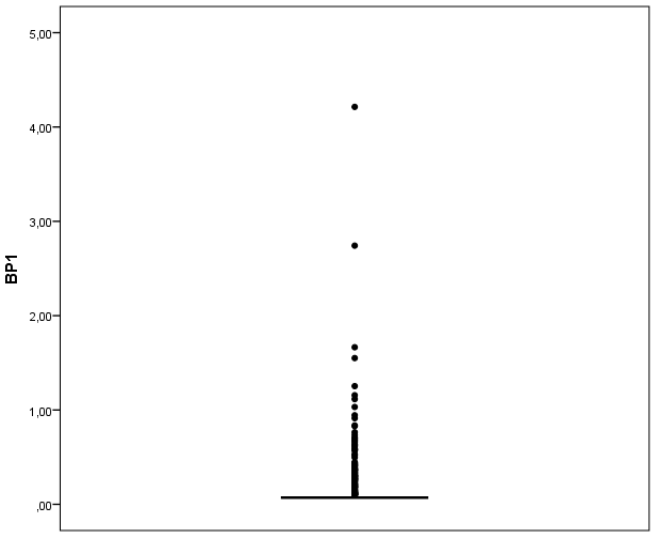


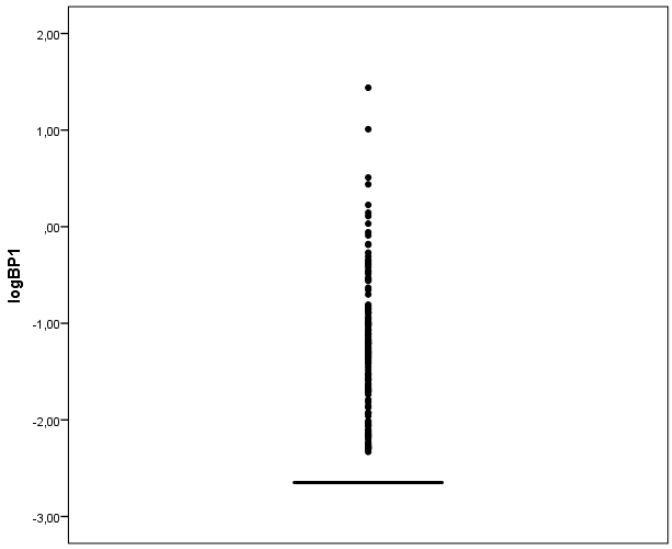

PP
10,00
8,00
6,00
4,00
2,00
,00

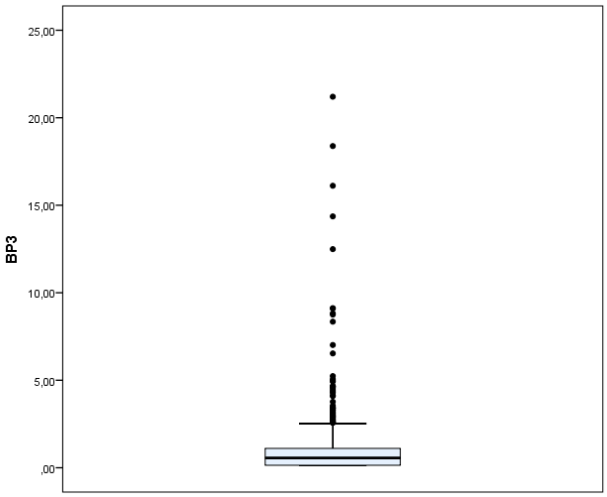

logPP
3,00
2,00
1,00
,00
-1,00
-2,00

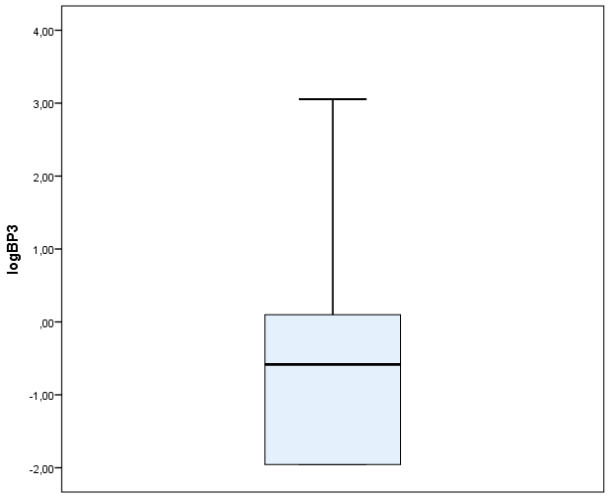

BP
6,00
4,00
2,00
,00


logBP
2,00
1,00
,00
-1,00
-2,00


BP1
5,00
4,00
3,00
2,00
1,00
,00


logBP1
2,00
1,00
,00
-1,00
-2,00
-3,00


BP3
25,00
20,00
15,00
10,00
5,00
,00


logBP3
4,00
3,00
2,00
1,00
,00
-1,00
-2,00

## 7.4 Objetivo específico 4

4. Evaluar la exposición a contaminantes no persistentes y riesgo de hipertensión arterial en la sub-cohorte de EPIC-Granada al seguimiento.

### 7.4.1 Exposición a contaminantes no persistentes y riesgo de hipertensión arterial

**Serum levels of non-persistent environmental pollutants and risk of incident hypertension in a sub-cohort from the EPIC study**

Elena Salamanca-Fernández E, Fernando Vela-Soria F, Miguel Rodríguez-Barranco M, Antonio Arrebola-Moreno, Luz Iribarne-Durán, Nicolás Olea N, María José Sánchez, Juan Pedro Arrebola*

En revisión

## ABSTRACT

*Background:* The prevalence of arterial hypertension (AHT), a well-known risk factor for cardiovascular disease, has considerably increased over last decades. Non-persistent environmental pollutants (npEPs) are a group of ubiquitous chemicals, widely used in consumer products such as food packaging and cosmetics, which have been identified as endocrine disrupting chemicals and obesogens. The aim of this study was to assess the potential associations of serum levels of three groups of npEPs with the risk of incident AHT.

*Methods:* We designed a cohort study in a sub-cohort of Granada EPIC-Spain centre (n=670). We quantified serum concentrations of three groups of npEPs, i.e., bisphenol A (BPA), four parabens: methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butylparaben (BP), and two benzophenones: benzophenone 1 (BP1), benzophenone 3 (BP3), in samples extracted at recruitment. Statistical analyses were performed by means of Cox Proportional Hazard Models.

*Results:* Median follow-up time was 23 years. BPA and MP were found in >80% of the study population. Individuals within the 4$^{th}$ PP quartile (0.53-9.24 ng/ml) showed a statistically significant increased risk of AHT (HR=1.40, p=0.015), as well as men within the 5$^{th}$ BP3 quintile (1.46-21.20 ng/ml) (HR=1.63, p=0.033). No associations were found for the rest of pollutants.

*Conclusions:* Overall, we evidenced no associations of most npEPs with AHT risk, with the exception of an increased risk in the highest PP percentiles. Further research on the potential contribution of npEPs on the development of AHT risk is warranted.


*Keywords:* non-persistent environmental pollutants; Parabens; Benzophenones; Bisphenol A, Arterial hypertension

## Funding sources

This research was supported in part by research grants from the ISCIII (PI14/00067). The authors are grateful to Instituto de Salud Carlos III (Miguel Servet Type I Program CP15/00193) for the research contract. Dr. J.P. Arrebola is under contract within Ramon y Cajal program (RYC-2016-20155, Ministerio de Economía, Industria y Competitividad, Spain). Funding sources had no involvement in the conduct of the research.

## Ethics statement

All participants were informed at recruitment and they signed an informed consent. This study was approved by Ethics Committee of Granada (Comité de Ética de la Investigación Biomédica de Granada).

## 1. Introduction

The global prevalence of Arterial hypertension (AHT) has markedly increased over last decades, reaching 40% of adults >25 years old in 2010 [148,149]. The global age-standardized prevalence of raised blood pressure (SBP ≥140 mm Hg or diastolic BP [DBP] ≥90 mm Hg) in adults was estimated as ≥ 20% in 2015 [320,321]. In addition, systolic blood pressure (SBP) >115 mm Hg has been considered as the leading risk factor for the global burden of disease in 2017[320].

External and potentially-modifiable risk factors affecting the development of AHT include unhealthy diet (e.g. high salt intake), lack of physical activity, obesity, smoking, and exposure to persistent stress [148]. Among environmental aspects the role of chronic exposure to low doses of ubiquitous environmental pollutants on the development of metabolic disorders is increasingly studied [124–134]. Specifically, there is a group of widely-used chemicals, including substances with very heterogonous chemical structures which are non-persistent environmental pollutants (npEPs) [51]. In this regard, Bisphenol A (BPA) is considered a npEP as it does not persist in human organisms. BPA is an industrial chemical originally developed as a synthetic oestrogen, widely used in the manufacture of polymers and epoxy resins, as well as in polycarbonate and polysulfone plastics. BPA is one of the highest volume chemicals produced worldwide [72] and, consequently, the general population is frequently and inadvertently exposed to this chemical [73,74]. In this regard, it has been estimated that >90% of the general population in the US, Europe and Asia present detectable values of BPA [75–78]. The main exposure route to BPA is mainly through diet, since it is frequently present in food packaging, e.g., tins, cans, or plastic boxing, from which BPA can migrate to the food under certain environmental conditions [73,87–91]. On the other hand, parabens (PBs) are widely used as antimicrobial preservatives in cosmetics, pharmaceuticals, food and beverages [94] and Benzophenones (BPs) are synthetic chemicals frequently used in personal care products UV filters [101]. BPs have been detected in urine and some other biological matrices, such as placenta [50], human breast milk [47] and menstrual blood [52].

In general, npEPs are rapidly eliminated from humans. Indeed, once absorbed, BPA is readily glucuro-conjugated or sulfo-conjugated in the liver, and finally excreted in urine, once absorbed in the intestine (half-life in humans: 7-8 hours) [74]. In the same way, PBs and BPs are also

rapidly metabolized and excreted from the body, mainly during the first 24h after exposure [95,293,322].

These npEPs are considered potential metabolic and endocrine disruptors, since they can induce metabolic syndrome–related conditions [294,295]. In this regard, these pollutants can act as obesogens, which are functionally defined as chemicals that promote obesity and, thus, obesity-related diseases, including insulin resistance, cardiovascular disease or even cancer [133,136,158] in humans or animals [135]. Suspected biological mechanisms that are known to be associated with increased risk of AHT includes thyroid dysfunction [151], weight gain [152], insulin resistance [153], hyperlipidaemia [154], oxidative stress [155], and higher systemic inflammation [156]. However, the implications of npEP exposure and AHT to the general population are still controversial due to the lack of homogeneous results [157–163].

The present study, which is framed within the European Prospective Investigation into Cancer and Nutrition (EPIC) study, aims to assess the potential associations of serum concentrations of selected npEPs at recruitment with the risk of incident AHT.

## 2. Methods

*Study design and Study population: The EPIC cohort*

This research is designed as a longitudinal study within a sub-cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC), recruited in Granada, Spain. Information at baseline and data collection were explained elsewhere [177]. Approval for our study was obtained from the ethical review boards of the International Agency for Research on Cancer and from the Granada EPIC center.

Recruitment in Granada was described elsewhere [291]. For this study we selected on a sub-cohort of 795 participants (10.09%), with no prevalent AHT diagnoses at recruitment using stratified random sampling by sex and age. Because of missing information in their clinical records or in the covariates, we excluded 125 individuals (Supplementary material, Table 1). Consequently, our study population involved 670 participants.

*Outcome and covariate assessment*

Ascertainment of incident AHT cases was performed retrospectively by reviewing the datasets of Andalusian CMBD (minimum basic Hospital Data Set) and the patient´s clinical records at the specialized care databases as all the study participants were residents in Granada province and users of the Andalusian Public Health System. A participant was considered as hypertensive when he/she had been diagnosed of AHT during follow-up time and/or when continuous prescription of anti-hypertensive medication had been registered in the clinical records. We excluded prevalent AHT cases from the statistical analyses that were identified by baseline self-report, previous diagnosis by a clinician, and/or chronic prescription of anti-hypertensive medication. Follow-up time started at EPIC recruitment date and continued until AHT diagnosis or patient's death or 31st July 2017, whatever happened first.

Information on educational level, history of previous illnesses, history of tobacco use, physical activity, occupation, and reproductive history (for women) were gathered during a personal interview at recruitment, as well as measurements of height, weight, and hip and waist circumferences [177]. Classification of covariates was explained elsewhere [291].

*Chemical analysis*

One spot blood sample was drawn from each participant at recruitment (1992-1996). Blood samples were subsequently centrifuged, and 0.5 mL serum aliquots were stored in liquid nitrogen (-196ºC). Serum concentrations of three groups of npEPs (bisphenol A (BPA), four parabens (methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butylparaben (BP)), and two benzophenones (benzophenone 1 (BP1), benzophenone 3 (BP3)), were quantified in 2018-2019 in serum samples extracted at recruitment. Detailed chemical analysis was described elsewhere [291].

*Statistical analysis:*

Serum concentrations of npEP below the LOD were assigned a value of LOD/√2. The npEP concentrations ((ng/mL) serum sample) were expressed as geometric mean (GM), 95% Confidence Interval (95% CI), median and $25^{th}$ and 75th percentiles in the descriptive analysis. Differences between categorical variables were calculated using the Chi-square test. An analysis of the correlation between pollutants was performed by Spearman correlation coefficients (Supplementary Table 2). Detailed statistical analysis was described elsewhere [291].

## 3. Results

The average age of the participants in our study population, was 53.04 years (range 35.19-67.58), and 52.1% were women. At baseline, 58.7% of participants were considered to be moderately active, 46.5% of them had overweight and 60.7% had never smoked (Table 1). During the study period (Median follow-up time was 22.8 years), there were 482 (71.9%) incident AHT diagnoses in our sub-cohort (Table 1).

Table 2 depicts a description of npEP serum levels. BPA and MP were found in 83.8% and 83.5% of the study population respectively. More than a half of the population presented detectable levels of EP and BP3 (55.7% and 68.4%, respectively). The highest GM concentrations were found in MP, BPA and BP3, with 2.03 ng/ml, 1.76 ng/ml and 0.93 ng/ml respectively. PP was detected in 37.54% of the individuals, with a GM of 0.93 ng/ml (Table 2).

Those individuals within the 4$^{th}$ quartile of PP concentrations showed and increased risk of AHT in comparison to those within the 1$^{st}$ quartile, which was consistent with the observed trend in GAM models (Figure 1). Interestingly, we observed this association in females (HR for log[PP]= 1.21, p=0.021) but not in males (Figure 2 and Supplementary Table 3). Notably, women showed both increased percentage of PP detection (46.3% *vs* 29.1% respectively) and GM concentrations (0.24 *vs* 0.20 ng/ml) (Table 2). Moreover, it is noteworthy that our results suggested an inverted U-Shape-like effect of BP on AHT risk, that was only evident in females (Figure 2). On the other hand, men within the 5$^{th}$ BP3 percentile (P5: 1.46-21.20 ng/ml) showed a statistically significant increased risk of AHT (HR=1.63, p=0.033), although no trend in the other percentiles of this chemical were observed (Supplementary Table 4). Regarding BP1, we found a negative trend with AHT risk in GAM models (Figure 1), although not significant in the analyses using percentiles or log-transformed concentrations. No trend nor significant association with AHT risk was for the rest of npEPs (Figure 1, Table 3). The observed associations for PP and BP1 did not substantially changed after their inclusion in the multi-pollutant model (Supplementary Table 5).

Due to the low detection rate of some npEPs (Table 2), we also performed Cox-regression analyses considering dichotomized independent variables (> LOD / <LOD). The results were consistent with those from models using log-transformed concentrations and percentiles (Table 3).

## 4. Discussion

Our results suggested an increased risk of incident AHT in those individuals with the highest PP serum concentrations at recruitment showed an increased risk of incident AHT. However, we found no evident associations to AHT risk for the rest of the included pollutants (BPA, MP, EP, BP, BP1 and BP3), neither in global models nor after sex-stratification, with the exception of a positive association of BP3 for P2 and P5 and AHT risk in males, and an inverted U-shape for BP in females (Figure 1). Our results are in consonance with previous findings in EPIC-Granada subcohort of an increased risk of Type-2 Diabetes Mellitus in those individuals with the highest serum PP concentrations[291]. To the best of our knowledge, no previous studied have reported similar associations with PP exposure. In fact, Shiue et al. reported no clear associations between environmental urinary parabens and high blood pressure in the NHANES study [162,163,323]. In this regard, parabens are still poorly explored as a cardiovascular risk factor. Our results for BPA, MP, EP, BP, BP1 and BP3 are in consonance with those of Wang et al.[324] that reported no association of urinary BPA with the risk of HTA in a cross-sectional study with Chinese population.

When stratifying by sex, our associations with PP were only evident in females. Interestingly, our female participants showed increased detection rates and GM concentrations in serum PP concentrations. These differences in the magnitude of the exposure might be behind the observed differential effect, but it could also be related to underlying biological differences in npEP metabolism and AHT risk between sexes [325]. It is noteworthy that parabens and benzophenones are frequently used in cosmetics [94,306,307], which likely account for a percentage of our sex-related differences in the effects npEP exposure. Beyond the abovementioned gender-related differential use of cosmetics, men commonly present increased liver enzyme activity and, therefore, tend to more readily eliminate npEPs [227]. This is consistent with the increased levels and detection rates found in women for PP MP, EP, PP, BP, BP1 and BP3 (Supplementary Table 1). Conversely, an increased risk of AHT was found in the

5th quintile of BP3 concentrations, although this association was only observed in males and no trend over the rest of percentiles was evidenced (Supplementary Table 4).

The analysis of the 2009–2010 NHANES data yielded to a lack of significant associations between urinary BPA levels and high blood pressure (OR 1.12, 95 % CI 0.93–1.35) [162], which is in agreement with our findings. However, a number of studies have previously reported associations of BPA exposure with the risk of AHT [157–160] and/or associated comorbidities/risk factors, e.g., heart rate variability, peripheral vascular disease, abnormal liver function enzymes, increased fasting glucose levels, insulin resistance, general and central obesity, and diabetes mellitus [119,189,259,268,270,326]. On the light of these results, we conclude that the inclusion of several metabolic outcomes in further epidemiologic studies will considerably improve the robustness of the findings. Furthermore, the conflicting results in epidemiologic studies are also likely due to different experimental designs, timing of exposure, and uncontrolled or residual confounding factors, such as the route of the administration of these pollutants, the degradation time of npEPs or low exposure doses [132,137,146]. Further mechanistic studies in conjunction with epidemiologic approaches should test if the abovementioned mechanisms are sufficient to produce a relevant metabolic effect in the general populations at current exposure levels.

Potential common mechanisms of action for the target pollutants might include endocrine and/or metabolic disruption, since animal studies have reported that abnormal levels of endogenous oestrogens or environmental oestrogen exposure enhances the risk of developing type 2 diabetes mellitus, hypertension, and dyslipidaemia [327]. However, the underlying npEPs potential mechanisms of action still remain unclear [146,275]. The vast majority of the mechanistic studies focus on BPA, but since benzophenones and parabens have also endocrine disruption potential, they might have similar and/or converging mechanisms [256] and, therefore, warrant further research. Those mechanisms may involve alteration of ion channel inhibition/activation, oxidative stress, and genome/transcriptome modifications[160]. Moreover, BPA and paraben exposure may have a role in the development of AHT through their obesogenic potential in humans [136,255–258,265,276,277,328], mainly by means of their influence on preadipocytes [329,330]. In this regard, a case-control study in US population revealed an association of BPA with general and abdominal obesity [255]. Similarly, a cross-sectional study found important associations of MP with energy balance and metabolic health [277]. Another suspected mechanism of action of BPA and parabens is by disrupting epigenetic

signalling (e.g. DNA methylation, histone marks, chromatin remodelling and noncoding RNAs) [275], which might affect normal tissue development, eventually leading to obesity and related conditions [278]. Epigenetic changes are known to be associated with cardiovascular disease-related pathologies as hypertension [331,332]. Another potential mechanism of action is through endocrine disruption [189], as EDs have shown agonistic and antagonistic effects on oestrogen receptors [333]. Since a number of oestrogen receptors are located in the cardiovascular system and are involved in vasodilation, modulating the response lipid profiles, some EDs may be affecting blood pressure through its actions on oestrogen receptors [334,335]. In addition, BPA was associated with oxidative stress [336] and oxidative stress-related endothelial cell injury in animal studies [337,338]. Accordingly, previous human studies found positive associations between maternal urinary EP levels (but not MP or PP) and urinary malondialdehyde [310], which strengthens the hypothesis of oxidative stress induction by EDs. Because elevated levels of oxidative stress and inflammation markers are found in many cardiovascular disorders, they may play an important role in hypertension pathogenesis caused by BPA exposure [339]. It should be considered that the abovementioned mechanisms of action may not be applicable for all the selected npEPs, since there is still scant research in this field, particularly for some of the chemicals (e.g., parabens and benzophenones).

Our study has several strengths, including our longitudinal design and relatively large sample size, with >20 years of follow-up. Although the study population may not be entirely representative of the Spanish general population, we evidenced a similar AHT incidence in comparison to previous reports in Spanish adult population[340]. Recruitment and follow-up processes were performed by using previously-validated protocols, which allowed us to have a precise characterization of the covariates. Moreover, our cohort was entirely covered by the Public Health System of Andalusia and, thus, was accessible by our clinical record review. Furthermore, the selected chemicals were analysed by using validated analytical methodologies [175].

Nevertheless, our study has also some limitations, since npEP exposure was estimated by using serum concentrations at recruitment, and we do not have information on possible changes in npEPs concentrations and covariates during the follow-up time. In addition, the use of a npEP point measurement might not take into account intra-day fluctuations, which might be relevant [284,285]. On the other hand, some authors acknowledge urine concentrations as the best biomarker of BPA exposure, since metabolites in blood can be several orders of magnitude

lower than in urine and it can be indicative of a relatively longer exposure period in comparison to other matrices [197]. All biological matrices have their own variability and characteristics that can interfere in the stability of the exposure measurement and, consequently, their concentrations might have dissimilar biological meanings. In this regard, our approach would assume low fluctuations in exposure levels over follow-up or, at least, a proportional inter-individual increase or decrease, which might not represent the real scenario of our cohort. Moreover, and despite our multipollutant models, we cannot exclude that the associations found from one single contaminant may be due to other correlated (and unmeasured) co-exposures, potentially including both persistent and non-persistent pollutants, or even a result of interactions among different co-exposures [289,290]. It is noteworthy that, as abovementioned, AHT belongs to a cluster of conditions closely related to Metabolic Syndrome. Indeed, we previously reported positive associations between PP and T2DM risk in our cohort [291]. Although we adjusted for prevalent T2DM diagnoses, we cannot exclude a potential residual confounding. Therefore, more research considering subclinical disease markers would shed light on the causality of the observed associations.

## 5. Conclusions

Overall, we evidenced no associations of most npEPs with AHT risk, with the exception of an increased risk in the highest PP percentiles. In view of the high prevalence of the exposure as well as the epidemic burden of the disease in current society, we consider that our results are relevant and contribute to the scientific knowledge in the area. Further research on the potential contribution of npEPs on the development of AHT risk is warranted.

## Conflict of interest

The authors disclose any financial or personal conflict of interest that could inappropriately influence the work.

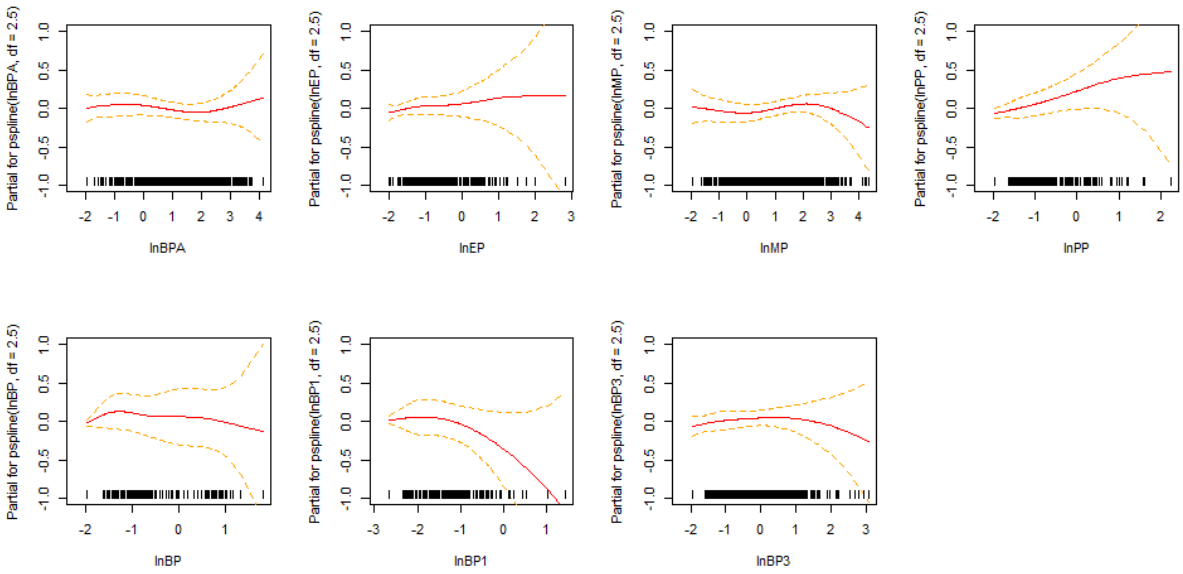
## Acknowledgments

This work would not have been possible without the generous collaboration of the EPIC participants. This paper will be part of the doctoral thesis developed by Elena Salamanca-

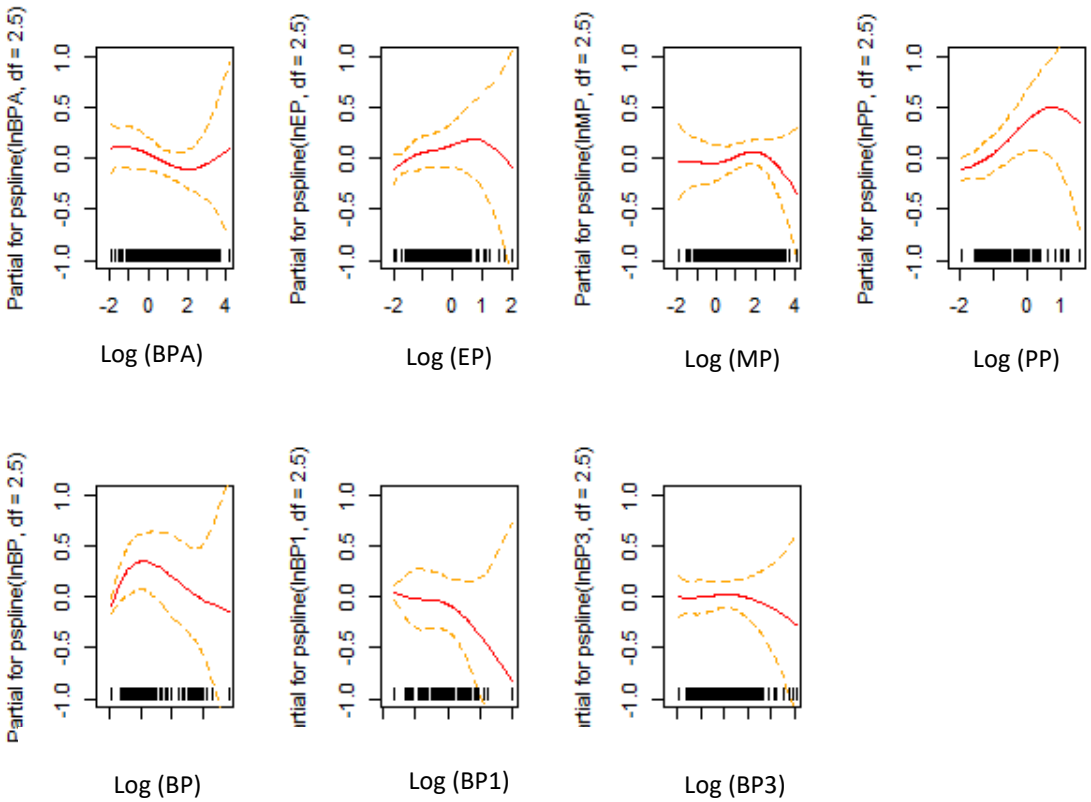
Fernández in the context of the "Clinical Medicine and Public Health Program" of the University of Granada (Spain).

**Figure 1. Serum levels of npEPs and risk of incident arterial hypertension. Generalized Additive Models.**

**Total population**




**Females**



## Males

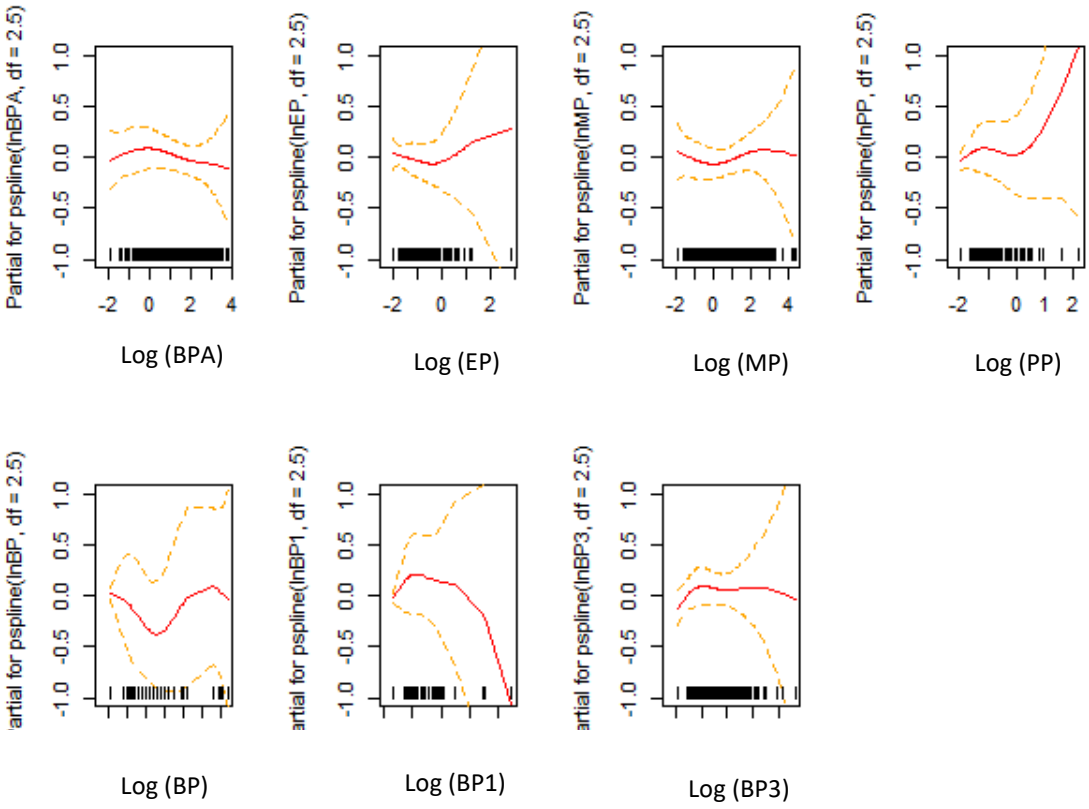
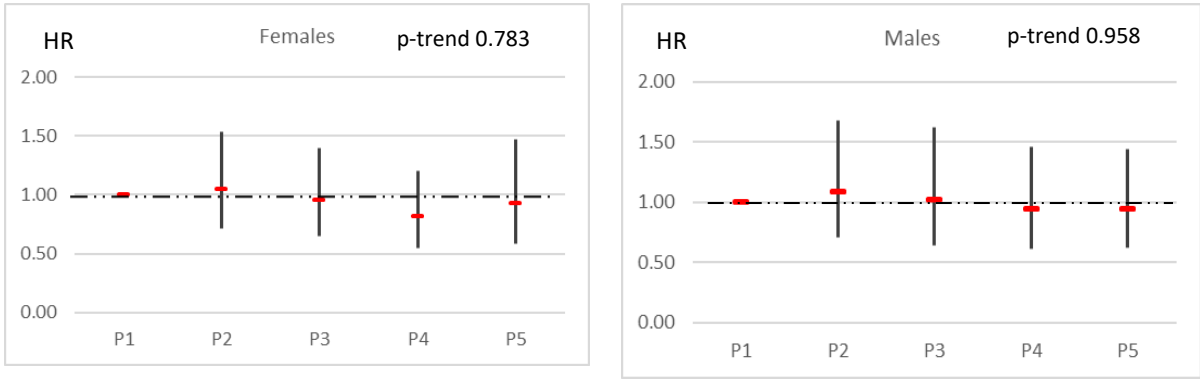
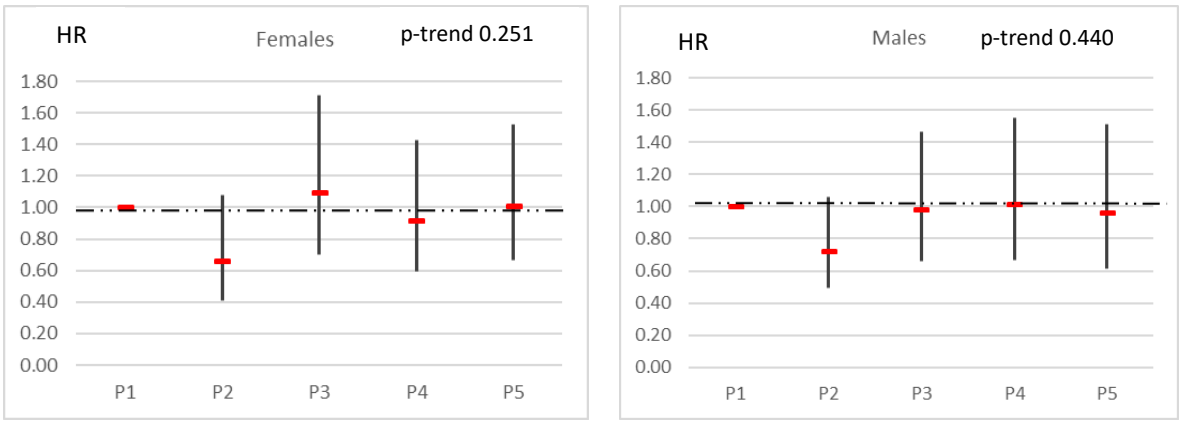

Partial for pspline(lnBPA, df = 2.5)
Log (BPA)
Partial for pspline(lnEP, df = 2.5)
Log (EP)
Partial for pspline(lnMP, df = 2.5)
Log (MP)
Partial for pspline(lnPP, df = 2.5)
Log (PP)
artial for pspline(lnBP, df = 2.5)
Log (BP)
artial for pspline(lnBP1, df = 2.5)
Log (BP1)
artial for pspline(lnBP3, df = 2.5)
Log (BP3)

**Figure 2. . Serum levels of npEPs and risk of incident arterial hypertension in males and females. Hazard ratios and 95% Confidence Intervals.**

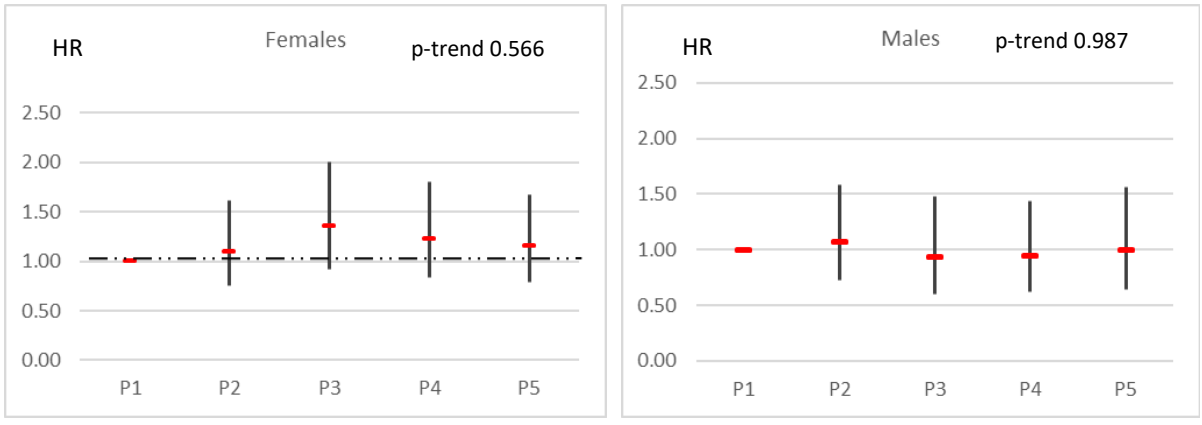
BPA



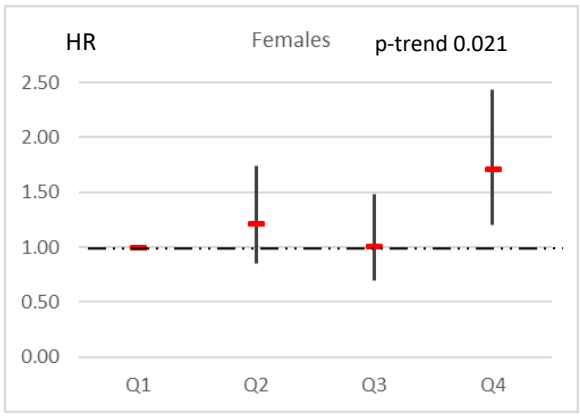

MP

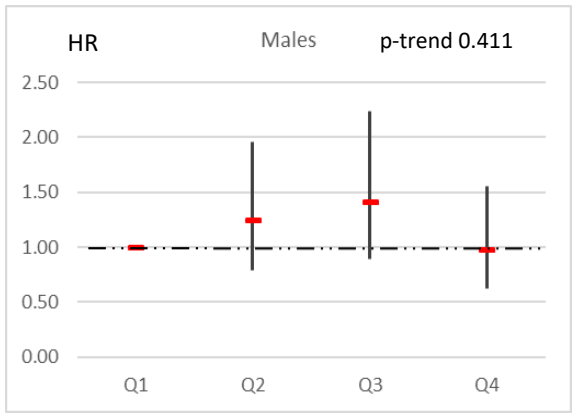



EP

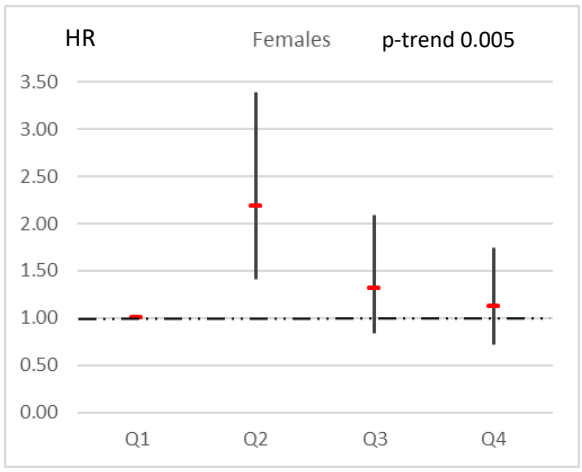


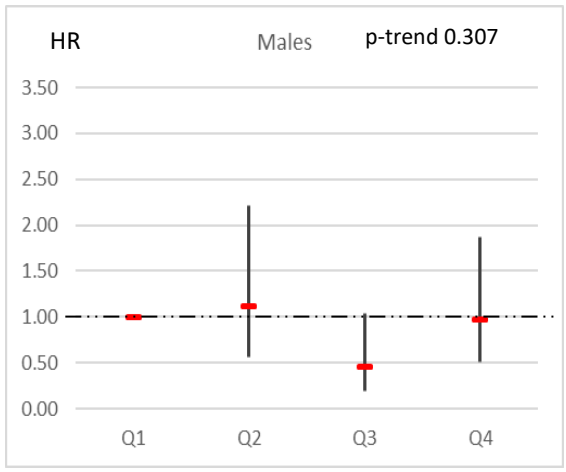
PP


HR
Females
p-trend 0.021
2.50
2.00
1.50
1.00
0.50
0.00
Q1
Q2
Q3
Q4

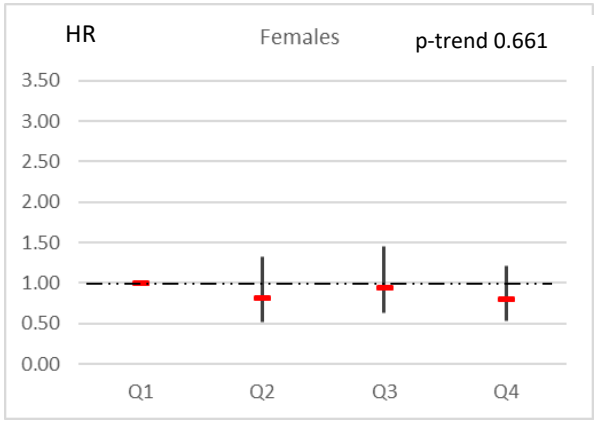

HR
Males
p-trend 0.411
2.50
2.00
1.50
1.00
0.50
0.00
Q1
Q2
Q3
Q4

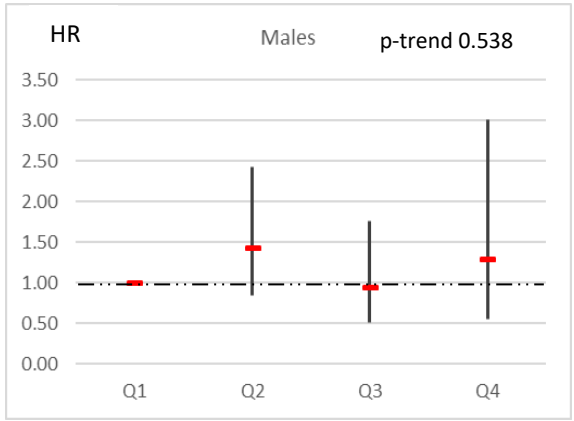
BP


HR
Females
p-trend 0.005
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Q1
Q2
Q3
Q4


HR
Males
p-trend 0.307
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Q1
Q2
Q3
Q4

BP1


HR
Females
p-trend 0.661
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Q1
Q2
Q3
Q4


HR
Males
p-trend 0.538
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Q1
Q2
Q3
Q4

BP3

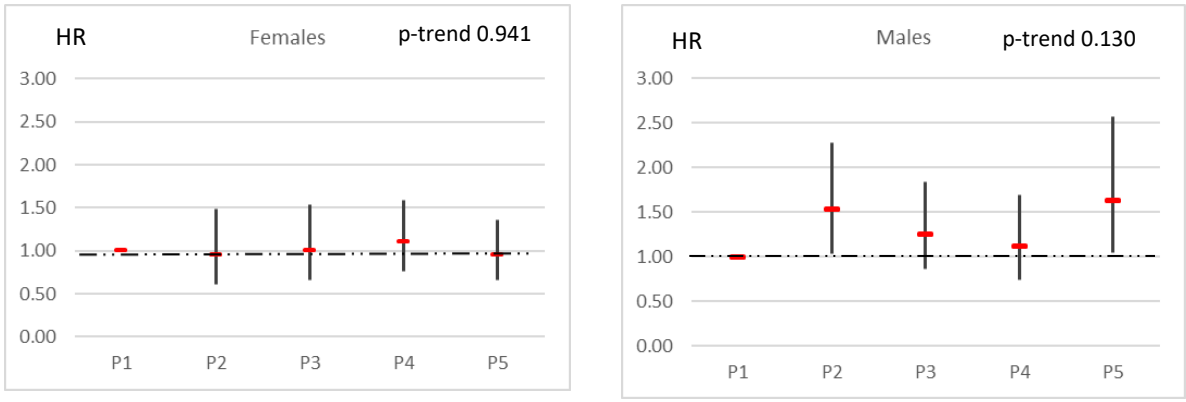

HR
Females
p-trend 0.941
3.00
2.50
2.00
1.50
1.00
0.50
0.00
P1
P2
P3
P4
P5
HR
Males
p-trend 0.130
3.00
2.50
2.00
1.50
1.00
0.50
0.00
P1
P2
P3
P4
P5

**Table 1. Baseline characteristics of the study population**

| | N (%) | AHT (%) | p-value* | Male (%) | Female (%) |
|---|---|---|---|---|---|
| | 670 (100) | 482 (71.9) | | | |
| **Sex** | | | 0.285 | | |
| Male | 320 (47.8) | 224 (70.0) | | - | - |
| Female | 350 (52.2) | 258 (73.7) | | - | - |
| **Age** | | | <0.001 | | |
| <45 | 142 (21.2) | 62 (43.7) | | 65 (20.3) | 77 (22.0) |
| 45-54 | 233 (34.8) | 169 (72.5) | | 110 (34.4) | 123 (35.1) |
| 55-59 | 126 (18.8) | 107 (84.9) | | 63 (19.7) | 63 (18.0) |
| ≥ 60 | 169 (25.2) | 144 (85.2) | | 82 (25.6) | 87 (24.9) |
| **Education level** | | | <0.001 | | |
| None | 352 (52.5) | 279 (79.3) | | 135 (42.2) | 217 (62.3) |
| Primary school | 172 (25.7) | 113 (65.7) | | 80 (25.0) | 92 (26.3) |
| Technical school | 25 (3.7) | 15 (60.0) | | 13 (4.1) | 12 (3.4) |
| Secondary school | 43 (6.4) | 26 (60.5) | | 31 (9.7) | 12 (3.4) |
| University | 78 (11.6) | 49 (62.8) | | 61 (19.1) | 17 (4.9) |
| **BMI** | | | <0.001 | | |
| Normal weight | 93 (13.9) | 38 (40.9) | | 34 (10.6) | 59 (16.9) |
| Overweight | 311 (46.4) | 213 (68.5) | | 172 (53.8) | 139 (39.7) |
| Obese | 266 (39.7) | 231 (86.8) | | 114 (35.6) | 152 (43.4) |
| **Smoke status** | | | 0.132 | | |
| Never | 410 (61.2) | 304 (74.1) | | 102 (31.9) | 308 (88.0) |
| Former | 144 (21.5) | 103 (71.5) | | 129 (40.3) | 15 (4.3) |
| Smoker | 116 (17.3) | 75 (64.7) | | 89 (27.8) | 27 (7.7) |
| **Physical activity** | | | 0.569 | | |
| Inactive | 93 (13.9) | 67 (72.0) | | 82 (25.6) | 11 (3.1) |
| Moderately inactive | 143 (21.3) | 99 (69.2) | | 112 (35.0) | 31 (8.9) |
| Moderately active | 394 (58.8) | 290 (73.6) | | 111 (34.7) | 283 (80.9) |
| Active | 40 (6.0) | 26 (65.0) | | 15 (4.7) | 25 (7.1) |

* Pearson chi-square

**Table 2. Serum npEPs concentrations (ng/mL) and rates of detection (%) in the study population.**

| | | | | | | | Female | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **> LOD (%)** | **GM** | **95% CI** | **M** | **P 25th** | **P75th** | **> LOD (%)** | **GM** | **95% CI** | **M** | **P 25th** | **P 75th** | **> LOD (%)** |
| BPA | 81.6 | 1.76 | (1.56-1.99) | 2.14 | 0.58 | 5.80 | 80.0 | 1.43 | (1.22-1.68) | 1.736 | 0.45 | 4.25 | 83.4 |
| MP | 88.5 | 2.03 | (1.82-2.28) | 2.27 | 0.73 | 6.52 | 92.9 | 2.81 | (2.42-3.27) | 3.306 | 1.30 | 8.75 | 83.8 |
| EP | 56.4 | 0.29 | (0.27-0.31) | 0.24 | < LOD | 0.50 | 58.0 | 0.30 | (0.28-0.33) | 0.249 | < LOD | 0.54 | 54.7 |
| PP | 38.1 | 0.22 | (0.21-0.24) | < LOD | < LOD | 0.32 | 46.3 | 0.24 | (0.23-0.26) | < LOD | < LOD | 0.39 | 29.1 |
| BP | 19.6 | 0.18 | (0.17-0.19) | < LOD | < LOD | < LOD | 26.0 | 0.20 | (0.19-0.22) | < LOD | < LOD | 0.21 | 12.5 |
| BP1 | 21.6 | 0.09 | (0.09-0.10) | 0.07 | < LOD | < LOD | 29.4 | 0.10 | (0.10-0.11) | 0.070 | < LOD | < LOD | 13.1 |
| BP3 | 68.7 | 0.51 | (0.47-0.56) | 0.55 | < LOD | 1.10 | 73.1 | 0.63 | (0.55-0.71) | 0.706 | < LOD | 1.37 | 63.7 |

LOD: Limit of detection; GM: Geometric Mean (ng/ml); M: Median (ng/ml); P: Percentile (ng/ml); 95% CI: Confidence Interval (ng/m
Bisphenol A (BPA), Methylparaben (MP), Ethylparaben (EP), Propylparaben (PP), Butilparaben (BP), Benzophenone 1 (BP1), Benzop

## Table 3. Serum npEP exposure and risk of incident arterial hypertension. Cox Proportional Hazard Models

| | | | | Parabens | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **BPA** | | | **MP** | | | **EP** | | | **PP** | | | **BP** | |
| | HR | SE | p | HR | SE | p | HR | SE | p | HR | SE | p | HR | SE | p |
| **Percentiles of npEPs** | | | [a]0.847 | | | [a] 0.044 | | | [a] 0.742 | | | [a] 0.085 | | | [a] 0.102 |
| **P2**[b] | 1.052 | 1.155 | 0.725 | 0.661 | 1.164 | 0.006 | 1.108 | 1.149 | 0.461 | 1.197 | 1.153 | 0.205 | 1.520 | 1.201 | 0.022 |
| **P3**[b] | 1.059 | 1.158 | 0.695 | 0.974 | 1.156 | 0.858 | 1.191 | 1.158 | 0.235 | 1.074 | 1.158 | 0.629 | 0.872 | 1.220 | 0.490 |
| **P4**[b] | 0.909 | 1.158 | 0.516 | 0.876 | 1.162 | 0.376 | 1.142 | 1.153 | 0.351 | 1.403 | 1.149 | 0.015 | 1.109 | 1.201 | 0.573 |
| **P5**[b] | 0.993 | 1.165 | 0.964 | 0.963 | 1.160 | 0.797 | 1.098 | 1.154 | 0.511 | - | - | - | - | - | - |
| **>LOD *vs* <LOD** | 0.979 | 1.127 | 0.862 | 0.990 | 1.156 | 0.946 | 1.131 | 1.100 | 0.192 | 1.220 | 1.102 | 0.039 | 1.128 | 1.124 | 0.303 |
| **Log-transformed variable (ng/ml)** | 0.986 | 1.030 | 0.643 | 1.001 | 1.033 | 0.980 | 1.060 | 1.057 | 0.288 | 1.143 | 1.064 | 0.031 | 1.020 | 1.068 | 0.760 |
| **Unadjusted model** | 0.966 | 1.028 | 0.229 | 1.022 | 1.031 | 0.487 | 1.057 | 1.055 | 0.304 | 1.192 | 1.061 | 0.003 | 1.188 | 1.066 | 0.007 |

BPA: Bisphenol A; MP: Methylparaben; EP: Ethylparaben; PP: Propylparaben; BP: Butilparaben; BP1: Benzophenone 1; BP3:Benzophenone 3; HR: Hazard ratio; Detection

Adjusted by sex, age, BMI, fasting, energy intake, physical activity, educational level, extraction time and smoking status

[a] p trend

[b] reference category: P1

Ranges of concentrations (ng/mL) in each percentile:

BPA: P1 (<LOD - 0.31), P2 (0.32- 1.47), P3 (1.49 - 3.34), P4 (3.38- 7.42), P5 (7.51- 62.95)

MP: P1 (<LOD - 0.48), P2 (0.49- 1.54), P3 (1.55- 3.42), P4 (3.44- 8.05), P5 (8.16-75.55)

EP: P1 (<LOD - 0.18), P2 (0.20- 0.29), P3 (0.30-0.45), P4 (0.45-0.75), P5 (0.76-16.91)

PP: P1 (<LOD - 0.20), P2 (0.20-0.31), P3 (0.31-0.52), P4 (0.53-9.24)

BP: P1 (<LOD - 0.20), P2 (0.20-0.36), P3 (0.37-0.57), P4 (0.61-6.21)

BP1: P1 (<LOD - 0.10), P2 (0.10-0.20), P3 (0.21-0.37), P4 (0.37-4.46)

BP3: P1 (<LOD - 0.14), P2 (0.21-0.48), P3 (0.49-0.85), P4 (0.86-1.43), P5 (1.46-21.20)

**Supplementary material**

**Table S1. Comparison between selected and non-selected participants from the entire EPIC-Granada cohort.**

| | Selected | Non-selected |
|---|---|---|
| | N (%) | |
| Granada | 670 (83.02) | 125 (16.98) |
| **Age** | | |
| <45 | 142 (21.2) | 34 (27.2) |
| 45-54 | 233 (34.8) | 55 (44.0) |
| 55-59 | 126 (18.8) | 17 (13.6) |
| 60+ | 169 (25.2) | 19 (15.2) |
| **Education level** | | |
| None | 352 (52.5) | 24 (19.2) |
| Primary school | 172 (25.7) | 29 (23.2) |
| Technical school | 25 (3.7) | 4 (3.2) |
| Secondary school | 43 (6.4) | 11 (8.8) |
| University | 78 (11.6) | 57 (45.6) |
| **BMI** | | |
| <25 kg/m2 | 93 (13.9) | 17 (13.6) |
| 25-<30 kg/m2 | 311 (46.4) | 81 (64.8) |
| ≥30 kg/m2 | 266 (39.7) | 27 (21.6) |
| **Smoke status** | | |
| Never | 410 (61.2) | 71 (56.8) |
| Former | 144 (21.5) | 36 (28.8) |
| Smoker | 116 (17.3) | 18 (14.4) |
| **Physical activity** | | |
| Inactive | 93 (13.9) | 33 (26.4) |
| Moderately inactive | 143 (21.3) | 40 (32.0) |
| Moderately active | 394 (58.8) | 47 (37.6) |
| Active | 40 (6.0) | 5 (4.0) |

**Table S2. Spearman correlation among npEPs included in the study**

| | | BPA | MP | EP | PP | BP | BP1 | BP3 |
|---|---|---|---|---|---|---|---|---|
| BPA | Correlation coefficient | - | -0.130 | -0.131 | -0.115 | -0.178 | -0.127 | -0.078 |
| | p-value | | 0.001 | 0.001 | 0.003 | <0.001 | 0.001 | 0.044 |
| MP | Correlation coefficient | | - | 0.436 | 0.315 | 0.370 | 0.116 | 0.268 |
| | p-value | | | <0.001 | <0.001 | <0.001 | 0.003 | <0.001 |
| EP | Correlation coefficient | | | - | 0.448 | 0.244 | 0.068 | 0.200 |
| | p-value | | | | <0.001 | <0.001 | 0.078 | <0.001 |
| PP | Correlation coefficient | | | | - | 0.382 | 0.253 | 0.193 |
| | p-value | | | | | <0.001 | <0.001 | <0.001 |
| BP | Correlation coefficient | | | | | - | 0.165 | 0.107 |
| | p-value | | | | | | <0.001 | 0.006 |
| BP1 | Correlation coefficient | | | | | | - | 0.271 |
| | p-value | | | | | | | <0.001 |
| BP3 | Correlation coefficient | | | | | | | - |
| | p-value | | | | | | | |

## Table S3. Serum npEP concentrations and risk of incident arterial hypertension in females. Cox Proportional H

| | | | | Parabens | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **BPA** | | | **MP** | | | **EP** | | | **PP** | | | **BP** | | |
| | HR | SE | P | HR | SE | p | HR | SE | p | HR | SE | p | HR | SE | |
| **Percentiles of npEPs** | | | [a]0.783 | | | [a]0.251 | | | [a]0.566 | | | [a]0.021 | | | [a]0. |
| **P2**[b] | 1.043 | 1.215 | 0.830 | 0.664 | 1.280 | 0.098 | 1.097 | 1.217 | 0.634 | 1.217 | 1.200 | 0.282 | 2.189 | 1.251 | <0 |
| **P3**[b] | 0.953 | 1.214 | 0.804 | 1.093 | 1.256 | 0.696 | 1.360 | 1.219 | 0.120 | 1.010 | 1.214 | 0.959 | 1.320 | 1.262 | 0. |
| **P4**[b] | 0.814 | 1.220 | 0.301 | 0.920 | 1.250 | 0.710 | 1.230 | 1.215 | 0.290 | 1.715 | 1.196 | 0.003 | 1.121 | 1.252 | 0. |
| **P5**[b] | 0.923 | 1.266 | 0.736 | 1.009 | 1.235 | 0.968 | 1.149 | 1.210 | 0.467 | - | - | - | - | - | |
| **>LOD *vs* <LOD** | 0.884 | 1.178 | 0.452 | 1.261 | 1.281 | 0.349 | 1.199 | 1.139 | 0.161 | 1.282 | 1.137 | 0.053 | 1.429 | 1.155 | 0. |
| **Log-transformed variable (ng/ml)** | 0.957 | 1.045 | 0.317 | 1.004 | 1.044 | 0.934 | 1.091 | 1.076 | 0.230 | 1.211 | 1.087 | 0.021 | 1.084 | 1.080 | 0. |
| **Unadjusted model** | 1.006 | 1.043 | 0.892 | 1.013 | 1.046 | 0.769 | 1.071 | 1.073 | 0.325 | 1.245 | 1.081 | 0.005 | 1.210 | 1.080 | 0. |

BPA: Bisphenol A; MP: Methylparaben; EP: Ethylparaben; PP: Propylparaben; BP: Butilparaben; BP1: Benzophenone 1; BP3:Benzophenone 3; HR: Hazard ratio; Detection

Adjusted by sex, age, BMI, fasting, energy intake, physical activity, educational level, extraction time and smoking status

[a] p trend

[b] reference category: P1

Ranges of concentrations (ng/mL) in each percentile:

BPA: P1 (<LOD - 0.31), P2 (0.32- 1.47), P3 (1.49 - 3.34), P4 (3.38- 7.42), P5 (7.51- 62.95)

MP: P1 (<LOD - 0.48), P2 (0.49- 1.54), P3 (1.55- 3.42), P4 (3.44- 8.05), P5 (8.16-75.55)

EP: P1 (<LOD - 0.18), P2 (0.20- 0.29), P3 (0.30-0.45), P4 (0.45-0.75), P5 (0.76-16.91)

PP: P1 (<LOD - 0.20), P2 (0.20-0.31), P3 (0.31-0.52), P4 (0.53-9.24)

BP: P1 (<LOD - 0.20), P2 (0.20-0.36), P3 (0.37-0.57), P4 (0.61-6.21)

BP1: P1 (<LOD - 0.10), P2 (0.10-0.20), P3 (0.21-0.37), P4 (0.37-4.46)

BP3: P1 (<LOD - 0.14), P2 (0.21-0.48), P3 (0.49-0.85), P4 (0.86-1.43), P5 (1.46-21.20)

**Table S4. Serum npEPs exposure and risk of incident arterial hypertension in males. Cox Proportional Hazard**

| | | | | Parabens | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **BPA** | | | **MP** | | | **EP** | | | **PP** | | | **BP** | |
| | HR | SE | p | HR | SE | p | HR | SE | p | HR | SE | p | HR | SE | p |
| **Percentiles of npEPs** | | | [a]0.958 | | | [a]0.440 | | | [a]0.987 | | | [a]0.411 | | | [a]0.30 |
| **P2**[b] | 1.090 | 1.247 | 0.695 | 0.724 | 1.214 | 0.096 | 1.070 | 1.223 | 0.737 | 1.244 | 1.259 | 0.342 | 1.112 | 1.419 | 0.76 |
| **P3**[b] | 1.015 | 1.270 | 0.95 | 0.986 | 1.224 | 0.944 | 0.937 | 1.261 | 0.778 | 1.411 | 1.264 | 0.142 | 0.455 | 1.527 | 0.06 |
| **P4**[b] | 0.946 | 1.249 | 0.802 | 1.017 | 1.242 | 0.940 | 0.944 | 1.240 | 0.788 | 0.979 | 1.265 | 0.928 | 0.973 | 1.398 | 0.93 |
| **P5**[b] | 0.946 | 1.239 | 0.796 | 0.965 | 1.259 | 0.876 | 0.996 | 1.256 | 0.987 | - | - | - | - | - | - |
| **>LOD *vs* <LOD** | 0.993 | 1.202 | 0.969 | 0.933 | 1.151 | 0.707 | 0.992 | 1.154 | 0.953 | 1.188 | 1.163 | 0.253 | 0.798 | 1.242 | 0.29 |
| **Log-transformed variable (ng/ml)** | 0.977 | 1.043 | 0.573 | 1.013 | 1.048 | 0.786 | 0.993 | 1.093 | 0.940 | 1.076 | 1.103 | 0.455 | 0.880 | 1.141 | 0.33 |
| **Unadjusted model** | 0.945 | 1.041 | 0.157 | 0.992 | 1.047 | 0.852 | 1.008 | 1.088 | 0.927 | 1.090 | 1.099 | 0.362 | 1.050 | 1.133 | 0.69 |

BPA: Bisphenol A; MP: Methylparaben; EP: Ethylparaben; PP: Propylparaben; BP: Butilparaben; BP1: Benzophenone 1; BP3:Benzophenone 3; HR: Hazard ratio; Detection

Adjusted by sex, age, BMI, fasting, energy intake, physical activity, educational level, extraction time and smoking status

[a] p trend

[b] reference category: P1

Ranges of concentrations (ng/mL) in each percentile:

BPA: P1 (<LOD - 0.31), P2 (0.32- 1.47), P3 (1.49 - 3.34), P4 (3.38- 7.42), P5 (7.51- 62.95)

MP: P1 (<LOD - 0.48), P2 (0.49- 1.54), P3 (1.55- 3.42), P4 (3.44- 8.05), P5 (8.16-75.55)

EP: P1 (<LOD - 0.18), P2 (0.20- 0.29), P3 (0.30-0.45), P4 (0.45-0.75), P5 (0.76-16.91)

PP: P1 (<LOD - 0.20), P2 (0.20-0.31), P3 (0.31-0.52), P4 (0.53-9.24)

BP: P1 (<LOD - 0.20), P2 (0.20-0.36), P3 (0.37-0.57), P4 (0.61-6.21)

BP1: P1 (<LOD - 0.10), P2 (0.10-0.20), P3 (0.21-0.37), P4 (0.37-4.46)

BP3: P1 (<LOD - 0.14), P2 (0.21-0.48), P3 (0.49-0.85), P4 (0.86-1.43), P5 (1.46-21.20)

**Table S5. Serum npEP concentrations and risk of incident arterial hypertension. Adjusted Multi-pollutant Cox**

| | | | | Parabens | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **BPA** | | | **MP** | | | **EP** | | | **PP** | | | **BP** | |
| | HR | SE | p | HR | SE | p | HR | SE | p | HR | SE | p | HR | SE |
| **Multi-pollutant model** | 0.984 | 1.030 | 0.59 | 0.979 | 1.037 | 0.553 | 1.019 | 1.069 | 0.775 | 1.163 | 1.074 | 0.034 | 0.983 | 1.078 |

BPA: Bisphenol A; MP: Methylparaben; EP: Ethylparaben; PP: Propylparaben; BP: Butilparaben; BP1: Benzophenone 1; BP3:Benzophenone 3; HR: Hazard ratio;

# 8 Discusión

## 8.1 Niveles de exposición a BPA

Los resultados de esta tesis sugieren que la exposición a los contaminantes no persistentes es frecuente en las poblaciones estudiadas. En concreto, BPA se encontró en el 70% de la población en la cohorte EPIC-España. Las concentraciones séricas de BPA en nuestros participantes (GM = 1.19 ng / mL) fueron algo menores (aunque de un orden de magnitud similar) que las observadas en muestras de población de otros países, en distintas fechas como, Japón en 1998 (GM = 2,24 ng / ml), Japón en 2004 (GM = 2,5 ng / ml), EE. UU. en 2008 (GM = 5,9 ng / ml), Italia en 2009 (GM = 2,91 ng / ml), China en 2015 (GM = 9,73 ng / ml con tiempo trabajado ≤ 5 años y 27,18 ng / ml con tiempo trabajado > 5 años), China en 2014 (GM = 1,50 ng / ml), Corea del Sur en 2012 (GM = 1,56 ng / ml) y China en 2015 (GM = 3,2 ng / ml) [33–40]. También ha habido, sin embargo, varios estudios anteriores que han mostrado concentraciones más bajas que las encontradas en nuestra población de estudio, incluyendo Tailandia en 2009 (GM = 0.34 ng / ml), Japón en 2003 (GM = 0.46 ng / ml), Japón en 2004 (GM = 1.17 ng / ml) [205–207] y España (GM= 0.58 ng/ml) [204]. Para comparar diferentes estudios, se debe tener en cuenta la fecha de reclutamiento y toma de la muestra, ya que la mayoría de las investigaciones anteriores se han realizado en poblaciones reclutadas años o décadas después del período de estudio correspondiente a nuestra cohorte (1992-1996). La fecha de reclutamiento es relevante porque durante los años 90 los envases de plástico y los alimentos enlatados eran menos habituales que en los años posteriores. Los alimentos son la principal ruta de exposición al BPA, y por ello cabe destacar que los hábitos alimenticios también han cambiado gradualmente al incrementar el consumo de comidas precocinadas.

En cuanto a los determinantes de la exposición a BPA en el reclutamiento analizados en esta tesis, en la sub-cohorte de estudio, los principales determinantes de los niveles séricos de BPA fueron el sexo, el centro de reclutamiento y la dieta. Con respecto a los predictores dietéticos, el consumo de azúcar y productos de confitería se asociaron positivamente con los niveles séricos de BPA y las grasas y aceites añadidos se asociaron negativamente con el BPA. El consumo de grasas y aceites añadidos en la cohorte era principalmente de aceites vegetales, particularmente el aceite de oliva, que representó el 70.4% del total de grasas y aceites añadidos consumidos, seguido del aceite de girasol con el 12.2%. Estos

alimentos junto con aceites mixtos y no especificados, constituyeron el 93% del consumo en este grupo principal. En nuestra opinión, es difícil evaluar la asociación negativa entre los niveles de BPA y el consumo de aceite de oliva, ya que no hay información sobre el envasado de aceite de oliva en el momento del reclutamiento. Con respecto al envasado, un estudio de BPA en el aceite de oliva en el Líbano reveló niveles más altos de BPA en muestras almacenadas en envases plásticos frente a envases no plásticos ($\beta = 121.56$, IC 95% 53.44-194.39, valor $p = 0.009$) [209]. Se obtuvieron hallazgos similares en un estudio en atún enlatado en Italia, donde los niveles de bisfenoles detectados fueron más altos que los valores medios para el aceite [210]. Como se ha demostrado previamente, el BPA es ligeramente lipofílico [211,212] y, de hecho, un estudio previo encontró concentraciones de BPA detectables en el 86.8% de las muestras de tejido adiposo de una cohorte adulta (cohorte GraMo) [51].

Por otro lado, en nuestra población de estudio, la ingesta de azúcar y productos de repostería se asoció positivamente con los niveles séricos de BPA. En este sentido, nuestros resultados parecen concordar con los de Larsson et al. que encontraron niveles más altos de BPA en niños que a menudo comían chocolate y sugirieron que esto podría reflejar un consumo más frecuente de alimentos contaminados por sus envoltorios [216]. En esta misma línea, Rivas et al. [215] concluyeron en su estudio dietético que la adherencia a una dieta mediterránea (con un consumo muy bajo o nulo de productos de repostería) estaba inversamente asociada con los niveles de BPA en las matrices humanas. Sin embargo, la evidencia muestra que la exposición dietética al BPA está más determinada por el envasado de alimentos que por la comida en sí misma. Por lo tanto, esta asociación podría reflejar algunos hábitos alimenticios más relacionados con la exposición a BPA, ya que las personas que consumen más productos de confitería pueden estar consumiendo más alimentos enlatados, comidas precocinadas o refrescos, que son productos relacionados con la exposición a BPA [217–219].

Nuestros resultados también destacaron la existencia de una asociación positiva con el consumo de alimentos ultraprocesados. Esta relación corrobora lo observado para el consumo de azúcar, ya que los alimentos ultraprocesados generalmente incluyen altos niveles de azúcares [220–222]. Sin embargo, aunque el azúcar y los productos de confitería generalmente están envueltos en plástico, también se ha demostrado que los

alimentos enlatados suponen una importante contribución dietética a los niveles de BPA [223].

Encontramos diferencias significativas en la exposición relacionadas con el sexo, ya que los hombres tenían niveles significativamente más altos de BPA en suero que las mujeres en nuestro estudio. Estudios previos también han mostrado concentraciones de BPA significativamente más altas en plasma masculino que en las mujeres [224,225]. Por otro lado, González et al. [204] encontraron niveles de BPA dos veces más altos en trabajadoras (0,68 y 1,20 μg / L en hombres y mujeres, respectivamente), pero esta diferencia no alcanzó el nivel de significación estadística ($p < 0,05$) y puede estar relacionada con el diferente lugar de trabajo entre sexos en su estudio. Sin embargo, otros estudios no han encontrado diferencias significativas a este respecto [40,226]. Las diferencias de sexo en los niveles de BPA podrían explicarse a través de los mecanismos por los cuales el BPA se metaboliza en el hígado. Biológicamente, la fisiología hepática difiere según el sexo; los hombres tienen más actividad enzimática hepática que las mujeres [227], por lo que deberían eliminar el BPA más rápido. Además, a parte de las razones biológicas, se debe suponer que otras variables como los hábitos de vida o la ocupación, desempeñan un papel en este contexto. El hábito de fumar, los altos niveles de andrógenos en la sangre y los hábitos alimenticios son otras posibles explicaciones de las mayores concentraciones de BPA observadas en los hombres. En otro estudio, Takeuchi et al. [228] analizaron las concentraciones séricas y el metabolismo de BPA en ratas, encontrando concentraciones significativamente más altas en los machos que en las hembras. Estos autores comentaron que la diferencia de sexo en las concentraciones séricas de BPA podría explicarse por diferencias en la depuración, de acuerdo con las actividades enzimáticas resultantes. En este sentido, construimos modelos ajustados por sexo para estudiar la asociación con grasas y aceites añadidos, observando que el consumo de grasas y aceites añadidos estaba inversamente asociado con los niveles séricos de BPA en las mujeres. Sin embargo, cuando el grupo de estudio se estratificó por sexo, esta asociación no se observó en los hombres. El efecto protector de las grasas y aceites añadidos contra los niveles de BPA que observamos solo en las mujeres podría interpretarse como un reflejo de las diferencias en el consumo de aceite vegetal entre los participantes de nuestra cohorte, donde el aceite de oliva se consume en un porcentaje mayor en mujeres que en hombres (52.3% vs 47.7 %, respectivamente). Además, como se indicó anteriormente, el aceite de oliva podría desempeñar un papel en el metabolismo

hepático y ejercer un efecto protector [214]. Por otro lado, y a pesar de las diferencias observadas, al estratificar a los participantes por sexo, se redujo el poder estadístico de nuestro análisis y, por lo tanto, decidimos combinar ambos sexos en los modelos dietéticos construidos.

La edad y el BPA se asociaron positivamente en nuestra población de estudio. Sin embargo, un estudio anterior no observó diferencias significativas relacionadas con la edad en la concentración de BPA en sangre en personas de Shanghai, China [225]. Algunas publicaciones han mostrado niveles urinarios más altos de BPA en adultos más jóvenes [51,229], probablemente reflejando su mayor consumo de agua embotellada [230,231] y alimentos envasados en recipientes de plástico [232]. Además, otros estudios han sugerido que los contaminantes no persistentes como el BPA pueden no excretarse por completo y que una proporción puede almacenarse en compartimentos corporales [233,234]. A este respecto, un estudio farmacocinético reveló que los niveles de BPA no conjugado permanecieron hasta 20 horas en el tejido adiposo, mientras que las concentraciones séricas se convirtieron rápidamente (<5 h) en la forma iso-monoglucurónido de BPA no estrogénica [234].

En definitiva, debido a la creciente preocupación por el BPA, durante los últimos años se ha aumentado la utilización de algunos análogos estructurales durante los últimos años (BPS y BPF) y, por lo tanto, se podría esperar que en las nuevas medidas de exposición más recientes, los niveles biológicos de BPA puedan ser más bajos [208].

En general, nuestros resultados sugieren que la exposición dietética al BPA va más allá de los alimentos individuales y podría verse afectada por los diferentes métodos de cocción, envasado o preparación de alimentos, que, de hecho, podría ser la ruta clave de exposición al BPA.

## 8.2 Relación de la exposición a contaminantes no persistentes con componentes de síndrome metabólico: enfermedad coronaria, diabetes tipo 2 e hipertensión arterial.

En cuanto a la posible asociación entre los contaminantes no persistentes y el riesgo de síndrome metabólico, los resultados de esta tesis no encontraron asociaciones evidentes

entre las concentraciones séricas de BPA y el riesgo de ECI incidente aunque el BPA se considere un DE con potencial obesogénico [257,258,265]. En este sentido, nuestros resultados concuerdan con algunos estudios epidemiológicos previos que no observaron asociación entre la exposición urinaria a BPA y las enfermedades cardiovasculares, ataque cardíaco o enfermedad coronaria [266,267]. Específicamente, LaKind et al. examinaron los datos de NHANES de 2003–04 a 2009–10 en adultos de ≥20 años, y no encontraron asociaciones significativas entre enfermedad coronaria y BPA en orina [267].

Sin embargo, otros estudios epidemiológicos mostraron un mayor riesgo de enfermedad coronaria en una población aparentemente sana expuesta a BPA [189,268–270]. El estudio de Shankar et al. mostró que los niveles de BPA en orina se asociaron significativamente con la enfermedad arterial periférica, independientemente de los factores de riesgo de ECV tradicionales [157]. Un meta-análisis concluyó que el BPA en orina a los niveles encontrados en la población general está asociado con una mayor prevalencia de diabetes, obesidad general y abdominal e hipertensión [271]. Esto coincide con el estudio NHANES 2003–04 [268], donde los autores encontraron que una mayor concentración de BPA urinario se asoció con un mayor riesgo de ECV (infarto de miocardio, angina o enfermedad coronaria) (OR = 1.39, IC 95%: 1.18– 1.63), pero no de accidente cerebrovascular (OR = 0.97, IC 95%: 0.74–1.27). Melzer et al. mostraron asociaciones similares con los datos de NHANES 2005–06 [270] y Casey y Neidell revelaron asociaciones positivas significativas entre el BPA urinario y la enfermedad coronaria en NHANES 2003–04, pero los resultados no fueron consistentes en los siguientes ciclos 2005/06 y 2007/08 [272]. En otro estudio de Melzer et al. mostraron que las concentraciones de BPA en orina se asociaron significativamente con enfermedad coronaria grave (OR = 1.43, IC 95%: 1.03–1.98; n = 385), y se asociaron casi significativamente con enfermedad coronaria intermedia (OR = 1.69, IC 95%: 0.98 –2,94; n = 86) [273]. El estudio prospectivo de casos y controles anidados dentro de la cohorte EPIC-Norfolk, mostró una asociación positiva entre las concentraciones de BPA en la orina y la incidencia de enfermedad coronaria: OR = 1.11, IC 95% = 1.00-1.23 [269]. Por lo tanto, varios estudios epidemiológicos transversales han obtenido relaciones positivas entre los niveles de BPA (orina) con factores de riesgo de ECV, como presión arterial, colesterolemia, etc. [86].

Las diferencias entre resultados de los diversos estudios epidemiológicos pueden deberse a varios factores: la heterogeneidad de las poblaciones incluidas en los estudios, las diferentes formas de medición de la enfermedad, la diferente evaluación de la exposición a BPA y la matriz biológica que puede tener una influencia sustancial en los niveles de exposición. En este sentido, una revisión sistemática realizada por Lakind et al. concluyó que debido al diseño epidemiológico de los estudios, la comprensión de los efectos sobre la salud asociados con la exposición al BPA es limitada [266]. Además, la exposición humana a muchos posibles disruptores endocrinos puede confundirse porque la mayoría de las cohortes y estudios epidemiológicos existentes fueron diseñados para medir el impacto de un solo químico, sin tener en cuenta los efectos de las mezclas [274]. Todos estos factores deben tenerse en cuenta al considerar los resultados de los estudios porque las diferentes metodologías pueden dar lugar a resultados inconsistentes. Específicamente, Lakind et al. sugirieron que las conclusiones deben hacerse con precaución con respecto a las encuestas NHANES [267].

A pesar de que todos los npEPs incluidos en nuestro estudio se consideran disruptores endocrinos con potencial diabetogénico [133,138,266,287,299,300], y que la literatura científica reciente sugiere que el BPA y los parabenos tienen un potencial obesogénico en humanos [255–258,265,276,277], en esta tesis no se encontraron asociaciones evidentes para la mayoría de los contaminantes no persistentes y el riesgo de diabetes tipo 2. Sin embargo, los individuos con las concentraciones séricas más altas de PP mostraron un mayor riesgo de incidencia de DT2. Además, se observó una tendencia negativa entre el riesgo de BP1 y DT2, que solo fue evidente en mujeres.

Nuestros resultados respecto a DT2 coinciden con los de un estudio prospectivo de casos y controles anidado en una cohorte, que no encontró una asociación entre los niveles de BPA en suero con la incidencia de DT2 a 5 años entre los participantes chinos: OR 0,93 (IC del 95%: 0,41 a 2,13) [301]. Por el contrario, un estudio transversal en una población tailandesa sí encontró asociaciones positivas entre las concentraciones séricas de BPA y el riesgo de diabetes (OR para el 3 cuartil y 4 cuartil de BPA = 1.88 [IC 95% = 1.18, 2.99] y 1.83 [IC 95% = 1.12 , 2,95], respectivamente) [205]. Con respecto a los estudios epidemiológicos que utilizan la orina como matriz para la evaluación de la exposición, Li et al. observaron un aumento de 6 veces en las probabilidades de diabetes en aquellos individuos con las concentraciones urinarias más altas de MP y EP, en comparación con

aquellos con las concentraciones más bajas [302]. Del mismo modo, Kim et al. observaron una asociación significativa entre los niveles de BPA y el riesgo de DM2 en un estudio de casos y controles (OR = 1.71; IC 95%: 0.89-3.26; p = 0.374) [303]. Sorprendentemente, el estudio de Wang et al. encontró un efecto protector del BPA sobre el riesgo de diabetes gestacional (OR = 0,73; IC del 95% = 0,56 a 0,97) [304]. Además, un estudio prospectivo en 200 mujeres embarazadas concluyó que el uso de filtros UV (incluidos los BP) podría interrumpir la homeostasis endocrina y afectar negativamente el desarrollo de fetos y niños [305]. En general, hay una serie de estudios epidemiológicos [301,303] y revisiones [266,287] que no encontraron una asociación clara entre las npEP y la DT2. A la luz de lo anterior, actualmente hay poca evidencia científica sobre los posibles efectos diabétogénicos para la mayoría de los npEP.

Por otro lado, los parabenos y las benzofenonas se usan con frecuencia en cosméticos (aparte de los alimentos) [94,306,307], lo que probablemente representa un porcentaje relevante de nuestras diferencias relacionadas con el sexo en los efectos de la exposición a npEP. En nuestros resultados, las concentraciones séricas de la mayoría de las npEP fueron más altas en las mujeres que en los hombres, con la excepción del BPA. A pesar de que el uso de cosméticos no se abordó en los cuestionarios del estudio EPIC, los hallazgos antes mencionados sugieren marcadas diferencias en su uso, relacionadas con el sexo en su uso. Además, en las mujeres encontramos un mayor número de valores extremos y, por lo tanto, esta mayor variabilidad podría hacer que su efecto potencial sea más evidente en las mujeres.

Entre los resultados de esta tesis, se encuentra un mayor riesgo de HTA incidente en aquellos individuos con las concentraciones séricas más altas de PP en el reclutamiento. Sin embargo, no se encuentran asociaciones evidentes con el riesgo de HTA para el resto de los contaminantes incluidos (BPA, MP, EP, BP, BP1 y BP3), ni en modelos globales ni después de la estratificación por sexo, con la excepción de una asociación positiva de BP3 para P2 y P5 y el riesgo de HTA en hombres, y una forma de U invertida para BP en mujeres.

En este sentido, Shiue et al. concluyeron que había asociaciones claras entre los niveles de parabenos en orina y la presión arterial alta en el estudio NHANES [162,163,323].

Aun así, los parabenos todavía son poco explorados como un factor de riesgo cardiovascular.

Nuestros resultados para BPA están en consonancia con los de Wang et al. [324] que no mostraron asociación entre los niveles de BPA en orina con el riesgo de HTA en un estudio transversal con población china, ni con los resultados de NHANES 2009–2010 que arrojaron una falta de asociaciones significativas entre los niveles urinarios de BPA y la presión arterial alta (OR 1.12, IC 95% 0.93–1.35) [162]. Sin embargo, varios estudios han mostrado previamente asociaciones de exposición a BPA con el riesgo de HTA [157–160] y / o comorbilidades / factores de riesgo asociados, por ejemplo, variabilidad de la frecuencia cardíaca, enfermedad vascular periférica, enzimas anormales de la función hepática, aumento de la glucosa en ayunas, resistencia a la insulina, obesidad general y central, y diabetes mellitus [119,189,259,268,270,326].

En esta tesis, la tendencia observada para HTA es similar a aquella para el riesgo de padecer DT2, lo que puede explicarse también por la alta inter-relación de estas patologías componentes del Síndrome Metabólico. Las personas con diabetes tienen mayor probabilidad de tener presión arterial alta, o hipertensión arterial, que las personas sin diabetes (entre un 40% y un 60% de los diabéticos padecen hipertensión y las personas con presión arterial alta tienen un aumento del riesgo de desarrollar diabetes tipo 2 del 50%) [341]. Con lo cual, que los resultados para ambas patologías sean similares puede ser explicable por esta correlación.

Por otro lado, los posibles mecanismos de acción de los npEP seleccionados aún no están claros [146,275]. Aun así, se sospecha que estos mecanismos pueden incluir la alteración endocrina y / o metabólica, ya que los estudios en animales muestran que los niveles anormales de estrógenos endógenos o la exposición ambiental al estrógeno aumenta el riesgo de desarrollar diabetes tipo 2, hipertensión y dislipidemia [327]. En concreto, algunos posibles mecanismos de acción del BPA sobre el riesgo de síndrome metabólico podrían ser los siguientes: daños genéticos, efectos epigenéticos, alteración endocrina, estrés oxidativo, disfunción mitocondrial y señalización celular [146,189,275].

Sin embargo, el mecanismo más ampliamente explorado es el potencial de los disruptores endocrinos para actuar como obesógenos. La obesidad tiene componentes genéticos,

epigenéticos y ambientales, incluidos los contaminantes ambientales [146]. La literatura científica reciente señala que la exposición a BPA y parabenos puede tener un potencial obesogénico en humanos [255–258,265,276,277] y desempeñar un papel en el desarrollo de HTA [136,255–258,265,276,277,328], principalmente por su influencia en los preadipocitos [329,330]. En este sentido, un estudio de casos y controles en población de Estados Unidos reveló una asociación de BPA con obesidad general y abdominal [255]. Del mismo modo, un estudio transversal encontró importantes asociaciones de MP con el equilibrio energético y la salud metabólica [277]. Se ha sugerido que la exposición al BPA puede ser un promotor de los factores de riesgo de ECI como la obesidad al alterar los circuitos neuronales que regulan el comportamiento de alimentación o alteran la diferenciación de los adipocitos [259]. De esta forma, los estudios realizados en Estados Unidos encontraron una asociación positiva entre el BPA en orina y la obesidad en la población adulta general [259], así como en niños y adolescentes [136]. El estudio de Amin et al. mostró asociaciones significativas de exposición a BPA con obesidad y algunos factores de riesgo cardio-metabólicos en niños y adolescentes [125]. Además, la exposición a BPA se asoció con una disminución de la variabilidad de la frecuencia cardíaca (que se sabe que aumenta el riesgo de eventos cardíacos) y un aumento de la presión arterial [161], que son otros factores de riesgo de ECI.

Ciertos npEP también pueden alterar la señalización epigenética (p. ej., metilación del ADN, marcas de histonas, remodelación de la cromatina y ARN no codificantes) [275], lo que podría afectar el desarrollo normal de los tejidos y, finalmente, provocar obesidad y afecciones relacionadas [278]. Se sabe que los cambios epigenéticos están asociados con patologías relacionadas con enfermedades cardiovasculares como la hipertensión arterial [331,332] .

La disrupción endocrina [189] presenta otra posible vía de acción, ya que los DE han mostrado efectos agonistas y antagonistas sobre los receptores de estrógenos [333]. Dado que varios receptores de estrógenos se encuentran en el sistema cardiovascular y están involucrados en la vasodilatación, modulando los perfiles de lípidos de respuesta, algunos DE pueden estar afectando la presión arterial a través de sus acciones en los receptores de estrógenos [334,335]. También hay evidencia experimental e *in vivo* que respalda que la exposición a BPA y BP puede producir una sobreestimulación del receptor de estrógeno α (ERα) en las células β pancreáticas, lo que eventualmente conduce a la alteración de la

biosíntesis y a la secreción de insulina [107,313–315]. Otros estudios sugirieron que el BPA puede contribuir a la resistencia a la insulina al suprimir la adiponectina [316,317], una hormona involucrada en el mantenimiento de la sensibilidad a la insulina [318] y, por lo tanto, inducir el deterioro de la señalización de los receptores de insulina en el músculo esquelético y el hígado [319]. Sin embargo, los estudios experimentales en general han arrojado resultados contradictorios, muy probablemente debido a diferentes diseños experimentales, tiempos de exposición y factores de confusión no controlados o residuales, como la ruta de administración de estos contaminantes, el tiempo de degradación de npEP o dosis bajas de exposición. [132,137,146].

Otro mecanismo potencial es el estrés oxidativo, que se ha implicado en el envejecimiento y en muchos trastornos patológicos como las enfermedades isquémicas, las enfermedades neurodegenerativas, la diabetes y el cáncer, aunque los mecanismos subyacentes no se conocen completamente [279]. La evidencia creciente sugiere que el daño inducido por BPA está asociado con el estrés oxidativo [189,280,281,336] ya que el BPA puede alterar la homeostasis oxidativa a través de vías directas o indirectas, incluida la función mitocondrial [282] y la modulación de las enzimas antioxidantes de los ratones expuestos a lo largo de la ruta embrionaria / fetal [283], así como la lesión de células endoteliales relacionadas con el estrés oxidativo en estudios con animales [337,338]. En consecuencia, estudios previos en humanos [309] plantearon la hipótesis de que la exposición a los parabenos durante el embarazo podría causar disfunción de las células beta pancreáticas al inducir radicales libres de oxígeno y, por lo tanto, aumentar el riesgo de diabetes gestacional. En este sentido, un estudio transversal en una cohorte coreana de madre e hijo encontró asociaciones positivas de los niveles de EP en la orina materna (pero no MP o PP) con los niveles de malondialdehído en la orina [310], lo que refuerza la hipótesis antes mencionada de inducción de estrés oxidativo. El estrés oxidativo y los marcadores de inflamación que se encuentran en muchos trastornos cardiovasculares pueden desempeñar un papel importante en la patogenia de la hipertensión causada por la exposición al BPA [339].

La gran mayoría de los estudios que se centan en desentrañar los mecanismos de acción, se ocupan del BPA, por ello, debe considerarse que los mecanismos de acción mencionados anteriormente pueden no ser aplicables para todas los npEP seleccionados, ya que todavía hay poca investigación en este campo, particularmente para algunos de los

productos químicos (como parabenos y benzofenonas). De cualquier forma, entendemos que son precisos más estudios sobre mecanismos de acción, en conjunto con enfoques epidemiológicos, que deberían probar si los mecanismos mencionados anteriormente son suficientes para producir un efecto metabólico relevante en las poblaciones generales y en los niveles de exposición actuales.

Por otro lado, en los estudios incluidos en esta tesis se han analizado las concentraciones de npEP en muestras de suero, que pueden ser relativamente inestables, siendo más representativas de exposiciones recientes [197,241]. Algunos estudios señalan que, entre las matrices de biomonitorización, la orina contiene las concentraciones más altas de BPA, seguida por el suero [39], lo que con probabilidad implica una capacidad superior para detectar niveles bajos de exposición, siendo también un estimador más adecuado de la exposición a medio plazo.

Hay que tener en cuenta, además, que la literatura científica concerniente a este tipo de asociaciones es relativamente escasa y los estudios epidemiológicos hasta la fecha proporcionan evidencias altamente contradictorias sobre el potencial de alteración metabólica de estos npEP. Estos resultados, muy probablemente son debidos a los diferentes diseños de los estudios, los tiempos de exposición y los factores de confusión no controlados o residuales, como la ruta de administración de estos contaminantes, el tiempo de degradación de los contaminantes o las bajas dosis de exposición [132,137,146]. Por ello, pensamos que la inclusión de varios resultados metabólicos en estudios epidemiológicos adicionales mejorará considerablemente la solidez de los hallazgos.

Los resultados de esta tesis pueden presentar, en algunos casos, asociaciones débiles pero que creemos que pueden resultar relevantes debido a la ubicuidad de la exposición de estos contaminantes en millones de personas en el mundo [75–78].

# 9 Conclusiones

El análisis global de los resultados obtenidos en la presente tesis doctoral nos permite extraer las siguientes conclusiones:

1. La exposición de la población general a disruptores endocrinos no persistentes, tales como Bisfenol A, parabenos y benzofenonas es un fenómeno frecuente, que ocurre de forma inadvertida para la población no profesionalmente expuesta y que puede tener consecuencias nocivas para la salud. El conocimiento de los niveles de exposición y los factores asociados podría ser de ayuda para identificar a grupos de población particularmente expuestos y, de esta forma, contribuir a mejorar futuras campañas enfocadas a la prevención de la exposición. Casi el 70% de la población del estudio tenía niveles detectables de BPA. Se han evidenciado asociaciones entre el consumo de grasas y aceites añadidos con niveles séricos de BPA un 43% más bajos, mientras que el azúcar y los productos de confitería se asociaron con niveles un 25% más altos de BPA sérico. Estos resultados necesitan ser confirmados en futuras investigaciones. La caracterización histórica de la exposición a BPA obtenida en el presente estudio representa un primer paso hacia una evaluación de las implicaciones a largo plazo de la exposición a BPA en la cohorte EPIC.

2. No se observaron asociaciones significativas entre las concentraciones séricas de BPA y el riesgo de enfermedad coronaria isquémica, infarto agudo de miocardio o angina de pecho. Sin embargo, y debido al enfoque metodológico utilizado, es necesario un abordaje más profundo de la influencia de la exposición a BPA en el riesgo de enfermedad coronaria isquémica.

3. No se evidenciaron asociaciones entre las concentraciones séricas de contaminantes no persistentes en el reclutamiento y el riesgo de diabetes tipo 2, con la excepción de un incremento del riesgo en el quintil más alto de exposición al PP. Se observa una tendencia similar para el riesgo de hipertensión arterial, lo que puede explicarse también por la alta inter-relación de estas patologías componentes del Síndrome Metabólico. A pesar de las limitaciones metodológicas inherentes al diseño epidemiológico, y en vista de la plausibilidad biológica de las asociaciones encontradas, la alta prevalencia de la

exposición, así como la carga epidémica de estas patologías en la sociedad actual, consideramos que nuestros resultados son relevantes para la salud pública y requieren confirmación en futuros estudios científicos de carácter multidisciplinar.

# 10 Referencias bibliográficas


[1] Goldman L. Preventing pollution? U.S. toxic chemicals and pesticides policies and sustainable development. ELR News Anal 2002;32:11018–41.

[2] Toxic Chemicals released by industries - Worldometer n.d. https://www.worldometers.info/view/toxchem/ (accessed May 12, 2020).

[3] UN. UN report: Urgent action needed to tackle chemical pollution as global production is set to double by 2030 | UNEP - UN Environment Programme n.d. https://www.unenvironment.org/news-and-stories/press-release/un-report-urgent-action-needed-tackle-chemical-pollution-global (accessed May 12, 2020).

[4] Plastics Europe. Plastics–the facts: an analysis of European plastics production, demand and waste data. 2017.

[5] FAO. Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety. Rome: 2017.

[6] Geyer R, Jambeck JR, Law KL. Production, use, and fate of all plastics ever made. Sci Adv 2017;3:e1700782. doi:10.1126/sciadv.1700782.

[7] UNEP. Single-use plastics: A road map for Sustainability. 2018.

[8] WHO. Microplastics in drinking-water. 2019.

[9] Gallo F, Fossi C, Weber R, Santillo D, Sousa J, Ingram I, et al. Marine litter plastics and microplastics and their toxic chemicals components: the need for urgent preventive measures. Environ Sci Eur 2018;30:13. doi:10.1186/s12302-018-0139-z.

[10] Wagner M, Scherer C, Alvarez-Muñoz D, Brennholt N, Bourrain X, Buchinger S, et al. Microplastics in freshwater ecosystems: what we know and what we need to know. Environ Sci Eur 2014;26:1–9. doi:10.1186/s12302-014-0012-7.

[11] Olea N, Pazos P, Exposito J. Inadvertent exposure to xenoestrogens. Eur J Cancer Prev 1998;7 Suppl 1:S17-23.

[12] Maffini M V., Rubin BS, Sonnenschein C, Soto AM. Endocrine disruptors and reproductive health: The case of bisphenol-A. Mol Cell Endocrinol 2006;254–255:179–86. doi:10.1016/j.mce.2006.04.033.

[13] El-Shahawi MS, Hamza A, Bashammakh AS, Al-Saggaf WT. An overview on the accumulation, distribution, transformations, toxicity and analytical methods for the monitoring of persistent organic pollutants. Talanta 2010;80:1587–97. doi:10.1016/j.talanta.2009.09.055.

[14] WHO. State of the science of endocrine disrupting chemicals - 2012. World Health Organization; 2016.

[15] Carson R. Silent Spring. Houghton M. New York: 1962.

[16] Colborn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 1993;101:378–84. doi:10.1289/ehp.93101378.

[17] Miller WR, Sharpe RM. Environmental oestrogens and human reproductive cancers. Endocr Relat Cancer 1998;5:69–96. doi:10.1677/erc.0.0050069.

[18] Comisión Europea. Hacia un marco de la Unión Europea más exhaustivo en materia de alteradores endocrinos COM(2018). Bruselas: 2018.

[19] Comisión de las Comunidades Europeas. Aplicación de la estrategia comunitaria en materia de alteradores endocrinos - Sustancias de las que se sospecha interfieren en los sistemas hormonales de seres humanos y animales- COM. Bruselas: 2001.

[20] Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Lee DH, et al. Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses. Endocr Rev 2012;33:378–455. doi:10.1210/er.2011-1050.

[21] Buka I, Osornio-Vargas A, Walker R. Canada declares bisphenol A a “dangerous substance”: Questioning the safety of plastics. Paediatr Child Health (Oxford) 2009;14:11–3. doi:10.1093/pch/14.1.11a.

[22] Bisphenol A (BPA) - Canada.ca n.d. https://www.canada.ca/en/health-canada/services/home-garden-safety/bisphenol-bpa.html (accessed September 30, 2020).
[23] Canada Consumer Product Safety Act n.d. https://laws-lois.justice.gc.ca/eng/acts/C-1.68/index.html (accessed September 30, 2020).
[24] LOI n° 2012-1442 du 24 décembre 2012 visant à la suspension de la fabrication, de l'importation, de l'exportation et de la mise sur le marché de tout conditionnement à vocation alimentaire contenant du bisphénol A (1) - Légifrance n.d. https://www.legifrance.gouv.fr/loda/id/JORFTEXT000026830015/2020-09-29/ (accessed September 29, 2020).
[25] Scientific topic: Bisphenol A | European Food Safety Authority n.d. https://www.efsa.europa.eu/en/topics/topic/bisphenol (accessed September 29, 2020).
[26] REACH: Reglamento de registro, evaluación, autorización y restricción de sustancias químicas - Salud y seguridad en el trabajo - EU-OSHA n.d. https://osha.europa.eu/es/themes/dangerous-substances/reach (accessed September 29, 2020).
[27] UE. REGLAMENTO (UE) No 10/2011 DE LA COMISIÓN de 14 de enero de 2011 sobre materiales y objetos plásticos destinados a entrar en contacto con alimentos. Bruselas: 2011.
[28] UE. DIRECTIVA 2011/8/UE DE LA COMISIÓN. n.d.
[29] UE. REGLAMENTO (UE) 2018/ 213 DE LA COMISIÓN - de 12 de febrero de 2018 - sobre el uso de bisfenol A en los barnices y revestimientos destinados a entrar en contacto con los alimentos y por el que se modifica el Reglamento (UE) n.o 10/ 2011 por lo que respect. n.d.
[30] Unión europea. Parabenos en cosméticos, Green facts n.d. https://ec.europa.eu/health/scientific_committees/docs/citizens_parabens_es.pdf (accessed September 30, 2020).
[31] UE. REGLAMENTO (UE) No 1004/2014 DE LA COMISIÓN - de 18 de septiembre de 2014 - por el que se modifica el anexo V del Reglamento (CE) no 1223/2009 del Parlamento Europeo y del Consejo, sobre los productos cosméticos -. n.d.
[32] Louro H, Heinälä M, Bessems J, Buekers J, Vermeire T, Woutersen M, et al. Human biomonitoring in health risk assessment in Europe: Current practices and recommendations for the future. Int J Hyg Environ Health 2019;222:727–37. doi:10.1016/j.ijheh.2019.05.009.
[33] Yamada H, Furuta I, Kato EH, Kataoka S, Usuki Y, Kobashi G, et al. Maternal serum and amniotic fluid bisphenol A concentrations in the early second trimester. Reprod Toxicol 2002;16:735–9. doi:10.1016/S0890-6238(02)00051-5.
[34] Hiroi H, Tsutsumi O, Takeuchi T, Momoeda M, Ikezuki Y, Okamura A, et al. Differences in serum bisphenol a concentrations in premenopausal normal women and women with endometrial hyperplasia. Endocr J 2004;51:595–600. doi:10.1507/endocrj.51.595.
[35] Padmanabhan V, Siefert K, Ransom S, Johnson T, Pinkerton J, Anderson L, et al. Maternal bisphenol-A levels at delivery: a looming problem? J Perinatol 2008;28:258–63. doi:10.1038/sj.jp.7211913.
[36] Cobellis L, Colacurci N, Trabucco E, Carpentiero C, Grumetto L. Measurement of bisphenol A and bisphenol B levels in human blood sera from healthy and endometriotic women. Biomed Chromatogr 2009;23:1186–90. doi:10.1002/bmc.1241.
[37] Zhuang W, Wu K, Wang Y, Zhu H, Deng Z, Peng L, et al. Association of Serum Bisphenol-A Concentration and Male Reproductive Function Among Exposed Workers. Arch Environ Contam Toxicol 2015;68:38–45. doi:10.1007/s00244-014-0078-7.
[38] Ye Y, Zhou Q, Feng L, Wu J, Xiong Y, Li X. Maternal serum bisphenol A levels and risk of pre-eclampsia: a nested case–control study. Eur J Public Health 2017;27:1102–7. doi:10.1093/eurpub/ckx148.
[39] Lee JJJ, Choi K, Park J, Moon H-B, Choi G, Lee JJJ, et al. Bisphenol A distribution in serum, urine, placenta, breast milk, and umbilical cord serum in a birth panel of mother–neonate pairs. Sci Total Environ 2018;626:1494–501.

doi:10.1016/j.scitotenv.2017.10.042.
[40] Song S, Duan Y, Zhang T, Zhang B, Zhao Z, Bai X, et al. Serum concentrations of bisphenol A and its alternatives in elderly population living around e-waste recycling facilities in China: Associations with fasting blood glucose. Ecotoxicol Environ Saf 2019;169:822–8. doi:10.1016/j.ecoenv.2018.11.101.
[41] Teeguarden JG, Twaddle NC, Churchwell MI, Doerge DR. Urine and serum biomonitoring of exposure to environmental estrogens I: Bisphenol A in pregnant women. Food Chem Toxicol 2016;92:129–42. doi:10.1016/j.fct.2016.03.023.
[42] Cutanda F, Koch HM, Esteban M, Sánchez J, Angerer J, Castaño A. Urinary levels of eight phthalate metabolites and bisphenol A in mother–child pairs from two Spanish locations. Int J Hyg Environ Health 2015;218:47–57. doi:10.1016/j.ijheh.2014.07.005.
[43] Honda M, Robinson M, Kannan K. Parabens in human urine from several Asian countries, Greece, and the United States. Chemosphere 2018;201:13–9. doi:10.1016/j.chemosphere.2018.02.165.
[44] Karzi V, Tzatzarakis MN, Vakonaki E, Alegakis T, Katsikantami I, Sifakis S, et al. Biomonitoring of bisphenol A, triclosan and perfluorooctanoic acid in hair samples of children and adults. J Appl Toxicol 2018;38:1144–52. doi:10.1002/jat.3627.
[45] Martín J, Santos JL, Aparicio I, Alonso E. Exposure assessment to parabens, bisphenol A and perfluoroalkyl compounds in children, women and men by hair analysis. Sci Total Environ 2019;695:133864. doi:10.1016/j.scitotenv.2019.133864.
[46] Liu J, Li J, Wu Y, Zhao Y, Luo F, Li S, et al. Bisphenol A Metabolites and Bisphenol S in Paired Maternal and Cord Serum. Environ Sci Technol 2017;51:2456–63. doi:10.1021/acs.est.6b05718.
[47] Schlumpf M, Kypke K, Vökt CC, Birchler M, Durrer S, Faass O, et al. Endocrine Active UV Filters: Developmental Toxicity and Exposure Through Breast Milk. Chim Int J Chem 2008;62:345–51. doi:10.2533/chimia.2008.345.
[48] Park N-Y, Cho YH, Choi K, Lee E-H, Kim YJ, Kim JH, et al. Parabens in breast milk and possible sources of exposure among lactating women in Korea. Environ Pollut 2019;255:113142. doi:10.1016/j.envpol.2019.113142.
[49] Strakovsky RS, Schantz SL. Impacts of bisphenol A (BPA) and phthalate exposures on epigenetic outcomes in the human placenta. Environ Epigenetics 2018;4:dvy022. doi:10.1093/eep/dvy022.
[50] Vela-Soria F, Jiménez-Díaz I, Rodríguez-Gómez R, Zafra-Gómez A, Ballesteros O, Navalón A, et al. Determination of benzophenones in human placental tissue samples by liquid chromatography-tandem mass spectrometry. Talanta 2011;85:1848–55. doi:10.1016/j.talanta.2011.07.030.
[51] Artacho-Cordón F, Fernández MFF, Frederiksen H, Iribarne-Durán LMM, Jiménez-Díaz I, Vela-Soria F, et al. Environmental phenols and parabens in adipose tissue from hospitalized adults in Southern Spain. Environ Int 2018;119:203–11. doi:10.1016/j.envint.2018.05.052.
[52] Jiménez-Díaz I, Iribarne-Durán LM, Ocón O, Salamanca E, Fernández MF, Olea N, et al. Determination of personal care products -benzophenones and parabens- in human menstrual blood. J Chromatogr B Anal Technol Biomed Life Sci 2016;1035:57–66. doi:10.1016/j.jchromb.2016.09.035.
[53] EEA. Environment and human health — European Environment Agency 2013. https://www.eea.europa.eu/publications/environment-and-human-health (accessed May 12, 2020).
[54] CDC. Fourth National Report on Human Exposure to Environmental Chemicals. 2013.
[55] Ibarluzea J, Aurrekoetxea JJ, Porta M, Sunyer J, Ballester F. La biomonitorización de sustancias tóxicas en muestras biológicas de población general. Gac Sanit 2016;30:45–54. doi:10.1016/j.gaceta.2016.02.012.
[56] HBM4EU - science and policy for a healthy future n.d. https://www.hbm4eu.eu/ (accessed September 30, 2020).
[57] DEMOCOPHES — COPHES n.d. http://www.eu-hbm.info/democophes/ (accessed September 30, 2020).

[58] Pérez-Gómez B, Pastor-Barriuso R, Cervantes-Amat M, Esteban M, Ruiz-Moraga M, Aragonés N, et al. BIOAMBIENT.ES study protocol: Rationale and design of a cross-sectional human biomonitoring survey in Spain. Environ Sci Pollut Res 2013;20:1193–202. doi:10.1007/s11356-012-1320-3.
[59] German Environmental Survey for Children 2003/06 - GerES IV - | Umweltbundesamt n.d. https://www.umweltbundesamt.de/en/publikationen/german-environmental-survey-for-children-200306 (accessed September 30, 2020).
[60] Proyecto INMA n.d. https://www.proyectoinma.org/ (accessed September 30, 2020).
[61] NHANES - National Health and Nutrition Examination Survey Homepage n.d. https://www.cdc.gov/nchs/nhanes/index.htm (accessed October 1, 2020).
[62] N. Olea M.F. Fernández P. Araque F. Olea-Serrano. Perspectivas en disrupción endocrina. Gac Sanit 2002;16.
[63] Information on OECD Work Related to Endocrine Disrupters. 2012.
[64] Gelbke HP, Kayser M, Poole A. OECD test strategies and methods for endocrine disruptors. Toxicology, vol. 205, Elsevier; 2004, p. 17–25. doi:10.1016/j.tox.2004.06.034.
[65] Rüegg J, Penttinen-Damdimopoulou P, Mäkelä S, Pongratz I, Gustafsson JA. Receptors mediating toxicity and their involvement in endocrine disruption. EXS 2009;99:289–323. doi:10.1007/978-3-7643-8336-7_11.
[66] Schug TT, Abagyan R, Blumberg B, Collins TJ, Crews D, DeFur PL, et al. Designing Endocrine Disruption Out of the Next Generation of Chemicals. Green Chem 2013;15:181–98. doi:10.1039/C2GC35055F.
[67] Anway MD, Cupp AS, Uzumcu N, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science (80- ) 2005;308:1466–9. doi:10.1126/science.1108190.
[68] Vilahur N, Bustamante M, Byun HM, Fernandez MF, Santa Marina L, Basterrechea M, et al. Prenatal exposure to mixtures of xenoestrogens and repetitive element DNA methylation changes in human placenta. Environ Int 2014;71:81–7. doi:10.1016/j.envint.2014.06.006.
[69] Jansen MS, Nagel SC, Miranda PJ, Lobenhofer EK, Afshari CA, McDonnell DP. Short-chain fatty acids enhance nuclear receptor activity through mitogen-activated protein kinase activation and histone deacetylase inhibition. Proc Natl Acad Sci U S A 2004;101:7199–204. doi:10.1073/pnas.0402014101.
[70] Frederiksen H, Skakkebæk NE, Andersson AM. Metabolism of phthalates in humans. Mol Nutr Food Res 2007;51:899–911. doi:10.1002/mnfr.200600243.
[71] Søeborg T, Frederiksen H, Andersson AM. Considerations for estimating daily intake values of nonpersistent environmental endocrine disruptors based on urinary biomonitoring data. Reproduction 2014;147:455–63. doi:10.1530/REP-13-0458.
[72] Mcgroup. World BPA Production Grew by Over 372,000 Tonnes in 2012 2013:1–3. http://mcgroup.co.uk/news/20131108/bpa-production-grew-372000-tonnes.html.
[73] Geens T, Aerts D, Berthot C, Bourguignon J-P, Goeyens L, Lecomte P, et al. A review of dietary and non-dietary exposure to bisphenol-A. Food Chem Toxicol 2012;50:3725–40. doi:10.1016/j.fct.2012.07.059.
[74] vom Saal FS, Hughes C. An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. Environ Health Perspect 2005;113:926–33. doi:10.1289/ehp.7713.
[75] Vandenberg LN, Maffini M V., Sonnenschein C, Rubin BS, Soto AM. Bisphenol-a and the great divide: A review of controversies in the field of endocrine disruption. Endocr Rev 2009;30:75–95. doi:10.1210/er.2008-0021.
[76] Hormann AM, Vom Saal FS, Nagel SC, Stahlhut RW, Moyer CL, Ellersieck MR, et al. Holding thermal receipt paper and eating food after using hand sanitizer results in high serum bioactive and urine total levels of bisphenol A (BPA). PLoS One 2014;9:e110509. doi:10.1371/journal.pone.0110509.
[77] Liao C, Kannan K. Determination of free and conjugated forms of bisphenol A in human urine and serum by liquid chromatography-tandem mass spectrometry. Environ Sci

Technol 2012;46:5003–9. doi:10.1021/es300115a.
[78] Vandenberg LN, Chahoud I, Heindel JJ, Padmanabhan V, Paumgartten FJR, Schoenfelder G. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ Health Perspect 2010;118:1055–70. doi:10.1289/ehp.0901716.
[79] Wang W, Abualnaja KO, Asimakopoulos AG, Covaci A, Gevao B, Johnson-Restrepo B, et al. A comparative assessment of human exposure to tetrabromobisphenol A and eight bisphenols including bisphenol A via indoor dust ingestion in twelve countries. Environ Int 2015;83:183–91. doi:10.1016/J.ENVINT.2015.06.015.
[80] Zhang T, Xue J, Gao C, Qiu R, Li Y, Li X, et al. Urinary Concentrations of Bisphenols and Their Association with Biomarkers of Oxidative Stress in People Living Near E-Waste Recycling Facilities in China. Environ Sci Technol 2016;50:4045–53. doi:10.1021/acs.est.6b00032.
[81] Mandrup K, Boberg J, Isling LK, Christiansen S, Hass U. Low-dose effects of bisphenol A on mammary gland development in rats. Andrology 2016;4:673–83. doi:10.1111/andr.12193.
[82] Castro B, Sánchez P, Torres JM, Preda O, del Moral RG, Ortega E. Bisphenol A Exposure during Adulthood Alters Expression of Aromatase and 5α-Reductase Isozymes in Rat Prostate. PLoS One 2013;8:e55905. doi:10.1371/journal.pone.0055905.
[83] Wu JH, Jiang XR, Liu GM, Liu XY, He GL, Sun ZY. Oral exposure to low-dose bisphenol A aggravates testosterone-induced benign hyperplasia prostate in rats. Toxicol Ind Health 2011;27:810–9. doi:10.1177/0748233711399310.
[84] Betancourt AM, Wang J, Jenkins S, Mobley J, Russo J, Lamartiniere CA. Altered carcinogenesis and proteome in mammary glands of rats after prepubertal exposures to the hormonally active chemicals bisphenol a and genistein. J Nutr 2012;142:1382S-8S. doi:10.3945/jn.111.152058.
[85] Acevedo N, Davis B, Schaeberle CM, Sonnenschein C, Soto AM. Perinatally administered bisphenol A as a potential mammary gland carcinogen in rats. Environ Health Perspect 2013;121:1040–6. doi:10.1289/ehp.1306734.
[86] Rochester JR. Bisphenol A and human health: A review of the literature. Reprod Toxicol 2013;42:132–55. doi:10.1016/j.reprotox.2013.08.008.
[87] Hahladakis JN, Velis CA, Weber R, Iacovidou E, Purnell P. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. J Hazard Mater 2018;344:179–99. doi:10.1016/j.jhazmat.2017.10.014.
[88] López-Cervantes J, Paseiro-Losada P. Determination of bisphenol A in, and its migration from, PVC stretch film used for food packaging. Food Addit Contam 2003;20:596–606. doi:10.1080/0265203031000109495.
[89] Grumetto L, Montesano D, Seccia S, Albrizio S, Barbato F. Determination of Bisphenol A and Bisphenol B Residues in Canned Peeled Tomatoes by Reversed-Phase Liquid Chromatography. J Agric Food Chem 2008;56:10633–7. doi:10.1021/jf802297z.
[90] Ćwiek-Ludwicka K. Bisphenol A (BPA) in food contact materials - new scientific opinion from EFSA regarding public health risk. Rocz Państwowego Zakładu Hig 2015;66:299–307.
[91] Huang R-P, Liu Z-H, Yuan S-F, Yin H, Dang Z, Wu P-X. Worldwide human daily intakes of bisphenol A (BPA) estimated from global urinary concentration data (2000-2016) and its risk analysis. Environ Pollut 2017;230:143–52. doi:10.1016/j.envpol.2017.06.026.
[92] General Court of the European Union. Confirmation of the inclusion of Bisphenol A as a substance of very high concern on account of its properties as a substance toxic for reproduction. Luxembourg, 11 July 2019: 2019.
[93] Ma Y, Liu H, Wu J, Yuan L, Wang Y, Du X, et al. The adverse health effects of bisphenol A and related toxicity mechanisms. Environ Res 2019;176:108575. doi:10.1016/j.envres.2019.108575.
[94] Cosmetic Ingredient Review. Final amended report on the safety assessment of

Methylparaben, Ethylparaben, Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben, and Benzylparaben as used in cosmetic products. Int J Toxicol 2008;27 Suppl 4:1–82. doi:10.1080/10915810802548359.
[95] Soni MG, Carabin IG, Burdock GA. Safety assessment of esters of p-hydroxybenzoic acid (parabens). Food Chem Toxicol 2005;43:985–1015. doi:10.1016/j.fct.2005.01.020.
[96] Oishi S. Effects of propyl paraben on the male reproductive system. Food Chem Toxicol 2002;40:1807–13. doi:10.1016/S0278-6915(02)00204-1.
[97] Charles AK, Darbre PD. Combinations of parabens at concentrations measured in human breast tissue can increase proliferation of MCF-7 human breast cancer cells. J Appl Toxicol 2013;33:390–8. doi:10.1002/jat.2850.
[98] Darbre PD, Harvey PW. Paraben esters: Review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks. J Appl Toxicol 2008;28:561–78. doi:10.1002/jat.1358.
[99] Darbre PD, Aljarrah A, Miller WR, Coldham NG, Sauer MJ, Pope GS. Concentrations of Parabens in human breast tumours. J Appl Toxicol 2004;24:5–13. doi:10.1002/jat.958.
[100] Careghini A, Filippo Mastorgio A, Saponaro S, Sezenna E. Bisphenol A, nonylphenols, benzophenones, and benzotriazoles in soils, groundwater, surface water, sediments, and food: a review n.d. doi:10.1007/s11356-014-3974-5.
[101] Louis GMB, Kannan K, Sapra KJ, Maisog J, Sundaram R. Urinary concentrations of benzophenone-type ultraviolet radiation filters and couples' fecundity. Am J Epidemiol 2014;180:1168–75. doi:10.1093/aje/kwu285.
[102] León Z, Chisvert A, Tarazona I, Salvador A. Solid-phase extraction liquid chromatography-tandem mass spectrometry analytical method for the determination of 2-hydroxy-4-methoxybenzophenone and its metabolites in both human urine and semen. Anal Bioanal Chem 2010;398:831–43. doi:10.1007/s00216-010-3947-6.
[103] IARC. Benzophenone. IARC Monographs 101. 2010.
[104] Toxicology and carcinogenesis studies of benzophenone (CAS No. 119-61-9) in F344/N rats and B6C3F1 mice (feed studies). Natl Toxicol Program Tech Rep Ser 2006:1–264.
[105] Chen TH, Wu YT, Ding WH. UV-filter benzophenone-3 inhibits agonistic behavior in male Siamese fighting fish (Betta splendens). Ecotoxicology 2016;25:302–9. doi:10.1007/s10646-015-1588-4.
[106] Kinnberg KL, Petersen GI, Albrektsen M, Minghlani M, Awad SM, Holbech BF, et al. Endocrine-disrupting effect of the ultraviolet filter benzophenone-3 in zebrafish, Danio rerio. Environ Toxicol Chem 2015;34:2833–40. doi:10.1002/etc.3129.
[107] Schlumpf M, Cotton B, Conscience M, Haller V, Steinmann B, Lichtensteiger W. In vitro and in vivo estrogenicity of UV screens. Environ Health Perspect 2001;109:239–44. doi:10.1289/ehp.109-a359.
[108] Mykkänen H. TJ. PT. HO. Fecal Bacterial Enzyme Activities in Infants Increase with A... : Journal of Pediatric Gastroenterology and Nutrition. J Pediatr Gastroenterol Nutr 1997;25:312–6.
[109] Inoue H, Yuki G, Yokota H, Kato S. Bisphenol A glucuronidation and absorption in rat intestine. Drug Metab Dispos 2003;31:140–4. doi:10.1124/dmd.31.1.140.
[110] Völkel W V., Bittner N, Dekant W. Quantitation of bisphenol A and bisphenol A glucuronide in biological samples by high performance liquid chromatography-tandem mass spectrometry. Drug Metab Dispos 2005;33:1748–57. doi:10.1124/dmd.105.005454.
[111] Kim YH, Kim CS, Park S, Han SY, Pyo MY, Yang M. Gender differences in the levels of bisphenol A metabolites in urine. Biochem Biophys Res Commun 2003;312:441–8. doi:10.1016/j.bbrc.2003.10.135.
[112] Calafat AM, Kuklenyik Z, Reidy JA, Caudill SP, Ekong J, Needham LL. Urinary concentrations of bisphenol A and 4-Nonylphenol in a human reference population. Environ Health Perspect 2005;113:391–5. doi:10.1289/ehp.7534.
[113] Völkel W, Colnot T, Csanády GA, Filser JG, Dekant W. Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. Chem Res Toxicol

2002;15:1281–7. doi:10.1021/tx025548t.
[114] Ye X, Bishop AM, Reidy JA, Needham LL, Calafat AM. Parabens as Urinary Biomarkers of Exposure in Humans.pdf. Environ Health Perspect 2006;114:1843–6.
[115] Shin MY, Shin C, Choi JW, Lee J, Lee S, Kim S. Pharmacokinetic profile of propyl paraben in humans after oral administration. Environ Int 2019;130. doi:10.1016/j.envint.2019.104917.
[116] Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global Burden of Disease and Risk Factors. The International Bank for Reconstruction and Development / The World Bank; 2006.
[117] Branca F.Nikogosian H. LT. The challenge of obesity in the WHO European Region and the strategies for response. 2007.
[118] Ruzzin J, Lee D-H, Carpenter DO, Jacobs DR. Reconsidering metabolic diseases: The impacts of persistent organic pollutants. Atherosclerosis 2012;224:1–3. doi:10.1016/j.atherosclerosis.2012.02.039.
[119] DeFronzo RA. Insulin resistance: A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidaemia and atherosclerosis. Neth J Med 1997;50:191–7. doi:10.1016/S0300-2977(97)00012-0.
[120] Goncharov A, Haase RF, Santiago-Rivera A, Morse G, McCaffrey RJ, Rej R, et al. High serum PCBs are associated with elevation of serum lipids and cardiovascular disease in a Native American population. Environ Res 2008;106:226–39. doi:10.1016/j.envres.2007.10.006.
[121] BMJ Best Practice. Type 2 diabetes in adults - Aetiology n.d. https://bestpractice.bmj.com/topics/en-gb/24/aetiology (accessed May 12, 2020).
[122] Kahn SE, Cooper ME, Del Prato S. Pathophysiology and treatment of type 2 diabetes: Perspectives on the past, present, and future. Lancet 2014;383:1068–83. doi:10.1016/S0140-6736(13)62154-6.
[123] Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract 2017;128:40–50. doi:10.1016/j.diabres.2017.03.024.
[124] Duan Y, Yao Y, Wang B, Han L, Wang L, Sun H, et al. Association of urinary concentrations of bisphenols with type 2 diabetes mellitus: A case-control study. Environ Pollut 2018;243:1719–26. doi:10.1016/j.envpol.2018.09.093.
[125] Amin MM, Ebrahim K, Hashemi M, Shoshtari-Yeganeh B, Rafiei N, Mansourian M, et al. Association of exposure to Bisphenol A with obesity and cardiometabolic risk factors in children and adolescents. Int J Environ Health Res 2019;29:94–106. doi:10.1080/09603123.2018.1515896.
[126] Silver MK, O'Neill MS, Sowers MR, Park SK. Urinary Bisphenol A and Type-2 Diabetes in U.S. Adults: Data from NHANES 2003-2008. PLoS One 2011;6:e26868. doi:10.1371/journal.pone.0026868.
[127] Yang C, Kong APS, Cai Z, Chung ACK. Persistent Organic Pollutants as Risk Factors for Obesity and Diabetes. Curr Diab Rep 2017;17:132. doi:10.1007/s11892-017-0966-0.
[128] Gong H, Zhang X, Cheng B, Sun Y, Li C, Li T, et al. Bisphenol A Accelerates Toxic Amyloid Formation of Human Islet Amyloid Polypeptide: A Possible Link between Bisphenol A Exposure and Type 2 Diabetes. PLoS One 2013;8:e54198. doi:10.1371/journal.pone.0054198.
[129] Ruzzin J, Petersen R, Meugnier E, Madsen L, Lock E-J, Lillefosse H, et al. Persistent Organic Pollutant Exposure Leads to Insulin Resistance Syndrome. Environ Health Perspect 2010;118:465–71. doi:10.1289/ehp.0901321.
[130] Grice BA, Nelson RG, Williams DE, Knowler WC, Mason C, Hanson RL, et al. Associations between persistent organic pollutants, type 2 diabetes, diabetic nephropathy and mortality. Occup Environ Med 2017;74:521–7. doi:10.1136/oemed-2016-103948.
[131] Ngwa EN, Kengne A-P, Tiedeu-Atogho B, Mofo-Mato E-P, Sobngwi E. Persistent organic pollutants as risk factors for type 2 diabetes. Diabetol Metab Syndr 2015;7:41. doi:10.1186/s13098-015-0031-6.
[132] Provvisiero D, Pivonello C, Muscogiuri G, Negri M, de Angelis C, Simeoli C, et al.

Influence of Bisphenol A on Type 2 Diabetes Mellitus. Int J Environ Res Public Health 2016;13:989. doi:10.3390/ijerph13100989.
[133] Hwang S, Lim J, Choi Y, Jee SH. Bisphenol A exposure and type 2 diabetes mellitus risk: a meta-analysis. BMC Endocr Disord 2018;18:81. doi:10.1186/s12902-018-0310-y.
[134] Ahmadkhaniha R, Mansouri M, Yunesian M, Omidfar K, Jeddi M, Larijani B, et al. Association of urinary bisphenol a concentration with type-2 diabetes mellitus. J Environ Heal Sci Eng 2014;12:64. doi:10.1186/2052-336X-12-64.
[135] Grün F, Blumberg B. Environmental Obesogens: Organotins and Endocrine Disruption via Nuclear Receptor Signaling. Endocrinology 2006;147:s50–5. doi:10.1210/en.2005-1129.
[136] Trasande L, Attina TM, Blustein J. Association between urinary bisphenol A concentration and obesity prevalence in children and adolescents. JAMA - J Am Med Assoc 2012;308:1113–21. doi:10.1001/2012.jama.11461.
[137] Mirmira P, Evans-Molina C. Bisphenol A, obesity, and type 2 diabetes mellitus: genuine concern or unnecessary preoccupation? Transl Res 2014;164:13–21. doi:10.1016/j.trsl.2014.03.003.
[138] Lind PM, Lind L. Endocrine-disrupting chemicals and risk of diabetes: an evidence-based review. Diabetologia 2018;61:1495–502. doi:10.1007/s00125-018-4621-3.
[139] Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. J Am Coll Cardiol 2017;70:1–25. doi:10.1016/j.jacc.2017.04.052.
[140] GBD 2017 Causes of Death Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2018;392:1736–88. doi:10.1016/S0140-6736(18)32203-7.
[141] Alwan A, Armstrong T, Bettcher D, Branca F, Chisholm D, Ezzati M, et al. Global status report on noncommunicable diseases 2010. 2010.
[142] ECIS European Cancer Information System n.d. https://ecis.jrc.ec.europa.eu/ (accessed November 22, 2018).
[143] Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, et al. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. Eur J Cancer 2013;49:1374–403. doi:10.1016/j.ejca.2012.12.027.
[144] INE. Causas de muerte en España. 2018 n.d.
[145] Coronary Artery Disease | cdc.gov n.d. https://www.cdc.gov/heartdisease/coronary_ad.htm (accessed May 12, 2020).
[146] Heindel JJ, Blumberg B. Environmental Obesogens: Mechanisms and Controversies. Annu Rev Pharmacol Toxicol 2019;59:89–106. doi:10.1146/annurev-pharmtox-010818-021304.
[147] Chobanian A V., Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. JAMA 2003;289:2560. doi:10.1001/jama.289.19.2560.
[148] WHO. A global brief on hypertension. 2013.
[149] Mills KT, Bundy JD, Kelly TN, Reed JE, Kearney PM, Reynolds K, et al. Global Disparities of Hypertension Prevalence and Control: A Systematic Analysis of Population-Based Studies From 90 Countries. Circulation 2016;134:441–50. doi:10.1161/CIRCULATIONAHA.115.018912.
[150] WHO. Hypertension n.d. https://www.who.int/news-room/fact-sheets/detail/hypertension (accessed September 30, 2020).
[151] Streeten DH, Anderson GH, Howland T, Chiang R, Smulyan H. Effects of thyroid function on blood pressure. Recognition of hypothyroid hypertension. Hypertension 1988;11:78–83. doi:10.1161/01.HYP.11.1.78.
[152] Juhaeri, Stevens J, Chambless L, Tyroler H, Rosamond W, Nieto F, et al. Associations between weight gain and incident hypertension in a bi-ethnic cohort: the Atherosclerosis Risk in Communities Study. Int J Obes 2002;26:58–64. doi:10.1038/sj.ijo.0801846.

[153] Sowers JR. Insulin resistance and hypertension. Am J Physiol Circ Physiol 2004;286:H1597–602. doi:10.1152/ajpheart.00026.2004.
[154] Laaksonen DE, Niskanen L, Nyyssonen K, Lakka TA, Laukkanen JA, Salonen JT. Dyslipidaemia as a predictor of hypertension in middle-aged men. Eur Heart J 2008;29:2561–8. doi:10.1093/eurheartj/ehn061.
[155] Rodrigo R, Prat H, Passalacqua W, Araya J, Guichard C, Bächler JP. Relationship between Oxidative Stress and Essential Hypertension. Hypertens Res 2007;30:1159–67. doi:10.1291/hypres.30.1159.
[156] Lakoski SG, Herrington DM, Siscovick DM, Hulley SB. C-Reactive Protein Concentration and Incident Hypertension in Young Adults. Arch Intern Med 2006;166:345. doi:10.1001/archinte.166.3.345.
[157] Shankar A, Teppala S, Sabanayagam C. Bisphenol A and peripheral arterial disease: Results from the NHANES. Environ Health Perspect 2012;120:1297–300. doi:10.1289/ehp.1104114.
[158] Shankar A, Teppala S. Urinary bisphenol A and hypertension in a multiethnic sample of US adults. J Environ Public Health 2012;2012:481641. doi:10.1155/2012/481641.
[159] Bae S, Hong Y-C. Exposure to Bisphenol A From Drinking Canned Beverages Increases Blood Pressure. Hypertension 2015;65:313–9. doi:10.1161/HYPERTENSIONAHA.114.04261.
[160] Gao X, Wang H-S. Impact of Bisphenol A on the Cardiovascular System — Epidemiological and Experimental Evidence and Molecular Mechanisms. Int J Environ Res Public Health 2014;11:8399–413. doi:10.3390/ijerph110808399.
[161] Bae S, Kim JH, Lim Y-HH, Park HY, Hong Y-CC. Associations of bisphenol a exposure with heart rate variability and blood pressure. Hypertension 2012;60:786–93. doi:10.1161/HYPERTENSIONAHA.112.197715.
[162] Shiue I. Higher Urinary Heavy Metal, Phthalate, and Arsenic but Not Parabens Concentrations in People with High Blood Pressure, U.S. NHANES, 2011–2012. Int J Environ Res Public Health 2014;11:5989–99. doi:10.3390/ijerph110605989.
[163] Shiue I. Higher urinary heavy metal, arsenic, and phthalate concentrations in people with high blood pressure: US NHANES, 2009–2010. Blood Press 2014;23:363–9. doi:10.3109/08037051.2014.925228.
[164] EPIC study n.d. http://epic.iarc.fr/.
[165] González CA, Navarro C, Martínez C, Quirós JR, Dorronsoro M, Barricarte A, et al. El estudio prospectivo Europeo sobre cáncer y nutrición (EPIC). Rev Esp Salud Publica 2004;78:167–76.
[166] Gonzalez C, Navarro C, Martinez C, Quiros J, Dorronsoro M, Barricarte A, et al. El estudio prospectivo Europeo sobre cancer y nutricion (EPIC). Rev Esp Salud Publica 2004;78:167–76.
[167] Slimani N, Kaaks R, Ferrari P, Casagrande C, Clavel-Chapelon F, Lotze G, et al. European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study: rationale, design and population characteristics. Public Health Nutr 2002;5:1125. doi:10.1079/PHN2002395.
[168] Langenberg C, Sharp S, Forouhi NG, Franks PW, Schulze MB, Kerrison N, et al. Design and cohort description of the InterAct Project: An examination of the interaction of genetic and lifestyle factors on the incidence of type 2 diabetes in the EPIC Study. Diabetologia 2011;54:2272–82. doi:10.1007/s00125-011-2182-9.
[169] Langenberg C, Sharp SJ, Schulze MB, Rolandsson O, Overvad K, Forouhi NG, et al. Long-term risk of incident type 2 diabetes and measures of overall and regional obesity: The epic-interact case-cohort study. PLoS Med 2012;9:17. doi:10.1371/journal.pmed.1001230.
[170] Sluik D, Boeing H, Bergmann MM, Schaütze M, Teucher B, Kaaks R, et al. Alcohol consumption and mortality in individuals with diabetes mellitus. Br J Nutr 2012;108:1307–15. doi:10.1017/S0007114511006532.
[171] Ekelund U, Palla L, Brage S, Franks PW, Peters T, Balkau B, et al. Physical activity reduces the risk of incident type 2 diabetes in general and in abdominally lean and obese

men and women: The EPIC-InterAct study. Diabetologia 2012;55:1944–52. doi:10.1007/s00125-012-2532-2.
[172] Danesh J, Saracci R, Berglund G, Feskens E, Overvad K, Panico S, et al. EPIC-Heart: The cardiovascular component of a prospective study of nutritional, lifestyle and biological factors in 520,000 middle-aged participants from 10 European countries. Eur J Epidemiol 2007;22:129–41. doi:10.1007/s10654-006-9096-8.
[173] EPIC group of Spain. Relative validity and reproducibility of a diet history questionnaire in Spain. I. Foods. EPIC Group of Spain. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997;26:91S – 99. doi:10.1093/ije/26.suppl_1.s91.
[174] WHO Library Cataloguing-in-Publication Data. Toxicological and Health Aspects of Bisphenol A. Ottawa, Canada: 2010.
[175] Vela-Soria F, Ballesteros O, Zafra-Gómez A, Ballesteros L, Navalón A. A multiclass method for the analysis of endocrine disrupting chemicals in human urine samples. Sample treatment by dispersive liquid–liquid microextraction. Talanta 2014;129:209–18. doi:10.1016/j.talanta.2014.05.016.
[176] Relative validity and reproducibility of a diet history questionnaire in Spain. II. Nutrients. EPIC Group of Spain. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997;26 Suppl 1:S100-9.
[177] Riboli E, Hunt KJK, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2003;5:1113–24. doi:10.1079/PHN2002394.
[178] Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr 2003;6:407–13. doi:10.1079/PHN2002439.
[179] Crino M, Barakat T, Trevena H, Neal B. Systematic Review and Comparison of Classification Frameworks Describing the Degree of Food Processing. Nutr Food Technol Open Access 2017;3. doi:10.16966/2470-6086.138.
[180] Monteiro CA, Cannon G, Moubarac J-C, Levy RB, Louzada MLC, Jaime PC. The UN Decade of Nutrition, the NOVA food classification and the trouble with ultra-processing. Public Health Nutr 2018;21:5–17. doi:10.1017/S1368980017000234.
[181] R Core Team. R: A language and environment for statistical computing. 2018.
[182] Ripley B. pspline: Penalized Smoothing Splines. 2017.
[183] Therneau T. A Package for Survival Analysis in R_. R package version 3.1-11 2020.
[184] Therneau TM, Grambsch PM. Modeling Survival Data: Extending the Cox Model_. New York: Springer; 2000.
[185] Barrera-Gomez J, Basagana X, Barrera-Gómez J, Basagaña X. Models with transformed variables: interpretation and software. Epidemiology 2015;26:e16-7. doi:10.1097/ede.0000000000000247.
[186] Te Grotenhuis M, Pelzer B, Eisinga R, Nieuwenhuis R, Schmidt-Catran A, Konig R. When size matters: advantages of weighted effect coding in observational studies. Int J Public Health 2017;62:163–7. doi:10.1007/s00038-016-0901-1.
[187] Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27. doi:10.1093/oxfordjournals.aje.a114366.
[188] Salamanca-Fernández E, Rodríguez-Barranco M, Arrebola JP, Vela F, Díaz C, Chirlaque MD, et al. Bisphenol-A in the European Prospective Investigation into Cancer and Nutrition cohort in Spain: Levels at recruitment and associated dietary factors. Environ Res 2020;182:109012. doi:10.1016/j.envres.2019.109012.
[189] Rezg R, El-Fazaa S, Gharbi N, Mornagui B. Bisphenol A and human chronic diseases: Current evidences, possible mechanisms, and future perspectives. Environ Int 2014;64:83–90. doi:10.1016/j.envint.2013.12.007.
[190] Bloom MS, vom Saal FS, Kim D, Taylor JA, Lamb JD, Fujimoto VY. Serum unconjugated bisphenol A concentrations in men may influence embryo quality indicators during in vitro fertilization. Environ Toxicol Pharmacol 2011;32:319–23. doi:10.1016/j.etap.2011.06.003.

[191] Prins GS, Ye SH, Birch L, Ho S mei, Kannan K. Serum bisphenol A pharmacokinetics and prostate neoplastic responses following oral and subcutaneous exposures in neonatal Sprague-Dawley rats. Reprod Toxicol 2011;31:1–9. doi:10.1016/j.reprotox.2010.09.009.
[192] Fujimoto VY, Kim D, Vom Saal FS, Lamb JD, Taylor JA, Bloom MS. Serum unconjugated bisphenol A concentrations in women may adversely influence oocyte quality during in vitro fertilization. Fertil Steril 2011;95:1816–9. doi:10.1016/j.fertnstert.2010.11.008.
[193] Ye X, Zhou X, Hennings R, Kramer J, Calafat AM. Potential external contamination with bisphenol A and other ubiquitous organic environmental chemicals during biomonitoring analysis: An elusive laboratory challenge. Environ Health Perspect 2013;121:283–6. doi:10.1289/ehp.1206093.
[194] Fénichel P, Déchaux H, Harthe C, Gal J, Ferrari P, Pacini P, et al. Unconjugated bisphenol A cord blood levels in boys with descended or undescended testes. Hum Reprod 2012;27:983–90. doi:10.1093/humrep/der451.
[195] Vandenberg LN, Hunt PA, Myers JP, Vom Saal FS. Human exposures to bisphenol A: Mismatches between data and assumptions. Rev Environ Health 2013;28:37–58. doi:10.1515/reveh-2012-0034.
[196] Niwa T, Fujimoto M, Kishimoto K, Yabusaki Y, Ishibashi F, Katagiri M. Metabolism and interaction of bisphenol A in human hepatic cytochrome P450 and steroidogenic CYP17. Biol Pharm Bull 2001;24:1064–7.
[197] Calafat AM, Koch HM, Swan SH, Hauser R, Goldman LR, Lanphear BP, et al. Misuse of blood serum to assess exposure to bisphenol A and phthalates. Breast Cancer Res 2013;15:403. doi:10.1186/bcr3494.
[198] Dickerson SM, Gore AC. Estrogenic environmental endocrine-disrupting chemical effects on reproductive neuroendocrine function and dysfunction across the life cycle. Rev Endocr Metab Disord 2007;8:143–59. doi:10.1007/s11154-007-9048-y.
[199] Keri RA, Ho SM, Hunt PA, Knudsen KE, Soto AM, Prins GS. An evaluation of evidence for the carcinogenic activity of bisphenol A. Reprod Toxicol 2007;24:240–52. doi:10.1016/j.reprotox.2007.06.008.
[200] Martínez MA, Rovira J, Sharma RP, Nadal M, Schuhmacher M, Kumar V. Prenatal exposure estimation of BPA and DEHP using integrated external and internal dosimetry: A case study. Environ Res 2017;158:566–75. doi:10.1016/j.envres.2017.07.016.
[201] Dualde P, Pardo O, Corpas-Burgos F, Kuligowski J, Gormaz M, Vento M, et al. Biomonitoring of bisphenols A, F, S in human milk and probabilistic risk assessment for breastfed infants 2019;668:797–805. doi:10.1016/j.scitotenv.2019.03.024.
[202] Perez-Lobato R, Mustieles V, Calvente I, Jimenez-Diaz I, Ramos R, Caballero-Casero N, et al. Exposure to bisphenol A and behavior in school-age children. Neurotoxicology 2016;53:12–9. doi:10.1016/j.neuro.2015.12.001.
[203] Mustieles V, Ocón-Hernandez O, Mínguez-Alarcón L, Dávila-Arias C, Pérez-Lobato R, Calvente I, et al. Bisphenol A and reproductive hormones and cortisol in peripubertal boys: The INMA-Granada cohort. Sci Total Environ 2018;618:1046–53. doi:10.1016/j.scitotenv.2017.09.093.
[204] González N, Cunha SC, Monteiro C, Fernandes JO, Marquès M, Domingo JL, et al. Quantification of eight bisphenol analogues in blood and urine samples of workers in a hazardous waste incinerator. Environ Res 2019;176:108576. doi:10.1016/j.envres.2019.108576.
[205] Aekplakorn W, Chailurkit L, Ongphiphadhanakul B. Relationship of serum bisphenol A with diabetes in the Thai population, National Health Examination Survey IV, 2009. J Diabetes 2015;7:240–9. doi:10.1111/1753-0407.12159.
[206] Kuroda N, Kinoshita Y, Sun Y, Wada M, Kishikawa N, Nakashima K, et al. Measurement of bisphenol A levels in human blood serum and ascitic fluid by HPLC using a fluorescent labeling reagent. J Pharm Biomed Anal 2003;30:1743–9.
[207] Takeuchi T, Tsutsumi O, Ikezuki Y, Takai Y, Taketani Y. Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction. Endocr J 2004;51:165–9. doi:10.1507/endocrj.51.165.

[208] 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;123:643–50. doi:10.1289/ehp.1408989.
[209] Abou Omar TF, Sukhn C, Fares SA, Abiad MG, Habib RR, Dhaini HR. Bisphenol A exposure assessment from olive oil consumption. Environ Monit Assess 2017;189:341. doi:10.1007/s10661-017-6048-6.
[210] Fattore M, Russo G, Barbato F, Grumetto L, Albrizio S. Monitoring of bisphenols in canned tuna from Italian markets. Food Chem Toxicol 2015;83:68–75. doi:10.1016/j.fct.2015.05.010.
[211] Oz F, Seyyar E. Formation of Heterocyclic Aromatic Amines and Migration Level of Bisphenol-A in Sous-Vide-Cooked Trout Fillets at Different Cooking Temperatures and Cooking Levels. J Agric Food Chem 2016;64:3070–82. doi:10.1021/acs.jafc.5b05716.
[212] Liao C, Kannan K. A survey of bisphenol A and other bisphenol analogues in foodstuffs from nine cities in China. Food Addit Contam Part A 2014;31:319–29. doi:10.1080/19440049.2013.868611.
[213] Geens T, Neels H, Covaci A. Distribution of bisphenol-A, triclosan and n-nonylphenol in human adipose tissue, liver and brain. Chemosphere 2012;87:796–802. doi:10.1016/J.CHEMOSPHERE.2012.01.002.
[214] Stupans I, Stretch G, Hayball P. Olive Oil Phenols Inhibit Human Hepatic Microsomal Activity. J Nutr 2000;130:2367–70. doi:10.1093/jn/130.9.2367.
[215] Rivas A, Monteagudo C, Heras-Gonzalez L, Mariscal-Arcas M, Lorenzo-Tovar ML, Olea-Serrano F. Association of bisphenol A exposure with dietary quality indices in Spanish schoolchildren. Food Chem Toxicol 2016;94:25–30. doi:10.1016/j.fct.2016.05.010.
[216] Larsson K, Ljung Björklund K, Palm B, Wennberg M, Kaj L, Lindh CH, et al. Exposure determinants of phthalates, parabens, bisphenol A and triclosan in Swedish mothers and their children. Environ Int 2014;73:323–33. doi:10.1016/j.envint.2014.08.014.
[217] Cao X-L, Corriveau J, Popovic S. Levels of Bisphenol A in Canned Soft Drink Products in Canadian Markets. J Agric Food Chem 2009;57:1307–11. doi:10.1021/jf803213g.
[218] Cao X-L, Perez-Locas C, Dufresne G, Clement G, Popovic S, Beraldin F, et al. Concentrations of bisphenol A in the composite food samples from the 2008 Canadian total diet study in Quebec City and dietary intake estimates. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2011;28:791–8. doi:10.1080/19440049.2010.513015.
[219] Schecter A, Malik N, Haffner D, Smith S, Harris TR, Paepke O, et al. Bisphenol A (BPA) in U.S. Food. Environ Sci Technol 2010;44:9425–30. doi:10.1021/es102785d.
[220] Latasa P, Louzada MLDC, Martinez Steele E, Monteiro CA. Added sugars and ultra-processed foods in Spanish households (1990–2010). Eur J Clin Nutr 2018;72:1404–12. doi:10.1038/s41430-017-0039-0.
[221] Neri D, Martinez-Steele E, Monteiro CA, Levy RB. Consumption of ultra-processed foods and its association with added sugar content in the diets of US children, NHANES 2009-2014. Pediatr Obes 2019;14:e12563. doi:10.1111/ijpo.12563.
[222] Martínez Steele E, Baraldi LG, Louzada ML da C, Moubarac J-C, Mozaffarian D, Monteiro CA. Ultra-processed foods and added sugars in the US diet: evidence from a nationally representative cross-sectional study. BMJ Open 2016;6:e009892. doi:10.1136/bmjopen-2015-009892.
[223] Oldring PKT, Castle L, O'Mahony C, Dixon J. Estimates of dietary exposure to bisphenol A (BPA) from light metal packaging using food consumption and packaging usage data: a refined deterministic approach and a fully probabilistic (FACET) approach. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2014;31:466–89. doi:10.1080/19440049.2013.860240.
[224] Jin H, Zhu J, Chen Z, Hong Y, Cai Z. Occurrence and Partitioning of Bisphenol Analogues in Adults' Blood from China. Environ Sci Technol 2018;52:812–20. doi:10.1021/acs.est.7b03958.
[225] He Y, Miao M, Herrinton LJ, Wu C, Yuan W, Zhou Z, et al. Bisphenol A levels in blood

and urine in a Chinese population and the personal factors affecting the levels 2009;109. doi:10.1016/j.envres.2009.04.003.
[226] Santhi VA, Sakai N, Ahmad ED, Mustafa AM. Occurrence of bisphenol A in surface water, drinking water and plasma from Malaysia with exposure assessment from consumption of drinking water. Sci Total Environ 2012;427–428:332–8. doi:10.1016/j.scitotenv.2012.04.041.
[227] Uno Y, Takata R, Kito G, Yamazaki H, Nakagawa K, Nakamura Y, et al. Sex- and age-dependent gene expression in human liver: An implication for drug-metabolizing enzymes. Drug Metab Pharmacokinet 2017;32:100–7. doi:10.1016/j.dmpk.2016.10.409.
[228] Takeuchi T, Tsutsumi O, Nakamura N, Ikezuki Y, Takai Y, Yano T, et al. Gender difference in serum bisphenol A levels may be caused by liver UDP-glucuronosyltransferase activity in rats. Biochem Biophys Res Commun 2004;325:549–54. doi:10.1016/j.bbrc.2004.10.073.
[229] Adoamnei E, Mendiola J, Vela-Soria F, Fernández MF, Olea N, Jørgensen N, et al. Urinary bisphenol A concentrations are associated with reproductive parameters in young men. Environ Res 2018;161:122–8.
[230] Engel LS, Buckley JP, Yang G, Liao LM, Satagopan J, Calafat AM, et al. Predictors and variability of repeat measurements of urinary phenols and parabens in a cohort of Shanghai women and men. Environ Health Perspect 2014;122:733–40. doi:10.1289/ehp.1306830.
[231] Li X, Ying GG, Zhao JL, Chen ZF, Lai HJ, Su HC. 4-Nonylphenol, bisphenol-A and triclosan levels in human urine of children and students in China, and the effects of drinking these bottled materials on the levels. Environ Int 2013;52:81–6. doi:10.1016/j.envint.2011.03.026.
[232] Nahar MS, Soliman AS, Colacino JA, Calafat AM, Battige K, Hablas A, et al. Urinary bisphenol A concentrations in girls from rural and urban Egypt: A pilot study. Environ Heal A Glob Access Sci Source 2012;11. doi:10.1186/1476-069X-11-20.
[233] Stahlhut RW, Welshons W V., Swan SH. Bisphenol A Data in NHANES Suggest Longer than Expected Half-Life, Substantial Nonfood Exposure, or Both. Environ Health Perspect 2009;117:784–9. doi:10.1289/ehp.0800376.
[234] Doerge DR, Twaddle NC, Vanlandingham M, Fisher JW. Pharmacokinetics of bisphenol A in serum and adipose tissue following intravenous administration to adult female CD-1 mice. Toxicol Lett 2012;211:114–9. doi:10.1016/j.toxlet.2012.03.008.
[235] Sungur Ş, Köroğlu M, Özkan A. Determinatıon of bisphenol a migrating from canned food and beverages in markets. Food Chem 2014;142:87–91. doi:10.1016/J.FOODCHEM.2013.07.034.
[236] González-Castro MI, Olea-Serrano MF, Rivas-Velasco AM, Medina-Rivero E, Ordoñez-Acevedo LG, De León-Rodríguez A. Phthalates and Bisphenols Migration in Mexican Food Cans and Plastic Food Containers. Bull Environ Contam Toxicol 2011;86:627–31. doi:10.1007/s00128-011-0266-3.
[237] Ferrer E, Santoni E, Vittori S, Font G, Mañes J, Sagratini G. Simultaneous determination of bisphenol A, octylphenol, and nonylphenol by pressurised liquid extraction and liquid chromatography–tandem mass spectrometry in powdered milk and infant formulas. Food Chem 2011;126:360–7. doi:10.1016/J.FOODCHEM.2010.10.098.
[238] Sajiki J, Miyamoto F, Fukata H, Mori C, Yonekubo J, Hayakawa K. Bisphenol A (BPA) and its source in foods in Japanese markets. Food Addit Contam 2007;24:103–12. doi:10.1080/02652030600936383.
[239] Kang J-H, Kondo F. Bisphenol A migration from cans containing coffee and caffeine. Food Addit Contam 2002;19:886–90. doi:10.1080/02652030210147278.
[240] Errico S, Bianco M, Mita L, Migliaccio M, Rossi S, Nicolucci C, et al. Migration of bisphenol A into canned tomatoes produced in Italy: Dependence on temperature and storage conditions. Food Chem 2014;160:157–64. doi:10.1016/J.FOODCHEM.2014.03.085.
[241] LaKind JS, Sobus JR, Goodman M, Barr DB, Fürst P, Albertini RJ, et al. A proposal for assessing study quality: Biomonitoring, Environmental Epidemiology, and Short-lived

Chemicals (BEES-C) instrument. Environ Int 2014;73:195–207. doi:10.1016/j.envint.2014.07.011.
[242] Völkel W, Colnot T, Csanády GA, Filser JG, Dekant W. Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. Chem Res Toxicol 2002;15:1281–7.
[243] Sanchis Y, Coscollà C, Yusà V. Analysis of four parabens and bisphenols A, F, S in urine, using dilute and shoot and liquid chromatography coupled to mass spectrometry. Talanta 2019;202:42–50. doi:10.1016/j.talanta.2019.04.048.
[244] Artacho-Cordón F, Arrebola JP, Nielsen O, Hernández P, Skakkebaek NE, Fernández MF, et al. Assumed non-persistent environmental chemicals in human adipose tissue; matrix stability and correlation with levels measured in urine and serum. Environ Res 2017;156:120–7. doi:10.1016/j.envres.2017.03.030.
[245] Pérez-Lobato R, Mustieles V, Calvente I, Jiménez-Díaz I, Ramos R, Caballero-Casero N, et al. Exposure to bisphenol A and behavior in school-age children. Neurotoxicology 2016;53:12–9. doi:10.1016/j.neuro.2015.12.001.
[246] Fernández MF, Arrebola JP, Jiménez-Díaz I, Sáenz JM, Molina-Molina JM, Ballesteros O, et al. Bisphenol A and other phenols in human placenta from children with cryptorchidism or hypospadias. Reprod Toxicol 2016;59:89–95. doi:10.1016/j.reprotox.2015.11.002.
[247] Casas M, Valvi D, Luque N, Ballesteros-Gomez A, Carsin AE, Fernandez MF, et al. Dietary and sociodemographic determinants of bisphenol A urine concentrations in pregnant women and children. Environ Int 2013;56:10–8. doi:10.1016/j.envint.2013.02.014.
[248] Casas M, Valvi D, Ballesteros-Gomez A, Gascon M, Fernández MF, Garcia-Esteban R, et al. Exposure to bisphenol a and phthalates during pregnancy and ultrasound measures of fetal growth in the INMA-sabadell cohort. Environ Health Perspect 2016;124:521–8. doi:10.1289/ehp.1409190.
[249] Gascon M, Casas M, Morales E, Valvi D, Ballesteros-Gómez A, Luque N, et al. Prenatal exposure to bisphenol A and phthalates and childhood respiratory tract infections and allergy. J Allergy Clin Immunol 2015;135:370-378.e7. doi:10.1016/j.jaci.2014.09.030.
[250] Covaci A, Hond E Den, Geens T, Govarts E, Koppen G, Frederiksen H, 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.
[251] Salamanca-Fernández E, Rodríguez-Barranco M, Petrova D, Larrañaga N, Guevara M, Moreno-Iribas C, et al. Bisphenol A exposure and risk of ischemic heart disease in the Spanish European Prospective Investigation into cancer and nutrition study. Chemosphere 2020;261:127697. doi:10.1016/j.chemosphere.2020.127697.
[252] INE. Defunciones por causas (lista reducida), sexo, tamaño de municipio y capital de residencia y edad . 2017. https://ine.es/jaxi/Datos.htm?path=/t15/p417/a2017/l0/&file=01002a.px (accessed January 20, 2020).
[253] Patel BB, Raad M, Sebag IA, Chalifour LE. Lifelong exposure to bisphenol a alters cardiac structure/function, protein expression, and DNA methylation in adult mice. Toxicol Sci 2013;133:174–85. doi:10.1093/toxsci/kft026.
[254] Asano S, Tune JD, Dick GM. Bisphenol A activates Maxi-K (K Ca1.1) channels in coronary smooth muscle. Br J Pharmacol 2010;160:160–70. doi:10.1111/j.1476-5381.2010.00687.x.
[255] Liu B, Lehmler H-J, Sun Y, Xu G, Sun Q, Snetselaar LG, et al. Association of Bisphenol A and Its Substitutes, Bisphenol F and Bisphenol S, with Obesity in United States Children and Adolescents. Diabetes Metab J 2019;43:59. doi:10.4093/dmj.2018.0045.
[256] Pereira-Fernandes A, Demaegdt H, Vandermeiren K, Hectors TLM, Jorens PG, Blust R, et al. Evaluation of a Screening System for Obesogenic Compounds: Screening of Endocrine Disrupting Compounds and Evaluation of the PPAR Dependency of the Effect. PLoS One 2013;8:e77481. doi:10.1371/journal.pone.0077481.

[257] Legeay S, Faure S. Is bisphenol A an environmental obesogen? Fundam Clin Pharmacol 2017;31:594–609. doi:10.1111/fcp.12300.
[258] Rubin BS, Schaeberle CM, Soto AM. The Case for BPA as an Obesogen: Contributors to the Controversy. Front Endocrinol (Lausanne) 2019;10:30. doi:10.3389/fendo.2019.00030.
[259] Carwile JL, Michels KB. Urinary bisphenol A and obesity: NHANES 2003-2006 ☆. Env Res 2011;111:825–30. doi:10.1016/j.envres.2011.05.014.
[260] Wang Z, Liu H, Liu S. Low-Dose Bisphenol A Exposure: A Seemingly Instigating Carcinogenic Effect on Breast Cancer. Adv Sci (Weinheim, Baden-Wurttemberg, Ger 2017;4:1600248. doi:10.1002/advs.201600248.
[261] Proposition de loi. Chir Dent Fr 1975;45:23–4.
[262] Baena-Díez JM, Alzamora-Sas MT, Grau M, Subirana I, Vila J, Torán P, et al. Validez del cuestionario cardiovascular MONICA comparado con la historia clínica. Gac Sanit 2009;23:519–25. doi:10.1016/j.gaceta.2009.01.009.
[263] Borgan ØrnulfSven, Ove Samuelsen. A review of cohort sampling designs for Cox's regression model: Potentials in epidemiology. Nor Epidemiol 2003;13:239–48.
[264] Barlow WE. Robust Variance Estimation for the Case-Cohort Design. Biometrics 1994;50:1064. doi:10.2307/2533444.
[265] Wang J, Sun B, Hou M, Pan X, Li X. 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 2013;37:999–1005. doi:10.1038/ijo.2012.173.
[266] Lakind JS, Goodman M, Mattison DR. Bisphenol A and indicators of obesity, glucose metabolism/type 2 diabetes and cardiovascular disease: A systematic review of epidemiologic research. Crit Rev Toxicol 2014;44:1040–8444. doi:10.3109/10408444.2013.860075.
[267] LaKind JS, Goodman M, Naiman DQ. Use of NHANES Data to Link Chemical Exposures to Chronic Diseases: A Cautionary Tale. PLoS One 2012;7:e51086. doi:10.1371/journal.pone.0051086.
[268] Lang IA, Galloway TS, Scarlett A, Henley WE, Depledge M, Wallace RB, et al. Association of Urinary Bisphenol A Concentration With Medical Disorders and Laboratory Abnormalities in Adults. Jama 2008;300:1303–10. doi:10.1001/jama.300.11.1303.
[269] Melzer D, Osborne NJ, Henley WE, Cipelli R, Young A, Money C, et al. Urinary bisphenol A concentration and risk of future coronary artery disease in apparently healthy men and women. Circulation 2012;125:1482–90. doi:10.1161/CIRCULATIONAHA.111.069153.
[270] Melzer D, Rice NE, Lewis C, Henley WE, Galloway TS. Association of urinary bisphenol A concentration with heart disease: Evidence from NHANES 2003/06. PLoS One 2010;5:e8673. doi:10.1371/journal.pone.0008673.
[271] Rancière F, Lyons JG, Loh VHYY, Botton J, Galloway T, Wang T, et al. Bisphenol A and the risk of cardiometabolic disorders: a systematic review with meta-analysis of the epidemiological evidence. Environ Health 2015;14:46. doi:10.1186/s12940-015-0036-5.
[272] Casey MF, Neidell M. Disconcordance in statistical models of Bisphenol a and chronic disease outcomes in NHANES 2003-08. PLoS One 2013;8. doi:10.1371/journal.pone.0079944.
[273] Melzer D, Gates P, Osborne NJ, Osborn NJ, Henley WE, Cipelli R, et al. Urinary bisphenol a concentration and angiography-defined coronary artery stenosis. PLoS One 2012;7:e43378. doi:10.1371/journal.pone.0043378.
[274] Braun JM, Gennings C, Hauser R, Webster TF. What can epidemiological studies tell us about the impact of chemical mixtures on human health? Environ Health Perspect 2016;124:A6–9. doi:10.1289/ehp.1510569.
[275] Heindel JJ, Blumberg B, Cave M, Machtinger R, Mantovani A, Mendez MA, et al. Metabolism disrupting chemicals and metabolic disorders. Reprod Toxicol 2017;68:3–33. doi:10.1016/j.reprotox.2016.10.001.

[276] Nowak K, Ratajczak-Wrona W, Górska M, Jabłońska E. Parabens and their effects on the endocrine system. Mol Cell Endocrinol 2018;474:238–51. doi:10.1016/j.mce.2018.03.014.
[277] Kolatorova L, Sramkova M, Vitku J, Vcelak J, Lischkova O, Starka L, et al. Parabens and their relation to obesity. Physiol Res 2018;67:S465–72.
[278] Martínez JA, Milagro FI, Claycombe KJ, Schalinske KL. Epigenetics in adipose tissue, obesity, weight loss, and diabetes. Adv Nutr 2014;5:71–81. doi:10.3945/an.113.004705.
[279] Lenaz G. Mitochondria and reactive oxygen species. Which role in physiology and pathology? Adv Exp Med Biol 2012;942:93–136. doi:10.1007/978-94-007-2869-1_5.
[280] Tiwari D, Kamble J, Chilgunde S, Patil P, Maru G, Kawle D, et al. Clastogenic and mutagenic effects of bisphenol A: An endocrine disruptor. Mutat Res - Genet Toxicol Environ Mutagen 2012;743:83–90. doi:10.1016/j.mrgentox.2011.12.023.
[281] Anjum S, Rahman S, Kaur M, Ahmad F, Rashid H, Ansari RA, et al. Melatonin ameliorates bisphenol A-induced biochemical toxicity in testicular mitochondria of mouse. Food Chem Toxicol 2011;49:2849–54. doi:10.1016/j.fct.2011.07.062.
[282] Ooe H, Taira T, Iguchi-Ariga SMM, Ariga H. Induction of Reactive Oxygen Species by Bisphenol A and Abrogation of Bisphenol A-Induced Cell Injury by DJ-1. Toxicol Sci 2005;88:114–26. doi:10.1093/toxsci/kfi278.
[283] Kabuto H, Amakawa M, Shishibori T. Exposure to bisphenol A during embryonic/fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice. Life Sci 2004;74:2931–40. doi:10.1016/j.lfs.2003.07.060.
[284] Jian L, Po ALW. Kinetic Evaluation of the Ciliotoxicity of Methyl- and Propyl-p-hydroxybenzoates Using Factorial Experiments. J Pharm Pharmacol 1993;45:98–101. doi:10.1111/j.2042-7158.1993.tb03691.x.
[285] Martínez MA, Rovira J, Prasad Sharma R, Nadal M, Schuhmacher M, Kumar V. Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure: A Catalonia (Spain) case study. Environ Res 2018;166:25–34. doi:10.1016/j.envres.2018.05.008.
[286] Kang J-H, Kondo F, Katayama Y. Human exposure to bisphenol A. Toxicology 2006;226:79–89. doi:10.1016/j.tox.2006.06.009.
[287] Sowlat MH, Lotfi S, Yunesian M, Ahmadkhaniha R, Rastkari N. The association between bisphenol A exposure and type-2 diabetes: a world systematic review. Environ Sci Pollut Res 2016;23:21125–40. doi:10.1007/s11356-016-7525-0.
[288] Artacho-Cordón F, Ríos-Arrabal S, León J, Frederiksen H, Sáenz JM, Martín-Olmedo P, et al. Adipose tissue concentrations of non-persistent environmental phenols and local redox balance in adults from Southern Spain. Environ Int 2019;133:105118. doi:10.1016/j.envint.2019.105118.
[289] Lenters V, Iszatt N, Forns J, Čechová E, Kočan A, Legler J, et al. Early-life exposure to persistent organic pollutants (OCPs, PBDEs, PCBs, PFASs) and attention-deficit/hyperactivity disorder: A multi-pollutant analysis of a Norwegian birth cohort. Environ Int 2019;125:33–42. doi:10.1016/j.envint.2019.01.020.
[290] Lenters V, Portengen L, Rignell-Hydbom A, Jönsson BAG, Lindh CH, Piersma AH, et al. Prenatal Phthalate, Perfluoroalkyl Acid, and Organochlorine Exposures and Term Birth Weight in Three Birth Cohorts: Multi-Pollutant Models Based on Elastic Net Regression. Environ Health Perspect 2016;124:365–72. doi:10.1289/ehp.1408933.
[291] Salamanca-Fernández E, Iribarne-Durán LM, Rodríguez-Barranco M, Vela-Soria F, Olea N, Sánchez-Pérez MJ, et al. Historical exposure to non-persistent environmental pollutants and risk of type 2 diabetes in a Spanish sub-cohort from the European Prospective Investigation into Cancer and Nutrition study. Environ Res 2020;185:109383. doi:10.1016/j.envres.2020.109383.
[292] Décision n° 2015-480 QPC du 17 septembre 2015. Article 1 2015. https://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000031183673&categorieLien=id (accessed January 21, 2020).
[293] Błędzka D, Gromadzińska J, Wąsowicz W, Błedzka D, Gromadzińska J, Wasowicz W. Parabens. From environmental studies to human health. Environ Int 2014;67:27–42.

doi:10.1016/j.envint.2014.02.007.
[294] Heindel JJ, Saal FS vom, Blumberg B, Bovolin P, Calamandrei G, Ceresini G, et al. Parma consensus statement on metabolic disruptors. Environ Heal 2015;14:54. doi:10.1186/S12940-015-0042-7.
[295] Casals-Casas C, Desvergne B. Endocrine Disruptors: From Endocrine to Metabolic Disruption. Annu Rev Physiol 2011;73:135–62. doi:10.1146/annurev-physiol-012110-142200.
[296] Wang J, Pan L, Wu S, Lu L, Xu Y, Zhu Y, et al. Recent advances on endocrine disrupting effects of UV filters. Int J Environ Res Public Health 2016;13. doi:10.3390/ijerph13080782.
[297] Darbre PD. Endocrine Disruptors and Obesity. Curr Obes Rep 2017;6:18–27. doi:10.1007/s13679-017-0240-4.
[298] World Health Organization. Obesity and overweight 2018. https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight (accessed September 25, 2019).
[299] Song YY, Chou EL, Baecker A, You N-CY, Song YY, Sun Q, et al. Endocrine-disrupting chemicals, risk of type 2 diabetes, and diabetes-related metabolic traits: A systematic review and meta-analysis. J Diabetes 2016;8:516–32. doi:10.1111/1753-0407.12325.
[300] Ehrlich S, Lambers D, Baccarelli A, Khoury J, Macaluso M, Ho S-M. Endocrine Disruptors: A Potential Risk Factor for Gestational Diabetes Mellitus. Am J Perinatol 2016;33:1313–8. doi:10.1055/s-0036-1586500.
[301] Shu X, Tang S, Peng C, Gao R, Yang S, Luo T, et al. Bisphenol A is not associated with a 5-year incidence of type 2 diabetes: a prospective nested case–control study. Acta Diabetol 2018;55:369–75. doi:10.1007/s00592-018-1104-4.
[302] Li AJ, Xue J, Lin S, Al-Malki AL, Al-Ghamdi MA, Kumosani TA, et al. Urinary concentrations of environmental phenols and their association with type 2 diabetes in a population in Jeddah, Saudi Arabia. Environ Res 2018;166:544–52. doi:10.1016/j.envres.2018.06.040.
[303] Kim K, Park H. Association between urinary concentrations of bisphenol A and type 2 diabetes in Korean adults: A population-based cross-sectional study. Int J Hyg Environ Health 2013;216:467–71. doi:10.1016/j.ijheh.2012.07.007.
[304] Wang X, Wang X, Chen Q, Luo Z-C, Zhao S, Wang W, et al. Urinary Bisphenol A Concentration and Gestational Diabetes Mellitus in Chinese Women. Epidemiology 2017;28:S41–7. doi:10.1097/EDE.0000000000000730.
[305] Krause M, Klit A, Blomberg Jensen M, Søeborg T, Frederiksen H, Schlumpf M, et al. Sunscreens: Are they beneficial for health? An overview of endocrine disrupting properties of UV-filters. Int J Androl 2012;35:424–36. doi:10.1111/j.1365-2605.2012.01280.x.
[306] Schlumpf M, Schmid P, Durrer S, Conscience M, Maerkel K, Henseler M, et al. Endocrine activity and developmental toxicity of cosmetic UV filters—an update. Toxicology 2004;205:113–22. doi:10.1016/J.TOX.2004.06.043.
[307] Pastor-Nieto MA, Alcántara-Nicolás F, Melgar-Molero V, Pérez-Mesonero R, Vergara-Sánchez A, Martín-Fuentes A, et al. Conservantes en productos de higiene y cosméticos, medicamentos tópicos y productos de limpieza doméstica en España. Actas Dermosifiliogr 2017;108:758–70. doi:10.1016/j.ad.2017.04.003.
[308] Selevan SG, Kimmel CA, Mendola P. Identifying critical windows of exposure for children's health. Environ Health Perspect 2000;108:451–5. doi:10.1289/ehp.00108s3451.
[309] Liu W, Zhou Y, Li J, Sun X, Liu H, Jiang Y, et al. Parabens exposure in early pregnancy and gestational diabetes mellitus. Environ Int 2019;126:468–75. doi:10.1016/j.envint.2019.02.040.
[310] Kang S, Kim S, Park J, Kim H-J, Lee J, Choi G, et al. Urinary paraben concentrations among pregnant women and their matching newborn infants of Korea, and the association with oxidative stress biomarkers. Sci Total Environ 2013;461–462:214–21.

doi:10.1016/J.SCITOTENV.2013.04.097.
[311] Mahadevan J, Parazzoli S, Oseid E, Hertzel A V, Bernlohr DA, Vallerie SN, et al. Ebselen treatment prevents islet apoptosis, maintains intranuclear Pdx-1 and MafA levels, and preserves β-cell mass and function in ZDF rats. Diabetes 2013;62:3582–8. doi:10.2337/db13-0357.
[312] Bast A, Wolf G, Oberbäumer I, Walther R. Oxidative and nitrosative stress induces peroxiredoxins in pancreatic beta cells. Diabetologia 2002;45:867–76. doi:10.1007/s00125-002-0846-1.
[313] Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A. The Estrogenic Effect of Bisphenol A Disrupts Pancreatic β-Cell Function *In Vivo* and Induces Insulin Resistance. Environ Health Perspect 2006;114:106–12. doi:10.1289/ehp.8451.
[314] Nadal A, Alonso-Magdalena P, Soriano S, Ropero AB, Quesada I. The role of oestrogens in the adaptation of islets to insulin resistance. J Physiol 2009;587:5031–7. doi:10.1113/jphysiol.2009.177188.
[315] Livingstone C, Collison M. Sex steroids and insulin resistance. Clin Sci (Lond) 2002;102:151–66.
[316] Ben-Jonathan N, Hugo ER, Brandebourg TD. Effects of bisphenol A on adipokine release from human adipose tissue: Implications for the metabolic syndrome. Mol Cell Endocrinol 2009;304:49–54. doi:10.1016/j.mce.2009.02.022.
[317] Hugo ER, Brandebourg TD, Woo JG, Loftus J, Alexander JW, Ben-Jonathan N. Bisphenol A at Environmentally Relevant Doses Inhibits Adiponectin Release from Human Adipose Tissue Explants and Adipocytes. Environ Health Perspect 2008;116:1642–7. doi:10.1289/ehp.11537.
[318] Whitehead JP, Richards AA, Hickman IJ, Macdonald GA, Prins JB. Adiponectin--a key adipokine in the metabolic syndrome. Diabetes Obes Metab 2006;8:264–80. doi:10.1111/j.1463-1326.2005.00510.x.
[319] Trujillo ME, Scherer PE. Adiponectin - journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. J Intern Med 2005;257:167–75. doi:10.1111/j.1365-2796.2004.01426.x.
[320] Stanaway JD, Afshin A, Gakidou E, Lim SS, Abate D, Abate KH. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Stu. Lancet 2018;392:1923–94. doi:10.1016/S0140-6736(18)32225-6.
[321] Zhou B, Bentham J, Di Cesare M, Bixby H, Danaei G, Cowan MJ. Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19·1 million participants. Lancet 2017;389:37–55. doi:10.1016/S0140-6736(16)31919-5.
[322] Watanabe Y, Kojima H, Takeuchi S, Uramaru N, Sanoh S, Sugihara K, et al. Metabolism of UV-filter benzophenone-3 by rat and human liver microsomes and its effect on endocrine-disrupting activity. Toxicol Appl Pharmacol 2015;282:119–28. doi:10.1016/j.taap.2014.12.002.
[323] Shiue I, Hristova K. Higher urinary heavy metal, phthalate and arsenic concentrations accounted for 3–19% of the population attributable risk for high blood pressure: US NHANES, 2009–2012. Hypertens Res 2014;37:1075–81. doi:10.1038/hr.2014.121.
[324] Wang T, Xu M, Xu Y, Lu J, Li M, Chen Y, et al. Association of bisphenol A exposure with hypertension and early macrovascular diseases in Chinese adults: A cross-sectional study. Med (United States) 2015;94. doi:10.1097/MD.0000000000001814.
[325] Gillis EE, Sullivan JC. Sex Differences in Hypertension: Recent Advances. Hypertension 2016;68:1322–7. doi:10.1161/HYPERTENSIONAHA.116.06602.
[326] Mouneimne Y, Nasrallah M, Khoueiry-Zgheib N, Nasreddine L, Nakhoul N, Ismail H, et al. Bisphenol A urinary level, its correlates, and association with cardiometabolic risks in Lebanese urban adults. Environ Monit Assess 2017;189:517. doi:10.1007/s10661-017-6216-8.
[327] Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A. The estrogenic effect

of bisphenol A disrupts pancreatic beta-cell function in vivo and induces insulin resistance. Environ Health Perspect 2006;114:106–12. doi:10.1289/ehp.8451.
[328] Newbold RR, Padilla-Banks E, Jefferson WN, Heindel JJ. Effects of endocrine disruptors on obesity. Int. J. Androl., vol. 31, 2008, p. 201–7. doi:10.1111/j.1365-2605.2007.00858.x.
[329] Phrakonkham P, Viengchareun S, Belloir C, Lombès M, Artur Y, Canivenc-Lavier M-C. Dietary xenoestrogens differentially impair 3T3-L1 preadipocyte differentiation and persistently affect leptin synthesis. J Steroid Biochem Mol Biol 2008;110:95–103. doi:10.1016/j.jsbmb.2008.02.006.
[330] Masuno H, Iwanami J, Kidani T, Sakayama K, Honda K. Bisphenol a accelerates terminal differentiation of 3T3-L1 cells into adipocytes through the phosphatidylinositol 3-kinase pathway. Toxicol Sci 2005;84:319–27. doi:10.1093/toxsci/kfi088.
[331] Millis RM. Epigenetics and hypertension. Curr Hypertens Rep 2011;13:21–8. doi:10.1007/s11906-010-0173-8.
[332] Ordovás JM, Smith CE. Epigenetics and cardiovascular disease. Nat Rev Cardiol 2010;7:510–9. doi:10.1038/nrcardio.2010.104.
[333] Hiroi H, Tsutsumi O, Momoeda M, Takai Y, Osuga Y, Taketani Y. Differential interactions of bisphenol A and 17β-estradiol with estrogen receptor α (ERα) and ERβ. Endocr J 1999;46:773–8. doi:10.1507/endocrj.46.773.
[334] Mastin JP. Environmental Cardiovascular Disease. Cardiovasc Toxicol 2005;5:091–4. doi:10.1385/CT:5:2:091.
[335] Mendelsohn ME. Protective effects of estrogen on the cardiovascular system. Am J Cardiol 2002;89:12–7. doi:10.1016/S0002-9149(02)02405-0.
[336] Iman M Mourad, Yasser Khadrawy. The sensetivity of Liver, Kidney and testis of rats to oxidative stress induced by different doses of Bisphenol A | Request PDF 2012.
[337] Stegeman JJ, Hahn ME, Weisbrod R, Woodin BR, Joy JS, Najibi S, et al. Induction of cytochrome P4501A1 by aryl hydrocarbon receptor agonists in porcine aorta endothelial cells in culture and cytochrome P4501A1 activity in intact cells. Mol Pharmacol 1995;47:296–306.
[338] Ooe H, Taira T, Iguchi-Ariga SMM, Ariga H. Induction of reactive oxygen species by bisphenol A and abrogation of bisphenol A-induced cell injury by DJ-1. Toxicol Sci 2005;88:114–26. doi:10.1093/toxsci/kfi278.
[339] Aboul Ezz HS, Khadrawy YA, Mourad IM. The effect of bisphenol A on some oxidative stress parameters and acetylcholinesterase activity in the heart of male albino rats. Cytotechnology 2013;67:145–55. doi:10.1007/s10616-013-9672-1.
[340] Beunza JJ, Martínez-González MÁ, Serrano-Martínez M, Alonso Á. Incidence of Hypertension in a Cohort of Spanish University Graduates: The SUN Study. Rev Española Cardiol (English Ed 2006;59:1331–4. doi:10.1016/s1885-5857(07)60090-5.
[341] Lastra G, Syed S, Kurukulasuriya LR, Manrique C, Sowers JR. Type 2 diabetes mellitus and hypertension: An update. Endocrinol Metab Clin North Am 2014;43:103–22. doi:10.1016/j.ecl.2013.09.005.

# 11 Anexo

## Lista de tablas



**Artículo 1**



**Artículo 2**

**Artículo 3**

- **Table 1.** Baseline characteristics of the study population
- **Table 2.** Serum npEP concentrations (ng/mL) in the study population.
- **Table 3.** Serum npEP concentrations and risk of incident type 2 diabetes. Cox Proportional Hazard Models
- **Supplementary Table 1.** Comparison between selected and non-selected participants from the entire cohort.
- **Supplementary Table 2.** Spearman correlation among npEPs included in the study
- **Supplementary Table 3**. Serum npEPs exposure and risk of incident type 2 diabetes. Cox Proportional Hazard Models stratified by sex: Women
- **Supplementary Table 4.** Serum npEPs exposure and risk of incident type 2 diabetes. Cox Proportional Hazard Models stratified by sex: Men
- **Supplementary Table 5.** Serum npEPs exposure and risk of incident type 2 diabetes. Cox Proportional Hazard Models stratified by BMI: Normal weight
- **Supplementary Table 6.** Serum npEPs exposure and risk of incident type 2 diabetes. Cox Proportional Hazard Models stratified by BMI: Overweight
- **Supplementary Table 7.** Serum npEPs exposure and risk of incident type 2 diabetes. Cox Proportional Hazard Models stratified by BMI: Obese
- **Supplementary Table 8.** Serum npEP concentrations (ng/mL) in women and men.

**Artículo 4**

- **Table 1.** Baseline characteristics of the study population
- **Table 2.** Serum npEPs concentrations (ng/mL) and rates of detection (%) in the study population.
- **Table 3.** Serum npEP exposure and risk of incident arterial hypertension. Cox Proportional Hazard Models
- **Supplementary Table 1.** Comparison between selected and non-selected participants from the entire EPIC-Granada cohort.

## Lista de figuras



**Artículo 2**



**Artículo 3**



**Artículo 4**

- **Figure 2.** Serum levels of npEPs and risk of incident arterial hypertension in males and females. Hazard ratios and 95% Confidence Intervals.