TESIS DOCTORAL INTERNACIONAL

UNIVERSIDAD
DE GRANADA

CARACTERIZACIÓN DE LA EXPOSICIÓN A DISRUPTORES ENDOCRINOS NO PERSISTENTES EN MADRES LACTANTES Y NEONATOS INGRESADOS EN LA UCIN

FACULTAD DE MEDICINA

Programa de Doctorado en Medicina Clínica y Salud Pública

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A mis padres

“Fuertes razones, hacen fuertes acciones”

William Shakespeare

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1. RESUMEN

El parto prematuro es una de las razones más comunes de hospitalización en la Unidad de Cuidados Intensivos Neonatales (UCIN) y representa el 75% de las muertes perinatales y el 50% de la morbilidad perinatal. A nivel global, durante los últimos 20 años, se han registrado un aumento en las tasas de prematuridad, con aproximadamente 15 millones de bebés nacidos prematuros anualmente en el mundo, lo que supone el 10% de los recién nacidos vivos. Concretamente, España registra una de las tasas más altas de Europa, con unos 28,000 nacimientos prematuros cada año. En este sentido, se ha sugerido que el elevado riesgo de retraso en el desarrollo, disfunción cognitiva, trastorno de déficit de atención e hiperactividad y autismo que presentan los neonatos que han estado ingresados en la UCIN podría no explicase exclusivamente por el grado de prematuridad o la gravedad de la enfermedad acompañante, sino que podrían atribuirse a exposiciones ocurridas en el medio hospitalario. Así, este medio, y en particular la UCIN, podría suponer un riesgo de exposición inadvertida a disruptores endocrinos (DEs).

En particular, dicha exposición podría derivarse de la amplia variedad de material sanitario empleado, fundamentalmente de origen plástico, del uso de productos para el cuidado personal (PCPs) usados para la higiene de estos neonatos. Es por ello que el primer objetivo específico de esta tesis doctoral fue caracterizar la exposición a DEs no persistentes, bisfenoles y parabenos (PBs), a través de los procedimientos médicos empleados en niños prematuros ingresados en la UCIN. Para ello, se determinaron las concentraciones de BPA y PBs [metil- (MeP), etil- (EtP), propil- (PrP) y butilparabeno (BuP)] y la actividad (anti-)estrogénica y (anti-)androgénica de 52 elementos de la UCIN, incluyendo sondas nasogástricas, endotraqueales, pañales, gafas de fototerapia o catateres intravenosos, entre otros. Se encontró BPA y PBs en aproximadamente el 60% y el 80% de los elementos de la UCIN analizados, respectivamente, con concentraciones medias entre 7,000 – 100 ng/g para BPA y >100 ng/g para PBs. Además, alrededor del 25% de extractos evidenciaron actividad estrogénica y el 10% actividad antiandrogénica. En conjunto, estos resultados indican claramente que los dispositivos y productos médicos usados rutinariamente en la UCIN pueden contribuir a la exposición de los niños prematuros ingresados en la UCIN a través de múltiples vías, respiratoria, digestiva, dérmica y IV/parenteral, durante su estancia hospitalaria.

Además, la alimentación de elección en estos prematuros institucionalizados en la UCIN es la leche materna, procediendo en muchos casos de Bancos de leche materna, la cual se ha reportado que puede ser una vía de excreción de estos químicos. Es por ello que, para dar respuesta al segundo objetivo específico de esta tesis doctoral, se recopiló la evidencia científica disponible sobre el grado de exposición a bisfenoles, PBs y BPs a través de la leche materna. Además, se caracterizó la exposición a dichos contaminantes en 83 muestras procedentes del Banco de leche materna del Hospital Universitario Virgen de las Nieves de Granada (España), explorando además, los diferentes determinantes de la exposición relacionados con las concentraciones obtenidas. Tras el análisis de los estudios encontrados hasta la fecha, hemos observado que a pesar de que existen diferencias regionales y temporales en la frecuencia y concentración de los contaminantes, la leche materna contiene cantidades detectables bisfenoles, PBs y BPs. Por otro lado, los resultados de las muestras del Banco de leche materna de Granada mostraron que 9 de cada 10 muestras analizadas contienen niveles detectables de DEs no persistentes. Los compuestos más frecuentemente detectados fueron MeP (90.5%), BP-3 (75.0%), EtP (51.2%), n-PrP (46.4%) y BPA (41.7%), con concentraciones medianas que oscilaron entre <0.1 ng/mL para n-PrP, n-BuP y BP-1, y 0.6 ng/mL para BP-3. Además, se hallaron asociaciones positivas entre las concentraciones obtenidas con ciertas características del estilo de vida de la madre donante, especialmente factores relacionados con la dieta y el uso regular de determinados PCPs, tales como el enjuague bucal, el desodorante y la crema facial, entre otros. Los resultados obtenidos confirman que la leche materna contribuye a la exposición a DEs del niño prematuro a través de la vía alimentaria, evidenciando la necesidad de implementar sistemas de control para reducir 0la carga química en la leche de los Bancos de leche materna.

Por otro lado, se ha evidenciado la presencia de DEs en prendas textiles. Por ello, para explorar el tercer y último objetivo específico de esta tesis doctoral sobre la existencia de exposición a DEs a través de los textiles infantiles, se analizaron 32 pares de calcetines para bebés y niños pequeños de tres tiendas de Granada (España). El BPA estuvo presente en el 90.6% de las muestras en concentraciones medias que oscilaban entre <0.7 y 3,736.0 ng/g. En relación a PBs, se encontró EtP en el 100% de las muestras, seguido de MeP (81.0%) y PrP (43.7%). Se detectó actividad estrogénica en 13 de los 32 pares de calcetines analizados (40.6%; rango = 48.2-6,051.0 pM E_2Eq/g), en su mayoría

procedentes de una tienda local de bajo coste, mientras que la actividad antiandrogénica fue detectada en 6 de los 32 calcetines estudiados (18.7%; rango = 94.4-2,989 μM Proceq/g), todos de la tienda locales de bajo coste. La exposición dérmica estimada a BPA fue mayor en calcetines para niños de 36 a 48 meses (mediana = 17.6 pg/kg/día), y la exposición dérmica a PBs fue mayor en calcetines para niños de 24 a 36 meses (mediana = 0.6 pg/kg/día). Así, estos hallazgos confirman la exposición del niño a estos DEs a través de textiles infantiles.

En conjunto, los resultados obtenidos en esta tesis doctoral ponen de manifiesto la necesidad de abordar las implicaciones a corto, medio y largo plazo, de la exposición a DEs no persistentes sobre la salud de los recién nacidos, especialmente de aquellos extremadamente vulnerables. Además, requiere de la necesidad de adoptar medidas preventivas con carácter urgente.

2. LISTA DE ABREVIACIONES, ACRÓNIMOS Y SIGLAS

ACRÓNIMOS Y SIGLAS

AAP	Academia Americana de Pediatras
EEUU	Estados Unidos de América
EFSA	European Food Safety Authority
EMBA	European Milk Bank Association
OMS	Organización Mundial de la Salud
SCCS	Scientific Committee on Consumer Safety
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
UCIN	Unidad de Cuidados Intensivos Neonatales
UE	Unión Europea

ABREVIACIONES

ADN	Ácido desoxirribonucleico
AM	Media aritmética
BPs	Benzofenonas
BPA	Bisfenol A
BPA-D_{16}	Etilparaben marcado $^{13}C_6$
BPF	Bisfenol F
BPS	Bisfenol S
BPAF	Bisfenol AF
BPN	Recién nacido de bajo peso al nacer
BP-1	Benzofenona 1
BP-2	Benzofenona 2
BP-3	Benzofenona 3
BP-6	Benzofenona 6
BP-8	Benzofenona 8
BuP	Butilparabeno
COPs	Compuestos orgánicos persistentes
CV	Coeficiente de variación
DDT	Diclorodifeniltricloroetano
DDE	Diclorodifenildicloroetileno
DEs	Disruptores endocrinos
DEHF	Di-(2-etilhexil)ftalato
ECN	Enterocolitis necrotizante
EI	Impacto por electrones
EMA	Agencia Europea de Medicamentos
EPA	United States Environmental Protection Agency
E-Screen	Bioensayo de estrogeneicidad
ESI	Fuente de ionización por electropulverización
EtP	Etilparabeno
FBS	Suero bovino fetal

FD	Frecuencia de detección
GC-MS/MS	Cromatografía de gases acoplada a espectrometría de masas en tándem
hAR	Receptores de androgenos humanos
HCB	Hexaclorobenceno
IARC	Internacional Agency for Research on Cancer
IDA	Ingesta diaria admisible
IDT	Ingesta diaria tolerable
IMC	Índice de masa corporal
IV	Infusión intravenosa
LC-MS/MS	Cromatografía líquida con espectrofotometría de masas
LOD	Límite de detección
LOQ	Límite de cuantificación
MBPN	Recién nacidos de muy bajo peso al nacer
MeP	Metilparabeno
MeOH	Metanol
npDEs	Disruptores endocrinos no persistentes
NOAEL	Nivel sin efecto adverso
PALM	Bioensayo de anti-androgeneicidad
PBDE	Éter de difenilo polibromado
PBs	Parabenos
PCB	Bisfenilos policlorados
PCPs	Productos de cuidado personal
PHBA	Ácido parahidroxibenzoico
PHHA	Ácido p-hidroxihipúrico
Proc	Procimidona
PrP	Propilparabeno
PTFE	Politetrafluoroetileno
P25	Percentil 25
P75	Percentil 75
RN	Receptores nucleares
RSD	Desviación estándar relativa
SIDA	Síndrome de Inmunodeficiencia Adquirida
SRB	Sulforrodanina B
SRM	Monitorización de reacción única
TBBPA	Tetrabromobisfenol A
TCA	Ácido tricloroacético
UHPLC-MS/MS	Cromatografía líquida de ultra rendimiento: espectrometría de masas en tándem
VIH	Virus de la Inmunodeficencia Humana
ϒ-HCH	ϒ- hexaclorociclohexano
4-OH-BP	Benzofenona 4 (4-hidroxi-benzofenona)

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TABLAS

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4. INTRODUCCIÓN

4.1. Recién nacidos prematuros.

4.1.1. Definición y epidemiología

Atendiendo a la definición más aceptada de 'recién nacido prematuro', se incluyen bajo este término a todos aquellos neonatos cuyo nacimiento ocurre antes de las 34 semanas gestación. Se estima que aproximadamente el 10% de los nacidos vivos son prematuros, siendo la principal causa de mortalidad y morbilidad entre los niños menores de 5 años (Liu et al., 2012; Rysavy et al., 2015). En este sentido, los recién nacidos prematuros representan el 75% de las muertes perinatales [partos muy prematuros (entorno al 10% de los nacidos vivos), bajo peso (4-9% de los nacidos vivos con un peso < 2,500 g) y anomalías congénitas (26 por 1,000 nacimientos)] y más de la mitad de morbilidad perinatal (Blencowe et al., 2012; Goldenberg et al., 2008; McCormick, 1985). Se calcula que alrededor del 40% de todas las muertes neonatales se deben a bebés nacidos antes de las 28 semanas de edad gestacional (Ministerio de Sanidad, 2014).

El parto prematuro es una de las razones más comunes de hospitalización en la Unidad de Cuidados Intensivos Neonatales (UCIN) (Axelin et al., 2021), que suele ser inesperada y, generalmente, muy estresante tanto para los padres como para el propio recién nacido. El período de hospitalización en la UCIN puede variar en función de la patología y el tamaño del neonato. En cuanto al peso, los recién nacidos de muy bajo peso al nacer (MBPN) (<1,500 g) y los recién nacidos de bajo peso al nacer (BPN) (<2,500 g) requieren de un entorno de atención complejo para simular las condiciones en el útero hasta el correcto desarrollo de su piel inmadura y sus sistemas gastrointestinal, inmunológico, nervioso o respiratorio (Harrison and Goodman, 2015; Polanska et al., 2006).

Además, durante los últimos 20 años, a nivel global, se han registrado un aumento en las tasas de nacimientos prematuros. Cada año, aproximadamente 15 millones de bebés prematuros nacen en el mundo (Vogel et al., 2016). En el caso de EEUU, cada año 45,000 bebés con muy bajo peso al nacer requieren atención en una UCIN (Liu et al., 2021a), mientras que en España lo hacen 28,000 bebés, siendo una de las tasas más altas de Europa (APNP, 2018).

A lo largo del documento se usará el término neonato, ya que el período neonatal (desde el punto de vista asistencial) abarca los primeros 28 días de vida en los nacidos a

término y hasta las 46 semanas de edad postmenstrual en los nacidos prematuramente (Ministerio de Sanidad, 2014).

4.1.2. Características de los recién nacidos prematuros.

La expresión de prematuridad hace referencia a la inmadurez de todos los órganos y sistemas, materializada ésta como alteraciones o anomalías en su funcionamiento. Los problemas asociados a la prematuridad dependen de la edad gestacional y el peso del neonato en su nacimiento, acentuándose cuando son MBPN, ya que los órganos y sistemas pasan a desarrollarse en un ambiente completamente diferente al que tendrían dentro del útero materno. De hecho, cuanto mayor es su grado de inmadurez, más acusadas son las diferencias externas en comparación con un neonato a término (Aguilar-Cordero, 2005).

Las características físicas más destacadas que diferencian a un recién nacido prematuro de uno a término son (Aguilar-Cordero, 2005; GREYDANUS and PATEL, 2008; Pérez Rodríguez et al., 2001):

- Piel: es delgada, laxa y arrugada, con vasos sanguíneos visibles, lanugo en la cara y en los hombros, coloración rojiza y falta de arrugas en las plantas de los pies. La temperatura es inestable ante la poca grasa subcutánea y por la mayor superficie corporal en relación con el peso. El control metabolómico y la producción de calor son menores y existe un escaso control vasomotor del flujo sanguíneo hasta los capilares cutáneos.
- Tronco: El tórax es muy pequeño, mientras que el abdomen es redondeado, prominente y más voluminoso que el tórax y los nódulos mamarios no se encuentran o son muy pequeños.
- Miembros superiores e inferiores: son cortos en relación con el tronco, las uñas de las manos y de los pies son blandas y alargadas. La posición fisiológica de estos neonatos es la de decúbito supino, en posición de "rana" de los miembros inferiores y la cabeza hacia un lado.
- Cabeza: la del recién nacido prematuro es característica, ya que tiene un tamaño mayor en proporción al cuerpo, los ojos son prominentes y las orejas laxas, debido a que los cartílagos son todavía blandos.
- Sistema nervioso central: es inmaduro y presenta una actividad mínima.

- El llanto es débil y apagado, y se aprecian movimientos incordinados y asimétricos.
- Los reflejos de náuseas, deglución y succión son débiles, e incluso nulos, y algunos reflejos arcaicos no se manifiestan.
- Aparato respiratorio: suele ser uno de los órganos más afectados, puesto que la maduración de los alvéolos se inicia a partir de las 20 semanas de gestación y los músculos respiratorios se desarrollan posteriormente. La pared del tórax tiene poca estabilidad y la producción de líquido tensioactivo es mínima. Por ello, la respiración suele ser irregular, superficial, rápida y con tendencia a las respiraciones periódicas e incluso apnea (Eichenwald EC, 1995).
- Aparato excretor: el riñón es inmaduro, por lo que aumenta la excreción de sodio, que puede provocar una hiponatremia y también, es menor la acidificación de la orina. La inmadurez glomerular impide la retención de azúcares, proteínas, aminoácidos y sodio, lo que genera un déficit en sangre de estos compuestos.
- Sistema inmunológico: está poco desarrollado. Los niveles de IgM son nulos, ya que se transmiten por la placenta en los dos últimos meses de embarazo. La reacción de las células a los estímulos químicos, o quimiotaxis, es limitada y la fagocitosis inmadura.
- Aparato digestivo: es inmaduro, puesto que su desarrollo se inicia a las 20 semanas de gestación, aunque sus funciones no son eficaces hasta las 28-29 semanas de gestación (**Tabla 1**). Por ello, es habitual una escasa utilización de los recursos calóricos que estos bebés necesitan. La tolerancia digestiva es menor, así como la capacidad de absorber grasas y vitaminas liposolubles, especialmente la vitamina D. Es muy característica la enterocolitis necrotizante (ECN), que consiste en un síndrome de necrosis intestinal cuya incidencia es 3-7% de los recién nacidos ingresados en la UCIN (Aguilar-Cordero, 2003). Sin embargo, la leche humana es bien tolerada por estos niños, e incluso parece que favorece la maduración intestinal, pues algunas enzimas del tubo digestivo son fundamentales para la digestión y la absorción de la leche. La función hepática es incompleta, no sabe utilizar los azúcares, por lo que la

hipoglucemia es frecuente (Haninger and Farley, 2001). La formación de vitamina K es insuficiente, lo que se traduce en trastornos hemorrágicos. En este sentido, la tasa de crecimiento de los recién nacidos durante los primeros meses posparto debe ser alrededor de 15-25 g/día, es decir, entre 140-200 g/semana y de 0.8-1.0 cm de talla por semana durante los primeros tres meses. De los 3 a los 12 meses, la tasa puede oscilar entre 10-20 g/día y la talla 5-6 cm por mes. Esto requiere que los niños tengan una mayor tasa de consumo de calorías por kg de masa corporal que los adultos (Aguilar-Cordero, 2005; Smith et al., 2003).

Tabla 1. Desarrollo del tracto gastrointestinal del recién nacido prematuro

Órgano	Crecimiento y desarrollo	Gestación (semanas)	Peso (g)
Succión	*Movimiento aislado de la boca*	25	600-650
Deglución	*Succión y deglución inmaduras*	24-26	1,000-1,500
Estómago	*Formación de las glándulas gástricas*	14-16	110
	Motilidad y secrección gástricas	20-22	300-350
	Relación del píloro y del fundus	14-16	110
Páncreas	*Gránulos de cimógeno*	20-22	300-350
	Disimilitud de los tejidos endocrino y exocrino	14-16	110
Hígado	*Formación de los lobulillos*	11-12	14-20
	Metabolismo biliar	11-15	80-100
	Secrección biliar	20-22	300-350
Esófago	*Desarrollo de las glándulas del esófago*	20-22	300-350
	Aparición de las células escamosas	26-28	1,000-1,200
Intestino delgado	*Formación de vellosidades y de criptas*	14-16	110
	Desarrollo de los ganglios linfáticos	14-16	110
	Traslado de aminoácidos	14-16	110
	Conducción de glucosa	18-20	300-350
	Utilización y absorción de los ácidos grasos	22-24	600-1,000
Func. enzimático	*Lactasa, dipeptidasas, alfaglucosidasas*	10-12	14-20
	Enterocinasa	24-26	700-1,000

Fuente: Aguilar-Cordero et al., 2005

4.1.3. Factores relacionados con el ingreso de los neonatos en la UCIN

Existen muchos factores que pueden conllevar al ingreso de un recién nacido en la UCIN, pudiéndose resumir en factores relacionados con la madre, factores relacionados con el momento y las características del parto y factores relacionados propiamente con el recién nacido (SCH, 2022). Dichos factores aparecen recogidos en la **Tabla 2.**

Tabla 2. Factores por los que un neonato ingresa en la UCIN

Factores	Motivos
Maternos	Edad menor de 16 o mayor de 40
	Exposición a la droga o el alcohol
	Diabetes
	Hipertensión
	Hemorragias
	Enfermedades de transmisión sexual
	Embarazo múltiple
	Muy poco o mucho líquido amniótico
	Ruptura del saco amniótico
Del parto	Sufrimiento fetal /asfixia durante el parto
	Parto en presentación pelviana (de nalgas) u otra posición anormal
	Meconio
	Circular de cordón (cordón alrededor del cuello del bebé)
	Parto con fórceps o césarea
Del bebé	Nacimiento a la edad gestacional menor que 37 semanas o mayor a 42 semanas
	Peso de nacimiento menor de 2,500 g o más de 4,000 g
	Pequeño para la edad gestacional (Esto varía según el neonato)*
	Administración de medicamentos o reanimación en la sala de partos
	Defectos de nacimiento
	Dificultad respiratoria, incluidas la respiración rápida, los quejidos o la apnea
	Infección por herpes, estreptococo del grupo B, clamidia
	Convulsiones
	Hipoglucemia
	Necesidad de oxígeno adicional o monitoreo, terapia intravenosa (IV) o medicamentos
	Necesidad de tratamientos o procedimientos especiales

**De manera orientativa, en la semana 40 un neonato suele tener un tamaño de 51.2 cm y un peso 3,462 g, aproximadamente*

4.2. Unidades de Cuidados Intensivos Neonatales

Según la Ley 16/2003 de 28 mayo de 2003 y el Real Decreto 1277/2003 de 10 de octubre de 2014 (Ministerio de Sanidad, 2014), las Unidades Asistenciales de Neonatos son las organizaciones de profesionales sanitarios ubicadas en el hospital que, cumpliendo unos requisitos funcionales, estructurales y organizativos, ofrecen asistencia multidisciplinar de forma que garantizan las condiciones de seguridad, calidad y eficiencia adecuadas para atender las necesidades sanitarias asistenciales de los neonatos. Se trata de un área que, bajo la responsabilidad de un médico especialista en pediatría y sus áreas específicas, se realiza la atención del recién nacido con patología médico-quirúrgica, con compromiso vital, que precisa de medios y cuidados especiales de forma continuada. Dentro de estas unidades se pueden diferenciar una sección (cuidados

intermedios) que, de nuevo bajo la responsabilidad de un médico especialista en pediatría y sus áreas específicas, se realiza la atención del recién nacido de edad gestacional superior a 32 semanas o peso superior a 1,500 g con patología leve que necesita técnicas especiales de cuidados medios.

4.2.1. Características de una UCIN

Las UCIN deben localizarse dentro del Área Materno Infantil del Hospital y en proximidad física con el Bloque Obstétrico y la Unidad de Maternidad Hospitalaria. Ambas unidades deben compartir el mismo nivel de planta, facilitando el traslado urgente de pacientes, en caso necesario, de manera rápida e independiente de medios mecánicos. En el caso de que la localización de las unidades mencionadas se diera en plantas diferentes, debe existir un bloque de ascensores específicos, con capacidad adecuada a la demanda de traslados urgentes de pacientes (Ministerio de Sanidad, 2014). A su vez, deben disponer de una ubicación próxima al Bloque Quirúrgico de Pediatría/Cirugía Neonatal, Unidad de Medicina Fetal (seguimiento embarazos de alto riesgo), Unidad de Pediatría y Urgencias (Ministerio de Sanidad, 2014).

Internamente, las UCIN deben disponer de zonas diferenciadas (zona de acceso público, zona clínica de atención al neonato, zona de estar y descanso de los padres, zona de apoyo a la actividad clínica, zona de personal y zona de servicios centrales), siendo el área clínica de atención al neonato la zona central de la Unidad (en términos funcionales y físicos) y debe estar comunicada con el resto de las zonas de la misma.

4.2.2. Instalaciones

La forma y proporciones de los diferentes locales incluidos en cada una de las zonas que definen la Unidad de Neonatología no deben ser muy diferentes del cuadrado o rectángulo de proporciones 1:1.5 m. La superficie útil para cada puesto de cuidados intensivos (en box individual o en sala múltiple) no debe ser inferior a 16 m², aunque lo más recomendado es de 20 m², incluyendo el espacio para el lavado del neonato y el frigorífico destinado al almacenamiento de alimento (leche). Además, se considera necesaria una superficie útil mínima de 20 m^2, para el box individual/de aislamiento, considerando las necesidades derivadas de la puerta de acceso y circulación interior, así como el lavabo y preparación de medicamentos.

La superficie de cada puesto de incubadora debe considerar:

– necesidades derivadas de la atención del recién nacido, entre las que hay que incluir aspectos como instalación para el lavado de manos clínico o de preparación de medicamentos.

– la localización de equipos de soporte vital, atención en situación crítica, equipos portátiles (radiología).

– necesidades derivadas del desarrollo de la atención centrada en la familia (como un sillón reclinable, cómodo, exclusivo para cada puesto).

- disponer de frigorífico para alimentación (leche), espacio para los efectos personales del neonato y pequeño depósito para residuos.

– necesidades derivadas de circulación y almacenamiento.

En la **figura 1**, se muestra un plano de la distribución general de una UCIN.

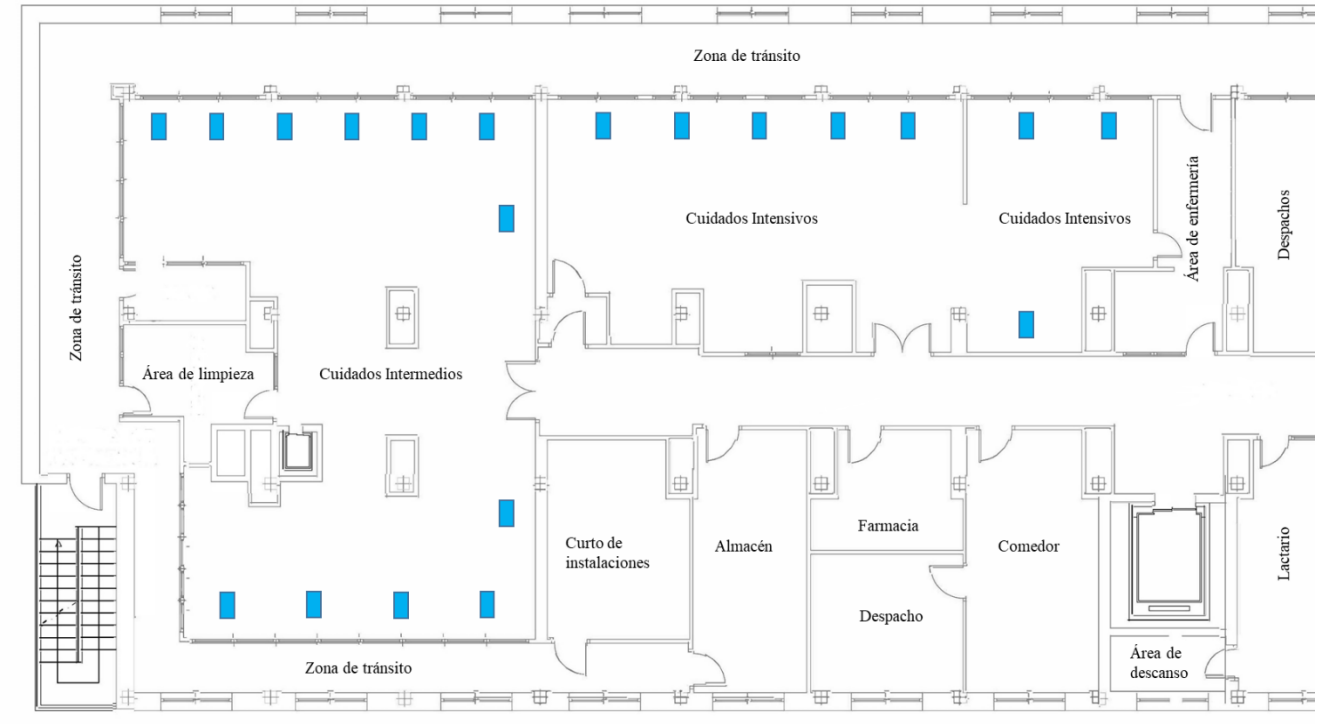

Figura 1. Distribución general de una UCIN

4.2.3. Instrumental médico en la UCIN

Existe una gran variedad de equipos y materiales en la UCIN, algunos son comunes para otras áreas de los hospitales y otras especiales para esta unidad (**Tabla 3**) (Gracia et al., 2013; SCH, 2022).

Tabla 3. Instrumental médico en una UCIN

Material	Categoría	Disposición
Bombas de infusión enteral	Alimetación	Box
Cánula y jeringa alimentación	Alimentación	Box
Sonda alimentación enteral	Alimentación	Box
Fototerapias	Cutáneo	Box
Cunas (de calor radiante…)	Descanso	Box
Incubadoras	Descanso	Box
Analizador (pH, gases, iones, hematocrito, glucemia...)	General	Unidad
Bilirrubinómetro transcutáneo	General	Unidad
Bombas de infusión intravenosa	General	Box
Calentador de fluidos	General	Unidad
Carro de parada	General	Unidad
Sensor monitorización	General	Box
Sala de aislamiento con flujo de aire directo e invertido	General	Unidad
Analgésicos, antihistamínicos y sedantes	Medicación	Carro de parada
Betabloqueantes y antihipertensivos	Medicación	Carro de parada
Drogas cardiovasculares	Medicación	Carro de parada
Aparato portátil de radiografía	Radiología	Unidad
Ecógrafo con sonda neonatal/ Doppler	Radiología	Unidad
Potenciales evocados visuales, auditivos y somatosensoriales	Radiología	Unidad
Equipo de hipotermia activa	Regulación de temperatura	Unidad
Monitor de temperatura	Regulación de temperatura	Box
Alargadera sistema infusion (venosa, intravenoso…)	Sondajes	Box
Catéter (vascular, umbilical venoso…)	Sondajes	Box
Jeringa de medicación	Sondajes	Box
Desfibrilador	Soporte cardiaco	Unidad
Electrocardiógrafo	Soporte cardiaco	Box
Marcapasos externo	Soporte cardiaco	Unidad

Tabla 3. Instrumental médico en una UCIN (continuación)

Material	Categoría	Disposición
Monitores de presión invasiva	Soporte cardiaco	Box
Electroencefalografía convencional	Soporte neurológico	Unidad
Monitor función cerebral (EEGa)	Soporte neurológico	Unidad
Monitor presión intracraneal	Soporte neurológico	Unidad
Cribado auditivo	Soporte sensorial	Unidad
Protección nasal	Soporte sensorial	Unidad
Adaptador nasal oxigeno	Soporte respiratorio	Unidad
Aspirador de secrecciones	Soporte respiratorio	Unidad
Bolsa autoinflable tipo ambú	Soporte respiratorio	Unidad
Capnógrafo	Soporte respiratorio	Unidad
CPAP nasal	Soporte respiratorio	Unidad
ECMO	Soporte respiratorio	Unidad
Laringoscopios	Soporte respiratorio	Unidad
Mascarilla laríngea neonatal	Soporte respiratorio	Unidad
Mezclador aire-oxígeno	Soporte respiratorio	Unidad
Monitores FC-ECG-respiración	Soporte respiratorio	Unidad
Monitorización transcutánea O_2-CO_2	Soporte respiratorio	Unidad
Pulsioxímetro	Soporte respiratorio	Unidad
Respiradores de alta frecuencia	Soporte respiratorio	Unidad
Respiradores para recién nacidos	Soporte respiratorio	Unidad
Sensor pulsómetro	Soporte respiratorio	Unidad
Sistemas de administración NO inhalado	Soporte respiratorio	Unidad
Sistema aspiración tubo endotraqueal	Soporte respiratorio	Unidad
Sonda oxígeno	Soporte respiratorio	Unidad
Tubo endotraqueal	Soporte respiratorio	Unidad
Apósito (vía, epicutáneo, transparente…)	Vendas	Box
Gasas (estériles, no estériles…)	Vendas	Box

4.3. Banco de Leche Materna

La leche materna se considera la opción nutricional óptima para recién nacidos y lactantes, la cual se ha demostrado que juega un papel fundamental durante los primeros 1000 días de vida en la salud y desarrollo de un niño (DiMaggio et al., 2022). La Organización Mundial de la Salud (OMS) recomienda la lactancia exclusiva durante seis meses y la lactancia materna continua hasta dos años o más junto con la nutrición complementaria (Fonseca et al., 2021; OMS., 2003.). Sin embargo, algunos recién nacidos no tienen acceso a la leche materna en el período neonatal temprano. Existen, además otras razones para la no disponibilidad de leche materna como son: la presencia de VIH positivo o cáncer de mama en la madre (Murguia-Peniche and Kirsten, 2014; Zanganeh et al., 2021); la ausencia por enfermedad, muerte o abandono; o su incapacidad para producir suficiente leche o leche con suficiente contenido graso, entre otras causas (Tran et al., 2021; Tran et al., 2020).

Cuando los bebés prematuros no pueden recibir leche materna de sus madres, incluidas las ingresadas en una UCIN, la OMS y la Academia Americana de Pediatras (AAP) recomiendan la administración de leche humana pasteurizada de un banco de leche en lugar de fórmula infantil artificial (AAP, 2012; UNICEF, 2003).

4.3.1. Qué son los Bancos de Leche Materna y su historia

Los bancos de leche materna son instituciones sanitarias especializadas y responsables de la promoción y apoyo a la lactancia materna (Shenker et al., 2021), donde la leche donada por madres seleccionadas se recibe, procesa, analiza, almacena y posteriormente, distribuye a los centros hospitalarios para alimentar a recién nacidos hospitalizados que por cualquier razón no disponen de leche de su propia madre o son bebés prematuros (Klotz et al., 2021). La creación de Bancos de leche permite garantizar especialmente la alimentación con leche materna, al tiempo que supone un gran apoyo para las estrategias de fomento de la lactancia materna, contribuyendo así a una mejora de la salud materno-infantil en la población general (AEBLH, 2022).

A principios del siglo XX se pusieron en funcionamiento los primeros bancos de leche en Europa y Estados Unidos, dado el contexto social en el que existía un número creciente de huérfanos por guerras y, una disminución de las nodrizas por su incorporación al mercado de trabajo y gracias al desarrollo de la tecnología de alimentos. El primero de ellos se creó en Viena en el año 1909, pero no fue hasta mediados de siglo cuando tuvo lugar una primera expansión de los Bancos de Leche en Europa, Norteamérica y otros países como Brasil (AEBLH, 2022).

En la década de los ochenta, la aparición del SIDA y la constatación del paso del virus VIH a través de la leche, supuso un freno muy importante en el desarrollo de los bancos de leche, haciendo que muchos de ellos cerraran. Una década después tiene lugar una segunda expansión, especialmente en los países desarrollados, en base a las amplias investigaciones acerca de la seguridad en los procesos de pasteurización y congelación de la leche, así como de los beneficios de la leche humana, de madre o donada. El 15 de octubre de 2010 fue creada la Asociación Europea de Bancos de Leche Materna (EMBA), la cual se encarga de promover los bancos de leche en Europa y fomentar la cooperación internacional entre los bancos de leche humana de los países de Europa (AEBLH, 2022).

En España el primer Banco de leche se creó en el año 2001 en Palma de Mallorca, incluido en la Fundación Banco de Sangre y Tejidos de las Islas Baleares. En el año 2007

tuvo lugar la apertura del segundo Banco de leche, en este caso adscrito a una Unidad de Neonatología en el Hospital 12 de octubre de Madrid. Según la EMBA, actualmente hay 280 bancos de leche humana ubicados en 26 países europeos (EMBA, 2021) y concretamente, en España existen 16 centros en activo, con diferentes modelos: ubicados en las propias Unidades de Neonatología, integrados en los Centros de Donación de Sangre y Tejidos, y modelos mixtos (AEBLH, 2022).

4.3.2. Regulación de los Bancos de Leche Materna

En un banco de leche, todos los procesos deben garantizar la seguridad, la calidad y la trazabilidad de la leche materna donada y procesada (AEBLH, 2022). La EMBA encontró que los procedimientos operativos para el funcionamiento de los bancos de leche humana de donantes varían ampliamente entre los países europeos (Kontopodi et al., 2021). En parte, estas diferencias pueden deberse a la falta de regulación en conjunto, así como a las diferentes regulaciones en cada país. Esto supone un problema, ya que a pesar de que cada vez hay una mayor tendencia a la creación de nuevos bancos, en los últimos años se ha extendido la distribución ilegal de leche materna a través de Internet. Esta práctica expone a los receptores a importantes riesgos de salud, ya que reciben leche materna sin control de donantes ni del procedimiento. Los desafíos detallados anteriormente, entre otros, han llevado a un marco legislativo del uso de donaciones de leche materna, para evitar cualquier daño potencial tanto para los donantes como para los receptores (European Commission, 2019).

La Comisión Europea ha catalogado a la leche materna como una sustancia que no está regulada o está regulada de manera divergente (European Commission, 2019), lo que puede dar lugar a niveles de protección inferiores para los donantes y receptores de estos tratamientos en comparación a los de los donantes y receptores de otras sustancias de origen humano (European Commission, 2019). Por ello, recientemente se ha añadido un nuevo capítulo sobre las donaciones de leche materna a la Guía sobre la calidad y seguridad de tejidos y células para aplicaciones humanas publicada en 2019 por la Dirección Europea de Calidad de Medicamentos y Atención Sanitaria (EDQM, 2019). Además, los procedimientos para emitir un marco legislativo para donaciones de leche materna para los estados miembros de la Unión Europea están actualmente en curso (European Commission, 2021).

Desde un punto de vista práctico regulatorio las autoridades competentes en materia de supervisión, así como los proveedores de atención médica dependen de un marco claro que guía las buenas prácticas de fabricación, la armonización y la estandarización al usar donaciones de leche materna o establecer bancos de leche materna (Kostenzer, 2021). Este marco, sin embargo, está sujeto a la catalogación que cada país establece para esta leche materna. En este sentido, mientras países como Francia han catalogado a la leche materna como un producto médico de origen humano, otros países como Alemania, Italia o Dinamarca los consideran como un producto alimentario (o similar). Sin embargo, la mayoría de los países europeos o bien no tienen establecidos bancos de leche materna (es el caso de Irlanda) o bien la leche materna no está regulada (Klotz et al., 2021) (**Figura 2**).

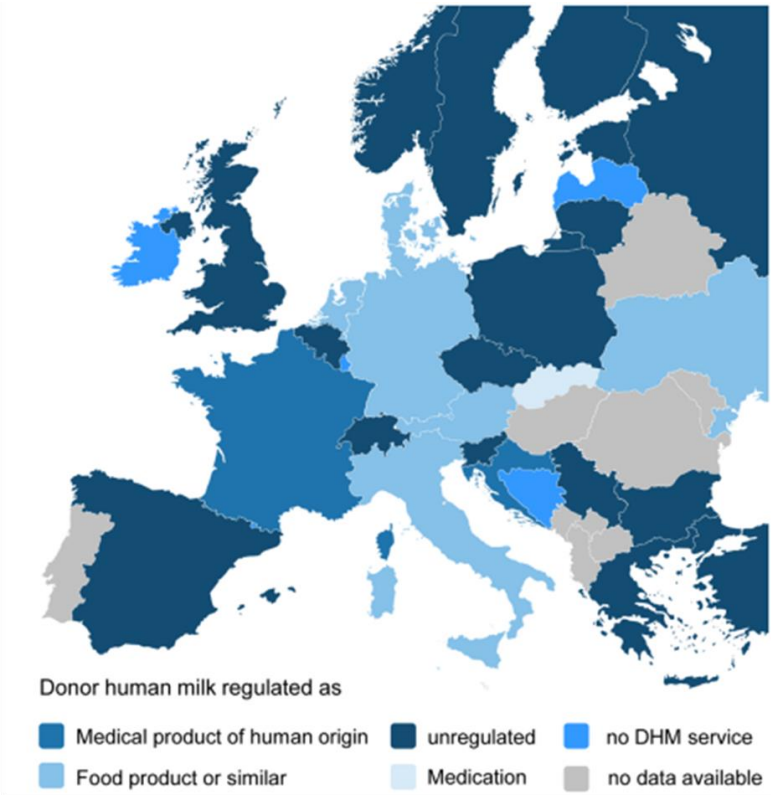

Figura 2. Clasificación de la leche humana de donante dentro de los países europeos.
Fuente: Klotz et al., 2021

Esta heterogeneidad en la regulación puede impedir la entrega de donaciones de leche materna entre diferentes jurisdicciones (internacional o nacional), lo que podría limitar su fuente de suministro y acceso para aquellas comunidades o países que actualmente no operan sus propios servicios de leche de donante (Klotz et al., 2021).

El uso de la leche humana en niños con prematuridad extrema ha sido etiquetado y percibido no solo como fuente de nutrición sino también como tratamiento médico

(WHO, 2017). En este contexto, Klotz et al. (2021) sostiene que hay una notable falta de directrices médicas que describen y orientan las respectivas normativas en torno al uso de la leche humana para bebés prematuros. Adicionalmente, el número de bancos de leche materna parece ser limitado y desigualmente distribuido dentro de cada uno de los países europeos de manera individual y en Europa en su conjunto lo que puede resultar en un acceso limitado a donaciones de leche materna en comparación con el número potencial de bebés que la requieren (Klotz et al., 2021). Una regulación y clasificación armonizada, así como normativas nacionales que implementen recomendaciones inequívocas en la política puede ser beneficiosa para una mayor implementación de donaciones de leche materna servicios en toda Europa.

4.3.3. Procedimientos y características de un Banco de Leche Materna

La leche materna es una sustancia fácilmente perecedera que puede deteriorarse durante su manipulación, pudiendo servir como vector de posibles sustancias orgánicas e inorgánicas peligrosas para los lactantes en riesgo (Blackshaw et al., 2021). Una donación implica un proceso que es impulsado por un motivo altruista y en base a una decisión informada y autónoma de mujeres y padres que no deben ser influenciados por ningún incentivo rentable (Miracle et al., 2011). Estas características de la leche humana de donante explican en parte su disponibilidad limitada, lo que ha contribuido a la mercantilización y comercialización en curso de la leche humana (Newman and Nahman, 2020).

En líneas generales, los procedimientos principales que se llevan a cabo dentro de un Banco de leche se podrían resumir en las siguientes actuaciones:

- Selección de las donantes y seguimiento de la donación.
- Recolección y conservación de la leche donada.
- Pasteurización y realización de análisis de control de calidad y microbiológicos de las leches donadas.
- Distribución de la leche donada a los centros hospitalarios.
- Administración de leche pasteurizada a recién nacidos hospitalizados bajo prescripción médica.

En cuanto a las características generales que deben cumplir las mujeres que deseen participar en un Banco de Leche materna, todas ellas deben tener la lactancia correctamente establecida. En este sentido, se recomienda esperar al menos 3 semanas

desde el parto para iniciar la donación. Además, deben encontrarse en alguno de los siguientes supuestos:

- Ser mujeres sanas con un estilo de vida saludable, que lactan satisfactoriamente a su hijo y que deciden, además donar su leche de forma altruista.
- Ser mujeres cuyos hijos están ingresados en las Unidades de Neonatología, y que tienen más leche de la que sus hijos necesitan.
- En las situaciones especiales como la muerte intrauterina del feto, del recién nacido o lactante, cada vez son más los bancos de leche que aceptan la posibilidad de recibir leche si la mujer así lo desea.

Por su parte, para ser receptor de esta leche donada, los sujetos deben reunir alguna de las siguientes características:

- Recién nacidos prematuros que han nacido antes de la semana 32 de gestación o con un peso inferior a 1,500 gramos.
- Recién nacido con riesgo de padecer ECN.
- Recién nacidos sometidos a cirugía abdominal, o con cardiopatías congénitas.

4.3.4. Actuación de un Banco de Leche Materna.

4.3.4.1. Donación de la leche materna.

A todas las mujeres que expresan su deseo de convertirse en donantes se le realiza una entrevista personal previa a la primera donación en la que se obtienen datos sobre el estado de salud y hábitos de vida, y un estudio serológico para descartar enfermedades potencialmente transmisibles a través de la leche como la sífilis, VIH, hepatitis B y C y según protocolos particulares para Chagas y HTLV.

El proceso de recogida de la leche materna puede tener pequeñas variaciones de unos centros a otros, pero en términos generales la madre donante se encarga de extraer la leche en su domicilio. El Banco de Leche les ofrece gratuitamente todo el material necesario para la extracción de la leche [sacaleches (manual o eléctrico), biberones, y nevera para el transporte hasta el banco]. En su domicilio, las donantes recogen la leche con el sacaleches, sin que haya un momento exacto de la recogida. Cada una de las tomas es recogida en un mismo biberón hasta que esté es completado, creando un pool de leche. Cada biberón debe ir marcado cada uno de ellos con su etiqueta correspondiente, en la que se indican: número de la donación, edad gestacional, número de historia clínica, fecha

del parto, fecha de extracción y volumen de extracción. Una vez recogida esta información, deben congelarse hasta el momento en el que se trasladen al banco. La madre donante tiene un plazo máximo de 4 semanas para llevar al banco de leche las muestras que haya podido recoger hasta ese momento. El personal del hospital recoge las muestras y completaran los datos restantes de las etiquetas (fecha y hora de recogida en el banco).

Figura 3. Imagen de las diferentes etiquetas, identificativa (izquierda) y datos de pasteurización (derecha) de las donaciones de leche materna.

4.3.4.2. Procesamiento de la leche materna.

Para garantizar la seguridad microbiológica de la leche donada es necesario eliminar las bacterias y virus contaminantes. Actualmente, el tratamiento térmico utilizado de manera generalizada por los Bancos de Leche es el método Holder, un método que ofrece un buen equilibrio entre la seguridad microbiológica y la preservación de la calidad nutricional/biológica de la leche (Caballero Martín et al., 2021). Sin embargo, se están realizando esfuerzos para implementar nuevas metodologías de pasteurización (Czank et al., 2010; Permanyer et al., 2010; Picaud and Buffin, 2017).

La metodología actual consiste en someter a la leche materna en un calentamiento rápido hasta alcanzar los 62.5ºC, seguido de una etapa "meseta" a 62.5ºC durante 30 minutos y a continuación, una fase de enfriamiento rápido hasta alcanzar los 4ºC (**Figura 4**). Para que el proceso se considere efectivo y seguro, los cambios de temperatura han de ser homogéneos. Los dispositivos empleados con mayor frecuencia son pasteurizadoras con calentamiento por aire o por agua. Este sistema permite destruir todos los microorganismos patógenos no esporulados y las partículas virales que pudieran estar presentes en la leche.

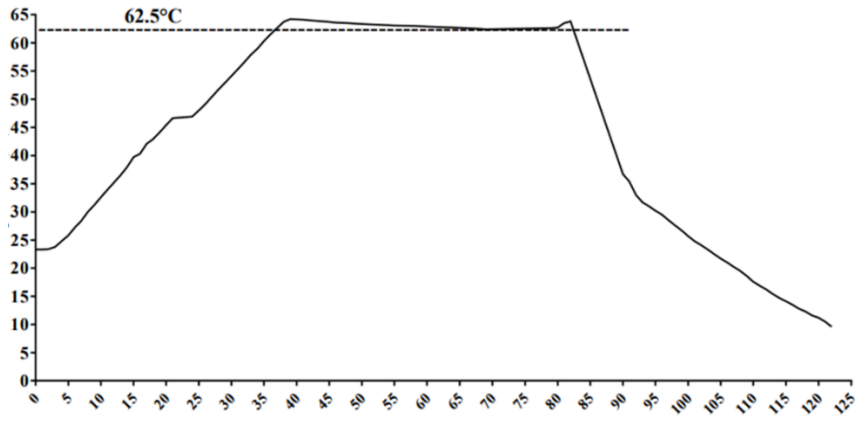

Figura 4. Curva de tiempo-temperatura para el método de pasteurización de Holder

Asimismo, durante el procesamiento de la leche se analiza el contenido nutricional (proteínas, grasa, lactosa y energía) y el nivel de acidez (acidez Dornic). El contenido de nutrientes y aminoácidos de la leche materna varía, dependiendo de las semanas de gestación y la edad postnatal del recién nacido de la donante. Todo esto permite seleccionar la leche nutricionalmente más adecuada para los recién nacidos que la recibirán, haciendo un suministro personalizado de leche materna, permitiendo ser la estrategia más beneficiosa para la nutrición de recién nacidos prematuros con necesidades diferentes (Arslanoglu et al., 2013). Por último, la leche se congela y se almacena hasta su distribución.

Finalmente, es importante destacar que solo los Bancos de Leche Humana disponen en la actualidad de controles que garantizan la seguridad y calidad de la leche materna donada, incluyendo (AEBLH, 2022):

- La estricta selección de donantes
- La recogida y el almacenamiento de la leche en condiciones óptimas.
- Su pasteurización y análisis nutricional previo al consumo.
- La trazabilidad del procedimiento desde la donante hasta el receptor.

4.4. Disruptores endocrinos

El sistema endocrino es una red de glándulas y órganos que producen y liberan hormonas, las cuales actúan como mensajeros químicos entre las diferentes partes del cuerpo. Cada hormona se dirige a un tipo (o grupo) particular de células capaces de recibir y responder incluso a dosis muy bajas, induciendo diferentes efectos en estos órganos y tejidos, tales como el crecimiento y el desarrollo, cambios en la función sexual o la

reproducción. Por esta razón, la homeostasis de la acción hormonal está estrictamente regulada en cualquier organismo. Pequeñas perturbaciones en los niveles pueden implicar graves consecuencias para la salud (Neal, 2016).

Cada vez es más conocida la presencia y el efecto de sustancias químicas de origen sintético que imitan el comportamiento de las hormonas, alterando el equilibrio del sistema endocrino (Dekant and Colnot, 2013). En este sentido, la Organización Mundial de la Salud (OMS) ha establecido la definición de disruptor endocrino (DE) como: "cualquier sustancia exógena o mezcla que altera la función del sistema endocrino y, por tanto, causa efectos adversos sobre la salud en un organismo intacto, en su progenie, o en la población" (WHO/PCS/EDC/02.2, 2002).

Por tanto, la disrupción endocrina se presenta como el efecto adverso derivado de la exposición a los DEs, cuyas características principales descritas hasta la fecha son (Anway and Skinner, 2006; Kortenkamp et al., 2007; Skinner and Guerrero-Bosagna, 2009; Soto et al., 1992):

- Actúan a concentraciones bajas y de forma combinada con las hormonas endógenas, por lo que es difícil establecer un nivel umbral de no efecto, lo que confiere a los DEs una especial peligrosidad ya que no existen dosis seguras.
- Cuando la exposición ocurre durante períodos del desarrollo del individuo con especial vulnerabilidad a la disrupción endocrina – embarazo, infancia, pubertad o lactancia- provocan daños que pueden manifestarse más tarde a lo largo de toda la vida.
- Las curvas que relacionan dosis de exposición con el efecto adverso no son lineales, es decir, la respuesta no siempre aumenta en la misma proporción que la dosis de exposición, y puede que tampoco sean monotónicas, por lo que puede cambiar el signo creciente/decreciente de la respuesta, al igual que se observa en las curvas dosis-efecto de las hormonas endógenas.
- Generalmente, los individuos no están expuestos a un solo tipo de DEs, sino a las mezclas de ellos (cóctel), por lo que los efectos son difícilmente predecibles dadas las posibles acciones sinérgicas, aditivas o antagónicas entre los residuos químicos.
- Como resultado de la exposición a DEs en un determinado individuo se pueden observar consecuencias en generaciones posteriores, ya sea por afectación genómica o mecanismos epigenéticos.

- Los efectos observados tras la exposición pueden ocurrir tras largos períodos de latencia, lo que distancia la exposición del efecto consecuente, y dificulta en gran medida el establecimiento de una asociación casual.

4.4.1. Clasificación de los disruptores endocrinos

Actualmente, el término DE define un grupo de sustancias químicas muy heterogéneo, que a priori puede considerarse que no tienen ninguna similitud estructural. Sin embargo, todos los DEs son moléculas con masa molecular pequeña (<1,000 Daltons) que frecuentemente, contienen sustituciones halogenadas (flúor, cloro o bromo), con un anillo fenólico que se cree que coincide con muchas de las hormonas naturales y, por lo tanto, estos compuestos son capaces de interactuar con los receptores nucleares (Diamanti-Kandarakis et al., 2009).

Existen múltiples clasificaciones para los DEs, atendiendo a diferentes características como su producción, su origen, estructura química, su actividad, persistencia en el medio, etc. Según su origen, los DEs pueden estar presentes de forma natural en plantas y/o animales, o bien puede tratarse de compuestos químicos generados en la industria (Marty et al., 2011). Dentro del grupo de los compuestos naturales, se distinguen entre los de procedencia animal y de procedencia vegetal (como los fitoestrogenos o los micoestrogenos). Algunos de estos compuestos no presentan la estructura típica y, sin embargo, muestran actividad hormonal, si bien este efecto puede ser agonista o antagonista dependiendo de las concentraciones tisulares alcanzadas y de los niveles de hormona endógenos.

La resistencia a la degradación física, química y biológica, así como su grado de liposolubilidad, son predictores útiles del comportamiento ambiental y biológica de estos compuestos. En este sentido, los DEs se clasifican en:

- Compuestos orgánicos persistentes (COPs): Estas sustancias persistentes fueron diseñadas para tener vidas medias largas para persistir en el suelo y, por lo tanto, se metabolizan muy lentamente en los organismos vivos, e incluso pueden descomponerse en compuestos más tóxicos que la molécula original. Debido a su persistencia y lipofilicidad, los COPs tienden a bioacumularse y biomagnificarse en la cadena alimentaria, dando como resultado exposición considerable de

organismos vivos (Arrebola et al., 2013; Botella et al., 2004; CDC., 2009.; Cerrillo et al., 2006; Fernandez et al., 2008; Frenich et al., 2000; Lopez-Espinosa et al., 2009; Schettgen et al., 2015). Comprenden una amplia variedad de plaguicidas organoclorados [incluido el diclorodifeniltricloroetano (DDT) y su metabolito diclorodifenildicloroetileno (DDE), hexaclorobenceno (HCB) y γ-hexaclorociclohexano (γ-HCH)]; bifenilos policlorados (PCB), dioxinas, furanos y éteres de difenilo polibromados (PBDE) utilizados como retardantes de llama. Además, también incluye compuestos perfluorados, como el ácido perfluorooctanoico (PFOA), entre otros (Glüge et al., 2020; Serrano et al., 2021), y algunos metales pesados como el mercurio (Porta et al., 2008a). La principal ruta de exposición a estos químicos es a través de la ingesta dietética (Porta et al., 2008b), mientras que las vías de exposición ambiental, como la exposición dérmica, representan menos del 2% de la exposición para la mayoría de ellos (Linares et al., 2010).

- Compuestos orgánicos no persistentes (npDEs). Son ubicuos en el ambiente y se eliminan del organismo de forma rápida, ya que son menos liposolubles que los COPs y, por lo tanto, tienden a ser metabolizados y excretados por la orina (Frederiksen et al., 2007; Søeborg et al., 2014), actuando durante periodos de tiempo cortos. Comprenden familias de compuestos tales como: bisfenoles, parabenos (PBs), benzofenonas (BPs) y ftalatos, entre otros. Las principales vías de exposición son la alimentaria y la dérmica, aunque también la oral no dietética e inhalatoria.

4.4.2. Compuestos orgánicos no persistentes

Esta tesis doctoral se centra principalmente en los npDEs y específicamente, en las familias de bisfenoles, PBs y BPs.

4.4.2.1. Bisfenoles

Los bisfenoles son compuestos aromáticos constituidos por dos anillos fenólicos que se unen a través de un grupo puente. El congénere más conocido de esta familia es el bisfenol A (BPA), aunque en la actualidad y debido a la regulación derivada de sus demostrados efectos en salud está siendo sustituido progresivamente por otros análogos

de la misma familia, entre los que se incluyen: bisfenol F (BPF), bisfenol S (BPS) y derivados halogenados como el bisfenol AF (BPAF) y tetrabromobisfenol A (TBBPA).

En la **figura 5**, se muestra la estructura química de algunos de los diferentes congéneres de la familia de los bisfenoles:

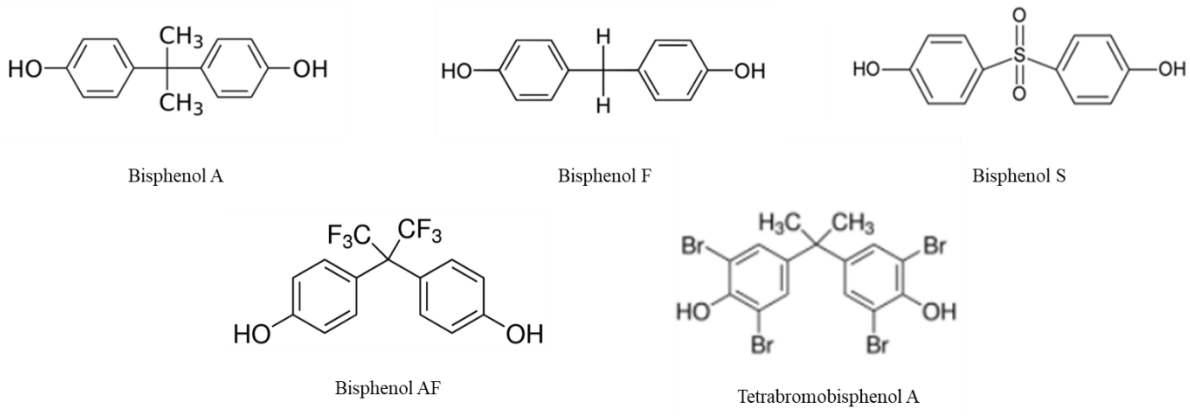

Figura 5. Estructura química de bisfenoles

Además, en la **tabla 4**, se muestran algunas de sus características principales, tales como su fórmula química y su peso molecular:

Tabla 4. Congéneres de bisfenoles

Sustancia	Abreviatura	Nombre	CAS	Fórmula	Peso molecular (g/mol)
Bisfenol A	BPA	Bisfenol A	80-5-7	$C_{15}H_{16}O_2$	228.29
Bisfenol F	BPF	Bisfenol F	620-92-8	$C_{13}H_{12}O_2$	200.23
Bisfenol S	BPS	Bisfenol S	80-09-1	$C_{12}H_{10}O_4S$	250.27
Bisfenol AF	BPAF	4,4´-(hexafluoroisopropilideno)difenol	1478-61-1	$C_{15}H_{10}F_6O_2$	336.23
Tetrabromobisfenol A	TBBPA	Tetrabromobisfenol A	79-94-7	$C_{15}H_{12}Br_4O_2$	543.87

El BPA [2, 2-bis (4-hidroxifenil) propano], se utiliza principalmente en la fabricación de policarbonato plásticos y resinas epoxi, que representan el 65% y el 30% de la producción de BPA, respectivamente (Xing et al., 2022). Su uso para recubrir los interiores de envases metálicos para alimentación, lo convierten en uno de los compuestos más producidos (Liu et al., 2021b). Además, teniendo en cuenta su empleo en otros materiales tales como el papel térmico, polisulfanato, dispersantes de colorantes, aditivos de fibra, dispositivos médicos y productos electrónicos, se prevé que la producción anual supere los 10 millones toneladas para 2022 (Huelsmann et al., 2021). El BPA ha sido

clasificado como tóxico para la reproducción de categoría 1B debido a la evidencia científica disponible en cuanto a sus efectos para la salud en general y a sus efectos reproductivos en particular (Xing et al., 2022). Por ello, ya en 2010, América del Norte prohibió la importación y venta de biberones de policarbonato que contuvieran BPA (Government of Canada, 2010). Un año más tarde, en 2011, la Unión Europea (UE) también prohibió el uso de BPA en biberones para bebés (European Commission, 2010). Fue entonces cuando en la UE y China se procedía a reemplazar el BPA, desarrollando y produciendo análogos de BPA como el bisfenol S (BPS), bisfenol F (BPF) (Frankowski et al., 2020a; Frankowski et al., 2020b).

Aún hay muy poca información sobre el efecto de estos análagos del BPA en la salud humana. Sin embargo, dado que BPS y BPF son estructuralmente similar al BPA, numerosos estudios han identificado que sean DEs y, por tanto, también podrían tener el mismo efecto tóxico que el BPA (Rochester and Bolden, 2015). En este sentido, son algunos los estudios publicados donde se relacionan con efectos adversos para la salud, tales como obesidad (Reina-Pérez et al., 2021), calidad espermática (Chen et al., 2022), trastornos de déficit de atención e hiperactividad (TDAH) (Kim et al., 2022) o criptorquidia en niños (Komarowska et al., 2021).

4.4.2.2. Parabenos

Los PBs son una familia de ésteres alquílicos del ácido p-hidroxibenzoico que incluyen cuatro cóngeneres principales: metilparabeno (MeP), etilparabeno (EtP), propilparabeno (PrP) y butilparabeno (BuP). Se trata de sustancias químicas ampliamente utilizadas como conservantes en muchos productos de consumo, tales como productos de salud y cuidado personal y alimentos (Darbre and Harvey, 2008; Fransway et al., 2019; Iribarne-Durán et al., 2020; Liao et al., 2013a; Liao et al., 2013b). En la **figura 6** y **tabla 5** se muestra la estructura química y características principales de los diferentes congéneres de PBs:

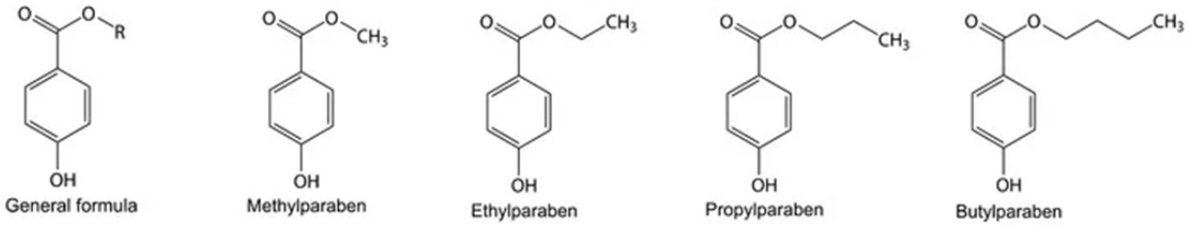

Figura 6. Estructura química de PBs

Tabla 5. Congéneres de PBs

Sustancia	Abreviatura	Nombre	CAS	Fórmula	Peso molecular (g/mol)
Metilparaben	MeP	Methyl 4-hydroxybenzoato	99-76-3	$C_8H_8O_3$	152.15
Etilparaben	EtP	Ethyl 4-hydroxybenzoato	120-47-8	$C_9H_{10}O_3$	166.17
Propilparaben	PrP	Propyl 4-hydroxybenzoato	94-13-3	$C_{10}H_{12}O_3$	180.21
Butilparaben	BuP	Butyl 4-hydroxybenzoato	94-26-9	$C_{11}H_{14}O_3$	194.23

Los PBs están regulados como conservantes en el Reglamento (UE) n.º 1004/2014 de la Comisión sobre productos cosméticos que establece un límite máximo del 0.4 % para ésteres simples y el 0.8 % mezclas de ésteres (EFSA, 2015b; Gálvez-Ontiveros et al., 2021). Hay una creciente preocupación por la presencia de estos conservantes en los productos farmacéuticos y productos cosméticos asociados con sus efectos estrogénicos demostrados por estudios *in vivo* e *in vitro* (Golden et al., 2005).

4.4.2.3. Benzofenonas

Desde un punto de vista físico-químico, la familia de las BPs son cetonas aromáticas e incluye diferentes cóngeneres, que entre los principales se encuentran: benzofenona 1 (BP-1), benzofenona 2 (BP-2), benzofenona 3 (BP-3), benzofenona 4 (4-OH-BP), benzofenona 6 (BP-6) y benzofenona 8 (BP-8). En la **figura 7** y en la **tabla 6** se muestran la estructura química y principales características de los diferentes congéneres de BPs:

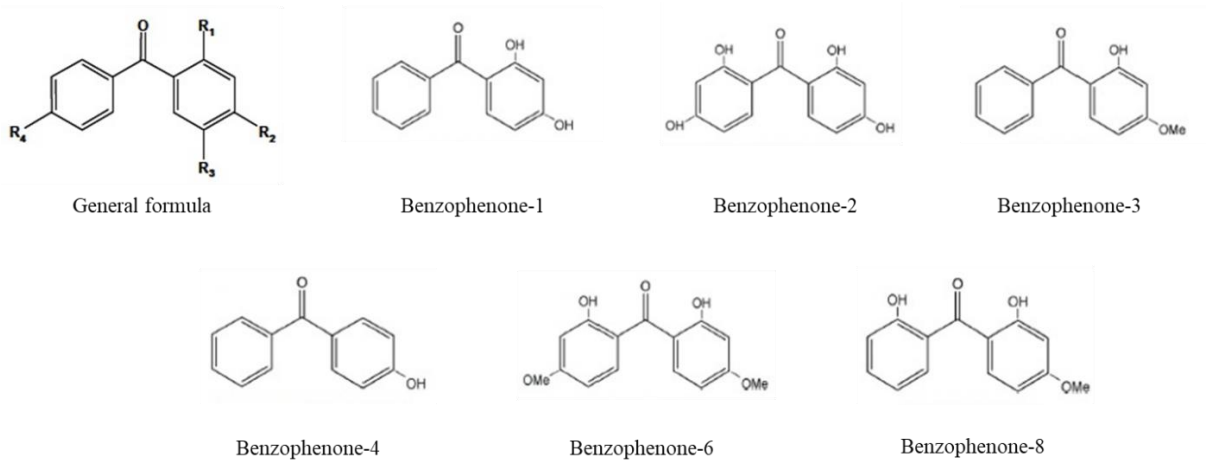

Figura 7. Estructura química BPs

Tabla 6. Congéneres de BPs

Sustancia	Abreviatura	Nombre	CAS	Fórmula	Peso molecular (g/mol)
Benzofenona 1	BP-1	Difenilmetanona	119-61-9	$C_{13}H_{10}O$	182.22
Benzofenona 2	BP-2	2-hidroxi-4(metacriloiloxi)benzofenona	2035-72-5	$C_{17}H_{17}O_4$	282.3
Benzofenona 3	BP-3	2-Hidroxi-4´metoxibenzofenona; Oxibenzona	131-57-7	$C_{14}H_{12}O_3$	228.25
Benzofenona 4	4-OH-BP	4-(allyoxy)benzofenona	42403-77-0	$C_{16}H_{14}O_2$	238.29
Benzofenona 6	BP-6	2, 2´-dihydroxy-4, 4´dimetoxibenzofenona	131-54-4	$C_{15}H_{14}O_5$	274.27
Benzofenona 8	BP-8	4-[8-(1,4-dioxa-8-azaspiro[4.5]decil) metil] benzofenona	898757-37-4	$C_{21}H_{23}NO_3$	337.419

Su naturaleza química las hace especialmente eficaces en la respuesta a la luz ultravioleta (λ: 280 - 400 nm), ya que son capaces de absorberla y disiparla en forma de calor. De esta propiedad deriva su aplicación industrial como filtros solares. De hecho, las BPs son los filtros ultravioleta más comúnmente utilizados en la industria, y se incluyen con frecuencia como componente en fórmulas de productos de cuidado personal (PCPs), envases de alimentos (Ding et al., 2018), estabilizadores UV o como tintas en materiales impresos en contacto con alimentos (IARC, 2013; Suzuki et al., 2005) y fotoiniciadores (Tsochatzis et al., 2020). De todos ellos, la BP-3 es el congénere más ampliamente utilizado, potencialmente en combinación con otras benzofenonas como la BP-2 (Asimakopoulos et al., 2016). Por su parte, se reconoce que la BP-1 es el principal metabolito de la BP-3 (Kim and Choi, 2014).

4.4.2.4. Vías de exposición a bisfenoles, parabenos y benzofenonas

Las principales vías de exposición de BPA en humanos son la digestiva, dérmica y respiratoria (Chen et al., 2016). La exposición humana al BPA a través de la dieta es aproximadamente diez veces mayor que la exposición al BPA a través de fuentes no

alimentarias en todas las edades (Geens et al., 2012), lo que supone el 94.9% de la ingesta diaria de BPA en humanos (Lu et al., 2018). Los estudios han demostrado que las personas consumen de 0.48 a 1.60 μg/kg/peso corporal/día de BPA a través de los alimentos (Vandenberg et al., 2007). El BPA administrado por vía oral se absorbe rápidamente en el tracto gastrointestinal y a través del metabolismo de primer paso en el hígado se convierte en BPA glucurónido y, en menor medida, BPA-sulfato. A diferencia de BPA libre, ambos conjugados no se unen al estrógeno receptor (Matthews et al., 2001; Shimizu et al., 2002). Se excretan en la orina y se pueden cuantificar como BPA después de la desconjugación en casi todas las muestras de orina de diferentes partes del mundo (Calafat et al., 2008; Frederiksen et al., 2013; Kim et al., 2011).

En relación a los PBs, derivado de su principal uso en los PCPs, se intuye que la principal vía de exposición a PBs es la dérmica (Guo and Kannan, 2013; Liao et al., 2013a), aunque la digestiva también constituye una fuente considerable de exposición a estas sustancias (Gálvez-Ontiveros et al., 2021; Liao et al., 2013b; Nobile et al., 2020).

Los seres humanos están expuestos principalmente a BPs a través de la vía dérmica (Hayden et al., 1997), aunque también se produce mediante las vías respiratorias (Wang et al., 2013) y la digestiva (IARC, 2013). La exposición por vía digestiva se produce como resultado de la migración de estos contaminantes a través de los materiales en contacto con los alimentos, dado que muchos suelen contener BPs por su capacidad como filtro UV. Aunque los niveles de BPs en los alimentos parecen ser bajos para constituir un riesgo per se, la exposición a largo plazo a BPs puede tener efectos adversos (Liu et al., 2022). La exposición por vía respiratoria ocurre por la presencia de BPs en el polvo doméstico,algunos perfumes y productos de limpieza que se volatilizan entre otros (Surana et al., 2018; Wang et al., 2013).

En general, se reconoce que las repercusiones para el organismo no son exactamente iguales en relación a la vía a través de la que se ha producido la exposición (Søeborg et al., 2014). En este sentido, se observa que los npDEs incorporados a través de la vía digestiva son rápidamente metabolizados por esterasas en el intestino y el hígado, transformándolos en metabolitos más hidrosolubles y excretándolos principalmente a través de la orina (aunque se produce alguna excreción en la bilis y las heces) (Boberg et al., 2010). Sin embargo, en el caso de los bebés prematuros, algunas enzimas hepáticas implicadas en el metabolismo de fase II (como las UDP-glucuronosiltransferasas) se pueden activar (Blake et al., 2005; Burchell et al., 1989;

Jansen et al., 1992; Leakey et al., 1987), pero las vías de glucuronidación no alcanzan el grado de funcionalidad del individuo adulto hasta meses después nacimiento (de Wildt et al., 1999), incrementando la presencia de BPA libre durante más tiempo en el organismo del individuo.

Los npDEs aplicados dérmicamente son absorbidos por la piel y metabolizados por esterasas dérmicas. Diversos factores influyen en el grado de absorción cutánea de los PBs. En primer lugar, la absorción depende de la longitud de la cadena del éster y de la formulación. En general, la penetración de la piel disminuye al aumentar longitud de la cadena. Por otro lado, los solubilizadores de lípidos reducen la absorción percutánea, mientras que los potenciadores de penetración aumentan la penetración. Se ha demostrado que la epidermis de los recién nacidos prematuros es especialmente frágil, lo que incrementa su susceptibilidad a la irritación química y las infecciones (Eichenfield and Hardaway, 1999; Oranges et al., 2015). Además, la inmadurez del estrato córneo puede incrementar la permeabilidad de la piel del prematuro a los agentes tópicos (Oranges et al., 2015). Una vez en el interior del organismo, mientras que los compuestos ingeridos por vía digestiva son generalmente convertidos a metabolitos más hidrofílicos, los asimilados a través de la piel suelen evitar el metabolismo de primer paso, por lo que el compuesto original entra directamente en la circulación sanguínea, incrementando su distribución por otros tejidos y retrasando su excreción a través de la orina (Søeborg et al., 2014).

En la f**igura 8**, se muestra una visión general de estos procesos.

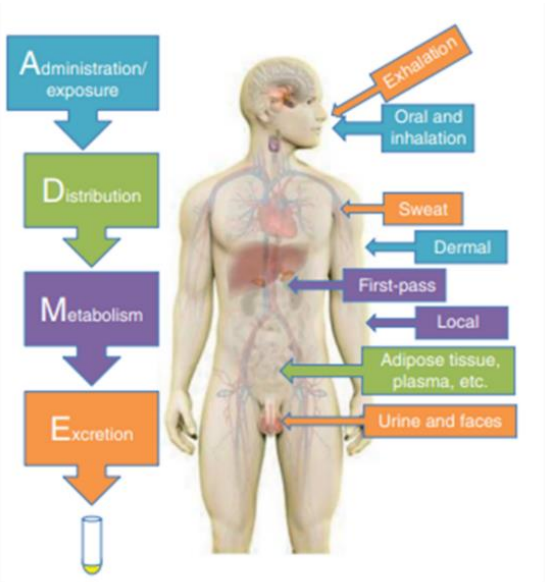

Figura 8. Propiedades desde la exposición hasta la excreción, la administración/exposición, distribución, metabolismo y excreción (ADME). *Fuente: Søeborg et al., 2014*

Además de las tres vías expuestas, en el caso de los individuos hospitalizados en general, y en el de los recién nacidos prematuros ingresados en la UCIN en particular, se reconoce una cuarta vía de exposición intravenosa y parenteral mediante el instrumental médico.

4.4.2.5. Estimación de la exposición a través de las diferentes vías:

- Estimación de la exposición digestiva en bebés (0-12 Meses).

Ingesta diaria (ng/ kg de peso del bebe Peso/día) = [(concentración de contaminante (ng/g) x ingesta de leche/día] / peso corporal promedio

- Estimación de la exposición dérmica

$$\mathbf{Exp_{derm}}\ (\text{pg/kg peso corporal/día}) = (C \times D \times SA \times F_{mig} \times F_{contact} \times F_{pen} \times T \times N) / BW$$

donde Exp_{derm} es la dosis de exposición cutánea diaria estimada (pg/kg peso corporal/día), C es la concentración de sustancias químicas en el calcetín (ng/g), D es la densidad de la fibra del calcetín (mg/cm^2), SA es el contacto con la superficie de la piel [estimándose 290, 330, 380 y 490 cm^2 para los grupos de edad 1-6, 6-12, 12-24, 24-36 y 36-48 meses, respectivamente (EPA, 2011)], F_{mig} es la tasa de migración de los productos químicos a la piel (valor predeterminado recomendado como escenario de exposición más adverso: 0.5%/día (BfR, 2012), $F_{contact}$ es la fracción del área de contacto con la piel (valor predeterminado recomendado como escenario más adverso: 100% (BfR, 2012), F_{pen} es la tasa de penetración de productos químicos en el cuerpo (valor predeterminado recomendado para el escenario más adverso: 1% (BfR, 2012), T es el tiempo de contacto entre el calcetín y la piel (se asume 1 día), N es el número de eventos/día (se asume 1), y BW es el peso corporal promedio de bebés/niños por edad, establecido previamente [6.60, 9.20, 11.40, 13.80 y 16.00 kg para los grupos de edad 1-6, 6-12, 12-24, 24-36 y 36-48 meses, respectivamente (EPA, 2011)].

- Estimación de la exposición respiratoria.

$$\mathbf{D\ (subst.x)_{inh}}\ (\text{mg/kg peso corporal/día}) = [(F_{resp} \times C_{inh} \times IH_{air} \times T_{contact}) / BW] \times n$$

donde $D\ (subst.x)_{inh}$ es la dosis diaria inhalada de una sustancia (mg/kg peso corporal/día), F_{resp} es la sustancia inhalada, es decir, fracción respirable (se asume 1, para

los contaminantes objetos de esta tesis doctoral), C_{inh} es Concentración de sustancia en el aire de la habitación (mg/m³), IH_{air} es el volumen de respiración de la persona (m^3/day), $T_{contact}$ es la duración de la exposición por incidente (horas), BW es el peso corporal (kg), y n es el número de exposiciones (incidentes) (por día) (EPA, 2012).

- Estimación de la exposición parenteral.

Los productos químicos administrados por vía parenteral evitan el primer paso del metabolismo y puede entrar así en la circulación sistémica y tejidos relevantes en el cuerpo como el compuesto original. Sin embargo, en la gran mayoría de los estudios con animales, la concentración de la sustancia química administrada y/o sus metabolitos en suero u orina, después de la exposición a una la dosis específica, no está cuantificada. (Søeborg et al., 2014).

A pesar de poder establecer una estimación de la exposición a DEs por las diferentes vías de exposición en la población en general, esto no debe aplicarse de forma literal en el caso de los recién nacidos prematuros. Dado que ciertas de sus características específicas, más concretamente el desarrollo más lento de sus enzimas digestivas y la mayor permeabilidad de la piel, permiten una mayor exposición de los contaminantes que en un recién nacido sano o en un adulto (Miodovnik et al., 2011).

4.4.3. Mecanismos de acción de los DEs

Los DEs actúan a muy diferentes niveles de complejidad interfiriendo en el mensaje hormonal a diferentes niveles del sistema endocrino (Scsukova et al., 2016) (**Figura 9**). Los DEs pueden modificar los niveles de hormona circulante actuando sobre su síntesis, metabolismo o degradación. También pueden reducir, incrementar o interferir sobre los receptores específicos de acción hormonal y, por lo tanto, afectar a la capacidad de respuesta a las hormonas naturales. En otros casos, un mismo compuesto puede influir en el sistema endocrino de diferentes maneras como es el caso del p, p'-DDT que puede activar el receptor de estrógenos o actuar competitivamente como el ligando natural del receptor androgénico (Kelce et al., 1995). En el caso particular de los DEs que interfieren con el funcionamiento de las hormonas esteroides (Colborn et al., 1993), los efectos observados parecen estar ligados a la activación/bloqueo de los receptores nucleares (RN), que son el modo más común de acción responsable de las curvas dosis-respuesta no-monotónicas en estudios experimentales (Cookman and Belcher, 2014) y por ello, también el mecanismo más estudiado (Rüegg et al., 2009). La función de estos RN es la

de actuar como ligandos para los factores de transcripción -actividad genómica-, que pasan al núcleo tras la unión con la hormona endógena o el DE. El complejo interactúa con secuencias específicas de ADN, los denominados elementos e respuesta hormonal y en cooperación con co-reguladores proteicos, regulan la transcripción de genes dependientes de la acción hormonal. Los co-reguladores incluyen co-activadores y co-represores que facilitan la unión del complejo con el RN para aumentar o reprimir la expresión de los genes diana (Kininis and Kraus, 2008). Los RN también puede modular la actividad transcripcional -actividad no genómica- mediante mecanismos independientes como puede ser la generación de un microambiente oxidativo e inflamatorio.

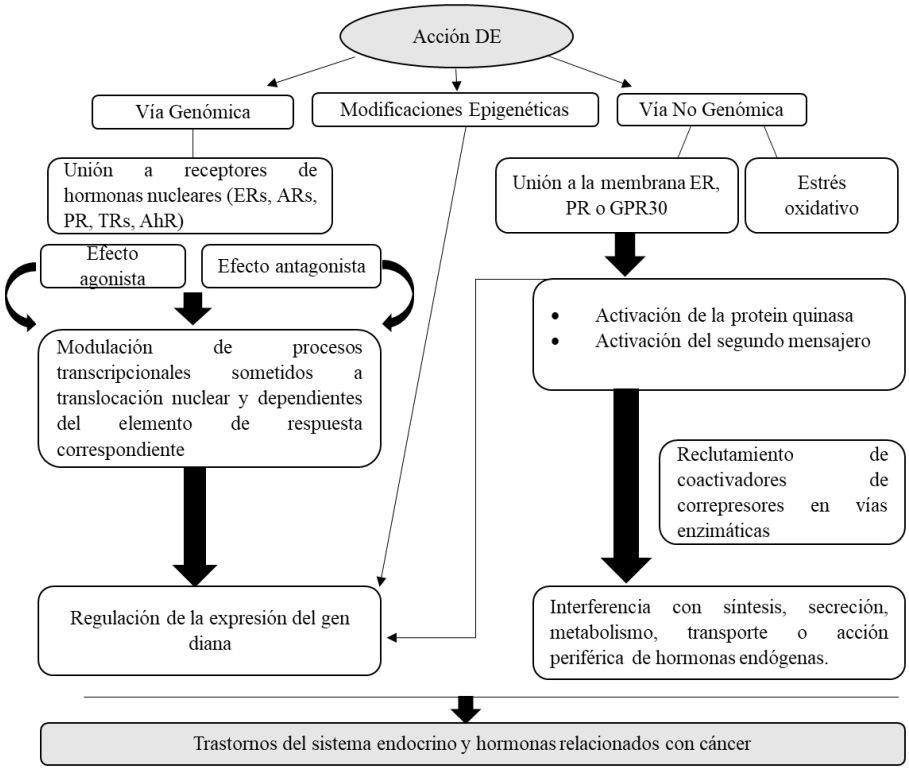

Figura 9. Mecanismos de acción de los DEs. *Fuente: Adaptado de Scukova et al., 2016*

También se han visto alteraciones epigenéticas inducidas por la exposición a DEs. Estos efectos epigenéticos representan un fenómeno molecular que regula la expresión génica sin presentar alteraciones en la secuencia de ADN (Jones and Takai, 2001). En términos biológicos, está demostrada la existencia de metilaciones a nivel de las histonas de la misma cadena de ADN, lo que provoca cambios en la estructura e impedimentos a nivel de expresión génica, que pueden derivar en diversas enfermedades. Aunque este es el mecanismo de acción más frecuente, hay otras formas de control epigenético como acetilaciones y otras modificaciones químicas (Skinner and Guerrero-Bosagna, 2009). Un

número creciente de estudios vinculan la exposición a DEs del tipo PCBs o plaguicidas organoclorados, como el mirex, clordano o p, p´-DDE, con cambios epigenéticos en humanos (Collotta et al., 2013; Perera and Herbstman, 2011). Un individuo no expuesto, puede mostrar cambios epigenéticos debido a la exposición a COPs, ocurrida en el útero materno o incluso desde óvulo alterado o espermatozoides de sus padres o abuelos. La exposición fetal a contaminantes del medio ambiente como los COPs, pueden causar cambios epigenéticos con efectos transgeneracionales (Titus-Ernstoff et al., 2008; Titus-Ernstoff et al., 2006).

Se ha demostrado que las familias de DEs de estudio de esta tesis doctoral (bisfenoles, PBs y BPs) ejercen efectos estrogénicos, tanto en estudios *in vivo* como *in vitro* (Charles and Darbre, 2013; Chen et al., 2007; Darbre and Harvey, 2008; Kerdivel et al., 2013; Oishi, 2002; Schlumpf et al., 2010; Suzuki et al., 2005). Además, existen algunos estudios que vinculan la exposición a npDEs con cambios epigenéticos como los observados en exposiciones prenatales al BPA y su asociación con la metilación del ADN en la preadolescencia (Goodrich et al., 2016). En esta misma línea, otros autores han reportado una asociación entre el BPA y PBs con cambios epigenéticos tras las exposiciones prenatales y de la adolescencia a estos productos químicos (Goodrich et al., 2016; Park et al., 2012). En relación a su potencial endocrino, se ha observado que los PBs de cadena larga, como el BuP y el PrP, tienen mayor potencia disruptora endocrina que los compuestos de cadena corta como el MeP y el EtP (Oishi, 2001).

Además, la inflamación y el estrés oxidativo también se han postulado recientemente como posibles mecanismos de acción de los npDEs (Artacho-Cordón et al., 2019; Mustafa et al., 2015; Thompson et al., 2015; Watkins et al., 2015). En este sentido, el estrés oxidativo, es decir, el desequilibrio entre la producción de radicales libres y la capacidad antioxidante del organismo, se ha demostrado que aumenta después de la exposición a una variedad de DEs, incluidos PBs y BPs (Artacho-Cordón et al., 2019; Thompson et al., 2015; Watkins et al., 2015). Por ejemplo, la exposición humana a PBs y BPs se ha relacionado con niveles más altos de peroxidación lipídica (Kang et al., 2013; Watkins et al., 2015) o el agotamiento del sistema antioxidante (Artacho-Cordón et al., 2019). En este sentido, aunque los mecanismos subyacentes aún no se conocen bien, se ha sugerido que, al menos en parte, los DEs podrían inducir estrés oxidativo a través de las vías de señalización del receptor de estrógenos α (Cho et al., 2018). Además, también se ha demostrado que la exposición a DEs desencadena un microambiente

inflamatorio (Peinado et al., 2020b; Watkins et al., 2015). Con una relación íntima, tanto las respuestas oxidativas como las inflamatorias también se han sugerido como mecanismos cruciales de la acción de los DEs en una variedad de enfermedades crónicas (Gupta et al., 2006; Lambrinoudaki et al., 2009; Peinado et al., 2020a).

Es importante destacar que el impacto de la exposición a DEs no se limitan únicamente a las hormonas esteroides. La relación entre las múltiples redes reguladoras hormonales que subyacen a un rasgo dado es crucial para interpretar los efectos de la exposición (Duarte-Guterman et al., 2014; Tilghman et al., 2010). Por ejemplo, se han encontrado estudios donde la exposición a BPA también alteró los niveles circulantes de gonadotrofina pituitaria, como la hormona luteinizante (LH), la hormona foliculoestimulante (FSH), las hormonas tiroideas como la tiroxina (T4) y triyodotironina (T3) (Kim and Park, 2019; Maffini et al., 2006; Rubin, 2011).

Además, algunos DEs se consideran obesogénicos (Brown et al., 2016; Gore et al., 2015; Grün and Blumberg, 2009; Morgen and Sørensen, 2014), ya que promueven el aumento de peso directamente al causar hipertrofia de adipocitos y/o hiperplasia (Baillie-Hamilton, 2002; Janesick and Blumberg, 2011) o indirectamente alterando el metabolismo y la regulación hormonal del apetito y saciedad. Además, la metilación del ADN de los genes relacionados con la adipogénesis podría verse afectado por la exposición a DEs (Kamstra et al., 2014). Numerosos estudios epidemiológicos han demostrado el efecto obesogénico del BPA (Gore et al., 2015; Khalil et al., 2014; Rochester, 2013; vom Saal and Hughes, 2005). La exposición al BPA causa disfunción de los adipocitos, ya que induce una disminución de la sensibilidad a la insulina y tolerancia a la glucosa para los efectos inhibitorios sobre la señalización de la insulina (Alonso-Magdalena et al., 2012; Dai et al., 2016; De Filippis et al., 2018; Moon et al., 2015). Además, conduce a una disfunción metabólica e inflamación de los adipocitos (Longo et al., 2019; Zatterale et al., 2019), dado que este promueve la expresión y liberación de ciertas citocinas proinflamatorias (Ariemma et al., 2016; De Filippis et al., 2018; Longo et al., 2019; Valentino et al., 2013). Por otro lado, los análogos del bisfenol A podrían inducir estrés oxidativo, alteración endocrina e interferencia inmunológica (Gao et al., 2021). Estudios recientes informaron que BPA y BPS podrían activar PPARγ en células THP-1 humanas y alterar el metabolismo de los lípidos (Gao et al., 2020). Por lo tanto, se especula que el receptor activado por proliferadores peroxisómicos β/δ (PPARβ/δ) podría ser un nuevo mecanismo para efectos de alteración de funciones

metabólicas de análogos de bisfenol. Por ejemplo, PPARβ/δ puede regular la homeostasis del sustrato de energía del músculo esquelético y hepático regulando el metabolismo de la glucosa y los ácidos grasos, que también podría influir en la hiperglucemia y la resistencia a la insulina (Krämer et al., 2007; Liu et al., 2011). Además, PPARβ/δ se ha implicado en múltiples vías proinflamatorias y ha sido considerado como un fármaco potencial diana para el tratamiento de la aterosclerosis (Barish et al., 2008), así como ejerce funciones biológicas esenciales en la carcinogénesis a través de la regulación de sus genes diana por tranactivación o represión inscriptiva (Müller, 2017; Peters et al., 2015).

4.4.4. Efectos adversos de los DEs en la población infantil

Las consecuencias de la exposición a DEs son distintas dependiendo de la edad y el sexo. El desarrollo fetal e infantil son períodos de especial vulnerabilidad que pueden verse afectados negativamente por la exposición a DEs. El feto o el bebé está expuesto a DEs a través de la transferencia placentaria y de lactancia de la madre (Liew and Guo, 2022; Sunderland et al., 2019). La exposición a los DEs se ha asociado con interferencia con el desarrollo sexual, neurológico y metabólico (Engdahl and Rüegg, 2020), bajo peso al nacer (Lizunkova et al., 2022), sobrepeso en la etapa del desarrollo infantil (Darbre, 2017), alteraciones tiroideas (Koutaki et al., 2021; Sun et al., 2022) o problemas respiratorios (Ghassabian et al., 2022), entre otros (**figura 10**).

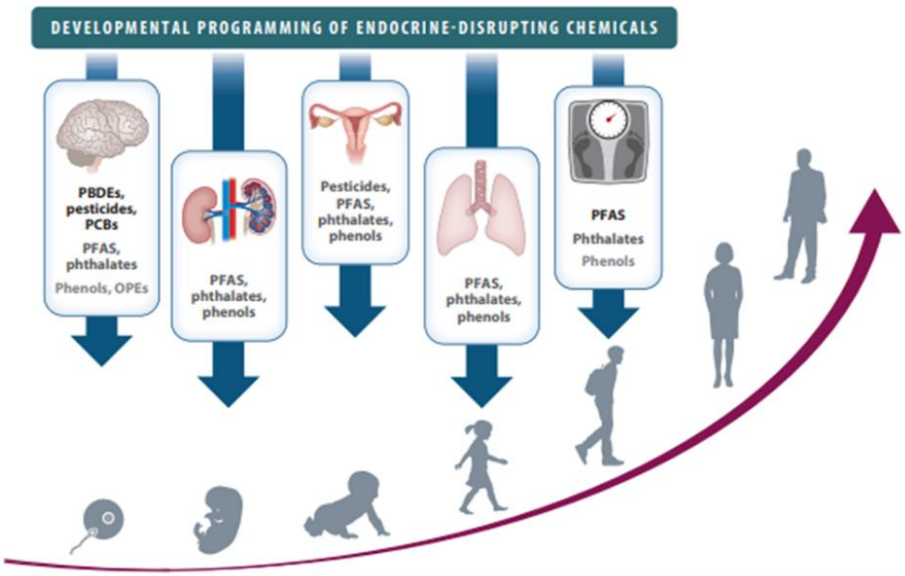

Figura 10. Evidencia epidemiológica sobre la exposición a DEs en el desarrollo y resultados. Texto más oscuro representa evidencia más fuerte. *Fuente: Ghassabian et al., 2021*

Por ejemplo, la señalización hormonal juega un papel vital en la diferenciación de las células madre y el desarrollo del tejido adiposo (Braun, 2017; Cohen, 2019; Engdahl and Rüegg, 2020; Gancz and Gilboa, 2013; Kopchick et al., 2020; Mota de Sá et al., 2017). Las exposiciones a DEs, pueden inducir retraso del crecimiento intrauterino y trastornos del desarrollo en la programación metabólica, produciendo efectos sobre el crecimiento fetal, así como efectos a largo plazo sobre la masa corporal y los resultados metabólicos y cardiovasculares en etapas posteriores de la vida (Philips et al., 2017). Los bisfenoles pueden reprogramar las células mesenquimales (Chin et al., 2018; Xin et al., 2018) y antagonizan la adiponectina (Hugo et al., 2008). Los ftalatos influyen en la expresión del peroxisoma, cruciales para el metabolismo de lípidos y carbohidratos (Desvergne et al., 2009; Gourlay et al., 2003). Numerosos datos epidemiológicos han observado una asociación entre el bajo peso al nacer y varias enfermedades que se manifiestan más tarde, como la obesidad, diabetes tipo II y enfermedades cardiovasculares (Barker, 2007; Kopec et al., 2017; Roberts and Cheong, 2014). El bajo peso al nacer es de hecho un indicador de cambios metabólicos más adelante en la vida y es respaldado por estudios que demuestran asociaciones entre la exposición a DEs durante el desarrollo y cambios metabólicos en niños (Blake and Fenton, 2020; Ghassabian et al., 2022; Heindel et al., 2017; Howard, 2018; Papalou et al., 2019). Las alteraciones epigenéticas podrían, al menos en parte, ser la base del vínculo entre la exposición a DEs del desarrollo y un la susceptibilidad del individuo a los trastornos metabólicos en períodos posteriores (Moore et al., 2013; Vilahur et al., 2016; Wadhwa et al., 2009; Zhu et al., 2019). Los PFAS han asociado con disminuciones en el peso al nacer (Steenland et al., 2018) así como aumentos en la adiposidad infantil.

Por otro lado, la exposición a estas sustancias se ha relacionado con el sobrepeso infantil. La obesidad en los niños está aumentando en los países occidentalizados, y en EEUU., alrededor del 20% de los niños de 3 a 17 años son obesos. Un período de tiempo especialmente sensible para la exposición a los obesógenos se ha encontrado que es anterior al nacimiento en el útero o en el período neonatal (Janesick and Blumberg, 2011). Estudios de recién nacidos de madres con hábito tabáquico durante el embarazo, dan a luz niños con bajo peso al nacer, pero que paradójicamente tienen un mayor riesgo de obesidad (Power and Jefferis, 2002). Algunos estudios han demostrado que los primeros años de vida a exposiciones de algunos COPs (Tang-Péronard et al., 2011) y BPA (Vafeiadi et al., 2016) están asociados con aumento de peso corporal en niños pequeños

(Darbre, 2017). Un metanálisis de diez estudios de cohortes encontró un aumento general del 25% en el sobrepeso infantil y aumentos en el índice de masa corporal debido a la exposición al PFOA (Liu et al., 2018).

La homeostasis de la hormona tiroidea es esencial para el desarrollo normal del cerebro del feto (Batistuzzo and Ribeiro, 2020), la organización y los efectos de activación de las hormonas sexuales (Prasanth et al., 2010; Richter et al., 2007). El hipotiroidismo congénito, que resulta en un nivel insuficiente de la hormona tiroidea, puede provocar problemas de desarrollo neurológico en los bebés (Chevrier et al., 2007), afectar la función y la estructura del cerebro en los niños (Alvarez-Pedrerol et al., 2007; Korevaar et al., 2016). Estudios recientes han demostrado que la exposición a DEs durante el embarazo se asocia con el compromiso de la homeostasis de la función tiroidea materna y, por lo tanto, puede conducir a resultados adversos en el neurodesarrollo fetal (Aker et al., 2018; Braun et al., 2018), el comportamiento y el efecto cognitivo de la descendencia (Batistuzzo and Ribeiro, 2020; Berbel et al., 2009; Pop et al., 2003). La exposición a DEs puede contribuir al desarrollo de cáncer de tiroides (Zhang et al., 2021). Existen estudios que revelan que la exposición de BPA durante la gestación y los primeros años de vida están correlacionados con una variedad de problemas del neurodesarrollo, entre los que se incluyen el trastorno por déficit de atención con hiperactividad, trastorno del espectro autista, depresión y ansiedad (de Cock et al., 2012; Harley et al., 2013; Tewar et al., 2016), que a menudo se presentan conjuntamente con trastornos del sueño (Hoban, 2000; Nesan et al., 2021; Robinson-Shelton and Malow, 2016). La modificación epigenética de genes que codifican el factor neurotrófico derivado del cerebro y otras proteínas también pueden tener implicaciones para la función cognitiva y otros criterios de valoración neuroconductuales (Atlas et al., 2014). Algunos estudios de exposición a PBDE, pesticidas organofosforados y bifenilos policlorados, han demostrado que estos compuestos producen disfunción cognitiva (Biasiotto et al., 2016).

En cuanto al efecto de DEs en el aparato respiratorio, se han examinado la asociación de la exposición a DEs con la respuesta de citoquinas, proponiendo que una mayor exposición prenatal a fenoles se asoció con niveles elevados de IL-6 en la circulación tanto de la madre como de la descendencia (Kelley et al., 2019; Watkins et al., 2015). Algunos estudios muestran que la exposición a DEs también está asociada con un desarrollo interrumpido y el desequilibrio en las vías T helper 1/T helper 2, lo que conduce a la progresión del asma y la alergia en los niños (Yang et al., 2014). A pesar de

estas primeras evidencias, los hallazgos de estudios epidemiológicos sobre la asociación prospectiva de DEs (principalmente ftalatos y bisfenoles) con enfermedades alérgicas no han sido concluyentes, dada la heterogeneidad en las evaluaciones de resultados (dermatitis alérgica o rinitis, sibilancias o asma) y la edad de los niños (demasiado pequeños para manifestar síntomas de asma y alergia) (Casas and Gascon, 2020; Ghassabian et al., 2022).

En lo que respecta al sistema reproductivo se han descrito diferencias en cuanto a los efectos de los DEs dependiendo del sexo. En el caso de los niños, el descenso testicular y la maduración de los órganos sexuales masculinos está mediada por las células de Leydig, que producen la hormona factor 3 (INSL3) (Thorup et al., 2010). Los DEs producen enzimas estrogénicas, antiandrogénicas y esteroidogénicas con efectos inhibidores que alteran la función de INSL3 (Xin et al., 2018). Criptorquidia, hipospadias y el cáncer testicular comparten varios mecanismos patogénicos comunes que resultan de la desregulación de INSL3 (Bray et al., 2006; Yu et al., 2019). Existen estudios que han mostrado asociaciones de exposición de ftalatos y algunos fenoles, con hipospadias y otras anomalías genitales (Fisher et al., 2020; Sathyanarayana et al., 2016), como el tamaño más corto del pene (Sunman et al., 2019), distancia anogenital disminuida (Barrett et al., 2016; Bornehag et al., 2015; Fisher et al., 2020; Sunman et al., 2019), anomalías hormonales que sugieren deterioro de las células de Leydig (Hart et al., 2018; Muerköster et al., 2020), así como, disminución de la calidad espermática y volúmenes testiculares (Hart et al., 2018).

Por otro lado, el efecto producido por la exposición a DEs en las niñas, no se pueden evaluar durante la niñez, pero sí, en etapas posteriores. Se ha comprobado que un incremento de la carga estrogénica puede aumentar el riesgo de pubertad temprana, desarrollo mamario prematuro, síndrome de ovario poliquístico (Costa et al., 2014; Kahn et al., 2021; Palioura and Diamanti-Kandarakis, 2015; Rutkowska and Diamanti-Kandarakis, 2016; Wang et al., 2017), fibromas (Pollack et al., 2015; Shen et al., 2013), riesgo de cáncer de mama (Chang et al., 2018; Kahn et al., 2020), infertilidad (Buck Louis et al., 2011), endometriosis (Kawa et al., 2021) , entre otras. El entorno hormonal prenatal y de los primeros años de vida también puede influir en el desarrollo de las glándulas mamarias y susceptibilidad al cáncer de mama (Rudel et al., 2011). En cuanto al desarrollo de las características sexuales durante la pubertad, un estudio reciente observó asociaciones entre los niveles de PBs y desarrollo más temprano de los senos y el vello

púbico en las niñas (Harley et al., 2019) o con la menarquía temprana (Harley et al., 2019). Asimismo, existen estudios que han demostrado que los niveles elevados de BPA están asociados con la anovulación (Costa et al., 2014), bajo recuento de folículos antrales (Souter et al., 2013; Ziv-Gal and Flaws, 2016), retraso de la menarquia (Berger et al., 2018; McGuinn et al., 2015), parto prematuro (Peretz et al., 2014) e infertilidad (Weinberger et al., 2014).

4.4.5. Límites de exposición humana a BPA, PBs y BPs

Las agencias reguladoras tienen el cometido de establecer unos niveles seguros de exposición para toda la gama de productos químicos que la población en general está expuesta. En este sentido, se establecen parámetros como la ingesta diaria admisible (IDA) o la ingesta diaria tolerable (IDT) (Pisanello, 2014). Aun así, numerosos estudios de biomonitorización asocian la exposición a varias sustancias químicas en dosis por debajo de los límites reglamentarios con ciertas enfermedades o problemas de salud (Docea et al., 2017; Fenga et al., 2017).

Múltiples factores contribuyen a la explicación de la asociación observada. En primer lugar, tal como se ha explicado anteriormente, los DEs actúan a bajas dosis, al igual que lo hacen las hormonas. Por otro lado, los límites de dosis se establecen usando estudios que evalúan la exposición a un solo químico, sin atender al efecto combinado que ejerce la exposición a múltiples sustancias con efecto similar (Kostoff et al., 2018). Estas exposiciones combinadas podrían, además iniciar varios tipos de interacciones, algunas de ellas dando lugar a efectos aditivos o sinérgicos, proceso que se demostró para las mezclas de DEs o para plaguicidas (Bergman et al., 2014; Colosio et al., 2012). En conjunto, esto hace que los límites establecidos por las agencias reguladoras sean, en muchas ocasiones, inapropiada para asegurar la inocuidad.

En la familia de los bisfenoles, el BPA está autorizado para su uso como monómero en materiales plásticos en contacto con alimentos, de acuerdo con el Reglamento (UE) n.º 10/2011/UE de la Comisión sobre materiales y objetos plásticos destinados a entrar en contacto con alimentos (European Commission Regulation, 2011). Su uso está sujeto a un límite de migración específico de 0.60 mg/kg. Además, el Reglamento de Ejecución (UE) n.º 321/2011 (European Commission Implementing Regulation, 2011) de la Comisión impuso una restricción al uso de BPA en la fabricación de biberones PC para lactantes que se introdujo en 2011.

En la evaluación del riesgo asociado al BPA de 2015 (EFSA, 2015a), la EFSA estableció una IDT de 4 μg/kg peso corporal/día. Tras su reevaluación en el año 2021, han decido disminuir IDT a 0.04 ng/kg de peso corporal/día (EFSA, 2021), considerablemente inferior a la IDT temporal anterior. Al comparar la nueva IDT con las estimaciones de la exposición de los consumidores al BPA en los alimentos, la EFSA concluye que las personas de todos los grupos de edad con una exposición media y elevada al BPA superan la nueva IDT (EFSA, 2021).

En el caso de los PBs, en 2010, el Comité Científico de Seguridad del Consumidor (SCCS) de la UE consideró que el uso de MeP y EtP al máximo concentraciones autorizadas es seguro, pero debido a la falta de datos científicos, el Comité no puede asegurar lo mismo para el PrP y el BuP (SCCS, 2011). La EFSA concluyó que se puede establecer una IDA de 0 -10 mg/ kg peso corporal/ día para MeP y EtP y sus sales de sodio (EFSA, 2004). En 2015, la Agencia Europea de Medicamentos (EMA) informó la evidencia de efectos adversos para la salud relacionados con la ingesta de PrP y una IDA de 1.25 mg/kg/día (EMA, 2015). A pesar de haber sido utilizado como conservante de alimentos durante muchos décadas, hay poca información disponible sobre la concentración de PBs en ciertos alimentos y exposición dietética (Gálvez-Ontiveros et al., 2021; Nobile et al., 2020; Pradhan et al., 2020). Según un comité de expertos CIR, se ha establecido un límite máximo de 1,000 mg/kg/día para los PBs. El margen de seguridad para la población humana adulta es de 1.20 mg/kg/día de múltiples PBs y de 0.30 mg/kg/día para la población infantil, aunque se han descrito algunos efectos adversos para el MeP, EtP, PrP y BuP en dosis por debajo de 1,000 mg/kg bw/día (Boberg et al., 2010).

Las BPs participan en la fabricación en las tintas de impresión para el etiquetado para alimentos, entre otros usos. En 2009, tras la evaluación de la EFSA determinó una IDT de 0.03 mg/kg de peso corporal (EFSA, 2009). En 2007, la EFSA valoró los posibles riesgos para la salud relacionados con la ITD, una sustancia utilizada en tintas aplicada a materiales de envasado, incluidos envases de cartón. La EFSA observó que la presencia de ITD en los alimentos, aunque no es deseable, no causa problemas de salud en los niveles notificados (EFSA, 2009).

En este sentido, los diferentes organismos reguladores están estableciendo límites de forma individual para cada compuesto, pero en ninguno de los casos, se está considerando el efecto combinado de la exposición a DEs. Es por ello, que los niveles

aparentemente seguros de exposición establecidos no responden a la realidad de la exposición a múltiples compuestos.

4.4.6. Principio de precaución.

El principio de precaución se recogió por primera vez en la convención internacional de la Declaración de Bergen para el desarrollo Sostenible (1990), y establece que «cuando una actividad o compuesto químico representa una amenaza o un daño para la salud humana o el medio ambiente, hay que tomar medidas precautorias a pesar de que la relación de causalidad no haya podido demostrarse científicamente de forma concluyente».

Esta declaración implica:

i) Actuar con cautela en presencia de incertidumbre,
ii) derivar la responsabilidad y la seguridad a quienes crean el riesgo,
iii) analizar las alternativas posibles y
iv) utilizar métodos participativos para la toma de decisiones.

Es por ello, que cuando se dispone de evidencias demostradas de riesgo para la salud o el medio ambiente, se aplican medidas preventivas; cuando no existe esa certeza, pero hay indicios de posibles efectos perjudiciales, deben instaurarse medidas de precaución para evitar el potencial daño (vom Saal et al., 2007).

Existe un alto grado de incertidumbre sobre el potencial efecto de los DEs sobre la salud. El conocimiento científico tiene limitaciones para definir tanto los aspectos relacionados con la exposición como los relativos a la variabilidad de la respuesta en individuos y poblaciones. Sin embargo, esta falta de evidencia científica no significa que dichos compuestos no supongan un riesgo para la salud humana, sino la necesidad de ser cautos disminuyendo la exposición, al menos, en periodos de mayor vulnerabilidad.

Mientras que la asociación certera entre exposición y efecto sobre la salud se consigue probar, el sentido común enunciado en el principio de precaución sugiere actuar con cautela, reduciendo la exposición, sobre todo en aquellas poblaciones más susceptibles de daño, tales como los neonatos ingresados en la UCIN.

4.5. Importancia de los DEs en la UCIN

Se ha sugerido que el elevado riesgo de niños con retraso en el desarrollo, disfunción cognitiva, trastorno de déficit de atención e hiperactividad y autismo que presentan los neonatos que han estado ingresados en la UCIN podría no explicase exclusivamente por el grado de prematuridad o la gravedad de la enfermedad acompañante (Reynolds et al., 2013). Por ejemplo, algunas de las condiciones médicas asociadas con la prematuridad como la enfermedad pulmonar crónica o la retinopatía del prematuro, tampoco son pronosticadas por la edad gestacional al nacer o la patología concomitante del recién nacido. De esta manera, se ha sugerido que algunas de las deficiencias neurocognitivas, sociales y somáticas vistas en los niños que abandonan la UCIN podrían atribuirse a exposiciones ocurridas en este medio y, por tanto, potencialmente prevenibles (Reynolds et al., 2013).

El medio hospitalario, especialmente la atención en la UCIN, supone un riesgo de exposición inadvertida a DEs en una etapa vital de especial susceptibilidad. Por ello, dadas las características particulares de los neonatos prematuros, el nivel aceptable de riesgo de exposición a DEs debería ser cero. Los niños prematuros de muy bajo peso al nacer son especialmente vulnerables a la presencia de DEs ya que les podría causar efectos irreversibles, debido a la susceptibilidad del cerebro y otros órganos a los estrógenos durante este período (Safe, 2005), la inmadurez de los sistemas de detoxificación, hepático y renal, y a las deficiencias en su sistema de inmunovigilancia (Vilahur et al., 2014), además de alteraciones en el comportamiento y la función ejecutiva (Jiang et al., 2019), pubertad acelerada (Berger et al., 2018; Harley et al., 2019) o enfermedad respiratoria (Agier et al., 2019; Buckley et al., 2018). La relación peso/talla/masa corporal es desfavorable de tal manera que, por ejemplo, el volumen de la ingesta o la superficie corporal es relativamente mayor que en los adultos, lo que significa una mayor exposición relativa a cualquier contaminante, ya sea por vía digestiva o dérmica. A ello se le suma la inmadurez de las enzimas digestivas o la mayor permeabilidad de la piel. Por tanto, la interacción entre contaminantes con actividad DE, solos o en combinación con otros residuos químicos, con los mecanismos de regulación de la homeostasis hormonal y con la expresión de genes específicos (Miodovnik et al., 2011) son especialmente críticos para el desarrollo del niño prematuro.

Numerosos materiales usados en la UCIN están hechos de materiales plásticos que pueden contener elevados porcentajes de bisfenoles y ftalatos (Pak et al., 2007), dejando de ser productos inertes, para convertirse en fuentes de exposición (Duty et al., 2013). Al

igual ocurre con la leche materna administradas desde los bancos de leche materna de las UCINs. A pesar de ser una fuente de elementos protectores para la salud, la leche materna tambié juega un papel relevante como fuente de exposición a contaminantes (Hadders-Algra et al., 2007; Koletzko et al., 2008). En este sentido, el estudio publicado por Calafat et al. (2004) sobre la exposición a di-(2-etilhexil)ftalato (DEHP) en niños prematuros atendidos en la UCIN de dos hospitales de New Yersey muestra que la concentración en orina de tres metabolitos del DEHP en niños prematuros sometidos a tratamiento en la UCIN, es muy superior a lo esperado en la población infantil y adulta. Años después, los mismos autores añadieron la determinación de BPA y otros fenoles, triclosan, BP-3, MeP, y PrP, a las mismas muestras de orina. Como ocurrió con DEHP, las concentraciones de BPA y el resto de fenoles fueron entre 10 y 100 veces superiores a las esperadas en la población no hospitalizada (Calafat et al., 2009).

Además, cada vez hay más debate sobre la necesidad de regular los bancos de leche materna, no solo para asegurar que la leche esté libre de contaminantes como la cafeína o las drogas (Weaver et al., 2019) sino también a monitorear su carga xenobiótica (Cohen, 2019). Este tema es de particular relevancia para los recién nacios, dado que la inmadurez del metabolismo de fase II es aún más pronunciada en estos bebés. (Calafat et al., 2009; Duty et al., 2013; Mulla et al., 2015; Nachman et al., 2014). Además, la inmadurez de sus órganos hace que los bebés sean muy susceptibles a las hormonas contenidas en la leche humana, que están regulados por mecanismos de retroalimentación fisiológica materna (Mazzocchi, 2019). Sin embargo, la exposición inadvertida de la madre a sustancias puede conducir a su migración en la leche materna y la consiguiente exposición de su bebé (Dualde et al., 2020) o del bebé receptor de esa leche.

La falta de trabajos que han abordado este tema, dados los datos mostrados anteriormente sobre el incremento los niños prematuros ingresados en UCIN en los últimos años, hace necesario actuar de manera urgente. En respuesta a las crecientes preocupaciones sobre la exposición a los DEs en el entorno hospitalario, la Comisión Europea publicó Opiniones sobre el riesgo de exposición oral, subcutánea e intravenosa a BPA (SCENIHR, 2015) y ftalatos (SCENIHR, 2016) de Dispositivos médicos hechos de materiales que potencialmente pueden filtrarse, describieron un riesgo particular de disponibilidad de BPA después de exposiciones no orales, como en el caso de recién nacidos en UCIN, bebés sometidos a procedimientos médicos prolongados y pacientes que reciben diálisis (SCENIHR, 2015). El informe indica que la exposición diaria más

alta ocurre en los recién nacidos en las UCIN (3µg/kg de peso corporal), mientras que la exposición diaria para pacientes adultos en diálisis fue de 57 ng/kg peso corporal, lo que llevó a este comité de expertos a y pedir una investigación urgente sobre la composición y liberación de BPA de dispositivos médicos SCENIHR (2015). Sin embargo, no se ha prestado atención a la presencia de PBs en el entorno de la UCIN.

5. HIPÓTESIS Y OBJETIVOS

El medio hospitalario, especialmente la atención intensiva en los servicios de neonatología, supone el empleo de multitud de material sanitario, fundamentalmente de origen plástico, en una fase crítica del desarrollo del recién nacido. Los niños prematuros son especialmente vulnerables a los efectos de los compuestos químicos debido a su bajo peso, la inmadurez de los sistemas de detoxificación, sistema hepático y renal, deficiencias en su sistema de inmunovigilancia, así como por una relación peso/talla/masa corporal desfavorable. Como consecuencia, el volumen de la ingesta, la capacidad respiratoria o la superficie corporal es relativamente mayor que en los adultos, lo que sumado a la inmadurez de los procesos de metabolización y la mayor permeabilidad de las barreras fisiológicas supone una mayor exposición relativa a cualquier contaminante.

No son muchos los estudios donde se explora de forma simultanea la identificación de fuentes y las vías de exposición a DEs en neonatos ingresados en la UCIN. Numerosos materiales que son usados en la UCIN, como incubadoras, dispositivos de asistencia respiratoria, sondas de alimentación enteral, catéteres intravenosos o adhesivos, entre otros, están hechos de materiales plásticos que pueden contener DEs. Además, los textiles y los productos utilizados para el cuidado personal de estos neonatos, como geles, cremas hidratantes, entre otros, pueden contener productos utilizados en la formulación de cosméticos tales como PBs y BPs. Además, los DEs incorporados por las madres donantes a través de la dieta o el uso de PCPs pueden ser excretados a través de la leche materna, siendo este otro vehículo más de exposición a estos contaminantes para el neonato institucionalizado.

Es por ello necesario, una aproximación holística para evaluar la exposición dérmica, parenteral y alimentaria mediante el análisis químico y/o biológico de: i) los materiales en contacto con el niño, incluidos sondas, catéteres, pegamentos y textiles, ii) el alimento suministrado y los materiales plásticos en contacto con su alimentación, procesado, conservación y administración, y iii) los productos cosméticos y de cuidado personal que se utilicen en su aseo. Sin embargo, es importante recordar que las exposiciones suelen ocurrir a bajos niveles de cada DE, y es importante considerar la exposición simultánea a múltiples DEs, y el efecto combinado o cóctel. La exposición combinada a múltiples compuestos químicos, que ocurre por diferentes vías podría resultar en efectos adversos observados durante el crecimiento y desarrollo del niño. Es

por ello que la identificación de las fuentes de exposición a DEs en la UCIN permitiría la sustitución de productos y la revisión de protocolos resultando en una disminución significativa de la carga hormonal exógena atribuida a los DEs en neonatos dada su especial vulnerabilidad.

Una vez revisada la literatura científica al respecto y en base a la hipótesis enunciada, se planteó este trabajo de tesis doctoral con el siguiente objetivo:

Evaluar la exposición perinatal de niños prematuros a disruptores endocrinos no persistentes.

Los objetivos específicos planteados para esta tesis doctoral son:

1. Caracterizar la exposición a DEs no persistentes, bisfenoles y parabenos, a través de los procedimientos médicos empleados en niños prematuros ingresados en la UCIN.

2. Estudiar la exposición a DEs no persistentes, bisfenoles, parabenos y benzofenonas, a través del alimento administrado a niños prematuros ingresados en la UCIN.

3. Caracterizar la exposición a DEs no persistentes, bisfenoles y parabenos, a través del empleo de textiles.

5. MATERIAL Y MÉTODOS

6.1.Objetivos 1 y 3.

6.1.1. Diseño de estudio

Estudio observacional

6.1.2. Colección de muestras

Para dar respuesta al objetivo 1, se recopilaron un total de 52 materiales (25 dispositivos médicos de plástico, 18 textiles y 9 productos semisólidos / líquidos) utilizados en la UCIN del Hospital Virgen de las Nieves (Granada, España), entre mayo y junio de 2018 que estaban en contacto íntimo con el neonato. En cuanto a las características de los materiales de la UCIN encontramos que el lugar de procedencia de fabricación, se distribuyó en 37 ítems en Europa, 8 en Estados Unidos, 6 en Asia y 1 en Australia. De los 25 artículos de plástico, solo 8 describieron la materia prima utilizada (polihexahidrotriazina en 1, polietileno en 1, PVC en 4, poliuretano en 1 y algodón / poliamida / poliuretano en 1). Con respecto a los aditivos, la presencia o ausencia de látex se indicó 17 ítems (16 ítems fueron declarados sin látex) y la presencia o ausencia de di-(2-etilhexil) -ftalato (DEHP) en 7 artículos, incluidos 4 declarados libres de DEHP.

De forma similar, para dar respuesta al objetivo 3, se recopilaron 32 pares de calcetines para bebés de 1 a 12 meses y niños pequeños de 12 a 48 meses en tres tiendas diferentes en la provincia de Granada. Los 32 pares de calcetines fueron comprados en tres tiendas diferentes: un minorista local de bajo costo (tienda 1), un minorista internacional de ropa de moda (tienda 2) y una marca minorista internacional de ropa de mayor calidad (tienda 3). Compramos un total de 10 paquetes que contenían tres pares de calcetines cada uno y solo un paquete contenía dos pares de calcetines (cuatro paquetes en las tiendas 1 y 2, respectivamente, y tres paquetes en la tienda 3). Las características de los calcetines fueron muy variables. El precio por paquete osciló entre 1.50-1.80 € para la tienda 1, 3.00- 4.50 € para la tienda 2, y 6.95- 7.95 € para la tienda 3. Los calcetines variaban en composición (% algodón, % poliamida, % poliéster, % elastano o "spandex"), color (negro, blanco, gris, azul marino, azul oscuro, azul claro, rojo, multicolor y estampado) y tamaño. Todas las etiquetas de los envases daban información sobre el "país de origen" (el 66.0% de los calcetines era fabricados en España, 25.0% en Turquía y 9.0% en Italia) pero no especificaban si el país de origen de la fibra era el mismo o diferente.

6.1.3. Productos químicos y reactivos

Todos los reactivos utilizados fueron de calidad analítica a menos que se especifique lo contrario. BPA, PBs (MeP, EtP, PrP y BuP), BPA marcado con deuterio-16 (BPA-D_{16}), y el EtP marcado con $^{13}C_6$ (EtP-$^{13}C_6$) se adquirieron de Sigma- Aldrich (Madrid, España). Los disolventes utilizados para los procedimientos de extracción -acetato de etilo, tetrahidrofurano, acetona y diclorometano- fueron suministrados por Merck (Darmstadt, Alemania). Para la cromatografía líquida (LC-MS)- metanol, agua y amoníaco (25.0%)- se adquirieron de Sigma-Aldrich (Madrid, España). El agua (18.20 MΩ cm) se purificó utilizando un sistema Milli-Q (Millipore, Bedford, MA, EEUU). Para los análisis químicos, las soluciones estándar madre (100 mg/L) de cada compuesto se prepararon en acetonitrilo y se almacenaron a 4°C en oscuridad. Las soluciones se mantuvieron estables durante al menos dos meses. Los estándares de trabajo se prepararon inmediatamente antes de su uso mediante dilución con acetonitrilo puro.

Para los ensayos de actividad biológica, los estándares de referencia 17β-estradiol (E_2), metiltrienolona (R1881), ICI 182780 (en adelante, ICI), puromicina, geneticina (G418), luciferina (sal sódica), sulforrodamina B (SRB) y el ácido tricloroacético (TCA) se obtuvo de Sigma-Aldrich Inc. (St Louis, MO). Las soluciones madre (10 mM) de E_2, R1881, procimidona e ICI, se prepararon en etanol y se realizaron sucesivas diluciones en medio de cultivo. Las soluciones madre se mantuvieron a -20 ° C y las series de dilución se prepararon justo antes de cada experimento. Finalmente, los medios de cultivo y el suero bovino fetal (FBS) fueron suministrados por Gibco (Invitrogen, Barcelona, España) y todos los plásticos de cultivo celular procedían de Falcon (VWR International Eurolab, Barcelona, España).

6.1.4. Instrumentación

Los análisis se realizaron mediante cromatografía líquida de ultra alta resolución acoplada a espectrometría de masas (UHPLC-MS/MS), utilizando un ACQUITYUPLC ™ H-Class (Waters, Manchester, Reino Unido) que consta de gestor binario de disolventes y gestor de muestras. Un espectrómetro de masas triple cuadrupolo Xevo TQS (Waters) equipado con una fuente de ionización por electropulverización (ESI) ortogonal Z-spray ™ fue utilizado para la detección de BPA y PBs. La separación cromatográfica de los compuestos se realizó utilizando un ACQUITYUPLCBEH ™ C_{18} (diámetro interno de 50.0 mm × 2.1 mm, tamaño de partículas de 1.7 µm) de Waters. El gradiente

de la fase móvil consistió en una solución acuosa de amoníaco al 0.025% (v/v) (disolvente A) y una solución amoniaco en metanol al 0.025% (v/v) (disolvente B). Las condiciones de gradiente fueron las siguientes: 0.00-3.50 min, 60% disolvente B; 3.50–4.00 min, 60-100% disolvente B; 4.00–6.50 min, 100% disolvente B y de regreso al 60% de disolvente B en 0.10 min. La velocidad de flujo fue de 0.25 ml/min. El volumen de inyección fue de 5 µl. La temperatura de columna se mantuvo a 40°C. El espectrómetro de masas se hizo funcionar en modo ESI tanto positivo como negativo, utilizando parámetros MS/MS optimizados como se definieron en un estudio anterior (Vela-Soria et al., 2014).

Para los ensayos de proliferación celular, la absorbancia se midió en un lector de absorbancia Titertek Multiscan (Flow, Irvine, CA, EEUU) a 492 nm para detectar la cantidad de SRB, y se utilizó un lector de luminiscencia M200 (Tecan, Barcelona, España) para detectar la actividad luciferasa en células intactas.

6.1.5. Extracción química, tratamiento de la muestra y condiciones de LC-MS

Materiales plásticos

Los materiales plásticos se dividieron en plásticos duros y blandos, variando a su vez, el proceso de extracción para cada tipo, que se detallan a continuación:

- Para materiales de plástico duro se pesaron con precisión 50 mg de la muestra y se colocó en un vial de vidrio de 7 mL. Seguidamente, se agregaron 3 mL de acetona y se dejaron en reposo durante 24 horas, pasado este tiempo, se procedió a sonicar durante 30 min. A continuación, las muestras se centrifugaron a 20°C durante 10 min a 5,000×g. Una vez estuvieron diferenciadas las distintas fases, se tomó la fase orgánica y se filtró a través de un filtro de politetrafluoroetileno (PTFE) de 0.20 µm y se secó bajo una corriente de nitrógeno. El residuo se disolvió con 200 µl acetonitrilo (que contiene 25 µg/L de BPA-d16 y 10 µg/L de EP-13C6) / Milli-Q® agua 1:1 v/v, y se centrifugó a 24,960×g durante 10 min a 4°C, para que este quedara más limpio. Finalmente, se inyectó 5 µL en el sistema LC-MS/MS.

- Para los materiales de plástico blando se utilizó una ligera modificación del método descrito por Genay et al. (2011). Para ello, se pesó con precisión 50 mg de cada muestra y se colocó en un vial de vidrio de 4 mL. Se añadió 1 ml de tetrahidrofurano y se dejó durante 30 min. A continuación, se vertieron 100 µl de la solución en otro vidrio y se añadieron 600 µl de MeOH. Después de mezclar, se tomaron 200 µL. Se procedió a secar

bajo una corriente de nitrógeno. Para limpiar el residuo, este se disolvió con 200 µl de acetonitrilo (que contenía 250 µg/L de BPA-D_{16} y 62.50 µg/L de EtP-$^{13}C_6$) / agua Milli-Q® 1:1 v/v y se centrifugó 24,960×g durante 10 min a 4°C. Por último, se inyectaron 5 µL en el sistema LC-MS/MS.

Cuando se detectó un alto contenido de BPA, PBs, y/o actividad estrogénica o actividad antiandrogénica elevada en los componentes plásticos, se investigó la concentración de BPA y PBs liberados en condiciones de extracción. En resumen, los componentes de plástico se sumergieron en una solución acuosa de cloruro de sodio (NaCl) al 0.90% (250, 500 o 1,000 mL con pH 7.5) durante 20 días a 37°C. El BPA y los PBs se extrajeron de las soluciones de NaCl mediante extracción en fase sólida como se describe en Real et al. (2015). Brevemente, los cartuchos Isolute® C18 se acondicionaron con 2 x 4 mL de MeOH y 2 x 4 mL de agua Milli-Q®, y las soluciones de NaCl a 12 mL/min, seguido de secado durante 1 h y elución con 4 mL de MeOH. El eluyente se secó bajo un corriente de nitrógeno y el residuo disuelto con 200 µL de acetonitrilo (conteniendo 25 µg/L BPA-D_{16} y 10 µg/L EP-$^{13}C_6$) / Agua Milli-Q®, 1:1 v/v. Se inyectaron 5 µL en el sistema LC-MS/MS.

Productos textiles.

La extracción de los materiales de origen textil se realizó mediante la metodología propuesta por Xue et al. (2017), con algunas modificaciones. Brevemente, se pesaron 0.50 g por cada textil y se colocaron en tubos de vidrio de centrifuga de 15 mL enriquecidos con 0.25 mL de una mezcla marcado con isótopos solución de mezcla (250 µg/L BPA-D_{16} y 62.50 µg/L EP-$^{13}C_6$ en acetonitrilo). La extracción se hizo con 7.5 mL de una mezcla de acetona y diclorometano (1:4, v/v). A continuación, se procedió a sonicar durante 20 min y se centrifugó a 5,000×g durante 5 min. Se recogió el disolvente, se filtró a través de un filtro de naylon de 0.20 µm, y fue transferido a otro tubo de vidrio. El solvente se evaporó a sequedad bajo una corriente de nitrógeno y el residuo se disolvió con 250 µL de acetonitrilo, inyectando 5 µL en el sistema LC-MS/MS.

Productos líquidos / semisólidos.

La extracción de BPA y PBs de las pomadas se realizaron como se describió previamente en Wang and Zhou (2013), con algunas modificaciones. Brevemente, se pesaron 0.50 g de cada pomada y se colocaron en tubos de centrífuga de vidrio de 15 mL,

seguido de calentamiento a 80°C durante 2 min para homogeneizar y se agregó 0.02 mL de la solución de mezcla sustituta marcada con isótopos (25 µg/L de BPA-D_{16} y 10 µg/L de EP-$^{13}C_6$, en acetonitrilo). A continuación, se añadieron 5 mL de MeOH, y se agitó durante 2 min. Seguidamente, se sonicó durante 20 min. Después de centrifugar a 5,000 x g durante 10 min, se recogió el disolvente y se filtró a través de un de PTFE de 0.20 µm. El disolvente filtrado se evaporó hasta sequedad bajo una suave corriente de nitrógeno y el residuo se disolvió con 200 µL de acetonitrilo/agua Milli-Q®, 1:1 v/v. A continuación, el extracto se colocó en un tubo Eppendorf® de 1.50 mL y se centrifugó a 24,960×g durante 10 min a 4°C. El extracto se llevó a un vial cromatográfico para inyección en el sistema LC-MS/MS.

En cuanto a la extracción de los líquidos almacenados en plástico y ampollas de vidrio, así como la cafeína intravenosa y el agua esterilizada, se usó una modificación del método de extracción descrito por Sanchez-Prado et al. (2011). Brevemente, se tomaron 0.50 mL de cada muestra, colocados en un tubo de centrífuga de vidrio y enriquecido con 0.02 mL de la solución de la mezcla marcada con isótopos (25 µg/L BPA-D_{16} y 10 µg/L EP-$^{13}C_6$ en acetonitrilo), seguido de la adición de 5 ml de acetato de etilo, agitación durante 2 min y sonicación en un baño ultrasónico durante 20 min. Después de la centrifugación a 5,000×g durante 10 min, el disolvente se recogió y se filtró a través de un filtro PTFE de 0.20 µm. El disolvente filtrado se evaporó hasta sequedad bajo una suave corriente de nitrógeno, y el residuo se disolvió con 200 µL de acetonitrilo/agua Milli-Q®, 1:1 v/v, con 5 µL siendo inyectado en el sistema LC-MS/MS.

En la extracción del líquido desinfectante de manos se utilizó una ligera modificación del procedimiento de extracción descrito por Zanganeh et al. (2021). Brevemente, se tomó 1 mL de cada muestra, colocado en un tubo de centrífuga de vidrio de 15 ml y enriquecido con 0.20 mL de la solución de la mezcla sustituta marcada con isótopos (25 µg/ L BPA-D_{16} y 10 µg/ L EP-$^{13}C_6$ en acetonitrilo), seguido de la adición de 5 mL de MeOH, agitación durante 2 min y sonicación en un baño ultrasónico durante 10 min. Luego se agregaron 5 mL más de MeOH y la solución se filtró a través de un filtro PTFE de 0.20 µm. Se tomaron 300 µL junto con 700 µL de agua Milli-Q® en un vial cromatográfico, inyectando 5 µL en el sistema LC-MS/MS.

Para los ensayos E-Screen y PALM, todos los materiales analizados se analizaron en duplicado utilizando el procedimiento de extracción antes mencionado (en función del tipo de material), pero sin añadir los patrones marcados.

6.1.6. Garantía de calidad y control de calidad de los análisis químicos

Las muestras de textiles, líquidos, plásticos duros y plásticos blandos se utilizaron como blancos para la calibración y validación del método, así como para la evaluación del control de calidad. Para ello, previamente fueron analizados para confirmar que los compuestos de interés no estaban presentes o estaban por debajo del límite de detección (LOD). Se utilizaron dos muestras de textiles diferentes, ya que todos ellos contenían al menos uno de los compuestos estudiados. Los LODs y los límites de cuantificación (LOQs) se establecieron a partir del punto más bajo de la curva de calibración con una relación señal-ruido (S/N) >3 y >10, respectivamente. Los LODs para textiles fueron de 0.70 ng/g para BPA, 0.50 ng/g para MeP y BuP, y 0.40 ng/g para EtP y PrP; Los LOQs fueron 2.20 ng/g para BPA, 1.80 ng/g para MeP y BuP, y 1.40 ng/g para EtP y PrP. LODs para los ungüentos fueron 0.30 ng/g para BPA, 0.15 ng/g para MeP y BuP y 0.10 ng/g para EtP y PrP. Los LODs para los plásticos fueron 0.10 ng/g para BPA y 0.03 ng/g para PBs.

Las muestras se analizaron por duplicado. La extracción se llevó a cabo en lotes de 15 muestras (12 muestras y 3 muestras de control de calidad). Las muestras de control de calidad fueron, un blanco de procedimiento (sin muestra) para comprobar si hay interferencias o contaminación del laboratorio, y dos muestras en blanco con la solución patrón (5.00 ng/g de BPA y 2.50 ng/g de PBs). Las muestras fueron congeladas después de la extracción hasta que se inyectaron en el LC-MS/MS. No se detectó BPA ni PBs en ningún blanco metodológico. Las recuperaciones para todos los controles de calidad oscilaron entre 82% y 107%, y el coeficiente de variación (CV) fue inferior al 20% en todos los casos.

6.1.7. Evaluación de la actividad hormonal (E-Screen y PALM)

El ensayo biológico E-Screen y el ensayo PALM se llevaron a cabo como se describió previamente (Molina-Molina et al., 2019; Molina-Molina et al., 2014), con algunas modificaciones.

6.1.8. Líneas celulares MCF-7 y PALM: condiciones de cultivo.

Las células de cáncer de mama MCF7 BUS (Soule et al., 1973) fueron cedidas por C. Sonnenschein (Universidad de Tufts, Boston, MA) y las células de cáncer de próstata PALM, procedentes de una línea celular andrógeno-dependiente con una transfección estable (Térouanne et al., 2000), fueron cedidas por P. Balaguer (DR2 at INSERMU896, Montpellier, Francia). Ambas líneas celulares se cultivaron como se describió anteriormente (Molina-Molina et al., 2014). De forma resumida, se cultivaron células MCF-7 en DMEM (Gibco, Invitrogen) con fenol rojo suplementado con 10% de FBS (medio de siembra), mientras que las células PALM fueron cultivadas en un medio Ham F_{12} suplementado con un 10% de FBS, 1.00 mg/ml de G418 y 1.00 µg/ml de puromicina (medio de siembra). Dada la actividad hormonal del rojo fenol y el FBS, los experimentos se realizaron en un medio de cultivo DMEM libre de rojo fenol (Gibco, Invitrogen) suplementado con FBS tratado con carbón y dextrano al 10% (10% DCC-FBS) para las células MCF-7 y medio Ham F_{12} suplementado con un 6% de DCC-FBS y antibiótico al 1% para las células PALM, en una atmósfera humificada a 37ºC con dióxido de carbono al 5%.

-*E-Screen.* Brevemente, se sembraron las células MCF-7 en placas de cultivo de 96 pocillos en concentraciones iniciales de 4×10^3 células por pocillo. Un día después, el medio de cultivo fue eliminado y reemplazado por 150 µL de medio de cultivo de siembra (sin rojo fenol). Los extractos secos de las muestras se resuspendieron en 1.25 mL de medio de cultivo, se agitaron vigorosamente, se dejaron reposar durante 30 min, y luego se filtró a través de un filtro de 0.22 µm (jeringa PALL® Acrodisc® Filtros con Membrana Supor®, 13 mm) y se testaron (50 µL añadidos por pocillo) en las células MCF-7 en diluciones de 1:1 a 1:10. La curva respuesta (0.10–1,000 pM) para el estradiol (E_2), el control negativo (células tratadas únicamente con medio sin rojo fenol) y control de disolventes (0.1% de etanol en medio de cultivo sin rojo fenol) se incluyeron en cada experimento. El ensayo finalizó el día 6 (fase exponencial tardía) quitando el medio de los pocillos, fijando las células con ácido tricloroacético (TCA) (10% wt /vol a 4°C, 30 min) y tiñendo con SRB [0.40% wt/vol en ácido acético (1% v/v), a temperatura ambiente, 30 min]. Finalmente, el colorante captado por las células se solubilizó con tris(hidroximetil)aminometano (10 mM, a temperatura ambiente, 30 min, pH 10.4) y la

absorbancia se midió a 492 nm. Este método se basa en la habilidad de la SRB para unirse estequiométricamente a las proteínas de membrana celulares bajo condiciones de acidez leve y de ser liberada en condiciones alcalinas. La cantidad de SRB unida puede servir, por tanto, de un indicador aproximado del número de células, que puede entonces extrapolarse como medida de proliferación celular. A continuación, se calculó la proporción de células teñidas con SRB entre las células tratadas y las células de control sin hormonas (controles negativos) para cada concentración. Los ensayos se realizaron por triplicado y los resultados se expresaron como efecto proliferativo (PE) o proliferación de células MCF-7 expresada sobre el control. La actividad antagonista de los extractos de las muestras fue determinada a través de la co-incubación con el agonista de E_2 a 100 pM. Debido a que el PE solo proporciona información sobre el efecto del extracto en el bioensayo E-Screen, este se transformó en equivalentes de E_2 (E_2Eq) o equivalentes de antiestrogeno (ICI 182780) (EqICI) referidas a 1 g de muestra mediante la lectura de las curvas dosis-respuesta de E_2 o ICI. De esta manera, el PE de cada extracto se refirió a la PE máxima obtenida con E_2 o ICI y se transformó en E_2Eq o EqICIeq. Los valores de EqE_2 y EqICI para cada extracto de muestra se calcularon utilizando la concentración que obtuvo la mayor inducción o inhibición de la proliferación celular, respectivamente. Los valores de EqE_2/g y EqICI se corrigieron para el factor de dilución y se reportaron como EqE_2/g o EqICI/g de la muestra original de la UCIN.

-*Ensayo PALM.* Se sembraron células PALM a una densidad de $5x10^4$ células por pocillo en placas de cultivo de tejidos blancas opacas de 96 pocillos con 150 µl de medio de cultivo suplementado con 6% de DCC-FBS. Los extractos secos de las muestras se diluyeron en serie (como se describió anteriormente para el bioensayo E-Screen) y se agregaron 50 µL por pocillo a las 8 h de la siembra. En cada placa se incluyeron las muestras de prueba y (i) diluciones en serie del agonista sintético del receptor de andrógenos humano metiltrienolona-R1881 (1-10,000 pM) y (ii) el medio de cultivo de prueba como controles positivos y negativos, respectivamente. Las células PALM se incubaron durante 40 h a 37ºC, y luego el medio se retiró y se reemplazó por medio de cultivo de prueba que contenía luciferina 0.30 mM (Sigma-Aldrich). A continuación, se introdujo la placa de 96 pocillos en un lector de luminiscencia durante 2 s para medir la luminiscencia de las células vivas intactas.

Las actividades agonísticas del receptor de andrógenos humanos (hAR) se testaron a diluciones de 1:1 a 1:10 de las muestras, realizando ensayos por cuadriplicado para cada

dilución. Los valores se normalizaron a las lecturas con solo R1881 (10 nM), y esto se tomó como la respuesta máxima o la actividad luciferasa máxima (100%). Se utilizaron controles negativos (medio de cultivo de prueba) para definir la respuesta mínima (10%). La actividad antagonista de los extractos se determinó por coincubación con el agonista de R1881 (0.30 nM). Los resultados se expresaron como un porcentaje de la actividad luciferasa máxima. Finalmente, la actividad luciferasa en cada extracto de muestra se expresó como porcentaje de la actividad luciferasa máxima obtenida con R1881 o procimidona (Proc) y se transformó en equivalentes de R1881 o procimidona (R1881Eq o EqProc, respectivamente) mediante la lectura de las curvas de dosis-respuesta para R1881 o procimidona (diluciones en serie estándar) incluidas en cada placa. Los valores de R1881eq y EqProc se calcularon a partir de la concentración que obtuvo la mayor inducción o inhibición de la actividad luciferasa, respectivamente. Los valores de EqR1881 y EqProc obtenidos se corrigieron para el factor de dilución y se informaron como EqR1881/g y EqProc/g de la muestra original.

6.1.9. Estimación de la exposición dérmica

Dentro del objetivo 3, se realizó específicamente una estimación de la exposición por vía dérmica a través de los calcetines a BPA y PBs para bebés de 1 a 6, 6 a 12, 12 a 24, 24 a 36 y 36 a 48 meses.

La dosis de exposición se calculó de acuerdo con las pautas de evaluación de la exposición de EPA de EEUU. (EPA, 2011) y estudios previos sobre productos químicos peligrosos en la ropa (Li and Kannan, 2018; Liu et al., 2017; Rovira et al., 2015; Xue et al., 2017), utilizando la siguiente fórmula:

$$Exp_{derm} = C \times D \times SA \times F_{mig} \times F_{contact} \times F_{pen} \times T \times N / BW$$

donde Exp_{derm} es la dosis de exposición cutánea diaria estimada (pg/kg peso corporal/día), C es la concentración de sustancias químicas en el calcetín (ng/g), D es la densidad de la fibra del calcetín (mg/cm^2), SA es el contacto con la piel superficie, F_{mig} es la tasa de migración de los productos químicos a la piel (valor predeterminado recomendado como escenario de exposición más adverso: 0.5%/día; BfR (2012), $F_{contact}$ es la fracción del área de contacto con la piel, valor predeterminado recomendado como escenario más adverso: 100%; BfR (2012), F_{pen} es la tasa de penetración de productos químicos en el cuerpo

(valor predeterminado recomendado para el escenario más adverso: 1%; BfR (2012), T es el tiempo de contacto entre el calcetín y la piel (se asume 1 día), N es el número de eventos/día, se asume 1, y BW es el peso corporal promedio de bebés/niños por edad (EPA, 2011). Como superficie de la piel cubierta por los calcetines se consideró la superficie total del pie de los bebés/niños para cada grupo de edad (230, 290, 330, 380 y 490 cm^2, respectivamente; (EPA, 2011). El peso corporal de los bebés/niños considerado en los cinco grupos de edad fue 6.60, 9.20, 11.40, 13.80 y 16.00 kg, respectivamente (EPA, 2011).

6.1.10. Análisis estadístico

Se calcularon concentraciones medias y coeficientes de variación (CV) de BPA, PBs y de las actividades estrogénicas y antiandrogénicas de dos extracciones independientes de todos los elementos recolectados. En el caso particular de los calcetines, también se reportaron las frecuencias, el rango de concentraciones y los valores medianos. Los resultados se organizaron de acuerdo con la ruta principal de exposición. Sin embargo, debe tenerse en cuenta que el contenido de DEs de algunos elementos de la UCIN pueden alcanzar a los compartimentos internos del cuerpo a través de múltiples rutas; por ejemplo, el tubo endotraqueal puede ser una ruta tanto respiratoria como dérmica de exposición aDEs. Además, dada la ausencia de estándares para el contenido máximo de DEs en la ropa, calculamos la frecuencia de muestras con concentraciones de BPA que excedían el estándar de migración de la UE para juguetes (0.1 ppm o µg/g) (Commission Directive 2014/81/EU). Se realizó un análisis de correlación de Spearman para examinar la relación entre las concentraciones de BPA y PBs. Además, se calculó el peso relativo de cada sustancia química en relación con la concentración total de DEs para cada vía de exposición. Para ello, se dividieron las concentraciones de cada compuesto por la suma de BPA y PBs encontrados en cada elemento, seguido del cálculo del peso relativo medio de cada compuesto en los elementos relacionados con cada vía de exposición, y los resultados se expresaron como porcentajes. El caso particular de los calcetines, se usaron diagramas de caja para mostrar la distribución de sustancias químicas en función de las características de calcetín con datos de BPA transformados logarítmicamente y niveles de parabenos no transformados. Se utilizaron pruebas no paramétricas de Kruskal-Wallis y Mann-Whitney para analizar las diferencias en las concentraciones en las diferentes tiendas, el contenido de algodón

(agrupado como <80%, 85% o >90%), contenido de poliéster (agrupado como 0%, 10-20% o >20%), color, sección de calcetines y país de origen. Finalmente, se calcularon los valores promedio, mediano y percentil 95 para las dosis estimadas de absorción dérmica. La significación estadística se estableció en $p < 0.05$. Para los análisis estadísticos se utilizó el software SPSS (versión 23.0; IBM).

6.2. Objetivo 2

6.2.1. Diseño de los estudios

Revisión sistemática + meta-análisis, y estudio transversal

6.2.2. Revisión sistemática + meta-análisis

6.2.2.1 Bases de datos y estrategia de búsqueda

Para llevar a cabo este objetivo, se realizó una revisión sistemática y un meta-análisis de la evidencia bibliográfica sobre la presencia generalizada de bisfenoles, PBs y BPs en leche materna. Este trabajo se llevó a cabo de acuerdo con la *check list Preferred Reporting Items for Systematic Reviews and Metaanalyses* (PRISMA) (Moher et al., 2009).

La búsqueda bibliográfica se realizó en las tres bases de datos de salud más utilizadas: Medline (PubMed), Web of Science (WoS) y Scopus. La estrategia de búsqueda se detalla en la Tabla 7, que se muestra a continuación:

Tabla 7. Estrategia de busqueda en Pubmed, Scopus y Web of Science

Base de datos	**Pubmed (Medline)**
Fecha	**16/06/2020**
Estrategia	**#1 AND #2**
#1	("bisphenols" [All fields] OR "bisphenol A" [All fields] OR "BPA" [All fields] OR "bisphenol S" [All fields] OR "BPS" [All fields] OR "bisphenol F" [All fields] OR "BPF" [All fields] OR "bisphenol A-glycidyl methacrylate" [Mesh] OR "bisphenol A-glycidyl methacrylate" [All fields] OR "BisGMA" [All fields] OR "bisphenol A diglycidyl ether" [All fields] OR "BADGE" [All fields] OR "bisphenol F diglycidyl ether" [All fields] OR "BFDGE" [All fields] OR "benzophenones" [All fields] OR "benzophenone 1" [All fields] OR "2,4-dihydroxy-benzophenone" [All fields] OR "BP1" [All fields] OR "benzophenone 2" [All fields] OR "2,2 ', 4,4'-tetrahydroxybenzophenone" [All fields] OR "BP2" [All fields] OR "benzophenone 3" [All fields] OR "oxybenzone" [All fields] OR "BP3" [All fields] OR "benzophenone 4" [All fields] OR "sulisobenzone" [All fields] OR "BP4" [All fields] OR "4-hydroxybenzophenone" [All fields] OR "4-OHBP" [All fields] OR "benzophenone 5" [All fields] OR "BP5" [All fields] OR "benzophenone 6" [All fields] OR "2,2'-dihydroxy-4,4'-dimethoxybenzophenone" [All fields] OR "BP6" [All fields] OR "benzophenone 7" [All fields] OR "5-chloro-2-hydroxybenzophenone" [All fields] OR "BP7" [All fields] OR "benzophenone 8" [All fields] OR "dioxybenzone" [All fields] OR "BP8" OR "benzophenone 9" [All fields] OR "BP9" [All fields] OR "benzophenone 10" [All fields] OR "BP10" [All fields] OR "BP11" [All fields] OR "benzophenone 12" [All fields] OR "octabenzone" [All fields] OR "BP12"[All fields] OR "parabens" [Mesh] OR "parabens" [All fields] OR "methylparaben" [All fields] OR "MeP" [All fields] OR "ethylparaben" [All fields] OR "EtP" [All fields] OR "prophylparaben" [All fields] OR "PrP" [All fields] OR "buthylparaben" [All fields] OR "BuP" [All fields])
#2	("milk, human" [Mesh] OR "human milk" [All fields] OR "breast milk" [All fields] OR "milk banks" [Mesh] OR "milk banks" [All fields] OR "lactation" [Mesh] OR "lactation" [All fields] OR "colostrum" [Mesh] OR "colostrum" [All fields])
Base de datos	**Scopus**
Fecha	**16/06/2020**
Estrategia	**#1 AND #2**
#1	("bisphenols" OR "bisphenol A" OR "BPA" OR "bisphenol S" OR "BPS" OR "bisphenol F" OR "BPF" OR "bisphenol A-glycidyl methacrylate" OR "BisGMA" OR "bisphenol A diglycidyl ether" OR "BADGE" OR "bisphenol F diglycidyl ether" OR "BFDGE" OR "benzophenones" OR "benzophenone 1" OR "2,4-dihydroxy-benzophenone" OR "BP1" OR "benzophenone 2" OR "2,2 ', 4,4'-tetrahydroxybenzophenone" OR "BP2" OR "benzophenone 3" OR "oxybenzone" OR "BP3" OR "benzophenone 4" OR "sulisobenzone" OR "BP4" OR "4-hydroxybenzophenone" OR "4-OHBP" OR "benzophenone 5" OR "BP5" OR "benzophenone 6" OR "2,2'-dihydroxy-4,4'-dimethoxybenzophenone" OR "BP6" OR "benzophenone 7" OR "5-chloro-2-hydroxybenzophenone" OR "BP7" OR "benzophenone 8" OR "dioxybenzone" OR "BP8" OR "benzophenone 9" OR "BP9" OR "benzophenone 10" OR "BP10" OR "BP11" OR "benzophenone 12" OR "octabenzone" OR "BP12" OR "parabens" OR "methylparaben" OR "MeP" OR "ethylparaben" OR "EtP" OR "prophylparaben" OR "PrP" OR "buthylparaben" OR "BuP")
#2	("human milk"OR "breast milk" OR "milk banks" OR "lactation" OR "colostrum")
Base de datos	**Web of Science**
Fecha	**16/06/2020**
Estrategia	**#1 AND #2**
#1	("bisphenols" OR "bisphenol A" OR "BPA" OR "bisphenol S" OR "BPS" OR "bisphenol F" OR "BPF" OR "bisphenol A-glycidyl methacrylate" OR "BisGMA" OR "bisphenol A diglycidyl ether" OR "BADGE" OR "bisphenol F diglycidyl ether" OR "BFDGE" OR "benzophenones" OR "benzophenone 1" OR "2,4-dihydroxy-benzophenone" OR "BP1" OR "benzophenone 2" OR "2,2 ', 4,4'-tetrahydroxybenzophenone" OR "BP2" OR "benzophenone 3" OR "oxybenzone" OR "BP3" OR "benzophenone 4" OR "sulisobenzone" OR "BP4" OR "4-hydroxybenzophenone" OR "4-OHBP" OR "benzophenone 5" OR "BP5" OR "benzophenone 6" OR "2,2'-dihydroxy-4,4'-dimethoxybenzophenone" OR "BP6" OR "benzophenone 7" OR "5-chloro-2-hydroxybenzophenone" OR "BP7" OR "benzophenone 8" OR "dioxybenzone" OR "BP8" OR "benzophenone 9" OR "BP9" OR "benzophenone 10" OR "BP10" OR "BP11" OR "benzophenone 12" OR "octabenzone" OR "BP12" OR "parabens" OR "methylparaben" OR "MeP" OR "ethylparaben" OR "EtP" OR "prophylparaben" OR "PrP" OR "buthylparaben" OR "BuP")
#2	("human milk"OR "breast milk" OR "milk banks" OR "lactation" OR "colostrum")

6.2.2.2. Selección y extracción de datos

Los criterios de inclusión de esta revisión sistemática fueron:

- Artículos científicos originales
- Artículos publicados en inglés y castellano durante los años 2000-2020.
- Artículos que reporten datos sobre las concentraciones de bisfenoles, PBs y/o BPs en leche materna.

Por el contrario, los criterios de exclusión fueron:

- Artículos que fueran capítulos de libro, revisiones sistemáticas, etc.
- Artículos que no utilizaran leche materna como matriz de estudio o utilizaran leche animal o de fórmula en su trabajo.

La revisión fue realizada por pares, es decir dos expertos procedieron a la selección y evaluación de los artículos de manera independiente y en caso de desacuerdo, un tercer experto externo, definía el resultado.

Los artículos fueron clasificados según el objetivo principal del estudio como epidemiológicos o metodológicos (es decir, artículos cuyo principal objetivo era validar una metodología de extracción y/o cuantificación del compuesto químico y/o su detección).

De cada uno de los artículos se extrajeron hasta 30 variables, que se clasificaron según:

Tabla 8. Variables obtenidas de cada artículo incluido en el estudio

Información de reclutamiento
1. País
2. Continente
3. Origen de las muestras
4. Período de recolección
5. Tamaño muestral
6. Frecuencia de detección
7. Unidades
Información estadística
8. Medias aritméticas
9. P25
10. Medianas
11. P75
12. Rango
Información química
13. LOD
14. LOQ
20. Metodología de extracción
22. Metodología de cuantificación
Información materna
15. Edad
16. Estado de salud
17. Paridad
18. IMC
19. Hábitos tabáquicos
Información leche
21. Cantida de leche
23. Pasteurización previa
24. Tipo de leche
25. Tiempo de lactancia acumulada
26. Tª congelación
27. Ciclos de congelación/descongelación de las muestras
28. Blancos metodológicos
29. Muestras simples/ pooled
Información epidemiológica
30. Factores relacionados con las [bisfenoles], [PBs] y [BPs] * Características antropométricas * Características sociodemográficas * Carácterísticas reproductivas * Hábitos dietéticos * Uso de PCPs

6.2.2.3. Evaluación de calidad y el riesgo de sesgos de los estudios.

La calidad de los informes de los estudios epidemiológicos se evaluó utilizando la lista STROBE (Strengthening the Reporting of Observational studies in Epidemiology) (von Elm et al., 2008). Esta lista de verificación consta de seis bloques y un total de 23 ítems:

- Título y resumen (2 ítems),
- Introducción (2 ítems),
- Método (9 ítems),
- Resultados (5 ítems),
- Discusión (4 ítems),
- Otra información (1 ítems).

La calidad reportada de los artículos fue categorizada de acuerdo con Alvarenga et al. (2021), como:

- Alto (≥16 ítems)
- Medio (15-8 ítems)
- Bajo (<8 ítems).

El riesgo de sesgo se estimó utilizando una versión modificada de ROBINS- I (Schünemann et al., 2019; Sterne et al., 2016) para estudios no aleatorizados (Morgan et al., 2018). El riesgo de sesgo en cada estudio se clasificó como:

- Bajo (⊕)
- Moderado (⊕⊕)
- Grave (⊕⊕⊕)
- Crítico (⊕⊕⊕⊕).

Dada la naturaleza de los artículos metodológicos, no se evaluó su calidad reportada ni el riesgo de sesgo.

6.2.3. Estudio transversal

6.2.3.1. Población de estudio

A finales de 2014 se inició un estudio de seguimiento hospitalario que tenía como propósito analizar la calidad química en la leche materna de madres donantes del banco

de leche materna del Hospital Universitario Virgen de las Nieves de Granada. Durante el período de 2015-2019, se consiguió la participación de un total de 83 madres donantes. El protocolo de investigación fue aprobado por el Comité de Ética en Investigación Biomédica de Granada (Anexo I).

Criterios de inclusión propios del Banco de Leche Materna del Hospital Universitario Virgen de las Nieves de Granada para ser donante de leche materna:

- Registro en el banco de leche después de que la lactancia materna está bien establecida (es decir, 2-3 semanas después del parto).
- No presentar serología positiva para VIH, sífilis o hepatitis B o C;
- No presentar factores de riesgo de enfermedades de transmisión sexual (por ejemplo, pareja inestable, no uso de preservativos, tatuajes / perforaciones en los 3 meses anteriores, acupuntura o transfusión de sangre); o la recepción de trasplante de órganos en los 6 meses anteriores.
- No hábitos tabáquicos o uso de drogas actual. El consumo elevado de alcohol (> 2 bebidas [20 g] / día) o bebidas que contienen cafeína (> 3 tazas [30 g] / día).

Criterios de inclusión propios del estudio:

- Consentimiento informado firmado.
- Ser donantes del banco de leche materna del Hospital Universitario Virgen de las Nieves de Granada.

Información y consentimiento de las madres donantes:

Una vez que las donantes habían realizado su inscripción en el banco de leche, fueron informadas de los objetivos y procedimiento del estudio, solicitando su participación. En el caso de que consintieran formar parte del mismo, se le ofrecía el consentimiento informado. Tras su firma, se les hacía entrega de todo el material necesario para el proceso de extracción de leche, en el que, entre otros, se incluía un tríptico y un manual con todo el procedimiento a seguir. Tras la primera donación de leche de la madre y superadas las diferentes pruebas de calidad de la leche, se le realizó un contacto telefónico para concretar una entrevista personal, adaptándose el equipo a la disponibilidad que las donantes dispusieran. En dicha entrevista, las mujeres completaron un cuestionario *ad*

hoc con información sobre características sociodemográficas y reproductivas y sobre estilo de vida, incluyendo información sobre dieta y uso de PCPs.

Se indicó a las madres que recolectaran las muestras de leche en casa, mediante extracción manual y/o extractor de leche y manteniéndolas congeladas a -20°C hasta el momento de la entrega. El período de recogida debía ser durante un mínimo de una semana y un máximo de cuatro semanas, recogiendo las muestras en biberones hasta el llenado del mismo (aunque fueran diferentes tomas), obteniendo un pool de leche. Las muestras recolectadas fueron sometidas a los procedimientos estándar del Banco de Leche del Hospital Virgen de las Nieves, proceso que se ha explicado en el apartado "procesamiento de la leche materna" (ver apartado 4.3.3). Para este estudio se obtuvo una alícuota de 5-30 mL de leche pre-pasteurizada. Se incluyeron blancos metodológicos para cada una de las muestras, incluyendo un tubo similar llenado con una cantidad semejante, pero con agua de calidad MS/MS. De esta forma, las muestras y los blancos pareados se sometieron a los mismos pasos, en lo que se refiere a condiciones de almacenamiento y procedimientos de extracción y cuantificación idénticos. Ambos tubos se almacenaron a -20ºC hasta su análisis. La fecha de pasteurización se registró como fecha de donación.

6.2.3.2. Productos químicos y reactivos

Bisfenoles [BPA, BPS, BPF y bisfenol A marcado (BPA-D_{16})], PBs [MeP, EtP, PrP, BuP y etilparabeno marcado (EP-$^{13}C_6$)] y BPs [BP-1, BP-2 BP-3, BP-6, BP-8, y 4-hydroxi-BP (4-OH-BP)] se adquirieron de Sigma-Aldrich (Madrid, España). Soluciones estándar en metanol (100 mg/L) se almacenaron a 4°C y se mantuvieron estables durante al menos cuatro meses. Las diluciones de trabajo se prepararon inmediatamente antes de su utilización. Sigma-Aldrich también suministró la β-glucuronidasa / sulfatasa (Helix pomatia, H1). La enzima se preparó diariamente disolviendo 6 mg de β-glucuronidasa/sulfatasa (3×10^6 U g/sólido) en 1.0 mL de solución tampón de acetato de amonio/ácido acético 1M (pH 5.0). El acetonitrilo (grado HPLC), el acetato de etilo y el triclorometano (TCM) se adquirieron en Merck (Darmstadt, Alemania). El cloruro de sodio y N, O-Bis(trimetilsilil)trifluoro-acetamida con trimetilclorosilano (BSTFA/1% TCMS) fueron suministrados por Sigma-Aldrich (Madrid, España). El acetato de zinc hidratado, el ácido fosfotúngstico polihidratado y el ácido acético glacial se compraron en Sigma-Aldrich, y una mezcla de estas sustancias (0.91 g, 0.55 g y 0.60 ml, respectivamente) se disolvió en 10.0 mL de agua desionizada, produciendo una solución

de precipitación de grasa/proteína (FPS) que se preparadó inmediatamente antes de su utilización.

6.2.3.3. Extracción de las muestras, tratamiento de muestras y condiciones GC-MS/MS

El diseño del protocolo de extracción fue adaptado del original (Vela-Soria et al., 2016), con pequeñas modificaciones. Brevemente, las muestras de leche materna se descongelaron completamente a temperatura ambiente y se analizaron por triplicado. A cada alícuota (1.0 mL) se le añadió (i) 10 ng/g de BPA-D16 y EP-$^{13}C_6$ mediante la adición de 20 µL de una solución madre a 1.0 mg/L y (ii) 30 µL de solución enzimática (β-glucuronidasa / sulfatasa). Después de mezclar, la muestra se incubó a 37°C durante 24 h, y luego se agregaron 20 µL de FPS y 0.75 mL de acetonitrilo. La mezcla se agitó con vórtex durante 30s y se centrifugó a 11,357g durante 10 min a 4ºC. El sobrenadante se transfirió a un tubo de vidrio cónico y se diluyó con 10.0 mL de solución acuosa de NaCl al 10% (p/v) a pH 2.0. Después de usar una jeringa para inyectar rápidamente 1.0 mL de TCM en la muestra acuosa, la mezcla se agitó manualmente durante 30s y luego se centrifugaron a 3,226g durante 10 min a 4ºC. La fase sedimentada completa se transfirió a un vial de vidrio limpio y se evaporó bajo una corriente de nitrógeno. A continuación, el residuo se derivatizó con 0.10 mL de BSTFA: acetato de etilo (60:40 v/v) y se incubó durante 15 min a 60ºC. Finalmente, la mezcla se enfrió a temperatura ambiente y se transfirió a un vial de vidrio cromatográfico. Las muestras fueron congeladas después de la extracción y se inyectaron en el GC-MS / MS en un solo lote en el mismo orden que el de su preparación. La extracción se llevó a cabo en lotes de 121 muestras [33 muestras de calibrado, (24 muestras y 6 muestras de control de calidad) * 3] (**Figura 11**).

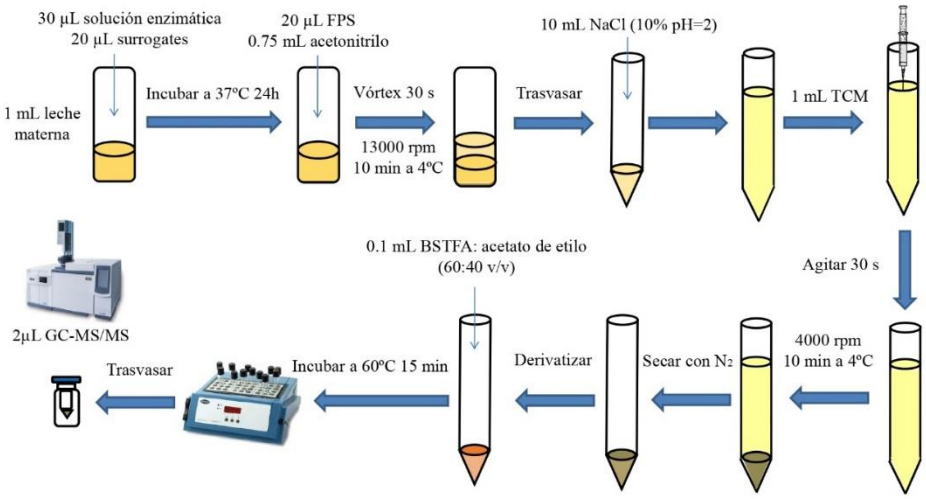

Figura 11. Esquema del protocolo de extracción y tratamiento de muestras de leche materna

6.2.3.4. Instrumentación y análisis GC-MS/MS

En relación al método instrumental, fue adaptado con pequeñas modificaciones de un procedimiento ya existente (Vela-Soria et al., 2014). Los análisis se llevaron a cabo mediante cromatografía de gases acoplada a espectometría de masas en tándem (GC-MS/MS), utilizando un cromatógrafo de gases Agilent 7890 (Agilent Technologies, Palo Alto, CA, EE. UU.) con inyector split-splitless y un inyector automático 7693 ALS. El detector era un espectrómetro de masas de triple cuadrupolo Agilent 7000D con fuente de iones de impacto de electrones inertes, que funcionaba en modo de monitorización de reacción única (SRM, 'single reaction monitoring'), e informaba de dos transiciones MS/MS para cada analito. La ionización por impacto de electrones (EI) se fijó en 70 eV. Para el control del instrumento se utilizó el software Agilent MassHunter B.03.02. Los analitos se separaron en una columna capilar HP–5MS-UI (30 m × 0.25 mm de d.i.; espesor de película de 0.25 µm) de Agilent. El puerto de inyección del cromatógrafo se fijó a 250°C. Las muestras se inyectaron automáticamente en modo de inyección splitless utilizando un Ultra Inert Liner 5,190-3,163 de Agilent. El volumen de inyección fue de 2 µL y se utilizó una jeringa Agilent 5,181-3,354 de 10 µL. El flujo de gas portador helio (99,999 % de pureza) se mantuvo a 1.2 ml/min. La temperatura inicial del horno se fijó en 70ºC y se mantuvo durante 2.0 min, se aumentó a 120ºC a 25ºC/min, se mantuvo durante 0.5 min, se aumentó a 250ºC a 10ºC/min y, finalmente, a 280ºC a 120ºC/min y luego se mantuvo durante 4.0 min (tiempo total: 22 min). La resolución se ajustó a 1.0 Da para los cuadrupolos 1 y 3. Las temperaturas de la línea de transferencia, la fuente de

iones y los cuadrupolos fueron de 280ºC, 280ºC y 150ºC, respectivamente. El espectrómetro de masas se autosintonizó semanalmente.

6.2.3.5. Garantía de calidad y control de calidad de análisis químicos

La linealidad, sensibilidad, precisión y selectividad del método se probaron de acuerdo con las pautas de la FDA para la validación de métodos bioanalíticos (Guidance for Industry, 2001). Los LODs fueron de 0.20 ng / mL para BPA y BP-3 y 0.10 ng / mL para MeP, EtP, PrP, BuP y BP-1. Los LOQs fueron 0.50 ng / mL para BPA y BP-3 y 0.30 ng / mL para MeP, EtP, PrP, BuP y BP-1. Mediante un pool de tres muestras de leche sin analitos, se preparó una curva de calibración con 10 concentraciones de cada analito (tres réplicas) desde 0.2 a 40.0 ng/g, enfrentando el ratio entre el área del compuesto y el compuesto marcado, y la concentración del compuesto. Un rango de concentraciones desde el LOQ hasta 40 ng/g se estableció como el rango lineal dinámico (LDR), con coeficientes de determinación (%R^2) entre 99.1%-99.7%. Los p-valores del test lack-of-fit (% P_{lof}) fue >0.050 en todos los casos, confirmando la linealidad dentro del rango establecido. La selectividad del método fue evaluada mediante el análisis de los cromatogramas del blanco procedimental y la muestra blanco del pool. No se observó ninguna interferencia de sustancias endógenas en los tiempos de retención de los compuestos. La contaminación de fondo se controló mediante el análisis de blancos procedimentales cada 24 inyecciones, usando agua milliQ como muestra. No se detectaron concentraciones cuantificables de los compuestos analizados. Además, las alícuotas del blanco pool dopadas con 1.0, 20.0 y 40.0 ng/g fueron analizadas por triplicado cada 24 inyecciones. Los valores de precisión para las muestras del control de calidad estuvieron dentro de los valores nominales±15%, y, por tanto, cada tanda de muestras pudo fue aceptada. La variación intra-día, expresad como la desviación estándar relativa (RSD, %) fluctuó entre 4.9 y 8.7% para el grupo completo de compuestos analizados. La variación inter-día fluctuó entre 10.7 y 12.9%.

6.2.3.6. Variables independientes

Se obtuvo información sobre características sociodemográficas, antropométricas y reproductivas y de estilo de vida (incluyendo hábitos dietéticos y uso de PCPs) a través de un cuestionario *ad hoc* diseñado para el proyecto y del formulario de datos inicial que

toda donante del Banco debe rellenar al inicio. Se muestra un resumen a continuación, en la **tabla 9:**

Tabla 9. Información recogida de las madres donantes de leche materna

Características sociodemográficas	
	Lugar de residencia (urbano / semiurbano / rural)
	Tiempo en el lugar de residencia
	Tipo de residencia (calle / Avenida)
	Estudios (universitaria / no universitaria)
	Ocupación (desempleado / trabajador manual / trabajador no manual)
Características antropométricas y reproductivas	
	Edad
	Peso
	Altura
	Paridad (primíparas o multíparas)
	Edad gestacional (semanas)
	Duración de la lactancia materna (meses) (actual y anterior si procede)
	Cambio de peso desde antes del embarazo (ganancia / pérdida / sin cambio)
	Transfusión intrauterina (sí / no)
	Diabetes gestacional (sí / no)
Estilo de vida	
	Hábito tabáquico (exfumador / nunca fumador)
	Ingesta de alcohol (≥1 bebida al mes / nunca)
	Ingesta de café (1 taza al día / menos)
	Ingesta regular de medicación (sí / no)
	Empastes (sí / no). Tipos
Cuestionario dietético	
	Tipo de agua potable utilizada (agua del grifo o embotellada)
	Frecuencia de consumo de: pescado (pescado graso y magro), productos lácteos (yogur, leche, queso y mantequilla), embutidos, carnes (todas), carnes rojas, legumbres, verduras (crudas y cocidas), frutas, huevos, pasta, pan, cereales, chocolate, alimentos enlatados, alimentos fritos, alimentos orgánicos, vitaminas y otros suplementos nutricionales.
	Variaciones realizadas en la dieta durante los últimos 12 meses
Cuestionario sobre uso de PCPs y cosmética	
	Frecuencia de uso durante los 12 meses anteriores de: protector solar (aplicación total, sólo cabeza y brazos y/o piernas o solo cabeza), protector labial, crema facial, tónico facial, tratamiento facial, loción corporal, crema de manos, mascarilla para el cabello, productos de maquillaje (base, lápiz labial, sombra de ojos, delineador de ojos), esmalte de uñas, manicura, pedicura, tinte para el cabello, champú, crema de ducha, desodorante, laca / mousse / gel para el cabello, perfume, pasta de dientes y enjuague bucal
Relacionadas con la leche	
	Número de días tras el parto (diferencia entre el parto y la fecha de la donación de la muestra)
	Características nutricionales de la leche [proteínas (g/100 mL), lípidos (g/100 mL), lactosa (g/100 mL), y calorías (kcal/100 mL)

6.2.4. Análisis estadístico

A) Revisión sistemática y metaanálisis

En este trabajo se recogieron todas las concentraciones expresadas como ng/mL. En caso de trabajos donde no se usaran estas unidades, se usó el factor de conversión 1.02 g/mL de leche para los resultados dados en "ng/g de leche" y el factor 2.17 g de lípidos/100 mL para los resultados dados en ng/g de lípidos, de acuerdo con bibliografía previa (Kelishadi et al., 2012).

Para llevar a cabo el meta-análisis, se llevó a cabo el cálculo de la frecuencia de detección ponderada mediante la siguiente fórmula:

$$\text{FD ponderada (\%)} = \sum [(n_1 * FD_1) + \ldots + (n_x * FD_x)] / \sum n_x$$

donde FD es la frecuencia de detección, n es el número de muestras y x es el número de estudios.

De la misma forma, se calculó la media aritmética ponderada de las concentraciones reportadas en cada estudio mediante la siguiente fórmula:

$$\text{AM ponderada} = \sum [(n_1 * AM_1) + \ldots + (n_x * AM_x)] / \sum n_x$$

donde AM es la media aritmética, n es el número de muestras, y x es el número de estudios.

Nota: Los estudios cuyos resultados que expresaron sus resultados mediante los valores medianos o las medias geométricas, no fueron incluidos en el meta-análisis.

B) Estudio transversal

Se usó la media aritmética de los triplicados para los análisis estadísticos. Se calcularon las frecuencias de detección de bisfenoles, PBs y BPs en muestras de leche. A las concentraciones por debajo del LOD se les asignó un valor de LOD/√2. No se detectó BPF, BPS, BP-2, BP-6, BP-8 o 4-OH-BP en ninguna muestra de leche. Las concentraciones de fenoles se resumieron mediante las medias aritméticas con sus desviaciones estándar (DE), medianas, percentiles 25, 75 y 95 y valores mínimos/máximos. También se determinaron las concentraciones totales de PBs (∑PBs),

expresadas en ng/mL, como la suma de las concentraciones molares de PBs en leche materna multiplicadas por el peso molecular del MeP (peso molecular=152.15 ng/nmol), siguiendo estudios previos (Deierlein et al., 2017; Quirós-Alcalá et al., 2018). Una aproximación similar se llevó a cabo para las BPs (∑BPs) multiplicadas por el peso molecular de la BP-3 (peso molecular=228.25 ng/nmol). La suma de todos los fenoles estudiados (∑EDCs) se calculó como BPA + ∑PBs + ∑BPs. Las concentraciones de bisfenol, PBs y BPs no presentaron una distribución normal de acuerdo con la prueba de Kolmogorov-Smirnoff y, por lo tanto, se transformaron en logaritmo neperiano (ln) para minimizar la influencia de los valores extremos. Se utilizó la prueba de correlación de Spearman para explorar las relaciones entre los fenoles. Se realizaron análisis bivariados y de regresión múltiple para identificar predictores de las concentraciones de BPA, PBs y BPs en muestras de leche materna, utilizando una combinación de procedimientos por pasos hacia atrás y hacia adelante para asegurar la robustez de los modelos. Se construyeron modelos de regresión lineal para aquellas sustancias químicas detectadas en ≥75% de las muestras (es decir, MeP, ΣPBs, BP-3, ΣBPs y ΣEDC) y modelos de regresión logística para aquellas detectadas en <75% de las muestras (es decir, BPA, EtP, PrP, y BP-1). Dado el pequeño tamaño de la muestra, el nivel de significancia se estableció en un p-valor=0.050, aunque los resultados con un p-valor entre 0.100 y 0.050 también se discutieron con cautela. Se utilizaron los softwares estadísticos R 3.0 (http://www.r-project.org/) y SPSS v23.0 (IBM SPSS, Armonk, NY) para los análisis de datos.

7. RESULTADOS Y DISCUSIÓN

7.1. Objetivo 1. Caracterizar la exposición a DEs no persistentes (Bisfenoles y parabenoss) a través de los procedimientos médicos, en niños prematuros ingresados en la UCIN.

PRESENCE OF BISPHENOL A AND PARABENS IN A NEONATAL INTENSIVE CARE UNIT: AN EXPLORATORY STUDY OF POTENTIAL SOURCES OF EXPOSURE

Iribarne-Durán LM, *Artacho-Cordón F, Peña-Caballero M, Molina-Molina JM, Jiménez-Díaz I, Vela-Soria F, Serrano L, Hurtado JA, Fernández MF, Freire C, Olea N.*

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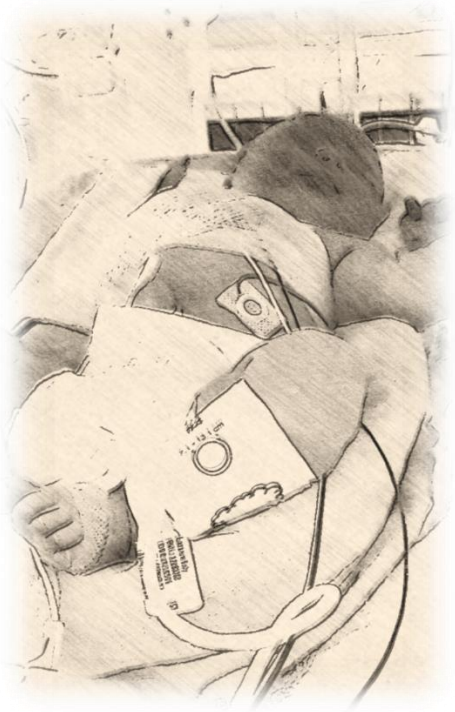
ABSTRACT GRÁFICO



ABSTRACT

Antecedentes: Los recién nacidos en las unidades de cuidados intensivos neonatales (UCIN) están en contacto con una variedad de productos médicos cuyacomposición podría incluir sustancias químicas sintéticas con actividad hormonal.

Objetivos: Nuestro objetivo fue evaluar el contenido de bisfenol A (BPA) y parabenos (PB) y las actividades (anti-)estrogénicas y (anti-)androgénicas de productos médicos comúnmente utilizados en las UCIN en contacto íntimo prolongado con recién nacidos de la UCIN.

Métodos: Se analizaron 52 elementos de la UCIN, determinando las concentraciones de BPA y PBs [metil- (MeP), etil- (EtP), propil- (PrP) y butilparabeno (BuP)] y utilizando el E-Screen y PALM -Ensayos de luciferasa para medir *in vitro* la actividad (anti-) estrogénica y (anti-) androgénica, respectivamente, de los extractos. Los elementos que se encontraron con un contenido elevado de BPA, PBs o actividades similares a las hormonas se extrajeron mediante metodologías de lixiviación.

Resultados: Se encontró BPA en tres de cada cinco y PBs en cuatro de cada cinco de los elementos de la UCIN testados. Aproximadamente el 25% y el 10% de los extractos mostraron actividad estrogénica y antiandrogénica, respectivamente. El contenido más alto de BPA se encontró en la llave de paso de tres vías (>7,000 ng/g), seguida del apósito de película transparente estampado, sondas de alimentación gastro-duodenales, guantes estériles, catéteres umbilicales de un solo luz y conjuntos para intravenosa (IV), las concentraciones variaron de 100 a 700 ng/g de BPA. Se observó una concentración total de PBs (∑PBs) >100 ng/g en varios artículos, incluidos las gafas de protección para la fototerapia, el apósito de película transparente estampado, los catéteres intravenosos con alas, los conjuntos para infusión IV y el esparadrapo textil. La actividad estrogénica más alta, >450 pM de equivalente de estradiol (EqE_2), se encontró en las tetinas de los chupetes pequeños, las llaves de paso de tres vías y los apósitos de película transparente estampados, y la actividad antiandrogénica más alta [>5 mM de unidades equivalentes de procimidona por gramo (Proceq/g)] se encontró en las tetinas de los chupetes pequeños y en las llaves de paso de tres vías.

Discusión: De acuerdo con estos hallazgos, los recién nacidos pueden estar expuestos a múltiples fuentes de BPA y PBs en las UCIN por vía inhalatoria, dérmica, oral e IV/parenteral. Es necesario abordar las posibles implicaciones para la salud de estos pacientes extremadamente vulnerables y adoptar medidas preventivas de precaución con carácter de urgencia.

Introduction

Bisphenol A (BPA) is a high-production volume chemical commonly used in the manufacturing of epoxy resins and polycarbonate plastics and as an additive in many other plastics, e.g. polyvinyl chloride (PVC) (Gimeno et al. 2015). Parabens (PBs) are a family of alkyl esters of p-hydroxybenzoic acid [methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP), and butylparaben (BuP) congeners]. They are widely included in personal care products and pharmaceuticals as antimicrobial preservatives and as an additive in plastics for food packaging and beverages (Darbre and Harvey 2008). Over the past few decades, it has been shown that BPA and PBs demonstrate estrogen-like effects, and these compounds have therefore been described as endocrine disrupting chemicals (EDCs) (Boberg et al. 2010; Darbre and Harvey 2008; Perez et al. 1998). Moreover, numerous studies have associated BPA and PB exposure with metabolic disorders in adults and with impaired neurodevelopment and disrupted sexual maturation in children (Giulivo et al. 2016). Particular concerns have been raised about early-life EDC exposure, because of its potential to cause adverse health consequences throughout life. Furthermore, the pharmacokinetics of xenobiotics differ between neonates and older children/adults and can also be affected by underlying medical conditions (Nellis et al. 2015). Thus, it has been suggested that exposure to EDCs such as BPA and PBs during susceptible periods of child development (e.g., perinatal period) may be associated with various adverse effects in children, including alterations in behavior and executive function (Ghassabian et al. 2018; Jiang et al. 2019), accelerated pubertal timing (Berger et al. 2018; Harley et al. 2019), or respiratory disease (Agier et al. 2019; Buckley et al. 2018).

Very-low-birth-weight (VLBW) newborns (<1500g) and low-birth-weight (LBW) newborns (<2500g) often require a complex care environment in a

neonatal intensive care unit (NICU) to simulate in utero conditions until the proper development of their immature skin and gastrointestinal, immune, nervous, and/or respiratory systems (Harrison and Goodman 2015). LBW infants are also more likely to require medical appliances in intimate contact with them, including: (1) bags of intravenous (IV) fluids and total parenteral nutrition, (2) nasogastric and enteral feeding tubes, respiratory masks/endotracheal tubes, and venous catheters; and/or (3) cardiopulmonary bypass circuits, among others. Many of these medical devices are made of polycarbonate and/or PVC plastics, in which residual non-polymerized BPA can remain after the polymerization process and may leach from the product (Gimeno et al. 2015). BPA may also leach after hydrolysis of the polymer under certain conditions (EU 2010; Mercea 2009), and its release is greater with longer contact time, higher temperature, and elevated pH (hydroxide aqueous solutions) (Geens et al. 2011; Geens et al. 2012). To date, only two studies have addressed the exposure of NICU neonates (Calafat et al. 2009; Duty et al. 2013). One of these (Calafat et al. 2009) found that urinary BPA concentrations were 3.42- to 8.75-fold higher when the intensity of medical device utilization was medium or high versus low. The other study (Duty et al. 2013) observed 16- to 32-fold higher BPA concentrations in NICU infants than in those from the general population. Concerning PBs, Calafat et al. (2009) also found higher urinary MeP levels among newborns with a greater use of medical devices, although potential sources of exposure were not identified. In addition, infants are also potentially exposed to BPA and PB exposures from personal care products, clothing and other baby products, including socks, diaper-changing mats, and baby mattresses (Asimakopoulos et al. 2016; Freire et al. 2019; Xue et al. 2017). Thus, NICU-admitted infants can be inadvertently exposed to BPA and PBs via dermal, ingestion, inhalation, intravenous (IV), and parenteral routes. Furthermore, the combined exposure of NICU infants to other EDCs besides BPA and BPs, such as phthalates, should be taken in consideration. Previous studies have reported the exposure of VLBW and LBW newborns to phthalates in NICUs (Calafat et al. 2004; Green et al. 2005; Stroustrup et al. 2018b; Weuve et al. 2006), suggesting that it impacts on neurobehavioral performance (Stroustrup et al. 2018a).

In response to increasing concerns about EDC exposure in the hospital environment, the European Commission published Opinions on the risk of oral, subcutaneous, and intravenous exposure to BPA (SCENIHR 2015) and phthalates (SCENIHR 2016) from medical devices made of materials that may potentially leach these chemicals. They described a particular risk of systemic BPA availability after non-oral exposures, as in the case of neonates in NICUs, infants undergoing prolonged medical procedures, and patients receiving dialysis (SCENIHR 2015). The highest daily exposure was reported to be for neonates in NICUs, at 3000 ng/kg bw, while the daily exposure for adult dialysis patients was 57 ng/kg bw, and SCENIHR (2015) called for urgent research on the composition and release of BPA from medical devices. However, no attention has been paid to the presence of PBs in the NICU environment. The present study is part of a wider project that aims to assess the potential adverse health impact on neonates in a NICU of exposure to EDCs from their medical care, diet, and environment. The general aim of this first study was to identify potential sources of exposure to EDCs in neonates admitted to NICUs. For this purpose, we assessed the content of BPA and PBs in an extensive array of (1) plastic medical devices, (2) textiles, and (3) semisolid/liquid products (including ointments and nutritional supplements) that are commonly used in NICUs and are in intimate contact with newborns, and we measured both the estrogenic and anti-estrogenic activities and the androgenic and anti-androgenic activities of extracts from these products.

Material and methods

Sample collection

In June 2018, we collected a total of 52 unused items habitually employed in the NICU of the Virgen de las Nieves Hospital, in Granada (Spain). This convenience sample was selected by three pediatricians of the unit (JAH, MPC and LS), who together compiled a list of all items used in the unit that were in intimate contact with neonates. It included 25 plastic medical devices, 18 textiles, and 9 semisolid/liquid products, as detailed in Table 1, which also displays the information on their composition reported by the manufacturers on the packaging or on their website. We report the extraction method, concentrations of BPA and PBs (MeP, EtP, PrP, BuP and ∑PBs), and the average length of time in contact with the neonate for each item (estimated by the pediatricians). The country of

manufacture was in Europe for 37 items, America for 8, Asia for 6, and Australia for 1. Out of the 25 plastic items, only 8 described the raw material used (polyhexahydrotriazine in 1, polyethylene in 1, PVC in 4, polyurethane in 1, and cotton/polyamide/polyurethane in 1). With regard to additives, the presence/absence of latex was indicated in 17 items (16 items declared latex-free) and the presence/absence of di-(2-ethylhexyl)-phthalate (DEHP) in 7 items, including 4 declared as DEHP-free.

Chemicals and reagents

All reagents were analytical grade unless otherwise specified. BPA, MeP, EtP, PrP, BuP, labeled deuterium BPA (BPA-d16), and labeled EtP ring 13C6 (EtP-13C6) were purchased from Sigma-Aldrich (Madrid, Spain). Solvents for extraction procedures, ethyl acetate, tetrahydrofuran, and dichloromethane, were purchased from Merck (Darmstadt, Germany), and methanol and acetone were supplied by Sigma-Aldrich. LC-MS grade acetonitrile, water, and ammonia (25%) were purchased from Sigma-Aldrich. Water (18.2 MΩ cm) was purified using an in-house Milli-Q system (Millipore, Bedford, MA, USA).

For chemical analyses, stock standard solutions (100 mg/L) of each compound were prepared in acetonitrile and stored at 4 ºC in the dark. The solutions remained stable for at least two months.

Working standards were prepared immediately before use by dilution with pure acetonitrile. For in vitro cell assays, reference standards 17β-estradiol (E2), methyltrienolone (R1881), ICI 182780 (henceforth, ICI), procymidone, puromycin, geneticin (G418), luciferin (sodium salt), sulforhodamine B (SRB), and trichloroacetic acid (TCA) were obtained from Sigma-Aldrich Inc. (St Louis, MO). Stock solutions (10 mM) of E2, R1881, procymidone, and ICI were prepared in ethanol, and successive dilutions were performed in culture medium. Stock solutions were kept at -20 °C, and dilution series were freshly prepared before each experiment. The culture medium and fetal bovine serum (FBS) were supplied by Gibco (Invitrogen, Barcelona, Spain) and all cell culture plastics by Falcon (VWR International Eurolab, Barcelona, Spain).

Instrumentation and UHPLC-MS/MS conditions

Analyses were performed by ultra-high-performance liquid chromatography coupled to mass spectrometry (UHPLC-

MS/MS), using an ACQUITY UPLC™ H-Class (Waters, Manchester, UK) consisting of ACQUITY UPLC™ binary solvent manager and ACQUITY UPLC™ sample manager. A Xevo TQS tandem quadrupole mass spectrometer (Waters) equipped with an orthogonal Z-spray™ electrospray ionization (ESI) source was used for BPA and PBs detection. Chromatographic separation of compounds was performed using an . Flow rate was 0.25 mL/min. The injection volume was 5 µL. The column temperature was maintained at 40 °C. The mass spectrometer was operated in negative ESI mode, using optimized MS/MS parameters as defined in a previous study (Vela-Soria et al. 2014).

Plastic medical devices. BPA and PBs from hard plastic materials in medical devices were studied as follows: 50 mg of sample were accurately weighted and placed in a 7 mL glass vial, and 3 mL of acetone were then added and left for 24 h, followed by sonication for 30 min. Samples were then centrifuged at 20 ºC for 10 min at 5000 g, and the organic phase was taken, filtered through 0.2 µm PTFE filter, and dried under a nitrogen stream. The residue was dissolved with 200 µL acetonitrile (containing 25 µg/L of BPA-d16 and 10 µg/L of EP-13C6): Milli-Q water 1:1 v/v, and centrifuged at 4 ºC for 10 min at 24960 g for cleaning. BPA and PBs from soft plastic materials were studied using a slight modification of the method described by Genay et al. (2011). Briefly, 50 mg of sample were accurately weighted and placed in a 4 mL glass vial, and 1 mL tetrahydrofuran was added and left for 30 min. Next, 100 µL of the solution were poured into another glass vial and 600 µL of MeOH were added. After mixing, 200 µL were taken and dried under a nitrogen stream. The

ACQUITY UPLC BEH™ C18 (50 mm × 2.1 mm I.D., 1.7 µm particle size) from Waters. The gradient mobile phase consisted of 0.025% (v/v) ammonia aqueous solution (solvent A) and 0.025% (v/v) ammonia in acetonitrile (solvent B). Gradient conditions were as follows: 0.0–3.5 min, 60% B; 3.5–4.0 min, 60–100% B; 4.0–6.5 min, 100% B and back to 60% in 0.1 min

Sample extraction, treatment, and LC-MS conditions

Samples were extracted from the selected items using different methodologies according to the nature of the material. The extraction procedure for each type of material is described below.

residue was then dissolved with 200 µL acetonitrile (containing 250 µg/L of BPA-d16 and 62.5 µg/L of EtP-13C6): Milli-Q water 1:1 (v/v) and centrifuged at 4 ºC for 10 min at 24960 g for cleaning, followed by the injection of 5 µL into the LC system.

When high BPA/PB content or elevated estrogenic/anti-androgenic activity was detected in plastic components, the concentration of released BPA and/or PBs was then studied under soft extraction conditions. In brief, plastic components were fully immersed in 0.9% NaCl aqueous solution (250 mL, 500 mL, or 1000 mL with pH of 7.5) for 20 days at 37 ºC, followed by analysis of the solutions to determine the presence of BPA and/or PBs. BPA and PBs were extracted from the NaCl solutions by solid phase extraction as described elsewhere (Real et al. 2015). Briefly, Isolute C18 cartridges were conditioned with 2 x 4 mL methanol and 2 x 4 mL Milli-Q water, and NaCl solutions were then loaded at 12 mL/min, followed by drying for 1 h and elution with 4 mL of methanol. The eluent was dried under a nitrogen stream and the residue dissolved with 200 µL acetonitrile (containing 25 µg/L BPA-d16 and 10 µg/L EP-13C6): Milli-Q water, 1:1 v/v. 5 µL was injected into the LC system

Textile products. Extraction of BPA and PBs from textile products was performed as previously described (Xue et al. 2017) with some modifications. Briefly, 0.5 g of each textile was accurately weighed, cut, placed in 15 mL glass centrifuge tubes, and spiked with 0.25 mL of an isotope-labeled surrogate mixture solution (250 µg/L BPA-d16 and 62.5 µg/L EP-13C6 in acetonitrile), while 7.5 mL of a mixture of acetone and dichloromethane (1:4, v/v) was used for the extraction. After sonication for 20 min and centrifugation at 5,000 g for 5 min, the solvent was collected, filtered through an 0.2 µm nylon filter and transferred to another glass tube. The solvent was evaporated to dryness under a gentle stream of nitrogen and the residue dissolved with 250 µL of acetonitrile for injection into the LC system.

Table 1. NICU item detail information

Item no.	Description	Route of exposure	Country[a]	Contact time with neonates[b]	Material	Extraction method	Raw material[c]	Details
1	Feeding syringe I	Oral	France	Hours	Plastic	Own methodology	n.f.	
2	Feeding syringe II	Oral	France	Hours	Plastic	Own methodology	n.f.	
3	Gastro-duodenal feeding tube	Oral	France	Week	Plastic	Genay et al. 2011	n.f.	
4	Extension tube for feeding syringe	Oral	France	Days	Plastic	Genay et al. 2011	PVC	
5	Feeding sampling straw	Oral	France	Minutes	Plastic	Genay et al. 2011	n.f.	
6	Small dummy	Oral	Germany	Week	Plastic	Own methodology	n.f.	Latex
7	Large dummy	Oral	Germany	Week	Plastic	Own methodology	n.f.	
8	Human milk fortifier	Oral	Switzerland	Minutes	Semisolid/ liquid	Wang et al. 2013	n.f.	
9	Pulse oximeter adhesive sensor I	Dermal	USA	Days	Plastic /	Own methodology	n.f.	Latex-free
10	Pulse oximeter adhesive sensor II	Dermal	China	Days	Textile	Own methodology	n.f.	Latex-free
11	ECG electrode	Dermal	Malaysia	Days	Textile	Xue et al. 2017	n.f.	Latex-free
12	Light therapy protection glasses	Dermal	USA	Days	Textile	Xue et al. 2017	Cotton/ PA/PUR	
13	Occlusive skin wrap	Dermal	New	Minutes	Plastic	Genay et al. 2011	PE	
14	Sterile gloves	Dermal	Malasia	Minutes	Plastic	Genay et al. 2011	n.f.	Latex-free
15	Latex gloves	Dermal	Malasia	Minutes	Plastic	Genay et al. 2011	n.f.	
16	Patterned transparent film dressing	Dermal	USA	Weeks	Textile	Xue et al. 2017	n.f.	Latex-free
17	White hypoallergenic paper tape	Dermal	Spain	Weeks	Textile	Xue et al. 2017	n.f.	
18	Textile tape	Dermal	France	Weeks	Textile	Xue et al. 2017	n.f.	
19	Surgical tape	Dermal	Germany	Weeks	Textile	Xue et al. 2017	n.f.	
20	Self-adhesive dressing pad	Dermal	Spain	Weeks	Textile	Xue et al. 2017	n.f.	Latex-free
21	Wound dressing transparent with paper frame	Dermal	China	Weeks	Textile	Xue et al. 2017	n.f.	Latex-free
22	Transparent adhesive film dressing	Dermal	England	Weeks	Textile	Xue et al. 2017	n.f.	
23	Hydrocolloid transparent dressing	Dermal	Denmark	Weeks	Textile	Xue et al. 2017	n.f.	
24	White cohesive bandage	Dermal	Spain	Weeks	Textile	Xue et al. 2017	n.f.	Latex-free
25	Infant flow LP headgear	Dermal	Mexico	Weeks	Textile	Xue et al. 2017	n.f.	Latex-free,
26	Sterile non-woven swabs	Dermal	Spain	Minutes	Textile	Xue et al. 2017	n.f.	Latex-free
27	Non-sterile non-woven swabs	Dermal	Spain	Minutes	Textile	Xue et al. 2017	n.f.	Hypoalergen
28	Absorbent bed underpad	Dermal	Portugal	Days	Textile	Xue et al. 2017	n.f.	
29	XS-sized diaper	Dermal	Germany	Hours	Textile	Xue et al. 2017	n.f.	
30	S-sized diaper	Dermal	Germany	Hours	Textile	Xue et al. 2017	n.f.	
31	Chlorhexidine	Dermal	Spain	Minutes	Semisolid/ liquid	Wang and Zhou 2013	n.f.	2g 100mL
32	Hand sanitizer	Dermal	Germany	Minutes	Semisolid/ liquid	Zhang et al. 2005	n.f.	45g 2-propanol,
33	Talcum and zinc oxide cream	Dermal	Spain	Minutes	Semisolid/ liquid	Wang and Zhou 2013	n.f.	Lanoline, dimethicone,
34	Proteolytic enzyme cream	Dermal	United Kingdom	Minutes	Semisolid/ liquid	Wang and Zhou 2013	n.f.	Collagenase A, liquid
35	Winged IV catheter (transparent section)	IV/parenteral	Belgium	Days	Plastic	Own methodology	n.f.	
36	Winged IV catheter	IV/parenteral	Germany	Week	Plastic	Genay et al. 2011	n.f.	
37	Single lumen umbilical vein catheter	IV/parenteral	France	Week	Plastic	Genay et al. 2011	PVC	DEHP-free
38	Double lumen umbilical vein catheter	IV/parenteral	France	Week	Plastic	Genay et al. 2011	PUR	
39	Extension set for the intravenous infusion	IV/parenteral	United	Week	Plastic	Genay et al. 2011	PVC	Latex-free,
40	Extension set for the intravenous infusion	IV/parenteral	Italy	Week	Plastic	Genay et al. 2011	PVC	Latex-free,
41	Three-way stopcock	IV/parenteral	Israel	Week	Plastic	Own methodology	n.f.	
42	Disinfecting cap for needle-free connectors	IV/parenteral	USA	Week	Plastic	Own methodology	n.f.	
43	Hypodermic injection needle	IV/parenteral	USA	Week	Plastic	Own methodology	n.f.	
44	Syringe	IV/parenteral	Spain	Day	Plastic	Own methodology	n.f.	
45	Caffeine perfusion 20 mg/ml	IV/parenteral	Italy	Minutes	Semisolid/ liquid	Sánchez-Prado et al. 2011	n.f.	
46	Water for injection solvent for parenteral use	IV/parenteral	Spain	Minutes	Semisolid/ liquid	Sánchez-Prado et al. 2011	n.f.	
47	0,9% Sodium chloride solution for IV flush (syringe)	IV/parenteral	Germany	Minutes	Semisolid/ liquid	Sánchez-Prado et al. 2011	n.f.	
48	0,9% Sodium chloride solution for IV (ampoule)	IV/parenteral	Spain	Minutes	Semisolid/ liquid	Sánchez-Prado et al. 2011	n.f.	
49	Endotracheal tube	Respiratory	Malaysia	Weeks	Plastic	Genay et al. 2011	PHT	Latex-free,
50	Closed suction system	Respiratory	Mexico	Weeks	Plastic	Genay et al. 2011	n.f.	Latex-free,
51	Nasal cannula	Respiratory	Lithuania	Weeks	Plastic	Genay et al. 2011	n.f.	Latex-free,
52	Nasal prong	Respiratory	USA	Weeks	Plastic	Genay et al. 2011	n.f.	Latex-free,

PVC: polyvinyl chloride; PA: polyamide; PUR: polyurethane; PE: polyethylene; DEHP: di-(2-ethylhexyl)-phthalate; PHT: polyhexahydrotriazine; n.f.: not found; [a] country in which the item was manufactured; [b] average time that the item is in contact with newborns, based on a survey of the pediatricians; [c] information obtained from packaging and/or manufacturer's website.

Liquid/semisolid products. Extraction of BPA and PBs from ointments was performed as previously described (Wang and Zhou 2013) with some modifications. Briefly, 0.5 g of each ointment was accurately weighed and placed in 15 mL glass centrifuge tubes, followed by heating at 80 ºC for 2 min to homogenize and spiking with 0.020 mL of the isotope-labeled surrogate mixture solution (25 µg/L of BPA-d16 and 10 µg/L of EP-13C6, in acetonitrile). Next, 5 mL of MeOH was added, followed by shaking for 2 min and sonication in an ultrasonic bath for 20 min. After centrifugation at 5,000 g for 10 min, the solvent was collected and filtered through 0.2-µm PTFE filter. The filtered solvent was evaporated to dryness under a gentle stream of nitrogen and the residue dissolved with 200 µL of acetonitrile: Milli-Q water, 1:1 v/v. Next, the extract was placed in a 1.5 mL Eppendorf tube and centrifuged at 24960 g for 10 min at 4 ºC. The extract was placed in a chromatographic vial for injection into the LC system.

BPA and PBs were extracted from sodium chloride (plastic and glass ampoules), IV caffeine, and sterile water using a modification of the extraction method described by Sanchez-Prado et al. (2011). Briefly, 0.5 mL of sample was taken, placed in 15 mL glass centrifuge tubes and spiked with 0.020 mL of the isotope-labeled surrogate mixture solution (25 µg/L BPA-d16 and 10 µg/L EP-13C6 in acetonitrile), followed by the addition of 5 mL ethyl acetate, shaking for 2 min and sonication in an ultrasonic bath for 20 min. After centrifugation at 5,000 g for 10 min, the solvent was collected and filtered through 0.2 µm PTFE filter. The filtered solvent was evaporated to dryness under a gentle nitrogen stream, and the residue was dissolved with 200 µL of acetonitrile: Milli-Q water, 1:1 v/v, with 5 µL being injected into the LC system.

BPA and PBs were extracted from hand sanitizer liquid using a slight modification of the extraction procedure described by Zhang et al. (2005). Briefly, 1 mL of sample was taken, placed in a 15 mL glass centrifuge tube and spiked with 0.20 mL of the isotope-labeled surrogate mixture solution (25 µg/L BPA-d16 and 10 µg/L EP-13C6 in acetonitrile), followed by the addition of 5 mL methanol, shaking for 2 min, and sonication in an ultrasonic bath for 10 min. A further 5 mL methanol was then added, and the solution was filtered through 0.2 µm PTFE filter. Next, 300 µL was taken, placed in a chromatographic vial, and 700 µL of

Milli-Q water was added, with 5 µL being injected into the LC system.

Quality assurance and quality control in chemical analyses

Matrix-matched calibration was performed for the analysis of textiles and ointments. Samples used as blanks were previously analyzed to confirm that the compounds of interest were absence or below the limit of detection (LOD). Standard addition calibration was used for samples of liquids, hard plastics, and soft plastics.

LODs and limits of quantification (LOQs) were determined on the basis of the lowest point of the calibration standard with a signal-to-noise (S/N) ratio of >3 and >10, respectively. LODs for textiles were 0.7 ng/g for BPA, 0.5 ng/g for MeP and BuP, and 0.4 ng/g for EtP and PrP. LODs for ointments were 0.3 ng/g for BPA, 0.15 ng/g for MeP and BuP, and 0.1 ng/g for EtP and PrP. LODs for the standard addition calibration were 0.1 ng/g for BPA and 0.03 ng/g for PBs.

Samples were analyzed in duplicate. Extraction was carried out in batches of 15, with each batch containing 12 samples as well as the following 3 quality control samples: procedural blank (no sample) to test for interference or laboratory contamination and two spiked blank samples (5.0 ng/g of BPA and 2.5 ng/g of PBs). Samples were frozen after extraction until injection into the LC system. No BPA or PBs were detected in any procedural blank. Recoveries for all target compounds in the quality-control spiked samples ranged between 86 and 104%, and the coefficient of variation (CV) was below 20% in all cases.

Hormone-like activity assessment

The E-Screen bioassay and PALM luciferase assay were performed as previously described (Molina-Molina et al. 2014; Molina-Molina et al. 2019) with some modifications.

MCF-7 and PALM cell lines: culture conditions. MCF7 BUS human breast cancer cells (Soule et al. 1973) were a gift from Dr. C. Sonnenschein (Tufts University, Boston). PALM human prostate cancer cells, from a human androgen-dependent stable transfected cancer line (Terouanne et al. 2000), were provided by Dr. P. Balaguer (DR2 at INSERM U896, Montpellier, France). Both cell lines were cultured as previously described (Molina-Molina et al. 2014). In brief, MCF-7 cells were

cultured in DMEM (Gibco, Invitrogen, Barcelona, Spain) with phenol red supplemented with 10% FBS (seeding medium), while PALM cells were cultured in Ham's F12 supplemented with 10% FBS, 1 mg/mL G418, and 1 µg/mL puromycin (seeding medium). Given the hormonal activity of phenol red and FBS, experiments were performed in a test culture medium of phenol red-free DMEM (Gibco, Invitrogen, Barcelona, Spain) supplemented with 10% dextran-coated charcoal-FBS (10% DCC-FBS) for MCF-7 cells and Ham's F12 supplemented with 6% DCC-FBS and 1% antibiotic for PALM cells, in a 5% CO2 humidified atmosphere at 37 °C.

E-Screen. Briefly, MCF-7 cells were trypsinized and plated in 96-well culture plates at initial concentrations of 4 x 103 cells per well. One day later, the seeding medium was removed and replaced with 150 µL test culture medium. For agonistic assays, dry extracts of the samples were resuspended in 1.25 mL test culture medium, vigorously shaken, left at rest for 30 min, and then filtered through a 0.22 µm filter and tested (50 µL added per well) on MCF-7 cells at 1:1 to 1:10 dilutions. A dose-response curve (0.1-1000 pM) for estradiol (E2) and a negative control of cells treated solely with hormone-free medium (test culture medium) and a solvent control (0.1% ethanol in test culture medium) were included in each experiment. The bioassay was ended on day 6 (late exponential phase) by removing the media from wells, fixing the cells with TCA [10 % (w/v) at 4 ºC, 30 min], and staining them with SRB [0.4 % (w/v) in acetic acid (1% v/v), at room temperature, 30 min]. Finally, bound dye was solubilized using tris(hydroxymethyl)aminomethane (10 mM, at room temperature, 30 min, and pH 10.4) and the absorbance read at 492 nm. This method relies on the ability of SRB to bind stoichiometrically to cell membrane proteins under mild acidic conditions and to be removed under basic conditions. The amount of bound dye can therefore serve as a proxy for cell number, which can then be extrapolated to measure cell proliferation. Next, the ratio of SRB-stained cells between treated cells and hormone-free control cells (negative controls) was calculated for each concentration. Tests were done in triplicate, and results were expressed as proliferative effect (PE) [MCF-7 cell proliferation (-fold over control)]. The antagonistic activities of sample extracts were determined by co-incubation with the agonist E2 at 100 pM. Because the

PE only provides information on the effect of the extract in the E-Screen bioassay, this was transformed into E2 equivalent (E2eq) or anti-estrogen (ICI 182780) equivalent (ICIeq) units related to 1 g of sample by reading from dose-response curves of E2 or ICI (Supplementary Figure 1A and 1B). In this manner, the PE of each extract was referred to the maximal PE obtained with E2 or ICI and transformed into E2eq or ICIeq. E2eq and ICIeq values for each sample extract were calculated by using the concentration that obtained the greatest induction or inhibition of cell proliferation, respectively. E2eq and ICIeq values were corrected for the dilution factor and reported as E2eq/g or ICIeq/g of the original NICU sample.

PALM assay. PALM cells were seeded at a density of 5 x 104 cells per well in 96-well white opaque tissue culture plates in 150 µL test culture medium. Dry extracts of the samples were serially diluted (as described above for the E-Screen bioassay), and 50 µL per well were added at 8 h after seeding. Serial dilutions of the synthetic human androgen receptor agonist methyltrienolone-R1881 (1-10,000 pM) and the test culture medium alone were included on each plate with the test samples as positive and negative controls, respectively. PALM cells were incubated for 40 h at 37 °C, and the medium was then removed and replaced by test culture medium containing 0.3 mM luciferin (Sigma-Aldrich Inc). Next, the 96-well plate was introduced into a luminometer for 2 s to measure luminescence from intact living cells.

Human androgen receptor (hAR)-agonistic activities were tested at 1:1 to 1:10 dilutions of the samples, performing tests in quadruplicate for each dilution. Values were normalized to the readings with R1881 alone (10 nM), and this was taken as the maximum response or maximal luciferase activity (100%). Negative controls (test culture medium alone) were used to define the minimum response (10%). The antagonistic activity of extracts was determined by co-incubation with R1881 agonist (0.3 nM). Results were expressed as percentage of maximal luciferase activity. Finally, the luciferase activity in each sample extract was expressed as percentage of the maximal luciferase activity obtained with R1881 or procymidone (Proc) and transformed into R1881 or procymidone equivalent units (R1881eq or Proceq, respectively) by reading from dose-response curves for R1881 or procymidone (standard serial dilutions) included on each plate

(Anexo II, Supplementary Figure 1C and 1D). R1881eq and Proceq were calculated from the concentration that obtained the greatest induction or inhibition of luciferase activity, respectively. R1881eq and Proceq values obtained were corrected for the dilution factor and reported as R1881eq/g and Proceq/g of the original sample.

Statistical data analysis

Mean concentrations and coefficients of variation (CVs) of BPA, MeP, EtP, PrP, BuP, and total paraben compounds (∑PBs) were calculated, as well as the estrogenic and anti-androgenic activities from two separate extractions. Results for EDC content and hormone-like activities were summarized according to the main route of exposure. Nevertheless, it should be taken into account that the EDC content of some NICU items (e.g., BPA and/or PBs) may plausibly reach internal body compartments via multiple routes; for instance, the endotracheal tube might be both a respiratory and dermal route of exposure to hormone-like chemicals. Spearman correlation analysis was performed to examine the relationship between log-transformed concentrations of BPA and PBs. The relative weight of each chemical in relation to the total EDC concentration was calculated for each exposure route. For this purpose, the concentrations of each compound were divided by the sum of BPA and PBs found in each item, followed by calculation of the mean relative weight of each compound in the items related to each exposure route, expressing the results as percentages. Statistical significance was set at $p<0.05$. SPSS v.23.0 (IBM, New York, USA) was used for the statistical analyses

Results

Data on the BPA and PBs content and (anti-)estrogen and (anti-)androgenic activities of extracts are exhibited in Tables 2 to 5 according to the exposure route (oral, dermal, IV/parenteral, and inhalation). Figure 1 depicts the mean relative concentration of each compound in items in contact with newborns via the same exposure route. As shown, BPA concentrations represented ~30% of the total concentration of the studied EDCs via all routes of exposure, while MeP represented 40-60% of total BPA and PB content via all routes except for inhalation. In addition, BPA levels were significantly and positively correlated with ∑PBs in the samples (rho coefficient 0.411, $p = 0.017$). Estrogenic and anti-androgenic activities were detected in 13 (25%) and 5 (10%) of the 52 tested samples, respectively (Tables

2-5). With the exception of the winged IV catheter, all NICU items that exhibited estrogenic activity contained BPA at detectable levels. No anti-estrogenic or androgenic activity was observed in any sample. Estrogenic activity was observed in two out of five DEHP-free items (40.0%) and in one out of three DEHP-containing items (33.3%).

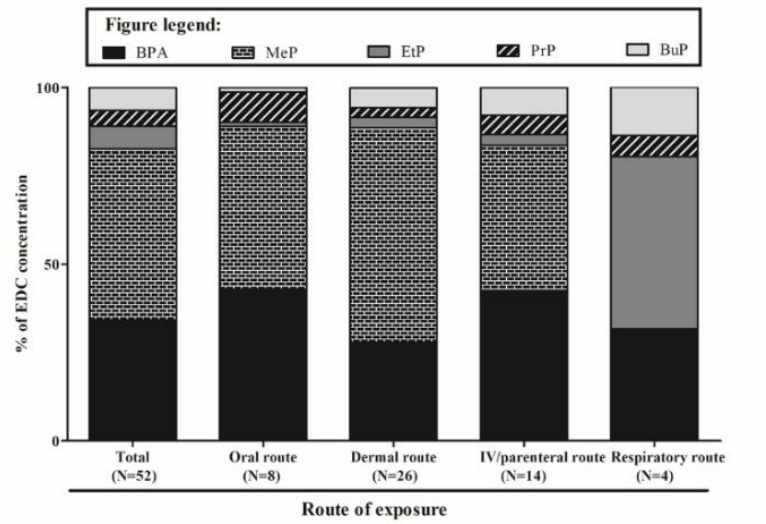


Figure 1. Distribution profiles of the BPA and paraben content found in NICU items according to the main route of exposure. Bars represent the mean relative concentration of each compound in items in contact with neonates via the same exposure route

Oral route of exposure

BPA was found in five (62.5 %) of the eight oral-use items at concentrations ranging up to 301.1 ng/g (Table 2). The highest BPA concentration was recorded in the gastro-duodenal feeding tube (301.1 ng/g), followed by the feeding sampling straw (107.7 ng/g). PBs were detected in five (62.5%) of the oral-use items, observing the highest concentrations in the small dummy nipple (90.8 ng/g) and gastro-duodenal feeding tube (73.8 ng/g). The most frequently detected PB congener was MeP, observing the highest concentrations in the small dummy nipple (63.7 ng/g) and gastro-duodenal feeding tube (64.8 ng/g), which were also positive for estrogenicity (2403.8 pM E2eq/g and 193.9 pM E2eq/g, respectively). The highest anti-androgen activity was recorded in the small dummy nipple (9.8 mM Proceq/g). The large dummy leached detectable levels of BPA (0.12 ng/g) and the small dummy exhibited anti-androgenic activity (0.03mM ProcEq/g) (Anexo II, Supplemental Table 1).

Dermal route of exposure

Table 3 displays the BPA and PB content and hormonal activity of the NICU items in dermal contact with neonates. BPA

was detected in 17 (65.4%) of the 26 items at concentrations ranging from 0.90 to 688.1 ng/g. The highest BPA concentration was in patterned transparent film dressing (688.1 ng/g), followed by sterile gloves (140.5 ng/g) and the hard section of the pulse oximeter adhesive sensor I (73.6 ng/g).

Detectable concentrations of at least one PB congener were found in 24 (92.3%) of the 26 items, and ∑PBs values ranged from 0.30 to 484.9 ng/g. The most frequently detected PB was MeP (88.5%), which showed the highest concentration among PBs (mean ± SD= 47.0 ± 104.2 ng/g), followed by EtP (34.6%; 7.9 ± 10.7 ng/g). The highest MeP concentration was found in light therapy protection glasses (480.7 ng/g), followed by patterned transparent film dressing (208.0 ng/g), textile tape (108.0 ng/g), hard section of the pulse oximeter adhesive sensor I (81.9 ng/g), and self-adhesive dressing pad (79.1 ng/g).

As in the case of BPA and PB concentrations, the highest estrogenic activity was found in patterned transparent film dressing, followed by hard and adhesive sections of the pulse oximeter adhesive sensor I, light therapy protection glasses, textile tape, transparent wound dressing with paper frame, and ECG electrode. Anti-androgenic activity was detected by the PALM assay in non-sterile and sterile gloves and in patterned transparent film dressing, with values ranging from 1.0 to 2.4 mM ProcEq/g.

Intravenous and parenteral routes of exposure

Table 4 displays the BPA and PB content and hormonal activity of the NICU items related to the IV/parenteral exposure route. BPA was detected in 7 (50.0%) of the 14 items. The highest BPA concentration (among all 52 items in the study) was found in the three-way stopcock (clear section) (7052.7 ng/g), followed by the single-lumen umbilical vein catheter (130.4 ng/g), IV infusion system extension set (112.7 ng/g), and double-lumen umbilical vein catheter (49.0 ng/g). PBs were detected in 11 items (78.6%), with the highest concentrations being 149.5 ng/g (96.9 ng/g PrP) for the winged IV catheter (item no. 36) and 125.8 ng/g (106.4 ng/g MeP) for the IV infusion system extension set. Detectable levels of PBs were also observed in some liquid solutions, including 0.9% sodium chloride (in commercial syringe or ampoule preparations)

In addition to the presence of BPA and/or PBs, estrogen-like activity was detected in the clear section of the three-

way stopcock (489.8 pM E2eq/g), the winged IV catheter (item no. 36) (300.0 pM E2eq/g), the single-lumen umbilical vein catheter (281.0 pM E2eq/g), and the IV infusion system extension set (192.2 pM E2eq/g). Anti-androgenic activity was observed in the clear section of the three-way stopcock (5.4 mMProceq/g). Both the three-way stopcock and the single-lumen umbilical vein catheter leached detectable levels of BPA (4.7 and 0.9 ng/g, respectively) and exhibited estrogenic activity (18.3 and 9.3 pM E2eq/g, respectively) (Anexo II, Supplemental Table 1).

Inhalation exposure route

Table 5 displays the BPA and PB content and hormonal activity of the four NICU items related to the inhalation route. BPA was detected in two of these, at a concentration of 95.4 ng/g in the endotracheal tube and 33.9 ng/g in the nasal prong. At least two PB congeners were detected in each item, with ∑PBs ranging up to 116.6 ng/g (nasal cannula). The PB congener with the highest concentration was BuP in the nasal prong and EtP in the remaining three items. The endotracheal tube, which contained 95.4 ng/g BPA and 72.1 ng/g ∑PBs, also showed estrogenic activity (148.1 pM E2eq/g). No anti-androgenic activity was observed in any of these items. Endotracheal tube samples were found to leach EtP (0.87 ng/g) and PrP (0.43 ng/g), and the extracts exhibited estrogenic activity (7.28 pM E2eq/g) (Anexo II, Supplemental Table 1).

Table 2. Concentrations of BPA and PBs and hormone-like activities in NICU items related to oral routes of exposure (N=8).

		EDC content (ng/g)											Hormone-like activity	
		BPA		MeP		EtP		PrP		BuP		ΣPBs	E-Screen	PALM assay
Item no.	Description	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)		E_2 eq/g(pM)[a]	Proc eq/g(mM)[b]
1A	Feeding syringe I	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
1B	Feeding syringe I (piston)	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
2A	Feeding syringe II	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
2B	Feeding syringe II (piston)	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
3	Gastro-duodenal feeding tube	301.1	13.2	64.8	15.7	5.5	6.7	3.5	18.1	<0.03	-	73.8	193.9	ND
4	Extension tube for feeding syringe	11.9	6.7	17.4	2.1	<0.03	-	<0.03	-	<0.03	-	17.4	ND	ND
5	Feeding sampling straw	107.7	15.3	5.0	12.9	<0.03	-	17.4	2.1	2.4	7.4	24.8	ND	ND
6A	Small dummy (nipple)	6.5	12.3	63.7	5.4	5.3	11.5	20.4	5.4	1.4	10.6	90.8	2,403.8	9788.0
6B	Small dummy (hard section)	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
7A	Large dummy (nipple)	7.8	8.6	24.2	14.8	<0.03	-	6.3	11.5	1.7	16.6	32.2	ND	ND
7B	Large dummy (hard section)	3.6	5.6	9.4	4.1	<0.03	-	<0.03	-	<0.03	-	9.4	ND	ND
8	Human milk fortifier	<0.3	-	<0.15	-	<0.1	-	<0.1	-	<0.15	-	-	ND	ND
	N (%) NICU items	5 (62.5%)		5 (62.5%)		2 (25.0%)		4 (50.0%)		3 (37.5%)		6 (75.0%)	2 (25.0%)	1 (12.5%)

Note: Values below the limit of detection are represented by the symbol < followed by the limit of detection. -, not applicable; BPA, bisphenol A; BuP, butyl-paraben; CV, coefficien of variance, E_2, estradiol: EDC: endocrine disrupting chemical; EtP: ethyl-paraben; MeP: methyl-paraben; ND, not detected; NICU, neonatal intensive care unit; PrP: propyl-paraben; ∑PBs: total concentrations of parabens.

[a]Concentrations equivalent to E2 per gram;

[b]Concentrations equivalent to procymidone per gram.

Table 3. Concentrations of BPA and PBs and hormone-like activities in NICU items related to dermal routes of exposure (N=26).

		EDC content (ng/g)										Hormone-like activity	
		BPA		MeP		EtP		PrP		BuP		ΣPBs	E-Screen	PALM assay
Item no.	Description	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)		E_2 eq/g (pM)[a]	Proc eq/g(mM)[b]
9A	Pulse oximeter adhesive sensor I (hard section)	73.6	13.5	81.9	15.7	<0.03	-	11.5	17.1	<0.03	-	93.4	1, 400.0	ND
9B	Pulse oximeter adhesive sensor I (adhesive section)	4.1	8.4	8.3	12.3	<0.04	-	<0.04	-	<0.05	-	8.3	346.2	ND
10	Pulse oximeter adhesive sensor II	2.7	5.1	12.7	16.8	0.8	5.1	<0.04	-	<0.05	-	13.5	ND	ND
11	ECG electrode	33.1	9.0	<0.05	-	0.3	1.1	<0.04	-	<0.05	-	0.3	126.2	ND
12	Light therapy protection glasses	4.6	9.0	480.7	4.1	4.2	12.3	<0.04	-	<0.05	-	484.9	197.0	ND
13	Occlusive skin wrap	67.0	10.2	4.3	8.4	<0.04	-	<0.04	-	9.2	12.3	13.5	ND	ND
14	Sterile gloves	140.5	12.3	19.4	14.0	<0.04	-	14.3	12.8	<0.05	-	33.5	ND	1.8
15	Non-sterile gloves	17.8	10.6	32.4	2.1	<0.04	-	13.0	4.4	<0.05	-	45.4	ND	2.4
16	Patterned transparent film dressing	688.1	11.2	208.0	4.8	11.7	6.1	1.4	12.3	<0.05	-	221.1	2, 107.4	1.0
17	White hypoallergenic paper tape	1.4	0.9	13.4	6.2	2.1	11.1	<0.04	-	<0.05	-	15.5	ND	ND
18	Textile tape	6.4	8.0	108.0	12.7	3.4	11.7	<0.04	-	1.6	13.4	113.0	171.4	ND
19	Surgical tape	0.9	13.4	7.5	1.4	<0.04	-	<0.04	-	<0.05	-	7.5	ND	ND
20	Self-adhesive dressing pad	1.2	8.0	79.1	13.0	32.8	13.0	<0.04	-	<0.05	-	111.9	ND	ND
21	Transparent wound dressing with paper frame	4.3	13.9	40.5	15.0	7.1	2.2	<0.04	-	<0.05	-	47.6	144.0	ND
22	Transparent adhesive film dressing	1.1	16.6	6.1	5.4	<0.04	-	<0.04	-	<0.05	-	6.1	ND	ND
23	Hydrocolloid transparent dressing	26.7	13.1	1.5	5.4	<0.04	-	<0.04	-	<0.05	-	1.5	ND	ND
24	White cohesive bandage	2.7	5.9	5.4	11.5	0.9	3.6	<0.04	-	<0.05	-	6.3	ND	ND
25	Infant flow LP headgear	<0.07	-	4.3	6.7	<0.04	-	1.7	10.4	<0.05	-	6.0	ND	ND
26	Sterile non-woven swabs	<0.07	-	1.1	3.6	<0.04	-	<0.04	-	<0.05	-	1.1	ND	ND
27	Non-sterile non-woven swabs	<0.07	-	3.8	8.0	<0.04	-	<0.04	-	<0.05	-	3.8	ND	ND
28	Absorbent bed underpad	<0.07	-	0.3	0.1	<0.04	-	<0.04	-	<0.05	-	0.3	ND	ND
29	XS-sized diaper	<0.07	-	<0.05	-	<0.04	-	<0.04	-	<0.05	-	-	ND	ND
30	S-sized diaper	<0.07	-	1.1	10.6	<0.04	-	<0.04	-	<0.05	-	1.1	ND	ND
31	Chlorhexidine	1.1	11.3	3.2	16.4	<0.1	-	<0.1	-	<0.15	-	3.2	ND	ND
32	Hand sanitizer	<0.3	-	<0.15	-	<0.1	-	<0.1	-	<0.15	-	-	ND	ND
33	Talcum and zinc oxide cream	<0.3	-	2.6	2.1	<0.1	-	0.2	10.1	1.1	6.1	3.9	ND	ND
34	Proteolytic enzyme cream	<0.3	-	1.9	13.4	<0.1	-	<0.1	-	10.7	12.1	12.6	ND	ND
	N (%) NICU items	17 (65.4%)		23 (88.5%)		9 (34.6%)		6 (23.1%)		4 (15.4%)		24 (92.3%)	7 (26.9%)	3 (11.5%)

Note: Values below the limit of detection are represented by the symbol < followed by the limit of detection. -, not aplicable; BPA, bisphenol A; BuP, butyl-paraben; CV coefficient of variance; E_2, estradiol; EDC: endocrine disrupting chemical; ECG, electrocardiograph; EtP: ethyl-paraben; MeP: methyl-paraben; ND, not detected; NICU, neonatal intensive care unit; PrP: propyl-paraben; S, small; XS, extra small; ∑PBs: total concentrations of parabens;

[a]Concentrations equivalent to E_2 per gram;

[b]Concentrations equivalent to procymidone per gram.

Table 4. Concentrations of BPA and PBs and hormone-like activities in NICU items related to IV/parenteral routes of exposure (N=14).

		EDC content (ng/g)											Hormone-like activity	
		BPA		MeP		EtP		PrP		BuP		ΣPBs	E-Screen	PALM assay
Item no.	Description	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)		E_2 eq/g(pM)[a]	Proc eq/g(mM)[b]
35A	Winged IV catheter (disinfecting cap for needle)	<0.1	-	21.5	14.0	<0.03	-	<0.03	-	<0.03	-	21.5	ND	ND
35B	Winged IV catheter (colored section)	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
36	Winged IV catheter	<0.1	-	43.8	8.5	7.0	10.6	96.9	14.8	1.8	1.5	149.5	300.0	ND
37	Single lumen umbilical vein catheter	130.4	13.1	<0.03	-	10.9	3.6	<0.03	-	<0.03	-	10.9	281.0	ND
38	Double lumen umbilical vein catheter	49.0	5.9	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
39	Extension set for the IV infusion system	112.7	4.8	106.4	5.4	<0.03	-	19.4	9.3	<0.03	-	125.8	192.2	ND
40	Extension set for IV infusion system (light resistant)	29.8	20.2	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
41A	Three-way stopcock (clear section)	7, 052.7	18.2	10.0	13.1	1.2	5.9	<0.03	-	<0.03	-	11.2	489.8	5.4
41B	Three-way stopcock (cap)	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
42	Disinfecting cap for needle	5.2	14.0	17.6	2.8	<0.03	-	<0.03	-	<0.03	-	17.6	ND	ND
43	Hypodermic injection needle (plastic protector)	6.7	11.4	12.2	15.7	<0.03	-	<0.03	-	<0.03	-	12.2	ND	ND
44A	Syringe	<0.1	-	4.7	7.3	<0.03	-	<0.03	-	<0.03	-	4.7	ND	ND
44B	Syringe (plunger)	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
45	Caffeine perfusion 20 mg/mL infant	<0.3	-	<0.15	-	<0.1	-	<0.1	-	0.8	15.7	0.8	ND	ND
46	Water for injection solvent for parenteral use	<0.3	-	<0.15	-	<0.1	-	<0.1	-	<0.15	-	-	ND	ND
47	0.9% Sodium chloride solution for IV flush (syringe)	<0.3	-	5.7	6.7	2.6	10.2	<0.1	-	0.8	9.3	9.1	ND	ND
48	0.9% Sodium chloride solution for IV flush (ampoule)	<0.3	-	3.2	6.1	<0.1	-	<0.1	-	<0.15	-	3.2	ND	ND
	N (%) NICU items	7 (50.0%)		9 (62.3%)		4 (28.6%)		2 (14.3%)		3 (21.4%)		11 (78.6%)	4 (28.6%)	1 (7.1%)

Note: Values below the limit of detection are represented by the symbol < followed by the limit of detection. -, not applicable, BPA, bisphenol A; BuP, butyl-paraben; CV, coefficient of variance; E_2, estradiol; EDC: endocrine disrupting chemical; EtP: ethyl-paraben; IV: intravenous; MeP: methyl-paraben; NaCl, sodium chloride; ND, not detected; NICU, neonatal intensive care unit; PB, paraben; PrP: propyl-paraben; BuP: butyl-paraben; ∑PBs: total concentrations of parabens.

[a]Concentrations equivalent to E2 per gram;

[b]Concentrations equivalent to procymidone per gram.

Table 5. Concentrations of BPA and PBs and hormone-like activities in NICU items related to respiratory routes of exposure (N=4).

		EDC content (ng/g)											Hormone-like activity	
		BPA		MeP		EtP		PrP		BuP		ΣPBs	E-Screen	PALM assay
Item no.	Description	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	-	pM E_2 eq/g(pM)[a]	Proc eq/g(mM)[b]
49	Endotracheal tube	95.4	16.6	<0.03	-	58.6	14.0	5.6	11.1	7.9	10.2	72.1	148.1	ND
50	Closed suction system	<0.1	-	<0.03	-	8.4	7.3	<0.03	-	<0.03	-	8.4	ND	ND
51	Nasal cannula	<0.1	-	<0.03	-	76.2	3.6	18.1	9.8	22.3	19.3	116.6	ND	ND
52	Nasal prong	33.9	18.4	<0.03	-	<0.03	-	3.4	12.3	15.6	7.6	19.0	ND	ND
	N (%) NICU items	2 (50.0%)		0 (0.0%)		3 (75.0%)		3 (75.0%)		6 (75.0%)		4 (100%)	1 (25.0%)	0 (0.0%)

Note: Values below the limit of detection are represented by the symbol < followed by the limit of detection. -, not applicable, BPA, bisphenol A; BuP, butyl-paraben; CV, coefficient of variance; E_2, estradiol; EDC: endocrine disrupting chemical; EtP: ethyl-paraben; ND, not detected; NICU, neonatal intensive care unit; PB, paraben; PrP: propyl-paraben; BuP: butyl-paraben; ∑PBs: total concentrations of parabens.

[a]Concentrations equivalent to E2 per gram;

[b]Concentrations equivalent to procymidone per gram.

Discussion

This study reports, to our knowledge for the first time, the presence of BPA and PBs in materials in contact with newborns in NICUs. It also presents the first evidence that the contents of NICU materials exert hormonal activities. Thus, BPA was detected in 59.6% of the 52 NICU items tested and PBs in 86.5%, while estrogenic activity was observed in 26.9% of item extracts and anti-androgenic activity in 9.6%. These findings indicate that various NICU materials may act as potential sources of exposure to BPA and PBs for the extremely vulnerable neonates admitted to this type of unit.

Diet is the main route of BPA human exposure in the general population. Although we did not assess nutritional samples (breast milk or formula) in the present investigation, a previous study revealed that nutritional intake might not be a crucial contributor to urinary BPA concentrations in NICU newborns (Duty et al. 2013). With regard to the oral intake of PBs, only limited evidence has been published on the PB content of medication administered in NICUs (Nellis et al. 2015). Interestingly, we found that some medical devices related to the nutrition system have detectable levels of BPA and PBs and show estrogenic and anti-androgenic activities. In particular, we observed a high concentration of BPA and PBs in the gastro-duodenal feeding tube and of BPA in the feeding sampling straw. Higher urinary BPA concentrations were previously reported in NICU neonates who required feeding-related medical devices (e.g., nasogastric tube) in comparison to those who did not (Duty et al. 2013). Estrogenic activity was also shown by two of the items related to oral exposure. The highest estrogenic and anti-androgenic activities in any studied item were observed in extracts from the small dummy used in the NICU, likely due not only to the BPA and PB content but also to non-tested EDCs such as DEHP, whose presence is suggested by the plasticity of the material. In this context, authors using a water and methanol migration technique found that baby teethers leached BPA and PBs (Asimakopoulos et al. 2016; Potouridis et al. 2016).

As in the case of adult patients, the skin of hospitalized neonates is habitually in direct contact with dressings, tapes, bandages, electrodes, or wraps, among others. The epidermis of preterm neonates is especially fragile due to incomplete maturation of the skin barrier, exacerbating their susceptibility

to chemical irritation and local or systemic infections (Eichenfield and Hardaway 1999; Oranges et al. 2015). Moreover, incomplete development of the stratum corneum can increase the permeability of neonatal skin to topical agents (Oranges et al. 2015). In the present study, some of the NICU items were identified as putative sources of dermal exposure to BPA and PBs, including patterned transparent film dressing, textile tapes, and light therapy protection glasses, whose extracts also evidenced estrogenic and/or anti-androgenic activity, as did extracts from pulse oximeter adhesive sensor I, ECG electrodes, and sterile and non-sterile gloves. The range of BPA concentrations in these dermal-contact items (range <LOD-688.1 ng/g) is similar to that reported in infant clothing (<2.21-111 ng/g) (Xue et al. 2017) and lower than that observed in infant socks (186-13300 ng/g) (Freire et al. 2019; Xue et al. 2017). However, the dermal exposure of neonates to these EDCs from NICU items is likely to be higher, given the aforementioned immaturity of their skin.

Several IV/parenteral tubing items contained BPA and PBs (e.g., three-way stopcock, umbilical vein and winged catheters, and IV infusion system extension set), and their extracts also showed hormonal (estrogenic and/or anti-androgenic) activity. In this regard, detectable amounts of BPA were reported to leach from hemodialyzers (Haishima et al., 2001; Murakami et al., 2007), and plasma BPA levels were lower in hemodialysis patients using polynephron (BPA-free) versus conventional polysulfone dialyzer membranes (Mas et al., 2018). Likewise, BPA concentrations were increased after a single hemodialysis session in patients with diabetes (Turgut et al. 2016) or uremia (Shintani 2001), and urinary BPA levels were significantly higher in patients using BPA-free dialyzers in comparison to BPA-containing dialyzers (Bosch-Panadero et al., 2016). Similar studies have reported increased serum DEHP levels in hemodialysis patients (Wahl et al. 2004). We also found that BPA was leached by the IV/parenteral tubing and accessories, and the eluted extracts exhibited estrogenic activity.

With regard to the inhalation exposure route, high BPA and PBs concentrations (>100 ng/g) were observed in the endotracheal tube and nasal cannula. In this line, Duty et al. (2013) found significantly higher median BPA urinary concentrations in newborns who required nasal cannula (40 μg/L vs 26 μg/L) or continuous positive airway

pressure (38 μg/L vs 13 μg/L) in comparison to those who did not. The extract of endotracheal tube assayed in the present study exhibited estrogenic activity, a novel finding. Endotracheal tube, nasal cannulas, and nasal prong were made of plasticized PVC, and it is known that products made of plasticized PVC and/or polycarbonate and epoxy resins may contain BPA (Lopez-Cervantes and Paseiro-Losada 2003; Sun et al. 2001). Chiellini et al. (2011) reported detectable levels of DEHP in PVC endotracheal tubes.

Our findings reveal the widespread presence of hormonally active chemicals in medical items used in NICUs and related to inhalation, oral, dermal and IV/parenteral routes. Given the extreme vulnerability of these neonates, these findings are of major concern. In adults, the toxicokinetics of BPA and PB vary widely according to the exposure route (Soeborg et al. 2014), whereas the metabolism of BPA and PB in neonates appears to be similar for all routes of exposure (Taylor et al. 2008). For instance, first-pass metabolism considerably reduces the bioavailability of free BPA and PBs after oral exposure in adults (Shin et al. 2019; Soeborg et al. 2014), the capacity for phase II metabolism is slowly maturing in neonates and infants (<3 months of age), increasing the bioavailability of free BPA and PBs (Mulla et al. 2015; Nachman et al. 2014) might be even more pronounced in VLBW and LBW pre-term neonates. In contrast, BPA and PBs are systemically bioavailable after dermal or IV exposure, even in adults (SCENIHR 2015; Soeborg et al. 2014). Furthermore, in the case of preterm neonates, the absorption fraction of EDCs after dermal exposure is likely to be higher than the range estimated for adults (10 – 30 %), given their increased skin permeability (SCENIHR 2015) and higher skin surface area-to-body weight and weight-to-intake ratios (Guzeilan et al. 1992). Alongside the immaturity of their xenobiotic metabolism capacity, this means that the bioavailability of EDCs may be substantively greater in neonates than in older children or adults for the same exposure doses.

The European Union (EU) has banned the use of BPA in baby bottles (EU 2011) and in materials in contact with foods intended for infants and young children (EU 2018). The EU has also set a maximum BPA migration limit of 0.1 mg/mL in toys designed for under 3-year-old children or to be placed in the mouth (EU 2017). With regard to PBs, the sole restriction in EU legislation is

that personal care products for under 3-year-olds should carry a label warning against their use in the diaper area (EU 2014). The evidence presented in the present study indicates the need to regulate the presence of BPA and PBs in materials commonly used in hospital, especially in NICUs. In this regard, SCENIHR (2015) recommended the selection of medical devices that do not leach EDCs when possible. However, current EU regulations on the prohibition of BPA and PBs do not include hospital materials in intimate contact (oral and non-oral) with extremely vulnerable VLBW and LBW neonates admitted to NICUs.

The EDC concentrations observed in plastic NICU items should not be considered as leaching levels, although the leaching of plastic items with high BPA/PB content or estrogenic/anti-androgenic activity was further examined by using softer extraction methods that may be closer to physiological temperature and pH conditions. Likewise, concentrations in textile and liquid/semisolid products should not be interpreted as absorbed concentrations, and the variability in skin permeability (SCENIHR 2015) prevents estimation of the daily dermal exposure dose. A second study limitation was the failure to measure other potential EDCs such as phthalates, although the presence/absence of phthalates was indicated on the label of some items. We highlight that no item indicated the presence/absence of BPA and/or PBs. However, we cannot rule out the presence in these extracts of other hormonally-active compounds, including phthalates as well as hitherto unidentified compounds with hormonal potency, or the presence of mixtures of various compounds. Furthermore, the possible effect of chemical mixtures, including additive, synergistic, or antagonistic interactions, cannot be predicted from individual chemical contents. Of special interest in this regard was NICU item 36 (Winged IV catheter) in the IV/parenteral route of exposure, in which no BPA or PB was detected but a high level of estrogenic activity was recorded. It should be borne in mind that this study was designed to explore the presence of these compounds in a range of NICU medical devices but not to provide exhaustive analyses of all materials used in NICUs or to compare products among different suppliers. Finally, the results cannot be generalized to NICUs worldwide, given potential differences in hospital protocols and procedures. However, the findings of our exploratory investigation have revealed

the widespread presence of hormonally active chemicals in several NIC items, indicating the need for the careful examination by competent authorities of all materials/devices in contact with NICU neonates, assessing not only their EDC content and leaching rates but also their biological activity.

Neonates in NICUs may be potentially exposed to hormonally-active chemicals from multiple sources. There is a need for further research to verify these findings, to support the development of preventive measures, and to ensure that health care professionals are aware of the EDC content/leaching of materials and devices. It is also crucial to include data on the utilization of medical devices in clinical-epidemiological studies on adverse effects in NICU neonates.

Conclusions

We contribute the first report, to our best knowledge, of direct measurements of the BPA/PB content and hormone-like activity of medical devices and products widely used in NICUs and in prolonged intimate contact with newborns. Our findings suggest that NICU newborns may be exposed to BPA and/or PBs via inhalation, oral, dermal, and intravenous/parenteral routes, with the possibility that other hospitalized infants may be similarly exposed. There is an urgent need to investigate the potential short-, mid- and long-term implications of our findings for the health of these highly vulnerable neonates.

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7.2. Objetivo 2. Estudiar la exposición a DEs no persistentes (bisfenoles, parabenos y benzofenonas) a través del alimento en niños prematuros ingresados en la UCIN.

CONCENTRATIONS OF BISPHENOLS, PARABENS, AND BENZOPHENONES IN HUMAN BREAST MILK: A SYSTEMATIC REVIEW AND META-ANALYSIS

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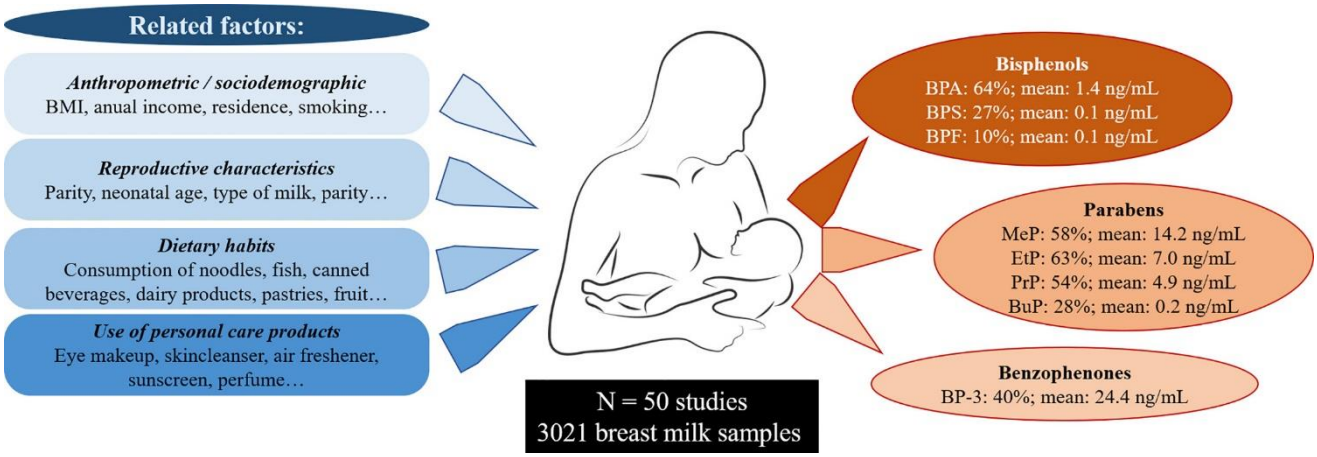
ABSTRACT GRÁFICO



ABSTRACT

Antecedentes: La leche materna es la principal fuente de nutrición de los lactantes, pero puede ser responsable de su exposición a sustancias químicas ambientales, incluidas aquellas sustancias que alteran el sistema endocrino.

Objetivo: Revisar la evidencia disponible sobre la presencia y concentraciones de bisfenoles, parabenos (PBs) y benzofenonas (BPs) en la leche materna y explorar factores relacionados con los niveles de exposición.

Métodos: Se llevó a cabo una revisión sistemática utilizando las bases de datos Medline, Web of Science y Scopus, realizando una búsqueda exhaustiva de artículos originales revisados por pares publicados durante el período 2000– 2020, incluyendo estudios epidemiológicos y metodológicos. Los criterios de inclusión se cumplieron en 50 estudios, que se compilaron calculando frecuencias de detección ponderadas y concentraciones medias aritméticas de las sustancias químicas. El riesgo de sesgo se evaluó mediante la lista de verificación ROBINS-I.

Resultados: Entre los 50 estudios revisados, las concentraciones de bisfenoles se evaluaron en 37 (74.0%), PBs en 21 (42.0%) y BPs en 10 (20.0%). Las frecuencias de detección ponderadas fueron 63.6% para bisfenol-A (BPA), 27.9-63.4% para PBs y 39.5% para benzofenona-3 (BP-3). Las concentraciones medias ponderadas fueron 1.4 ng/mL para BPA, 0.2 a 14.2 ng/mL para PB y 24.4 ng/mL para BP-3. Las concentraciones medias variaron entre los estudios de 0.1 a 3.9 ng/mL para BPA, 0.1 a 1063.6 ng/mL para PBs y 0.5 a 72.4 ng/mL para BP-3. Las concentraciones más altas de BPA y PBs se encontraron en muestras de Asia, frente a América y Europa. Se observaron concentraciones más altas de BPA y más bajas de MeP en las muestras recolectadas después de 2010. Las concentraciones elevadas de compuestos químicos se asociaron con factores sociodemográficos y de estilo de vida en ocho estudios (16.0%). Dos estudios epidemiológicos mostraron un riesgo de sesgo moderado / grave.

Conclusiones: Esta revisión sistemática constituye la primera visión de conjunto de la presencia generalizada y las concentraciones de bisfenoles, PBs y BPs en leche materna humana, revelando variaciones temporales y geográficas. La heterogeneidad

metodológica de los estudios publicados enfatiza la necesidad de estudios bien diseñados que analicen la magnitud de la exposición a estos compuestos en la leche humana.

1. Introduction.

Human breast milk is the main and sometimes sole source of nutrition for infants. Banks have been created to offer this essential nutrition to infants when insufficient milk is available from the mother (e.g., through maternal illness or admission of a premature baby to intensive care), reducing the incidence and severity of perinatal disease (Henderson et al., 2008). The immaturity of their organs means that babies are highly susceptible to hormones contained in human milk (e.g., leptin, ghrelin, insulin growth factor 1, adiponectin, or insulin), which are regulated by maternal physiological feedback mechanisms (Mazzocchi et al., 2019). However, inadvertent exposure of the mother to environmental chemicals with hormone-mimicking properties, known as endocrine disrupting chemicals (EDCs), may lead to their migration into her breast milk and the consequent exposure of her infant (Dualde et al., 2020). Human breast milk provides the nutrients, growth factors, and antibodies needed by infants and is known to be their best source of nutrition (Shah et al., 2020). Nevertheless, it is also considered to be a vehicle for excreting environmental pollutants from the body of the lactating woman and for transmitting them to the breastfeeding infant (LaKind et al., 2001; Mondal et al., 2014). Particular concerns have been raised about the potential short-, medium-, and long-term adverse effects of early exposure to these EDCs on infant health, such as impaired hormone balance or infant neurodevelopment and obesity (Brulport et al., 2021; Ruiz-Pino et al., 2019; Zhang et al., 2021).

It is well known that breast milk can be a pathway for the maternal excretion of environmental chemicals, including persistent organic pollutants such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), endosulfan, and lindane, among others (Hu et al., 2021; Naqvi et al., 2020). However, limited research data are available on the presence in breast milk of less persistent environmental phenolic compounds such as bisphenols, parabens (PBs), or benzophenones (BPs), despite their wide utilization in food packaging supplies, personal care products (PCPs), and cosmetics (Daniel, 1986; Panico et al., 2019; Rastogi, 2002; Yazar et al., 2011). Bisphenols comprise: bisphenol

A (BPA), the most common congener, and its analogs bisphenol S (BPS) and bisphenol F (BPF); bisphenol A-glycidyl methacrylate (BisGMA); bisphenol A diglycidyl ether (BADGE); and bisphenol F diglycidyl ether (BFDGE); among others (Pelch et al., 2017). Exposure to bisphenols is largely dietary (Ćwiek-Ludwicka, 2015; Geens et al., 2012; Grumetto et al., 2008; Huang et al., 2017; Salamanca-Fernández et al., 2020), but exposure to PB and BP also takes place via respiratory and dermal routes (Benech-Kieffer et al., 2000; Díaz-Cruz et al., 2012; Fent et al., 2010; Janjua et al., 2008; Janjua et al., 2004; Johns et al., 2000; Rocío-Bautista et al., 2015; Schlumpf et al., 2010). Although they are readily degraded and rapidly excreted, individuals are considered to be continually exposed to these compounds, which are widely present in everyday items (Nicolopoulou-Stamati et al., 2015). It has also been demonstrated that the octanol-water partition coefficient (Kow) for these compounds is between 1 to 5, so that they should be considered at least 'partially' lipophilic. Interestingly, Stahlhut et al. (2009) reported that the release of BPA via urine was lower than expected in the National Health and Nutrition Examination Survey (NHANES). This suggests that repeated exposure might lead to the partial distribution of BPA in body compartments with different percentages of lipids, such as adipose tissue or breast milk, from which it would be more slowly released (Artacho-Cordón et al., 2018; Stahlhut et al., 2009). A similar dynamic may also affect other npEDCs with comparable physicochemical properties (Wang et al., 2015), including BP-3, TCS, and 2-PP, with log Kow=3.18, 4.76, and 2.84, respectively. These environmental phenols are considered EDCs because they exert (anti-)estrogenic, (anti-) androgenic and/or (anti-)thyroid activities (Boberg et al., 2010; Darbre and Harvey, 2008; Perez et al., 1998). For instance, BPA can interfere with steroid signaling via human estrogen and androgen receptors (Molina-Molina et al., 2013), bind to thyroid receptors (Moriyama et al., 2002), and change the expression of various thyroid-specific genes (Gentilcore et al., 2013). PBs exert weak estrogenic (Karpuzoglu et al., 2013; Lange et al., 2014) and antiandrogenic (Molina-Molina et al., 2013) activities, and some BPs were found to exert estrogenic, antiestrogenic, and antiandrogenic activity in vitro and in vivo (Kawamura et al., 2005; Nakagawa and Tayama, 2001; Schreurs et al., 2005; Suzuki et al., 2005).

There is increasing discussion about the need to regulate human breast milk repositories, not only to ensure that the milk is free of contaminants such as caffeine or drugs (Weaver et al., 2019) but also to monitor its xenobiotic burden (Cohen, 2018). This issue is of particular relevance for premature low-birth-weight infants, given that the immaturity of phase II metabolism is even more pronounced in these infants (Mulla et al., 2015; Nachman et al., 2014), suggesting that the bioavailability of these compounds may be substantively greater in preterm low-birth-weight infants than in older children or adults for the same exposure doses (Calafat et al., 2009; Duty et al., 2013). Given the above background, the aim of this study was to conduct a systematic review of published scientific evidence on concentrations of bisphenols, PBs, and BPs in human breast milk and to identify differences in findings among continents and between samples collected before and after 2010. In addition, we also explored sociodemographic, anthropometric, reproductive, and lifestyle factors related to this exposure.

2. Material and Methods

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement (Moher et al., 2009).

2.1 Data sources and search strategy

Medline (PubMed), Web of Science (WoS), and Scopus databases were used to search for published studies reporting on concentrations of bisphenols, PBs and BPs in human breast milk. The last search was performed on June 16 2020. The detailed search strategy is displayed in supplementary material (Anexo III, Table S1).

2.2 Study selection and data extraction

Review inclusion criteria were: original scientific article, publication in English since 2000, and the reporting of data on the presence/concentration of bisphenols, PBs and/or BPs in human breast milk.

The review was conducted by two researchers (LMID and FMP). First, the titles and abstracts of retrieved articles were screened to exclude duplicates and those not meeting inclusion criteria. In case of disagreement between reviewers, a third external reviewer (FAC) participated to make a decision about the inclusion or exclusion of the article. Articles were then classified according to the main study goal as epidemiological or methodological (i.e., related to chemical extraction and/or

detection of these chemicals. The following data were collected from each article: 1) country; 2) continent; 3) origin of milk samples; 4) sample collection period; 5) sample size; 6) detection frequencies, 7) units, 8) arithmetic means, 9) percentile 25 (P25), 10) median concentrations, 11) percentile 75 (P75), and 12) range of concentrations; 13) limit of detection (LOD) and 14) limit of quantification (LOQ); 15) age, 16) health, 17) parity, 18) body mass index (BMI), and 19) smoking habits of the women; 20) chemical extraction methodology; 21) amount of milk; 22) chemical quantification methodology; 23) previous pasteurization (yes/no); 24) type of milk; 25) accumulated lactation time; 26) freezing temperature; 27) freeze/thaw cycles of samples; 28) methodological blanks of materials used; 29) spot/pooled samples; and 30) factors related to breast milk concentrations of bisphenols, PBs, and BPs (e.g., anthropometric and sociodemographic characteristics, reproductive characteristics, dietary habits, and use of PCPs).

In the case of bisphenols, information on concentrations of free, conjugated and/or total bisphenols was collected when available.

2.3. Assessment of reporting quality and risk of bias

The reporting quality of the epidemiological studies was assessed using the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) checklist (von Elm et al., 2008). This checklist consists of six blocks and a total of 23 items: 1) title and summary (2 items), 2) introduction (2 items), 3) method (9 items), 4) results (5 items), 5) discussion (4 items), and 6) other information (1 item). The reporting quality of articles was categorized according to Alvarenga et al. (2021 as high (≥16 items checked), medium (15-8 items), or low (<8 items).

The risk of bias was estimated by using a modified version of ROBINS-I (Schünemann et al., 2019; Sterne et al., 2016) for non-randomized studies (Morgan et al., 2018). The risk of bias in each study was classified as low (⊕), moderate (⊕⊕), serious (⊕⊕⊕) or critical (⊕⊕⊕⊕)".

Given the nature of the methodological articles, there was no evaluation of their reporting quality or risk of bias.

2.4. Statistical analysis

For the purposes of conducting a meta-analysis, all concentrations were expressed as ng/mL, using the

conversion factor 1.02 g/mL milk for results given in ng/g milk and the conversion factor 2.17 g lipid/100 mL for results given in ng/g lipid (Kelishadi et al., 2012) when there was no information on lipid content in the studies.

Detection frequencies were compared among studies using the following formula:

Weighted FD (%) = ∑ [(n1 * FD1) + … + (nx * FDx)] / ∑nx

where FD is the frequency of detection, n is the number of samples, and x is the number of studies.

The weighted arithmetic mean of reported concentrations was calculated as follows:

Weighted AM = ∑ [(n1 * AM1) + … + (nx * AMx)] / ∑nx

where AM is the arithmetic mean, n is the number of samples, and x is the number of studies

Weighted FD and AM values include data from all the studies reporting the analysis of each specific compound, regardless whether or not the compound was actually detected. Data from studies reporting geometric mean or median concentrations rather than arithmetic mean concentrations were not included in the weighted analysis.

3. Results

Figure 1 depicts the flow of articles through the study. A total of 3164 articles were retrieved (133 from Medline, 472 from WoS, and 2559 from Scopus), 2803 were excluded in the initial screening process for failing to meet inclusion criteria and 294 for being duplicates. Out of the 67 full-text articles considered for the review, 17 were excluded because they did not evaluate bisphenols, PBs, or BPs or did not assess them in human breast milk (e.g., in serum, urine, adipose tissue, or non-human milk). Finally, the systematic review contained 50 articles: 24 epidemiological studies and 26 methodological studies.

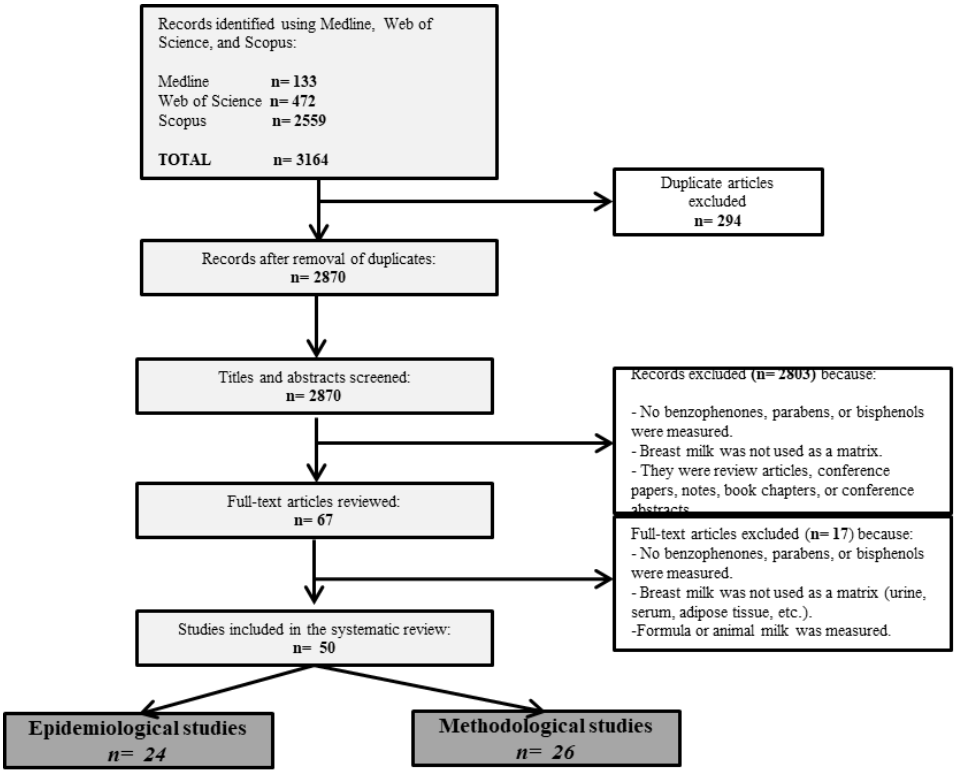


Fig. 1. PRISMA flowchart of article selection

3.1 Characteristics of studies

3.1.1. Epidemiological studies.

Table 1A exhibits the main characteristics of the 24 epidemiological studies (Arbuckle et al., 2015; Carignan et al., 2012; Cariou et al., 2008; Chang et al., 2019; Dualde et al., 2019a; Dualde et al., 2020; Fisher et al., 2017; Fujii et al., 2018; Fujii et al., 2014; Hines et al., 2015; Huang et al., 2020; Jin et al., 2020; Kim et al., 2020; Kuruto-Niwa et al., 2007; Lee et al., 2018; Martinez et al., 2019; Mendonca et al., 2014; Migeot et al., 2013; Molins-Delgado et al., 2018; Nakao et al., 2015; Park et al., 2019; Schlumpf et al., 2010; Yi et al., 2013; Zimmers et al., 2014). Most of them reported the origin of the samples (hospital-based recruitment) and the years of their collection [10 (41.7%) in 2000-2010 and 11 (45.8%) in 2011-2020; 3 (12.5%) not reported]. None of these studies used samples from a breast milk bank. Six (25.0%) of the studies were conducted in America, seven (29.2%) in Europe, and eleven (45.8%) in Asia. The reporting quality was classified as high in eight studies (33.3%), medium in fifteen (62.5%), and low in one (4.2%). The risk of bias was classified serious in one study (Fujii et al., 2014), moderate in another (Fujii et al., 2018), and low in twenty-two studies (Table 1A).

Table 1A. General characteristics, reporting quality, and risk of bias of epidemiological studies

Reference	Country	Continent	Origin of milk samples	Sample collection period	Reporting Quality*	Risk of bias**
Arbuckle et al., 2015	Canada	America	Hospital	2009-2010	High	⊕
Carignan et al., 2012	USA	America	NR	2004-2005	High	⊕
Fisher et al., 2017	Canada	America	Hospital	2009-2010	Medium	⊕
Hines et al., 2015	USA	America	Hospital	2004-2005	Medium	⊕
Mendonca et al., 2014	USA	America	Hospital	2006-2008	High	⊕
Zimmers et al., 2014	USA	America	NR	NR	Medium	⊕
Chang et al., 2019	Taiwan	Asia	Hospital	2014-2015	High	⊕
Fujii et al., 2014	Japan	Asia	Human Specimen Bank	2004-2009	Medium	⊕⊕⊕
Fujii et al., 2018	Japan	Asia	Human Specimen Bank	2008-2010	Medium	⊕⊕
Huang et al., 2020	China	Asia	Hospital	2014	Medium	⊕
Jin et al., 2020	China	Asia	Hospital	2018-2019	High	⊕
Kurutu-Niwa et al., 2007	Japan	Asia	NR	2000–2001	Medium	⊕
Kim et al., 2020	Korea	Asia	Nationwide breastfeeding clinics	2018	High	⊕
Lee et al., 2018a	Korea	Asia	Hospital	2011-2012	Medium	⊕
Nakao et al., 2015	Japan	Asia	NR	2012-2013	Medium	⊕
Park et al., 2019	Korea	Asia	Hospital	2013	High	⊕
Yi et al., 2013	Korea	Asia	Care centers	NR	Low	⊕
Cariou et al., 2008	France	Europe	Hospital	2004-2006	Medium	⊕
Dualde et al., 2019	Spain	Europe	Hospital	2015	Medium	⊕
Dualde et al., 2020	Spain	Europe	Hospital	2015	High	⊕
Martínez et al., 2019	Spain	Europe	Hospital	NR	Medium	⊕
Migeot et al., 2013	France	Europe	Hospital	2011	Medium	⊕
Molins-Delgado et al., 2018	Spain	Europe	Blood and Tissue Bank	2014	Medium	⊕
Schlumpf et al., 2010	Switzerland	Europe	Hospital	2004-2006	Medium	⊕

*USA: United States of America. *STROBE checklist items <8: "low quality"; 14-8: "moderate quality"; ≥15: "high quality". ** Risk of bias ⊕ (low risk of bias), ⊕⊕ (moderate risk of bias), ⊕⊕⊕ (serious risk of bias), ⊕⊕⊕⊕ (critical risk of bias). N.R.: Not reported*

Data on the characteristics of the women was displayed in Supplementary Table S2 (Anexo III). Most studies reported the age of participants (ranging from 18 to 43 years) and their parity status. Half of the studies described their health status (usually good) and BMI (arithmetic mean of 21.0 to >30.0 kg/m2). Around one-third of studies gathered information on smoking habit (n=10, 41.7%) or type of milk (usually mature) (n=8, 33.3%) (data not shown). Less than one-fifth of studies describe the nutritional content of the milk (e.g., proteins, lipids, lactose, energy, or carbohydrates), health status of the infants, or accumulated lactation time (data not shown). No study indicated whether the milk samples were pasteurized or not (data not shown).

3.1.2 Methodological studies

Table 1B displays the main characteristics of the 26 methodological studies included in the review (Azzouz et al., 2016a; Azzouz et al., 2016b; Cao et al., 2015; Cariot et al., 2012; Deceuninck et al., 2015; Dualde et al., 2019b; Fotouhi et al., 2017; Grecco et al., 2019; Manouchehri et al., 2020; Melo and Queiroz, 2013; Niu et al., 2017; Otaka et al., 2003; Rodriguez-Gomez et al., 2015; Rodriguez-Gomez et al., 2014a; Rodriguez-Gomez et al., 2014b; Rodriguez-Gomez et al., 2014c; Rodríguez-Gómez et al., 2015; Souza et

al., 2016; Sun et al., 2004; Tuzimski et al., 2019; Tuzimski and Szubartowski, 2019; Vela-Soria et al., 2018; Vela-Soria et al., 2016; Yang et al., 2018; Ye et al., 2008; Ye et al., 2006b). Only ten (38.5%) studies reported the origin of samples and seven (26.9%) the year of The main characteristics of participating women are summarized in Supplementary Table S3 (Anexo III). Eleven studies (42.3%) described the women's health status (good in all cases). Less than one-fifth of studies reported data on the age, BMI, smoking habits, or parity. One study reported the cumulative lactation time of the women and one the type of milk sample, while no study reported the nutritional content of milk samples, the health status of the infant, or whether the milk was pasteurized (data not shown).

milk collection [3 in 2000-2010 and 4 in 2011-2020]. Only one study (3.8%) obtained samples from a breast milk bank. Six studies (23.1%) were conducted in America, six (23.1%) in Asia, and fourteen (53.8%) in Europe.

3.2 Concentrations of bisphenols, PBs, and BPs in breast milk samples

The studies in this review analyzed 3021 breast milk samples collected over the past two decades from women in America, Asia, or Europe. Bisphenols were analyzed in 2,384 samples from 37 studies, PBs in 875 samples from 21 studies, and BPs in 251 samples from 10 studies. Only 5 out of the 50 articles included in this systematic review simultaneously measured all three families of compounds. Tables 2A and 2B summarize the data extracted from each study and Table 3 lists the weighted detection frequencies and arithmetic mean concentrations.

Table 1B. General characteristics, reporting quality, and risk of bias of methodological studies.

Reference	Country	Continent	Origin of samples	Sample collection period
Cao et al., 2015	Canada	America	N.R.	2009-2011
Grecco et al.,2019	Brazil	America	University	N.R.
Melo et al., 2014	Brazil	America	N.R.	N.R.
Souza et al., 2016	Brazil	America	Hospital	N.R.
Ye et al., 2006	USA	America	N.R.	N.R.
Ye et al.,2008	USA	America	Maternal Milk Bank	2007
Fotouhi et al., 2017	Iran	Asia	Hospital	N.R.
Manouchehri et al., 2020	Iran	Asia	Hospital	N.R.
Niu et al., 2017	China	Asia	N.R.	2014
Otaka et al., 2003	Japan	Asia	N.R.	N.R.
Sun et al., 2004	Japan	Asia	N.R.	N.R.
Yang et al., 2018	China	Asia	N.R.	2009
Azzouz et al., 2016a	Spain	Europe	Hospital	N.R.
Azzouz et al., 2016b	Spain	Europe	Hospital	N.R.
Cariot et al., 2012	France	Europe	N.R.	N.R.
Deceuninck et al., 2015	France	Europe	N.R.	N.R.
Dualde et al., 2019b	Spain	Europe	Hospital	2015
Rodríguez-Gómez et al., 2014a	Spain	Europe	N.R.	N.R.
Rodríguez-Gómez et al., 2014b	Spain	Europe	N.R.	N.R.
Rodríguez-Gómez et al., 2014c	Spain	Europe	N.R.	N.R.
Rodríguez-Gomez et al., 2015a	Spain	Europe	N.R.	N.R.
Rodríguez-Gómez et al., 2015b	Spain	Europe	N.R.	N.R.
Tuzimski et al., 2019	Poland	Europe	N.R.	2018
Tuzimski and Szubartowski., 2019	Poland	Europe	N.R.	2018-2019
Vela-Soria et al., 2016	Spain	Europe	Hospital	N.R.
Vela-Soria et al., 2018	Spain	Europe	Biobank	N.R.

*USA: United States of America. *N.R.: Not reporrted*

Table 2A. Summary of bisphenol, PB, and BP concentrations in milk samples from epidemiological studies.

Reference	Number of milk samples	Frequency of detection (%)	Concentrations					Units	LOD	LOQ
			Arithmetic Mean	P25	Median	P75	Range			
Arbuckle et al., 2015	80	BPA free: 4.00 BPA total: 5.00	BPA free: N.R. BPA total: N.R.	N.R.	<LOD	N.R.	N.R.	µg/L	BPA free: 0.30 BPA total: 0.30	N.R.
Carignan et al., 2012	43	TBBPA: 35.00	TBBPA: N.R.	N.R.	<LOD	N.R.	TBBPA: <30.00 – 550.00	ng/g lipid	N.R.	TBBPA: 30.00-550.00
Fisher et al., 2017	56	MeP: 82.00 EtP: 57.00 PrP: 66.00 BuP: 0.00 iBP: 0.00 BzP:0 .00	MeP: 0.99 EtP: 0.12 PrP: 0.33 BuP: 0.05 iBP: N.R. BzP: N.R.	MeP: <LOD EtP: <LOD PrP: <LOD BuP: N.R. iBP: N.R. BzP: N.R.	MeP: 0.22 EtP: <LOD PrP: <LOD BuP: <LOD iBP: ND BzP:ND	MeP: 0.80 EtP: <LOD PrP: 0.29 BuP: N.R. iBP: N.R. BzP: N.R.	MeP: <LOD -16.33 EtP: <LOD-2.18 PrP: <LOD-4.59 BuP: N.R. iBP: N.R. BzP: N.R.	µg/L	MeP: 0.10 EtP: 0.10 PrP: 0.10 BuP: 0.10 iBP: 0.10 BzP: 0.10	N.R.
Hines et al., 2015	34	BPA: 89.00 MeP: 100.00 EtP: 50.00 PrP: 100.00 BuP: 0.00 BP-3: 56.00	BPA: 0.30 MeP: 20.58 EtP: N.R. PrP: 0.30 BuP: N.R. BP-3: 1.90	N.R.	BPA: 0.22 MeP: 1.1 EtP: N.R. PrP: 0.30 BuP: N.R. BP-3: 0.40	N.R.	N.R.	µg/L	BPA: 0.28 MeP: 0.10 EtP: 0.10 PrP: 0.10 BuP: N.R. BP-3: 0.51	N.R.
Mendonca et al., 2014	27	BPA free: 20.00 BPA total:75.00	BPA free: 1.70 BPA total: 2.10	BPA free: <LOD BPA total: 0.40	BPA free: <LOD BPA total: 0.80	BPA free: BPA total:	N.R.	µg/L	BPA free: 0.30 BPA total: 0.30	N.R.
Zimmers et al., 2014	21	BPA free: 62.00	BPA free: 3.13	BPA free: <LOD	BPA free: 0.68	BPA free: 6.04	N.R.	ng/mL	BPA free: 0.22	N.R.
Chang et al., 2019	186	BPA: 74.40	BPA: N.R.	BPA: 1.56	BPA: 2.18	BPA: 4.58	BPA: 0.42-112.44	ng/g lipid	N.R.	N.R.
Fujii et al., 2014	9	TBBPA: 100.00	TBBPA: 1.04	N.R.	TBBPA: 0.72	N.R.	TBBPA: 0.39–2.22	ng/g lipid	N.R.	TBBPA: 1.00-200.00
Fujii et al., 2018	64	TBBPA: 97.00	TBBPA: 48.00	N.R.	TBBPA: 3.20	N.R.	TBBPA: 0-2200	ng/g lipid	N.R.	TBBPA: 34.00
Huang et al., 2020	111	TBBPA: 64.00	TBBPA: 5.59	TBBPA: <LOD	TBBPA: 1.57	TBBPA: 7.30	TBBPA: <LOD-42.00	ng/g lipid	TBBPA: 5.00	N.R.
Jin et al., 2020	190	BPA:53.00 BPS: 44.00 BPAF: 21.00 BPF: 0.00	BPA: 2.50 BPS: 0.19 BPAF: 0.09 BPF: <LOD	BPA:<LOD BPS: <LOD BPAF: <LOD BPF: <LOD	BPA:0.21 BPS: <LOD BPAF: <LOD BPF: <LOD	BPA:1.60 BPS: 0.11 BPAF: <LOD BPF: <LOD	BPA:<LOD-15.00 BPS: <LOD-1.30 BPAF: <LOD-0.58 BPF: <LOD-<LOD	ng/mL	BPA: 0.37 BPS: 0.37 BPAF: 0.37 BPF: 0.37	N.R.
Kurutu-Niwa et al., 2007	101	BPA: 100.00	BPA: 3.41	N.R.	BPA: 3.41	N.R.	BPA: 1.0–7.0	ng/mL	N.R.	N.R.
Kim et al., 2020	221	BPA: 0.08 MeP: 0.10 ETP: 0.04 PrP: 0.13	N.R.	BPA: <LOD MeP: <LOD ETP: 0.14 PrP: <LOD	BPA: <LOD MeP: 0.18 ETP: 0.62 PrP: <LOD	BPA: 0.23 MeP: 1.12 ETP: 1.62 PrP: 0.39	N.R.	µg/L	BPA: 0.04 MeP: 0.04 ETP: 0.04 PrP: 0.04	N.R.
Lee et al., 2018a	127	BPA: 79.50	N.R.	BPA: 0.34	BPA: 0.74	BPA: 1.79	BPA: <LOD-43.20	µg/L	BPA: 0.30	N.R.
Nakao et al., 2015	19	BPA:100.00 TBBPA: 94.70 TriBBPA: 94.70 DiBBPA: 5.30 MoDiBBPA: 10.60	BPA: 0.78 TBBPA: 1.94 TriBBPA: 5.49 DiBBPA: <LOQ MoDiBBPA: <LOQ	N.R.	BPA: 6.60 TBBPA: 1.70 TriBBPA: 4.20 DiBBPA: <LOQ MoBBPA:	N.R.	N.R.	ng/g lipid	N.R.	BPA: 0.00 TBBPA: 0.02 TriBBPA: 0.01 DiBBPA: 0.01 MoBBPA: 0.01

Table 2A. Summary of bisphenol, PB, and BP concentrations in milk samples from epidemiological studies (continuación).

Park et al., 2019	260	MeP: 26.90 EtP: 65.80 PrP: 35.80 BuP: 25.00	MeP: 0.62 EtP: 1.03 PrP: 0.17 BuP: 0.05	MeP: <LOD EtP: <LOD PrP: <LOD BuP: <LOD	MeP: <LOD EtP:0.24 PrP:<LOD BuP: <LOD	MeP: 0.10 EtP:1.16 PrP:0.10 BuP: 0.02	MeP: <LOD-29.60 EtP:LOD-17.80 PrP:<LOD-7.99 BuP: <LOD-1.12	µg/L	MeP: 0.04 EtP:0.02 PrP:0.02 BuP:0.04	N.R.
Yi et al., 2013	325	BPA free: 39.80 BPA conjugate: 70.60 BPA total: 70.60	N.R.	N.R.	BPA free: <LOD BPA conjugated: 4.20 BPA total: 7.80	N.R.	BPA free: <LOD–54.20 BPA conjugated: BPA total: LOD–57.30	ng/mL	BPA free: 10.00 BPA conjugate: BPA total: 10.00	BPA free: 30.00 BPA conjugate: 30.00 BPA total: 30.00
Cariou et al., 2008	93	TBBPA: 43.00	TBBPA: 4.11	N.R.	TBBPA: 0.48	N.R.	TBBPA: 0.06-37.34	ng/g lipid	N.R.	N.R.
Dualde et al., 2019a	120	BPA free: 77.40 BPA total: 83.00 BPF: 22.00 BPS: 1.10	BPA free: 1.10 BPA total: 1.60 BPF: N.R. BPS: N.R.	BPA free: 0.1 0 BPA total: 0.1 0 BPS: N.R. BPF: <LOQ	BPA free: 0.10 BPA total: 0.26 BPS: N.R. BPF: <LOQ	BPA free: 0.18 BPA total: 0.57 BPS: N.R. BPF: <LOQ	BPA free: <LOQ-41.00 BPA total: <LOQ-42.00 BPS: N.R. BPF: <LOQ-0.46	ng/mL	N.R.	0.37
Dualde et al., 2020	120	MeP free: 60.00 MeP total: 89.00 EtP free: 41.00 EtP total: 70.00 PrP free: 47.00 PrP total: 72.00 BuP free: 53.00 BuP total: 61.00	MeP free: 0.95 MeP total: 2.70 EtP free: 0.17 EtP total: 0.44 PrP free: 0.25 PrP total: 0.52 BuP free: 0.11 BuP total: 0.13	MeP free: <LOQ MeP total: 0.10 EtP free: <LOQ EtP total: <LOQ PrP free: <LOQ PrP total: <LOQ BuP free:<LOQ BuP total: <LOQ	MeP free: 0.10 MeP total: 0.19 EtP free: <LOQ EtP total: 0.10 PrP free: <LOQ PrP total: 0.10 BuP free: 0.10 BuP total: 0.10	MeP free: 0.36 MeP total: 1.00 EtP free: 0.10 EtP total: 0.23 PrP free: 0.10 PrP total: 0.18 BuP free: 0.10 BuP total: 0.10	MeP free: <LOQ-31 .00 MeP total: <LOQ-49.00 EtP free: <LOQ-5.20 EtP total: <LOQ-9.00 PrP free: <LOQ-6.50 PrP total:<LOQ-8.00 BuP free: <LOQ-1.10 BuP total: <LOQ-1.30	ng/mL	N.R.	MeP free: 0.10 MeP total: 0.10 EtP free: 0.10 EtP total: 0.10 PrP free: 0.10 PrP total: 0.10 BuP free: 0.10 BuP total: 0.10
Martínez et al., 2019	53	BPA free: 38.00 BPA total:76.00 TBBPA: 8.00	BPA free: 0.26 BPA total: 1.30 TBBPA: 0.58	N.R.	BPA free: 0.26 BPA total: 1.30 TBBPA: 0.58	N.R.	N.R.	µg/L	BPA free: 0.04 BPA total: 0.04 TBBPA: 0.02	BPA free: 0.05 BPA total: 0.05 TBBPA: 1.00
Migeot et al., 2013	21	BPA: 90.50 CBPA: 0.00 2,2′-DCBPA: 52.38 2,6-DCBPA: 100 .00 TCBPA: 80.95	BPA: 1.70 CBPA: <LOD 2,2′-DCBPA: 1.87 2,6-DCBPA: 1.56 TCBPA: 0.08	N.R.	BPA: 1.41 CBPA: <LOD 2,2′-DCBPA: 0.40 2,6-DCBPA: 1.27 TCBPA: <LOD	N.R.	N.R.	ng/mL	BPA: 0.09 CBPA: 0.01 2,2′-DCBPA: 2,6-DCBPA: TCBPA: 0.04	BPA: 0.04 CBPA: 0.04 2,2′-DCBPA: 0.04 2,6-DCBPA: 0.04 TCBPA: 0.04
Molins-Delgado et al., 2018	79	BP-1: 0.00 BP-3: 23.00 4-OH-BP: 3.00 4-DH-BP: 10.00	BP-1: N.R. BP-3: 72.40 4-OH-BP: 4.30 4-DH-BP: 27.90	N.R.	BP-1: N.R. BP-3: 72.40 4-OH-BP: 4.30 4-DH-BP: 27.90	N.R.	BP-1: N.R. BP-3: <LOQ-799.90 4-OH-BP: 3.90-4.70	ng/g lipid	BP-1: 0.10 BP-3: 0.10 4-OH-BP: 0.10 4-DH-BP: 0.6	BP-1: 0.30 BP-3: 0.30 4-OH-BP: 0.30 4-DH-BP: 2.00
Schlumpf et al., 2010	54	MeP: 35.00 EtP: 20.00 PrP: 15.00 BuP: 0.00 BP-2: 0.00 BP-3: 13.00	MeP: 2.18 EtP: 1.26 PrP: 1.42 BuP: ND BP-2: ND BP-3: 52.23	N.R.	MeP: 1.00 EtP: 1.30 PrP: 1.50 BuP: 0.00 BP-2: 0.00 BP-3: 26.70 ng/g lipid (milk fat)	N.R.	MeP: 1.0-8.00 EtP: 1.00-1.50 PrP: 1.00-2.00 BuP: 0 BP-2: 0 BP-3: 7.30–121.40	ng/mL	MeP: 4.00 EtP: 4.00 PrP: 4.00 BuP: 4.00 BP-2: 4.00 BP-3: 4.00	MeP: 2.00 EtP: 2.00 PrP: 2.00 BuP: 2.00 BP-2: 2.00 BP-3: 2.00

BPA: bisphenol A, BPF: bisphenol F, BPS: bisphenol S, BPAF: bisphenol AF, MoBBPA: monobromobisphenol A, DiBBPA: dibromobisphenol A, TriBBPA: tribromobisphenol A, TBBPA: tetrabromobisphenol A, CBPA: chlorobisphenol A, 2,2'-DCBPA: 2,2'-dichlorobisphenol A, 2,6-DCBPA: 2,6-dichlorobisphenol A, TCBPA: trichlorobisphenol A, MeP: methylparaben, EtP: ethylparaben, PrP: propylparaben, BuP: buthylparaben, IBP: isobutylparaben, BzP: benzylparaben, BP-1: benzophenone 1, BP-2: benzophenone 2, BP-3: benzophenone 3, 4-OH-BP: 4-hydroxibenzophenone, 4-DH-BP: 4-dihydroxibenzophenone, LOD: limit of detection, LOQ: limit of quantification. P25: percentile 25. P75: percentile 75. ND: not detected. N.R.: not reported.

Table 2B. Summary of bisphenol, PB, and BP concentrations in milk samples from methodological studies.

Reference	Number of milk samples	Frequency of detection (%)	Concentrations			Units	LOD	LOQ
			Arithmetic Mean	Median	Range			
Cao et al., 2015	278	BPA free: 46.00 BPA total: 72.00	BPA free: N.R. BPA total: N.R.	BPA free: 0.10 BPA total: 0.11	BPA free: 0.036-2.30 BPA total: 0.036-2.50	ng/g	BPA free: 0.21 BPA total: 0.21	N.R.
Grecco et al.,2019	3	MeP: 100.00 EtP: 100.00 PrP: 6.67 BuP: 0.00	MeP: 24.15 EtP: 12.07 PrP: 2.05 BuP: 0.50	MeP: 12.30 EtP: 11.10 PrP: <LOQ BuP: ND	N.R.	ng/mL	N.R.	MeP: 5.00 EtP: 5.00 PrP: 5.00 BuP: 5.00
Melo et al., 2014	3	MeP: <LOQ EtP: <LOQ PrP: <LOQ	MeP: 5.00 EtP: 10.00 PrP: 10.00	MeP: <LOQ EtP: <LOQ PrP: <LOQ	N.R.	ng/mL	N.R.	MeP: 10.00 EtP: 20.00 PrP: 20.00
Souza et al., 2016	16	MeP: 93.75 EtP: 50.00 PrP: 0.00 BuP: 25.00	MeP: 21.27 EtP: 11.54 PrP: 5.00 BuP: 1.26	MeP: 18.45 EtP: 5.75 PrP: <LOQ BuP: ND	N.R.	ng/mL	N.R.	MeP: 10.00 EtP: 10.00 PrP: 10.00 BuP: 3.00
Ye et al., 2006	20	BPA free: 60.00 BPA total: 90.00 BP-3 free: 15.00 BP-3 total: 60.00	BPA free: 1.30 BPA total: 1.90 BP-3 free: <LOD BP-3 total: 0.90	BPA free: 0.40 BPA total: 1.10 BP-3 free: <LOD BP-3 total: 0.07	BPA free: <LOD–6.30 BPA total: <LOD–7.30 BP-3 free: <LOD–1.50 BP-3 total: <LOD–3.20	ng/mL	BPA free: 1.00 BPA total: 1.00 BP-3 free: 1.00 BP-3 total: 1.00	N.R.
Ye et al.,2008	4	BPA free: 100.00 BPA total: 100.00 MeP free: 50.00 MeP total: 100.00 PrP free: 25.00 PrP total: 25.00 BP-3 free: 25.00 BP-3 total: 25.00	BPA free: 0.80 BPA total: 1.02 MeP free: 0.87 MeP total: 1.24 PrP free: 0.12 PrP total: 0.12 BP-3 free: 0.46 BP-3 total: 0.47	BPA free: 0.62 BPA total: 0.86 MeP free: 0.19 MeP total: 0.72 PrP free: <LOD PrP total: <LOD BP-3 free: <LOD BP-3 total: <LOD	BPA free: 0.41-1.54 BPA total: 0.73-1.62 MeP free: <LOD-3.04 MeP total: 0.53-3.00 PrP free: <LOD-0.32 PrP total: <LOD-0.33 BP-3 free: <LOD-1.24 BP-3 total: <LOD-1.28	ng/mL	BPA free: 0.30 BPA total: 0.30 MeP free: 0.10 MeP total: 0.10 PrP free: 0.10 PrP total: 0.10 BP-3 free: 0.40 BP-3 total: 0.40	N.R.
Fotouhi et al., 2017	6	MeP: 83.30 EtP: 100.00 PrP: 100.00	MeP: 1063.60 EtP: 526.35 PrP: 392.63	MeP: 857.00 EtP: 477.40 PrP: 288.50	N.R.	ng/mL	MeP: 25.00 EtP: 25.00 PrP: 25.00	MeP: 50.00 EtP: 50.00 PrP: 50.00
Manouchehri et al., 2020	5	MeP: 50.00 EtP: 100.00 PrP: 66.70	MeP: 41.68 EtP: 42.02 PrP: 31.18	MeP: 16.20 EtP: 27.00 PrP: 18.80	N.R.	µg/L	MeP: 3.00 EtP: 5.00 PrP: 5.00	MeP: 10.00 EtP: 15.00 PrP: 15.00
Niu et al., 2017	20	BPA: 85.00 BPS: 5.00 BPF: 60.00 BPAF: 15.00	BPA: 0.14 BPS: 0.04 BPF: 0.03 BPAF: 0.01	BPA: 0.13 BPS: ND BPF: 0.02 BPAF: ND	N.R.	µg/L	BPA: 0.02 BPS: 0.00 BPF: 0.02 BPAF: 0.00	BPA: 0.05 BPS: 0.01 BPF: 0.00 BPAF: 0.05
Otaka et al., 2003	3	BPA: 66.70	BPA: 0.47	BPA: 0.65	N.R.	ng/g	BPA:0.09	BPA: N.R.
Sun et al., 2004	23	BPA: 100.00	BPA: 0.61	BPA: 0.61	BPA: 0.28–0.97	ng/mL	BPA: 0.11	BPA: N.R.
Yang et al., 2018	20	BADGE: 100.00 BADGE·H2O:100.00 BADGE·HCl: 100.00 BADGE·2HCl: 100.00 BFDGE: 100.00 BFDGE·2HCl:100.00 BADGE·2H2O: 100.00 BADGE·HCl·H2O:100.00 BFDGE·2H2O: 100.00	BADGE: N.R. BADGE·H2O: N.R. BADGE·HCl: N.R. BADGE·2HCl: N.R. BFDGE: N.R. BFDGE·2HCl: N.R. BADGE·2H2O: N.R. BADGE·HCl·H2O: N.R. BFDGE·2H2O: N.R.	BADGE: 62.18 BADGE·H2O:48.83 BADGE·HCl: 51.40 BADGE·2HCl: 54.84 BFDGE: 26.43 BFDGE·2HCl:28.26 BADGE·2H2O: 23.91 BADGE·HCl·H2O:20.9 BFDGE·2H2O: 24.56	N.R.	µg/L	BADGE: 0.07 BADGE·H2O: 0.17 BADGE·HCl: 0.17 BADGE·2HCl: 0.50 BFDGE: 0.13 BFDGE·2HCl: 0.13 BADGE·2H2O: 0.27 BADGE·HCl·H2O: 0.27 BFDGE·2H2O: 0.03	BADGE: 0.20 BADGE·H2O: 0.50 BADGE·HCl: 0.50 BADGE·2HCl: 1.50 BFDGE: 0.40 BFDGE·2HCl: 0.40 BADGE·2H2O: 0.80 BADGE·HCl·H2O: 0.80 BFDGE·2H2O: 0.10
Azzouz et al., 2016a	7	BPA free: 0.00 BPA total: 57.10 MeP free: 57.10 MeP total: 85.70 EtP free: 71.40 EtP total: 85.70 PrP free: 14.29 PrP total: 28.60 BuP free: 0.00 BuP total: 71.40 iPrP free: 28.60 iPrP total: 14.29 BzP free: N.R. BzP total: N.R	BPA free: ND BPA total: 3.90 MeP free: 0.31 MeP total: 1.80 EtP free: 2.91 EtP total: 14.43 PrP free: 0.14 PrP total: 0.80 BuP free: ND BuP total: 0.32 iPrP free: 0.10 iPrP total: 0.30 BzP free: N.R. BzP total: N.R.	BPA free: ND BPA total: 2.70 MeP free: 0.53 MeP total: 3.00 EtP free: 3.20 EtP total: 14.00 PrP free: ND PrP total: ND BuP free: ND BuP total: ND iPrP free: ND iPrP total: ND BzP free: N.R. BzP total: N.R	N.R.	µg/L	BPA free: 2.10 BPA total: 2.10 MeP free: 16.00 MeP total: 16.00 EtP free: 16.00 EtP total: 16.00 PrP free: 15.00 PrP total: 15.00 BuP free: 16.00 BuP total: 16.00 iPrP free: 11.00 iPrP total: 11.00 BzP free: 14.00 BzP total: 14.00	N.R.
Azzouz et al., 2016b	6	BPA: 33.30 MeP: 50.00 EtP: 66.70 PrP: 0.00 iPrP: 33.30 BuP: 16.70 iBuP: 33.30 BzP: 0.00	BPA: 1.05 MeP: 4.17 EtP: 3.53 PrP: <LOD iPrP: 2.03 BuP: 3.81 iBuP: 1.67 BzP: <LOD	BPA: <LOD MeP: 0.60 EtP: 1.60 PrP: <LOD iPrP: <LOD BuP: <LOD iBuP: <LOD BzP: <LOD	N.R.	µg/L	BPA: 1.00 MeP: 8.00 EtP: 8.30 PrP: 8.40 iPrP: 5.50 BuP: 9.00 iBuP: 5.50 BzP: 8.80	BPA: 3.30 MeP: 26.40 EtP: 27.39 PrP: 27.72 iPrP: 18.13 BuP: 29.70 iBuP: 18.13 BzP: 29.04
Cariot et al., 2012	3	BPA: 100.00 CBPA: 0.00 2,2'-DCBPA: 100.00 2,6'-DCBPA: 100.00 TCBPA: 33.30	BPA: 2.39 CBPA: <LOD 2,2'-DCBPA: 1.81 2,6'-DCBPA: 0.86 TCBPA: 0.36	BPA: 3.07 CBPA: ND 2,2'-DCBPA: 1.09 2,6'-DCBPA: 0.97 TCBPA: ND	BPA: 0.80-3.07 CBPA: <LOD 2,2'-DCBPA: <LOQ-4.13 2,6'-DCBPA: <LOQ-1.40 TCBPA: <LOD-0.68	ng/mL	BPA: 0.09 CBPA: 0.01 2,2'-DCBPA: 0.05 2,6'-DCBPA: 0.05 TCBPA: 0.04	BPA: 0.40 CBPA: 0.40 2,2'-DCBPA: 0.40 2,6'-DCBPA: 0.40 TCBPA: 0.40
Deceuninck et al., 2015	30	BPA: 86.70 BPB: N.R BPC: N.R BPE: N.R. BPF: N.R. BPM: N.R. BPP: N.R. BPS: N.R. BPZ: N.R. BPAP: N.R. BPAF: N.R. BPBP: N.R. BPCl2: N.R BPFL: N.R. BPPH: N.R.	BPA: 0.27 BPB: N.R. BPC: N.R. BPE: N.R. BPF: N.R. BPM: N.R. BPP: N.R. BPS: N.R. BPZ: N.R. BPAP: N.R. BPAF: N.R. BPBP: N.R. BPCl2: N.R. BPFL: N.R. BPPH: N.R.	BPA: 0.11 BPB: N.R BPC: N.R BPE: N.R. BPF: N.R. BPM: N.R. BPP: N.R. BPS: N.R. BPZ: N.R. BPAP: N.R. BPAF: N.R. BPBP: N.R. BPCl2: N.R BPFL: N.R. BPPH: N.R.	N.R.	µg/kg	BPA: 0.01 BPB: N.R BPC: N.R BPE: N.R. BPF: N.R. BPM: N.R. BPP: N.R. BPS: N.R. BPZ: N.R. BPAP: N.R. BPAF: N.R. BPBP: N.R. BPCl2: N.R BPFL: N.R. BPPH: N.R.	BPA: 0.05 BPB: N.R BPC: N.R BPE: N.R. BPF: N.R. BPM: N.R. BPP: N.R. BPS: N.R. BPZ: N.R. BPAP: N.R. BPAF: N.R. BPBP: N.R. BPCl2: N.R BPFL: N.R. BPPH: N.R.

Table 2B. Summary of bisphenol, PB, and BP concentrations in milk samples from methodological studies (continuación).

Reference	Number of milk samples	Frequency of detection (%)	Concentrations			Units	LOD	LOQ
			Arithmetic Mean	Median	Range			
Dualde et al., 2019b	10	BPA: 80.00 BPF: 40.00 BPS: 10.00 MeP: 80.00 EtP: 40.00 PrP: 60.00 BuP: 20.00	BPA: 0.44 BPF: 0.12 BPS: 0.15 MeP: 1.60 EtP: 0.70 PrP: 0.22 BuP: 0.09	BPA: 0.20 BPF: <LOQ BPS: <LOQ MeP: 1.24 EtP: <LOQ PrP: 0.15 BuP: <LOQ	N.R.	ng/mL	N.R.	BPA: 0.10 BPF: 0.13 BPS: 0.25 MeP: 0.10 EtP: 0.10 PrP: 0.10 BuP: 0.10
Rodríguez-Gómez et al., 2014a	10	BPA: 60.00 CBPA: 20.00 DCBBPA: 20.00 MeP: 90.00 EtP: 90.00 PrP: 90.00 BuP: 90.00 BP-1: 80.00 BP-2: 40.00 BP-3: 90.00 4-OH-BP: 90.00	BPA: 2.76 CBPA: N.R. DCBBPA: 0.08 MeP: 1.49 EtP: 0.77 PrP: 2.03 BuP: 0.46 BP-1: 0.23 BP-2: 0.23 BP-3: 6.20 4-OH-BP: 2.34	BPA: 0.65 CBPA: ND DCBBPA: ND MeP: 1.00 EtP: 0.30 PrP: 0.80 BuP: 0.30 BP-1: >LOD-<LOQ BP-2: ND BP-3: 3.60 4-OH-BP: 2.50	N.R.	ng/mL	BPA: 0.05 CBPA: 0.04 DCBBPA: 0.04 MeP: 0.03 EtP: 0.03 PrP: 0.03 BuP: 0.03 BP-1: 0.03 BP-2: 0.03 BP-3: 0.03 4-OH-BP: 0.03	BPA: 0.15 CBPA: 0.12 DCBBPA: 0.12 MeP: 0.09 EtP: 0.09 PrP: 0.09 BuP: 0.10 BP-1: 0.09 BP-2: 0.09 BP-3: 0.09 4-OH-BP: 0.09
Rodríguez-Gómez et al., 2014b	10	MeP: 80.00 EtP: 60.00 PrP: 90.00 BuP: 80.00	MeP: 2.72 EtP: 2.37 PrP: 8.02 BuP: 2.75	MeP: 1.30 EtP: >LOD-<LOQ PrP: 1.60 BuP: 1.60	MeP: 0.90-11.30 EtP: 0.50-13.20 PrP: 0.50-37.00 BuP: 0.60–11.30	ng/mL	MeP: 0.20 EtP: 0.10 PrP: 0.10 BuP: 0.20	MeP: 0.50 EtP: 0.50 PrP: 0.40 BuP: 0.50
Rodríguez-Gómez et al., 2014c	10	BPA: 80.00 MeP: 50.00 EtP: 80.00 PrP: 90.00 BuP: 50.00 BP-1: 60.00 BP-3: 90.00 BP-6: 20.00 BP-8 : 15.00 4-OH-BP: 70.00	BPA: 3.61 MeP: 1.55 EtP: 2.95 PrP: 9.97 BuP: 2.43 BP-1: 0.45 BP-3: 1.80 BP-6: 0.31 BP-8 : 0.31 4-OH-BP: 3.50	BPA: 3.35 MeP: 0.40 EtP: 0.80 PrP: 1.00 BuP: ND BP-1: >LOD-<LOQ BP-3: 1.15 BP-6: ND BP-8: ND 4-OH-BP: >LOD-<LOQ	N.R.	ng/mL	BPA: 0.15 MeP: 0.10 EtP: 0.10 PrP: 0.15 BuP: 0.15 BP-1: 0.20 BP-3: 0.10 BP-6: 0.25 BP-8: 0.10 4-OH-BP: 0.10	BPA:0.40 MeP: 0.30 EtP: 0.35 PrP: 0.50 BuP: 0.25 BP-1: 0.60 BP-3: 0.40 BP-6: 0.80 BP-8: 0.35 4-OH-BP: 0.30
Rodríguez-Gomez et al., 2015a	10	BP-1: 90.00 BP-3: 90.00 BP-6: 40.00 BP-8: 40.00 4-OH-BP: 60.00	BP-1: 0.27 BP-3: 5.69 BP-6: 0.17 BP-8: 0.16 4-OH-BP: 0.71	BP-1: >LOD-<LOQ BP-3: 5.85 BP-6: ND BP-8: ND 4-OH-BP: 0.18	BP-1: 0.31-1.92 BP-3: 4.50-15.70 BP-6: ND BP-8: ND 4-OH-BP: 0.31-1.92	ng/mL	BP-1: 0.10 BP-3: 0.20 BP-6: 0.10 BP-8: 0.10 4-OH-BP: 0.10	BP-1: 0.50 BP-3: 0.60 BP-6: 0.50 BP-8: 0.40 4-OH-BP: 0.30
Rodríguez-Gómez et al., 2015b	10	BPA: 80.00 MeP: 90.00 EtP: 90.00 PrP: 80.00 BuP: 90.00	BPA: 0.70 MeP: 3.27 EtP: 2.58 PrP: 2.37 BuP: 2.24	BPA: >LOD and <LOQ MeP: 1.82 EtP: 1.05 PrP: 1.22 BuP: 0.53	N.R.	ng/mL	BPA: 0.20 MeP: 0.20 EtP: 0.10 PrP: 0.10 BuP: 0.20	BPA: 0.50 MeP: 0.50 EtP: 0.50 PrP: 0.40 BuP: 0.70
Tuzimski et al., 2019	20	BPA: 100.00 BPS: 100.00	BPA: 3.44 BPS: 0.14	BPA: 1.13 BPS: 0.11	N.R.	ng/mL	BPA: 0.10 BPS: 0.54	BPA: 0.20 BPS: 1.35
Tuzimski andc and Szubartowski ., 2019	50	BPA: 64.00 BPS: 34.00 BPF: 10.00 BPB: 14.00	BPA: 0.33 BPS: 0.19 BPF: 0.08 BPB: 147.04	BPA: 0.34 BPS: ND BPF: ND BPB: ND	BPA: ND-0.69 BPS: ND-0.68 BPF: ND-0.55 BPB: ND->LOD-	ng/mL	BPA: 92.00 BPS: 140.00 BPF: 53.00 BPB: 237.00	BPA: 277.00 BPS: 423.00 BPF: 159.00 BPB: 720.00
Vela-Soria et al., 2016	15	BPA: 26.70 BPS: 0.00 BPF: 0.00 MeP: 73.30 EtP: 33.30 PrP: 40.00 BuP: 6.70 BP-1: 6.70 BP-2: 0.00 BP-3: 53.30 BP-6: 0.00 BP8: 0.00 4-OH-BP: 0.00 BP-10: 0.00	BPA: 0.35 BPS: 0.05 BPF: 0.05 MeP: 5.22 EtP: 0.88 PrP: 0.39 BuP: 0.06 BP-1: 0.09 BP-2: N.R. BP-3: 0.48 BP-6: N.R. BP8: N.R. 4-OH-BP: N.R. BP-10: N.R.	BPA: ND BPS: N.R. BPF: N.R. MeP: 2.50 EtP: ND PrP: ND BuP: ND BP-1: ND BP-2: N.R BP-3: >LOD-<LOQ BP-6: N.R BP8: N.R 4-OH-BP: N.R BP-10: N.R	N.R.	ng/mL	BPA: 0.10 BPS: N.R. BPF: N.R. MeP: 0.10 EtP: 0.10 PrP: 0.10 BuP: 0.10 BP-1: 0.10 BP-2: N.R. BP-3: 0.20 BP-6: N.R. BP8: N.R. 4-OH-BP: N.R. BP-10: N.R.	BPA: 0.50 BPS: N.R. BPF: N.R. MeP: 0.50 EtP: 0.50 PrP: 0.50 BuP: 0.50 BP-1: 0.40 BP-2: N.R. BP-3: 0.60 BP-6: N.R. BP8: N.R. 4-OH-BP: N.R. BP-10: N.R.
Vela-Soria et al., 2018	15	MeP: 100.00 EtP: 46.70 PrP: 86.70 BuP: 0.00 BP-1: 60.00 BP-3: 100.00 BP-6: 0.00 BP-8: 0.00 4-OH-BP: 26.70	MeP: 4.71 EtP: 0.13 PrP: 1.69 BuP: 0.10 BP-1: 0.21 BP-3: 0.88 BP-6: N.R. BP-8: N.R. 4-OH-BP: N.R.	MeP: 2.20 EtP: ND, PrP: >LOD -<LOQ BuP: ND BP-1: >LOD -<LOQ BP-3: >LOD -<LOQ BP-6: ND BP-8: ND 4-OH-BP: ND	MeP: 0.6–4.3 EtP: ND PrP: 0.4–12 BuP: ND BP-1: 0.7–0.8 BP-3: 0.6–4.3 BP-6: ND BP-8: ND 4-OH-BP: ND	ng/mL	MeP: 0.10 EtP: 0.10 PrP: 0.10 BuP: 0.20 BP-1: 0.10 BP-3: 0.20 BP-6: 0.10 BP-8: 0.20 4-OH-BP: 0.10	MeP: 0.40 EtP: 0.30 PrP: 0.40 BuP: 0.50 BP-1: 0.40 BP-3: 0.50 BP-6: 0.40 BP-8: 0.50 4-OH-BP: 0.40

ND: Not detected, BPA: bisphenol A, BPB: bisphenol B, BPC: bisphenol C, BPE: bisphenol E, BPF: bisphenol F, BPM: bisphenol M, BPP: bisphenol P, BPS: bisphenol S, BPZ: bisphenol Z, BPAP; bisphenol AP, BPAF: bisphenol AF, BPBP: bisphenol BP, BPCl $_2$: dichlorobisphenol, BPFL: bisphenol FL, BPPH: bisphenol PH, TeCBPA: tetrachlorobisphenol A, CBPA: chlorobisphenol A, DCBPA: dichlorobisphenol A, TCBPA: trichlorobisphenol A, MeP: methylparaben, EtP: ethylparaben, PrP: propylparaben, BuP: buthylparaben, iBuP: isobutylparaben, BzP: benzylparaben, BP-1: benzophenone 1, BP-2: benzophenone 2, BP-3: benzophenone 3, 4-OH-BP: 4-hydroxibenzophenone. [a] Range of age, LOD: limit of detection, LOQ: limit of quantification. ND: not detected. N.R.: not reported.

Table 3. Weighted analyses of the number of samples, frequencies of detection and concentrations of bisphenols, parabens, and benzophenones in the selected studies.

	Total studies (N=50)									
	BPA	**TBBPA**	**BPS**	**BPF**	**BPAF**	**MeP**	**EtP**	**PrP**	**BuP**	**BP-3**
Cumulative samples	2044	392	455	435	240	846	853	857	617	251
FD (%)	63.6	56.8	27.4	10.1	17.9	57.9	63.4	53.9	27.9	39.5
Arithmetic mean*	1.4	0.5	0.1	0.1	0.1	14.2	7.0	4.9	0.2	24.4
	Epidemiological studies (N=24)									
	BPA	**TBBPA**	**BPS**	**BPF**	**BPAF**	**MeP**	**EtP**	**PrP**	**BuP**	**BP-3**
Cumulative samples	1525	392	310	310	190	703	714	714	493	167
FD (%)	58.4	56.8	27.3	7.1	21.0	34.8	42.0	34.1	25.8	26.5
Arithmetic mean*	2.2	0.5	0.1	0.1	0.1	2.6	0.8	0.4	0.1	35.7
	Methodological studies (N=26)									
	BPA	**TBBPA**	**BPS**	**BPF**	**BPAF**	**MeP**	**EtP**	**PrP**	**BuP**	**BP-3**
Cumulative samples	519	N.R.	145	125	50	143	139	143	124	84
FD (%)	50.7	N.R.	27.6	16.8	6.0	81.8	72.7	69.2	36.3	65.5
Arithmetic mean*	0.6	N.R.	0.1	0.1	0.0	53.4	29.0	20.6	0.9	2.1

*BPA: bisphenol A, BPF: bisphenol F, BPS: bisphenol S, BPAF: bisphenol AF, TBBPA: tetrabromobisphenol A, MeP: methylparaben, EtP: ethylparaben, PrP: propylparaben, BuP: buthylparaben, BP-3: benzophenone 3. FD: frequency of detection. N.R.: not reported. *:Calculated from studies reporting arithmetic mean.*

3.2.1 Epidemiological studies

A total of 2414 cumulative breast milk samples were analyzed in the 24 selected epidemiological studies, ranging from 9 to 325 sample per study; 14 studies (58.3%) examined less than 100 samples. In relation to bisphenol congeners, concentrations of BPA were described by 14 studies (n=1525 cumulative samples), 5 of these reported free and/or conjugated BPA as well as total BPA. TBBPA was measured in 7 studies (n=392), BPS in 2 (n=310), BPF in 2 (n=310), and BPAF in 1 (n=190). Weighted detection frequencies were: 58.4% for BPA, although it was >70.0% in all except three studies (62.0%, 53.0%, and 5.0%, respectively); 56.8% for TBBPA, ranging from 35 to 100% in all except one study; 27.3% for BPS; 7.1% for BPF; and 21.0% for BPAF, although this was only detected in one study. Weighted mean concentrations were: 2.2 ng/ml for BPA (<1.0 ng/mL in 2 studies, 1.0-2.0 ng/mL in 3, and >2.0 in 4; 5 studies did not express concentrations as arithmetic means); 0.5 ng/mL for TBBPA, <1.0 ng/mL in all studies except two (Carignan et al., 2012; Fujii et al., 2018) and 0.1 ng/mL for BPS, BPF, and BPAF.

With regard to PBs, MeP, EtP, and PrP were assessed in 703-714 samples and BuP in 493 samples. Weighted detection frequencies were 34.8%, 42.0%, 34.1%, and 25.8% for MeP, EtP, PrP, and BuP, respectively. This frequency ranged in studies from 26.9 to 100.0% for MeP, 20 to 70.0% for EtP, 15.0 to 100.0% for PrP, and 0.0 to 61.0% for BuP. MeP showed the highest weighted mean concentration (2.6 ng/mL), followed by EtP (0.8 ng/mL), PrP (0.4 ng/mL), and BuP (0.1 ng/mL). This concentration ranged from <2.0 ng/mL (2 studies) to 2.1-20.6 ng/mL (3 studies) for MeP, from <1.0 ng/mL (2 studies) and 1.0-5.3 ng/mL (2 studies) for EtP, and from <1.0 ng/mL (4 studies) to 1.42 ng/mL (1 study) for PrP, while it was <0.2 ng/mL for BuP (in all 4 studies).

In relation to BPs, BP-3 was quantified in 154 samples from three studies, and the mean weighted detection frequency was 26.5% (56.0%, 23.0%, and 13.0%, respectively). The weighted mean concentration of BP-3 was 35.7 ng/mL.

The storage and cryopreservation of collected samples was described in 22 of the 24 studies (91.7%), and 13 (54.2%) of these stored samples at -20 ºC. No information was given by any study on possible freeze-thaw cycles of samples (data not shown). Only nine studies (37.5%) used EDC-free tubes to avoid external contamination of the samples. Although most of the studies offered details their sampling procedure, such as the volume of samples or the timeframe of collections, none of them explicitly stated whether the analysis was performed in spot or pooled samples. This is crucial for the interpretation of results, given that even samples collected at different time points within the same day should be considered pooled samples. Extraction and quantification methodologies of the target compounds were heterogeneous (detailed in Supplementary table S2). Liquid-liquid (LLE) and solid-phase extractions (SPE) were the most common extraction procedures (n=17, 70.8%), and liquid chromatography-mass spectrometry (LC-MS/MS) was the most frequent quantification method, used in 16 (66.7%) of the studies. Only four studies (16.7%) did not report information on the volume/amount of milk used for analyses; among studies reporting these data, the sample volume ranged from 0.1 to 10 mL and the weight from 2 to 5 g.

3.2.2 Methodological studies

The total cumulative number of analyzed breast milk samples in the methodological studies was 620, ranging among studies from 3 to 278; 14 studies

(53.9%) examined ≤10 samples, 11 (45.8%) 11-50 samples, and 1 study (Cao et al., 2015) 278 samples. BPA was explored by 17 studies (n=519 cumulative samples) and 4 of these reported data on free and/or conjugated BPA as well as total BPA. BPS was measured in 7 studies (n=145), BPF in 6 (n=125), and BPAF in 2 (n=50). None of these studies analyzed TBBPA. BPA was detected in 50.7%, BPS in 27.6%, BPF in 16.8%, and BPAF in 6.0% of all samples. The weighted mean concentration was 0.6 ng/mL for BPA and ≤0.1 ng/mL for BPS, BPF, and BPAF.

With regard to PBs, MeP and PrP concentrations were assessed in 15 studies (n=143 cumulative samples), EtP concentrations in 14 (n=139), and BuP concentrations in 11 (n=124). Weighted detection frequencies of the four PB congeners ranged from 81.8% for MeP to 36.3% for BuP. MeP showed the highest weighted mean concentration (53.4 ng/mL), followed by EtP (29.0 ng/mL), PrP (20.6 ng/mL), and BuP (0.9 ng/mL).

In relation to BPs, six studies (n=84) evaluated the presence of BP-3, reporting a weighted detection frequency of 65.5% and weighted mean concentration of 2.1 ng/mL.

Storage conditions were described by all of the methodological studies, and 17 (65.4%) of them stored samples at -20 ºC. No study gave information on possible freeze-thaw cycles of samples (data not shown). Only five studies (19.2%) reported the use of field blanks to ensure that there was no external source of contamination in their samples. SPE was used for extraction in 13 (50.0%) of the studies and QuEChERS in three (11.5%). LC-MS/MS was used as measurement system in 18 (69.2%) of the studies. The sample volume was reported in all articles, as shown in Supplementary Table S3, ranging between 0.1 and 9.9 mL or between 1 and 25 g.

3.3 Geographical and temporal differences in the breast milk content of bisphenols, PBs and BPs

Results for bisphenols, PBs and BPs in breast milk were classified by continent and by sampling period (Figures 2A, 2B and Anexo III, Supplementary table S4). As shown in Figure 2A, weighted mean concentrations of BPA in American samples were 2.6-fold lower than in European samples and 4.5-fold lower than in those from Asia. MeP, EtP and PrP concentrations were 6- to 10-fold higher in Asian versus European samples and 1- to 4-fold higher in American

versus European samples. Finally, BP-3 concentrations were >20-fold higher in European versus American samples.

As depicted in Figure 2B, BPA concentrations were 1.8-fold higher in samples collected after versus before 2010, MeP concentrations were 5.4-fold higher in samples collected before versus after 2010, and BP-3 concentrations were >50-fold higher in samples collected after versus before 2010.

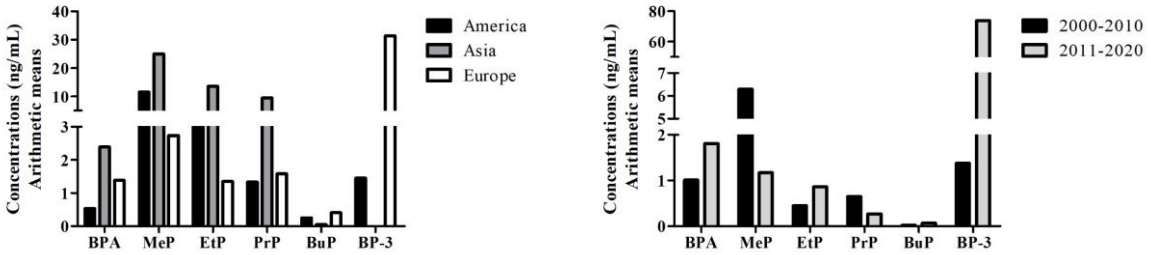


Fig. 2. Geographical and temporal differences in concentrations of bisphenols, parabens and benzophenones in human breast milk. A) Weighted concentrations by continents. B) Weighted concentrations by sampling period

3.4 Factors related to concentrations of bisphenols, PBs, and BPs in breast milk.

Factors associated with breast milk concentrations of these EDCs were studied by eight (33.3%) of the epidemiological studies. Most of these found that concentrations of bisphenols and/or PBs were related to certain anthropometric, socio-demographic, reproductive, and lifestyle characteristics. For instance, higher breast milk concentrations of BPA were observed in multiparous women, those living in a rural area, and those with a higher annual household income. Higher concentrations of some PBs were associated with a greater use of plastic food containers or consumption of canned beverages. Finally, concentrations of BPA, MeP, EtP, and/or PrP were related to the utilization of PCPs and cosmetics, including skin care products, air freshener, body lotion, makeup, eye shadow, face cleanser, perfume, and sunscreen products (Anexo III, Supplementary Table S5).

4. Discussion

This systematic review constitutes the first attempt to summarize current evidence on the presence of common non-persistent EDCs (bisphenols, PBs, and BPs) in human milk samples. The 50 articles selected for review studied a total of 3021 human milk samples; 24 were classified as epidemiological and 26 as methodological studies. Data were gathered on at least one bisphenol congener by 37 (74.0%) of the epidemiological studies, one PB

congener by 21 (42.0%), and one BP congener by 10 (20.0%). A serious/critical risk of bias was observed in two of the 24 epidemiological studies (8.3%).

Globally, four out of ten samples were positive for BPA, EtP, MeP, PrP, and/or BP-3. BP-3 showed the highest mean concentration, followed by MeP, EtP, and PrP. Mean concentrations of BPA ranged from 0.1 ng/mL (Cao et al., 2015; Niu et al., 2017) to 3.9 ng/mL (Azzouz et al., 2016a) and were 3.6-fold higher in the epidemiological versus methodological studies. Mean concentrations of PBs varied much more widely among studies, ranging from <0.1 ng/mL for BuP (Azzouz et al., 2016b; Dualde et al., 2019b; Vela-Soria et al., 2016) to 1063.6 ng/mL for MeP (Fotouhi et al., 2017), while weighted PB concentrations were higher in methodological versus epidemiological studies. Mean concentrations of BP-3 ranged from 0.5 ng/mL (Ye et al., 2008) to >73.5 ng/mL (Molins-Delgado et al., 2018); however, these results should be interpreted with caution given the very small number of samples analyzed. Taken together, these results in breast milk reflect the widespread presence of these chemicals reported in the general population (Pollack et al., 2018). These values have not been interpreted in terms of the tolerable daily intakes (TDIs) established by the European Food and Safety Authority (EFSA, 2017), because any concentration of some EDCs can have harmful repercussions in infants. For instance, the very few studies on this issue suggest that human milk concentrations of BPA might have a negative influence on the weight and/or length gain of the breastfeeding infant (Jin et al., 2020). There is also considerable research evidence on the impact of postnatal EDC exposure on the health of children, including its association with neurodevelopmental disturbances (Braun et al., 2011; Roen et al., 2015), obesity (Li et al., 2013; Wang et al., 2012), thyroid dysregulation (Heimeier et al., 2009), and asthma (Yang et al., 2020; Youssef et al., 2018).

BPA concentrations were highest in samples from Asia, consistent with data showing that 53% of this product is sold in Asia Pacific and 36% in Western Europe or the USA (Almeida et al., 2018; Industry-Experts, 2016). PB concentrations were also highest in Asia, where there has been a marked increase in the utilization of PBs and other preservatives (Market-Analysis-Report, 2016). A large difference in BP concentrations was observed between

samples from Europe and America, which may be in part attributable to differences in the composition of European and American sunscreens (Osterwalder et al., 2014) or in the proportion of lactating women who use BP-containing products in prevalence of lactating women who use BP-containing products. However, these findings should be interpreted with caution given the very small number of samples analyzed for BP (8.6% of cumulative samples). With regard to sampling dates, higher breast milk BPA concentrations were observed after than 2010, in agreement with the increased global production of this chemical over the past decade (Almeida et al., 2018).

Very limited information has been published on the influence of lifestyle patterns on the concentrations of bisphenols and PBs in breast milk and none on their relationship with BP concentrations. Among the eight studies that addressed potential determinants of exposure, certain sociodemographic and reproductive characteristics were associated with the breast milk burden of bisphenols, PBs and/or BPs, although most of the evidence was related to dietary habits and the utilization of PCPs and cosmetics. Some positive associations were found with the consumption of noodles (Kim et al., 2020), canned drinks (Park et al., 2019), and food in disposable containers (Kim et al., 2020), which may be attributable to the presence of these chemicals in the food packaging materials. For instance, containers often possess an inner coating of BPA to isolate the product (Brotons et al., 1995; Vandenberg et al., 2007) and can incorporate PBs for their preservative properties (Ye et al., 2006a). However, the aforementioned studies could not confirm whether these chemicals derived from the food itself or were leached from the food packaging material (Artacho-Cordón et al., 2018). As noted above, exposure to these chemicals has also been related to PCPs (Dualde et al., 2019a; Dualde et al., 2020; Fisher et al., 2017; Kim et al., 2020; Park et al., 2019; Yi et al., 2013). Thus, PBs in breast milk have been associated with hygiene and cosmetic products, including lotions (Kim et al., 2020), facial cleansers (Fisher et al., 2017), sun creams (Dualde et al., 2020), and makeup/skin care products (Park et al., 2019). Various studies have demonstrated the presence of these compounds in cosmetic products and PCPs (Gao and Kannan, 2020; Guo et al., 2014; Yazar et al., 2011).

4.1. Gaps of knowledge

Only a few epidemiological studies were found that addressed the presence of these chemicals in breast milk, and most of these had small sample sizes, limiting extrapolation of the data. In addition, only limited information is available on potential influential factors, and no study reported whether the milk was pasteurized or whether spot or pooled samples were used. Spot samples are considered to be more influenced by recent exposure, while pooled samples better reflect the average magnitude of exposure (Shin et al., 2019). In this regard, it has been reported that the absorbed dose of BPA is rapidly transferred to the breasts of lactating women, producing a high concentration of BPA within hours of the exposure (Tateoka, 2015).

Less than one-third of studies reported on the cumulative lactation time, lactational stage (colostrum, intermediate, or mature milk), and parity of the mothers, which are key data for this research. For instance, it has been suggested that BPA concentrations in mature milk are more related to recent maternal exposure and those in colostrum to accumulated exposure during the second half of pregnancy (Migeot et al., 2013). Finally, data on the nutritional content of breast milk samples and the use of EDC-free materials (e.g., breast pumps, bottles, laboratory material, etc.) were provided by very few studies.

Comparisons among studies are hampered by the expression of results in different units. Moreover, the reporting quality of more than half of the epidemiological studies was only medium or low. Researchers need to consider all items in the STROBE checklist before submitting studies for publication.

4.2. Limitations and strengths

This systematic review was based on a search of the three major public databases in the health field, and any publications only available in other databases would have been missed. The search was limited to the past two decades, because little or no attention was paid by researchers to non-persistent EDCs before this time. In addition, only three families of environmental phenols with endocrine-disrupting properties were selected for investigation; however, these include the phenolic EDCs in widest daily use, and a review has already been published on breast milk concentrations of organochlorine pesticides and PCBs (Pirsaheb et al., 2015). Future studies are warranted to

explore evidence on the occurrence of other non-persistent EDCs not included in this systematic review, such as phthalates in breast milk. There remains a need to take full account of the biological implications of simultaneous exposure to multiple pollutants.

One strength of the present systematic review is its inclusion of all studies in which a congener of interest was detected in breast milk, whether of methodological or epidemiological design. Care was also taken to harmonize data and facilitate comparisons, calculating weighted detection frequencies and arithmetic means of concentrations to obtain an average value for all samples, although this harmonization might also carry include an additional risk of bias. A further strength is the consideration of factors related to the breast milk burden of bisphenols, PBs, and BPs.

International biomonitoring programs have highlighted the widespread exposure of humans to numerous environmental chemicals; however, little attention has been paid to infant exposure during pregnancy and breastfeeding, and most studies have focused on developing analytical methods.

5. Conclusions

This review confirms the widespread presence of bisphenols, PBs and BPs in human milk and the need to investigate further the potential health risks they pose to the breastfeeding infant. Differences in mean concentrations of these chemicals were observed among continents and over the past two decades. However, some research has a higher risk of bias, failing to take account of key factors such as the use of spot or pooled samples, the type of milk (colostrum, intermediate, or mature), the parity of the mother, or the accumulated lactation time, among others. Further research with low risk of bias is urgently needed to elucidate the factors that influence the concentrations of these chemicals in human milk and to explore the health implications for the breastfeeding infant over the short, medium, and long term.

6. Declaration of competing interest

The authors declare no conflicts of interest.

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8. Figure legends

Figure 1. PRISMA flowchart of article selection.

Figure 2: Geographical and temporal differences in concentrations of bisphenols, parabens and benzophenones in human breast milk. A) Weighted concentrations by continents. B) Weighted concentrations by sampling period.

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BIOMONITORING BISPHENOLS, PARABENS, AND BENZOPHENONES IN BREAST MILK FROM A HUMAN MILK BANK IN SOUTHERN SPAIN

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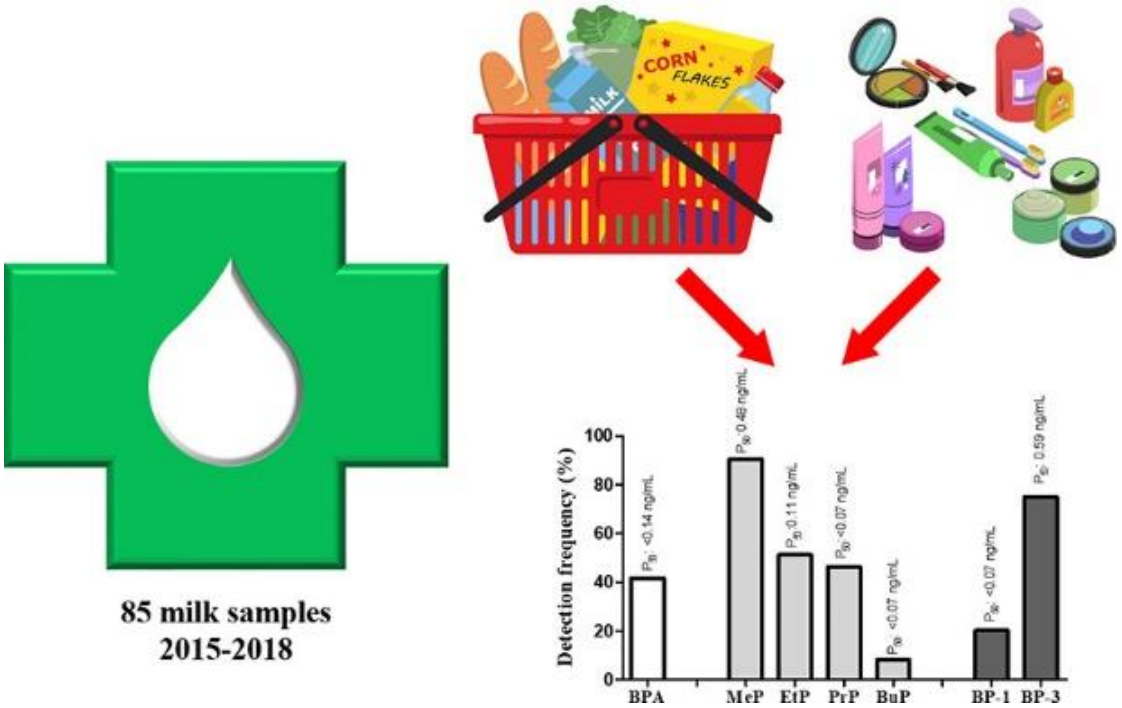
ABSTRACT GRÁFICO



ABSTRACT

Antecedentes: la leche materna humana se considera la fuente óptima de nutrición para los lactantes. Los bancos de leche materna ofrecen una alternativa a las fórmulas infantiles para los recién nacidos hospitalizados más vulnerables que en mayor medida se beneficiarán de la alimentación exclusiva con leche materna. No obstante, la leche materna también puede ser una fuente de exposición a contaminantes ambientales, incluidos los compuestos químicos disruptores endocrinos (DEs).

Objetivo: Evaluar las concentraciones de EDCs fenólicos, incluidos bisfenoles, parabenos (PBs) y benzofenonas (BPs) en muestras de leche materna donadas de un Banco de Leche Humana en Granada, sur de España, y explorar factores sociodemográficos, reproductivos y de estilo de vida, relacionados con sus concentraciones en la leche.

Métodos: Las concentraciones de tres bisfenoles, bisfenol A (BPA), bisfenol F (BPF) y bisfenol S (BPS); cuatro PBs, metil- (MeP), etil- (EtP), propil- (PrP) y butil -paraben (BuP); y seis BPs, BP-1, BP-2, BP-3, BP-6, BP-8 y 4-hidroxi-BP, se determinaron en muestras de leche de 83 donantes. La información sobre posibles variables explicativas se recopiló mediante un formulario del banco de leche y un cuestionario *ad hoc*. Se crearon modelos de regresión múltiple lineal y logística.

Resultados: Se encontraron concentraciones detectables de al menos uno de los compuestos analizados en todas las muestras de leche materna de donantes y al menos cinco compuestos en una quinta parte de ellas. Los compuestos detectados con mayor frecuencia fueron MeP (90.5 %), BP-3 (75.0 %), EtP (51.2 %), n-PrP (46.4 %) y BPA (41.7 %). Las concentraciones medianas oscilaron entre <0.10 ng/mL (n-PrP, n-BuP, BP-1) y 0.59 ng/mL (BP-3). Ninguna muestra contenía concentraciones detectables de BPF, BPS o la mayoría de los BPs (BP-2, BP-6, BP-8 y 4-hidroxi-BP). Las concentraciones de fenoles en la leche materna se asociaron con la paridad, el uso de desodorantes, enjuagues bucales, productos para el cuidado de la piel y cosméticos, y la ingesta de suplementos nutricionales.

Conclusiones: Los resultados revelan la presencia generalizada de BPA, PBs y BP-3 en muestras de leche materna de donantes, destacando la necesidad de implementar medidas

preventivas para incrementar los beneficios de la leche materna de los bancos de leche y de las mujeres lactantes, en general.

1. Introduction

Breast milk is considered the optimal nutritional choice for newborns and infants. The World Health Organization (WHO) recommends exclusive breastfeeding for six months and continuous breastfeeding for up to two years or more alongside complementary nutrition (Fonseca et al., 2021; WHO, 2003). Breast milk provides nutrients and multiple immune factors that can enhance host defenses, neurodevelopment, gastrointestinal function (Ballard and Morrow, 2013; Dussault et al., 2021; Eidelman, 2012), and immune system maturation (Fonseca et al., 2021; Palmeira et al., 2009). However, some newborns do not have access to maternal breast milk in the early neonatal period, such as premature infants and those with very low (<1,500 g) or low (<2,500 g) birth weight. Other reasons for the non-availability of maternal milk include: the presence of HIV positivity or breast cancer in the mother (Murguia-Peniche and Kirsten, 2014; Zanganeh et al., 2021); her absence due to illness, death, or abandonment; or her inability to produce enough milk or milk with sufficient fat content, among other causes (Tran et al., 2021; Tran et al., 2020). Despite advances in infant formulas, human breast milk provides benefits that cannot be replicated by any other source of nutrition (Kim and Unger, 2010). Hence, the best solution for infants unable to receive milk from their own mother, especially for premature infants in neonatal intensive care units (NICUs), is to supply them with milk from a breast milk bank (Agier et al., 2019; Tran et al., 2021; Tran et al., 2020). In fact, it has been reported that breast milk from this source is provided in up to 65.7% of newborns in level 3 NICUs and up to 73.3% of those in level 4 (highest-level) NICUs (Colaizy, 2021).

Breast milk in human milk banks undergoes multiple nutritional, microbiological, and biochemical procedures to minimize any possible risk to the newborn (Arslanoglu et al., 2013; Dussault et al., 2021). However, there are no standardized measures to evaluate the presence of environmental chemicals in donor milk. Breast milk is known to be a potential vehicle for the excretion of common environmental chemicals from mother to infant, especially persistent

chemicals such as organochlorine compounds (van den Berg et al., 2017) and perfluoroalkyl substances (PFAS) (Serrano et al., 2021; Thomsen et al., 2010). In addition, there is growing evidence that less persistent chemicals such as bisphenols, parabens (PBs), and benzophenones (BPs) are also present in breast milk Iribarne-Durán et al. (2021), leading to potential exposure of the breastfeeding infant (Dualde et al., 2020). Bisphenol A (BPA) and its substitutes bisphenol S (BPS) and F (BPF) are non-persistent phenolic compounds commonly used in the manufacture of epoxy resins and polycarbonate plastics and as an additive in numerous consumer products (Gimeno et al., 2015; Iribarne-Durán et al., 2019; Molina-Molina et al., 2019). Diet is considered the main source of exposure to bisphenols for the general population (Morgan and Clifton, 2021; Robles-Aguilera et al., 2021), although non-dietary sources (e.g., thermal paper and textile products) can also make a contribution (Freire et al., 2019; Molina-Molina et al., 2019). PBs, including methyl- (MeP), ethyl- (EtP), propyl- (n-PrP), and butyl-paraben (n-BuP) congeners, are a family of alkyl esters of p-hydroxybenzoic acid used in a wide range of personal care products (PCPs), pharmaceuticals, food, and beverages for their antimicrobial and preservative properties (Błędzka et al., 2014; Darbre and Harvey, 2008; Iribarne-Durán et al., 2020; Moos et al., 2015). BPs are frequently added as synthetic UV filters in PCPs, food packaging materials, and textiles, among their other uses (Molins-Delgado et al., 2016). The main routes of human exposure to PBs and BPs are reported to be the dermal absorption of PCPs and the consumption of pharmaceuticals and foodstuffs (Benech-Kieffer et al., 2000; Díaz-Cruz et al., 2012; Fent et al., 2010; Janjua et al., 2008; Janjua et al., 2004; Rocío-Bautista et al., 2015; Schlumpf et al., 2010). These environmental phenols are considered endocrine-disrupting chemicals (EDCs) because of their (anti-)estrogenic, (anti-)androgenic and/or (anti-)thyroid actions (Boberg et al., 2010; Darbre and Harvey, 2008; Perez et al., 1998). Infants, especially premature newborns, are particularly susceptible to these effects (Safe, 2005), and their exposure to phenolic EDCs has been associated with more behavior disorders, worse executive function (Jiang et al., 2019), precocious puberty (Berger et al., 2018; Harley et al., 2019), thyroid imbalances (Aker et al., 2016), and poorer respiratory function (Agier et al., 2019; Buckley et al., 2018).

This study is part of a wider project designed to assess the potential adverse health impact on NICU neonates of exposure to EDCs from their medical care, diet, and environment, including exposure to PFAS, heavy metals, and other toxic elements (Iribarne-Durán et al., 2019; Serrano et al., 2021). Only limited research has been published on the concentrations of environmental chemicals in milk from human breast milk banks and this is crucial information, given that donated milk is primarily given to fragile premature infants. Hence, the specific objectives of the present study were to evaluate the concentrations of bisphenols, PBs, and BPs in breast milk samples donated to a human milk bank in Granada (Southern Spain) and to explore sociodemographic, reproductive, and lifestyle factors related to this exposure.

2. Material and methods

2.1. Study population and sample collection

This study included 83 donors recruited between 2015 and 2018 at the Regional Human Milk Bank of the Virgen de las Nieves University Hospital (HUVN) in Granada. As customary in milk banks, the donors are volunteers who have delivered healthy full-term infants, have extra milk available, and meet stringent health criteria (Colaizy, 2021; HMBAoN, 2018). In general, donors are registered at the milk bank after breastfeeding is well established (i.e., 2–3 weeks post-delivery). Milk bank exclusion criteria for donor selection include: positive serology for Human Immunodeficiency Virus (HIV), syphilis or hepatitis B or C; risk factors for sexual transmitted diseases (e.g., unstable partner, non-use of condoms, tattooing/piercing in previous 3 months, acupuncture, or blood transfusion); receipt of organ transplantation in previous 6 months; current smoking or drug use; and elevated consumption of alcohol (>2 drinks [20 g]/day) or caffeine-containing drinks (>3 cups [30 g]/day). Mothers who met these eligibility criteria were invited to participate in the study and received detailed instructions on the collection, storage, and delivery of their milk samples. Mother who signed their informed consent to participation completed an ad hoc questionnaire on their socio-demographic and reproductive characteristics and lifestyle habits, including information on their diet and PCP utilization. The research protocol was approved by the Biomedical Research Ethics Committee of Granada.

Mothers were instructed to collect milk samples at home, pooling mature milk over a minimum of one week and a maximum of four weeks by manual expression and/or breast pump and keeping them frozen at −20 °C. Although donated milk samples might in part differ from the milk used by donors to feed their infants (given that breast milk bank does not recommend the precise timing for obtaining the smaples), tested samples are wholly comparable to that offered to the NICU infants, which is the objective of this study. Pooled samples were pasteurized at the bank within two weeks, and an aliquot of 5-30 mL of pre-pasteurized milk was obtained for the present study. Milk samples were stored at -20 ºC. Tubes used to collect samples were pre-tested to ensure that they did not contain or leach any target compound. For that purpose, each sample was accompanied by a similar tube filled with a similar amount of MS/MS water quality, and the samples and paired blanks underwent the same conditions for storage, extraction and quantification. until analysis. No blank showed any contamination. The date of pasteurization was recorded as the donation date.

2.2. Chemicals and reagents

Bisphenols [BPA, BPS, BPF, and labeled deuterium bisphenol A (BPA-D16)], PBs [MeP, EtP, n-PrP, n-BuP, and ethylparaben ring 13C6 labeled (EtP-13C6)], and BPs [BP-1, BP-2 BP-3, BP-6, BP-8, and 4-hydroxy-BP (4-OH-BP)] were purchased from Sigma-Aldrich (Madrid, Spain). Methanolic standard solutions (100 mg/L) were stored at 4 °C and stable for at least four months. Diluted solutions were prepared immediately before utilization. β-glucuronidase/sulfatase (Helix pomatia, H1) was also supplied by Sigma-Aldrich. The enzyme was prepared daily by dissolving 6 mg of β-glucuronidase/sulfatase (3 × 106 U g/solid) in 1.0 mL of 1 M ammonium acetate/acetic acid buffer solution (pH 5.0). Acetonitrile (HPLC-grade), ethyl acetate, and trichloromethane (TCM) were purchased from Merck (Darmstadt, Germany). Sodium chloride and N, O-Bis(trimethylsilyl)trifluoro-acetamide with trimethylchlorosilane (BSTFA/1% TCMS) were supplied by Sigma-Aldrich (Madrid, Spain). Hydrated zinc acetate, polyhydrated phosphotungstic acid, and glacial acetic acid were purchased from Sigma-Aldrich, and a mixture of these substances (0.91 g, 0.55 g, and 0.60 mL, respectively) was dissolved in 10.0 mL

of deionized water, yielding a fat/protein precipitation solution (FPS) prepared immediately before utilization.

2.3. Sample extraction and treatment

The extraction protocol design was adapted from Vela-Soria et al. (2016). Briefly, human milk samples were completely thawed at room temperature and analyzed in triplicate. Each aliquot (1.0 mL) was spiked at 10.0 ng/g with 20 µL of a solution containing BPA-D16 and EP-13C6 at 1.0 mg/L, adding 30 µL of enzyme solution (β-glucuronidase/sulfatase). After mixing, the sample was incubated at 37 °C for 24 h, and 20 µL of FPS and 0.75 ml of acetonitrile were then added. The mixture was vortexed for 30 s and centrifuged 11357 g for 10 min at 4 ºC. The supernatant was transferred to a conical glass tube and diluted with 10.0 mL of 10% NaCl aqueous solution (w/v) at pH 2. After using a syringe to rapidly inject 1.0 mL of TCM into the aqueous sample, the mixture was manually shaken for 30 s and then centrifuged 3226 g for 10 min at 4 ºC. The entire sedimented phase was transferred to a clean glass vial and evaporated under a nitrogen stream. The residue was then derivatized using 0.1 mL of BSTFA: ethyl acetate (60:40 v/v) and incubated for 15 min at 60 ºC. Finally, the mixture was cooled to room temperature, transferred into a chromatographic glass vial, and then analyzed by gas chromatography/tandem mass spectrometry (GC-MS/MS).

2.4. Instrumentation and GC-MS/MS conditions

Regarding the instrumental method, an already existing procedure was adapted with minor modifications (Vela-Soria et al., 2014). Thus, GC–MS/MS analysis was performed using an Agilent 7890 GC (Agilent Technologies, Palo Alto, CA, USA) with split-splitless inlet and 7693 ALS autosampler. The detector was an Agilent 7000D triple quadrupole mass spectrometer with inert electron-impact ion source, operated in single reaction monitoring (SRM) mode, reporting two MS/MS transitions for each analyte. Electron impact (EI) ionization was set at 70 eV. Agilent MassHunter B.03.02 software was used for instrument control. Analytes were separated in a HP–5MS-UI capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness) from Agilent. The injection port of the GC was set at 250 °C. Samples were automatically injected in splitless-injection mode using an Ultra Inert Liner 5190-3163 from Agilent. The injection volume was 2 µL, and an Agilent 5181-3354 10 µL syringe was

used. The flow of helium carrier gas (99.999% purity) was maintained at 1.2 mL/min. The initial oven temperature was set at 70 ºC and held for 2.0 min, ramped to 120 ºC at 25 ºC/min, held for 0.5 min, ramped to 250 ºC at 10 ºC/min and, finally, to 280 ºC at 120 ºC/min and then held for 4 min (total time of 22 min). The resolution was adjusted to 1.0 Da for quadrupoles 1 and 3. Temperatures of the transfer line, ion source, and quadrupoles were 280 ºC, 280 ºC, and 150 ºC, respectively. The mass spectrometer was auto-tuned weekly.

2.5. Analytical performance and quality control (QC)

The linearity, sensitivity, accuracy, and selectivity of the method was tested according to US Food and Drugs Administration guidelines for bioanalytical method validation (Guidance for Industry, 2001). The limits of detection (LOD) and quantification (LOQ), expressed as LOD-LOQ in ng/mL, were 0.2-0.5 for BPA and BP-3, 0.1-0.3 for BP-1, and 0.1-0.3 for PBs. A calibration curve (matrix-matched calibration using a pool of three blank milks) was constructed for each analyte with ten concentration levels (three replicates) from 0.2 to 40 ng/g, plotting the analyte/mass-labeled surrogate peak area ratio against the analyte concentration. A range of concentrations from the LOQ to 40 ng/g was established as the linear dynamic range (LDR), with determination coefficients (% R2) ranging from 99.1% to 99.7%. P-values for the lack-of-fit test (% Plof) were >5% in all cases, confirming linearity within the stated range. The selectivity of the method was evaluated by analyzing chromatograms of the procedure blank and the corresponding pooled blank sample. No interference from endogenous substances was observed at the analyte retention times. Background contamination was controlled for by analyzing procedural blanks every 24 injections, using milliQ water as sample. No quantifiable concentrations of target analytes were detected. In addition, aliquots of the blank pool spiked at 1.0, 20 and 40 ng/g were analyzed in triplicate every 24 injections. The resulting accuracy values for the quality control samples were within ±15% of the nominal values, and each batch sample could be accepted. The intra-day variation, expressed as relative standard deviation (RSD, %), ranged between 4.9 and 8.7% for the entire group of targeted compounds. Values for the inter-day variation ranged from 10.7 to 12.9%.

2.6. Independent variables

Independent variables were gathered from each participant using the standardized data form employed by the milk bank for prospective milk donors and an ad hoc questionnaire. Information was recorded on the donor's age (years), schooling (university/non-university), occupation (unemployed/manual worker/non-manual worker), place of residence (urban/semi-urban/rural), current weight and height, smoking habit (ex-smoker/never smoker), alcohol intake (≥1 drink monthly/never), coffee intake (1 cup per day/less), regular receipt of medication (including folic acid supplementation) (yes/no), and presence of composite filling (yes/no). The BMI was calculated as weight/height squared (kg/m2). Data were also collected on parity (primiparous or multiparous), gestational age (weeks), lifetime duration of breastfeeding (months), weight change since before pregnancy (gain/loss/no change), intrauterine transfusion (yes/no), and gestational diabetes (yes/no). In addition, participants were asked about their diet, including the type of drinking water used (tap or bottled water) and the consumption frequency over the previous 12 months of: fish (oily and lean fish), dairy products (yoghurt, milk, cheese, and butter), cold meats, meat (all), red meat, pulses, vegetables (raw and cooked), fruit, eggs, pasta, bread, cereals, chocolate, canned food, fried food, organic food, vitamins (other than folic acid), and other nutritional supplements. Information was also collected on the frequency with which participants had used the following PCPs over the previous 12 months: sunscreen, lip protector, face cream, face tonic, face treatment, body lotion, hand cream, hair mask, makeup products (foundation, lipstick, eye shadow, eyeliner), nail polish, manicure, pedicure, hair dye, shampoo, shower cream, deodorant, hairspray/mousse/gel, perfume, toothpaste, and mouthwash. Finally, data were gathered on the number of days post-delivery (difference between delivery and milk donation dates) and the nutritional characteristics of unpasteurized pooled milk samples as potential explanatory variables, measuring their protein (g/100 mL), lipid (g/100 mL), lactose (g/100 mL), and caloric (kcal/100 mL) contents.

2.7. Statistical analysis

The arithmetic mean value of the triplicates was used for statistical analyses. Detection frequencies of bisphenols, PBs, and BPs in milk

samples were calculated. Concentrations below the LOD were assigned a value of LOD/√2. No BPF, BPS, BP-2, BP-6, BP-8, or 4-OH-BP was detected in any milk sample. Concentrations of phenols were summarized as means with standard deviations (SD), medians, 25th, 75th, and 95th percentiles, and minimum/maximum values. Total concentrations of PBs (∑PBs), were also determined as the sum of the molar concentrations of breast milk PBs based on molecular weight, expressed as MeP (molecular weight=152.15 g/mol), following previous reports (Deierlein et al., 2017; Quirós-Alcalá et al., 2018). A similar approach was adopted for BPs (∑BPs) expressed as BP-3 (molecular weight=228.25 g/mol). The sum of all studied phenols (∑EDCs) was calculated as BPA + ∑PBs + ∑BPs. Bisphenol, PB, and BP concentrations were non-normally distributed according to the Kolmogorov-Smirnoff test and were therefore natural log(ln)-transformed to minimize the influence of extreme values. The Spearman correlation test was used for relationships between phenols. Bivariate and multiple regression analyses were conducted to identify predictors of bisphenol, PB, and BP concentrations in breast milk samples, using a combination of backward and forward stepwise procedures to ensure the robustness of the models. Linear regression models were constructed for chemicals detected in ≥75% of samples (i.e., MeP, ΣPBs, BP-3, and ΣEDCs) and logistic regression models for those detected in <75% of samples (i.e., BPA, EtP, n-PrP, and BP-1). Given the small sample size, the significance level was set at p=0.050, although results with p-values between 0.100 and 0.050 were also cautiously discussed. All tests were two-tailed, and the R-statistical computing environment 3.0 (http://www.r-project.org/) and SPSS v23.0 (IBM SPSS, Armonk, NY) were used for data analyses.

3. Results

3.1. Characteristics of study participants

Table 1 summarizes the characteristics of the study participants. The mean (±SD) age of the 83 donors was 33.7 (±4.8) years and their mean BMI was 23.5 (±3.9) kg/m2; 30.1%, of participants lived in a rural area, 66.3% were non-manual workers, 61.9% had completed university education; 48.0% were ex-smokers, 3.6% consumed ≥ 1 alcohol drink/month, 56.0% were primiparous, 47.6% had gained weight since before the pregnancy and 20.2% had lost weight. The mean (±SD) gestational age in the most recent pregnancy was 37.9 (±3.8) weeks, and

the mean lifetime breastfeeding duration was 6.5 (±10.2) months. Gestational diabetes developed in 7.1% of donors, and there were no cases of intrauterine transfusion during delivery. The median interval between delivery and milk donation was 98 days (3.3 months), ranging from 20 days (<1 month) to 273 days (9 months). The dietary habits and use of PCPs were previously reported (Serrano et al., 2021) and are summarized in Supplementary Tables S1 and S2 (Anexo IV).

The median protein content of milk samples was 1.10 g/100 mL (range, 0.20-6.80 g/100 mL), median fat content 3.70 g/100 mL (range, 1.16-8.30 g/100 mL), median lactose content 7.36 g/100 mL (range, 6.54-8.00 g/100 mL), and median energy content 68 kcal/100 mL (range, 44-110 kcal/100 mL).

Table 1. Sociodemographic, anthropometric, and reproductive characteristics of the study population (N = 83) and nutritional content of the milk samples

		N (%)
Age= *Years*		33.65 ± 4.8
BMI= *kg/m* ^{2}		23.46 ± 3.9
Occupational class	*Housewife*	5 (6.0)
	Manual worker	23 (27.7)
	Non Manual worker	55 (66.3)
Educational level	*Non-Universitary*	27 (32.1)
	Universitary	52 (61.9)
Smoking habit	*Never smoker*	44 (53.0)
	Ex-smoker	36 (47.6)
Alcohol	*Never*	80 (96.4)
	At least one/month	3 (3.6)
Coffee intake	*<1cup/day*	17 (20.7)
	1 cup/day	66 (79.5)
Residence	*Rural*	25 (30.1)
	Sub-urban	25 (30.1)
	Urban	32 (39.8)
Use of regular medication	*No*	43 (51.8)
	Yes	40 (48.2)
Parity	*Uniparas*	47 (56.0)
	Multiparas	37 (44.0)
Lifetime duration of breastfeeding= *Months*		6.48 ± 10.2
Gestational age *=Weeks*		37.9 ± 3.8
Weight change since before pregnancy	*Same*	22 (26.2)
	Loss	17 (20.2)
	Gain	40 (47.6)
Gestational diabetes	*No*	73 (86.9)
	Yes	6 (7.1)
Use of composite filling	*No*	19 (22.6)
	Yes	57 (67.9)
Proteins		1.10 ± 0.7
Lipids		3.65 ± 1.2
Kcal		68.00 ± 10.9
Lactose		7.37 ± 0.3

3.2. Concentrations of bisphenols, PBs, and BPs in breast milk samples

As shown in Figure 1, detectable levels of ≥1 analyzed compound were found in all breast milk samples, detectable levels of ≥3 compounds in 60 samples (72.3%), and detectable levels of ≥5 chemicals in 16 (19.3%).

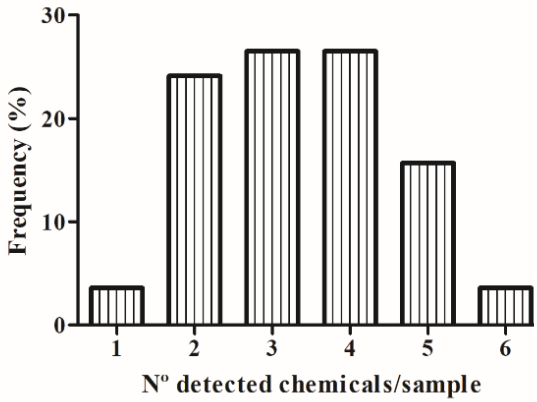


Figure 1. Frequency of detection of BPA, PBs, and BPs in breast milk samples

Table 2 exhibits the detection frequencies and concentrations of bisphenols, PBs, and BPs. BPA was detected in 41.7% of samples, with a median concentration of 0.14 ng/mL. The detection frequency of PBs ranged from 8.3% (n-BuP) to 90.5% (MeP), and their median concentration from <0.10 ng/mL (n-PrP and n-BuP) to 0.48 ng/mL (MeP). BP-1 was detected in 20.2% of samples and BP-3 in 75.0% (median of 0.59 ng/mL). Significant positive correlations were found between PB congeners (MeP-n-PrP, MeP-n-BuP, EtP-n-PrP, EtP-n-BuP, and n-PrP-n-BuP; Spearman's rho >0.242, $p<0.05$) and between BP-1 and BP-3 (rho=0.322; $p<0.010$), whereas no correlations were observed between BPA and any PB or BP congener or between PB and BP congeners.

Table 2. Concentrations of BPA, PBs, and BPs in human milk (ng/mL) (N=83)

	N(%) >LOD	Mean	SD	Mín.	Percentiles				Max.
					25	50	75	95	
BPA	35 (41.7)	0.72	1.31	0.14	<0.14	<0.14	1.11	2.21	8.73
MeP	76 (90.5)	1.48	4.12	0.07	0.24	0.48	0.69	9.78	29.49
EtP	43 (51.2)	0.69	1.31	0.07	<0.07	0.11	0.45	4.14	6.45
n-PrP	39 (46.4)	0.28	0.71	0.07	<0.07	<0.07	0.26	0.83	5.64
n-BuP	7 (8.3)	0.11	0.29	0.07	<0.07	<0.07	<0.07	0.13	2.62
ΣPBs	-	2.44	5.32	0.25	0.59	0.85	1.65	12.86	36.96
BP-1	17 (20.2)	0.09	0.04	0.07	<0.07	<0.07	<0.07	0.19	0.33
BP-3	63 (75.0)	0.82	1.12	0.14	0.27	0.59	0.95	2.21	8.20
∑BPs	-	0.93	1.14	0.23	0.36	0.71	1.10	2.30	8.44
ΣEDCs	-	4.09	5.34	0.75	1.68	2.52	3.46	13.69	37.33

BPA: bisphenol A; MeP: methylparaben; EtP: ethylparaben; n-PrP: n-propylparaben; n-BuP: n-buthylparaben; PBs: parabens; BP-1: benzophenone 1; BP-3: benzophenone 3; BPs: benzophenones; EDCs: endocrine disrupting compounds; LOD: limit of detection; SD: standard desviation; Min: minimum; Max: maximum. LODs for BPA and BP-3 = 0.2 ng/mL; LODs for MeP, EtP, n-PrP, n-BuP, and BP-1 = 0.1 ng/mL.

3.3. Factors related to bisphenol, PB, and BP concentrations in breast milk

Bivariate associations between potential predictors of exposure and concentrations of BPA, PBs, and BPs in breast milk are summarized in Supplementary Tables S3 and S4 (Anexo IV). In the multiple regression models (Table 3), age was positively associated with BP-3 concentration and BP-1 detection. Donors with a university degree had higher n-PrP concentrations, and those reporting regular use of medication had higher MeP concentrations. In contrast, donors living in sub-urban areas had lower n-PrP concentrations, and multiparas had lower MeP concentrations. No associations were found between the protein, lipid, lactose, or caloric content of the milk and concentrations of BPA, PBs, or BPs.

Some associations were found between dietary habits and breast milk phenol concentrations. In this way, a positive association was found between the consumption of vitamins and EtP, cold meat and concentrations of ΣPBs and ΣEDCs (close-to-significant association; p-value = 0.063), chocolate and concentrations of BP-3 (close-to-significant association; p-value = 0.054), and ΣEDCs; and the consumption of other (non-vitamin) nutritional supplements was associated with higher concentrations of BP-3, ΣBPs, and ΣEDCs. Associations close to statistical significance were also found between bread or pasta consumption and detectable BPA concentrations (p=0.092 and 0.084, respectively). In contrast, the consumption of fresh vegetables was inversely related to detectable BP-1 concentrations.

The utilization of various PCPs was positively associated with breast milk phenol concentrations. Specifically, the frequency of body lotion use was positively associated with detectable levels of BP-1, mouthwash use with detectable levels of BPA and with higher MeP, BP-3, ΣPB, and ΣEDC concentrations. Hair dye use was associated with detectable levels of BPA and n-PrP (close-to-significant for n-PrP; p-value=0.078) and deodorant use was associated with higher BP-3, ΣPB, and ΣEDC concentrations. The use of hairspray, hair wax, and/or hair mousse was associated with higher ΣEDC concentrations, the use of cologne/perfume with higher MeP concentrations, and the use of facial cream and artificial nails with higher ΣBP concentrations. Associations close to statistical significance were observed between the use of hand cream and MeP

concentrations (p-value=0.069), between the use of hair mask or eyeshadow and detectable BP-1 concentrations (p-values<0.070), and between the use of sunscreen and BP-3 concentrations (p=0.080).

Considered together, a more frequent intake of cold meat, chocolate, and other nutritional supplements and the utilization of facial cream, artificial nails, hair products, deodorant, and mouthwash were associated with a higher total concentration of phenols, explaining up to 64.5% of the variability in exposure levels.

4. Discussion

This study is one of the first to characterize the presence of bisphenols, PBs, and BPs in breast milk samples from a human milk bank. The results indicate that environmental phenols are widely present in donor milk, with all samples containing detectable concentrations of at least one of the chemicals under study. The most frequently detected compound was MeP (90.5% of samples), followed by BP-3 (75.0%), EtP (51.2%), and BPA (41.7%). The study also identified sociodemographic and reproductive factors, dietary habits, and the use of certain PCPs that may influence the concentrations of environmental phenols in donor milk.

Few human biomonitoring studies have addressed the contamination of breast milk with bisphenols, PBs, and BPs, especially BPA substitutes and BPs [recently reviewed in: (Iribarne-Durán et al., 2021)]. Comparisons between the present findings and previous epidemiological data are discussed below

4.1. Bisphenols

In general, the detection frequency of total BPA (41.7%) in milk from these Spanish donors is lower than the mean frequency (64%) observed in a previous meta-analysis by Iribarne-Durán et al. (2021). Furthermore, no sample evidenced a detectable amount of BPS or BPF, which were detected in 27.4% and 10.1% of samples, respectively, in the meta-analysis by Iribarne-Durán et al. (2021). Restrictions imposed on the use of BPA in the past decade have led manufacturers to use alternative substances, such as BPS and BPF, associated with a progressive decline in urinary BPA levels in the general population of certain European countries over the last decade (Frederiksen et al., 2020; Gyllenhammar et al., 2017). The absence of BPS and BPF in the present samples may suggest that less effective

measures to phase out BPA were implemented in Spain (Molina-Molina et al., 2019). In addition, the recent meta-analysis (Iribarne-Durán et al., 2021) observed much higher breast milk BPA concentrations between 2010 and 2020 (>6.0 ng/mL) than in the previous decade (~1.0 ng/mL), indicating a continued increase in BPA exposure worldwide (Almeida et al., 2018).

The mean concentration of total BPA in this study (0.72 ng/mL) was half the overall mean level of 1.40 ng/mL calculated in the aforementioned meta-analysis (Iribarne-Durán et al., 2021), although it is worth mentioning that none of these previous studies were focused on milk donors. Given differences in lifestyle habits between lactating women in general and milk donors, comparisons should be interpreted with caution. Globally, the highest BPA concentrations have been described in samples from Asia, consistent with the fact that 53.0% of this compound is sold in Asia Pacific versus 36.0% in Western Europe or the USA (Almeida et al., 2018; Industry-Experts, 2016). In comparison to the present Spanish donors, BPA concentrations were higher in breast milk from women in the USA (Mendonca et al., 2014; Zimmers et al., 2014), Taiwan (Chang et al., 2019), Japan (Kuruto-Niwa et al., 2007; Nakao et al., 2015), Korea (Lee et al., 2018; Yi et al., 2013), and France (Migeot et al., 2013), they were similar in breast milk from women in the USA (Hines et al., 2015) and China (Jin et al., 2020), and slightly lower in samples from women from Canada (Arbuckle et al., 2015) and Korea (Kim et al., 2020). Among Spanish studies, BPA concentrations in the present donors are similar to those in 120 milk samples collected in 2015 from women in Valencia (median=0.26 ng/mL) (Dualde et al., 2019) and lower than those in 53 milk samples collected in Madrid (median of 1.30 ng/mL) (Martínez et al., 2019). However, most previous studies in Spain and other countries did not specify the period between the delivery and milk donation or whether colostrum or mature milk was collected, hampering a more precise comparison with the present results. The type of breast milk may be a relevant issue, given suggestions that BPA concentrations in mature milk are more related to recent maternal exposure and those in colostrum more related to exposure accumulated during the second half of the pregnancy (Migeot et al., 2013).

Table 3. Factors associated with human milk levels of BPA, PBs, and BPs (N=83). Multivariate regression analyses

	BPA (R^2 = 0.292)*				BP-1 (R^2 = 0.269)*				BP-3 (R^2 = 0.313)**			
	OR	95% CI		P-value	OR	95% CI		P-value	exp(β)	95% CI		P-value
Age= *years*	-	-	-	-	1.38	1.11	1.72	0.004	4.88	1.40	17.04	0.014
Frequency of fresh vegetable consumption= *>2 times/week* [a]	-	-	-	-	0.10	0.02	0.59	0.011	-	-	-	-
Frequency of bread consumption= *>1 time/day* [b]	3.52	0.81	15.24	0.092	-	-	-	-	-	-	-	-
Frequency of pasta consumption= *≥1 time/week* [c]	4.27	0.82	22.13	0.084	-	-	-	-	-	-	-	-
Frequency of chocolate consumption= *≥1 time/day* [d]	-	-	-	-	-	-	-	-	1.51	0.99	2.29	0.054
Frequency of other nutritional supplement consumption= *Yes* [e]	-	-	-	-	-	-	-	-	1.75	1.11	2.75	0.016
Use of sunscreen= *Yes* [e]	-	-	-	-	-	-	-	-	1.41	0.96	2.07	0.080
Frequency of body lotion use = *≥1 time/day* [d]	-	-	-	-	7.94	1.18	53.28	0.033	-	-	-	-
Frequency of hair mask use = *≥1 time/week* [c]	-	-	-	-	4.67	0.97	22.50	0.055	-	-	-	-
Frequency of eyeshadow use = *≥1 time/day* [d]	-	-	-	-	6.73	0.88	51.43	0.066	-	-	-	-
Frequency of deodorant consumption= *>1 time/day* [b]	-	-	-	-	-	-	-	-	1.61	1.01	2.58	0.045
Frequency of mouthwash use = *≥1 time/day* [d]	10.50	2.46	44.79	0.001	-	-	-	-	2.18	1.38	3.44	0.001
Frequency of hair dye use = *Monthly* [f]	9.48	2.32	38.67	0.002	-	-	-	-	-	-	-	-

	MeP (R^2 = 0.376)**				EtP (R^2 = 0.011)*				PrP (R^2 = 0.014)*			
	exp(β)	95% CI		P-value	OR	95% CI		P-value	OR	95% CI		P-value
Schooling level= *University* [g]	-	-	-	-	-	-	-	-	3.11	1.03	9.37	0.044
Residence = *Sub-Urban* [h]	-	-	-	-	-	-	-	-	0.26	0.08	0.81	0.021
Use of regular medication= *Yes* [e]	1.50	0.94	2.39	0.092	-	-	-	-	-	-	-	-
Parity= *Multipara* [i]	0.53	0.33	0.85	0.009	-	-	-	-	-	-	-	-
Frequency of multivitamin consumption =*Daily* [j]	-	-	-	-	7.93	1.64	38.32	0.010	-	-	-	-
Frequency of handcream use = *≥1 time/day* [d]	1.56	0.97	2.51	0.069	-	-	-	-	-	-	-	-
Frequency of hair dye use = *Monthly* [f]	-	-	-	-	-	-	-	-	2.89	0.89	9.38	0.078
Frequency of cologne and/or perfume use = *≥1 time/day* [d]	2.00	1.25	3.20	0.005	-	-	-	-	-	-	-	-
Frequency of mouthwash use = *≥1 time/day* [d]	2.35	1.46	3.79	0.001	-	-	-	-	-	-	-	-

Table 3. Factors associated with human milk levels of BPA, PBs, and BPs (N=83). Multivariate regression analyses (continuación)

	ΣPBs (R^2 = 0.493)**				ΣBPs (R^2 = 0.393)**				ΣEDCs (R^2 = 0.645)**			
	exp(β)	95% CI		P-value	exp(β)	95% CI		P-value	exp(β)	95% CI		P-value
Frequency of cold meat consumption= *>2 times/week* [k]	2.08	0.13	1.33	0.019	-	-	-	-	1.45	-0.02	0.77	0.063
Frequency of chocolate consumption= *≥1 time/day* [d]	-	-	-	-	-	-	-	-	1.84	0.20	1.02	0.005
Frequency of other nutritional supplement consumption= *Yes* [e]	-	-	-	-	2.12	0.27	1.23	0.003	1.72	0.09	1.00	0.021
Frequency of facial cream use= *≥1 time/day* [d]	-	-	-	-	1.81	0.19	1.00	0.005	-	-	-	-
Frequency of artificial nail use= *Monthly* [f]	-	-	-	-	4.07	0.46	2.35	0.005	-	-	-	-
Frequency of hair spray, hair wax, and/or hair mousse use= *Daily* [j]	-	-	-	-	-	-	-	-	1.78	0.17	0.98	0.006
Frequency of deodorant use= *>1 time/day* [b]	2.70	0.13	1.86	0.025	-	-	-	-	1.86	0.05	1.19	0.034
Frequency of mouthwash use= *≥1 time/day* [d]	5.13	0.98	2.29	<0.001	-	-	-	-	3.85	0.91	1.79	<0.001

CI: confidence intervals; BPA: bisphenol A; BP-1: benzophenone 1; BP-3: benzophenone 3; MeP: methylparaben; EtP: ethylparaben; n-PrP: n-propylparaben; ΣPBs: sum of parabens; ΣBPs: sum of benzophenones;

[a] *reference category=≤ 2 times/week*

[b] *reference category=≤1 times/day*

[c] *reference category=<1 times/week*

[d] *reference category=<1 times/day*

[e] *reference category=No*

[f] *reference category=<1 time/month*

[g] *reference category=Non-university*

[h] *reference category=Rural*

[i] *reference category= Uniparas*

[j] *reference category=<1 time/day*

4.2. Parabens

Globally, MeP and EtP are the most frequently detected PBs in breast milk. MeP was detected in 90.5% of the present samples, a higher detection frequency than the mean (58%) in previous studies with similar LODs (Iribarne-Durán et al., 2021). However, the mean MeP concentration (1.48 ng/mL) was 10-fold lower than the mean (14.2 ng/mL) calculated in the meta-analysis of previous studies (Iribarne-Durán et al., 2021). EtP, n-PrP, and n-BuP were less frequently detected in the present study (63.4 vs. 51.2%, 53.9 vs. 46.4%, and 27.9 vs. 8.3%, respectively) than in the previous studies and their concentrations were generally lower (means of 7.0, 4.9, and 0.2 ng/mL, respectively) (Iribarne-Durán et al., 2021). As in the case of BPA, the highest PB concentrations have been observed in milk samples from Asia, where there has been a marked increase in the utilization of PBs and other preservatives in the past decade (Market-Analysis-Report, 2016). In comparison to the Spanish donors, MeP concentrations were higher in milk samples from the USA (Hines et al., 2015) and Switzerland (Schlumpf et al., 2010), similar in samples from Canada (Fisher et al., 2017), and lower in those from Korea (Kim et al., 2020; Park et al., 2019). Higher EtP concentrations were reported in Korean samples (Kim et al., 2020; Park et al., 2019) and lower concentrations in Canadian samples (Fisher et al., 2017). Among Spanish studies, MeP concentrations were higher and EtP concentrations lower in 120 milk samples collected in Valencia in 2015 (Dualde et al., 2020). n-PrP and n-BuP concentrations in the present donors are in the lower range of those reported in other studies (Fisher et al., 2017; Kim et al., 2020; Park et al., 2019; Schlumpf et al., 2010), including the Spanish study by Dualde et al. (2020).

4.3. Benzophenones

Very few studies have analyzed BP concentrations in human breast milk (Fisher et al., 2017; Hines et al., 2015; Molins-Delgado et al., 2018; Schlumpf et al., 2010), reporting much higher BP-3 concentrations (mean of 24.4 ng/mL) (Iribarne-Durán et al., 2021) than the mean concentration in the present donor samples (0.82 ng/mL) which was also several times lower than the mean observed in 79 milk samples collected in Catalonia (Spain) in 2014 (Molins-Delgado et al., 2018) and in 54 samples collected in Switzerland in 2004-2006 (Schlumpf et al., 2010). BP-1 and BP-2 were not detected in any sample in the aforementioned studies, while 4-OH-BP

was only found in a small number of samples from Catalonia (Molins-Delgado et al., 2018). Fisher et al. (2017) did not detect the presence of any BP in 56 breast milk samples from women in Canada, and Hines et al. (2015) found similar concentrations of BP-3 to those in the present samples in 34 milk samples from women in the in the USA. However, the detection frequency of BP-3 was markedly higher in the present milk samples than the mean calculated in our previous meta-analysis (75.0 vs. 39.5%) (Iribarne-Durán et al., 2021), likely attributable due to the high LOD in some studies (Hines et al., 2015; Schlumpf et al., 2010). No other BPs besides BP-1 and BP-3 were detected in the present study, which may reflect their limited industrial use (Mikkelsen et al., 2015).

4.4. Factors associated with breast milk concentrations of phenols

Very limited information has been published on factors associated with bisphenol, PB, or BP concentrations in breast milk (Dualde et al., 2019; Dualde et al., 2020; Fisher et al., 2017; Jin et al., 2020; Kim et al., 2020; Park et al., 2019; Yi et al., 2013). No correlations were found between BPA and PB or BP concentrations in the donor milk samples, as previously observed, indicating that sources of BPA exposure may differ from sources of exposure to PBs and BPs (Asimakopoulos et al., 2016; Frederiksen et al., 2013; Jiménez-Díaz et al., 2016). This study suggests that sociodemographic factors, the intake of certain food items, and the use of some PCPs may contribute to increasing breast milk concentrations of BPA, PBs, and/or BPs.

Epidemiological studies on BPA exposure in pregnant women and children have described the consumption of canned food/beverages and packaged/processed food as major predictors of urinary BPA levels (Casas et al., 2013; Covaci et al., 2015). The intake of canned food was not associated with BPA in the present study, although BPA concentrations were higher in donors with a more frequent intake of bread and pasta. This association may possibly be explained by the migration of BPA from plastic containers used when bread and pasta are still at high temperatures or by the presence of BPA in the yeast used, as previously suggested (Beltifa et al., 2017; Cao et al., 2011; Mervish et al., 2014). Regarding non-dietary sources, BPA in donor milk was associated with use of mouthwash and hair dye, in line with the Spanish study by Dualde et al. (2019) that found

higher BPA concentrations in breast milk from women who used skin care and makeup products every day. Some studies of urinary and serum BPA concentrations also found associations with the utilization of PCPs (Peinado et al., 2020; Wiraagni et al., 2020). There is increasing evidence of the presence of BPA in the formula of certain cosmetics and PCPs, including skin lotions (Liao and Kannan, 2014).

PBs are considered non-persistent chemicals but have been found in human adipose tissue (Artacho-Cordón et al., 2018). In this regard, MeP and total PB concentrations were lower in multiparous donors, suggesting a firstborn depuration effect, which is also consistent with the finding by Park et al. (2019) of higher EtP concentrations in breast milk from primiparas. The reason for the higher PB levels observed in donors who had gained weight from before pregnancy remains unclear but deserves further attention, given that the BMI has been associated with both higher and lower PB concentrations in breast milk (Dualde et al., 2020; Kim et al., 2020; Park et al., 2019). The use of hand cream, perfume, deodorant, mouthwash, and hair dye was also associated with higher PB concentrations in the donor breast milk samples. In the same line, previous studies have found the application of cosmetics and skin care products to be associated with higher concentrations of PBs in breast milk (Dualde et al., 2020; Fisher et al., 2017; Kim et al., 2020; Park et al., 2019) and in urine from young females (Berger et al., 2018). A recent study found at least one PB in all feminine hygiene products analyzed (wipes, creams, bactericide solutions, deodorant sprays, and powders) and detected MeP and EtP in >80% of them, (Gao and Kannan (2020). PBs were also detected in several dental hygiene products (Guo and Kannan, 2013), exemplifying the generalized presence of PBs in different types of PCP. The regular intake of medication and vitamins may plausibly be associated with EtP and MeP concentrations, given the widespread use of PBs as excipients in pharmaceuticals (Dodge et al., 2015; Lu et al., 2014; Nellis et al., 2016), and the reported endocrine disrupting effects of some medicines, including painkillers (Addo et al., 2021). Consequently, breast milk bank recommendations should highlight the need to minimize the consumption of medication during the donation period.

As observed for PBs, BP concentrations were higher in milk from donors with a

more frequent intake of nutritional supplements and a more frequent use of various PCPs, including cosmetics, body lotion, hair mask (BP-1), sunscreen, mouthwash, and deodorant (BP-3). This is explained by the incorporation of BPs, especially BP-3, as sunscreen agents in numerous PCPs as well as pharmaceuticals (Liao and Kannan, 2014). In this way, the use of various PCPs was associated with urinary BP concentrations in adults (Ko et al., 2016) and young females (Berger et al., 2018). It should be noted that the European Commission reduced the maximum authorized concentration of BP-3 as UV filter in all PCPs in 2017 (Commission-Regulation-EU, 2017). Interestingly, age was positively associated with both BP-1 and BP-3, which might be partially explained by a certain degree of retention in body compartments and/or a lower metabolic activity in older individuals that may delay the metabolism and clearance of these chemicals, as previously suggested for other environmental phenols (Artacho-Cordón et al., 2018; Aubert et al., 2012; Doerge et al., 2012; Stahlhut et al., 2009). However, these results should be interpreted with caution, because BP-1 was detected in only 20.2% of samples, and no previous study has associated BP exposure with age.

In general, it was not possible to identify a consistent pattern for the contribution of food intake to PB or BP concentrations. Food additives and packaging may be implicated in the exposure of humans to these compounds, but contradictory findings have been published on the relationship between the diet and the presence of PBs and BPs in different matrices (Artacho-Cordón et al., 2018; Husøy et al., 2019; Iribarne-Durán et al., 2020; Larsson et al., 2014; Mervish et al., 2014).

4.5. Limitations and strengths

Our study has several limitations. First, the reduced sample size limited our ability to identify relevant predictors of exposure to phenolic EDCs, although most other studies assessing these compounds in breast milk have also relied on a small number of samples. Second, breast milk donors may not be representative of lactating women in general, because donors tend to have higher socioeconomic status (e.g., two-thirds of our donors had university education). It should also be taken into account that geographical and temporal trends in BPA, PB and BP exposure may lead to differences among concentrations in samples stored in other breast milk banks. Third, unlike some other studies, we did not distinguish between free

(bioactive) and total BPA and PBs, which have been detected in human milk in both unconjugated and conjugated forms (Iribarne-Durán et al. (2021). Data on free and conjugated compounds would contribute to understanding the toxicokinetics of the biologically active compounds and the potential health risks for the infant. Fourth, dietary and PCP habits were gathered from self-reported information, which may imply a potential bias, although it appears highly unlikely that misclassification would be driven by exposure. In addition, the assessment of multiple explanatory factors may have led to some spurious statistically significant associations. Finally, the wide time frame for sample collection (ranging from 20 days to 9 months after delivery) hampers the comparison of results with those of other studies. In this regard, our research group is investigating time-dependent variations in the concentrations of bisphenols, PBs, and BPs in breast milk over the lactation period.

Despite these limitations, this is the first study to investigate the concentrations of environmental phenols in breast milk samples from a human milk bank. Assessment of pooled rather than spot samples may be considered a study strength, given that the latter may be more influenced by recent exposure, whereas pooled samples better reflect the average magnitude of exposure (Shin et al., 2019). In this regard, it has been reported that the absorbed dose of BPA is rapidly transferred to the breast of lactating women, resulting in its excretion via breast milk within a few hours after exposure (Tateoka, 2015).

Given that breast milk from milk bank is administered to highly vulnerable hospitalized infants, it is vital to collect breast milk with the lowest burden of EDCs and other potentially hazardous environmental chemicals. Identification of the main lifestyle contributors to the burden of EDCs in breast milk is useful to design preventive measures, including restrictions on the use of cosmetics and other PCPs during pregnancy and breastfeeding, among other changes.

5. Conclusions

This study contributes the first published evidence on the presence of phenolic EDCs in breast milk from a human milk bank. BPA, PBs, and BP-3 were all frequently detected in donor milk samples, and exploratory analysis revealed associations between their concentrations and some lifestyle factors, notably the regular utilization of certain PCPs. These preliminary findings, which need to be verified in

wider studies with larger sample sizes, point to an urgent need to monitor the concentrations of environmental chemicals in milk banks and to implement preventive measures to reduce the exposure of all breastfeeding mothers, especially breast milk donors. In addition to the unquestionable benefits of breast milk, these preventive measures would make donors aware of the extra benefit of reducing the use of certain PCPs for the quality of their breast milk.

6. Declaration of competing interest

The authors declare no conflicts of interest to disclose.

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7.3. Objetivo 3. Caracterizar la exposición a DEs no persistentes, bisfenoles y parabenos, a través de los textiles.

CONCENTRATIONS OF BISPHENOL A AND PARABENS IN SOCKS FOR INFANTS AND YOUNG CHILDREN IN SPAIN AND THEIR HORMONE-LIKE ACTIVITIES.

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ABSTRACT GRÁFICO

ABSTRACT

Antecedentes: Se dispone de poca información sobre el contenido de bisfenol A (BPA) y otros disruptores endocrinos (DEs) como es el caso de los parabenos (PBs) en los textiles y la ropa infantil.

Objetivos: 1) Determinar las concentraciones de BPA y parabenos en calcetines para bebés y niños pequeños, comprados en España, 2) evaluar la (anti-) estrogenicidad y (anti-) androgenicidad de los extractos de los calcetines, y 3) estimar las dosis de exposición cutánea a estos productos químicos.

Métodos: Treinta y dos pares de calcetines para bebés y niños pequeños (1-48 meses) se compraron en 3 tiendas en Granada (España). Se cortó el material textil del pie, la punta y la pierna de cada calcetín (n = 96 muestras) para el análisis químico. Las actividades hormonales se determinaron en secciones de pie (n = 32 muestras) utilizando el ensayo E-Screen para (anti-)estrogenicidad y el ensayo de luciferasa PALM para (anti-)androgenicidad.

Resultados: El BPA estuvo presente en el 90.6% de las muestras en concentraciones que van desde <0.70 a 3736 ng/g. Los niveles de BPA fueron unas 25 veces más altos en los calcetines de la tienda 1, que tenían un contenido de algodón más alto en comparación con las tiendas 2 y 3. Se encontró etil-paraben en el 100% de las muestras, seguido del metil-paraben (81.0%) y propil-paraben (43.7%). No se detectó butil-paraben en ninguna muestra. Se detectó actividad estrogénica en el 83.3% de los calcetines de la tienda 1 (rango = 48.2 a 6,051 pM E_2eq/g), pero solo en tres calcetines de las tiendas 2 y 3. Se detectó actividad antiandrogénica en seis de los 32 calcetines estudiados (rango = 94.4–2,989 μM Proceq/g), todos de la tienda 1. La exposición dérmica estimada al BPA fue mayor en los calcetines para niños de 36 a 48 meses (mediana = 17.6 pg/kg/día), y la exposición a parabenos fue mayor en los calcetines para niños de 24 a 36 meses (mediana = 0.60 pg/kg/día).

Discusión: Este es el primer trabajo en Europa sobre la amplia presencia de BPA y parabenos en calcetines comercializados para bebés y niños. El BPA parece contribuir a la actividad hormonal que se observa en los extractos de calcetines.

1. Introduction

Bisphenol A (BPA, 4,4'-isopropylidenediphenol) is a high-production-volume chemical mostly employed in the manufacture of polycarbonate plastics and in epoxy resins used for the inner coatings of food and beverage cans. BPA is a well-known endocrine-disrupting chemical (EDC) that has the ability to bind to the nuclear estrogen receptor (ER) (ANSES 2018; Wetherill et al. 2007), to modify gene expression under the control of estrogens (Chianese et al. 2018), to bind to other membrane ER families (Alonso-Magdalena et al. 2012), and to induce anti-androgenic activity in vitro (Molina-Molina et al. 2013). Various human studies have reported that prenatal and postnatal/childhood urinary levels of BPA are associated with adverse health outcomes in children, including obesity (Valvi et al. 2013), asthma (Gascón et al. 2015), behavioral problems (Braun 2017; Mustieles et al. 2015), and alterations in puberty timing (Berger et al. 2018a; Leonardi et al. 2017), blood pressure (Sanders et al. 2018), and serum hormones (Aker et al. 2016; Scinicariello and Buser 2016).

Parabens are alkyl esters of p-hydroxybenzoic acid and mainly used as preservatives in personal care products, cosmetics, and pharmaceuticals (Ashrap et al. 2018; Haman et al. 2015). Parabens are also classified as EDCs and have been found to exert weak estrogenic activity (Karpuzoglu et al. 2013; Lange et al. 2014) and to stimulate the proliferation of breast cancer cells in vitro (Pan et al. 2016). Although the number of human studies has been limited, there is an increasing body of epidemiological evidence associating early-life exposure to parabens with adverse health outcomes. Thus, maternal urinary levels of parabens have been linked to adverse pregnancy outcomes (Aker et al. 2018), reduced neonatal thyroid hormones (Berger et al. 2018b), altered puberty timing (Harley et al. 2019), behavioral problems (Philippat et al. 2017), and respiratory and allergic disorders (Berger et al. 2018c). Childhood exposure to methyl-paraben (MPB) and propyl-paraben (PPB) has also been associated with puberty timing (Harley et al. 2019).

Diet is considered the main pathway for BPA exposure in humans, but other

sources are now known to make a substantial contribution, especially in infants and children (Healy et al. 2015; Xue et al. 2017). For instance, BPA is commonly present as a plasticizer in food-packaging materials, baby bottles, electronics, and other household plastics (Healy et al. 2015). It can be found in automobiles, sports equipment and bicycle helmets, dental composites and sealants and is also widely used as an additive in thermal paper products, including receipts and magazines (Healy et al. 2015; Geens et al. 2012b; Molina-Molina et al. 2019; Pulgar et al. 2000; Vandenberg et al. 2010). For their part, parabens are used as food additives to inhibit microbial growth and have also been detected in paper products, baby teethers, and other consumer products (Ashrap et al. 2018; Asimakopoulos et al. 2016; Berger et al. 2015; Liao and Kannan 2014). Since the seminal article by R.H. Barker (Barker 1975), increasing concerns have been raised about EDCs such as BPA and parabens in textiles and clothing, especially when these contain synthetic fibers (e.g., nylon, polyester, polypropylene, and spandex), and about the potential for human exposure through dermal absorption (Li and Kannan 2018; Liu et al. 2017; Xue et al. 2017).

A large number of chemicals (~1,900) are used in industrial textile production, and many of these (~165) are classified as potentially toxic to humans and/or the environment, including antioxidants, plasticizers, dyes, flame retardants, surfactants, and pesticides (Lacasse and Baumann 2004; Swedish Chemicals Agency 2013). These chemicals are used in several processes (e.g., fiber and tissue preparation, washing, dyeing, and finishing) to achieve a variety of effects, including softening, stiffening, wrinkling, shrinking, UV resistance, antifading, repellence (against water, oil, stains, etc.), non-slip finishing, anti-microbiotic finishing, and antistatic protection (Baker 1975; Papaspyrides et al. 2009; Swedish Chemicals Agency 2013). Some of these chemicals may remain within the final textile product, either intentionally or unintentionally, and wearers of the product, including children, can be directly or indirectly exposed to them. In fact, several recent studies have demonstrated the presence of BPA and other bisphenols, parabens, benzophenones, benzothiazoles, benzotriazoles, antimicrobial compounds (triclocarban), phthalates, and flame retardants in textiles and clothing from various countries (Avagyan et al. 2013, 2015; Li and Kannan 2018; Liu et al. 2017; Negev et

al. 2018; Xue et al. 2017). Among these, Xue et al. (2017) detected the presence of BPA in 82% of infant sock samples at a mean concentration of 366 ng/g, finding the highest BPA concentrations (up to 13,300 ng/g) in socks made of polyester and spandex. Likewise, Liu et al. (2017) studied different items of infant clothing and found that socks were responsible for the highest proportion of dermal exposure to benzotriazoles and benzothiazoles. In another study, BPA and the parabens MPB and PPB were detected in pantyhose samples at concentrations of 100-600 ng/g, finding the greatest concentrations of bisphenols and EPB in the samples with highest spandex content (Li and Kannan 2018).

Epidemiological studies on determinants of BPA exposure in pregnant women and children have reported that the consumption of canned food/beverages and packaged and processed food is associated with urinary BPA levels (Casas et al. 2013; Covaci et al. 2015; Quirós-Alcalá et al. 2013; Snoj Tratnik et al. 2019). For instance, the intake of canned tuna was a major predictor of urinary BPA in 4-year-old children and their mothers from the Spanish Environment and Childhood (INMA) birth cohort (Casas et al. 2013). In general, exposure to BPA from dietary sources is thought to represent >90% of overall exposure, with exposure from non-food sources being considered at least one order of magnitude lower than that from food sources (Geens et al. 2012a). However, no epidemiologic evidence has yet been published on the relationship between non-dietary exposure and urinary BPA levels. With regard to parabens, the utilization of cosmetics and personal care products may be major determinants of urinary biomarkers in children (Larsson et al. 2014; Sakhi et al. 2018).

Early-life exposure to BPA and other EDCs is associated with a number of health risks, and little information is available on potentially toxic chemicals in textiles and clothes, particularly in those intended for infants and children. Therefore, this study was designed to determine concentrations of BPA and four paraben compounds (MPB, EPB, PPB, and butyl-paraben [BPB]) in socks for infants and young children purchased in Spain and to estimate the resulting dermal exposure to these chemicals. Children may be simultaneously exposed to multiple EDCs from textiles and clothing, but most available toxicity data derive from single-exposure studies, and their combined effects are poorly understood. For this reason, a further

study objective was to assess the (anti-)estrogenic and (anti-)androgenic activities of extracts from the socks in order to determine the combined effect of the hormonally active compounds present.

2. Material and methods

2.1. Sample collection

In May 2018, we purchased 32 pairs of socks for infants aged 1-12 months and young children aged 12-48 months from three stores in Granada, Southern Spain: a local low-cost retailer (store 1), a low-cost, fast-fashion clothing international retailer (store 2), and a higher-quality international retailer clothing brand (store 3). We purchased 10 packs containing three pairs of socks each and one pack containing two pairs of socks (four packs in stores 1 and 2, respectively, and three packs in store 3). The price per pack ranged between 1.50€ and 1.80€ for store 1, 3.00€ and 4.50€ for store 2, and 6.95€ and 7.95€ for store 3. The socks varied in composition (% cotton, % polyamide, % polyester, % elastane or spandex), color (black, white, grey, navy blue, dark blue, light blue, red, multi-color, and patterned), and size. All pack labels gave information on the "country of origin" (66% of socks from Spain, 25% from Turkey, and 9% from Italy) but did not specify whether the country of origin of the fiber was the same or different. Socks were stored in sealed polyethylene bags at 4°C in the dark until their analysis. Textile samples were cut from three sections of each sock (foot, toe, and leg), yielding a total of 96 samples for chemical analyses. Hormone-like activities were determined in samples from the foot section of each sock. A detailed description of the sock samples is displayed in Tables S1-S3 (Anexo V, Supplementary material).

2.2. Chemicals and reagents

All reagents were analytical grade unless otherwise specified. BPA, parabens (MPB, EPB, PPB, and BPB), labelled deuterium BPA (BPA-d16), and labelled EPB ring 13C6 (EP-13C6) were purchased from Sigma-Aldrich (Madrid, Spain). High-performance liquid chromatography (HPLC)-grade acetone and dichloromethane used for the extraction step were supplied by Merck (Darmstadt, Germany). Liquid chromatography-mass spectrometry (LC-MS) grade methanol, water, and ammonia (25%) were purchased from Sigma-Aldrich. Water (18.2 MΩ cm) was purified using an in-house Milli-Q system (Millipore, Bedford, MA, USA). For chemical analyses, stock standard solutions (100 mg/L) of each compound were prepared in acetonitrile and stored

at 4 ºC in the dark. The solutions remained stable for at least two months. Working standards were prepared immediately before use by dilution with pure acetonitrile.

For in vitro cell assays, reference standards 17β-estradiol (E2), methyltrienolone (R1881), ICI 182780 (henceforth, ICI), puromycin, geneticin (G418), luciferin (sodium salt), sulforhodamine B (SRB), and trichloroacetic acid (TCA) were obtained from Sigma-Aldrich Inc. (St Louis, MO). Stock solutions (10 mM) of E2, R1881, procymidone, and ICI were prepared in ethanol, and successive dilutions were performed in culture medium. Stock solutions were kept at -20 °C and dilution series were freshly prepared before each experiment. Finally, culture medium and fetal bovine serum (FBS) were supplied by Gibco (Invitrogen, Barcelona, Spain) and all cell culture plastics by Falcon (VWR International Eurolab, Barcelona, Spain).

2.3. Instrumentation

Ultra performance liquid chromatography - tandem mass spectrometry (UPLC-MS/MS) analyses were performed using an ACQUITY UPLC™ H-Class (Waters, Manchester, UK), consisting of ACQUITY UPLC™ binary solvent manager and ACQUITY UPLC™ sample manager. A Xevo TQS tandem quadrupole mass spectrometer (Waters) equipped with an orthogonal Z-spray™ electrospray ionization (ESI) source was used for BPA and parabens detection. Chromatographic separation of compounds was performed using an ACQUITY UPLC BEH™ C18 (50 mm × 2.1 mm I.D., 1.7 μm particle size) from Waters. The gradient mobile phase consisted of 0.025% (v/v) ammonia aqueous solution (solvent A) and 0.025% (v/v) ammonia in methanol (solvent B). Gradient conditions were as follows: 0.0-3.5 min, 60% B; 3.5-4.0 min, 60-100% B; 4.0-6.5 min, 100% B, and back to 60% in 0.1 min. Flow rate was 0.25 mL/min. The injection volume was 5 μL. The column temperature was maintained at 40 °C. The mass spectrometer was operated in both positive and negative ESI mode, using optimized MS/MS parameters as defined in a previous study (Vela-Soria et al. 2014).

For cell proliferation assays, the absorbance was measured in a Titertek Multiscan plate reader (Flow, Irvine, CA, USA) at 492 nm, and an infinite M200 luminometer (Tecan, Barcelona, Spain) was used to detect luciferase activity in intact cells.

2.4. Sample extraction, treatment, and LC-MS conditions

Extraction of BPA and parabens from the textile samples was performed following the methodology used by Xue et al. (2017) with some modifications. Briefly, approximately 0.5 g of each textile sample were accurately weighed, cut, placed in 15 mL glass centrifuge tubes, and spiked with 0.25 mL of the isotope-labelled surrogate mixture solution (250 µg/L of BPA-d16 and 62.5 µg/L of EP-13C6 in acetonitrile). Extraction was done with 7.5 mL of a mixture of acetone and dichloromethane (1:4, v/v). After sonication for 20 min and centrifugation at 5000 g for 5 min, the solvent was collected, filtered through 0.2 µm nylon filter, and transferred to another glass tube. The solvent was evaporated to dryness under a gentle stream of nitrogen and the residue dissolved with 250 µL of acetonitrile, injecting 5 µL into the LC system. For the E-Screen and PALM assays, samples were analyzed in duplicate using the aforementioned extraction procedure but without adding the isotope-labeled surrogate solution.

2.5. Quality assurance and quality control in chemical analyses

Textile samples used as blanks for matrix-matched calibration, method validation, and quality control assessment were previously analyzed to confirm that compounds of interest were not present or were below the limit of detection (LD). Two different textile samples were used, because all of them contained at least one of the studied compounds. LDs and limits of quantification (LQs) were based on the lowest point of the calibration standard with a signal-to-noise (S/N) ratio of >3 and >10, respectively. LDs were 0.7 ng/g for BPA, 0.5 ng/g for MPB and BPB, and 0.4 ng/g for EPB and PPB; LQs were 2.2 ng/g for BPA, 1.8 ng/g for MPB and BPB, and 1.4 ng/g for EPB and PPB.

Textile samples were analyzed in duplicate. Extraction was carried out in batches of 15 (12 samples and 3 quality control samples). Quality-control samples were a procedural blank (no textile sample) to check for interferences or laboratory contamination and two spiked blank samples (5 ng/g of BPA and 2.5 ng/g of parabens). Samples were frozen after extraction and injected into the LC-MS/MS in a single batch in the same order as that of their preparation. No BPA or parabens was detected in any procedural blank. Recoveries for all target compounds in the quality-control spiked samples ranged between 82 and 107%, and the coefficient of variation (CV) was under 20% in all cases.

2.6. E-screen bioassay

The E-Screen bioassay and data analysis were performed as previously described (Molina-Molina et al. 2013, 2014) with some modifications. Briefly, MCF-7 cells were trypsinized and plated in 96-well culture plates at initial concentrations of 4 x 103 cells per well. One day later, the seeding medium was removed and replaced with 150 µL test culture medium. For agonistic assays, dry extracts of the textile samples were resuspended in 1.25 mL of experimental medium, vigorously shaken, left at rest for 30 min, and then filtered through a 0.22 µm filter and tested (50 µL added per well) on MCF-7 cells at 1:1 to 1:10 dilutions. A dose-response curve (0.1-1000 pM) for estradiol (E_2) and a negative control (cell treated only with hormone-free medium) and solvent controls (blank and solvent) was included in each experiment. The bioassay was ended on day 6 (late exponential phase) by removing the media from wells, fixing the cells, and staining them with SRB. Finally, bound dye was solubilized and the absorbance read at 492 nm. Next, the ratio between the cell yield obtained and the proliferation of hormone-free control cells (negative control) was calculated for each concentration. Tests were done in triplicate and results were expressed as proliferative effect (PE) [MCF-7 cell proliferation (-fold over control)]. The antagonistic activities of sample extracts were determined by co-incubation with the agonist E2 at 100 pM. Because the PE only provides information on the effect of the extract in the E-Screen bioassay, this was transformed into E_2 equivalent ($_{E2eq}$) or anti-estrogen (ICI 182780) equivalent (ICIeq) units related to 1 g of textile sample by reading from dose-response curves of E2 or ICI. In this manner, the PE of each extract was referred to the maximal PE obtained with E_2 or ICI and transformed into E_2eq or ICIeq. E_2eq and ICIeq values for each sample extract were calculated by using the concentration that obtained the greatest induction or inhibition of cell proliferation, respectively. E_2eq and ICIeq values were corrected for the dilution factor and reported as E_2eq/g or ICIeq/g of the original textile sample.

2.7. PALM cell luciferase assay

PALM cells were seeded at a density of 5 x 104 cells per well in 96-well white opaque tissue culture plates in 150 µL test culture medium, following a protocol reported elsewhere (Molina-Molina et al. 2013, 2014). Dry extracts of the textile samples were serially diluted (as described above for the E-

Screen bioassay), and 50 µL per well were added at 8 h after seeding. Serial dilutions of the agonist methyltrienolone-R1881 (1-10,000 pM) and the test culture medium alone were included on each plate with the test samples as positive and negative controls, respectively. PALM cells were incubated for 40 h at 37 °C, and the medium was then removed and replaced by test culture medium containing 0.3 mM luciferin. Next, the 96-well plate was introduced into a luminometer for 2 s to measure luminescence from intact living cells.

Human androgen receptor (hAR)-agonistic activities were tested at 1:1 to 1:10 dilutions of the textile samples, performing tests in quadruplicate for each dilution. Maximal luciferase activity (100%) was obtained in the presence of 10 nM R1881. The antagonistic activity of extracts was determined by co-incubation with R1881 agonist (0.3 nM). Results were expressed as percentage of maximal luciferase activity. Finally, the luciferase activity in each sample extract was expressed as percentage of the maximal luciferase activity obtained with R1881 or procymidone (Proc) and transformed into R1881 or procymidone equivalent units (R1881eq or Proceq, respectively) by reading from dose-response curves of R1881 or procymidone (standard serial dilutions) included on each plate. R1881Eq and Proceq were calculated from the concentration that obtained the greatest induction or inhibition of luciferase activity, respectively. R1881eq and Proceq values obtained were corrected for the dilution factor and reported as R1881eq/g and Proceq/g of the original textile sample.

2.8. Estimation of dermal exposure

Dermal exposure of feet to BPA and parabens (individual compounds and sum of parabens) from wearing the socks was estimated for infants aged 1-6, 6-12, 12-24, 24-36, and 36-48 months. Exposure doses were calculated according to exposure assessment guidelines of the USA EPA (US EPA Exposure Factors Handbook 2011) and based on previous studies on hazardous chemicals in clothing (Li and Kannan 2018; Liu et al. 2017; Rovira et al. 2015; Xue et al. 2017), using the following formula:

$$Expderm = C \times D \times SA \times Fmig \times Fcontact \times Fpen \times T \times N / BW$$

where Expderm is the estimated daily dermal exposure dose (pg/kg body weight/day), C is the concentration of chemicals in the sock (ng per g), D is the

density of sock fiber (mg per cm^2), SA is the skin contact surface area, Fmig is the migration rate of chemicals to the skin (recommended default value as worst-case exposure scenario: 0.5% per day; Federal Institute for Risk Assessment 2012), Fcontact is the fraction of the skin contact area (recommended default value as worst-case assumption: 100%; Federal Institute for Risk Assessment 2012), Fpen is the penetration rate of chemicals into the body (recommended default value as worst-case assumption: 1%; Federal Institute for Risk Assessment 2012), T is the contact time between sock and skin (assumed to be 1 day), N is the number of events per day (assumed to be 1), and BW is the average body weight of infants/children by age (US EPA Exposure Factors Handbook 2011). The skin surface covered by socks was assumed to be the total foot surface area of the infants/children for each age group (230, 290, 330, 380, and 490 cm2, respectively; EPA Exposure Factors Handbook 2011). The body weight of the infants/children in the five age groups was considered to be 6.6, 9.2, 11.4, 13.8, and 16.0 kg, respectively (EPA Exposure Factors Handbook 2011).

2.9. Statistical data analysis

The frequency of samples with detected concentrations of BPA and/or parabens was analyzed, calculating the median value and range of their concentrations in the sock samples and the total concentration of paraben compounds (∑PBs). Given the absence of standards for the maximum content of EDCs in clothing, we calculated the frequency of samples with BPA concentrations that exceeded the EU migration standard for toys (0.1 ppm or μg/g) (Commission Directive 2014/81/EU). Estrogenic and anti-androgenic activities were reported as the frequency of positive samples and the range of activity. Spearman correlation analysis was applied to examine the relationship between concentrations of BPA and parabens. We used box plots to display the distribution of chemicals by sock characteristics with log-transformed BPA data and non-transformed parabens levels. Non-parametric Kruskal-Wallis and Mann-Whitney tests were used to analyze differences in chemical concentrations by store, cotton content (grouped as <80%, 85%, or >90%), polyester content (grouped as 0%, 10-20%, or >20%), color, sock section, and country of origin. Finally, mean, median, and 95th percentile values were calculated for estimated dermal absorption doses. Statistical significance was set at $p<0.05$. SPSS v.23.0 (IBM, Chicago, IL, USA) was used for statistical analyses.

3. Results

BPA was detected in 90.6% of samples, of which 35.4% exceeded the EU standard of 0.1 ppm for toys, and was undetectable in only three socks for

Table 1.
BPA and parabens concentrations (ng/g) and estrogenic and anti-androgenic activities in the total sample of socks and by store.

Chemicals and hormone-like activities	Total	Store 1	Store 2	Store 3
n (%)	96 (100)	36 (37.5)	36 (37.5)	24 (25.0)
BPA				
% detection	90.6	100	77.7	100
Median	20.5	255	8.17	7.6
Range	<0.70 - 3,736	75.6 - 3,739	<0.70 - 27.6	4.44 - 49.6
% >EU standard for toys[a]	35.4	94.4	0	0
MPB				
% detection	81.2	75	100	62.5
Median	3.09	1.31	7.12	3.26
Range	<0.50 - 23.8	<0.50 - 3.56	1.94 - 23.8	<0.50 - 16.9
EPB				
% detection	100	100	100	100
Median	2.44	2.4	2.44	2.43
Range	1.01 - 9.21	1.01 - 3.92	1.12 - 4.23	1.13 - 9.21
PPB				
% detection	43.7	50	0	100
Median	<0.40	0.27	<0.40	0.97
Range	<0.40 - 2.45	<0.40 - 2.45	<0.40	0.74 - 1.69
∑PBs				
Median	5.75	4.13	9.53	5.65
Range	2.56 - 27.6	2.56 - 7.48	4.07 - 26.2	3.17 - 27.6
Estrogenic activity				
% positive	40.6	83.3	8.3	25
Range[b] (pM E_2eq/g)[c]	48.2 - 6,051	48.2 - 6,051	62.5	151 - 230
Anti-androgenic activity				
% positive	18.7	50	0	0
Range[b] (µM Proceq/g)[d]	94.4 - 2,989	94.4 - 2,989	–	–

BPA: bisphenol A; MPB: methyl-paraben; EPB: ethyl-paraben; PPB: propyl-paraben; ∑PBs: total concentrations of parabens.

[a]Percent of samples exceeding the EU migration standard of 0.1 ppm for toys

[b]Range of positive values

[c]Concentrations equivalent to E2 per gram

[d]Concentrations equivalent to procymidone per gram.

infants aged 1-6 months purchased from store 2 (Table 1 and Anexo V, Table S2). BPA concentrations ranged between 75.6 and 3,736 ng/g in socks from store 1, including 15 socks with concentration >1,000 ng/g; between undetected and 27.6 ng/g in store 2; and between 4.44 and 49.6 in store 3 (Table 1). The median BPA concentration was around 25-fold higher in socks from store 1 than in those from stores 2 and 3 (p-value<0.001). With regard to parabens, EPB was found in all samples at concentrations ranging between 1.01 and 9.21 ng/g, with no significant differences between the stores (p-value=0.77); MPB was detected in 81% of samples at concentrations ranging between undetected and 23.8 ng/g, observing a higher median concentration in socks from store 2 (p-value<0.001); and PPB was detected in 43.7% of samples at concentrations ranging between undetected and 2.45 ng/g, observing a higher median concentration in socks from store 3 (p<0.001) (Table 1). BPB was not detected in any sample.

The E-screen test demonstrated estrogenic activity in almost all of the socks from store 1 (10 out of 12 samples), with values ranging from 48.2 to 6,051 pM E_2eq/g, but in only one sample from store 2 and two samples from store 3 (Table 1 and Anexo V, Tables S1-S3). The PALM luciferase assay revealed anti-androgenic activity in only 6 out of the 12 samples from store 1, ranging between 94.4 and 2,989 µM Proceq/g (Table 1 and Anexo V, Tables S1-S3). No anti-estrogenic or androgenic activity was observed in any sample.

BPA concentrations were inversely and significantly correlated with MPB concentrations (Spearman rho, r=−0.24, p-value=0.02) and EPB (r=−0.20, p-value=0.05) but not with PPB or ∑PBs concentrations. Higher median concentrations of BPA were observed in socks composed of 85% and >90% cotton (store 1) than in those with lower percentages of cotton (p-value<0.001) (Figure 1), in socks with 0% or 10-20% vs. >20% polyester (p-value<0.001) (Figure 1), in socks without polyamide (p-value<0.001), and in those with 0% or 5% elastane (p-value<0.001). In relation to the fabric color, higher BPA concentrations were found in grey and black, grey and white, and grey and navy-blue socks (p-value<0.001) (Figure 2). With regard to parabens, MPB and EPB concentrations were higher in samples with lower cotton content (p-value<0.001) and 0% polyester (p-value=0.003) (Figure 1), and in those with <20% polyamide (p-value<0.001).

By contrast, PPB and ∑PBs concentrations were higher in socks composed of 85% and >90% cotton than in those with <80% cotton (p-value=0.10 and <0.001, respectively) and were higher in socks with greater polyester content (p-value<0.001) (Figure 1). We observed no significant differences in parabens levels by sock color (data not shown). Among the countries of origin, BPA concentrations were higher in socks made in Spain (p-value<0.001), and parabens concentrations were higher in those from Turkey (p-value<0.001) (Figure 3). No significant differences in BPA or parabens concentrations were found among the three different sock sections studied.

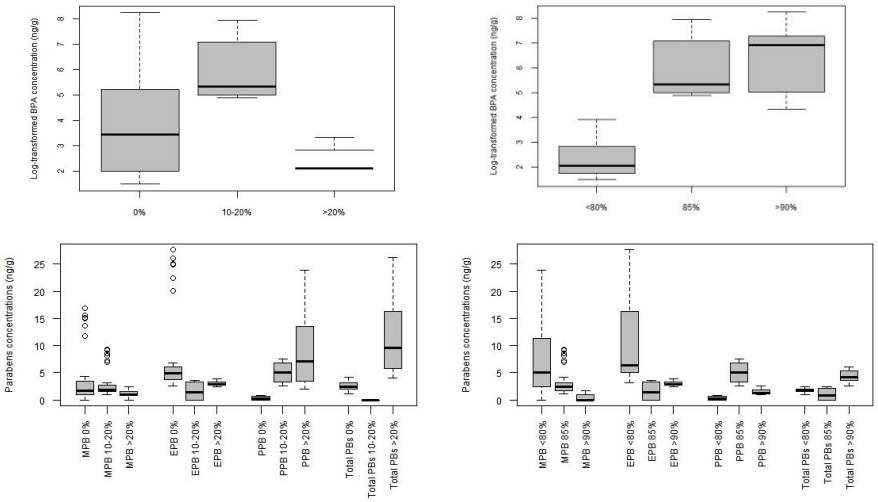

Fig. 1. Concentrations of BPA and parabens according to percent composition of cotton (left) and polyester (right) (BPA: bisphenol A, MPB: methyl-paraben; EPB: ethyl-paraben, PPB: propil-paraben; PBs: parabens).

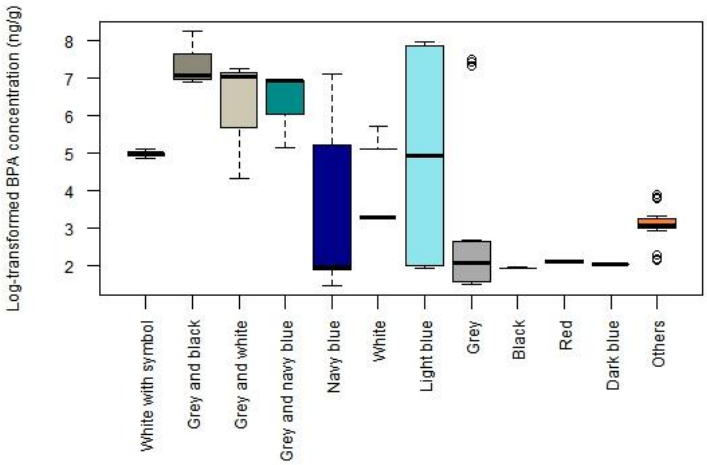

Figure 2. BPA concentrations according to color of sock. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Table 2 exhibits the estimated daily mean dermal exposure doses to BPA and parabens by age. Median and 95th percentile values for dermal exposure doses from socks were, respectively, 0.93 and 129 pg/kg/day for BPA, 0.14 and 0.95 pg/kg/day for MPB, 0.12 and 0.38 pg/kg/day for EPB, 0.00 and 0.10 pg/kg/day for PPB, and 0.27 and 1.19 pg/kg/day for ∑PBs. The median BPA dermal exposure dose was highest for children aged 36-48 months (17.6 pg/kg/day), followed by those aged 24-36 months (0.75 pg/kg/day), 6-12 months (0.46 pg/kg/day), 12-24 months (0.22 pg/kg/day), and 1-6 months (BPA not detected). The median dose of dermal exposure to the sum of parabens was highest for children aged 24-36 months (0.60 pg/kg/day), followed by those aged 6-12 months (0.39 pg/kg/day), 1-6 months (0.33 pg/kg/day), 12-24 months (0.23 pg/kg/day), and 36-48 months (0.22 pg/kg/day).

Table 2. Estimated dermal exposure doses (pg/kg/day) to BPA and parabens.

Chemicals	Mean	Median	95th percentile	Maximum
All samples (N=96)				
BPA	19.6	0.93	129.0	206.0
MPB	0.26	0.14	0.95	1.03
EPB	0.14	0.12	0.38	0.45
PPB	0.03	0.00	0.10	0.16
ΣPBs	0.43	0.27	1.19	1.34
Age 1-6 months (N=9)				
BPA	0.00	0.00	0.00	0.00
MPB	0.27	0.26	–	0.35
EPB	0.08	0.07	–	0.12
PPB	0.00	0.00	–	0.00
ΣPBs	0.35	0.33	–	0.45
Age 6-12 months (N=33)				
BPA	12.1	0.46	118.0	125.0
MPB	0.27	0.08	0.81	0.82
EPB	0.19	0.16	0.44	0.44
PPB	0.06	0.05	0.16	0.16
ΣPBs	0.51	0.39	1.32	1.34
Age 12-24 months (N=9)				
BPA	0.23	0.22	–	0.33
MPB	0.14	0.14	–	0.17
EPB	0.06	0.06	–	0.07
PPB	0.04	0.04	–	0.06
ΣPBs	0.24	0.23	–	0.28
Age 24-36 months (N=18)				
BPA	0.72	0.75	–	1.19
MPB	0.52	0.47	–	1.03
EPB	0.11	0.11	–	0.15
PPB	0.00	0.00	–	0.00
ΣPBs	0.63	0.60	–	1.15
Age 36-48 months (N=27)				
BPA	54.4	17.6	199.0	206.0
MPB	0.11	0.10	0.24	0.24
EPB	0.15	0.16	0.24	0.25
PPB	0.01	0.00	0.05	0.05
ΣPBs	0.27	0.22	0.49	0.50

BPA: bisphenol A; MPB: methyl-paraben; EPB: ethyl-paraben; PPB: propyl-paraben; ∑PBs: total concentrations of parabens.

4. Discussion

The present study evidenced for first time in Europe the wide presence of BPA in textile samples from socks marketed for infants and young children in Spain. It is noteworthy that 15 out of 96 samples (16%) had BPA concentrations above 1,000 ng/g (or 1 µg/g) and more than one-third had a concentration above 0.1 µg/g. Among parabens, EPB was detected in all samples, followed by MPB and PPB, at several-fold lower concentrations in comparison to BPA. The estimated dermal exposure doses to BPA and parabens were relatively low (of the order of pg/kg/day) but may be relevant, as discussed below. This is also the first study to determine the combined hormonal activity of extracts from consumer textile products. Interestingly, estrogenic and anti-androgenic activities were found in the sock samples with highest concentrations of BPA.

4.1. Bisphenol A

In general, our data are in agreement with previous reports on BPA in clothing. Xue et al. (2017) detected BPA in all 14 infant socks from China purchased in Albany, USA, at higher concentrations than observed in our study (range: 186-13,300 vs. <0.70-3,736 ng/g), although the median BPA concentration (396 ng/g) was of the same order as in socks from store 1, the low-cost retailer (255 ng/g). The same authors found BPA in other infant textiles at similar concentrations to the present findings (<2.21-1,830 ng/g), reporting that BPA concentrations were much higher in socks than in other items of clothing or in raw textiles (Xue et al. 2017). In a study of pantyhose, Li and Kannan (2018) detected BPA in 96% of samples from China, Japan, Korea, Portugal, Chile, and the USA at concentrations ranging from <1.3 to 504 ng/g, reporting a similar median BPA concentration (14.3 ng/g) to that in the present socks (20.5 ng/g). Another recent study found that BPA was present, at concentrations below 0.5 ppm (500 ng/g), in two out of seven baby textile items purchased in Israel (Negev et al. 2018).

Unlike in the present study, Xue et al. (2017) found higher mean BPA concentrations in clothing made of 97-98% polyester vs. 100% cotton and in colored vs. white clothing. They also reported that BPA concentrations were up to 72-fold higher in clothing made of synthetic fibers vs. 100% or 60% cotton. In the same line, Li and Kannan (2018) found higher concentrations of several bisphenols in black pantyhose vs. tan or khaki and in those made of 21-50% vs.

0-20% spandex. The authors of both studies proposed that the high concentrations of bisphenols in socks and pantyhose were related to their high content of spandex (or elastane). The elasticity and strength of this synthetic fiber has led to its utilization in socks, active wear, hosiery, elastic waistbands, gloves, underwear, and other skin-tight clothing.

The source of BPA in the present textile samples cannot be readily identified, given that the highest concentrations of BPA were observed in socks with no or scant polyester content and high % cotton. It is possible that BPA was introduced to improve the performance and durability of the socks. BPA ethoxylate diacrylate has been used in the hydrophilization of synthetic polyester fabric (https://www.lookchem.com/cas-644/64401-02-1.html?countryid=0). Moreover, BPA derivatives are employed as an intermediate chemical in the manufacture of antioxidants and dyes (Xue et al. 2017) that are added to fibers to prevent photo-degradation and to color the original raw material, respectively. In this context, Cesen et al. (2018) reported higher concentrations of BPA and other bisphenol residues in wastewater samples from two textile cleaning companies than in those from other industrial activities. They suggested that BPA may originate from textile packaging and that bisphenols are washed during textile cleaning into the sewerage system. Furthermore, recycled plastic bottles made of polyethylene and polycarbonate are increasingly used by the textile industry to produce polyester fibers (Al-Salem et al. 2009; Rochman et al. 2013). Hence, the source of BPA in socks containing polyester may be recycled plastic bottles used as raw materials in its production.

BPA and bisphenol B are known to be employed as "proton donors" in color developers. However, there was no clear association of BPA concentrations with the color of the socks, suggesting that the presence of BPA in these items was not related to fiber dyeing. Regarding the country of origin, it is very likely that the fiber in the socks from store 1 derived from an Asian country (e.g., China), which may have contributed to the high BPA concentrations observed. In this regard, Li and Kannan (2018) reported that the highest concentrations of all studied EDCs were in pantyhose samples from Asian countries. In addition, our findings indicate that BPA is not yet being replaced by BPA analogues (e.g., bisphenol S) in socks made in Spain,

Italy, or Turkey. In Japan, where the use of BPA has been restricted in many consumer products since 2001, elevated concentrations of BPS and other BPA analogues have been found in pantyhose (Li and Kannan 2018), as well as in thermal paper receipts (Li and Kannan 2013).

4.2. Parabens

To our knowledge, the presence of parabens in clothing has only been examined in one study (Li and Kannan 2018), which found higher concentrations of MPB, EPB, PPB, and BPB in pantyhose than those observed in the present socks. In comparison to findings in the socks, the frequency of their detection in pantyhose samples was higher for EPB (100 vs 63%) but lower for MPB (81 vs 94%), PPB (44 vs 85%), and BPB (0 vs 32%). As in our study, parabens concentrations in the pantyhose were several-fold lower than BPA concentrations. The same authors found that parabens concentrations were 100-fold higher in pantyhose samples purchased from China than in those from other countries (Li and Kannan 2018). Because the country in which the fiber was produced was not specified on the labels of the socks, we were unable to determine the influence of this variable. We found no clear pattern in parabens concentrations according to the composition and color of the socks. The presence of parabens in socks is most likely attributable to their utilization as antimicrobials in textile production (Goldade and Vinidiktova 2017).

4.3. Hormone-like activity

BPA is known to interfere with steroid signaling via human estrogen (hER) and human androgen (hAR) receptors (Molina-Molina et al. 2013; Wetherill et al. 2007), whereas parabens have been shown to exert weak estrogenic activity (Karpuzoglu et al. 2013). We previously demonstrated that BPA is a potent hER agonist and hAR antagonist using E-screen and PALM cell assays (Molina-Molina et al. 2013), supporting the present finding of a relationship between hormone-like activity and high BPA concentrations in the sock extracts. This observation is also consistent with the recent report by our group of significant positive correlations between BPA concentrations in thermal paper receipts and their estrogenic and anti-androgenic activity (Molina-Molina et al. 2019). In the same line, our group previously reported that the estrogenic activity of vegetables packed in lacquer-coated cans (ranging from 5.44 to 720 nM E2eq/L) was related to the amount of BPA in the liquid these contained (Brotons et al.

1995). Hence, BPA appears to have made a major contribution to the estrogenic and anti-androgenic activity of the socks. However, we cannot rule out the presence of other EDCs in the socks, which may also have played a role in their hormone-like effects. In comparison to findings previously published by our group, the estrogenic activity measured in the socks is comparable to the activity observed in paper and cardboard used as food containers (geometric mean [GM]=11.9 pM E2eq/g) (López-Espinosa et al. 2007) but much lower than recently detected in thermal paper receipts (GM=0.12 µM E2eq/g) (Molina-Molina et al. 2019). Likewise, anti-androgenic activity of the sock extracts was much lower than found in thermal paper receipts (GM=213 mM Proceq/g) (Molina-Molina et al. 2019) but markedly higher than recorded in commercial bottled waters (GM=1.61 nM Proceq/L) (Real et al. 2015).

4.4. Dermal exposure doses and risk assessment

The mean dermal exposure dose to BPA from the socks in our study (19.6 pg/kg/day) was higher in those marketed for older children. The mean dose estimated by Xue et al. (2017) from textile products and clothing for infants was several-fold higher (222 pg/kg/day), being highest in products for newborns aged <1 month (248 pg/kg/day). The dermal exposure dose from socks was several-fold lower for parabens than for BPA. Although the estimated dermal exposure doses were relatively low in our study, various factors should be taken into account. For instance, socks produce direct dermal exposure because they are worn in contact with the skin, and absorption may be increased by high temperatures and body moisture from the feet. In addition, infants and small children may be directly exposed to the chemicals in socks by putting them (or their feet) in their mouth. In addition, toxic chemicals can transfer from contaminated to uncontaminated clothes during washing and can disperse from textile fibers into indoor air, where they can bind to dust particles and cause indirect exposure though the inhalation and ingestion of dust. In this respect, indoor dust is believed to be the main route for the exposure of young children to brominated flame retardants and perfluorinated compounds (Björklund 2011), EDCs commonly present in household textiles.

In 2015, the European Food Safety Authority (EFSA) reduced the tolerable daily intake (TDI) of BPA from 50 to 4

μg/kg/day and concluded that BPA poses no health risk to consumers of any age group (including fetuses, infants, and children) at current exposure concentrations (EFSA 2015). Subsequently, in 2018, the EU Commission Regulation 2018/213 established that "no migration of BPA shall be permitted from varnishes or coatings applied to food contact materials and articles and similar products specifically intended for young children". Moreover, BPA may not be used in toys or in components of toys, except if inaccessible to children (Commission Directive 2014/81/EU). It remains unclear whether there is a no-effect concentration of BPA for its most sensitive endpoints (Vandenberg et al. 2012). There is consistent evidence that BPA affects the reproductive function, neurodevelopment, and metabolism (ANSES 2018). Hence, given that young children often suck their feet and put parts of their clothing in their mouth, it appears appropriate to propose a complete ban on BPA in clothing and textile products sold for children on public health grounds. In fact, the endocrine-disrupting properties of BPA led to its inclusion by the EU in the REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) list as a Substance of Very High Concern (SVHC) in January 2017. Moreover, the ecological criteria for awarding the EU Ecolabel to textile products include the absence from the manufacturing process of any substance on the REACH SVHC list (Regulation 2014/350/EU).

Overall, the estimated dermal exposure doses to BPA and parabens from these socks were not high. However, infants and young children are particularly susceptible to EDCs, and they are usually exposed to multiple chemicals; therefore, the potential cumulative health risk from their daily use of socks and other clothing items might not be negligible. There is need for epidemiological and risk assessment studies to quantify early-life exposure to chemicals from clothing. In the meantime, the precautionary principle should prevail in order to protect this vulnerable population.

4.5. Strengths and limitations

One study limitation was that the socks were purchased in one country and from a small number of stores, although different fabric qualities, colors, and compositions were represented, and samples were taken from three different sections of each sock. In addition, all socks were new and had not been washed, a process that is likely to remove

some of the chemical residues. Furthermore, the measurement of other hormonally active bisphenols (e.g., bisphenol S and bisphenol F) would have provided additional relevant information. However, there has been scant research on the potential for BPA exposure from non-dietary exposure sources in early childhood, and only toys, baby bottles, teethers, and teats have been analyzed to date (Healy et al. 2015). Besides being one of the few investigations into the presence of EDCs in consumer textile products, this is only the second study on the concentrations of BPA and parabens in clothing for infants and young children. Furthermore, the hormone-like activity of extracts from textile products has not been assessed in previous studies.

4.6. Conclusions

This study provides the first evidence of the utilization of BPA and parabens in European textile manufacturing and suggests that socks may be a relevant source of exposure to these EDCs for infants and young children. The findings also indicate that BPA contributes to the estrogenic and anti-androgenic activity of these socks. There is an urgent need for epidemiological research into the potential routes of exposure to chemicals used in textile products and clothing for newborns, infants, and children. Importantly, there are currently no environmental and health requirements for textiles in European regulations with the exception of Regulation 2014/350/EU on the EU Ecolabel for textile products. The present findings and previous data on the presence of hazardous chemicals in clothing and textiles, especially in those made of synthetic fibers, underscore the need for legal regulations that include the mandatory labeling of consumer textile products with their chemical content.

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Competing Financial Interests Declaration

The authors declare they have no actual or potential competing financial interests.

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8. DISCUSIÓN GENERAL

Esta tesis doctoral supone el estudio más amplio realizado hasta la fecha en relación a la caracterización de la exposición a DEs en niños prematuros de muy bajo peso al nacer ingresados en una Unidad Cuidados Intensivos Neonatales. Los resultados de esta tesis doctoral y su relación dentro del contexto del conocimiento científico más actual, muestran que (i) los dispositivos y productos médicos que están en contacto íntimo y prolongado con el neonato ingresado en la Unidad de Cuidados Intensivos Neonatales, contienen BPA y PBs; (ii) existe evidencia científica de la presencia generalizada de tres familias de compuestos analizadas, Bisfenoles, PBs y BPs, en leche materna humana, con concentraciones medias que dependen del lugar de residencia de las madres y de el período de colección de las muestras; (iii) la presencia de BPA, PBs y BP-3 demostrada en la mayor parte de las muestras de leche materna procedentes del banco de leche materna humana, se asocian positivamente, entre otros factores, con la utilización regular de determinados PCPs; (iv) la presencia de BPA y PBs en textiles, los convierten en una posible fuente de exposición relevante a DEs no persistentes en edad infantil; (v) y por último, la carga xenoestrogénica en ciertos dispositivos médicos y textiles infantiles puede contribuir a la exposición hormonal de los recién nacidos prematuros ingresados en la UCIN y a contaminantes que interaccionan con el equilibrio hormonal.

La caracterización de la exposición a DEs, particularmente a los contaminantes no persistentes, es un requisito indispensable para estudiar los posibles efectos en la salud de esa exposición, especialmente en los períodos de mayor vulnerabilidad, como es la infancia y la lactancia. De forma particular, es importante señalar que los datos humanos disponibles sobre el metabolismo de este tipo de químicos se basan en gran medida en estudios sobre individuos adultos sanos, que no reflejan la situación en poblaciones especiales y/o vulnerables, como la infancia (Søeborg et al., 2014), por lo que existe el riesgo de extrapolar datos de forma equivocada.

A pesar de la carencia de estudios, los resultados de esta tesis doctoral están en la línea de la escasez de trabajos previos que han demostrado la presencia de DEs en niños ingresados en UCIN (Calafat et al., 2004; Calafat et al., 2009; Duty et al., 2013). Sin embargo, en los trabajos publicados no se ha cuantificado el contenido de DEs en los distintos dispositivos y productos médicos, ni se ha medido la actividad biológica de dichas fuentes. Es cierto que existen diferentes estudios que han puesto de manifiesto la presencia de DEs, tales como BPA y ftalatos, a través de la orina de los recién nacidos en la UCIN (Calafat et al., 2009; Duty et al., 2013), incluyendo un estudio más reciente (Duty

et al., 2013), que observó de 16 a 32 veces mayores concentraciones de BPA en los bebés que habían estado ingresado en la UCIN que en los bebés de la población general. Calafat et al. (2009), determinó que las concentraciones urinarias de BPA eran entre 3.42 y 8.75 veces mayores cuando la frecuencia de la utilización de dispositivos médicos fue media o alta, frente a baja en niños que habían estado ingresados en la UCIN. En cuanto a los PBs, Calafat et al. (2009) también encontró niveles más altos de MeP en orina entre los recién nacidos con un mayor uso de dispositivos médicos. Además, otros estudios previos han observado la exposición a ftalatos en las orinas de recién nacidos prematuros de bajo y muy bajo peso al nacer en las UCIN (Calafat et al., 2004; Green et al., 2005; Stroustrup et al., 2020; Weuve et al., 2006). Entre los objetivos a más largo plazo del estudio epidemiológico en que se encuadra esta tesis doctoral, se encuentra la medida de estos compuestos químicos en la orina de los niños reclutados.

Nuestros resultados dan un paso más adelante, aportando información relevante sobre las fuentes y rutas de exposición, digestiva, dérmica, respiratoria e intravenosa, de los recién nacidos prematuros ingresados en la UCIN a los DEs de interés. Los principales hallazgos al respecto, han revelado la presencia generalizada de DEs en múltiples elementos de la maternidad empleados en la UCIN. Dada la extrema vulnerabilidad de los neonatos, estos hallazgos suponen una preocupación importante. Tras el análisis realizado en 52 artículos y dispositivos médicos, las concentraciones de BPA representan aproximadamente el 30% de la concentración total de los DEs estudiados a través de las diferentes vías de exposición, mientras que el MeP representó 40-60% del total. La toxicocinética de BPA y PBs en neonatos varía según la vía de exposición. En cuanto a la exposición sabemos que, el metabolismo de fase II está madurando lentamente en recién nacidos y lactantes, lo que supone un aumento de la biodisponibilidad de BPA libre y PBs (Mulla et al., 2015; Nachman et al., 2014), que podrían ser aún más pronunciada en los recién nacidos prematuros. Por el contrario, la exposición dérmica o intravenosa posibilitaria la biodisponibilidad sistémica de BPA y PBs, tanto en neonatos como en adultos, aunque la mayor tasa de permeabilidad de la piel de los prematuros y la mayor proporciónes de superficie cutánea en relación a su peso corporal implicaría que el grado de exposición sea mayor en éstos (Guzeilan PS, 1992.; SCENIHR, 2015; Søeborg et al., 2014). En este sentido, se ha estimado que la exposición dérmica en neonatos entre 10-30% mayor que en adultos.

En conjunto, los resultados de esta tesis doctoral unidos a las evidencias científicas encontradas en estudios previos, demuestran que los recién nacidos ingresados en la UCIN pueden estar potencial e inadvertidamente expuestos a DEs a través de materiales y dispositivos médicos. Creemos que un medio hospitalario tiene un gran control de la exposición ambiental neonatal de los protocolos biológicos, pero creemos que son insuficientes para monitorizar el estrés químico al que se someten los pacientes (Seltenrich, 2020). SCENIHR (2015) recomendó la selección de dispositivos médicos que no contengan y/o liberen DEs siempre que sea posible. Sin embargo, la normativa actual de la UE sobre la prohibición de BPA y los PBs no incluyen materiales hospitalarios en contacto íntimo (orales y no orales) con neonatos ingresados en UCIN. Es complicado eliminar el uso de materiales plásticos médicos, así como evitar la exposición de los niños ingresados en la UCIN, pero si que es recomendable identificar materiales más seguros y desarrollar nuevos protocolos de tratamiento, que puedan evitar la exposición (Seltenrich, 2020).

Esta tesis doctoral ha demostrado que, la información disponible hasta el momento sobre la exposición a bisfenoles, PBs y BPs a través de la leche materna, es bastante escasa, a pesar de que esta forma de alimentación es considerada como funtamental para los recién nacidos (DiMaggio et al., 2022; Fonseca et al., 2021; OMS., 2003.). Este estudio, coincidiendo con estudios previos (Pollack et al., 2018), ha confirmado la presencia generalizada de estos compuestos químicos en la leche materna. Este hallazgo es de extrema importancia ya que creemos que cualquier concentración de algún DEs puede tener repercusiones nocivas en los lactantes y más concretamente en aquellos recién nacidos prematuros con sistemas de detoxificación inmaduros (Darbre, 2017; Ghassabian et al., 2022; Koutaki et al., 2021; Liew and Guo, 2022; Lizunkova et al., 2022; Sun et al., 2022; Sunderland et al., 2019).

La revisión exhaustiva de la literatura científica ha puesto de manifiesto la dificultad para comparar los resultados presentados por los diferentes grupos que han abordado la exposición infantil a través de la lactancia materna.

De una parte hemos observado diferencias notables en los procedimientos de reclutamiento de las madres donantes en los diferentes estudios, en las distintas regulaciones de los bancos de leche materna y en las legislaciones sobre el uso e ingestas diarias recomendadas de estos compuestos entre los diferentes continentes, incluso entre

los países situados los mismos continentes (Klotz et al., 2021). Todas ellas podrían estar influyendo en los resultados obtenidos y dificultando la comparación entre trabajos encontrados. En este sentido, es pertinente aclarar que los datos generados en esta tesis doctoral, no permiten extrapolar resultados a la población general, pero sí que proporcionan información sobre el panorama actual del uso de estos compuestos químicos, así como de la eficacia de las regulaciones aplicadas, al menos en nuestro país. El trabajo ha puesto de manifiesto, por ejemplo, que las mujeres lactantes del continente asiático presentan unas mayores concentraciones de BPA y PBs con respecto a las de Europa y a América. A falta de estudios en Asia sobre BPs, las mujeres lactantes americanas presentan mayores concentraciones de BP-3, que pueden atribuirse en parte a las diferencias en la composición de los protectores solares europeos y americanos y a las formas de empleo (Osterwalder et al., 2014).

Este tipo de trabajos que comparan países y diferentes períodos de tiempo, también son útiles para estudiar, la evolución temporal de las concentraciones de bisfenoles, PBs y BPs descritas en leche materna. Sería esperable que, a medida que se establecen normativas de restricción o prohibición en el uso de estos compuestos químicos, se aprecie una disminución de la frecuencia y concentración de estos compuestos en muestras biológicas. Sin embargo, según los resultados obtenidos en esta tesis doctoral, aún no podemos señalar que este descenso se este produciendo de hecho, se observaron concentraciones más altas de BPA en la leche materna después de 2010. Esto está en concordancia con el aumento de la producción mundial de este compuesto químico durante la última década (Almeida et al., 2018). Posiblemente, los futuros estudios mostrarán la esperada disminución de los niveles dada la decisión de la EFSA, que pretende reducir la IDT para BPA de 4 μg/kg a 0.04 ng/kg de peso corporal/día (EFSA, 2021). La monitorización de los compuestos químicos en leche materna se presenta como un instrumento de la mayor utilidad para evaluar la eficacia de las medidas reguladas puestas en marcha.

También es de mayor interés señalar el origen de las muestras analizadas ya que, por ejemplo, son escasos los trabajos que han estudidado la presencia de los contaminantes de interés en las leches procedentes de un banco de leche materna. Aunque a priori podrían extrapolarse los resultados de las concentraciones reportadas en leche materna del banco leche a la población general, el hecho de que las donantes de leche materna presenten características particulares en cuanto a su estilo de vida y, por ende, de su grado

de exposición a estas sustancias, puede hacer que las exposiciones no sean comparables. En nuestro estudio de exposición a DEs empleando leche de un banco de leche materna, las concentraciones de bisfenoles, PBs y BPs están en concordancia con otros estudios previos que emplean poblaciones similares. Pero lo cierto es que en determinados casos se refieren concentraciones levemente elevados para los PBs o disminuidos para los bisfenoles y BPs. Además, en esta tesis doctoral se ha demostrado la presencia de al menos un DEs en todas las muestras analizadas en nuestro estudio, lo que es de extrema importancia, como anteriormente mencionábamos, dada la vulnerabilidad de estos recién nacidos ante cualquier concentración de DEs. El hecho de que la obtención de las muestras de leche se haga de forma poco reglada en lo que respecta a los criterios de inclusión de las donantes o sin información complementaria, impide comparar los resultados de muchos trabajos. De esta manera, la inclusión de los determinantes de la exposición a BPA, PBs y BPs en leche materna, es muy pobre. Nosotros hemos revisado los determinantes de la exposición que ejercen influencia sobre los niveles de estos contaminantes en leche materna (Carignan et al., 2012; Dualde et al., 2019; Dualde et al., 2020; Fisher et al., 2017; Jin et al., 2020; Kim et al., 2020; Park et al., 2019; Yi et al., 2013). En nuestro estudio, los determinantes de la exposición identificados coinciden con algunos reportados previamente, en donde se encontraron asociaciones con las características sociodemográficas y reproductivas, aunque la mayor evidencia se relaciona con los hábitos dietéticos, en concordancia con numerosos trabajos (Kim et al., 2020; Pak et al., 2007; Park et al., 2019) y la utilización de PCPs y cosméticos (Dualde et al., 2019; Dualde et al., 2020; Fisher et al., 2017; Gao and Kannan, 2020; Guo et al., 2014; Kim et al., 2020; Park et al., 2019; Yazar et al., 2011; Yi et al., 2013).

Por importante que sea el conocimiento de la exposición del niño a DEs por vía alimentaria, no podemos olvidar que los neonatos no solo están expuestos a bisfenoles, PBs y BPs, así como otros DEs, (Dualde et al., 2019; Dualde et al., 2020), PCPs (Fisher et al., 2017; Kim et al., 2020), sino que el material médico (Duty et al., 2013), los productos para bebés, tales como cambiadores de pañales y colchones para bebes (Asimakopoulos et al., 2016) y los juguetes infantiles (Souza et al., 2022), también contribuyen a la exposición (Li and Kannan, 2018; Xue et al., 2017).

Dentro de este contexto, esta tesis doctoral es el primer trabajo europeo donde se informa la presencia de bisfenoles y PBs en textiles de uso en la infancia. Existe un

estudio previo (Xue et al., 2017) que detectó concentraciones medias de BPA similares a las que obtuvimos en uno de los apartados de nuestro estudio, en materiales textiles empleados con niños. Lo mismo ha sido reportado en otros estudios realizados con textiles para adultos (Li and Kannan, 2018). Nuestro trabajo porporciona información adicional ya que profundiza en la composición y procede a una estimación de la absorción dérmica. Por ejemplo, encotramos mayores concentraciones de BPA en los textiles fabricados con algodón y de color. La fuente de BPA en las presentes muestras textiles no pudo determinarse fácilmente, aunque se sospecha que podría provenir del uso del BPA como producto químico intermedio en la fabricación de antioxidantes y colorantes, añadidos para evitar la fotodegradación y coloreado de la materia prima original (Xue et al., 2017). Con respecto al país de origen de los textiles, los resultados de los diferentes estudios coinciden en mayores concentraciones de BPA en los textiles procedentes de Asia, pese a su prohibición en 2001. Si consideramos la familia de PBs, sólo ha sido examinado previamente por Li and Kannan (2018), observando mayores concentraciones que los resultados obtenidos en nuestros resultados, pero coincidiendo en que son inferiores a los valores obtenidos para BPA y que siguen el mismo patrón para el origen de las muestras. Nosotros no encontramos un patrón claro en las concentraciones de PBs en relación a la composición y al color de los calcetines. Lo más probable es que la presencia de PBs en los calcetines se deba a su utilización como antimicrobianos en la producción textil.

Esta tesis doctoral es pionera al demostrar que algunos de los artículos médicos de uso en la UCIN y ciertos textiles, contribuyen a la carga hormonal atribuible a los DEs. A pesar de que la mayoría de los expertos en el campo están de acuerdo en que el efecto xenoestrogénico general es el resultado del efecto combinado de diversos DEs, la mayoría de los estudios abordan la exposición a un solo compuesto, o en el mejor de los casos, la suma de algunos compuestos. En nuestros estudios, la actividad (anti-)estrogénica y (anti-)androgénica se examinó mediante el uso de métodos de extracción disruptivos y suaves, simulando la temperatura fisiológica y condiciones de pH de un neonato, pero solo contemplamos el nivel individual de cada compuesto estudiado. A groso modo, los resultados obtenidos en el caso de los dispositivos y productos médicos, es que el 25% y 10% de las muestras analizadas evidenciaron actividad estrogénica y antiandrogénica, respetivamente. Por otro lado, la actividad estrogénica para los textiles fue detectada mucho más variable moviéndose entre el 83.3% y 2.8%. Por otra parte, la actividad

antiandrogénica solo se dectetcto para los textiles de una de las procedencias (18.8%). En cualquier caso, podemos considerar que la carga xenoestrogénica en ciertos dispositivos médicos y textiles infantiles puede contribuir a la carga hormonal en los recién nacidos prematuros ingresados en la UCIN y los nacidos a término.

En definitiva, esta tesis doctoral demuestra la presencia generaliza de estos tres grupos de compuestos DEs, bisfenoles, PBs y BPs, en el ambiente que rodea al recién nacido en la UCIN y sugiere la necesidad de llevar a cabo estudios que analicen los posibles efectos de los DEs, en la salud de los recién nacidos, especialmente de aquellos con una mayor vulnerabilidad. No obstante, los resultados aquí mostrados son suficientes para instaurar el pincipio de precaución, adoptando e implementando medidas reguladas, así como prácticas clínicas de carácter preventivo para reducir los niveles de exposición actuales, mientras que se genera nuevo conocimiento.

9. CONCLUSIONES

La revisión de la literatura científica más actual junto al análisis pormenorizado de los resultados experimentales y observacionales del presente trabajo nos permite enunciar las siguientes conclusiones

1. Los dispositivos y productos médicos usados rutinariamente en la Unidad de Cuidados Intensivos Neonatales que están en contacto íntimo y prolongado con el neonato contienen compuestos químicos como BPA y PBs, y sus extractos muestran actividad hormonal en los ensayos biológicos, por lo que los recién nacidos prematuros en la UCIN, especialmente los neonatos de muy bajo peso al nacer, pueden resultar expuestos a disruptores endocrinos a través de múltiples vías, respiratoria, digestiva, dérmica y IV/parenteral, durante su estancia hospitalaria.

2. La leche materna que es la principal fuente de alimentación de los recién nacidos y recomendada por sus incuestionables beneficios, contiene cantidades apreciables bisfenoles, PBs y BPs, por lo que contribuye a la exposición alimentaria del niño. Las diferencias regionales y temporales en la frecuencia y concentración de los contaminantes impiden la comparación entre los estudios.

3. Los Bancos de leche materna ofrecen una alternativa a las fórmulas infantiles para la alimentación de neonatos prematuros hospitalizados en las Unidades de Cuidados Intensivos Neonatales. La mayoría (90.5%) de las muestras de leche materna contienen niveles de DEs no persistentes, entre los que destacan el bisfenol A, PBs y BP-3, lo que evidencia la necesidad de implementar sistemas de control para reducir la carga química. Las concentraciones encontradas se relacionaron con ciertas características del estilo de vida de la madre donante en general y el uso regular de determinados PCPs en particular.

4. La presencia de bisfenoles y PBs en los textiles de uso infantil, así como la actividad hormonal asociada, es un importante hallazgo que tendría que ser sometido a investigación más profunda ya que que la frecuencia, concentración y actividad hormonal, parecen estar relacionada con el material del que estén fabricados y del color con el que se tiña la fibra.

5. Los resultados mostrados en esta tesis doctoral apoyan la necesidad de abordar las implicaciones a corto, medio y largo plazo, de la exposición a disruptores endocrinos no persistentes, sobre la salud de los recién nacidos, especialmente de aquellos extremadamente vulnerables. Además, requiere de la necesidad de adoptar medidas

preventivas con carácter urgente. Es por ello, que se hace necesario, entre otras actuaciones, incorpora recomendaciones a las guías de cuidados y guías clínicas para el personal sanitario, técnico y a las madres lactantes para reducir la exposición de los niños ingresados en las Unidades de Cuidados Intensivos Neonatales. También se deben incorporar regulaciones que incluyan la seguridad química en la leche materna de los Bancos de leche materna, así como en el etiquetado de los productos textiles.

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11. ANEXOS

ANEXO I. CERTIFICADO DE APROBACIÓN DEL COMITÉ DE ÉTICA

JUNTA DE ANDALUCIA

CONSEJERÍA DE IGUALDAD, SALUD Y POLÍTICAS SOCIALES
Dirección General de Calidad, Investigación, Desarrollo e Innovación
Comité Coordinador de Ética de la Investigación Biomédica de Andalucía

DICTAMEN ÚNICO EN LA COMUNIDAD AUTÓNOMA DE ANDALUCÍA

D/Dª: Juan Morales Arcas como secretario/a del CEI de Granada

CERTIFICA

Que este Comité ha evaluado la propuesta de (No hay promotor/a asociado/a) para realizar el estudio de investigación titulado:

TÍTULO DEL ESTUDIO:	Exposición del recién nacido de muy bajo peso a disruptores endocrinos (DEs) en la Unidad de Cuidados Neonatales (UCIN) y evaluación de las consecuencias sobre el desarrollo. ,(PI16/01820)
Protocolo, Versión:	
HIP, Versión:	2
CI, Versión:	2

Y que considera que:

Se cumplen los requisitos necesarios de idoneidad del protocolo en relación con los objetivos del estudio y se ajusta a los principios éticos aplicables a este tipo de estudios.

La capacidad del/de la investigador/a y los medios disponibles son apropiados para llevar a cabo el estudio.

Están justificados los riesgos y molestias previsibles para los participantes.

Que los aspectos económicos involucrados en el proyecto, no interfieren con respecto a los postulados éticos.

Y que este Comité considera, que dicho estudio puede ser realizado en los Centros de la Comunidad Autónoma de Andalucía que se relacionan, para lo cual corresponde a la Dirección del Centro correspondiente determinar si la capacidad y los medios disponibles son apropiados para llevar a cabo el estudio.

Lo que firmo en GRANADA a 29/03/2017

D/Dª. Juan Morales Arcas, como Secretario/a del CEI de Granada

Código Seguro De Verificación:	16a862a9f0e36e2d04b71770463a9f6da46bb934	Fecha	29/03/2017
Normativa	Este documento incorpora firma electrónica reconocida de acuerdo a la Ley 59/2003, de 19 de diciembre, de firma electrónica.		
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CERTIFICA

Que este Comité ha ponderado y evaluado en sesión celebrada el 28/11/2016 y recogida en acta 3/2017 la propuesta del/de la Promotor/a (No hay promotor/a asociado/a), para realizar el estudio de investigación titulado:

TÍTULO DEL ESTUDIO:	Exposición del recién nacido de muy bajo peso a disruptores endocrinos (DEs) en la Unidad de Cuidados Neonatales (UCIN) y evaluación de las consecuencias sobre el desarrollo. , (PI16/01820)
Protocolo, Versión:	
HIP, Versión:	2
CI, Versión:	2

Que a dicha sesión asistieron los siguientes integrantes del Comité:

Presidente/a
D/Dª. Fidel Fernández Quesada

Vicepresidente/a
D/Dª. Francisco Manuel Luque Martínez

Secretario/a
D/Dª. Juan Morales Arcas

Vocales
D/Dª. Jesús Martínez Tapias
D/Dª. José Expósito Hernández
D/Dª. Juan Ramón Delgado Pérez
D/Dª. Berta Gorlat Sánchez
D/Dª. José Dario Sánchez López
D/Dª. José Cabeza Barrera
D/Dª. José Uberos Fernández
D/Dª. Enrique Lopez Cordoba
D/Dª. MARIA ESPERANZA DEL POZO GAVILAN
D/Dª. ESTHER OCETE HITA
D/Dª. MAXIMILIANO OCETE ESPINOLA
D/Dª. Joaquina Martínez Galán
D/Dª. Maria José García Sánchez
D/Dª. AURORA BUENO CAVANILLAS
D/Dª. MARIA MERCEDES RODRIGUEZ MORALES
D/Dª. Paloma Muñoz de Rueda
D/Dª. Manuel Gálvez Ibáñez
D/Dª. JUAN ROMERO COTELO
D/Dª. Esther Espínola García
D/Dª. MARÍA DEL PILAR GONZÁLEZ CARRIÓN
D/Dª. Juan de Dios Luna del Castillo
D/Dª. Pilar Guijosa Campos
D/Dª. José Luis Martín Ruiz
D/Dª. FRANCISCO LUIS MANZANO MANZANO
D/Dª. MIGUEL LÓPEZ GUADALUPE

Que dicho Comité, está constituido y actua de acuerdo con la normativa vigente y las directrices de la Conferencia Internacional de Buena Práctica Clínica.

Lo que firmo en GRANADA a 29/03/2017

Código Seguro De Verificación:	16a862a9f0e36e2d04b71770463a9f6da46bb934	Fecha	29/03/2017
Normativa	Este documento incorpora firma electrónica reconocida de acuerdo a la Ley 59/2003, de 19 de diciembre, de firma electrónica.		
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Url De Verificación	https://www.juntadeandalucia.es/salud/portaldeetica/xhtml/ayuda/verificarFirmaDocumento.iface/code/16a862a9f0e36e2d04b71770463a9f6da46bb934	Página	2/2

ANEXO II. MATERIAL SUPLEMENTARIO ARTÍCULO *"Presence of Bisphenol A and Parabens in a Neonatal Intensive Care Unit: An Exploratory Study of Potential Sources of Exposure".*

Table S1. Concentrations of BPA and PBs and hormone-like activities released from plastic NICU items under soft extraction conditions

		EDC content (ng/g)											Hormone-like activity	
		BPA		MeP		EtP		PrP		BuP		ΣPBs	E-Screen	PALM Assay
Item	Description	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)		E_2eq/g (pM)[a]	Proceq/g(mM)[b]
3	Gastro-duodenal feeding tube	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
4	Extension tube for feeding syringe	<0.1	-	0.07	10.1	<0.03	-	<0.03	-	<0.03	-	0.07	ND	ND
5	Feeding sampling straw	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
6	Small dummy	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	$3*10^{-2}$
7	Large dummy	0.1	8.2	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
9A	Pulse oximeter adhesive sensor I (hard section)	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
14	Sterile gloves	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
15	Non-sterile gloves	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
35	Winged IV catheter (transparent section)	<0.1	-	0.53	5.5	<0.03	-	<0.03	-	<0.03	-	0.53	ND	ND
36	Winged IV catheter	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
37	Single lumen umbilical vein catheter	0.9	7.4	<0.03	-	0.18	8.9	<0.03	-	<0.03	-	0.18	18.32	ND
38	Double lumen umbilical vein catheter	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	n.d.	ND
39	Extension set for the intravenous infusion system	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	n.d.	ND
40	Extension set for the intravenous infusion system (light resistant)	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	n.d.	ND
41	Three-way stopcock	4.7	9.1	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	9.32	$4*10^{-2}$
49	Endotracheal tube	<0.1	-	<0.03	-	0.87	9.9	0.43	8.8	<0.03	-	1.3	7.28	ND
50	Closed suction system	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND
51	Nasal cannula	<0.1	-	<0.03	-	1.48	7.9	0.95	11.2	<0.03	-	2.42	ND	ND
52	Nasal prong	<0.1	-	<0.03	-	<0.03	-	<0.03	-	<0.03	-	-	ND	ND

EDC, endocrine disrupting chemical; BPA, bisphenol A; MeP, methyl-paraben; EtP, ethyl-paraben; PrP, propyl-paraben; ∑PBs, total concentrations of parabens; CV, coefficient of variation; ND, not detected. Values below the limit of detection were represented with the symbol "<" followed by the limit of detection.

[a]Concentrations equivalent to E2 per gram;

[b]Concentrations equivalent to procymidone per gram.

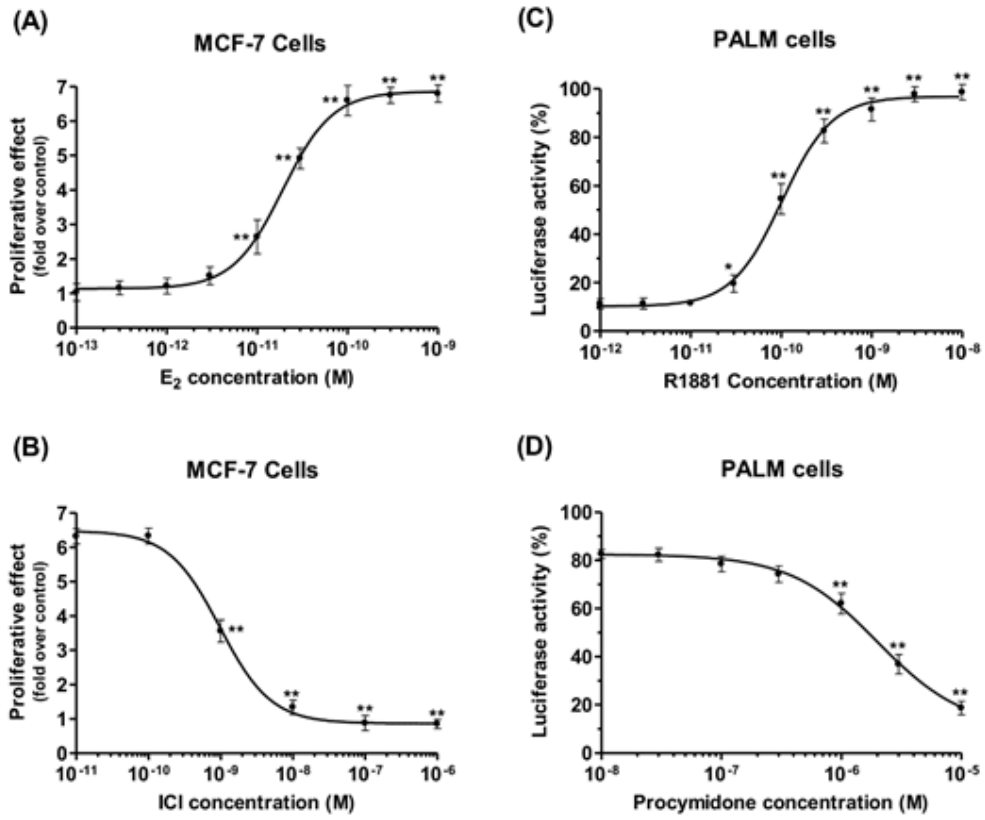

Figure S1. Dose-response curves of E2 and ICI on MCF-7cells and R1881 and procymidone on PALM cells. MCF-7 cells were incubated for 144 h at 37 °C with E2 (Panel A) or ICI in the presence of 100 pM E2 (Panel B) at the indicated concentrations. The values represent the mean ± SD of two independent experiments (in triplicate) and results are expressed as proliferative effect [MCF-7 cell proliferation (-fold over control)]. Data were analyzed for significant differences using one-way ANOVA followed by Dunnett's post-comparison test. *p< 0.05 and **p< 0.01 (versus hormone-free control or E2 100 pM). PALM cells treated with R1881 (Panel C) or procymidone in the presence of 0.2 nM R1881 (Panel D) at the indicated concentrations for 40 h at 37 °C. The values represent the mean ± SD of two independent experiments (in triplicate) and results are expressed as percentage of maximal R1881 induction. Data were analyzed for significant differences using one-way ANOVA followed by Dunnett's post-comparison test. *p< 0.05 and **p< 0.01 (versus R1881 10 or 0.2 nM). ICI: The estrogen receptor antagonist ICI 182780; E2: 17β-estradiol.

ANEXO III. MATERIAL SUPLEMENTARIO ARTÍCULO *"Concentrations of bisphenols, parabens, and benzophenones in human breast milk: A systematic review and meta-analysis"*

Supplementary Table S1. Search strategies for Pubmed, Scopus and Web of Science

Database	**Pubmed (Medline)**
Date	**16/06/2020**
Strategy	**#1 AND #2**
#1	("bisphenols" [All fields] OR "bisphenol A" [All fields] OR "BPA" [All fields] OR "bisphenol S" [All fields] OR "BPS" [All fields] OR "bisphenol F" [All fields] OR "BPF" [All fields] OR "bisphenol A-glycidyl methacrylate" [Mesh] OR "bisphenol A-glycidyl methacrylate" [All fields] OR "BisGMA" [All fields] OR "bisphenol A diglycidyl ether" [All fields] OR "BADGE" [All fields] OR "bisphenol F diglycidyl ether" [All fields] OR "BFDGE" [All fields] OR "benzophenones" [All fields] OR "benzophenone 1" [All fields] OR "2,4-dihydroxy-benzophenone" [All fields] OR "BP1" [All fields] OR "benzophenone 2" [All fields] OR "2,2 ', 4,4'-tetrahydroxybenzophenone" [All fields] OR "BP2" [All fields] OR "benzophenone 3" [All fields] OR "oxybenzone" [All fields] OR "BP3" [All fields] OR "benzophenone 4" [All fields] OR "sulisobenzone" [All fields] OR "BP4" [All fields] OR "4-hydroxybenzophenone" [All fields] OR "4-OHBP" [All fields] OR "benzophenone 5" [All fields] OR "BP5" [All fields] OR "benzophenone 6" [All fields] OR "2,2'-dihydroxy-4,4'-dimethoxybenzophenone" [All fields] OR "BP6" [All fields] OR "benzophenone 7" [All fields] OR "5-chloro-2-hydroxybenzophenone" [All fields] OR "BP7" [All fields] OR "benzophenone 8" [All fields] OR "dioxybenzone" [All fields] OR "BP8" OR "benzophenone 9" [All fields] OR "BP9" [All fields] OR "benzophenone 10" [All fields] OR "BP10" [All fields] OR "BP11" [All fields] OR "benzophenone 12" [All fields] OR "octabenzone" [All fields] OR "BP12"[All fields] OR "parabens" [Mesh] OR "parabens" [All fields] OR "methylparaben" [All fields] OR "MeP" [All fields] OR "ethylparaben" [All fields] OR "EtP" [All fields] OR "prophylparaben" [All fields] OR "PrP" [All fields] OR "buthylparaben" [All fields] OR "BuP" [All fields])
#2	("milk, human" [Mesh] OR "human milk" [All fields] OR "breast milk" [All fields] OR "milk banks" [Mesh] OR "milk banks" [All fields] OR "lactation" [Mesh] OR "lactation" [All fields] OR "colostrum" [Mesh] OR "colostrum" [All fields])
Database	**Scopus**
Date	**16/06/2020**
Strategy	**#1 AND #2**
#1	("bisphenols" OR "bisphenol A" OR "BPA" OR "bisphenol S" OR "BPS" OR "bisphenol F" OR "BPF" OR "bisphenol A-glycidyl methacrylate" OR "BisGMA" OR "bisphenol A diglycidyl ether" OR "BADGE" OR "bisphenol F diglycidyl ether" OR "BFDGE" OR "benzophenones" OR "benzophenone 1" OR "2,4-dihydroxy-benzophenone" OR "BP1" OR "benzophenone 2" OR "2,2 ', 4,4'-tetrahydroxybenzophenone" OR "BP2" OR "benzophenone 3" OR "oxybenzone" OR "BP3" OR "benzophenone 4" OR "sulisobenzone" OR "BP4" OR "4-hydroxybenzophenone" OR "4-OHBP" OR "benzophenone 5" OR "BP5" OR "benzophenone 6" OR "2,2'-dihydroxy-4,4'-dimethoxybenzophenone" OR "BP6" OR "benzophenone 7" OR "5-chloro-2-hydroxybenzophenone" OR "BP7" OR "benzophenone 8" OR "dioxybenzone" OR "BP8" OR "benzophenone 9" OR "BP9" OR "benzophenone 10" OR "BP10" OR "BP11" OR "benzophenone 12" OR "octabenzone" OR "BP12" OR "parabens" OR "methylparaben" OR "MeP" OR "ethylparaben" OR "EtP" OR "prophylparaben" OR "PrP" OR "buthylparaben" OR "BuP")
#2	("human milk"OR "breast milk" OR "milk banks" OR "lactation" OR "colostrum")
Database	**Web of Science**
Date	**16/06/2020**
Strategy	**#1 AND #2**
#1	("bisphenols" OR "bisphenol A" OR "BPA" OR "bisphenol S" OR "BPS" OR "bisphenol F" OR "BPF" OR "bisphenol A-glycidyl methacrylate" OR "BisGMA" OR "bisphenol A diglycidyl ether" OR "BADGE" OR "bisphenol F diglycidyl ether" OR "BFDGE" OR "benzophenones" OR "benzophenone 1" OR "2,4-dihydroxy-benzophenone" OR "BP1" OR "benzophenone 2" OR "2,2 ', 4,4'-tetrahydroxybenzophenone" OR "BP2" OR "benzophenone 3" OR "oxybenzone" OR "BP3" OR "benzophenone 4" OR "sulisobenzone" OR "BP4" OR "4-hydroxybenzophenone" OR "4-OHBP" OR "benzophenone 5" OR "BP5" OR "benzophenone 6" OR "2,2'-dihydroxy-4,4'-dimethoxybenzophenone" OR "BP6" OR "benzophenone 7" OR "5-chloro-2-hydroxybenzophenone" OR "BP7" OR "benzophenone 8" OR "dioxybenzone" OR "BP8" OR "benzophenone 9" OR "BP9" OR "benzophenone 10" OR "BP10" OR "BP11" OR "benzophenone 12" OR "octabenzone" OR "BP12" OR "parabens" OR "methylparaben" OR "MeP" OR "ethylparaben" OR "EtP" OR "prophylparaben" OR "PrP" OR "buthylparaben" OR "BuP")
#2	("human milk"OR "breast milk" OR "milk banks" OR "lactation" OR "colostrum")

Supplementary Table S2. Other characteristics of epidemiological studies

Reference	Characteristics of participating women					Extraction and quantification methodology		
	Women's age	Women's health	Parity	BMI (kg/m^2)	Smoker (Y/N/Ex)	Amount of milk (mL)	Extraction methodology	Quantification methodology
Arbuckle et al., 2015	32.40	Early pregnancy	Primiparous: 37 Multiparous: 43	>25: 22.40 women	Y/Ex: 68.00% N: 32.00%	0.10	LLE	GC–MS/MS
Carignan et al., 2012	>18.00	N.R.	Primiparous	23.20	Ex: 9.76% N: 90.24%	2.00[c]	LLE	LC-ESI-MS/MS
Fisher et al., 2017	>18.00	N.R.	N.R.	R.P.	R.P.	N.R.	LLE	LC-MS-MS
Hines et al., 2015	18.00-38.00[a]	Healthy	N.R.	N.R.	R.P.	N.R.	SPE	HPLC-MS/MS
Mendonca et al., 2014	36.50	N.R.	Uniparous: 23 Multiparous: 4	N.R.	N.R.	0.10	SPE	HPLC-MS/MS
Zimmers et al., 2014	26.00-41.00[a]	Breast cancer	Primiparous: 8 Secundiparous: 9 Tertiparous: 3 Quadriparous: 1	< 30 (Low BMI): 10 >30 (High BMI): 11	N.R.	1.00	SPE	LC-MS/MS
Chang et al., 2019	33.30	Healthy	Primiparous: 44 Multiparous: 141	21.80	Y: 0.00% Ex: NR	5.00[c]	LLE	UPLC-QTOF/MS
Fujii et al., 2014	31.40	N.R.	N.R.	R.P.	N.R.	10.00	GPC	GC/MS/ECNI
Fujii et al., 2018	19.00-40.00[a]	Healthy	Primiparous: 28 Secundiparous: 18 Tertiparous: 18	N.R.	Y/Ex: 34.40% N: 57.80%	5.00[c]	GPC	LC-ESI-MS/MS
Huang et al., 2020	29.70	N.R.	N.R.	N.R.	N.R.	N.R.	SLE	HPLC-MS/MS
Jin et al., 2020	29.00	N.R.	Primiparous: >133	21.00	N: 100%	5.00	LLE	UPLC-MS/MS
Kurutu-Niwa et al., 2007	29.00-40.00[a]	Healthy	Primiparous: 49 Secundiparous: 33 Tertiparous: 15 Quadriparous: 2	N.R.	N.R.	1.00	ELISA	HPLC
Kim et al., 2020	31.00	Healthy	Primiparous	21.30	R.P.	2.00	QuEChERS	LC-MS/MS
Lee et al., 2018a	< 40.00	N.R.	Uniparous: 124 Multiparous: 194	25.00	N.R.	1.00	SPE	HPLC-MS/MS
Nakao et al., 2015	19.00-39.00[a]	Healthy	Primiparous: 3 Multiparous: 16	N.R.	N.R.	5.00	SPE	GC/MS
Park et al., 2019	31.20	Lactating women[b]	Primiparous: 94 Multiparous: 165	23.59	Y: 0.00% N: 93.46% Ex: 6.54%	0.20	LLE	LC-MS/MS
Yi et al., 2013	30.67	Healthy: 89.2% Disease: 10.8%	N.R.	23.53	Y: 0.35% N: 88.07% Ex: 11.58%	2.00	LLE	LC-MS/MS
Cariou et al., 2008	32.50	Caesarean deliveries	N.R.	N.R.	N.R.	N.R.	LLE	GC-HRMS
Dualde et al., 2019	33.00	N.R.	Uniparouss: 69 Secundiparous: 40 Terctiparous: 11	21.90 (before pregnancy)	Y: 7.50% N: 52.50% Ex:40.00%	10.00	QuEChERS	LC-MS/MS
Dualde et al., 2020	33.00	N.R.	Uniparouss: 69 Secundiparous: 40 Terctiparous: 11	21.90 (before pregnancy)	Y: 7.50% N: 52.50% Ex:40.00%	10.00	QuEChERS	LC-MS/MS
Martínez et al., 2019	25.00-43.00[a]	Healthy	N.R.	24.50	N.R.	2.00[c]	SPE	HPLC
Migeot et al., 2013	33.00	Healthy	2	22.10	Y: 7.50% N: 52.50% Ex:40.00%	1.50	SPE	UPLC-MS/MS
Molins-Delgado et al., 2018	34.40	N.R.	Primiparous: 71 Multiparous: 8	N.R.	N: 100.00%	2.00[c]	TFC	TFC-HPLC-MS/MS
Schlumpf et al., 2010	32.30	N.R.	N.R.	27.21	N: 100.00%	0.20	LLE	HPLC–MS/MS

[a]: range of age, [b]: lactating women were advised not to apply body creams to their breasts before sampling. [c]: Grams of sample, QuEChERS: Quick, Easy, Cheap, Effective, Rugged & Safe method, SPE: solid phase extraction, LLE: liquid-liquid extraction, GPE: gel permeation chromatography, TFC: turbulent flow chromatography, C18: C18 extraction cartidgre, ELISA: Enzyme-Linked ImmunoSorbent Assay, SLE: solid-liquid extraction, HPLC: high performance liquid chromatography, LC-MS/MS: Liquid chromatography tandem-mass spectrometry, UPLC-MS/MS: liquid chromatography-hybrid mass spectrometry, HPLC-MS/MS: high performance liquid chromatography-tandem mass spectrometry , LC-ESI-MS/MS: electrospray ionization tandem mass spectrometry, TFC-HPLC-MS/MS: turbulent flow chromatography coupled with liquid chromatography-tandem mass spectrometry, GC/MS/ECNI: gas chromatography coupled with mass spectrometry in negative chemical ionization mode, GC-HRMS: gas chromatography-tandem high resolution mass spectrometry, GC/MS: gas chromatography mass spectrometry, GC-MS/MS: Gas chromatography- tandem mass spectrometry, UPLC-QTOF/MS: ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry; BMI: body mass index, Y: yes, N: No, Ex: ex-smoker. N.R.: not reported, R.P.: reported previously.

Supplementary Table S3. Other characteristics of methodological studies

Reference	Characteristics of participating women					Extraction and quantification methodology		
	Women's age	Women's health	Parity	BMI (kg/m^2)	Smoker (Y/N/Ex)	Amount of milk (mL)	Extraction methodology	Quantification methodology
Cao et al., 2015	N.R.	N.R.	N.R.	N.R.	N.R.	2.00[b]	SPE	GC-MS
Grecco et al.,2019	N.R.	N.R.	N.R.	N.R.	N.R.	1.00[b]	DLLME	UPLC-MS/MS
Melo et al., 2014	N.R.	N.R.	N.R.	N.R.	N.R.	0.10	SPE	MISPE/LC-UV
Souza et al., 2016	N.R.	N.R.	N.R.	N.R.	N.R.	0.20	SPME	UHPLC-MS/MS
Ye et al., 2006	N.R.	N.R.	N.R.	N.R.	N.R.	0.10	SPE	HPLC–MS/MS
Ye et al.,2008	N.R.	N.R.	N.R.	N.R.	N.R.	1.50	SPE	HPLC-MS/MS
Fotouhi et al., 2017	25.00-33.00[a]	Healthy	N.R.	N.R.	N.R.	0.20	MA-MSPD + DLLME	LC-UV and LC–MS/MS.
Manouchehri et al., 2020	N.R.	N.R.	N.R.	N.R.	N.R.	5.00	SPE	HPLC-UV
Niu et al., 2017	N.R.	N.R.	N.R.	N.R.	N.R.	0.20	SPE	LC-MS
Otaka et al., 2003	N.R.	N.R.	N.R.	N.R.	N.R.	25.00[b]	LLE + SPE	GC-MS
Sun et al., 2004	N.R.	Healthy	Primiparous: N.R Multiparous: N.R	N.R.	N.R.	0.10	SPE + LLE	HPLC-fluorescence detection
Yang et al., 2018	N.R.	Healthy	N.R.	N.R.	N.R.	0.20	SPE	UPLC-MS/MS
Azzouz et al., 2016a	N.R.	Healthy	N.R.	N.R.	N.R.	0.50	SPE	GC–MS
Azzouz et al., 2016b	N.R.	N.R.	N.R.	N.R.	N.R.	1.00	SPE	GC–MS
Cariot et al., 2012	N.R.	N.R.	N.R.	N.R.	N.R.	0.50	SPE	UPLC–MS/MS
Deceuninck et al., 2015	N.R.	N.R.	N.R.	N.R.	N.R.	3.00[b]	SPE	GC-MS/MS
Dualde et al., 2019b	28.00-40.00[a]	N.R.	N.R.	N.R.	N.R.	10.00	QuEChERS	HPLC-MS/MS
Rodríguez-Gómez et al., 2014a	N.R.	Healthy	N.R.	N.R.	N.R.	9.90	SPE	UHPLC-MS/MS
Rodríguez-Gómez et al., 2014b	N.R.	Healthy	N.R.	N.R.	N.R.	3.00	SM-SLLME	UHPLC-MS/MS
Rodríguez-Gómez et al., 2014c	N.R.	Healthy	N.R.	N.R.	N.R.	9.90	SBSE	GC–MS/MS and UHPLC–MS/MS
Rodríguez-Gomez et al., 2015a	N.R.	Healthy	N.R.	N.R.	N.R.	9.90	SPE	UHPLC–MS/MS
Rodríguez-Gómez et al., 2015b	N.R.	Healthy	N.R.	N.R.	N.R.	9.90	UAE	UHPLC–MS/MS
Tuzimski et al., 2019	N.R.	Healthy	N.R.	N.R.	N.R.	5.00	QuEChERS	LC-QqQ-MS
Tuzimski and Szubartowski., 2019	N.R.	Healthy	N.R.	N.R.	N.R.	5.00	QuEChERS/d-SPE	HPLC–DAD and HPLC-QqQ-MS
Vela-Soria et al., 2016	N.R.	N.R.	N.R.	N.R.	N.R.	1.00	DLLME	LC–MS/MS
Vela-Soria et al., 2018	N.R.	N.R.	N.R.	N.R.	N.R.	1.00	QuEChERS	UHPLC–MS/MS

[a]: range of age, [b]: grames of sample, QuEChERS: Quick, Easy, Cheap, Effective, Rugged & Safe method, SPE: solid phase extraction, LLE: liquid-liquid extraction, DLLME: dispersive liquid-liquid microextraction,SBSE: stir-bar sorptive extraction, MA-MSPD: Magnetically assisted matrix solid phase dispersion, d-SPE: dispersive SPE, UAE: combination of ultrasound-assisted extraction, SPME: solid phase microextraction, SM-LLME: Stir-membrane solid–liquid–liquid microextraction, C18: C18 extraction cartidgre, UHPLC-MS/MS: ultrahigh performance liquid chromatography mass spectrometry, LC-MS/MS: Liquid chromatography tandem-mass spectrometry, HPLC-MS/MS: high performance liquid chromatography-tandem mass spectrometry , GC/MS: gas chromatography mass spectrometry, GC-MS/MS: Gas chromatography- tandem mass spectrometry, HPLC-UV: ultraviolet high performance liquid chromatography, LC-UV: liquid chromatography ultraviolet, HPLC-DAD: high-performance liquid chromatography-diode array detection, HPLC-QqQ-MS: high-performance liquid chromatography method coupled with triple-quadrupole tandem mass spectrometry, MISPE/LC-UV: molecularly imprinted solid-phase extraction/ liquid chromatography ultraviolet , LC/MS:liquid chromatography mass spectrometry, LC-QqQ-MS: liquid chromatography method coupled with triple-quadrupole tandem mass spectrometry. Y: yes, N: No, Ex: ex-smoker. N.R.: not reported

Supplementary Table S4. Frequencies of detection and concentrations of bisphenols, parabens, and benzophenones in the selected studies, distributed by continent and sampling period.

	Total						Asia						Europe						America					
	BPA	**MeP**	**EtP**	**PrP**	**BuP**	**BP-3**	**BPA**	**MeP**	**EtP**	**PrP**	**BuP**	**BP-3**	**BPA**	**MeP**	**EtP**	**PrP**	**BuP**	**BP-3**	**BPA**	**MeP**	**EtP**	**PrP**	**BuP**	**BP-3**
Cumulative samples	1939	846	853	857	617	251	1134	493	493	493	260		345	225	236	236	236	193	460	128	124	128	121	58
FD (%)	56.33	42.76	46.96	39.98	27.92	39.53	58.59	15.81	37.14	20.91	25.00		78.06	75.08	58.31	60.00	42.05	34.81	34.46	89.78	64.45	76.54	6.62	55.24
Arithmetic mean*	1.46	14.22	6.96	4.92	0.23	24.45	2.39	24.97	13.52	9.52	0.05		1.38	2.74	1.35	1.58	0.41	31.36	0.53	11.55	3.25	1.33	0.25	1.45

	Total						2000-2010						2011-2020					
	BPA	**MeP**	**EtP**	**PrP**	**BuP**	**BP-3**	**BPA**	**MeP**	**EtP**	**PrP**	**BuP**	**BP-3**	**BPA**	**MeP**	**EtP**	**PrP**	**BuP**	**BP-3**
Cumulative samples	1403	717	724	728	503	171	520	135	131	135	131	92	883	582	593	593	372	79
FD (%)	59.69	51.77	60.36	49.43	45.82	26.45	43.94	72.49	43.60	57.86	0.00	29.41	72.05	44.02	66.26	46.38	34.74	23.00
Arithmetic mean*	1.40	2.56	0.75	0.37	0.06	34.86	1.01	6.29	0.45	0.65	0.02	1.38	1.81	1.17	0.86	0.27	0.07	73.85

*BPA: bisphenol A, BPF: bisphenol F, BPS: bisphenol S, BPAF: bisphenol AF, TBBPA: tetrabromobisphenol A, MeP: methylparaben, EtP: ethylparaben, PrP: propylparaben, BuP: buthylparaben, BP-3: benzophenone 3. FD: frequency of detection. *: Calculated from studies reporting arithmetic mean.*

Supplementary Table S5. Summary of factors related to breast milk concentrations of bisphenols, parabens, and benzophenones

	Anthropometric/Sociodemographic characteristics	Reproductive characteristics	Dietary habits	Use of PCPs
Carignan et al., 2012	N.R.	N.R.	N.R.	N.R.
Fisher et al., 2017	N.R.	N.R.	N.R.	PrP: Eye makeup (+), makeup (+), skinclean (+)
Jin et al., 2020	BPA: Annual household income (≥100000$) (+)	BPA: Parity (≥ 1 infant) (+)	N.R.	N.R.
Kim et al., 2020	EtP: BMI (+), moderate walking activity in previous week (+)	MeP: Neonatal age and neonatal sex (+) EtP: Delivery type (+), neonatal age (+) PrP: neonatal sex (+)	MeP: Plastic food containers (+) EtP: Cups for noodles (+) PrP: Fish consumption (+), disposable food container (+)	MeP: Air freshener (+), lotion (+)
Park et al., 2019	N.R.	MeP, EtP,and PrP: Type of milk (colostrum/transitional) (+) MeP: Pre-pregnancy BMI (kg/m2) (+) EtP and BuP: Parity (primipara) (+)	EtP: Drinking status (no) (+) PrP and BuP: Consumption of canned beverages (+)	MeP and PrP: Use of cosmetics (makeup) (+) EtP: Use of basic skin care products (+)
Yi et al., 2013	N.R.	N.R.	BPA: Consumption of dairy products (+) BPA: Use of detergents for food (+)	N.R.
Dualde et al., 2019	BPA: Rural residence (+)	N.R.	N.R.	BPA: Skin care products (used daily) (+)
Dualde et al., 2020	EtP: Smoker status: Ex-smoker (-) Smoker status: Non –smoker (+),	PrP: Maternal BMI before pregnancy (kg/m2) (+)	MeP: Protein (g/100 mL) (+) EtP: Pastries (g/month) (+) PrP: Protein (g/100 mL) (+) BuP: Fruit (g/month) (+)	EtP: Sunscreen frequency: (+) PrP: Perfume frequency: Never (+)

*BPA: bisphenol A, TBBPA: tetrabromobisphenol A, MeP: methylparaben, EtP.ethylparaben, PrP: propylparaben, BuP: buthylparaben, BMI: body mass index, PCPs: personal care products, *All associations were statistically significant, (+): Positive association, (-): Negative association. N.R.: not reported*

ANEXO IV. MATERIAL SUPLEMENTARIO ARTÍCULO *"Biomonitoring bisphenols, parabens, and benzophenones in breast milk from a human milk bank in Southern Spain"*

Supplementary Table S1. Dietary habits of the study population (N = 83)

	N (%)		N (%)
Frequency of fish and shellfish consumption		**Frequency of fruit consumption**	
<1 time/ week	12 (14.5)	≤2 times/ week	13 (15.7)
1 time/ week	21 (25.3)	>2 times/ week	70 (84.3)
>1 time/ week	50 (60.2)	**Frequency of egg consumption**	
Frequency of lean fish consumption		At least 1 time/ week	16 (19.3)
<1 time/ week	18 (21.7)	2 times/ week	31 (37.4)
1 time/ week	40 (48.2)	>2 times/ week	36 (43.3)
>1 time/ week	25 (30.1)	**Frequency of bread consumption**	
Frequency of oil fish consumption		<1 time/ day	15 (18.1)
<1 time/ week	32 (38.5)	1 time/ day	28 (33.8)
1 time/ week	37 (44.6)	>1 time/ day	40 (48.1)
>1 time/ week	14 (16.9)	**Frequency of pasta consumption**	
Frequency of dairy products consumption		1 time/ week	72 (86.7)
At least 1 time/day	50 (60.3)	>1 times/ week	11 (13.3)
<1 time/ day	33 (39.7)	**Frequency of chocolate consumption**	
Frequency of milk consumption		Never	10 (12.1)
<1 glass/ day	15 (18.1)	1 time/ day	47 (56.6)
At least 1 glass/ day	68 (81.9)	>1 time/ day	26 (31.3)
Frequency of sausage consumption		**Frequency of rice consumption**	
≤2 times / week	46 (55.4)	At least 1 time/ week	72 (86.8)
2 times/ week	37 (44.6)	>1 times/ week	11 (13.2)
Frequency of cheese consumption		**Frequency of cereals consumption**	
R/N	24 (28.9)	Never	30 (36.1)
>2 times/ week	38 (45.8)	<1 time/ day	37 (44.6)
At least 1 time/day	21 (25.3)	At least 1 time/ day	16 (19.3)
Frequency of meat consumption		**Consumption of canned food**	
At least 1 time/week	12 (14.5)	No	15 (18.1)
2 times/ week	15 (18.1)	Yes	68 (81.9)
>2 times/ week	56 (67.4)	**Consumption of organic food**	
Frequency of red meat consumption		No	29 (34.9)
Never	23 (27.7)	Yes	54 (65.1)
2 times/ week	35 (42.2)	**Consumption of fried food**	
>2 times/ week	25 (30.1)	<1 time/ week	40 (48.2)
Frequency of pulse consumption		1 time/ week	26 (31.3)
At least 1 time/ week	14 (16.9)	>1 time/ week	17 (20.5)
2 times/ week	32 (38.6)	**Frequency of multivitamins consumption**	
>2 times/ week	37 (44.5)	No	69 (83.1)
Frequency of fresh vegetable consumption		Yes	14 (16.9)
≤2 times/ week	17 (20.5)	**Frequency of other nutritional supplements consumption**	
>2 times/ week	66 (79.5)	No	18 (21.7)
Frequency of cooked vegetable consumption		Yes	65 (78.3)
≤2 times/ week	18 (21.7)		
>2 times/ week	65 (78.3)		

Supplementary Table S2. Use of PCPs by the study population (N = 83)

	N (%)		N (%)
Frequency of sunscreen use		**Frequency of shampoo use**	
No	43 (51.8)	<3 times/week	26 (31.3)
Yes	40 (48.2)	>3 times/week	57 (68.7)
Sun screen application use		**Frequency of bath gel use**	
No	44 (53.0)	<1 time/day	6 (7.2)
Face	28 (33.7)	>1 time/day	77 (92.8)
Yes	11 (13.3)	**Frequency of deodorant use**	
Protection factor use		<1 time/day	8 (9.6)
0	43 (51.8)	One time/day	58 (69.9)
50	28 (33.7)	>1 time/day	17 (20.5)
>50	12 (14.5)	**Frequency of hair conditioner use**	
Frequency of lip balm use		R/N	33 (39.8)
No	50 (60.2)	At least 1 time/ day	50 (60.2)
Yes	33 (39.8)	**Frequency of hairspray, hair wax, and/or hair mousse use**	
Frequency of facial cream use		R/N	60 (72.3)
<1 time/ day	26 (31.3)	At least 1 time/ day	23 (27.7)
1 time/ day	34 (41.0)	**Frequency of cologne and/or perfume use**	
>1 time/ day	23 (27.7)	R/N	12 (14.5)
Frequency of body lotion use		<1 time/day	26 (31.3)
R/N	29 (35.0)	>1 time/day	45 (54.2)
<1 time/ day	17 (20.5)	**Frequency of toothpaste use**	
At least 1 time/ day	37 (44.5)	At least 1 time/day	17 (20.5)
Frequency of handcream use		>1 time/day	66 (79.5)
<1 time/ day	48 (57.8)	**Frequency of mouthwash consumption use**	
1 time/ day	19 (22.9)	R/N	50 (60.2)
>1 time/ day	16 (19.2)	<1 time/day	13 (15.7)
Frequency of hair mask use		At least 1 time/ day	19 (23.0)
R/N	43 (51.8)	**Frequency of facial treatment use**	
At least 1 time/ week	40 (48.2)	Never	62 (74.7)
Frequency of makeup use		<1 time/month	15 (18.1)
R/N	45 (54.2)	Months	6 (7.2)
1 time/ day	20 (54.1)	**Frequency of manicure use**	
At least 1 time/ day	18 (24.7)	<1 time/month	73 (88.0)
Frequency of facial tonic use		Months	10 (12.0)
R/N	65 (78.3)	**Frequency of pedicure use**	
At least 1 time/ week	18 (21.7)	<1 time/month	72 (86.7)
Frequency of lipstick use		Months	11 (13.3)
R/N	44 (53.0)	**Frequency of artificial nails use**	
<1 time/day	25 (30.1)	<1 time/month	76 (91.6)
At least 1 time/ day	14 (16.9)	Months	7 (8.4)
Frequency of eye pencil use		**Frequency of massage use**	
R/N	40 (48.2)	Never	68 (81.9)
<1 time/day	22 (26.5)	<1 time/month	10 (12.0)
At least 1 time/ day	21 (25.3)	Months	5 (6.1)
Frequency of eyeshadow use		**Frequency of hair dye consumption use**	
R/N	54 (65.1)	Never	40 (48.2)
<1 time/day	19 (22.9)	<1 time/month	25 (30.1)
At least 1 time/ day	10 (12.0)	Months	18 (21.7)
Frequency of facial milk use		**Frequency of permanent, hair strengthening use**	
R/N	76 (91.6)	Never	75 (90.4)
At least 1 time/ week	7 (8.4)	Sometimes	8 (9.6)
Frequency of nail polish use			
R/N	70 (84.4)		
At least 1 time/ week	13 (15.6)		

Supplementary Table S3. Factors associated with human milk levels of BPA, EtP, PrP and BP-1 (N=83). Bivariate logistic regression analyses

		BPA				EtP				n-PrP				BP-1			
		OR	**95% CI**		**P-value**	**OR**	**95% CI**		**P-value**	**OR**	**95% CI**		**P-value**	**OR**	**95% CI**		**P-value**
Sociodemographic, antropometric and reproductive characteristics	**Age (years)**	1.32	0.07	24.58	0.854	4.05	0.21	76.37	0.350	4.89	0.24	97.93	0.299	**4.82**	**14.88**	**1.56**	**0.004**
	BMI	7.14	0.42	122.50	0.175	0.29	0.02	4.69	0.383	0.82	0.05	12.98	0.891	0.53	0.02	15.91	0.716
	Occupational class[a]																
	Manual worker	1.50	0.21	10.81	0.687	4.00	0.38	41.74	0.247	1.80	0.25	12.99	0.560	0.44	0.06	3.42	0.434
	Non Manual worker	0.88	0.14	5.74	0.896	5.39	0.56	51.50	0.143	1.29	0.20	8.37	0.787	0.34	0.05	2.32	0.271
	Schooling level[b]																
	Universitary	0.85	0.33	2.17	0.729	1.83	0.71	4.71	0.207	2.33	0.89	6.14	0.086	1.32	0.41	4.24	0.641
	Alcohol[c]																
	At least 1 time/month	0.44	0.04	4.43	0.487	2.92	0.29	29.34	0.362	0.36	0.04	3.61	0.385	0.00	0.00	1.00	0.999
	Residence[d]																
	Sub-urban	0.84	0.27	2.64	0.771	1.97	0.62	6.23	0.249	0.36	0.11	1.16	0.087	0.60	0.15	2.47	0.480
	Urban	0.75	0.25	2.20	0.596	2.31	0.77	6.88	0.133	0.43	0.15	1.29	0.133	0.88	0.25	3.05	0.834
	Use of regular medication[c]																
	Yes	1.25	0.52	3.00	0.615	0.87	0.37	2.06	0.751	1.54	0.64	3.65	0.333	1.27	0.44	3.70	0.661
	Parity[e]																
	Multiparas	**2.67**	**1.09**	**6.55**	**0.032**	0.59	0.25	1.42	0.241	0.83	0.35	2.00	0.685	1.21	0.41	3.52	0.731
	Accumulated lactation time= *Months*	1.83	0.80	4.19	0.153	0.72	0.32	1.58	0.409	0.50	0.21	1.16	0.106	0.77	0.01	93.40	0.914
	Gestacional age =*Weeks*	0.68	0.01	35.69	0.848	5.17	0.09	281.49	0.420	0.69	0.01	35.49	0.855	1.97	0.70	5.57	0.202
	Weight before pregnancy[f]																
	Loss	0.95	0.30	3.02	0.934	1.33	0.43	4.18	0.622	2.93	0.87	9.89	0.083	4.65	0.54	39.97	0.162
	Gain	1.19	0.33	4.28	0.789	0.41	0.11	1.53	0.187	2.00	0.52	7.63	0.310	7.47	0.82	68.10	0.075
	Gestational diabetes[c]																
	Yes	3.03	0.52	17.65	0.217	0.46	0.08	2.67	0.387	1.15	0.22	6.06	0.872	0.71	0.08	6.54	0.764
	Use of composite filling[c]																
	Yes	0.46	0.16	1.31	0.146	2.98	0.99	8.96	0.052	1.66	0.57	4.81	0.355	**0.13**	**0.04**	**0.45**	**0.001**
Nutricional content	**Proteins (g/100mL)**	1.98	0.64	6.15	0.239	1.08	0.58	2.02	0.803	1.30	0.63	2.68	0.478	0.89	0.36	2.20	0.806
	Lipids (g/100mL)	0.96	0.65	1.41	0.826	0.90	0.61	1.33	0.604	0.81	0.54	1.21	0.297	1.13	0.71	1.81	0.598
	Calories (Kcal/100mL)	1.00	0.95	1.04	0.828	0.99	0.95	1.03	0.571	0.97	0.93	1.02	0.188	1.01	0.96	1.07	0.677
	Lactose (g/100mL)	0.80	0.16	3.94	0.786	2.63	0.52	1.33	0.242	**0.21**	**0.04**	**1.11**	**0.066**	0.19	0.03	1.45	0.110

Supplementary Table S3. Factors associated with human milk levels of BPA, EtP, PrP and BP-1 (N=83). Bivariate logistic regression analyses (continuación)

		BPA				EtP				n-PrP				BP-1			
		OR	95% CI		P-value	OR	95% CI		P-value	OR	95% CI		P-value	OR	95% CI		P-value
Dietary habits	**Frequency of** 1 time/week	1.02	0.23	4.47	0.981	1.20	0.27	5.25	0.809	2.62	0.57	12.00	0.213	2.50	0.24	25.68	0.441
	>1 time/week	0.42	0.11	1.58	0.197	1.30	0.35	4.86	0.692	1.36	0.35	5.27	0.655	3.33	0.39	28.82	0.274
	Frequency of lean fish consumption[f]																
	1 time/week	1.13	0.36	3.47	0.838	0.52	0.16	1.62	0.255	1.39	0.45	4.29	0.568	2.86	0.56	14.70	0.209
	>1 time/week	0.55	0.15	1.98	0.358	0.69	0.20	2.43	0.568	0.80	0.23	2.81	0.732	2.22	0.38	13.08	0.377
	Frequency of oil fish consumption[f]																
	1 time/week	0.60	0.22	1.62	0.311	0.83	0.31	2.19	0.702	0.96	0.36	2.56	0.939	2.00	0.60	6.69	0.261
	>1 time/week	1.14	0.32	4.07	0.837	0.88	0.25	3.11	0.837	1.14	0.32	4.07	0.837	0.83	0.14	4.93	0.841
	Frequency of dairy products consumption[g]																
	<1 time/day	0.47	0.19	1.22	0.120	2.25	0.90	5.65	0.084	1.53	0.62	3.78	0.356	0.76	0.25	2.30	0.622
	Frequency of milk consumption[h]																
	At least 1 glass/day	0.95	0.29	3.05	0.928	1.46	0.46	4.69	0.523	1.22	0.38	3.90	0.742	1.80	0.36	8.95	0.473
	Frequency of sausage consumption[i]																
	2 times/week	0.99	0.40	2.43	0.986	1.17	0.48	2.84	0.727	1.55	0.64	3.79	0.334	0.42	0.13	1.32	0.138
	Frequency of cheese consumption[j]																
	>2 times/week	1.22	0.41	3.58	0.722	0.88	0.30	2.57	0.819	0.67	0.23	1.95	0.460	0.67	0.19	2.33	0.525
	At least 1 time/day	0.83	0.24	2.78	0.757	0.69	0.21	2.28	0.547	1.20	0.37	3.92	0.763	0.59	0.14	2.48	0.474
	Frequency of meat consumption[k]																
	2 times/week	1.31	0.26	6.64	0.742	0.63	0.13	3.07	0.562	0.90	0.18	4.41	0.897	0.48	0.09	2.52	0.386
	>2 times/week	1.30	0.34	4.97	0.703	0.90	0.24	3.30	0.871	1.11	0.30	4.09	0.871	**0.21**	**0.05**	**0.85**	**0.029**
	Frequency of red meat consumption[l]																
	<1 time/week	1.79	0.58	5.52	0.314	0.54	0.18	1.62	0.271	0.78	0.27	2.31	0.660	1.44	0.38	5.52	0.595
	At least 1 time/week	1.81	0.54	6.04	0.333	0.41	0.12	1.34	0.140	0.50	0.15	1.62	0.249	1.18	0.27	5.12	0.821
	Frequency of pulse consumption[k]																
	2 times/week	1.01	0.27	3.82	0.988	0.52	0.14	1.93	0.325	0.84	0.23	3.10	0.797	4.91	0.55	43.53	0.153
	>2 times/week	1.35	0.37	4.95	0.653	0.66	0.18	2.43	0.533	1.24	0.34	4.43	0.746	3.00	0.33	27.12	0.328
	Frequency of cooked vegetable consumption[m]																
	>2 times/week	0.65	0.23	1.87	0.422	1.38	0.48	3.97	0.551	0.64	0.22	1.83	0.401	0.95	0.27	3.37	0.934
	Frequency of fresh vegetable consumption[m]																
	>2 times/week	0.48	0.16	1.45	0.194	1.03	0.34	3.09	0.955	0.62	0.21	1.88	0.400	0.35	0.11	1.17	0.089
	Frequency of fruit consumption[m]																
	>2 times/week	0.56	0.17	1.84	0.338	0.86	0.26	2.82	0.800	1.03	0.31	3.41	0.957	1.62	0.32	8.12	0.559
	Frequency of egg consumption[k]																
	2 times/week	1.46	0.42	5.04	0.551	1.00	0.30	3.37	1.000	0.86	0.25	2.93	0.806	2.55	0.47	13.77	0.278
	>2 times/week	1.08	0.32	3.70	0.898	1.06	0.32	3.51	0.921	1.54	0.46	5.13	0.480	1.88	0.34	10.32	0.465
	Frequency of bread consumption[n]																
	1 time/day	2.55	0.65	10.06	0.180	0.72	0.20	2.58	0.612	1.87	0.52	6.76	0.336	0.63	0.14	2.80	0.539
	>1 time/day	2.10	0.56	7.81	0.271	0.57	0.17	1.92	0.361	1.14	0.34	3.87	0.830	0.76	0.19	3.03	0.696
	Frequency of chocolate consumption[l]																
	<1 time/day	1.00	0.25	4.05	1.000	0.64	0.16	2.57	0.527	2.44	0.56	10.65	0.236	1.29	0.24	7.03	0.765
	At least 1 time/day	1.27	0.28	5.68	0.755	0.67	0.15	2.98	0.596	1.97	0.41	9.52	0.397	0.80	0.12	5.27	0.817
	Frequency of rice consumption[k]																
	>1 time/week	1.82	0.51	6.57	0.359	1.20	0.33	4.31	0.780	0.38	0.09	1.54	0.173	0.33	0.04	2.74	0.301
	Frequency of cereals consumption[l]																
	<1 time/day	0.37	0.13	1.03	0.058	1.43	0.53	3.84	0.480	0.54	0.20	1.47	0.228	0.93	0.29	2.97	0.904
	At least 1 time/day	0.54	0.15	1.92	0.343	0.71	0.20	2.53	0.602	0.71	0.20	2.48	0.593	0.48	0.09	2.69	0.406
	Consumption of canned food[c]																
	Yes	1.09	0.35	3.44	0.877	1.22	0.39	3.75	0.733	0.52	0.17	1.63	0.261	0.70	0.19	2.56	0.591
	Consumption of organic food[c]																
	Yes	0.74	0.29	1.89	0.535	2.04	0.80	5.21	0.138	1.28	0.51	3.24	0.600	1.01	0.33	3.10	0.988
	Consumption of fried food[f]																
	1 time/week	0.86	0.30	2.45	0.781	1.41	0.51	3.90	0.503	0.97	0.35	2.68	0.954	0.70	0.21	2.36	0.566
	>1 time/week	1.97	0.60	6.43	0.261	1.11	0.35	3.58	0.860	1.59	0.49	5.15	0.441	0.40	0.08	2.08	0.276
	Frequency of multivitamins consumption[c]																
	Yes	1.06	0.33	3.39	0.928	**7.93**	**1.64**	**38.32**	**0.010**	1.17	0.37	3.71	0.794	0.99	0.24	4.06	0.993
	Frequency of other nutritional supplements consumption[c]																
	Yes	1.17	0.40	3.42	0.778	0.42	0.14	1.28	0.127	0.64	0.22	1.83	0.401	1.49	0.38	5.89	0.570

Supplementary Table S3. Factors associated with human milk levels of BPA, EtP, PrP and BP-1 (N=83). Bivariate logistic regression analyses (continuación)

		BPA				EtP				n-PrP				BP-1			
		OR	95% CI		P-value	OR	95% CI		P-value	OR	95% CI		P-value	OR	95% CI		P-value
PCP habits	**Frequency of sunscreen use**[c]																
	Yes	0.83	0.34	2.04	0.690	2.17	0.88	5.32	0.092	1.04	0.43	2.52	0.927	0.95	0.32	2.78	0.923
	Sun creen application use[c]																
	Face	0.75	0.28	2.03	0.574	2.05	0.76	5.51	0.153	1.08	0.41	2.84	0.884	1.02	0.32	3.27	0.979
	Yes	1.06	0.28	4.06	0.927	2.47	0.62	9.79	0.198	0.96	0.25	3.67	0.958	0.79	0.14	4.33	0.786
	Protection factor use[p]																
	50	0.91	0.25	3.36	0.891	1.98	0.54	7.29	0.306	0.83	0.23	3.04	0.775	0.32	0.04	2.85	0.309
	>50	0.80	0.29	2.18	0.660	2.26	0.83	6.17	0.112	1.16	0.43	3.10	0.770	1.31	0.42	4.09	0.642
	Frequency of lip balm use[c]																
	Yes	1.26	0.50	3.14	0.624	1.32	0.53	3.27	0.548	1.11	0.45	2.74	0.824	0.58	0.18	1.84	0.352
	Frequency of facial cream use[n]																
	1 time/day	0.48	0.17	1.41	0.184	1.23	0.43	3.50	0.701	0.63	0.22	1.81	0.393	**6.90**	**1.38**	**34.60**	**0.019**
	>1 time/day	0.64	0.20	2.03	0.448	1.08	0.34	3.41	0.891	0.92	0.29	2.90	0.891	1.82	0.27	12.01	0.536
	Frequency of body lotion use[j]																
	<1 time/day	0.52	0.14	1.91	0.328	1.15	0.34	3.95	0.820	0.83	0.24	2.89	0.772	5.00	1.04	23.98	0.044
	At least 1 time/day	0.87	0.32	2.35	0.777	1.37	0.51	3.71	0.536	0.30	0.10	0.84	0.022	2.47	0.59	10.36	0.217
	Frequency of handcream use[n]																
	1 time/day	1.16	0.39	3.45	0.796	1.01	0.35	2.98	0.979	0.99	0.34	2.90	0.979	2.44	0.69	8.60	0.165
	>1 time/day	1.59	0.50	5.03	0.431	0.71	0.22	2.25	0.560	0.85	0.27	2.69	0.785	1.76	0.44	7.08	0.425
	Frequency of hair mask use[j]																
	At least 1 time/week	1.43	0.58	3.51	0.436	1.05	0.44	2.54	0.909	1.16	0.48	2.81	0.741	1.63	0.55	4.82	0.381
	Frequency of makeup use[j]																
	>1 time/day	1.01	0.34	3.02	0.986	0.71	0.24	2.10	0.540	0.69	0.23	2.06	0.511	1.35	0.38	4.75	0.641
	At least 1 time/day	0.97	0.31	3.04	0.961	0.55	0.18	1.73	0.309	0.67	0.21	2.08	0.487	0.81	0.19	3.44	0.775
	Frequency of facial tonic use[j]																
	At least 1 time/week	0.97	0.33	2.88	0.955	0.45	0.15	1.37	0.159	0.75	0.25	2.21	0.598	0.73	0.18	2.93	0.662
	Frequency of lipstick use[j]																
	<1 time/day	1.93	0.69	5.39	0.211	0.95	0.35	2.61	0.924	0.68	0.25	1.88	0.458	1.62	0.47	5.54	0.443
	At least 1 time/day	1.93	0.56	6.60	0.296	0.95	0.28	3.20	0.937	0.71	0.21	2.43	0.590	1.94	0.47	8.01	0.358
	Frequency of eye pencil use[j]																
	<1 time/day	2.89	0.96	8.70	0.059	1.08	0.37	3.16	0.889	1.65	0.56	4.83	0.363	0.54	0.13	2.25	0.395
	At least 1 time/day	1.77	0.58	5.41	0.314	0.44	0.14	1.34	0.146	0.82	0.27	2.47	0.729	1.07	0.31	3.78	0.911
	Frequency of eyeshadow use[j]																
	<1 time/day	2.11	0.71	6.26	0.180	0.45	0.15	1.34	0.149	0.83	0.28	2.45	0.738	1.79	0.51	6.31	0.362
	At least 1 time/day	1.12	0.28	4.49	0.870	0.30	0.07	1.30	0.107	0.69	0.17	2.75	0.603	2.00	0.43	9.26	0.375
	Frequency of facial milk use[j]																
	At least 1 time/week	3.93	0.71	21.65	0.116	0.71	0.15	3.40	0.668	0.17	0.02	1.46	0.105	0.58	0.07	5.21	0.629
	Frequency of nail polish use[j]																
	At least 1 time/week	0.99	0.29	3.46	0.994	0.65	0.19	2.27	0.502	0.52	0.14	1.88	0.314	0.29	0.03	2.42	0.253
	Frequency of shampoo use[q]																
	>3 times/week	1.56	0.59	4.13	0.368	1.04	0.41	2.65	0.937	1.66	0.64	4.32	0.298	1.23	0.38	3.95	0.729
	Frequency of bath gel use[n]																
	>1 time/day	0.70	0.13	3.69	0.672	0.18	0.02	1.66	0.131	0.16	0.02	1.40	0.097	1.40	0.15	12.89	0.764
	Frequency of deodorant use[n]																
	1 time/day	2.59	0.48	13.98	0.270	1.08	0.24	4.76	0.922	0.86	0.20	3.81	0.845	0.60	0.10	3.46	0.568
	>1 time/day	1.64	0.25	10.77	0.608	0.89	0.17	4.78	0.891	0.89	0.17	4.78	0.891	1.64	0.25	10.77	0.608
	Frequency of hair conditioner use[j]																
	At least 1 time/week	**3.12**	**1.17**	**8.36**	**0.023**	1.16	0.47	2.86	0.748	0.90	0.37	2.23	0.824	1.24	0.41	3.78	0.707
	Frequency of hairspray, hair wax and/or hair mousse use[j]																
	At least 1 time/week	1.38	0.51	3.78	0.527	2.46	0.87	7.00	0.091	0.80	0.29	2.20	0.670	0.13	0.02	1.06	0.057
	Frequency of cologne and/or perfume use[j]																
	<1 time/day	1.00	0.23	4.35	1.000	2.80	0.66	11.92	0.164	1.96	0.48	7.99	0.348	1.00	0.16	6.42	1.000
	At least 1 time/day	1.91	0.50	7.30	0.345	2.10	0.55	8.01	0.280	1.01	0.28	3.69	0.990	1.72	0.32	9.09	0.524
	Frequency of toothpaste use[g]																
	>1 time/day	1.41	0.46	4.31	0.542	0.89	0.30	2.60	0.830	1.83	0.60	5.58	0.286	5.57	0.68	45.39	0.109
	Frequency of mouthwash consumption use[j]																
	<1 time/day	1.00	0.26	3.83	1.000	1.84	0.49	6.94	0.368	2.57	0.68	9.71	0.164	1.27	0.29	5.57	0.754
	At least 1 time/day	**5.20**	**1.58**	**17.15**	**0.007**	0.59	0.19	1.77	0.342	1.03	0.35	3.06	0.960	1.09	0.29	4.03	0.902
	Frequency of facial treatment use[l]																
	<1 time/month	0.58	0.16	2.08	0.406	1.29	0.40	4.17	0.672	1.58	0.49	5.12	0.446	2.42	0.68	8.67	0.173
	Months	7.29	0.80	66.40	0.078	0.48	0.08	2.84	0.421	0.59	0.10	3.49	0.563	0.87	0.09	8.24	0.905
	Frequency of manicure use[r]																
	Months	0.56	0.13	2.34	0.424	2.55	0.61	10.66	0.201	1.16	0.31	4.36	0.830	0.90	0.17	4.69	0.901
	Frequency of pedicure use[r]																
	Months	0.77	0.21	2.87	0.696	1.86	0.50	6.93	0.357	1.43	0.40	5.15	0.582	1.45	0.34	6.17	0.618
	Frequency of artificial nails use[r]																
	Months	1.05	0.22	5.04	0.951	0.71	0.15	3.40	0.668	0.42	0.08	2.32	0.322	0.58	0.07	5.21	0.629
	Frequency of massage use[l]																
	<1 time/month	0.55	0.13	2.33	0.417	1.00	0.26	3.79	1.000	0.46	0.11	1.92	0.285	0.82	0.16	4.27	0.810
	Months	0.86	0.13	5.48	0.871	1.50	0.23	9.59	0.668	1.60	0.25	10.21	0.621	0.00	0.00	1.00	0.999
	Frequency of hair dye use[l]																
	<1 time/month	1.62	0.54	4.87	0.390	0.54	0.19	1.54	0.252	0.79	0.28	2.25	0.656	3.20	0.90	11.38	0.072
	Months	**9.45**	**2.51**	**35.63**	**0.001**	0.76	0.25	2.36	0.637	2.62	0.81	8.51	0.108	1.83	0.43	7.85	0.417
	Months	1.45	0.34	6.26	0.620	0.65	0.07	6.17	0.704	0.65	0.15	2.94	0.579	2.44	0.52	11.47	0.258

Supplementary Table S3. Factors associated with human milk levels of BPA, EtP, PrP and BP-1 (N=83). Bivariate logistic regression analyses (continuación)

BPA: PA: bisphenol A; EtP: ethylparaben; n-PrP: n-propylparaben; BP-1: benzophenone 1;

[a]: reference category= housewife;

[b]: reference category= non-university;

[c]: reference category= no;

[d]: reference category= rural;

[e]: reference category= same;

[f]: reference category= <1 time/ week ;

[g]: reference category= At least 1 time/day;

[h]: reference category= <1 glass/ day

[i]: reference category= ≤2 times / week;

[j]: reference category= R/N ;

[k]: reference category= At least 1 time/week;

[l]: reference category= Never;

[m]: reference category= ≤2 times/ week;

[n]: reference category= <1 time/ day;

[o]: reference category= 1 time/ week;

[p]: category reference= 0;

[q]: category reference= <3 times/week.

[r]: category reference: <1 time/month.

Supplementary Table S4. Factors associated with human milk levels of MeP, ΣPBs, BP-3, ΣBPs and ΣEDCs (N=83). Bivariate linear regression analyses

		MeP				ΣPBs				BP-3				ΣBPs				ΣEDCs			
		exp(β)	95% CI		p-value	exp(β)	95% CI		p-value	exp(β)	95% CI		p-value	exp(β)	95% CI		p-value	exp(β)	95% CI		p-value
Sociodemographic, antropometric and reproductive characterisics	**Age (years)**	0.78	0.13	4.53	0.777	0.80	-2.88	2.44	0.868	3.44	0.91	12.97	0.068	2.32	-0.70	2.38	0.278	1.45	-1.58	2.33	0.701
	BMI	0.78	0.14	4.27	0.772	1.24	-2.17	2.56	0.857	0.76	0.20	2.83	0.676	0.37	-2.39	0.41	0.162	0.74	-2.07	1.47	0.734
	Occupational class[a]																				
	Manual worker	1.56	0.47	5.14	0.461	1.62	-1.36	2.32	0.598	0.77	0.30	1.94	0.572	1.01	-1.09	1.10	0.991	1.61	-0.88	1.84	0.482
	Non Manual worker	1.15	0.37	3.54	0.807	1.66	-1.25	2.27	0.564	0.89	0.37	2.13	0.788	1.20	-0.87	1.23	0.726	1.47	-0.92	1.70	0.551
	Schooling level[b]																				
	Universitary	0.62	0.35	1.08	0.091	0.58	-1.40	0.30	0.197	0.90	0.58	1.41	0.642	1.03	-0.50	0.56	0.909	0.57	-1.18	0.06	0.075
	Alcohol[c]																				
	At least one/month	0.32	0.10	1.08	0.065	0.94	-1.49	1.37	0.934	0.54	0.21	1.38	0.195	0.85	-1.01	0.67	0.691	0.87	-1.19	0.91	0.788
	Residence[d]																				
	Sub-urban	0.81	0.41	1.60	0.536	1.35	-0.72	1.32	0.555	0.84	0.49	1.44	0.511	0.94	-0.69	0.57	0.838	1.32	-0.48	1.04	0.463
	Urban	0.54	0.28	1.04	0.063	0.75	-1.26	0.70	0.559	0.75	0.45	1.25	0.260	1.05	-0.55	0.66	0.862	0.87	-0.87	0.59	0.705
	Use of regular medication[c]																				
	Yes	**1.92**	**1.16**	**3.18**	**0.012**	**2.67**	**0.312**	**1.65**	**0.005**	1.23	0.82	1.84	0.303	0.94	-0.50	0.37	0.767	**1.74**	**0.04**	**1.07**	**0.035**
	Parity[e]																				
	Multiparas	0.71	0.42	1.20	0.200	0.94	-0.839	0.72	0.873	1.47	0.99	2.20	0.057	1.47	-0.06	0.83	0.085	1.16	-0.42	0.72	0.601
	Accumulated lactation time= *Months*	0.71	0.48	1.06	0.091	1.03	-0.024	0.08	0.278	0.89	0.59	1.36	0.586	0.97	-0.06	0.00	0.053	1.01	-0.03	0.05	0.554
	Gestacional age =*Weeks*	0.10	0.01	1.04	0.054	**0.01**	**-8.563**	**-1.88**	**0.003**	0.35	0.06	2.21	0.261	1.83	-1.58	2.79	0.578	0.06	-5.42	-0.23	0.034
	Weight before pregnancy[f]																				
	Loss	1.62	0.75	3.54	0.218	1.65	-0.842	1.85	0.453	1.17	0.64	2.13	0.610	0.98	-0.84	0.81	0.962	1.85	-0.39	1.62	0.224
	Gain	1.40	0.74	2.66	0.298	1.69	-0.663	1.71	0.377	0.78	0.48	1.29	0.330	0.76	-0.99	0.46	0.458	1.83	-0.28	1.49	0.173
	Gestational diabetes[c]																				
	Yes	0.71	0.25	1.97	0.503	0.99	-1.788	1.77	0.991	0.69	0.31	1.53	0.361	0.57	-1.63	0.49	0.284	1.10	-1.23	1.42	0.883
	Use of composite filling[c]																				
	Yes	1.08	0.56	2.08	0.815	1.43	-0.654	1.38	0.476	0.73	0.44	1.21	0.218	0.99	-0.63	0.61	0.976	1.02	-0.74	0.78	0.958
Nutricional content	**Proteins (g/100mL)**	1.09	-0.27	0.44	0.649	1.40	-0.02	0.70	0.067	1.11	-0.18	0.39	0.471	1.11	-0.13	0.34	0.363	1.27	-0.02	0.50	0.067
	Lipids (g/100mL)	1.04	-0.18	0.26	0.725	0.92	-0.43	0.26	0.615	1.05	-0.13	0.22	0.618	1.00	-0.23	0.22	0.966	0.88	-0.37	0.12	0.298
	Calories (Kcal/100mL)	1.00	-0.02	0.03	0.792	0.99	-0.05	0.03	0.526	1.00	-0.02	0.02	0.669	1.00	-0.02	0.03	0.899	0.99	-0.04	0.01	0.281
	Lactose (g/100mL)	0.80	-1.14	0.69	0.627	0.48	-2.20	0.73	0.313	0.69	-1.10	0.37	0.326	1.73	-0.37	1.47	0.234	0.96	-1.10	1.01	0.932

Supplementary Table S4. Factors associated with human milk levels of MeP, ΣPBs, BP-3, ΣBPs and ΣEDCs (N=83). Bivariate linear regression analyses (continuación)

		MeP				ΣPBs				BP-3				ΣBPs				ΣEDCs			
		exp(β)	95% CI		p-value	exp(β)	95% CI		p-value	exp(β)	95% CI		p-value	exp(β)	95% CI		p-value	exp(β)	95% CI		p-value
Dietary habits	**Frequency of fish and shellfish consumption**[f]																				
	1 time/week	1.66	0.68	4.08	0.264	1.02	-1.15	1.20	0.968	0.95	0.47	1.93	0.887	0.89	-0.83	0.59	0.733	1.03	-0.87	0.92	0.950
	>1 time/week	1.97	0.88	4.38	0.097	1.69	-0.54	1.58	0.322	1.18	0.63	2.22	0.600	0.69	-1.02	0.26	0.241	1.06	-0.75	0.86	0.890
	Frequency of lean fish consumption[f]																				
	1 time/week	1.62	0.83	3.16	0.151	1.16	-0.68	0.98	0.721	1.08	0.63	1.85	0.776	0.86	-0.69	0.38	0.569	1.06	-0.60	0.71	0.863
	>1 time/week	**2.74**	**1.32**	**5.70**	**0.008**	**3.72**	**0.29**	**2.34**	**0.014**	1.43	0.79	2.57	0.235	0.59	-1.20	0.13	0.109	1.92	-0.16	1.46	0.113
	Frequency of oil fish consumption[f]																				
	1 time/week	1.35	0.74	2.46	0.321	1.28	-0.57	1.06	0.544	1.30	0.81	2.08	0.267	1.06	-0.45	0.57	0.818	1.20	-0.43	0.80	0.547
	>1 time/week	1.53	0.70	3.34	0.281	2.73	-0.34	2.34	0.137	1.22	0.67	2.25	0.510	0.99	-0.85	0.82	0.976	1.64	-0.52	1.51	0.326
	Frequency of dairy products consumption[g]																				
	<1 time/day	0.73	0.42	1.26	0.257	0.73	-1.09	0.46	0.416	1.05	0.68	1.62	0.819	0.91	-0.57	0.38	0.696	0.76	-0.86	0.30	0.332
	Frequency of milk consumption[h]																				
	At least 1 glass/day	1.58	0.78	3.19	0.204	1.70	-0.43	1.49	0.269	0.86	0.49	1.49	0.586	0.95	-0.63	0.54	0.871	1.27	-0.48	0.96	0.504
	Frequency of sausage consumption[i]																				
	2 times/week	1.08	0.62	1.86	0.792	1.31	-0.51	1.05	0.486	1.03	0.68	1.58	0.874	0.92	-0.56	0.39	0.715	1.14	-0.45	0.72	0.654
	Frequency of cheese consumption[j]																				
	>2 times/week	1.37	0.72	2.62	0.332	1.09	-0.85	1.03	0.849	0.92	0.55	1.54	0.748	0.73	-0.89	0.27	0.281	1.18	-0.53	0.87	0.633
	At least one glass/day	0.73	0.35	1.49	0.377	0.55	-1.61	0.40	0.231	0.78	0.44	1.38	0.386	0.87	-0.76	0.47	0.639	0.69	-1.12	0.37	0.316
	Frequency of meat consumption[k]																				
	2 times/week	1.82	0.69	4.81	0.223	0.97	-1.36	1.29	0.959	1.79	0.87	3.69	0.111	1.13	-0.71	0.95	0.773	1.06	-0.96	1.08	0.903
	>2 times/week	1.60	0.72	3.54	0.247	1.99	-0.33	1.71	0.177	0.80	0.44	1.44	0.446	0.87	-0.77	0.50	0.665	1.38	-0.46	1.10	0.408
	Frequency of red meat consumption[l]																				
	<1 time/week	1.14	0.58	2.23	0.700	0.76	-1.15	0.60	0.525	1.31	0.78	2.19	0.305	1.35	-0.23	0.83	0.255	0.86	-0.80	0.49	0.632
	At least 1 time/week	0.84	0.44	1.61	0.589	0.67	-1.47	0.66	0.446	0.77	0.47	1.28	0.312	0.69	-1.01	0.27	0.253	0.67	-1.19	0.39	0.311
	Frequency of pulse consumption[k]																				
	2 times/week	1.18	0.53	2.61	0.685	1.37	-0.78	1.40	0.565	0.75	0.41	1.39	0.356	0.79	-0.88	0.40	0.451	1.35	-0.49	1.08	0.450
	>2 times/week	0.77	0.35	1.68	0.504	0.89	-1.17	0.94	0.823	0.56	0.31	1.01	0.055	**0.59**	**-1.14**	**0.09**	**0.091**	0.77	-1.03	0.50	0.487
	Frequency of cooked vegetable consumption[m]																				
	>2 times/week	0.62	0.33	1.17	0.137	0.46	-1.71	0.17	0.106	0.93	0.56	1.54	0.766	0.86	-0.73	0.44	0.617	**0.55**	**-1.29**	**0.11**	**0.095**
	Frequency of fresh vegetable consumption[m]																				
	>2 times/week	**0.38**	**0.20**	**0.71**	**0.003**	**0.22**	**-2.57**	**-0.45**	**0.007**	0.79	0.47	1.34	0.381	1.06	-0.65	0.76	0.874	**0.28**	**-2.04**	**-0.52**	**0.002**
	Frequency of fruit consumption[m]																				
	>2 times/week	1.30	0.63	2.71	0.477	0.88	-1.06	0.80	0.777	1.04	0.59	1.85	0.882	0.63	-1.01	0.07	0.088	0.57	-1.23	0.10	0.094
	Frequency of egg consumption[k]																				
	2 times/week	1.19	0.56	2.51	0.647	1.45	-0.70	1.44	0.483	0.96	0.54	1.72	0.892	0.58	-1.17	0.07	0.082	1.43	-0.43	1.15	0.365
	>2 times/week	0.83	0.40	1.74	0.625	0.88	-1.19	0.93	0.805	1.22	0.69	2.17	0.487	0.86	-0.77	0.46	0.618	0.94	-0.84	0.73	0.883
	Frequency of bread consumption[n]																				
	1 time/day	0.99	0.45	2.16	0.981	1.12	-1.13	1.35	0.856	0.92	0.50	1.70	0.796	0.51	-1.43	0.07	0.073	1.01	-0.93	0.95	0.984
	>1 time/day	0.79	0.38	1.66	0.531	0.54	-1.80	0.58	0.307	0.90	0.50	1.60	0.712	0.56	-1.30	0.13	0.107	0.66	-1.32	0.48	0.350
	Frequency of chocolate consumption[l]																				
	<1 time/day	0.78	0.33	1.82	0.559	0.43	-2.15	0.48	0.205	1.71	0.90	3.25	0.100	0.89	-0.91	0.67	0.762	0.67	-1.37	0.57	0.403
	At least 1 time/day	0.74	0.30	1.86	0.522	0.64	-1.82	0.94	0.523	**2.19**	**1.10**	**4.38**	**0.026**	1.28	-0.58	1.08	0.546	1.09	-0.93	1.11	0.862
	Frequency of rice consumption[k]																				
	>1 time/week	**0.45**	**0.21**	**0.96**	**0.040**	0.42	-1.92	0.18	0.101	1.20	0.65	2.21	0.552	1.04	-0.62	0.69	0.908	0.66	-1.21	0.39	0.303
	Frequency of cereals consumption[l]																				
	<1 time/day	0.92	0.50	1.68	0.786	0.57	-1.42	0.28	0.182	1.18	0.73	1.89	0.499	0.97	-0.56	0.50	0.904	0.58	-1.18	0.09	0.090
	At least 1 time/day	0.55	0.26	1.18	0.123	0.39	-1.97	0.07	0.067	1.12	0.61	2.04	0.713	1.34	-0.35	0.93	0.360	0.57	-1.33	0.20	0.142
	Consumption of canned food[c]																				
	Yes	0.60	0.30	1.18	0.136	0.77	-1.44	0.91	0.650	**0.49**	**0.29**	**0.83**	**0.008**	0.79	-0.94	0.47	0.502	0.84	-1.05	0.69	0.680
	Consumption of organic food[c]																				
	Yes	0.60	0.34	1.04	0.068	0.58	-1.34	0.26	0.179	0.86	0.55	1.34	0.503	0.80	-0.71	0.27	0.363	0.62	-1.06	0.11	0.110
	Consumption of fried food[f]																				
	1 time/week	1.08	0.59	2.01	0.795	0.88	-1.00	0.75	0.774	1.04	0.64	1.70	0.859	0.93	-0.64	0.50	0.807	0.81	-0.82	0.41	0.506
	>1 time/week	1.80	0.88	3.67	0.107	**2.75**	**0.08**	**1.94**	**0.034**	0.98	0.56	1.72	0.938	1.09	-0.52	0.69	0.784	**2.44**	**0.23**	**1.55**	**0.010**
	Frequency of multivitamins consumption[c]																				
	Yes	0.72	0.35	1.46	0.356	0.95	-0.95	0.85	0.911	**0.48**	**0.28**	**0.82**	**0.008**	**0.49**	**-1.20**	**-0.22**	**0.006**	0.90	-0.77	0.57	0.757
	Frequency of other nutritional supplements consumption[c]																				
	Yes	1.00	0.52	1.92	1.000	0.84	-1.07	0.72	0.695	1.52	0.93	2.50	0.097	1.41	-0.19	0.88	0.196	1.09	-0.58	0.76	0.795

Supplementary Table S4. Factors associated with human milk levels of MeP, ΣPBs, BP-3, ΣBPs and ΣEDCs (N=83). Bivariate linear regression analyses (continuación)

		MeP				ΣPBs				BP-3				ΣBPs				ΣEDCs			
		exp(β)	95% CI		p-value	exp(β)	95% CI		p-value	exp(β)	95% CI		p-value	exp(β)	95% CI		p-value	exp(β)	95% CI		p-value
PCP habits	**Frequency of sunscreen use[c]**																				
	Yes	0.79	0.46	1.35	0.378	0.67	-1.17	0.38	0.308	1.44	0.95	2.18	0.088	1.18	-0.31	0.64	0.481	0.67	-0.97	0.17	0.166
	Sun creen application use[c]																				
	Face	0.78	0.43	1.42	0.415	0.66	-1.26	0.44	0.333	1.39	0.88	2.21	0.156	1.19	-0.34	0.69	0.492	0.68	-1.01	0.24	0.217
	Yes	1.02	0.43	2.43	0.963	0.71	-1.58	0.88	0.569	1.11	0.57	2.16	0.756	1.14	-0.62	0.88	0.720	0.65	-1.34	0.48	0.343
	Protection factor use[p]																				
	50	0.80	0.36	1.78	0.581	0.76	-1.36	0.80	0.603	1.09	0.60	2.00	0.777	1.08	-0.58	0.74	0.810	0.74	-1.09	0.50	0.456
	>50	0.97	0.42	2.27	0.946	0.84	-1.32	0.97	0.758	1.50	0.79	2.84	0.217	1.14	-0.56	0.83	0.700	0.86	-1.00	0.69	0.710
	Frequency of lip balm use[c]																				
	Yes	**0.55**	**0.32**	**0.94**	**0.030**	0.60	-1.27	0.26	0.192	0.91	0.59	1.40	0.650	1.03	-0.45	0.50	0.916	0.67	-0.96	0.18	0.169
	Frequency of facial cream use[n]																				
	1 time/day	1.18	0.62	2.25	0.611	0.61	-1.41	0.44	0.292	**1.79**	**1.11**	**2.89**	**0.017**	1.65	-0.02	1.02	0.059	0.83	-0.89	0.51	0.583
	>1 time/day	0.82	0.40	1.67	0.580	0.68	-1.38	0.60	0.427	**2.10**	**1.24**	**3.55**	**0.006**	**1.94**	**0.11**	**1.22**	**0.020**	0.80	-0.97	0.52	0.540
	Frequency of body lotion use[j]																				
	<1 time/ day	0.99	0.47	2.09	0.972	0.64	-1.44	0.54	0.362	0.67	0.38	1.19	0.169	0.62	-1.08	0.12	0.114	0.65	-1.16	0.29	0.230
	At least 1 time/day	1.62	0.88	2.97	0.118	1.63	-0.39	1.37	0.269	1.46	0.92	2.30	0.103	1.09	-0.45	0.61	0.755	1.48	-0.25	1.03	0.225
	Frequency of handcream use[n]																				
	1 time/day	1.79	0.95	3.40	0.073	1.32	-0.73	1.29	0.575	1.00	0.60	1.67	0.996	0.80	-0.85	0.41	0.486	1.24	-0.53	0.96	0.560
	>1 time/day	**2.28**	**1.15**	**4.50**	**0.018**	2.94	-0.08	2.23	0.066	1.39	0.80	2.41	0.235	0.88	-0.86	0.60	0.719	**2.33**	**-0.01**	**1.70**	**0.052**
	Frequency of hair mask use[j]																				
	at least once/week	0.82	0.48	1.42	0.476	0.94	-0.85	0.72	0.876	1.09	0.71	1.66	0.689	0.93	-0.55	0.40	0.755	0.80	-0.81	0.35	0.431
	Frequency of makeup use[j]																				
	<1 time/day	0.87	0.45	1.70	0.688	1.07	-0.90	1.04	0.883	1.32	0.79	2.22	0.283	1.11	-0.48	0.69	0.720	1.30	-0.45	0.98	0.455
	At least 1 time/day	0.66	0.33	1.32	0.235	0.65	-1.49	0.63	0.418	0.90	0.53	1.54	0.705	0.90	-0.75	0.54	0.745	0.72	-1.11	0.46	0.405
	Frequency of facial tonic use[j]																				
	At least 1 time/week	1.11	0.57	2.16	0.746	1.04	-1.05	1.13	0.939	1.27	0.76	2.12	0.361	0.85	-0.81	0.50	0.630	1.23	-0.60	1.01	0.614
	Frequency of lipstick use[j]																				
	<1 time/ day	1.09	0.58	2.02	0.788	1.37	-0.60	1.23	0.486	1.00	0.62	1.62	0.998	1.00	-0.56	0.56	0.990	1.51	-0.26	1.08	0.218
	At least 1 time/day	0.67	0.32	1.41	0.286	0.80	-1.30	0.84	0.668	1.40	0.78	2.50	0.257	1.00	-0.65	0.66	0.995	0.89	-0.90	0.67	0.769
	Frequency of eye pencil use[j]																				
	<1 time/ day	1.06	0.55	2.05	0.861	1.55	-0.45	1.32	0.322	0.85	0.51	1.42	0.537	0.91	-0.64	0.45	0.714	1.60	-0.18	1.12	0.153
	At least 1 time/day	0.73	0.38	1.43	0.358	0.77	-1.30	0.76	0.604	1.00	0.60	1.69	0.989	0.85	-0.80	0.47	0.603	0.97	-0.79	0.73	0.943
	Frequency of eyeshadow use[j]																				
	<1 time/ day	0.97	0.50	1.88	0.917	1.75	-0.52	1.64	0.300	1.16	0.69	1.94	0.576	1.11	-0.57	0.78	0.755	1.64	-0.32	1.31	0.222
	At least 1 time/day	0.65	0.28	1.49	0.303	0.57	-1.72	0.61	0.341	1.44	0.75	2.76	0.267	0.90	-0.84	0.62	0.761	0.84	-1.05	0.70	0.689
	Frequency of facial milk use[j]																				
	At least 1 time/week	0.68	0.26	1.78	0.431	0.79	-1.53	1.06	0.713	1.07	0.51	2.25	0.865	1.25	-0.55	1.00	0.561	1.12	-0.85	1.07	0.820
	Frequency of nail polish use[j]																				
	At least 1 time/week	0.66	0.31	1.40	0.275	0.41	-2.03	0.24	0.120	1.33	0.74	2.40	0.332	1.40	-0.36	1.04	0.335	0.73	-1.19	0.55	0.462
	Frequency of shampoo use[q]																				
	>3 times/week	1.20	0.67	2.13	0.542	1.14	-0.69	0.94	0.755	1.11	0.71	1.74	0.654	1.18	-0.33	0.66	0.502	0.86	-0.76	0.45	0.610
	Frequency of bath gel use[n]																				
	>1 time/day	0.84	0.30	2.34	0.733	0.98	-1.19	1.16	0.976	0.78	0.35	1.73	0.532	0.57	-1.25	0.12	0.105	0.99	-0.88	0.87	0.987
	Frequency of deodorant use[n]																				
	1 time/day	**0.38**	**0.16**	**0.92**	**0.031**	0.57	-1.98	0.84	0.419	1.20	0.60	2.43	0.600	1.14	-0.77	1.04	0.771	0.79	-1.32	0.84	0.653
	>1 time/day	0.88	0.33	2.36	0.802	1.82	-1.11	2.30	0.482	1.84	0.83	4.08	0.130	1.30	-0.83	1.36	0.629	1.63	-0.81	1.78	0.453
	Frequency of hair conditioner use[j]																				
	At least 1 time/week	1.21	0.69	2.11	0.498	0.96	-0.83	0.75	0.918	1.09	0.71	1.68	0.699	0.87	-0.61	0.34	0.560	1.06	-0.54	0.64	0.853
	Frequency of hairspray, hair wax and/or hair mousse use[j]																				
	At least 1 time/week	1.26	0.68	2.33	0.460	1.33	-0.53	1.10	0.483	0.81	0.50	1.31	0.381	1.03	-0.47	0.52	0.906	1.65	-0.08	1.09	0.090
	Frequency of cologne and/or perfume use[j]																				
	<1 time/day	1.35	0.59	3.06	0.475	1.57	-0.70	1.60	0.432	0.95	0.49	1.84	0.878	0.84	-0.88	0.55	0.633	1.08	-0.79	0.94	0.861
	At least 1 time/day	**2.46**	**1.15**	**5.25**	**0.021**	2.69	-0.12	2.10	0.078	1.35	0.73	2.49	0.328	1.19	-0.52	0.86	0.617	1.77	-0.26	1.40	0.171
	Frequency of toothpaste use[g]																				
	>1 time/day	1.12	0.58	2.18	0.725	0.98	-0.95	0.91	0.962	1.34	0.81	2.24	0.253	1.11	-0.46	0.67	0.706	1.08	-0.62	0.77	0.831
	Frequency of mouthwash consumption use[j]																				
	<1 time/ day	**3.00**	**1.48**	**6.10**	**0.003**	**3.59**	**0.37**	**2.19**	**0.007**	0.87	0.48	1.56	0.628	0.69	-1.04	0.29	0.263	**2.13**	**0.08**	**1.44**	**0.030**
	At least 1 time/day	**3.03**	**1.65**	**5.57**	**0.001**	**5.23**	**0.74**	**2.57**	**0.001**	**1.88**	**1.14**	**3.11**	**0.014**	1.10	-0.57	0.76	0.770	**3.82**	**0.66**	**2.02**	**0.000**
	Frequency of facial treatment use[l]																				
	<1 time/month	**2.33**	**1.16**	**4.67**	**0.018**	2.03	-0.45	1.87	0.222	1.67	0.96	2.89	0.067	0.95	-0.78	0.67	0.876	1.88	-0.21	1.47	0.136
	Months	0.76	0.28	2.08	0.595	2.33	-0.91	2.60	0.336	0.87	0.39	1.92	0.723	1.02	-1.07	1.12	0.968	2.65	-0.30	2.25	0.130
	Frequency of manicure use[r]																				
	Months	1.62	0.72	3.64	0.242	1.17	-1.02	1.33	0.790	1.10	0.58	2.09	0.758	0.84	-0.88	0.53	0.621	1.07	-0.80	0.95	0.868
	Frequency of pedicure use[r]																				
	Months	1.06	0.48	2.34	0.876	0.86	-1.32	1.02	0.798	1.19	0.64	2.18	0.581	0.95	-0.76	0.66	0.879	0.98	-0.90	0.85	0.962
	Frequency of artificial nails use[r]																				
	Months	1.12	0.43	2.92	0.815	0.91	-1.87	1.68	0.914	1.83	0.88	3.81	0.105	**2.82**	**0.02**	**2.06**	**0.046**	1.33	-1.04	1.61	0.664
	Frequency of massage use[l]																				
	<1 time/month	0.90	0.40	2.05	0.800	0.34	-2.22	0.04	0.059	1.01	0.54	1.91	0.975	0.77	-0.95	0.44	0.462	0.49	-1.57	0.14	0.096
	Months	0.55	0.18	1.70	0.295	0.53	-1.89	0.61	0.304	0.52	0.22	1.24	0.137	0.53	-1.40	0.14	0.105	0.61	-1.44	0.44	0.287
	Frequency of hair dye consumption use[l]																				
	<1 time/month	0.88	0.46	1.66	0.679	0.78	-1.22	0.71	0.597	1.52	0.93	2.47	0.091	0.86	-0.73	0.44	0.614	0.92	-0.80	0.64	0.824
	Months	0.79	0.39	1.59	0.501	0.75	-1.25	0.68	0.548	1.43	0.84	2.44	0.182	1.15	-0.44	0.72	0.627	1.10	-0.63	0.82	0.791
	Frequency of permanent, hair strengthening use[l]																				
	Months	0.48	0.09	2.71	0.404	2.57	-0.31	2.20	0.136	1.97	0.52	7.53	0.317	0.98	-0.80	0.76	0.956	2.18	-0.15	1.71	0.097

Supplementary Table S4. Factors associated with human milk levels of MeP, ΣPBs, BP-3, ΣBPs and ΣEDCs (N=83). Bivariate linear regression analyses (continuación)

MeP: methylparaben; PBs: parabens; BP-3: benzophenone 3; BPs: benzophenones; EDCs: endocrine disrupting compunds;

[a] *: reference category= housewife;*

[b] *: reference category= non-university;*

[c] *: reference category= no;*

[d] *: reference category= rural;*

[e] *: reference category= same;*

[f] *: reference category= <1 time/ week;*

[g] *: reference category= At least 1 time/day;*

[h] *: reference category= <1 glass/ day;*

[i] *: reference category= ≤2 times / week;*

[j] *: reference category= R/N;*

[k] *: reference category= At least 1 time/week;*

[l] *: reference category= Never;*

[m] *: reference category= ≤2 times/ week;*

[n] *: reference category= <1 time/ day;*

[o] *: reference category= 1 time/ week;*

[p] *: category reference= 0;*

ANEXO V. MATERIAL SUPLEMENTARIO ARTÍCULO *"Concentrations of bisphenol A and parabens in socks for infants and young children in Spain and their hormone-like activities"*

Table S1. Characteristics of samples, concentrations of BPA and parabens, and hormone-like activities in socks collected in store 1 (N=36).

	Characteristics of samples											Chemical concentrations (ng/g)					Hormone-like activity	
						Composition (%)						Parabens					E-screen	PALM
Sample no.	Pack	Price (€)	Age (months)	Country of origin[a]	Color	Cotton	Polyester	Polyamide	Elastane	Section	BPA	MPB	EPB	PPB	BPB	∑PBs	(pM E_2eq/g)	(μM Proceq/g)
1					White, blue symbol	85	10	0	5	Toe	131	<0.50	3.17	<0.40	<0.50	3.17	–	–
2					White, blue symbol	85	10	0	5	Foot	139	<0.50	3.38	<0.40	<0.50	3.38	57.3	n.s.
3					White, blue symbol	85	10	0	5	Leg	163	<0.50	3.92	<0.40	<0.50	3.92	–	–
4					White, red symbol	85	10	0	5	Toe	143	<0.50	2.79	<0.40	<0.50	2.79	–	–
5	1	1.5	36-48	Spain	White, red symbol	85	10	0	5	Foot	150	<0.50	3.55	<0.40	<0.50	3.55	75.8	n.s.
6					White, red symbol	85	10	0	5	Leg	158	<0.50	2.57	<0.40	<0.50	2.57	–	–
7					White, grey symbol	85	10	0	5	Toe	146	<0.50	2.71	<0.40	<0.50	2.71	–	–
8					White, grey symbol	85	10	0	5	Foot	131	<0.50	3.3	<0.40	<0.50	3.3	175	n.s.
9					White, grey symbol	85	10	0	5	Leg	149	<0.50	3.89	<0.40	<0.50	3.89	–	–
10					Grey and black	97	0	0	3	Toe	1,153	1.8	1.93	<0.40	<0.50	3.73	–	–
11					Grey and black	97	0	0	3	Foot	3,763	1.66	1.32	<0.40	<0.50	2.98	105	1,219
12					Grey and black	97	0	0	3	Leg	976	1.09	1.47	<0.40	<0.50	2.56	–	–
13					Grey and black	97	0	0	3	Toe	1,143	1.87	1.49	<0.40	<0.50	3.36	–	–
14	2	1.5	36-48	Italy	Grey and black	97	0	0	3	Foot	75.6	2.54	1.01	<0.40	<0.50	3.55	48.2	609
15					Grey and black	97	0	0	3	Leg	1,424	2.17	1.64	<0.40	<0.50	3.81	–	–
16					Grey and navy blue	97	0	0	3	Toe	1,038	1.45	1.87	<0.40	<0.50	3.32	–	–
17					Grey and navy blue	97	0	0	3	Foot	174	2.2	1.44	<0.40	<0.50	3.64	72.8	750
18					Grey and navy blue	97	0	0	3	Leg	1,020	2.41	1.48	<0.40	<0.50	3.89	–	–
19					Navy blue	85	15[b]	0[b]	0[b]	Toe	1,217	3.56	2.44	0.54	<0.50	6.54	–	–
20					Navy blue	85	15[b]	0[b]	0[b]	Foot	1,130	3.46	2.8	0.67	<0.50	6.93	94.4	496
21					Navy blue	85	15[b]	0[b]	0[b]	Leg	1,187	3.28	2.72	0.76	<0.50	6.76	–	–
22					White	85	15[b]	0[b]	0[b]	Toe	301	2.94	2.64	0.7	<0.50	6.28	–	–
23	3	1.8	36-48	Spain	White	85	15[b]	0[b]	0[b]	Foot	254	3.47	2.71	0.67	<0.50	6.85	196	n.s.
24					White	85	15[b]	0[b]	0[b]	Leg	256	3.37	2.53	0.6	<0.50	6.5	–	–
25					Light blue	85	15[b]	0[b]	0[b]	Toe	2,823	2.98	2.92	0.56	<0.50	6.46	–	–
26					Light blue	85	15[b]	0[b]	0[b]	Foot	2,533	3.31	3.09	0.81	<0.50	7.21	6,051	2,989
27					Light blue	85	15[b]	0[b]	0[b]	Leg	2,272	3.48	3.26	0.74	<0.50	7.48	–	–
28					Navy blue	95	0	0	5	Toe	153	0.91	1.72	1.72	<0.50	4.35	–	–
29					Navy blue	95	0	0	5	Foot	184	1.32	1.79	1.79	<0.50	4.9	n.s.	n.s.
30					Navy blue	95	0	0	5	Leg	183	1.3	2.19	2.19	<0.50	5.68	–	–
31					Grey	95	0	0	5	Toe	1,657	1.12	2.26	2.26	<0.50	5.64	–	–
32	4	1.5	06-dic	Spain	Grey	95	0	0	5	Foot	1,491	1	1.83	1.83	<0.50	4.66	245	859
33					Grey	95	0	0	5	Leg	1,819	1.22	2.37	2.37	<0.50	5.96	–	–
34					White	95	0	0	5	Toe	108	1.18	1.74	1.99	<0.50	4.91	–	–
35					White	95	0	0	5	Foot	97.1	1.07	1.96	2.31	<0.50	5.34	n.s.	n.s.
36					White	95	0	0	5	Leg	110	1.25	2.3	2.45	<0.50	6	–	–

[a] Label did not specify whether country of origin was the same as country of origin of the fiber.

[b] Composition given on the label differed among countries: Spain, 15% polyester; Germany, 15% polyamide; United Kingdom, 15% spandex; and France, 15% elastane.

n.s.: not significant.

Table S2. Characteristics of samples, concentrations of BPA and parabens, and hormone-like activities in socks collected in store 2 (N=36).

	Characteristics of samples											Chemical concentrations (ng/g)						Hormone-like activity	
						Composition (%)						Parabens						E-screen	PALM
Sample no.	Pack	Price (€)	Age (months)	Country of origin[a]	Color	Cotton	Polyester	Polyamide	Elastane	Section	BPA	MPB	EPB	PPB	BPB	∑PBs		(pM E_2eq/g)	(µM Proceq/g)
1					Black	76	22	0	2	Toe	7.33	2.48	3.25	<0.40	<0.50	5.73		–	–
2					Black	76	22	0	2	Foot	6.89	2.01	2.44	<0.40	<0.50	4.45		n.s.	n.s.
3					Black	76	22	0	2	Leg	6.61	2.37	2.81	<0.40	<0.50	5.18		–	–
4					Grey	76	22	0	2	Toe	14.6	2.15	1.92	<0.40	<0.50	4.07		–	–
5	5	3	24-36	Spain	Grey	76	22	0	2	Foot	11.8	1.94	2.57	<0.40	<0.50	4.51		n.s.	n.s.
6					Grey	76	22	0	2	Leg	13.6	2.43	2.34	<0.40	<0.50	4.77		–	–
7					Navy blue	76	22	0	2	Toe	7.12	2.64	2.34	<0.40	<0.50	4.98		–	–
8					Navy blue	76	22	0	2	Foot	6.37	2.33	2.78	<0.40	<0.50	5.11		n.s.	n.s.
9					Navy blue	76	22	0	2	Leg	6.95	2.14	1.98	<0.40	<0.50	4.12		–	–
10					Stripes and stars	72	22	4	2	Toe	23.6	18.8	2.53	<0.40	<0.50	21.4		–	–
11					Stripes and stars	72	22	4	2	Foot	20.7	16.3	2.08	<0.40	<0.50	18.3		n.s.	n.s.
12					Stripes and stars	72	22	4	2	Leg	21.3	20.5	2.29	<0.40	<0.50	22.8		–	–
13					Stars	72	22	4	2	Toe	20.1	22.7	3.19	<0.40	<0.50	25.9		–	–
14	6	3.5	24-36	Spain	Stars	72	22	4	2	Foot	18.9	19.1	2.43	<0.40	<0.50	21.5		n.s.	n.s.
15					Stars	72	22	4	2	Leg	24.6	22.5	2.92	<0.40	<0.50	25.4		–	–
16					Stripes	72	22	4	2	Toe	25.6	23.8	2.39	<0.40	<0.50	26.2		–	–
17					Stripes	72	22	4	2	Foot	21.9	21	3.11	<0.40	<0.50	24.1		n.s.	n.s.
18					Stripes	72	22	4	2	Leg	27.6	23.4	2.47	<0.40	<0.50	25.8		–	–
19					Grey	69	22	6	3	Toe	<0.70	4.59	1.34	<0.40	<0.50	5.93		–	–
20					Grey	69	22	6	3	Foot	<0.70	5.43	1.67	<0.40	<0.50	7.1		62.5	n.s.
21					Grey	69	22	6	3	Leg	<0.70	5.87	2.22	<0.40	<0.50	8.09		–	–
22					White	72	22	3	3	Toe	<0.70	4.97	1.12	<0.40	<0.50	6.09		–	–
23	7	4	01-jun	Spain	White	72	22	3	3	Foot	<0.70	5.17	1.16	<0.40	<0.50	6.33		n.s.	n.s.
24					White	72	22	3	3	Leg	<0.70	6.91	1.53	<0.40	<0.50	8.44		–	–
25					Black	69	22	6	3	Toe	<0.70	4.2	1.56	<0.40	<0.50	5.76		–	–
26					Black	69	22	6	3	Foot	<0.70	5.27	1.21	<0.40	<0.50	6.48		n.s.	n.s.
27					Black	69	22	6	3	Leg	<0.70	6.11	2.34	<0.40	<0.50	8.45		–	–
28					Grey	70	23	5	2	Toe	8.08	8.18	2.44	<0.40	<0.50	10.6		–	–
29					Grey	70	23	5	2	Foot	7.02	8.01	3.28	<0.40	<0.50	11.3		n.s.	n.s.
30					Grey	70	23	5	2	Leg	8.25	9.49	3.85	<0.40	<0.50	13.3		–	–
31					Red	70	23	5	2	Toe	7.95	7.34	3.6	<0.40	<0.50	10.9		–	–
32	8	4.5	06-dic	Spain	Red	70	23	5	2	Foot	8.28	8.11	3.95	<0.40	<0.50	12.1		n.s.	n.s.
33					Red	70	23	5	2	Leg	8.74	10.8	3.18	<0.40	<0.50	14		–	–
34					Stripes	70	23	5	2	Toe	8.78	8.45	4.23	<0.40	<0.50	12.7		–	–
35					Stripes	70	23	5	2	Foot	8.72	9.01	3.43	<0.40	<0.50	12.4		n.s.	n.s.
36					Stripes	70	23	5	2	Leg	9.76	10.1	4.02	<0.40	<0.50	14.2		–	–

[a] Label did not specify whether country of origin was the same as country of origin of the fiber.
n.s.: not significant.

Table S3. Characteristics of samples, concentrations of BPA and parabens, and hormone-like activities in socks collected in store 3 (N=24).

	Characteristics of samples										Chemical concentrations (ng/g)						Hormone-like activity	
						Composition (%)					Parabens						E-screen	PALM
Sample no.	Pack	Price (€)	Age (months)	Country of origin[a]	Color	Cotton	Polyester	Polyamide	Elastane	Section	BPA	MPB	EPB	PPB	BPB	∑PBs	(pM E_2eq/g)	(µM Proceq/g)
1					White	63	0	35	2	Toe	4.83	3.59	1.58	0.87	<0.50	6.04	–	–
2					White	63	0	35	2	Foot	5.01	3.11	1.29	1.26	<0.50	5.66	n.s.	n.s.
3					White	63	0	35	2	Leg	7.28	3.08	1.59	0.97	<0.50	5.64	–	–
4					Grey	63	0	35	2	Toe	4.55	3.18	1.13	0.95	<0.50	5.26	–	–
5	9	7.95	dic-24	Turkey	Grey	63	0	35	2	Foot	5.13	4.38	1.36	1.06	<0.50	6.8	n.s.	n.s.
6					Grey	63	0	35	2	Leg	8.04	3.41	1.52	0.82	<0.50	5.75	–	–
7					Navy blue	63	0	35	2	Toe	4.6	3.52	1.24	0.78	<0.50	5.54	–	–
8					Navy blue	63	0	35	2	Foot	4.44	4.04	1.27	0.95	<0.50	6.26	n.s.	n.s.
9					Navy blue	63	0	35	2	Leg	7.06	3.35	1.79	0.95	<0.50	6.09	–	–
10					Navy blue	79	0	20	1	Toe	7.1	<0.50	2.77	0.93	<0.50	3.7	–	–
11					Navy blue	79	0	20	1	Foot	6.7	<0.50	3.15	1.06	<0.50	4.21	n.s.	n.s.
12					Navy blue	79	0	20	1	Leg	7.68	<0.50	3.13	1.15	<0.50	4.28	–	–
13					Electric blue	79	0	20	1	Toe	7.87	<0.50	2.39	0.78	<0.50	3.17	–	–
14	10	7.95	06-dic	Turkey	Electric blue	79	0	20	1	Foot	7.78	<0.50	3.11	0.74	<0.50	3.85	n.s.	n.s.
15					Electric blue	79	0	20	1	Leg	7.79	<0.50	2.47	0.94	<0.50	3.41	–	–
16					Light blue	79	0	20	1	Toe	7.52	<0.50	2.78	1.06	<0.50	3.84	–	–
17					Light blue	79	0	20	1	Foot	6.88	<0.50	2.32	0.94	<0.50	3.26	n.s.	n.s.
18					Light blue	79	0	20	1	Leg	8.17	<0.50	2.4	0.96	<0.50	3.36	–	–
19					Stripes	79	0	20	1	Toe	43.5	15.2	8.17	1.49	<0.50	24.8	–	–
20					Stripes	79	0	20	1	Foot	45.9	13.6	7.17	1.69	<0.50	22.5	151	n.s.
21					Stripes	79	0	20	1	Leg	49.6	11.7	6.96	1.42	<0.50	20.1	–	–
22	11	6.95	06-dic	Turkey	Print	79	0	20	1	Toe	20.3	16.9	9.21	1.56	<0.50	27.6	–	–
23					Print	79	0	20	1	Foot	22	15	8.49	1.5	<0.50	25	230	n.s.
24					Print	79	0	20	1	Leg	21.3	15.6	9.11	1.38	<0.50	26.1	–	–

[a] Label did not specify whether country of origin was the same as country of origin of the fiber.
n.s.: not significant.