UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

INGESTA DE CONTAMINANTES TÓXICOS PERSISTENTES

RIESGO ASOCIADO AL CONSUMO DE ALIMENTOS DE ORIGEN ANIMAL

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IUSA Instituto Universitario de Sanidad Animal Seguridad Alimentaria

Anexo II

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Título de la Tesis

"Ingesta de Contaminantes Tóxicos Persistentes: Riesgo asociado al consumo de alimentos de origen animal."

Tesis Doctoral presentada por D. Ángel Rodríguez Hernández

Dirigida por el Dr. D. Octavio Pérez Luzardo

Codirigida por el Dr. D. Luis Domínguez Boada

El Director **El Codirector** **El Doctorando**

Las Palmas de Gran Canaria, a _____ de_____ de 2017

“Los que contemplan la belleza del mundo encuentran reservas de fortaleza que los acompañarán durante toda la vida.”

Rachel L. Carson

Agradecimientos

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En el año 2011, mientras cursaba el Máster en Sanidad y Seguridad Alimentaria de la Universidad de Las Palmas de Gran Canaria, recibí una charla sobre la contaminación química en el medio ambiente y sus efectos en la salud de los ecosistemas y del hombre. Este tema siempre me había llamado la atención y me había llevado a indagar en algunos de los aspectos relacionados a dicha problemática. Pero, fue en este periodo, cuando definitivamente me di cuenta de la verdadera importancia que tiene la interacción entre las sustancias químicas y el estado actual de nuestro planeta, así como sus consecuencias en los seres vivos y nuestra salud. Desde ese momento, continuamente he tratado de centrar y profundizar mis conocimientos en esta materia, teniendo siempre presente que es necesario poner una balanza para comparar los beneficios y riesgos asociados al uso y aplicación de estos compuestos a nivel global. Por ello, decidí realizar mi trabajo de tesina en la misma línea de investigación en la que se centra esta Tesis, el estudio de la dieta como fuente de exposición a algunos de estos contaminantes.

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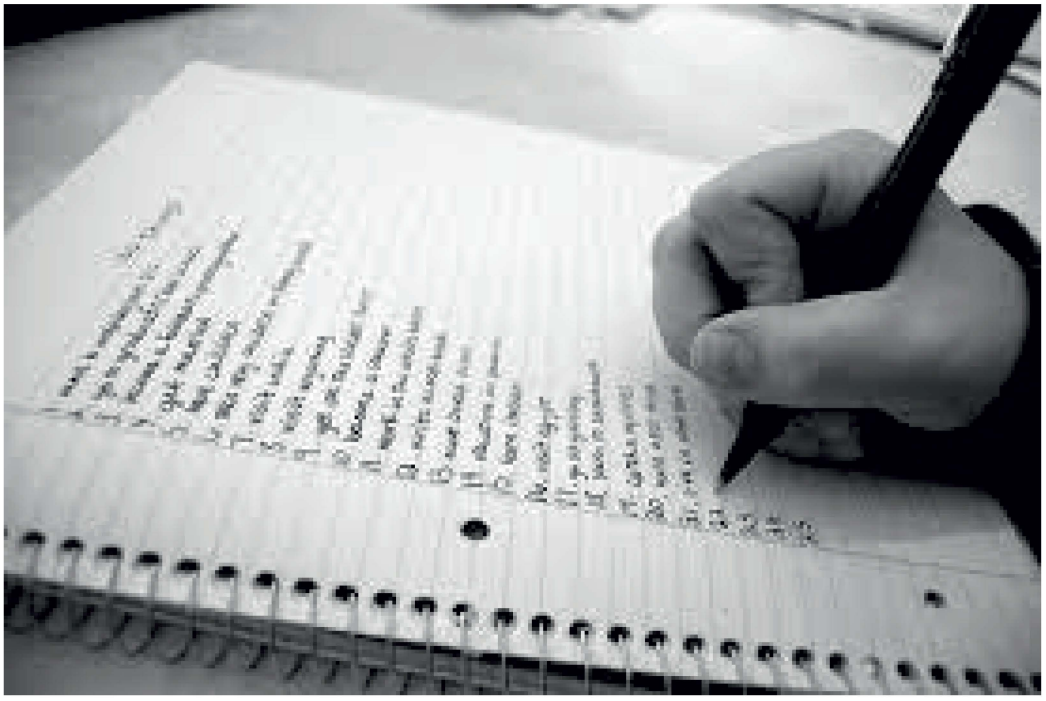

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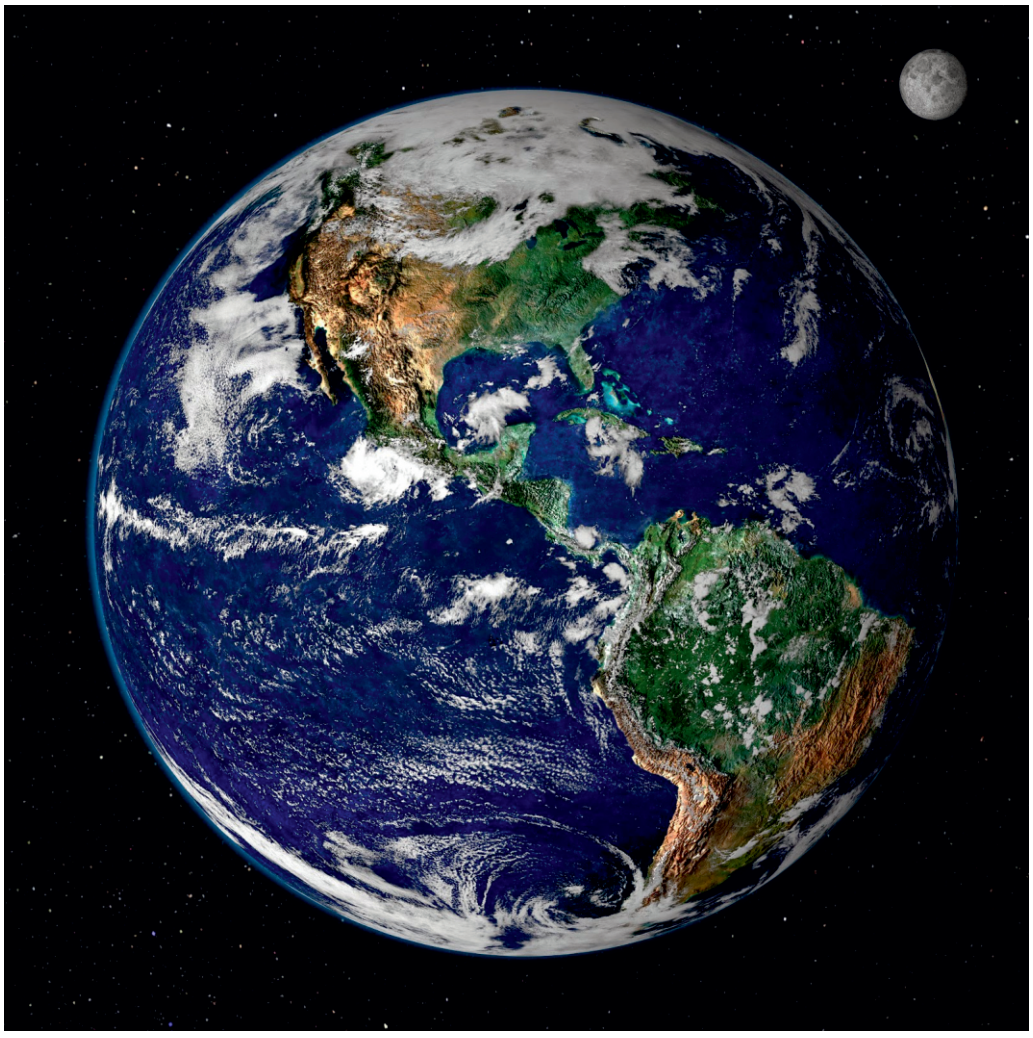
Introducción



INTRODUCCIÓN

1. Seguridad Alimentaria

1.1. Peligros y riesgos sanitarios asociados a los alimentos

Según la definición de la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO): "*Existe seguridad alimentaria cuando todas las personas tienen en todo momento acceso físico y económico a suficientes* ***alimentos inocuos y nutritivos*** *para satisfacer sus necesidades alimentarias*". La Seguridad Alimentaria es un derecho reconocido en la Declaración Universal de los Derechos Humanos, en su artículo 25, y la Constitución Española en su artículo 43 reconoce el derecho a la protección de la salud. En la Conferencia FAO/OMS sobe Nutrición de Roma (1992), se declaró que "el acceso a alimentos nutricionalmente adecuados y seguros, es un derecho de todo ser humano".

La seguridad alimentaria implica el cumplimiento de las siguientes condiciones:

- Oferta y disponibilidad de alimentos adecuados.
- La estabilidad de la oferta sin fluctuaciones ni escasez en función de la estación del año.
- El acceso a los alimentos o la capacidad para adquirirlos.
- La buena calidad e inocuidad de los alimentos.

En los países de la Unión Europea y de Occidente en general, las tres primeras premisas se alcanzan de forma generalizada, salvo excepciones ocasionales. Por lo tanto, el punto que se refiere a la inocuidad de los alimentos, es el que cobra relevancia y protagonismo y al que van dirigidas todas las políticas de control sanitario. De tal forma, se podría decir que en Europa, como en todos los países desarrollados, el término "Seguridad Alimentaria" hace referencia únicamente a los problemas de higiene e inocuidad de los alimentos. Es decir, las políticas gubernamentales, las medidas de control y sus correspondientes procesos, pretenden lograr que todo alimento que llega al consumidor, sea un **alimento "seguro" y de calidad**, libre de contaminantes que supongan una amenaza para la salud.

Los **riesgos asociados** a los alimentos han sido categorizados en 5 clases (Robert, 1986; Serra-Majem y Mata Albert, 2006),que ordenados de mayor a menor intensidad atendiendo a su gravedad, incidencia y período de incubación, son:

- Enfermedades de origen microbiano transmitidas por los alimentos.
- Trastornos nutricionales.
- Contaminantes ambientales.
- Sustancias tóxicas naturales presentes en los alimentos.
- Aditivos y colorantes alimentarios.

En el caso de España, garantizar la seguridad de los alimentos requiere el compromiso de instituciones públicas, desde la Comisión Europea, las Autoridades Sanitarias Nacionales y las Autonómicas y locales. La política de la Unión Europea engloba toda la cadena alimentaria y establece una amplia legislación, una de las más estrictas del mundo. Además, en el año 2000 se fundó la Autoridad Europea de Seguridad Alimentaria (EFSA por sus siglas en inglés), que trabaja en colaboración con diversas instituciones y organismos científicos de los países miembros de la UE.

1.2. Peligros químicos asociados a los alimentos

Los riesgos asociados a la contaminación de los alimentos por peligros químicos son una preocupación importante de la salud pública. La contaminación industrial del aire, el suelo y el agua y el uso de diversas sustancias químicas, como aditivos alimentarios, pesticidas y medicamentos veterinarios a lo largo de la cadena alimentaria pueden plantear peligros si dichas sustancias químicas no están reguladas correctamente o no se las utiliza adecuadamente (Serra-Majem y cols., 2001).

De origen biológico

- **Plantas y setas.** Las sustancias tóxicas de los vegetales comestibles y los vegetales venenosos que parecen comestibles son causas importantes de enfermedad en muchas áreas del mundo.
- **Micotoxinas.** Las micotoxinas son metabolitos tóxicos producidos por hongos de diversos géneros y suelen estar presentes en piensos, legumbres o cereales.
- **Biotoxinas marinas.** Las biotoxinas marinas que representan un problema de salud pública en el ámbito geográfico europeo son las que, producidas por varias especies de dinoflagelados, ocasionan intoxicaciones agudas tras el consumo de pescados y mariscos que se alimentan de estas algas y acumulan las toxinas sin verse afectados.

De origen no biológico

- **Plaguicidas.** Los plaguicidas pueden llegar a los alimentos por los tratamientos fitosanitarios o por la impregnación ambiental (en cuyo caso pasan a ser considerados contaminantes ambientales). Los problemas para la salud pública pueden deberse a la presencia de cantidades elevadas en los alimentos, con efectos patológicos agudos y, sobre todo, por la ingesta continuada de pequeñas cantidades con posibles efectos crónicos sobre la salud.
- **Antibióticos, antiparasitarios, tranquilizantes, AINES, hormonas y promotores del crecimiento.** Las vacunas y los medicamentos veterinarios son esenciales para proteger la salud de los animales de producción. Las condiciones de uso establecen un periodo de espera y unas dosis adecuadas para que sus residuos no sean ingeridos por las personas cuando se consumen productos de origen animal (carnes, huevos, pescado o leche). No obstante, en ocasiones estos plazos no se respetan y los residuos de estas sustancias pueden pasar a la cadena alimentaria.
- **Químicos originados en el procesado de alimentos.** Tal es el caso por ejemplo de las nitrosaminas, que son carcinógenos potentes, cuyas propiedades toxicológicas se han demostrado en animales de experimentación. También, por simple migración, aquellos compuestos de bajo peso molecular presentes en los materiales y objetos plásticos en contacto con los alimentos, pueden resultar en una contaminación de los mismos.

- **Aditivos alimentarios.** Los aditivos alimentarios comprenden un grupo amplio y variado de sustancias químicas utilizadas durante mucho tiempo y han sido evaluadas minuciosamente para asegurar su seguridad.
- **Contaminantes ambientales.** Varias sustancias químicas, consecuencia de la contaminación ambiental, pueden ser transmitidas por los alimentos. La exposición humana a los **metales pesados** como el plomo, el cadmio o el mercurio constituyen un problema de salud pública y se produce por diversos medios (aire, agua, suelo y alimentos). Por ejemplo, los **hidrocarburos aromáticos policíclicos** (PAH) constituyen uno de los grupos compuestos por varios compuestos carcinógenos y están ampliamente distribuidos en ecosistemas tales como el marino, estando también presentes en los alimentos. Las dioxinas, los **PCB** o los PBDE son **contaminantes orgánicos persistentes (COP)** que tienden a bioacumularse en la cadena alimentaria (Serra-Majem y Mata Albert, 2006; Weijs y cols., 2015).

Este trabajo de Tesis Doctoral se ha centrado en el estudio de la exposición de los consumidores a contaminantes químicos ambientales a través del consumo de alimentos de origen animal, por lo que dedicaremos una parte de esta introducción a la descripción del origen y las principales características de dicha contaminación química ambiental.

2. Contaminación química global

Uno de los componentes esenciales de la producción industrial y de los modelos económicos y sociales imperantes en muchas partes del mundo en las últimas décadas ha sido la síntesis de una gran cantidad de sustancias químicas. Muchas de ellas han tenido efectos socialmente beneficiosos, por ejemplo, a través de su uso en la asistencia sanitaria o en electrodomésticos, pero también muchas sustancias han demostrado tener efectos tóxicos, tanto para la salud de las personas como para el medio ambiente. Por tanto, debido a su liberación al medio ambiente, en la actualidad son miles las moléculas que están presentes en nuestro entorno en concentraciones significativas. Así, la literatura científica arroja frecuentemente evidencias de que muchas de estas sustancias, aún estando a concentraciones muy bajas, ejercen efectos nocivos para la salud del hombre , los animales y los ecosistemas; desde los pesticidas hasta los compuestos perfluorinados que han sido utilizados masivamente como antiadherentes y que actualmente han sido prohibidos por su elevada persistencia y su poder inmunosupresor; o los bifenilos policlorados, compuestos fabricados en grandes cantidades y ampliamente utilizados en aplicaciones industriales, dejando de fabricarse en todo el mundo en 1983, por citar algunos ejemplos (Crinnion, 2011; Linares y cols., 2015). Pero lo que resulta aún más preocupante, es que una gran cantidad de sustancias comercializadas no ha pasado ni una mínima evaluación sobre sus posibles efectos tóxicos a largo plazo para la salud humana (Porta y cols., 2009).

Por tanto, miles de estas sustancias se encuentran ampliamente distribuidas en el medioambiente y pueden tener un **origen natural**, como por ejemplo los hidrocarburos aromáticos o el arsénico liberado en los procesos de erupciones volcánicas e incendios forestales; o principalmente un **origen antropogénico** (liberadas al medio por la acción del hombre) mediante miles de procesos industriales, uso en cultivos agrícolas y hogares o la incineración de residuos

sólidos urbanos. Por todo ello, es fundamental recalcar que la **contaminación química global** interacciona con la población general del planeta, que está expuesta a diversos productos químicos que provienen del ambiente y como veremos, terminan incorporándose a los alimentos, que van a constituir la principal fuente de exposición no ocupacional a estas sustancias (EFSA, 2012d; Schecter y cols., 2010).

Mención especial merecen los desechos provenientes de equipos electrónicos (ordenadores, móviles, etc.) desde las industrias, los establecimientos o los hogares, ya que debido al consumo acelerado y progresivo de estos productos se estima que la producción global de **"basura electrónica"** es de 20-25 millones de toneladas por año, aportando España unas 200000 toneladas a dicha cantidad. La mayor parte de estos desechos se producen en Europa, Estados Unidos y Australia; pero China, los países de Europa del Este y latinoamericanos se han convertido también en grandes productores de basura electrónica durante los últimos años (Heacock y cols., 2016; Robinson, 2009)**.** La basura electrónica contiene metales valiosos como el cobre o el platino, pero también numerosos contaminantes ambientales potenciales, especialmente plomo, estaño, mercurio, cadmio, níquel, difeniléteres polibrominados (PBDE) y bifenilos policlorados (PCB). Además, la incineración de basura electrónica puede generar dioxinas, furanos e hidrocarburos aromáticos policíclicos (PAH), entre otros. Un gran porcentaje de esta basura electrónica se desecha en los vertederos, ya que la tecnología de reprocesamiento efectiva, que recupera la mayor parte de los materiales minimizando el impacto ambiental, es muy cara. En consecuencia, pese a ser una actividad ilegal en virtud del Convenio de Basilea (UNEP, 2009), los países ricos exportan cada año una cantidad desconocida de desechos electrónicos a los países pobres, donde las técnicas de reciclaje incluyen la quema y la disolución en ácidos fuertes con muy pocas medidas para proteger la salud humana y el medio ambiente (Cobbing, 2008)**.** Este tipo de "reprocesamiento" da como resultado inicial una fuerte contaminación local, que en breve plazo es seguida por la migración de los contaminantes al medio ambiente y finalmente **su incorporación a la cadena alimenticia**. El manejo y eliminación incorrecta de estos productos afecta a los trabajadores del sector de los desechos electrónicos, que sufren efectos negativos en la salud por el contacto con la piel y la inhalación de estas sustancias (Qu y cols., 2007), mientras que la población general del planeta se expone a través del humo, polvo, agua potable y sobre todo los alimentos (Chatterjee, 2007; Luzardo y cols., 2014).

Por otro lado, el sector de la industria química de los **productos plaguicidas**, ha contribuido enormemente en los últimos 70 años a incrementar los rendimientos del sector agrícola mediante el control de las plagas y enfermedades, y también en el control de las enfermedades transmitidas por insectos (paludismo, dengue, encefalitis, filariosis, etc.) (Abhilash y Singh, 2009). La necesidad de aumentar la producción de alimentos para una población mundial en rápido y continuo crecimiento es bien conocida. Una de las principales estrategias para aumentar la productividad de los cultivos es el manejo efectivo de plagas, ya que más del 45% de la producción anual de alimentos se pierde por la infestación de plagas. En los países tropicales las pérdidas en los cultivos son aún mayores debido a que las elevadas temperaturas y el alto grado de humedad dan lugar a la proliferación de plagas, por lo que la aplicación de productos plaguicidas es muy intensa en estos países (Abhilash y Singh, 2009). Debido al uso y abuso de este tipo de productos, se produce una considerable generación de

residuos, lo cual repercute en el coste de los productos, pero sobre todo produce consecuencias adversas sobre la salud de los ecosistemas y del hombre. La aplicación inadecuada de plaguicidas afecta al medioambiente en su conjunto, haciendo que los residuos contaminen el suelo, el aire y las aguas y finalmente alcancen la **cadena alimentaria** (UN/DESA, 2002). Por tanto, el ser humano está expuesto a residuos de plaguicidas por diferentes rutas (inhalatoria, digestiva y dérmica) y la exposición a dichas sustancias resulta en problemas agudos y crónicos de salud. Así, la exposición crónica a productos plaguicidas, aún a dosis muy bajas, ha sido relacionada con el aumento de la incidencia de cáncer, enfermedades renales crónicas, inmunosupresión, esterilidad masculina y femenina, desórdenes endocrinos, o desórdenes neurológicos y del comportamiento entre otros (Luzardo y cols., 2009; Mostafalou y Abdollahi, 2016). Obviamente, la contaminación por plaguicidas del medio ambiente también afecta a la vida de las aves, la fauna silvestre, animales domésticos, peces y el ganado.

De entre todos los grupos de insecticidas preocupan especialmente los insecticidas organoclorados como el Diclorodifeniltricloroetano (DDT), los derivados del hexaclorociclohexano (HCH), la aldrina y la dieldrina debido a su bajo coste, eficacia y versatilidad en la lucha contra plagas y los insectos vectores de enfermedades (Lallas y cols., 2001). Sin embargo, debido a su potencial de bioacumulación y efectos biológicos adversos, estos compuestos fueron prohibidos o se restringió su uso en los países desarrollados desde hace más de cuatro décadas (Bettinetti y cols., 2016; Rasmussen y cols., 2015; Zumbado y cols., 2005). Su resistencia a la degradación ha dado lugar a la contaminación universal por los mismos que hoy padecemos. Según el inventario de la FAO, más de 500.000 toneladas de plaguicidas obsoletos y no utilizados representan una amenaza real para el medio ambiente y la salud pública mundial. En sustitución de estos compuestos policlorinados, durante las tres últimas décadas, se impuso la utilización de los pesticidas del grupo de los organofosforados y carbamatos, de elevada eficacia plaguicida, pero también de elevada toxicidad. Por este motivo, cada vez se imponen más restricciones a su uso, en beneficio de la utilización de otros compuestos de menor toxicidad para los humanos, como los piretroides, los benzimidazoles o los nicotinoides, entre otros. Así, los esfuerzos de las industrias en busca de productos de máxima eficacia contra las plagas, han dejado un balance de más de 800 principios activos diferentes que pueden acabar contaminando el suelo, el aire y las aguas y aparecer como residuos en los **productos alimenticios.**

Si bien hemos mencionado dos de los grandes sectores de la industria química, estos representan sólo una parte de la cantidad de productos químicos que se utilizan hoy en día y que se liberan cotidianamente al medio, ya que hay que destacar que, a finales de la década de 1990, se habían registrado en la Unión Europea (UE) unos 100.000 productos químicos en el **Catálogo europeo de sustancias químicas comercializadas de la Agencia Europea del Medio Ambiente** (AEMA, 2007b), siendo especialmente grave el hecho de que se sabe muy poco de la toxicidad a largo plazo de la mayoría de ellos. Sólo el 14% de cerca de 100.000 compuestos, aquellos que se fabrican en mayor cantidad, disponen actualmente de suficiente cantidad de datos toxicológicos como para poder valorar que no suponen un riesgo real para la salud (AEMA, 2007a).

Así, muchas de estas sustancias químicas, una vez liberadas al medio tras su uso o producción indeseada en la agricultura, industrias o a nivel doméstico, entran a la cadena alimentaria

contaminando los pastos, el forraje o los piensos comerciales consumidos por los animales de producción; o también se acumulan en el suelo y sedimento del medio acuático y llegan a los peces. Estas sustancias pueden ser asimilados por ellos y, o bien son acumulados en su organismo (los más liposolubles) o bien son eliminados del mismo, tras un proceso de biotransformación, la cual puede ser completa o incompleta. Como resultado, se pueden detectar **residuos de todas estas sustancias y sus metabolitos** en **los productos alimenticios** procedentes de animales y también en los alimentos de origen vegetal por contaminación directa (EFSA, 2016; Hu y cols., 2016), lo que hace imprescindible investigar la presencia de residuos químicos en todos los alimentos.

Como ya se ha dicho, son muchas las fuentes de contaminantes a las que puede verse expuesto el ser humano. Algunas de estas sustancias, debido a sus características, se consideran **contaminantes tóxicos persistentes**, pudiendo permanecer inalterados en el medioambiente durante largos periodos de tiempo por su estabilidad y difícil degradación, siendo además capaces de transportarse a grandes distancias. En este proceso se disuelven en los tejidos grasos de los animales y vegetales y se van concentrando en ellos (bioacumulación). Esta concentración de los tejidos se biomagnifica, es decir, que se incrementa su concentración en la medida en que los organismos expuestos a estos compuestos sean devorados por sus depredadores. Por tanto, aumentan sus concentraciones y, como consecuencia, sus efectos tóxicos, en los eslabones más altos de la cadena alimentaria. De esta forma, el hombre al estar en lo más alto la cadena trófica, se expone a concentraciones significativas de estos compuestos. Por estas características, algunas de estas sustancias prohibidas o restringidas, se siguen cuantificando en muestras medioambientales como agua y suelos (David y cols., 2015; Zhao y cols., 2016), alimentos (Darnerud y cols., 2006; Mihats y cols., 2015) o muestras biológicas animales y humanas, como la sangre, leche, líquido amniótico o diversos tejidos (Beckmen y cols., 2016; Luzardo y cols., 2009; Torres y cols., 2015; Whitehead y cols., 2015), por lo que la exposición a contaminantes ambientales empieza desde la etapa prenatal o perinatal (Colborn, 2004).

Por este motivo, la investigación de residuos de sustancias ambientales se plantea como una necesidad para garantizar la seguridad alimentaria. El problema de los efectos sobre la salud de los contaminantes químicos tiene tal magnitud, que con el fin de proteger a los consumidores, a la vez que al Mercado, se han establecidos unos límites legales o recomendaciones para la presencia de todas estas sustancias en los alimentos de origen animal y vegetal por autoridades internacionales como la Organización Mundial de la Salud (OMS) o la FAO, destacando la EFSA en la UE. Dichos valores son constantemente revisados, normalmente a la baja, a partir de las investigaciones que arrojan datos sobre la peligrosidad de determinados niveles de dichas sustancias para la salud. A nivel internacional, se han puesto en marcha numerosas estrategias para minimizar los riesgos producidos y evaluar los efectos por estos contaminantes, destacando la creación del Convenio de Estocolmo sobre Contaminantes Orgánicos Persistentes, cuyo objetivo es la protección de la salud humana y del medio ambiente frente a estos contaminantes, reduciendo o eliminando sus emisiones al medio ambiente mediante medidas y planes de acción, como lo es la monitorización de estas sustancias en alimentos.

Por ello, el ser humano ingiere diariamente cantidades variables de estos contaminantes a través de la dieta, sufriendo una exposición crónica y continuada a dichas sustancias. Esta

exposición alimentaria ha sido extensamente estudiada para los contaminantes de mayor toxicidad. Así, por ejemplo, la exposición a las dioxinas (dibenzo-p-dioxinas policloradas y dibenzofuranos policlorados [PCDD/PCDF]) a través de diversos grupos de alimentos está bien caracterizada (Liem y cols., 2000). Se estima que el 90% de la exposición humana a estas sustancias químicas se produce a través de alimentos, principalmente los de origen animal ricos en grasa (lácteos, carne roja, pescado azul), tal y como lo han corroborado sucesivos informes (EFSA, 2007b; EFSA, 2010a; EFSA, 2012d) y numerosos estudios (Polder y cols., 2010; Yaktine y cols., 2006). Sin embargo, también se debe tener en cuenta el aporte de contaminantes en alimentos de origen vegetal, tal y como han puesto de manifiesto diferentes estudios de la EFSA.

De esta forma, numerosos estudios y publicaciones científicas a lo largo de los años han relacionado la exposición crónica a estas sustancias con efectos negativos en la salud de poblaciones animales y humanas, debido a su capacidad para actuar como disruptores del sistema endocrino (alteraciones tiroideas, diabetes), disruptores del metabolismo (obesidad y síndrome metabólico), disruptores inmunológicos (inmunodeficiencia, alergia, asma) o disruptores del sistema reproductivo (endometriosis, oligospermia, epi e hipospadias, infertilidad), y además por su capacidad carcinogénica (cáncer de mama, leucemias, linfomas) y de asociación con alteraciones neurológicas (déficit de aprendizaje en niños, enfermedades neurodegenerativas) (WHO/UNEP, 2013)**.**

Por tanto, el estudio de la exposición alimentaria a estos contaminantes es de enorme relevancia para conocer los niveles a los que se expone la población. Las investigaciones realizadas en este campo, permiten conocer los niveles de estos contaminantes en los alimentos consumidos por las diferentes poblaciones, pudiendo identificarse aquellos alimentos que aportan un mayor número y una mayor cantidad de estos compuestos (los que podríamos denominar “alimentos de riesgo”), y establecer una comparación de los resultados con estudios similares en otras poblaciones. Estos datos son de utilidad para las Autoridades en materia de Salud Pública y Seguridad Alimentaria, que pueden así establecer medidas y estrategias que minimicen la exposición de la población a estos contaminantes ambientales.

Dado que la dieta varía entre poblaciones, es obvio que la ingesta de contaminantes va a ser variable entre las diferentes poblaciones, dependiendo del **patrón de consumo** de alimentos en cada región. Por ejemplo, si observamos el consumo de lácteos en Holanda, su ingesta diaria es de 401 mL-g/p.c./día, mientras que en EEUU disminuye hasta 268 mL-g/p.c./día. Sin embargo, con la carne el patrón es inverso, con un mayor consumo por la población de EEUU frente a la de Holanda (197 y 111 g/p.c./día, respectivamente) (AECOSAN, 2011).

Los hábitos dietéticos, la composición química de los alimentos, así como el período en el que se hacen los estudios (como por ejemplo la estación del año), pueden explicar las diferencias en la contribución de los alimentos a la exposición química global entre las diversas poblaciones, por lo que los resultados de los estudios **no son fácilmente extrapolables** de una región a otra. Por tanto, los residuos de contaminantes ambientales en los alimentos pueden variar según los siguientes puntos:

Numerosos factores sociales y demográficos tales como el sexo, la edad, la raza o etnia, residencia urbana o rural, la situación económica o los niveles de estudios están fuertemente asociados con variaciones en los **hábitos alimentarios**. Así, si nos fijamos por ejemplo en el consumo de grasas, la variabilidad es enorme, existiendo países en los que se consumen menos de 30 g/día (ej: países en vías de desarrollo) hasta otros en los que se consumen más de 130 g/día (FAO/WHO, 1994). En nuestra región, el consumo medio de grasas es de unos 100 g/día (Garcia-Segovia y cols., 2006).

También, debido a los patrones locales de uso de sustancias químicas y a las prácticas medioambientales y de políticas de seguridad alimentaria, los **niveles de residuos presentes en los alimentos** varían de región en región y, por tanto, se modifica su exposición dietética (Chikuni y cols., 1997; Koopman-Esseboom y cols., 1994). Un ejemplo de la variabilidad de los residuos químicos en los productos alimenticios es el amplio rango de niveles de DDT, DDE y HCH detectados en la mantequilla en un estudio realizado en 23 países, asociándose los mayores niveles de residuos con un uso más reciente o continuado a estas sustancias (Kalantzi y cols., 2001).

El **momento en el que se realiza el estudio** también es una variable que puede influir en la exposición dietética de los seres humanos, debido a posibles cambios estacionales en los patrones de alimentación, la ingesta de grasas totales, o los propios niveles de contaminantes de los alimentos, que pueden variar debido a restricciones locales o al uso estacional de las sustancias químicas (Dejonckheere y cols., 1996). Por ejemplo, en un estudio de exposición dietética a plaguicidas organofosforados, los residuos de clorpirifós en alimentos vegetales se detectaban con más frecuencia en la primavera y verano que en el resto del año (MacIntosh y cols., 2001). No obstante, debido a su larguísima vida media, salvo que existan accidentes que provoquen una liberación masiva, es muy poco probable que pueda observarse este efecto estacional con los contaminantes químicos persistentes, como las dioxinas o los plaguicidas organoclorados (Ryan y cols., 1990). Por lo tanto, cuando hablamos de contaminantes químicos resistentes a la degradación, es necesario hacer un seguimiento prolongado para poder apreciar un cambio en la dosis interna y observar las posibles fluctuaciones temporales.

Esta situación de contaminación medioambiental y de los alimentos con sustancias que producen efectos adversos sobre la salud es altamente preocupante y lógicamente no ha pasado desapercibida a las autoridades gubernamentales. Por este motivo, en el año 2006 la UE promulgó una ambiciosa y monumental iniciativa legislativa para el **Registro, Evaluación, Autorización y Restricción de las sustancias químicas (REACH)**. Había muchas razones para la promulgación del REACH, pero en general es una extensión del deseo global de que se liberen al medio menos productos químicos industriales, de comprender los posibles riesgos humanos y ecológicos de los que ya existen, y de asegurarse de que no se produce ninguna nueva catástrofe o amenaza relacionada con el uso de las sustancias químicas (Williams y cols., 2009). De acuerdo con esta directiva comunitaria, salvo unas pocas excepciones, es necesario el registro de todas las sustancias químicas fabricadas o importadas en la UE. Esta inscripción en el registro, generará un expediente que contendrá datos sobre las características físico-químicas y las propiedades toxicológicas y ecotoxicológicas de cada una de las sustancias. Para

muchos productos químicos, el examen de los peligros y riesgos derivados del uso de estas sustancias, también se requerirá en forma de un informe de seguridad química.

Comenzando con el doble proceso de expediente y la evaluación de sustancias, la **Agencia Europea de Sustancias Químicas (ECHA)**, los Estados miembros de la UE, y la Comisión Europea identificarán los productos químicos que representan riesgos inaceptables para la salud humana y/o el medio ambiente, y en consecuencia reducirán o restringirán su uso. Así, de todos los químicos existentes en el mercado, cerca de 145000 se encuentran actualmente prerregistrados en el REACH (ECHA, 2011), de los cuales una gran parte son lo suficientemente persistentes para llegar a los humanos y la fauna pero no se bioacumulan.

No obstante, a pesar de que el REACH es una ambiciosa estrategia de cara al futuro, no resuelve el problema de los contaminantes que ya han sido vertidos y están en circulación en el medioambiente. Por esto, varias organizaciones internacionales han desarrollado estrategias encaminadas a eliminar o reducir la producción, uso y liberación de las sustancias persistentes y bioacumulativas con efectos adversos para la salud, los denominados **contaminantes tóxicos persistentes (CTP).**

3. Los contaminantes químicos como disruptores endocrinos

Gran parte de los efectos adversos que tiene sobre la salud humana la **exposición crónica** a dosis bajas de los CTP y su mecanismo de acción son muy complejos y han sido encuadrados en los fenómenos denominados **disrupción endocrina-metabólica**. Por tanto, los contaminantes químicos capaces de producir disrupción endocrina son denominados **disruptores endocrinos (DE).**

El **sistema endocrino** humano se compone de glándulas productoras de hormonas que regulan y controlan nuestro organismo de diferentes maneras, por lo que son fundamentales en el equilibrio fisiológico. Las hormonas juegan un papel crucial en la regulación del crecimiento del cuerpo, el metabolismo, el desarrollo y la función sexual. Algunos grupos poblacionales, como los bebés no nacidos, los bebés y los niños son especialmente sensibles a la actividad hormonal debido a que están en las **etapas críticas del desarrollo**, llamadas "ventanas de susceptibilidad" (EFSA, 2010d).

Es notable el hecho de que la tasa de incidencia de enfermedades y trastornos endocrinos se ha incrementando simultáneamente con el crecimiento de la industria química, lo que hace temer que estos factores pueden estar vinculados. Por ejemplo, el estado actual de la calidad del semen es muy pobre en los países europeos donde se han realizado estudios para evaluar sus parámetros, observándose que la fertilidad se ha visto mermada en aproximadamente el 40% de los hombres. Asimismo, otros estudios epidemiológicos revelan también un aumento de la incidencia de malformaciones genitales, como la criptorquidia o las hipospadias (asociado con cáncer de testículo y con infertilidad); además de un mayor número de nacimientos prematuros y menor peso al nacer; trastornos de la conducta asociada a disrupción tiroidea en niños; aumento de cánceres relacionados al sistema endocrino (de mama, endometrial, de ovario, prostático o testicular) y aumento de la prevalencia de obesidad y diabetes tipo 2, entre otros. Estos trastornos reproductivos y en el desarrollo relacionados con alteraciones en la función endocrina son más evidentes en especies de fauna

silvestre, especialmente en entornos contaminados por altas mezclas de sustancias químicas (WHO/UNEP, 2013).

Dicho esto, debemos definir a una **sustancia activa endocrina** como cualquier compuesto químico producido por el hombre o de forma natural, el cual puede interactuar directa o indirectamente con el sistema endocrino y posteriormente producir un efecto sobre el mismo, incluyendo los órganos y tejidos diana. Si el efecto es adverso ("disruptivo") o no, dependerá del tipo de efecto, la dosis y la situación fisiológica (EFSA, 2010d). Así, "un **disruptor endocrino** es una sustancia exógena o una mezcla de sustancias que altera las funciones del sistema endocrino y en consecuencia provoca efectos adversos en la salud de un organismo intacto, o a su progenie, o a las subpoblaciones" (ICPS, 2002). Según la EPA, un disruptor endocrino es "un agente exógeno que interfiere con la síntesis, secreción, transporte, metabolismo, unión o eliminación de las hormonas que de forma natural están presentes en el cuerpo y que son las responsables de la homeostasis, la reproducción y el desarrollo" (Diamanti-Kandarakis y cols., 2009).

El rango de **compuestos químicos** que se consideran DE es muy amplio y crece exponencialmente, comprendiendo desde productos químicos sintetizados por el hombre hasta sustancias que se encuentran de manera natural en los alimentos o en el medio ambiente. Se conoce o se sospecha que existen cerca de 800 productos químicos capaces de interferir con los receptores hormonales, su síntesis y su conversión. Sin embargo, sólo un pequeño porcentaje de estos productos químicos se han investigado en ensayos capaces de identificar efectos endocrinos manifiestos en organismos intactos (WHO/UNEP, 2013).

Como ejemplo de sustancias presentes de **forma natural** en los alimentos que pueden ejercer efectos hormonales, podemos citar a los fitoestrógenos vegetales, como las isoflavonas, que a menudo están presentes en los frutos secos, semillas oleaginosas y productos de soja. Otro ejemplo es el ácido glicirrético del regaliz, que puede alterar la regulación hormonal del equilibrio electrolítico en la sangre y varios órganos, por lo que puede afectar a la regulación de la presión arterial.

El abanico de moléculas de **origen antropogénico** identificadas hasta el momento como DE es enormemente amplio y heterogéneo. Así, un largo número de productos químicos de diversas clases se identifican actualmente como DE, entre los que se incluyen los aditivos de diversos materiales y bienes de consumo (como productos farmacéuticos, productos de cuidado personal, productos electrónicos, envases de alimentos, ropa, etc.); además de metales, promotores del crecimiento y plaguicidas de uso actual, por lo que sus fuentes de emisión, sus propiedades químicas y su distribución y comportamiento en el medioambiente varían ampliamente. Podemos citar a sustancias plásticas o plastificantes (como el Bisfenol A o los ésteres de ftalatos), fungicidas (como la vinclozolina), fármacos (como el dietilestilbestrol, DES), productos conservantes de higiene personal (como los parabenos) y gran parte de los CTP como PCB, dioxinas y furanos, PBDE, naftalenos policlorados, así como muchos de los POC (DDT, metoxicloro, clordano, entre otros), PAH (como benzo(a)pireno o antraceno) y algunos metales pesados y metaloides como el cadmio o el arsénico (WHO/UNEP, 2013).

Por tanto, estamos ante un fenómeno global que afecta a todos los seres vivos del planeta, expuestos a niveles enormemente variables de estas sustancias, con notables diferencias entre organismos y territorios. El escenario cambia constantemente, ya que muchos se consideran persistentes y han sido prohibidos desde hace décadas, mientras que otros aún están permitidos para algunos usos o prohibidos recientemente, como se observa con los mayores

niveles actuales de compuestos alquilofluorinados en sustitución de los retardantes de llama brominados prohibidos (Covaci y cols., 2011). Además, los efectos relacionados a los DE pueden complicarse si existe exposición a otros químicos que no sean DE y a otros factores de estrés medioambientales, biológicos o físicos. Por tanto, es determinante tener en cuenta que las relaciones que se establecen con el desarrollo de enfermedades y desórdenes son probabilísticas, a la vez que multicausales.

Los DE son muy diversos desde un punto de vista químico, lo que representa un desafío en el campo de estudio de la disrupción endocrina, ya que, aparte de su pequeño tamaño (< 1000 Dalton), no parecen compartir otras características que hagan predecir si una molécula química va a comportarse como un DE o no. Sin embargo, en términos muy amplios, DE como las PCDD/PCDF, PBDE, PCB y POC, contienen grupos halogenados en su estructura (con sustituciones por átomos de cloro y bromo). Además, muchas de estas moléculas contienen un grupo fenólico, que se piensa que es el responsable de conferirles similitud con las hormonas esteroideas, siendo así reconocidos como ligandos por los receptores de dichas hormonas, ya sea como agonistas o como antagonistas.

Por otra parte, según se concluye en el último informe emitido por la OMS y el Programa de las Naciones Unidas para el Medio Ambiente (UNEP), denominado Estado de la Ciencia de la Disrupción Química Endocrina, los efectos que aparecen en animales de vida silvestre o de experimentación, pueden ocurrir también en seres humanos si se exponen a los DE en un momento vulnerable y en concentraciones que llevan a alteraciones de la regulación endocrina (WHO/UNEP, 2013). Además, la velocidad con la que se ha producido el aumento de la incidencia de enfermedades descarta a los factores genéticos como la única explicación plausible, entrando en juego factores no genéticos, ambientales y otros; incluyendo la nutrición, la exposición materna, las enfermedades virales y la exposición a sustancias químicas. En las últimas décadas, algunas **asociaciones** entre la exposición a compuestos químicos y diversas patologías son evidentes, como por ejemplo: relación entre criptorquidia con el dietilestilbestrol (DES) y polibromodifenil éteres (PBDE); alta exposición a dioxinas y DL-PCB como factor de riesgo de cáncer de mama; exposición ocupacional a pesticidas, PCB y arsénico con cáncer de próstata; exposición a PCB con neurotoxicidad con impacto negativo en el desarrollo del cerebro; exposición ocupacional a pesticidas con cáncer de tiroides.

En los últimos años, se ha incrementado notablemente el conocimiento acerca de estas sustancias y sus mecanismos de acción, pero aún faltan más esfuerzos para la comprensión global de sus efectos sobre la salud. En un principio, se pensaba que los DE ejercían sus efectos principalmente a través de su unión con los **receptores nucleares** para hormonas, incluyendo los receptores de estrógenos (RE), los receptores de andrógenos (RA), los receptores de progesterona (RP), los receptores de hormonas tiroideas (RT) y los receptores de retinoides (RAR), entre otros. Actualmente, se sabe que el espectro de mecanismos de acción es mucho mayor y que actúan también mediante su unión a **receptores no nucleares** (o de membrana) para hormonas esteroideas, **receptores no esteroideos** (p.e. receptores para neurotransmisores como el de serotonina o dopamina), **receptores huérfanos** (p.e. el receptor de aril hidrocarburos [AhR]), **rutas enzimáticas implicadas en la biosíntesis y/o metabolismo de esteroides**, y otros mecanismos implicados en la afectación de los sistemas endocrino o reproductivo.

Si bien la mayor parte de los efectos netos de los DE podrían catalogarse como “efectos estrogénicos”, lo cual ha motivado que durante tiempo se haya hablado de los DE como sinónimo de xenoestrógenos, actualmente se sabe que no sólo afecta a la reproducción sino

también a otros sistemas endocrinos. Así, hoy sabemos que existen numerosas sustancias que tienen efectos androgénicos, o más frecuentemente antiandrogénicos, y también sustancias agonistas o antagonistas de las hormonas tiroideas. De esta manera, desde una perspectiva fisiológica, un DE es un compuesto, ya sea natural o sintético, que, a través de la exposición ambiental, altera los sistemas hormonales y homeostáticos que le permiten al organismo comunicarse y responder a su medioambiente (Diamanti-Kandarakis y cols., 2009).

En nuestro caso, es de importancia recalcar que los DE se detectan en **alimentos y aguas**, y por lo tanto, su ingesta constituye la principal fuente de exposición a DE en nuestro organismo. Evidentemente, las personas que trabajan con pesticidas, fungicidas y sustancias químicas industriales, tienen una exposición particularmente alta y por consiguiente, mayores probabilidades de desarrollar anormalidades reproductivas o endocrinas. Otras fuentes de exposición identificadas para los humanos son la contaminación en ambientes interiores, el reciclado electrónico y el tratamiento incorrecto de desechos en los basureros de países en desarrollo.

Hay una serie de **aspectos claves** a remarcar cuando se habla de **disrupción endocrina**, ya que pueden ser decisivos para entender los mecanismos de acción y las consecuencias de exposición a estas sustancias:

- **Lugar de unión:** Algunos DE pueden actuar directamente sobre los receptores hormonales actuando como agonistas o antagonistas de las hormonas. Otros pueden actuar directamente sobre cualquier proteína que controle el transporte de una hormona a su célula o tejido diana. Además, algunos DE pueden interactuar con múltiples receptores simultáneamente.

- **Edad de exposición:** A muchos de estos compuestos estamos expuestos durante toda nuestra vida, incluso antes de nacer, como lo demuestra el hecho de que hayan podido ser medidos en el líquido amniótico de mujeres gestantes (Luzardo y cols., 2009). Como hemos dicho, las consecuencias de la exposición a los DE pueden ser muy diferentes dependiendo de la edad a la que se produzca. Es decir, que la exposición de un adulto a un DE puede tener consecuencias muy diferentes a la de un feto en desarrollo o un niño, siendo el desarrollo fetal, la vida perinatal y la infancia los periodos más críticos a los efectos de los DE. Por tanto, sus efectos en el desarrollo ocurren a dosis más bajas que las requeridas por los adultos. En términos generales, las sustancias presentes en el medio ambiente en el que se desarrolla un organismo interactúan con los genes del individuo para determinar su propensión a desarrollar una enfermedad o disfunción en el futuro. La Endocrine Society abarca este principio a la infancia de cada individuo, denominándolo **"bases del desarrollo de las enfermedades del adulto"** (Barker, 2003).

- **Latencia desde la exposición:** Las consecuencias de la exposición a los DE pueden no aparecer inmediatamente ni de forma precoz en la vida, sino que pueden manifestarse con el transcurso del tiempo, muchos años incluso, durante la vida adulta (Barker, 2003). Por lo tanto, es fundamental la evaluación de los efectos latentes.

- **Importancia de las mezclas:** Si los individuos y las poblaciones están expuestos a un determinado DE, es muy probable que lo estén a otros muchos de forma simultánea, ya que rara vez los ecosistemas están contaminados por un único compuesto. Esto tiene

mucha relevancia de cara a los efectos clínicos, ya que, independientemente de los efectos que hayan podido ser demostrados en experimentos *in vitro* o *in vivo*, la presencia de otros contaminantes en las situaciones reales de exposición, puede inducir fenómenos de adición de efectos, pero también de sinergismo y de potenciación (Kortenkamp, 2007). Por lo tanto, el estudio de la relación entre una mezcla de DE y una enfermedad o disfunción es fisiológicamente más relevante que el estudio de vinculación en referencia a un solo DE, que por sí mismo puede estar asociado a múltiples enfermedades o síndromes.

- **Relación de dosis-respuesta no convencional:** Muchas de estas sustancias, a dosis muy pequeñas, pueden producir anormalidades endocrinas o reproductivas, particularmente si la exposición tiene lugar en el periodo crítico del desarrollo. Así, la afinidad de un DE por el receptor hormonal no es equivalente a su potencia, dependiendo de muchos factores y mecanismos de acción. Pero lo llamativo es que, en algunos casos, las dosis bajas pueden producir efectos más potentes que las dosis altas a través de múltiples mecanismos, dando lugar a curvas de dosis-respuesta en forma de "U" o de "U invertida" (Boada y cols., 2007; vom Saal y cols., 2007).

- **Efectos epigenéticos transgeneracionales:** Otra característica muy importante de los DE es que no sólo pueden afectar a la persona expuesta, sino también a las generaciones subsiguientes. Los mecanismos no están claros, aunque investigaciones recientes sugieren que los mecanismos pueden ser no genómicos, es decir, que no se transmitirían por una mutación en la secuencia de ADN, sino a través de modificaciones de factores que regulan la expresión génica, tales como la metilación del ADN o la acetilación de histonas.

Por lo tanto, el problema de la exposición humana a los DE y las consecuencias sobre la salud puede ser investigado desde diferentes enfoques y con propósitos muy distintos. Destacan los **estudios clínico-epidemiológicos** que tratan de establecer relaciones entre la exposición a disruptores endocrinos y la frecuencia de presentación de una determinada enfermedad. Este proceso parece sencillo, pero requiere la **definición de instrumentos** para la evaluación de la exposición y de las variables que una vez cuantificadas permitan clasificar a los pacientes de acuerdo a su grado de exposición (Diamanti-Kandarakis y cols., 2009).

De esta manera, la evaluación de la exposición a DE es muy compleja. Por una parte, porque la información sobre la producción, uso y aplicaciones de los compuestos químicos incluidos bajo esta denominación es muy escasa. Por otra, los **métodos de determinación validados** a nivel internacional para su identificación abarcan sólo un espectro reducido de sustancias. Por ello, los organismos nacionales e internacionales con intereses en la regulación y comercialización de compuestos químicos han lanzado propuestas para la estandarización de estos test y verificar las actividades hormonales/antihormonales para su aplicación sistemática a los compuestos de nueva síntesis y a aquellos preexistentes. Por tanto, la lista de mimetizadores hormonales aumenta con la misma velocidad que se expande nuestro conocimiento sobre las formas de exposición y reconocimiento de estas sustancias. Aún así, los test y ensayos adecuados son insuficientes y, como consecuencia, al no disponer de los suficientes medios, se obstaculiza seriamente el progreso de comprensión de los riesgos.

A continuación, se nombran algunos de los **efectos** en humanos y poblaciones animales (WHO/UNEP, 2013), debido a los diferentes mecanismos de acción por las características disruptivas de varios de los **contaminantes** incluidos en esta Tesis Doctoral:

- **DDT:** Posible causa de endometriosis y disrupción del ciclo menstrual en humanos. Disminución del grosor de la cáscara del huevo, alteración de la conducta sexual y disminución de la población en aves. Menores niveles de testosterona y desmasculinización en osos polares y aligátores, intersex en peces y ranas. El metilsulfonil DDE y el o,p'-DDD se asocian a hiperplasia adrenal y síndrome de Cushing en focas. Existen algunas evidencias de supresión de la hormona tiroidea en mamíferos marinos, aves y anfibios y son limitadas para el incremento de riesgo de cáncer de mama, leucemia y linfoma en humanos y el incremento de riesgo de obesidad mediante la exposición perinatal. Probable causa de disminución de la población de mamíferos y aves que se alimentan de peces.

- **PCB:** Posible endometriosis y fibromas en los seres humanos; fibromas, tumores de útero y problemas adrenales en focas. Claras evidencias en ensayos experimentales a nivel molecular de la supresión de la hormona tiroidea en todas las clases de vertebrados y datos epidemiológicos evidentes de la disminución de la función cognitiva en niños. Evidencias limitadas de un mayor riesgo de cáncer de próstata y de mama en humanos y de carcinomas genitales en leones marinos. Evidente disfunción inmune en humanos y mamíferos marinos y evidencias limitadas del incremento de riesgo de diabetes. Probable causa de disminución de la población de mamíferos y aves devoradoras de peces.

- **Benzo(a)pireno:** La exposición al benzo(a)pireno y a otros hidrocarburos aromáticos policíclicos en los estuarios se asocia con neoplasias en poblaciones de fauna silvestre.

- **Metilmercurio:** El mercurio atraviesa la barrera hematoencefálica y reduce los niveles de enzimas clave para la reproducción, la cognición, el crecimiento y el desarrollo de vertebrados de vida silvestre. Una exposición elevada al metilmercurio por los peces y anfibios también daña los comportamientos que son críticos para la reproducción y las conductas para ser evitados por depredadores, especialmente los animales más jóvenes. En las aves acuáticas, la exposición al metilmercurio a niveles relevantes para el medioambiente puede interferir con el éxito reproductivo debido a efectos disruptivos neuroendocrinos en el comportamiento del cortejo y la elección de la pareja. Estos efectos pueden ser relevantes para la sostenibilidad de las poblaciones de aves.

En otro sentido, recientemente se han descrito también las consecuencias a nivel metabólico debidas al papel de los disruptores endocrinos en el sistema tiroideo (Boas y cols., 2009; Jugan y cols., 2010; Patrick, 2009). Así, las dioxinas, los furanos y los PCB podrían actuar sobre el tiroides uniéndose a la transtiretina, proteína transportadora de la tiroxina (Lans y cols., 1994), y también parecen afectar adversamente a las enzimas que metabolizan las hormonas tiroideas presentes en el hígado y en el cerebro (Brouwer y cols., 1998).

Por otro lado, numerosos autores están convencidos de la **disrupción metabólica** que pueden producir los PCB, dando lugar a fenómenos tóxicos a varios niveles (Janesick y Blumberg, 2011a; Newbold y cols., 2009). Por tanto, la exposición a contaminantes ambientales entre los que se encuentran los PCB y algunos pesticidas organoclorados podría estar relacionada a la reciente epidemia de diabetes tipo 2, al aumentar la resistencia a la insulina (Lee y cols., 2007) y de obesidad y otros trastornos metabólicos relacionados con la obesidad (Baillie-Hamilton, 2002; Lee, 2012; Lee y cols., 2010).

Los metales pesados, disolventes, pesticidas, DDE, PCB, organofosforados, glutamato monosódico, ftalatos, compuestos orgánicos de estaño, dietilestilbestrol y el bisfenol A son conocidos disruptores endocrinos ahora denominados también **obesógenos**. Son sustancias que influyen en varios ejes endocrinos, donde generalmente participan receptores nucleares, incluyendo receptores esteroideos, receptores del ácido retinoico, receptores de los glucocorticoides y el receptor gamma proliferador de peroxisomas (PPARγ). La activación de este último receptor tiene gran importancia, ya que es el principal regulador de la adipogénesis. Todos afectan directa o indirectamente a la fisiología del adipocito y a la regulación del gasto energético (Decherf y Demeneix, 2011). Nos referimos a sustancias obesógenas cuando son capaces de producir alteraciones metabólicas y predisposición a la ganancia de peso (Janesick y Blumberg, 2011b; Newbold y cols., 2009), incluyéndose entre ellas, como hemos dicho, los PCB, algunos pesticidas organoclorados o el benzo(a)pireno.

Por todo lo mencionado, no cabe duda que la disrupción endocrina representa una forma especial de toxicidad y ello se debe tener cuenta para el diseño y la interpretación de estudios para evaluar sus efectos y riesgos. Por tanto, **se necesita un esfuerzo internacional coordinado e integrado para definir el papel de los disruptores endocrinos en la actual disminución de la salud de los seres humanos y las poblaciones animales.** Ante las lagunas que existen sobre la dimensión real de sus efectos, es fundamental que las autoridades en materia de salud pública articulen todas aquellas medidas preventivas necesarias para disminuir la exposición a las mismas, basándose siempre en **el principio de precaución**, ya que pese a ser insuficientes, en aquellos casos en los que se ha hecho, la experiencia y el tiempo han demostrado la efectividad de su aplicación. Ejemplos lo constituyen la prohibición o restricción de COP, clorpirifós (aún en periodo de moratoria de uso) o tributilestaño, que ha contribuido a la disminución de la frecuencia de trastornos relacionados en seres humanos y poblaciones animales.

4. Contaminantes tóxicos persistentes

Los CTP son sustancias químicas o subproductos que poseen riesgos muy significativos para la salud humana o de los ecosistemas, y que debido a su resistencia a la degradación, permanecen durante largos periodos de tiempo en el medioambiente. Se trata de sustancias altamente liposolubles, por lo que son capaces de bioacumularse en los tejidos humanos, animales o vegetales. Debido a esta **biocumulación**, las concentraciones medibles de estas sustancias aumentan según se suben eslabones en la cadena alimentaria, en un fenómeno conocido como **biomagnificación** (Gray, 2002).

Los criterios que debe cumplir un contaminante químico para ser considerado un CTP son:

- Ser extremadamente estable y persistente en el medioambiente.
- Bioacumularse en los organismos vivos y en la cadena alimentaria.
- Ser tóxico para el ser humano y los animales y tener efectos crónicos sobre la salud tales como disrupción de los sistemas endocrino, inmune o reproductivo, y/o ser carcinogénicos.
- Ser transportados en el medioambiente a grandes distancias del punto donde fueron liberados.

Como consecuencia de todas estas características, muchas especies, y en particular las situadas en los eslabones más altos de la cadena trófica, están crónicamente expuestas a estas sustancias, incluso durante toda la vida (Luzardo y cols., 2009). De esta manera, los CTP pueden alcanzar niveles muy altos en, por ejemplo, las focas y otros mamíferos marinos, y osos polares (Braune y cols., 2005; Leonards y cols., 2008). Al ser sustancias muy liposolubles, los alimentos de origen animal ricos en grasas como los productos lácteos, las carnes o pescados grasos como el salmón, el arenque y la anguila, tienen concentraciones más altas que los alimentos de origen vegetal o bajos en grasa (Brustad y cols., 2008).

Algunas poblaciones humanas, como los *inuit* (o esquimales) que comen alimentos extremadamente grasos como salmón y foca, ingieren niveles de estos compuestos muy superiores a la ingesta diaria admisible (IDA) establecida por la OMS. Una sola comida puede contener tanto como 100 veces la IDA (Bonefeld-Jorgensen y cols., 2006). Los bebés lactantes (o personas con un consumo excesivo de productos lácteos) también podrían llegar a superar fácilmente el valor de IDA (Trapp y cols., 2008). Los efectos adversos sobre la salud de los CTP pueden ser muy graves, incluidos los efectos nocivos sobre la fertilidad y el desarrollo embrionario, el daño al sistema nervioso (incluyendo el aprendizaje intelectual y deterioro de la función cognitiva) o el cáncer, por citar sólo algunos de los más graves (Noyes y cols., 2009).

En principio, un CTP liberado en cualquier parte del mundo puede con el tiempo llegar a alcanzar cualquier otro lugar del planeta. Sin embargo, en términos generales, se produce una redistribución a gran escala de los CTP desde las zonas más templadas a las zonas más frías del planeta, debido a un fenómeno denominado **"efecto saltamontes"**. Básicamente los CTP se propagan por evaporación a la atmósfera desde las regiones más templadas del planeta (los países tropicales, que es por ejemplo donde más pesticidas se utilizan) a los más fríos, donde se depositan por condensación. De esta manera, estos compuestos pueden viajar miles de kilómetros desde el lugar en que son emitidos (Scheringer, 2009). Debido a que en los países de las regiones circumpolares existen temperaturas anuales medias muy bajas, con una exposición muy limitada a la luz solar y una biosfera de pequeño tamaño, se produce una tasa muy pequeña de evaporación de los CTP, que quedan "atrapados" allí, de forma que los seres humanos y los animales que habitan esas regiones tienden a acumular concentraciones inusualmente altas de CTP en sus organismos, en particular en sus tejidos grasos (Scheringer, 2009). Por esta razón, las primeras iniciativas puestas en marcha para el control de estas sustancias, partieron de los países industrializados situados más al norte, como Canadá y Estados Unidos. De hecho, los riegos de los CTP fueron reconocidos por vez primera en 1978 por los gobiernos de los estos países en el Acuerdo para la Calidad del Agua de los Grandes Lagos (Davies, 2006). Desde entonces se han desarrollado numerosas **estrategias nacionales o regionales para minimizar los riesgos de los CTP**, encaminadas a la reducción/eliminación de diferentes CTP en el medioambiente durante su ciclo vital, en base a sus criterios de bioacumulación, persistencia y toxicidad.

Así, el problema de los CTP ha preocupado a los gobiernos de los países industrializados, que han desarrollado numerosas iniciativas a nivel nacional o regional con desigual resultado, pudiendo o no cumplir los objetivos marcados. Sin embargo, aunque la actuación local puede

disminuir la magnitud del problema, no puede solucionarlo completamente, ya que la contaminación química se distribuye globalmente y no conoce fronteras políticas.

De hecho, existe un desconocimiento muy grande acerca de las fuentes y emisiones de CTP en muchos de los países en vías de desarrollo. Resulta paradójico el hecho de que, a pesar de que la mayoría de estos países se encuentren localizados en las regiones templadas del planeta y que debido al efecto saltamontes deberían estar relativamente descargados de CTP, los datos recogidos a partir de poblaciones humanas o de muestras de fauna silvestre de África y otras regiones, muestran que en ellas existen niveles de contaminantes iguales o incluso superiores a las de las regiones más frías del planeta (Weber y cols., 2008; Wiktelius y Edwards, 1997). Hay varias razones que explican este hecho. En primer lugar, gran parte de los CTP son sustancias plaguicidas de enorme eficacia, muy baratos, fáciles de utilizar y además son percibidos como seguros, ya que curiosamente no son muy tóxicos de forma aguda. Por ello, son una alternativa rápida, barata y efectiva para la subsistencia de miles de familias de granjeros con bajo nivel cultural, y desprovistas de equipamiento y productos modernos. Este uso se ve facilitado además por el hecho de que los controles fronterizos de mercancías en estos países son habitualmente ineficaces, lo cual podría permitir un comercio ilegal de plaguicidas prohibidos (Mansour, 2004; Ssebugere y cols., 2010).

Otro problema con el que se encuentran muchos países tropicales es el de las graves enfermedades que son transmitidas por determinados insectos. Así, el DDT ha sido recomendado como parte esencial del arsenal de insecticidas disponibles para la fumigación de interiores mientras no haya otras alternativas igual de eficaces para el control de los vectores de enfermedades tan graves como la malaria.

Pero en muchos casos, el problema trasciende a estas cuestiones, porque aunque los gobiernos de estos países en vías de desarrollo quieran sumarse o se hayan sumado ya a las iniciativas internacionales que promulgan la prohibición y eliminación de los CTP, aún queda el problema de la gestión de los plaguicidas y productos prohibidos ya fabricados. Algunos países han establecido inventarios de existencias de **plaguicidas obsoletos** con el apoyo de la FAO. En algunos casos, estas reservas han sido destruidas o eliminadas, a veces por su exportación a países industrializados con las instalaciones de incineración apropiadas. Pero los productos químicos obsoletos son a menudo difíciles de identificar, debido a condiciones de almacenamiento inadecuadas o las fugas y pérdida de etiquetado, así que la cantidad real de estas sustancias sin controlar es desconocida, constituyendo una fuente constante de emisión medioambiental (Felsot y cols., 2003; Haylamicheal y Dalvie, 2009).

De todo lo expuesto, se deduce que mientras en algunas regiones del mundo se sigan utilizando, los CTP permanecerán en el medioambiente durante largo tiempo. Incluso si la producción de estas sustancias cesara de inmediato a escala global, el problema seguiría existiendo durante varios años o quizá décadas. Ello significa que ningún país puede solucionar el problema de los CTP por sí sólo, siendo fundamentales los acuerdos y medidas globales para poder resolver el problema.

Por este motivo, en el marco del Programa de Naciones Unidas para el Medio Ambiente (PNUMA), se elabora el ***Convenio de Estocolmo sobre Contaminantes Orgánicos Persistentes (2001)***. En este Convenio se habla exclusivamente de compuestos de naturaleza orgánica, y es que cuando se habla de CTP deben considerarse por separado aquellos que contienen átomos de carbono en su estructura molecular, llamados **contaminantes orgánicos persistentes (COP)** de aquellos cuya naturaleza es inorgánica (básicamente metales y metaloides), llamados **contaminantes inorgánicos persistentes (CIP)**, que se van a considerar también de forma individual en esta Introducción.

4.1 Contaminantes orgánicos persistentes

En esta Tesis nos hemos centrado principalmente en el estudio de los COP por su relevancia para la salud y su clara vinculación con la dieta, al ser ésta, como ya hemos mencionado, su principal fuente para el ser humano.

El 23 de mayo de 2001 España, junto con otros 90 países, firmó el **Convenio de Estocolmo sobre Contaminantes Orgánicos Persistentes.** Este Convenio entró en vigor el 17 de mayo de 2004 y el 6 de junio del año pasado (2016), firmó su pertenencia al mismo el participante número 180 (Iraq). El objetivo del mismo puede resumirse en esta frase:

"Proteger la salud humana y el medio ambiente de contaminantes orgánicos persistentes, reduciendo o eliminando sus emisiones en el medioambiente".

Bajo este Convenio, los países firmantes confían en reducir y/o eliminar la producción y el uso, en primera instancia, de los 12 COP más prioritarios, conocidos comúnmente como la "docena sucia". Además, establecen un mecanismo por el cual otros COP se puedan agregar al Convenio en el futuro, como ya se hizo en el año 2009, tras la cual la lista inicial quedó ampliada a 9 sustancias más, hasta la última inclusión de sustancias en mayo de 2015. El Convenio permite ciertas exenciones a la eliminación/reducción de la producción o uso de tales sustancias y, por tanto, a las normas relativas a la importación y exportación. Con el fin principal de reducir y/o eliminar los COP, el Convenio de Estocolmo contiene:

- Medidas para eliminar o para restringir la producción, el uso y el comercio de los COP producidos intencionadamente.

- Desarrollo de planes de acción para localizar la liberación de subproductos inintencionados considerados COP, junto con la obligación de utilizar las mejores técnicas disponibles para reducir sus emisiones mediante nuevas instalaciones.

- Medidas para reducir o eliminar la liberación de COP desde lugares de almacén y abandono.

- Posibilidad de ayuda técnica y financiera a los países en vías de desarrollo, y a los países con economías en transición para que ejecuten las obligaciones del Convenio.

- Criterios y procedimientos basados en la ciencia para la adición de otros COP al Convenio.

Así, los COP se clasifican en: **Anexo A**- Sustancias que deben ser eliminadas (las partes deben tomar medidas para eliminar la producción y el uso de las sustancias listadas en este anexo), **Anexo B**- Sustancias de uso restringido (las partes deben tomar medidas para restringir la producción y el uso de las sustancias incluidas en esta lista) y **Anexo C**- Disminución de las emisiones de sustancias de producción no intencionada (las partes deben tomar medidas para reducir la liberación de los productos químicos enumerados con el objetivo de minimizar la continuación de los mismos, y, cuando sea factible, la eliminación de dicha liberación).

Es destacable que este convenio recoge también la necesidad de realizar acciones tendentes a verificar la efectividad de las medidas propuestas. Así, el artículo 11.1.b. del Convenio expone que: *"las partes (países firmantes del convenio) alentarán y/o efectuarán las actividades de investigación, desarrollo, vigilancia y cooperación adecuadas respecto de los COP y su presencia, niveles y tendencias en las personas y el medio ambiente"*, lo que se traduce, en la **justificación de la determinación y cuantificación (monitorización) de estos COP en poblaciones, alimentos, matrices ambientales y animales.**

En su Guía para el plan de vigilancia mundial de COP, el PNUMA, considerando que puede no ser necesario, o ni siquiera posible, analizar todos y cada uno de las sustancias y congéneres, recomienda analizar las sustancias listadas en la **Tabla 1** (PNUMA, 2007). Se trata de los COP precursores o determinados congéneres y de algunos productos de transformaciones, que son de interés para que los programas de vigilancia sirvan de apoyo a la evaluación de la eficacia de las medidas instaladas.

Asimismo, la Guía recomienda que, para poder calcular el potencial de toxicidad de las mezclas de contaminantes encontradas, se registre la equivalencia de toxicidad de cada uno de ellos. Por ello, para las dioxinas y los PCB análogos a las dioxinas (DL-PCB) se utiliza el factor de equivalencia de toxicidad (TEF). Se recomienda registrar las concentraciones de 29 congéneres (12 congéneres de DL-PCB + 17 congéneres de dioxinas), y mostrar por separado las equivalencias de toxicidad (TEQ) obtenidas individualmente para cada congénere, así como los TEQ parciales de cada grupo (∑TEQ-PCDD, ∑TEQ-PCDF y ∑TEQ-PCB) y el TEQ total (∑TEQ).

De cara a facilitar la descripción de las características de los COP seleccionados para este trabajo de Tesis Doctoral, abordaremos su estudio por separado, dividiéndolos en: 1) Plaguicidas organoclorados, 2) Dioxinas y compuestos análogos y 3) Hidrocarburos Aromáticos Policíclicos.

Tabla 1. Analitos recomendados por la Guía para el Plan de Vigilancia Mundial de COP.

Sustancia	COP precursores	Productos de transformación
Aldrina	Aldrina	
Clordano	*cis*- y *trans*- clordano	*cis*- y *trans*nonacloro, oxiclordano
DDT	4,4'-DDT, 2,4'-DDT	4,4'-DDE, 2,4'-DDE, 4,4'-DDD, 2,4'-DDD
Dieldrina	Dieldrina	
Endrina	Endrina	
Heptacloro	Heptacloro	Heptacloro epóxido
Mirex	Mirex	
HCB	HCB	
Bifenilos policlorados (PCB)	∑M-PCB (7 congéneres: 28, 52, 101, 118, 138, 153 y 180) PCB con TEF (12 congéneres: 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 y 189)	
Dibenzo-para-dioxinas policloradas (PCDD) y dibenzofuranos policlorados (PCDF)	PCDD/PCDF sustituidos en 2,3,7,8 (17 congéneres)	
Toxafeno	Congéneres P26, P50, P62	

4.1.1 PLAGUICIDAS ORGANOCLORADOS

Bajo el nombre de plaguicidas organoclorados (POC), se agrupa un número considerable de compuestos sintéticos cuya estructura química, en general, corresponde a la de los hidrocarburos clorados, aunque, además de cloro, algunos poseen oxígeno o azufre o ambos elementos en su estructura. Fueron los primeros insecticidas orgánicos utilizados a gran escala y entre sus propiedades destacan su reducida volatilidad, alta estabilidad química y solubilidad en lípidos, lenta biotransformación y degradación en el medio ambiente, así como una notable resistencia al ataque de los microorganismos (Matsumoto y cols., 2009). Estas propiedades, que les hacían enormemente atractivos, son también las que han provocado su prohibición en numerosos países, pues constituyen el fundamento de los problemas que los COP plantean al medio ambiente: persistencia, bioacumulación y biomagnificación en seres vivos (animales y humanos), transporte de largo alcance y toxicidad.

Hay que destacar que las restricciones impuestas en el uso de estos compuestos se han traducido en un marcado descenso en la concentración media de estos plaguicidas en tejidos humanos. Sin embargo, los estudios realizados en nuestro país y otros territorios demuestran que, tras más de 30 años después de su prohibición, aún es posible medir su principal metabolito, el DDE, en la sangre de la práctica totalidad de los individuos adultos sanos y el DDT en cerca del 30% de la población (Jakszyn y cols., 2009; Koureas y cols., 2016; Luzardo y cols., 2006; Zumbado y cols., 2005), e incluso en el líquido amniótico (Luzardo y cols., 2009). No obstante, no es un hecho sorprendente, ya que su uso persiste o incluso ha sido reintroducido en países en vías de desarrollo tras un periodo de prohibición, donde desempeñan un importante papel como

agentes de control de organismos vectores (malaria en algunos países de África y Sudamérica) y aún como insecticida de uso agrícola (WHO, 2010).

Un episodio histórico de gran trascendencia sucedió entre 1954 y 1961, en Sheldon (Estados Unidos), donde se pulverizaron las tierras con DDT para acabar con una invasión de escarabajo japonés. Los escarabajos medio muertos atrajeron a los pájaros insectívoros, la lluvia arrastró los componentes químicos, los cuales afectaron a las lombrices y contaminaron los charcos donde bebían diferentes aves, afectando posteriormente a ardillas, ratas almizcleras o conejos, cuya población disminuyó notablemente. Estos sucesos quedaron reflejados en 1962 por la bióloga Rachel Carson en la **"Primavera Silenciosa",** best-seller que fundó las bases del ecologismo moderno (Carson y cols., 1962; Jaga y Brosius, 1999). Por primera vez se habló del peligro de usar DDT y otros plaguicidas, describiendo su toxicidad y capacidad de bioacumularse. La publicación de estos trabajos condujo finalmente a la prohibición del uso del DDT y otros POC en muchos países, así como su inclusión en todas las listas de regulación de CTP.

Al igual que el resto de COP considerados en este apartado, la tendencia a la biomagnificación y persistencia ambiental de los insecticidas organoclorados hace que estén sujetos a amplios ciclos de transporte en toda la biosfera. De esta forma, se han encontrado niveles relativamente altos de un cóctel de POC en el tejido adiposo de humanos y también animales como focas, morsas, ballenas y peces muestreados en el círculo polar ártico (Hoekstra y cols., 2005; Leonards y cols., 2008; Yasunaga y cols., 2015).

4.1.1.1 Clasificación y propiedades físico-químicas

Atendiendo a su estructura, modo de síntesis u otras propiedades comunes, se han propuesto varias clasificaciones para los POC. Aquí se presenta la clasificación y las propiedades físico-químicas (O'Neil, 2006) de los más utilizados, según su estructura química:

- **Compuestos poliaromáticos clorados (DDT, Dicofol, Metoxicloro, Clorobencilato):** El DDT es prácticamente insoluble en agua a 25°C, pero es moderadamente soluble en numerosos disolventes orgánicos, tales como hidrocarburos aromáticos, cetonas y alcoholes. Su presión de vapor es baja, por lo que se considera no volátil, pero puede pasar continuamente al aire a partir del suelo, sobre todo en presencia de agua. En presencia de luz ultravioleta, el DDT pierde HCl y se transforma en DDE, compuesto que carece de acción insecticida, pero conserva todo el resto de propiedades. El resto de plaguicidas poliaromáticos clorados tienen características similares a las del DDT.

- **Cicloalcanos clorados (Hexaclorociclohexano):** El hexaclorociclohexano técnico (HCH) es una mezcla de 8 isómeros, entre los cuales el isómero conocido como *gamma* HCH o lindano está presente en una proporción del 10-18% y es el que le confiere las propiedades insecticidas a la mezcla, pues los demás isómeros apenas poseen efecto tóxico agudo sobre los insectos y otros organismos. Sin embargo, numerosos estudios identifican al isómero *beta* HCH como promotor del cáncer de mama (Wong y Matsumura, 2007; Zou y Matsumura, 2003). El lindano (*gamma*

HCH) es moderadamente soluble en agua y más soluble en acetona, cloroformo y etanol y su presión de vapor es baja.

- **Compuestos ciclodiénicos clorados (Aldrina, Dieldrina, Endrina, Heptacloro, Endosulfán, Clordano, Mirex, Clordecona):** El clordano técnico es una mezcla de diferentes hidrocarburos clorados, estrechamente relacionados por sus estructuras. En teoría contiene 70% de *cis*-clordano, 25% de *trans*-clordano, 1% de heptacloro y el 4% restante de una mezcla de otros compuestos. Su volatilidad es intermedia entre la del lindano y la del DDT. Es prácticamente insoluble en agua y soluble en éteres, cetonas, hidrocarburos aromáticos y alifáticos. Es relativamente estable ante los ácidos, mientras que en medio alcalino libera ácido clorhídrico con facilidad.

  De manera similar, el heptacloro y el endosulfán tienen limitada solubilidad en agua y se disuelven preferentemente en disolventes orgánicos. Son estables ante la humedad, el aire y el calor y no se degradan por la acción de la luz ultravioleta. El heptacloro es moderadamente estable en presencia de agua, ácidos, bases y agentes oxidantes. En el ambiente se transforma para dar su epóxido, el cual es todavía más estable. El endosulfán es sensible a los ácidos y a las bases y, en presencia de agua, se hidroliza, dando lugar a endosulfandiol, el cual carece de acción insecticida.

  La aldrina tiene un punto de fusión entre 104 y 104,5°C, es muy poco soluble en agua y estable ante los ácidos débiles, las bases y el calor. Es sensible a la acción de la luz ultravioleta y en presencia de ácidos fuertes y tras su biotransformación se transforma en dieldrina, la cual es mucho más estable.

- **Bencenos policlorados (pentaclorobenceno, hexaclorobenceno):** El hexaclorobenceno (HCB) es un sólido blanco cristalino que no ocurre de forma natural en el medio ambiente. Tiene un punto de fusión de 231°C, es prácticamente insoluble en agua y muy soluble en disolventes orgánicos. Además de haber sido utilizado hasta el año 1965 como plaguicida, se forma espontáneamente a partir de otros compuestos orgánicos policlorados, como subproducto industrial y en la combustión de residuos sólidos urbanos.

Como comprobamos, todos los POC comparten varias características fisicoquímicas, principalmente su liposolubilidad, la estabilidad química y sobre todo su elevada resistencia a la degradación. Además, su vida media en el suelo varía según el compuesto, desde días hasta 3 años para el HCH, una media de 5 años en suelos templados para la aldrina o la dieldrina o hasta 10-15 años para el DDT, como algunos ejemplos (Ritter y cols., 1995).

4.1.1.2 Estado del uso de los POC

Desde principios de los años 70, un país tras otro ha ido restringiendo o prohibiendo el uso de la mayor parte de los POC, a menudo con el DDT como única excepción para el control

de vectores de enfermedades infecciosas. En 2009, 13 países del África subsahariana y 3 del sudeste asiático usaban DDT por spray para el control de la malaria y otros planteaban reintroducirlo (WHO, 2010). En la **Tabla 2,** recogemos un resumen de los últimos usos registrados de los POC (UNEP/FAO/WHO, 2002). Los datos sobre el uso real actual de determinados plaguicidas son muy difíciles de obtener y en ocasiones ofrecen poca fiabilidad.

A fecha de 2016, la producción y utilización de todos los plaguicidas que fueron incluidos en la primera lista del Convenio de Estocolmo, con la mencionada excepción del DDT, ha cesado prácticamente por completo en todos los países desarrollados. Otros, como el HCB, si bien ya ha dejado de fabricarse y utilizarse a nivel mundial, se sigue liberando al medio ambiente, ya que es un subproducto frecuente de numerosos procesos químicos industriales y, en menor medida, de la incineración de residuos sólidos urbanos (Barber y cols., 2005; Lysychenko y cols., 2015).

Tabla 2. Últimos usos registrados de los POC incluidos en el Convenio de Estocolmo.

Plaguicida	Últimos usos conocidos
Aldrina	Contra termitas y otras plagas rastreras, almacenamiento de cereales, control de vectores.
Camfeclor (Toxafeno)	Insecticida en cultivos de algodón.
Clordano	Contra termitas y otras plagas rastreras.
DDT	Control de vectores transmisores de enfermedades infecciosas para el hombre y animales (*Anopheles spp.*, mosca tse-tse, pulgas).
Dieldrina	Control de la langosta, termitas, y vectores de enfermedades humanas.
Endrina	Antiguamente utilizado para el control de roedores e insectos. No se conocen usos actuales ni recientes.
HCB	Antiguamente utilizada para el tratamiento de semillas contra enfermedades fúngicas, así como para fines industriales. No se conocen usos agrícolas o industriales actuales o recientes.
Heptacloro	Contra termitas y otras plagas rastreras.
Mirex	Contra hormigas cortadoras de hojas, termitas y como retardante de llama y otros fines industriales.
HCH	Uso agrícola general. Aplicaciones farmacéuticas. Aplicaciones veterinarias. Insecticida para instalaciones ganaderas.
Endosulfán	Uso agrícola común. Prohibido en España en 2008.

Posteriormente a los COP incluidos en primera instancia en el Convenio de Estocolmo, se incluyó el lindano y la clordecona, que por consiguiente han sido utilizados más recientemente. El último COP en ser incluido fue el endosulfán en el 2011 y el dicofol se encuentra bajo revisión. El endosulfán era ampliamente utilizado en nuestro país, por ejemplo en el cultivo de tomates en Almería, Murcia o Canarias y se empleaba en muchos países del mundo para el control de plagas agrícolas, preservación de madera, control de la mosca tse-tse, etc. (Srivastava y cols., 2009).

Tras la inclusión del lindano en el Convenio de Estocolmo en 2009, su uso agrícola ha cesado por completo en todos los países que han ratificado el Convenio, pero las aplicaciones farmacéuticas para el control de piojos y la sarna se permitieron como excepción durante 5 años más. Estados Unidos, que aún no ha ratificado el Convenio de Estocolmo, reserva los usos farmacéuticos como tratamiento de segunda elección para piojos/sarna (EPA, 2006b). Además, la síntesis de lindano es ineficaz, ya que por cada tonelada producida, se generan de 8 a10 toneladas de los isómeros *alfa* y *beta* del HCH.

4.1.1.3 Parámetros toxicocinéticos

Como ocurre con todas las sustancias químicas, la toxicidad de los organoclorados depende de su absorción, distribución, metabolización y eliminación:

- **Absorción:** Los POC (con algunas excepciones) pueden ser absorbidos de forma tópica u oral de forma muy rápida, debido a la alta liposolubilidad de estos compuestos (Marth y cols., 1989). Actualmente, la vía principal de exposición a estos compuestos es la oral, a través de los alimentos, principalmente los más ricos en grasas animales. Como hemos visto, no son altamente volátiles, así que la inhalación no es una ruta normal de exposición (Jaeger y cols., 1975).

- **Distribución:** En el organismo, se distribuyen al hígado, riñón, cerebro y principalmente al tejido adiposo (Tao y cols., 2009). En el medioambiente, al igual que todos los hidrocarburos tratados con cloro, lo que les confiere una alta liposolubilidad, se distribuyen igualmente a los tejidos grasos de los seres vivos; consecuentemente ocurre la bioacumulación en la cadena alimentaria, y por consiguiente en vegetales, animales (Backer y cols., 2001) y finalmente en seres humanos (Gerber y cols., 2016).

- **Metabolización:** Los compuestos poliaromáticos clorados, tales como el DDT, son declorinados mediante oxidasas de función mixta (MFO), si bien no todos con la misma eficacia. Así, el metoxicloro es rápidamente eliminado comparado con el DDT por decloración y oxidación. Los ciclodienos, tales como la endrina, son rápidamente convertidos en epóxidos mediante las MFO. Los bencenos policlorados, como el pentaclorobenceno, experimentan glucuronidación y sulfoconjugación. Como vemos, existen varias rutas de biotransformación de los POC, pero en general, la degradación metabólica de estos plaguicidas tiene lugar muy lentamente, debido a la presencia de múltiples átomos de cloro como sustituyentes, muy difíciles de eliminar por mecanismos enzimáticos, y a las estructuras aromáticas complejas que presentan algunos de estos compuestos. El lento ritmo de metabolismo junto con la elevada liposolubilidad dan lugar al almacenamiento prolongado en el tejido adiposo, del que se movilizan muy lentamente para restaurar el equilibrio entre tejidos y sangre a medida que progresa la transformación metabólica y la excreción.

- **Excreción:** La mayor ruta de excreción de los organoclorados es mediante la bilis al tracto digestivo y consecuentemente puede ocurrir la recirculación enterohepática. Los metabolitos son también lipofílicos, con lo que se almacenan en el tejido adiposo y

pueden distribuirse nuevamente (Tanabe y Kunisue, 2007). La vida media de algunos difenilalifáticos y los ciclodienos puede ser desde varios días hasta varios meses/años. La eliminación puede a veces ser explicada por un modelo de dos compartimentos, donde la primera fase es una eliminación rápida y la segunda, una eliminación más prolongada (Daly y cols., 2007; Tanabe y Kunisue, 2007).

4.1.1.4 Mecanismos de acción y toxicidad

Cuando hablamos del mecanismo de acción tóxica de los POC, hay que distinguir claramente entre el mecanismo que produce la toxicidad aguda, responsable de la amplia utilización durante décadas de estos compuestos en el control de plagas, de los mecanismos de toxicidad a largo plazo; comunes además a gran parte del resto de CTP, que se producen tras la exposición inadvertida a bajas concentraciones de estos compuestos durante toda la vida, como ya hemos visto, a través de la dieta principalmente (Luzardo y cols., 2009; Wang y cols., 2011). Describiremos de forma breve en este apartado los mecanismos de toxicidad aguda y algunos aspectos de la toxicidad crónica, ya que gran parte de los efectos atribuibles a la exposición crónica se encuadran en el concepto de disrupción endocrina, ya discutido en detalle.

Los **efectos tóxicos agudos** de los POC, al igual que ocurre con el resto de productos plaguicidas, se clasifican como primarios y secundarios. El efecto primario es aquel por el cual el plaguicida actúa directamente sobre una especie dada, matándola o reduciendo sensiblemente su población. El efecto es secundario si el plaguicida no actúa directamente sobre la especie, pero destruye su sustrato o su hábitat y la pone seriamente en peligro de desaparición (Milne, 1995). Así, hay varios mecanismos de toxicidad aguda de los POC. Un mecanismo común es la interferencia con el arco reflejo que se establece entre los nervios sensoriales, el sistema nervioso central y los nervios motores, que activan la respuesta muscular a los estímulos externos (Mariussen y Fonnum, 2006). En la intoxicación aguda en mamíferos y el hombre, se observa un cuadro sintomatológico derivado de su interferencia con los mecanismos de neurotransmisión: parestesia en la lengua, labios y cara, hipersensibilidad a estímulos externos, como la luz, sonido o tacto, irritabilidad, mareos, vértigo, temblores y convulsiones persistentes (Milne, 1995).

En la intoxicación en insectos o mamíferos por acción del DDT o compuestos similares, se observan temblores y convulsiones persistentes, lo que sugiere una descarga repetida de las neuronas. Los POC inducen un alargamiento considerable de la fase descendente del potencial de acción, manteniendo a la membrana de la neurona en un estado de despolarización parcial, muy susceptible a una despolarización total ante menores estímulos. Esta modificación del comportamiento eléctrico se produce por varios mecanismos, afectando la permeabilidad de la membrana de las células nerviosas, lo que reduce el transporte de iones potasio.

Por otra parte, los POC modifican la funcionalidad de los canales porosos por los que circulan los iones sodio, de forma que su apertura se produce de forma normal, pero se dificulta su cierre, que tiene lugar más lentamente que en ausencia de este compuesto. Por consiguiente, interfiere con el transporte activo de sodio hacia el exterior del axón durante la repolarización (Mariussen y Fonnum, 2006; Narahashi, 2002). El DDT, además, inhibe la calmodulina, necesaria para el transporte de iones calcio, a su vez imprescindible en la secreción de neurotransmisores en la sinapsis. También inhibe la adenosina trifosfatasa (ATPasa) de las neuronas, en particular las bombas de sodio, potasio y calcio, esenciales en la repolarización (Janik y Wolf, 1992).

Los clorobencenos, clorociclohexanos y clorociclodienos también son inhibidores potentes de las bombas de Na^+, K^+ y Ca^{2+} en las membranas de las neuronas, provocando potenciales de acción disminuidos (Mariussen y Fonnum, 2006; Narahashi, 2002). Pero estos plaguicidas también provocan una estimulación del sistema nervioso central, ya que son antagonistas del ácido γ-aminobutírico (GABA), con lo que bloquean el paso de iones cloruro inducido por este neurotransmisor (Coats, 1990; Sunol y cols., 1998). El mecanismo inhibitorio del GABA explica los efectos colinérgicos (sobreestimulación mediada por la acetilcolina) de la dieldrina y del lindano en varias especies. Esto produce una hiperpolarización que inhibe la liberación de más neurotransmisores al espacio sináptico, por lo que se limita la estimulación del nervio post-sináptico. A diferencia del DDT, para el que existen pocos casos de intoxicación aguda con resultado de muerte, los clorociclodienos han sido causantes de numerosas muertes. Se absorben rápida y eficazmente por vía cutánea, lo que contribuye a una mayor toxicidad en las exposiciones ocupacionales.

Además de los efectos ya comentados sobre el sistema nervioso, la exposición al lindano y al llamado lindano técnico (una mezcla de todos los isómeros del HCH) produce efectos tóxicos sobre el hígado y los túbulos renales (Reddy y cols., 1994; Srinivasan y cols., 1984).

Brevemente, estos son algunos efectos tóxicos a corto y largo plazo de los plaguicidas organoclorados, según su estructura química:

En relación a la **aldrina, la dieldrina** (metabolito de la aldrina y usado también como pesticida) y al **heptacloro époxido**, muestran toxicidad aguda moderada y sus efectos tóxicos son predominantes en el sistema nervioso y el hígado, siendo los efectos muy similares entre uno y otro para el caso de la aldrina y dieldrina (EFSA, 2005b; EFSA, 2007c). No son genotóxicos ni teratogénicos y se puede ver afectada la reproducción y el sistema inmune, y en el caso de la **endrina** puede ocurrir toxicidad fetal (EFSA, 2005a). El **clordano**, considerando sus dos isómeros principales, tiene efectos similares y causa tumores de hígado en ratones, probablemente por mecanismos no genotóxicos (EFSA, 2007a).

En el caso del **HCB**, se absorbe fácilmente en los seres humanos y animales. Tiene baja toxicidad aguda y el hígado es el órgano predominantemente afectado, provocando inducción enzimática y porfiria. Es inmunotóxico y se ha descrito toxicidad en ovarios de primates a dosis muy bajas. Se clasifica por la Agencia Internacional para la Investigación del Cáncer (IARC) como posible carcinógeno humano, basado en efectos observados en animales de experimentación, exhibiendo una actividad mutagénica débil y, por lo tanto, se podría tener en cuenta un modo de acción genotóxico (EFSA, 2006b).

El **DDT** tiene una toxicidad aguda baja para los mamíferos y la mayoría de las especies de aves. Los principales órganos diana son el sistema nervioso y el hígado y afecta también a los tejidos hormonales, a la reproducción, el desarrollo fetal y el sistema inmunológico. El DDT, incluyendo el p,p'-DDE, causa tumores, principalmente en el hígado de animales de experimentación (EFSA, 2006a).

En contraste al resto de plaguicidas organoclorados, altamente liposolubles, el **endosulfán** tiene una afinidad menos pronunciada por los lípidos. En consecuencia, es menos probable su biomagnificación y bioacumulación en la cadena alimentaria, por lo que la presencia de este compuesto o sus metabolitos en los alimentos se asocia normalmente a niveles bajos. Sin embargo, bajo prácticas agrícolas o de uso inadecuadas, se ha descrito ampliamente que puede producir efectos neurotóxicos en los seres humanos y los animales e inducir una serie de efectos que incluyen toxicidad hepática y renal, efectos hematológicos y alteraciones en el sistema inmune y órganos reproductivos (EFSA, 2005c).

En relación al **HCH**, su toxicidad varía según el isómero considerado, siendo el γ-HCH el más neurotóxico, mientras que El β-HCH penetra con menor facilidad en el cuerpo tras la absorción. Todos los isómeros causan hiperplasia del hígado y tumores hepáticos. A excepción de los animales de experimentación, existe poca disponibilidad de datos sobre la toxicidad en otras especies animales. El β-HCH posee una actividad estrogénica débil y los isómeros α- y β-HCH son promotores tumorales en hígados de rata (EFSA, 2005d). El HCH se clasificó por la IARC en el grupo 2B (posiblemente carcinógeno) en base a pruebas suficientes de carcinogenicidad para los seres humanos (por el isómero alfa) y actualmente el lindano técnico (γ-HCH), se encuentra en fase de preparación para ser incluido como carcinógeno humano (Grupo 1), siendo el primer POC en ser incluido en dicha categoría. Por ello, en la **Tabla 3** hemos decidido reflejar la clasificación actual de los POC según la clasificación de la IARC.

Tabla 3. Clasificación de los Plaguicidas Organoclorados según la IARC.

Plaguicida organoclorado	Carcinogenicidad IARC (Grupo)
Lindano	1
DDT	2A
HCB **HCH** **Clordano** **Heptacloro** **Mirex** **Toxafeno**	2B
Aldrín **Dicofol** **Dieldrín** **Endrín** **Metoxicloro**	3

4.1.1.5 Niveles de exposición alimentaria de los ciudadanos de la UE a POC

Como ya hemos comentado, actualmente la exposición humana a contaminantes ambientales como los COP se produce en un 90% a través del consumo de alimentos contaminados (Baars y cols., 2004; Liem y cols., 2000). Numerosos estudios nos describen la contaminación de los alimentos en general (Schecter y cols., 2010; Törnkvist y cols., 2011), y nos muestran al DDE como el pesticida más frecuentemente detectado en alimentos (Rylander y cols., 2012; Sarcinelli y cols., 2003), o como el de mayor concentración (Luzardo y cols., 2012). Podemos entonces considerar que el DDE encontrado en suero humano, se deba probablemente a la exposición dietética, ya que se ha comprobado que la capacidad humana para metabolizar DDT es muy limitada.

En el año 2009 Van Audenhaege y cols., realizaron una estimación de la ingesta diaria máxima teórica (IDMT) en grupos poblacionales con diferentes hábitos dietéticos en Europa, incluyendo 421 plaguicidas, entre ellos los POC (Van Audenhaege y cols., 2009). En este estudio se calculó la IDMT, expresada como porcentaje de la IDA, basándose en los LMR establecidos para cada uno de los plaguicidas y los datos sobre los hábitos nutricionales de la población. Los resultados para los POC se muestran en la **Tabla 4.**

No obstante, hay que remarcar que se trata sólo de una estimación teórica máxima, que implica asumir que los controles funcionan perfectamente y que no se comercializa en la UE ningún alimento que presente niveles de contaminación por POC que supere los Límites Máximos de Residuos (LMR) establecidos. También implica considerar que todos los alimentos consumidos presentan un nivel de residuos igual al LMR de cada contaminante.

Para poder hacer una estimación real del consumo de POC habría que apoyarse en los estudios de dieta total o como mínimo en un muestreo aleatorio de alimentos de todos los grupos. Un **estudio de dieta total** considera el total de alimentos que componen la dieta de un individuo de la población de estudio y analiza en ellos los niveles de cada uno de los contaminantes químicos. De esta forma, supervisa el nivel de consumo total de residuos de las sustancias tóxicas que habitualmente están presentes en los alimentos que componen dicha dieta. Por lo tanto, determinan la exposición global de un individuo a los contaminantes, analizando si esta exposición posee un riesgo inaceptable para la salud humana. Otra característica muy importante es que todos los análisis se realizan sobre los alimentos listos para consumir, dado que la preparación de los alimentos afecta a la concentración de los contaminantes y otras sustancias. Así, por ejemplo, un bistec de ternera debe ser preparado (frito, a la plancha, etc.) o el arroz cocinado, ya que de esta manera es como se consumen.

Cabe mencionar que existen estudios específicos emitidos por la EFSA en relación a los niveles de pesticidas organoclorados, como para el DDT o el HCB, pero éstos se refieren a los niveles en los alimentos de producción animal. El último informe de la EFSA emitido en cuanto a residuos de pesticidas en alimentos es de 2013, pero no se centra de forma concisa en la exposición a los POC mediante la ingesta dietética de los diferentes grupos alimentarios. Por tanto, a falta de informes globales a nivel europeo sobre la contaminación de alimentos por POC realizados a partir de muestreos aleatorios, como los disponibles para otros grupos de contaminantes (dioxinas, cadmio, mercurio, etc.), presentamos a modo orientativo los resultados del estudio de Van Audenahege y cols., del año 2009. Según esta estimación, en la UE se podrían consumir niveles de aldrina, dieldrina y heptacloro superiores al valor de IDA establecido por la OMS. En este trabajo se caracterizó la contribución de 10 grupos de alimentos (incluyendo la leche y productos lácteos, huevos, carne y otros productos de origen animal, agua y vino y alimentos de origen vegetal) como una relación entre el consumo de los grupos alimenticios y la ingesta total.

Tabla 4. Estimación teórica de la exposición (IDMT) a POC de la población europea a través de la dieta en función de sus hábitos alimentarios. Los resultados se expresan como porcentaje de la IDA para cada contaminante.

POC	IDA (mg/kg)	IDMT (% IDA)
Aldrina	0.0001	348.8
Dieldrina	0.0001	348.8
Clordano (suma de *cis*- y *trans*- clordano)	0.0005	38.5
DDT (suma de 4,4'-DDT, 2,4'-DDT, 4,4'-DDE y 4,4'-DDD, expresado como DDT)	0.01	17.5
Endosulfán (suma de los isómeros α- y β- endosulfán y endosulfán sulfato, expresados como endosulfán)	0.006	27.1
Endrina	0.0002	77.7
Heptacloro	0.0001	331.0
HCH (isómero γ) – lindano	0.005	4.2
Metoxicloro	0.1	0.1

4.1.2. DIOXINAS Y COMPUESTOS ANÁLOGOS

Bajo el nombre genérico de dioxinas y compuestos análogos se engloba a un grupo de sustancias químicas complejas que se caracterizan por tener varios aspectos en común, entre ellos, contener cloro en sus moléculas y una alta liposolubilidad, lo que las convierte en sustancias estructural, ambiental y biológicamente persistentes, además de acumularse y biomagnificarse a lo largo de la cadena trófica (Van den Berg y cols., 1998; Van den Berg y cols., 2006). Además, las dioxinas son ampliamente conocidas por su potente capacidad carcinogénica, estando incluidas en el Grupo I de sustancias de la IARC. En este trabajo únicamente se analizaron los PCB análogos de las dioxinas, en los cuales nos centraremos.

4.1.2.1 Clasificación y propiedades físico-químicas. Fuentes de emisión

Dentro de este complejo grupo nos podemos estar refiriendo a 4 tipos de sustancias: (1) Dibenzo-p-dioxinas policloradas (PCDD) o dioxinas propiamente dichas, (2) Dibenzofuranos policlorados (PCDF) o furanos, (3) Bifenilos policlorados (PCB) y (4) Bifenilos polibromados (PBB), aunque debido a la relativamente corta vida comercial de éstos últimos y su restringida distribución ambiental, habitualmente se les excluye de la consideración toxicológica de "análogos a las dioxinas".

- **Las dioxinas (PCDD/PCDF)** son un grupo amplio y estructuralmente relacionado de hidrocarburos aromáticos clorados (Institute of Medicine (U.S.). Committee on the Implications of Dioxin in the Food Supply. y Institute of Medicine (U.S.), 2003). Teóricamente, existen 210 congéneres posibles dentro de este grupo: 75 dibenzo-*p*-dioxinas policloradas (PCDD) y 135 dibenzofuranos policlorados (PCDF). Las 75 PCDD están formadas por dos anillos de benceno unidos a su vez por dos átomos de oxígeno y en su molécula pueden contener de cuatro a ocho átomos de cloro. El prototipo de dioxina está representado por el congénere más tóxico de todos, la 2,3,7,8 tetraclorodibenzodioxina (TCDD). Los 135 PCDF se componen de dos anillos de benceno, pero los anillos están unidos por un solo átomo de oxígeno. Cada anillo tiene cuatro sitios disponibles para la unión de los átomos de cloro.

  Se trata de microcontaminantes de origen antropogénico ampliamente distribuidos en el medio ambiente. No tienen uso alguno, pero se forman espontáneamente en multitud de procesos industriales, como la manufactura de ciertos productos químicos, procesos industriales como la fabricación de PVC o la incineración de residuos sólidos urbanos. Son sustancias que cumplen todas las características de los CTP.

- **Los bifenilos policlorados (PCB** por sus siglas en inglés**)** son una serie de compuestos organoclorados, que constituyen una serie de 209 congéneres, los cuales se forman mediante la cloración de diferentes posiciones del bifenilo, 10 en total. Cada posición puede ser sustituida por un átomo de cloro. Si las posiciones 2, 2', 6 y 6' no tienen ningún cloro los bifenilos se mantienen coplanares, hablando por tanto de PCB coplanares o *no-orto*. Si tenemos una posición sustituida en cada lado, son PCB *mono-orto* sustituidos, y el resto son los PCB no coplanares. Su fórmula empírica es $C_{12}H_{10}$-nCln, donde n puede variar entre 1 y 10, siendo mayoritarios los congéneres con 2 a 7 cloros.

Los PCB coplanares y los *mono-orto* sustituidos son los que tienen mayor importancia medioambiental y analítica debido a que su toxicidad puede ser asimilada a la de las PCDD/PCDF, posiblemente debido a la coplanaridad de la molécula (Headrick y cols., 1999; Huwe, 2002; Schecter y cols., 2006a), y son los que denominamos "compuestos análogos a las dioxinas" (**DL-PCB**, de la denominación anglosajona *dioxin-like PCBs*).

Las propiedades fisicoquímicas de estos compuestos también dependen del grado de cloración y de si son *no-orto*, *mono-orto* o no coplanares. Así, la presión de vapor disminuye con el grado de cloración, y lo mismo con su estabilidad en el medio ambiente. El periodo de semivida puede variar desde 10 días a un año y medio; por lo general estos compuestos son termoestables (punto de ebullición de 320-420°C), no los ataca la luz y son difícilmente biodegradables. Son ligeramente solubles en agua y muy liposolubles, por lo que se disuelven en su mayor parte en disolventes orgánicos (Headrick y cols., 1999; Huwe, 2002; Schecter y cols., 2006a).

A diferencia de las dioxinas, que aparecen como subproductos indeseables, los PCB fueron intencionadamente fabricados. Su producción comercial para una gran variedad de aplicaciones, comenzó mayoritariamente en 1929 por la compañía Monsanto en los Estados Unidos y se extendió hasta 1977 (Headrick y cols., 1999). Debido a sus características físico-químicas como son su alta estabilidad, inercia y características dieléctricas, que eran ventajosas para muchos propósitos industriales, los PCB fueron utilizados para una gran variedad de usos; en sistemas cerrados tales como transformadores eléctricos, condensadores y sistemas de traspaso térmico (en todos ellos como retardantes de llama para evitar el incendio de dichos aparatos), así como también, en sistemas hidráulicos. Durante un tiempo también tuvieron otra variedad de usos, ya que se utilizaron en formulaciones de pinturas, polímeros, pegamentos, lubricantes, plastificantes, formulaciones de plaguicidas y como agentes para la suspensión de pigmentos en el papel de copia sin carbón (Headrick y cols., 1999; Safe, 1990). La producción de PCB fue muy elevada en la mitad el siglo XX por empresas como **Monsanto (EEUU), Bayer (Alemania), Prodelec (Francia), Caffro (Italia) y Kanechlors (Japón)**, estimándose que entre 1930 y 1977 se fabricaron alrededor de 2 millones de toneladas de PCB (Kimbrough, 1987; Kimbrough, 1995; Safe, 1990).

Su fabricación está prohibida desde 1977 en Estados Unidos y desde entonces se fueron prohibiendo en todos los países en donde se fabricaba, siendo Alemania en 1983 el último país en dejar de fabricarlos (Schecter y cols., 2006a). Se estima que de los dos millones de toneladas que se fabricaron en el pasado, alrededor de 370.000 toneladas todavía están presentes en el medio ambiente, donde no han sufrido degradación. Además, unas 780.000 toneladas más siguen aún funcionando en equipos eléctricos antiguos, lo que significará si no se toman medidas, una futura fuente de descarga medioambiental cuando estos productos sean desechados.

4.1.2.2 Parámetros toxicocinéticos

Debido a que los productos comerciales son una mezcla de congéneres individuales que se diferencian entre sí en el número de átomos de cloro y en la posición que estos ocupan, no es fácil estudiar su absorción, distribución, metabolismo y eliminación. Para conocer la toxicocinética de los PCB, se han realizado numerosos experimentos en diferentes especies animales con una mezcla comercial de bifenilos polibromados (PBB) (FireMaster BP-6). La información encontrada para los PBB se puede aplicar a los PCB debido a su similitud estructural (Fries, 1985; Gupta, 2007).

- **Absorción:** Debido a la alta liposolubilidad de estos compuestos, la absorción es eficaz por todas las vías. No obstante, la principal vía de exposición es la oral, a través de los alimentos, siendo la absorción por el resto de vías prácticamente despreciable. La absorción gastrointestinal puede exceder el 75% de la dosis ingerida para los congéneres tratados con cloro en porcentajes más bajos. El incremento del tamaño molecular (proporcional al número de sustituciones con cloro) reduce significativamente la absorción intestinal, estimándose la absorción de los compuestos con 8 átomos de cloro en tan solo un 2-10%.

- **Distribución:** Las dioxinas y compuestos análogos se distribuyen extensa y eficazmente por el organismo. Una vez absorbidos, alcanzan fácilmente el compartimento central y se distribuyen principalmente unidos a los quilomicrones, lipoproteínas y otras proteínas plasmáticas. El hígado y de forma muy relevante el tejido adiposo son los principales sitios de depósito de estos compuestos en la mayor parte de las especies. En algunas especies son también relevantes como sitios de almacenamiento la piel y las glándulas suprarrenales. La concentración en los músculos y órganos suele ser menor que la del tejido adiposo.

  La transferencia placentaria de las dioxinas y análogos depende, al igual que para la absorción, del tamaño molecular. Los compuestos con menor cantidad de átomos de cloro son altamente retenidos en el feto, aunque la concentración del feto o del recién nacido es relativamente baja comparada con la concentración en los tejidos de la madre. En muchas especies de mamíferos, la transferencia de PCB de la madre al hijo a través de la lactancia es cuantitativamente más importante que el transporte hacia el feto a través de la placenta. La excreción a través de la lactancia disminuye conforme aumenta el contenido en cloro de la molécula, siendo más pronunciado para los congéneres hepta- y octacloro sustituidos (Pohjanvirta y Tuomisto, 1994; Van den Berg y cols., 1994). Como en los mamíferos, en las aves son el hígado y el tejido adiposo los sitios principales para el almacenaje y la acumulación de dioxinas y PCB, y el grado de deposición parece aumentar con el aumento del número de átomos de cloro, dando como resultado, una transferencia limitada de los congéneres más altamente tratados con cloro hacia los huevos (Van den Berg y cols., 1994).

- **Metabolismo:** Es necesario para la eliminación urinaria y biliar, desempeñando así un papel importante en la regulación del índice de la excreción de éstos compuestos. Por tanto, es un proceso de detoxificación y así la toxicidad es atribuible al compuesto padre sin cambios (Pohjanvirta y Tuomisto, 1994). En estudios in vivo, se ha sugerido que el metabolismo tiene lugar si hay dos posiciones colindantes vacías (Fries, 1985; Matthews y cols., 1978). En ratas, las reacciones metabólicas incluyen la oxidación, preferiblemente en las posiciones laterales y la decloración reductora, así como la rotura de las uniones de oxígeno del difenil éter.

- **Excreción:** La eliminación de los metabolitos polares de PCDD/PCDF y PCB ocurre predominantemente por vía biliar y fecal, donde la excreción urinaria desempeña un papel muy minoritario. Sin embargo, esto no es así para todas las especies, ya que por ejemplo la eliminación urinaria desempeña un papel importante en el hámster. La semivida de estos compuestos en humanos es de alrededor de 6 meses, dependiendo del índice de cloración de la sustancia (Leung y cols., 2006).

  Así, la leche y los huevos debido a su composición grasa, representan una vía muy eficaz de excreción en mamíferos y aves, respectivamente (Brambilla y cols., 2008; Hoogenboom y cols., 2016; Schecter y cols., 2006b). Este hecho no ha pasado desapercibido y el Convenio de Estocolmo recomienda que la leche (en particular la humana) sea utilizada con una periodicidad regular en la **biomonitorización de la presencia de CTP**, como los PCB, para comprobar la eficacia de las medidas articuladas (PNUMA, 2007).

4.1.2.3 Mecanismos de acción y toxicidad

El mecanismo de acción mejor conocido y estudiado de las dioxinas y compuestos análogos es el que está mediado principalmente por su capacidad de unirse al receptor de aril hidrocarburos (AhR), del que son agonistas, induciendo la síntesis de proteínas.

El AhR es un factor de transcripción activado por unión de ligando que está implicado en la regulación de varios genes, incluyendo muchos que codifican para enzimas que desempeñan un papel importante en el metabolismo de las sustancias tóxicas, así como en genes implicados en la regulación del crecimiento y la diferenciación celular (Denison y Nagy, 2003; Denison y cols., 2002; Hahn, 2002; Mandal, 2005). Parece ser que muchos de los efectos tóxicos atribuidos a estas sustancias cloradas requieren la activación del AhR, siendo más tóxicos los congéneres que se unen con mayor afinidad al AhR (Okey y cols., 1994). Hay diferencias relacionadas con la especie afectada, que se explican parcialmente por diferencias en la afinidad de unión al ligando debido a variantes polimórficas del AhR (Karchner y cols., 2006).

En ausencia de ligando, el AhR está como complejo soluble multiproteínico en el citoplasma celular. Cuando una dioxina o compuesto análogo atraviesa la membrana plasmática, se une al AhR y el complejo ligando-AhR experimenta un cambio conformacional que expone una secuencia de localización nuclear. El complejo se trasloca entonces al núcleo de la célula y las chaperonas se disocian del complejo. El complejo AhR-ligando es capaz ahora de unir varias proteínas nucleares y formar heterodímeros que son capaces de unirse a

determinadas regiones del ADN, conocidas como elementos de respuesta a las dioxinas (DRE). La unión de los heterodímeros activados por ligando al DRE estimula la transcripción de una serie de genes, destacando los que codifican para las enzimas de la subfamilia CYP1A1 del citocromo P450 (Denison y Nagy, 2003; Denison y cols., 2002). Se piensa que la modulación continua e inadecuada de la expresión de estos genes es la responsable de una serie de cambios bioquímicos, celulares y de los tejidos que dan lugar a parte de los efectos tóxicos de estos compuestos (Mandal, 2005).

Sin embargo, es destacable que la toxicidad de los diferentes congéneres de PCDD/PCDF y PCB difiere considerablemente entre sí. De los 210 posibles congéneres de PCDD y PCDF, sólo aquellos que tienen átomos de cloro en las posiciones 2-, 3-, 7- y 8- de los 2 anillos aromáticos son relevantes desde un punto de vista toxicológico. Son en total 17 congéneres que exhiben un perfil toxicológico similar, siendo el más tóxico de todos el que presenta átomos de cloro en las 4 posiciones: la 2,3,7,8 tetracloro-*p*-dibenzodioxina (2,3,7,8-TCDD) (Ahlborg, 1993). Por su parte, de los 209 congéneres posibles de PCB, los DL-PCB presentan la capacidad de adoptar una conformación coplanar y tienen la capacidad de unirse al receptor AhR (Safe, 2005), siendo así similares a la del 2,3,7,8 TCDD. Ello favorece que presenten una fuerte afinidad al AhR, lo que condiciona que estos 12 congéneres de PCB sean caracterizados como compuestos similares a las dioxinas por tener un potencial tóxico similar a éstas. Si bien las PCDD/PCDF son considerablemente más tóxicas que los DL-PCB, las cantidades liberadas de éstos al medioambiente son mayores y por lo tanto se encuentran habitualmente a concentraciones superiores en los alimentos.

Además, los congéneres de **PCB marcadores de exposición** (**M-PCB**, de la denominación anglosajona *marker PCBs*) frecuentemente detectados, como los 138, 153, y 180 tienen una menor toxicidad que los DL-PCB (McFarland y Clarke, 1989; Safe, 1990). Sin embargo, se consideran entre los 36 más importantes para el medio ambiente, por su prevalente potencial tóxico y su relativa abundancia en el tejido animal y son causantes de efectos biológicos adversos, basándose en su capacidad para inducir el sistema oxidasa de función mixta (Rignall y cols., 2013).

Se considera a las dioxinas y análogos entre las sustancias orgánicas más tóxicas que existen. Así, la exposición crónica a estas sustancias está relacionada con varios tipos de cáncer. Basándose tanto en estudios con animales como en evidencias epidemiológicas, la 2,3,7,8-TCDD ha sido catalogada por la IARC dentro del grupo 1: “carcinógenos para el ser humano”(IARC, 2012b). No obstante, según la misma IARC, no es un carcinógeno directo, es decir no afecta directamente al material genético, y se considera que existe un nivel de exposición por debajo del cual el riesgo de que produzca cáncer es despreciable.

Con la finalidad de poder evaluar correctamente el riesgo de la exposición a estas sustancias, se desarrolló el concepto de **Equivalencia de Toxicidad (TEQ)** para describir la toxicidad acumulada de las mezclas complejas de estos compuestos (Ahlborg y cols., 1992). El procedimiento consiste en la asignación a cada uno de los congéneres de PCDD, PCDF y DL-PCB de un **Factor de Equivalencia (TEF)** a la toxicidad del 2,3,7,8-TCDD, que se toma como referencia (TEF = 1). El TEQ de una mezcla se calcula multiplicando las concentraciones de cada congénere individual por su TEF, para obtener el TEQ de cada congénere y posteriormente sumando los TEQ individuales para obtener el TEQ total.

Mediante el Programa Internacional de Seguridad Química (ICPS), la OMS ha establecido y reevaluado los TEF a lo largo de las últimas décadas. Por este motivo, los valores de TEF han ido cambiando desde su establecimiento hasta la última reevaluación en el año 2005 (Van den Berg y cols., 2006). Para evitar confusiones, se recomienda que se especifique

como subíndice el año del valor de TEF usado. No obstante, para los artículos de esta Tesis Doctoral, hemos utilizado siempre los valores de la última revisión (2005), que para los DL-PCB son los que se muestran en la **Tabla 5** y, en consecuencia, se ha omitido el subíndice.

La utilización del modelo TEQ para estimar el riesgo de la exposición a dioxinas y compuestos análogos implica hacer varias asunciones:

- Los efectos de PCDD, de PCDF y/o de PCB individuales en una mezcla son aditivos.
- Solamente a los congéneres análogos en su toxicidad al 2,3,7,8-TCDD se les ha asignado un valor TEF.
- Todos estos compuestos se unen al AhR y manifiestan las mismas respuestas tóxico-bioquímicas receptor-mediadas.

Tabla 5. Factores TEF para los congéneres tóxicos de DL-PCB.

Compuesto	TEF
PCB *no-orto* sustituidos	
PCB-77	0.0001
PCB-81	0.0003
PCB-126	0.1
PCB-169	0.03
PCB *mono-orto* sustituidos	
PCB-105	0.00003
PCB-114	0.00003
PCB-118	0.00003
PCB-123	0.00003
PCB-156	0.00003
PCB-157	0.00003
PCB-167	0.00003
PCB-189	0.00003

Sin embargo, este modelo no toma en consideración la posibilidad de que en estas mezclas complejas los fenómenos no sean simplemente aditivos, sino que puedan darse además fenómenos de sinergismo o potenciación con otros contaminantes químicos, presentes en las mezclas de cada individuo. A pesar de todo, la aproximación TEQ se ha adoptado internacionalmente como la manera más apropiada para estimar el potencial de riesgo para la salud de mezclas de dioxinas y compuestos análogos (Pizarro-Aránguiz y cols., 2015; Schecter y cols., 2006b).

Los efectos tóxicos de los PCB dependen de distintos factores como el contenido en cloro, el tipo de exposición y la duración, la edad o el sexo. Si la exposición se produce en el útero materno, podemos encontrarnos a largo plazo una deficiencia de las células mediadoras del sistema inmune, con aumentos de la incidencia de infecciones de oído medio, disminución de los glóbulos blancos o con déficit neurológicos persistentes (déficit cognitivos, cambios de humor, alteraciones del comportamiento); y estudios in vitro indican que son potentes inductores de la apoptosis de monocitos (Shin y cols., 2000) y timocitos (Tan y cols., 2003). Pero si la exposición es dietética, la continua ingesta de estas sustancias se ha relacionado con dislipemias, obesidad (Lee, 2012; Lee y cols., 2011), diabetes tipo 2 (Crinnion, 2011; Lee, 2012; Lee y cols., 2011), síndrome metabólico (Lee y cols., 2007), complicaciones inflamatorias

vasculares y enfermedades como infarto, hipertensión o accidente cerebro vascular (Carpenter, 2011; Goncharov y cols., 2008; Uemura, 2012), incrementos de los anticuerpos nucleares y anticuerpos tiroides, problemas de aprendizaje, memoria y visión, endometriosis, desordenes menstruales o problemas reproductivos en las mujeres, o también hipotiroidismo (Crinnion, 2011).

Es destacable que la IARC considera al congénere 126 como carcinógeno para humanos (IARC, 2012b). Además, de acuerdo a los últimos valores de TEF establecidos por la OMS, actualmente el conjunto de DL-PCB se encuentra en fase de preparación para ser incluidos en el Grupo I tras una evaluación general que se apoya en datos relevantes que muestran claras evidencias para dicha inclusión. Asimismo, como ya nombramos, los PCB causan disrupción endocrina, por lo que la toxicidad crónica a estas sustancias ya se ha detallado en el apartado 3.

4.1.2.4 Niveles de exposición alimentaria de los ciudadanos de la UE a dioxinas y compuestos análogos

En términos globales, los distintos estudios internacionales concluyen que alrededor del 95% de la exposición humana a dioxinas y compuestos análogos se produce por el consumo de alimentos de origen animal, siendo la carne, los productos lácteos, los huevos y el pescado las fuentes principales (EFSA, 2010a). De hecho, actualmente se considera el consumo de pescado como la fuente más relevante de PCB en los humanos (Arnich y cols., 2009; Baars y cols., 2004; Malisch y Kotz, 2014).

Si bien las dioxinas no son incorporadas (o lo son en muy bajo grado) a los vegetales, con la excepción de algunas especies de la familia de las cucurbitáceas (White y cols., 2005), la exposición a dioxinas y compuestos análogos a través de la ingesta de frutas, verduras y cereales no es en absoluto despreciable, ya que pueden depositarse en la superficie de las hojas en forma de aerosoles provenientes de la atmósfera y entrar en la cadena alimentaria cuando los animales herbívoros comen dichas hojas. Así, si un rumiante ingiere vegetales contaminados por PCB, éstos se absorben rápidamente en el tracto gastrointestinal, acumulados en el hígado y en el tejido adiposo, y finalmente eliminados en la leche (Esposito y cols., 2009).

En un informe de la EFSA de 2010, se analizó el nivel de PCDD, PCDF y DL-PCB en muestras de alimentos recogidas entre 1999 y 2008 por 19 estados miembros (EFSA, 2010a). Los niveles más altos se encontraron en el hígado de pescado (32.6 pg TEQ/g), seguidos del hígado y vísceras de animales terrestres (5.7 pg TEQ/g) y la carne de pescado (3.98 pg TEQ/g), siendo en algunos de ellos más altos, como la carne de anguila (6.7 pg TEQ/g) (EFSA, 2010a). En otro informe centrado en evaluar los M-PCB, los congéneres más abundantes eran los 153 y 138. Los niveles de exposición más altos se observaron mediante la ingesta de pescado y productos derivados del pescado, seguido de los productos de animales terrestres, mientras que los valores más bajos se encontraron en las frutas y las verduras (EFSA, 2010b).

Para reflejar los resultados de los niveles de exposición de los ciudadanos de la UE a dioxinas y compuestos análogos mediante la dieta, hemos utilizado los datos de consumo de alimentos de la población adulta, emitidos por la EFSA en 2009, para la estimación de la

ingesta media diaria (EFSA, 2010a) (**Tabla 6**). Hemos utilizado el promedio de los consumos diarios de alimentos (expresados en g producto fresco/día) de los 16 países incluidos, obviando las diferencias entre los hábitos alimentarios de los diferentes países, que no son excesivamente relevantes. También hemos omitido particularizar por sexos, considerando como modelo un ciudadano sin género definido de un peso estándar de 60 kilos.

Tabla 6. Consumo estimado de Equivalentes Tóxicos de Dioxinas a través del consumo de diferentes grupos de alimentos por un consumidor adulto típico de la UE.

Grupo alimentario	Contenido medio de dioxinas en el alimento (pg $TEQ_{OMS\ 2005}$/g producto fresco)	Valor de mediana del consumo de alimentos en 16 países de la UE (g producto fresco/día)[a]	Consumo medio Dioxinas UE (pg $TEQ_{OMS\ 2005}$/kg/día)	% IDT[b]
Carnes y derivados cárnicos, incluidos los embutidos	0.31	216	0.95	47.8%
Vísceras de animales terrestres	0.17	24	0.06	3%
Carne de pescados	3.98	62	3.52	176%
Leche y productos lácteos	0.20	287	0.82	41%
Huevos y derivados	0.21	25	0.08	3.8%
Grasas animales y vegetales	1.49	38	0.80	40%
Frutas, verduras y cereales	0.54	580	4.48	224%
Repostería, incluyendo el chocolate	1.28	43	0.79	39.3%

[a] *Los valores de consumo para cada grupo alimentario se calcularon como la mediana de los valores en los Estados miembros para cada categoría de alimentos.*

[b] *Este valor representa el porcentaje de la ingesta diaria tolerable para dioxinas y compuestos análogos (2 pg/$TEQ_{OMS\ 2005}$/kg/día) que aporta cada grupo de alimentos.*

Es importante señalar que debido a que las contribuciones se calculan sólo para los consumidores de alimentos de cada categoría, y que dichos consumidores varían entre las diferentes categorías de alimentos, las distintas contribuciones no pueden sumarse para estimar la exposición total. Es muy llamativo el hecho de que varios de los grupos alimentarios, por sí solos, o bien sobrepasan o bien se acercan mucho al valor de consumo máximo diario de dioxinas y compuestos análogos recomendado por la OMS (2 pg $TEQ_{WHO2005}$/kg día), lo que nos permite concluir que en la UE se sobrepasa ampliamente dicho límite. Así, debemos fijarnos por ejemplo, en que sólo el grupo de frutas, verduras y cereales aporta por sí solo unas 2.2 veces el nivel de ingesta máxima recomendado. Al igual que ocurre en otros lugares del mundo, en la UE el segundo grupo alimentario en importancia es el pescado, que aporta también por sí solo más de 1.7 veces el nivel de consumo máximo recomendado, y en tercer

lugar varios grupos alimentarios como la carne o la leche y productos lácteos, que aportan cada uno cerca de 1 pg TEQ, casi un 50% de dicho límite.

Posteriormente, en el año 2012, la EFSA elaboró un nuevo informe que actualizaba la monitorización de los niveles de dioxinas, DL-PCB y M-PCB en alimentos y piensos. Al menos se cuantificó un congénere de dioxinas y DL-PCB en casi todas las muestras, mientras que al menos se cuantificó un M-PCB en el 82,6% de las muestras de alimentos (EFSA, 2012d). Los niveles más altos de contaminación se cuantificaron en la "carne de anguila" y de "hígado de pescado y productos derivados". Los niveles de dioxinas y DL-PCB y de M-PCB eran superiores a los niveles máximos permitidos en el 10 y el 3% de las muestras de alimentos, respectivamente.

Además, se calcularon los niveles medios de estos compuestos en diferentes categorías de alimentos de origen animal considerando sus diversos tipos de producción. Tal y como se recomienda, para los cálculos se estimaron las concentraciones según el LB (lower bound) y el UB (upper bound), por su terminología en inglés, y para los percentiles 50, 95 y 99. En la **Tabla 7,** hemos decidido expresar los datos de la contaminación media de diferentes categorías de alimentos incluidos para el global de los TEQ y los M-PCB, según su UB y LB (EFSA, 2012d).

Tabla 7. Distribución de la suma de los niveles de dioxinas, DL-PCB (expresados en TEQ) y M-PCB en grupos alimentarios de origen animal.

Categoría	TEQ-OMS (pg TEQ/g)		M-PCB (µg/kg)	
	LB[a]	UB[b]	LB	UB
Huevos				
Huevos en batería	0.18	0.25	0.90	2.29
Huevos camperos	0.69	0.79	7.90	9.75
Huevos ecológicos	1.68	1.73	4.36	7.56
Carne de las siguientes especies:				
Carne de bovino	2.25	2.34	9.55	11.00
Carne de ovino	1.17	1.24	4.49	5.31
Carne de pescado de:				
Arenque salvaje	4.80	4.81	20.29	20.89
Salmón y trucha	3.90	3.92	16.74	17.31
Otros pescados salvajes	1.76	1.78	18.79	19.42
Salmón y trucha de acuicultura	1.04	1.05	3.64	4.72
Otros pescado de acuicultura	6.43	6.45	7.94	8.27
Leche y productos lácteos				
Leche a granel	0.98	1.10	9.52	9.76
Leche de granja	0.92	1.00	1.63	6.70
Leche al por menor	0.91	1.12	5.53	7.82
Leche no especificada	4.20	4.33	9.13	9.23
Queso	1.00	1.10	4.28	4.80

[a] LB: los resultados analíticos por debajo del LD/LC se consideran 0.

[b] UB: a los resultados analíticos por debajo del LD/LC se les asigna dicho valor.

Dependiendo del grupo de población considerado, se estimó una exposición media a dioxinas y DL-PCB que oscilaba entre 0,57 y 2,54 pg TEQ/kg p.c./día y para el percentil 95 entre 1,2 y 9,9 pg TEQ/kg p.c./día. Los DL-PCB no-orto eran los compuestos que contribuían en mayor porcentaje a la ingesta total de TEQ, representando entre el 21,0 y el 74,5% de este

valor, seguido de las PCDD y los PCDF, que representaban entre el 12,4 y el 73,2%. Además, se estimó que entre el 1,0 y el 52,9% de los consumidores sobrepasaban la ingesta semanal tolerable (IST) de 14 pg de TEQ/ kg p.c./ semana (EFSA, 2012d). Por otra parte, la exposición media dietética a M-PCB oscilaba entre 4,3 y 25,7 ng/kg p.c./día. Los grupos alimentarios que más contribuían a la exposición dietética eran el pescado y los productos de la pesca (especialmente en la población adolescente, adulta y anciana), la carne y los productos cárnicos y la leche y productos lácteos (especialmente en lactantes y la población infantil).

4.1.3 HIDROCARBUROS AROMÁTICOS POLICÍCLICOS

4.1.3.1 Características y fuentes actuales de contaminación

Los Hidrocarburos Aromáticos Policíclicos (**PAH** por sus siglas en inglés), son un grupo de más de 100 compuestos orgánicos que derivan químicamente del benceno. Están formados por carbono e hidrógeno que se configuran en estructuras aromáticas con dos o más anillos fusionados y pueden existir en varias disposiciones isoméricas. Se generan cuando la materia orgánica que contiene carbono e hidrógeno es expuesta a temperaturas superiores a 700º C, lo que ocurre frecuentemente en procesos pirolíticos y de combustión incompleta, que unido a su estabilidad y persistencia explican su ubicuidad en todos los estratos medioambientales. En la atmósfera, se asocian principalmente con partículas, pero también se encuentran compuestos en fase gaseosa. Así, los PAH son resistentes a la degradación de los procesos naturales del medio ambiente, o se dividen o transforman en otras sustancias a través de la luz o mediante sustancias químicas o biológicas.

En los alimentos, se pueden formar in situ debido a la combustión incompleta de material orgánico (glúcidos y lípidos) a temperaturas elevadas (300-600 ºC), aunque su presencia puede también deberse a contaminaciones de origen medioambiental, en concreto por los humos de la combustión de motores de coches, industrias (producción de aluminio, hierro y acero), incineradoras, incendios, etc. (SCF, 2002). Igualmente pueden derivar de la impregnación directa con el humo generado en ciertos procedimientos culinarios o de conservación de alimentos.

Aunque algunos PAH presentes en el medio terrestre y marino tienen su origen en procesos naturales, la fuente de emisión principal es el resultado de numerosas actividades humanas, las cuales han contribuido al incremento general de las concentraciones en los últimos 100 años (Fernández y cols., 2002). Así, cerca de 43.000 toneladas métricas de PAH son descargadas a la atmósfera cada año, y otras 230.000 toneladas a los ambientes acuáticos. Aunque los PAH son ubicuos en la naturaleza como consecuencia de procesos de biosíntesis o la actividad volcánica, las cantidades formadas por procesos naturales son pequeñas en comparación con las producidas a partir de fuentes antropogénicas (USEPA, 1986).

Por tanto, entre las **fuentes naturales** mayoritarias se incluyen la **transformación** de determinados **compuestos orgánicos** en suelos y sedimentos (**PAH diagénicos**), donde en condiciones deficientes en oxígeno ciertos compuestos como quinonas y fenoles pueden ser reducidos a PAH (Gogou y cols., 2000; Pereira y cols., 1999) o los **incendios forestales**, con o sin intervención del hombre (Pereira y cols., 1999; Ribes y cols., 2003). También se incluyen las f**ugas naturales de petróleo** (aunque las concentraciones suelen ser bajas), ya que los PAH se encuentran de forma natural en los combustibles fósiles (el crudo de petróleo o el carbón) y las **erupciones volcánicas**. Más significativo es el aporte de **la combustión incompleta de petróleo y sus derivados**, pero también en la de todo **tipo de materia orgánica**, como carbón, madera, tabaco o vegetación en general.

Como **fuentes antropogénicas**, el **tráfico** representa una importante fuente de emisión de PAH, debido a los **vehículos a motor** que usan combustibles fósiles, ya que además de los constituyentes de éstos, las temperaturas de un motor de combustión son lo suficientemente altas para convertir una fracción del combustible en PAH vía pirolisis. En cuanto a los **procesos industriales** destacan las **plantas de producción de aluminio**; las emisiones de los procesos térmicos que usan carbón y coque en la **industria del hierro y acero**; las **plantas de generación de calor y electricidad (centrales térmicas)**; o las **incineradoras de residuos industriales y municipales,** describiéndose altas concentraciones de PAH por kilogramo de cenizas (Han y cols., 2012; Zhou y cols., 2015). Destaca también la combustión doméstica de madera y otro tipo de masa en condiciones deficientes y es reseñable la contaminación ocasional por la **navegación y vertidos accidentales de petróleo,** sin despreciar la contaminación del agua que producen las **plataformas petrolíferas en mar abierto**.

4.1.3.2 Clasificación y propiedades físico-químicas

Los PAH son poco solubles en agua y se adsorben fuertemente a las partículas, aunque los compuestos de bajo peso molecular (con 3 o menos anillos) son más solubles y se adsorben más débilmente a las partículas que los de alto peso molecular (4 o más anillos). Por lo tanto, los PAH unidos a partículas son menos degradables que los volátiles o los solubles en agua. Debido a la baja tasa de degradación, la menor volatilidad y la fuerte adsorción a partículas, los PAH de elevado peso molecular tienden a acumularse en los sedimentos, como el benzo(a)pireno, que tiene una estructura con cinco anillos aromáticos condensados.

Cuando estos compuestos son emitidos al aire, algunos están en fase gaseosa, otros adsorbidos a partículas, mientras que los semivolátiles están en parte, en forma gaseosa o adsorbidos a partículas, dependiendo de la temperatura y su concentración. Posteriormente, se transfieren desde la atmósfera al suelo o al agua superficial tanto por deposición seca como por sedimentación de las partículas y, dependiendo de la temperatura, se puede producir reemisión desde el suelo, sufriendo los compuestos de peso molecular más bajo una mayor dispersión atmosférica. A pesar de su carácter lipofílico, su vida media en el medio ambiente es corta, oscilando bajo condiciones atmosféricas controladas entre las 0,15 horas del antraceno y las 21,10 horas del benzo(a)pireno, aunque su liberación es continua y descontrolada. El transporte atmosférico puede llevar cantidades significativas de esos compuestos a lugares remotos, por lo que pueden encontrarse en sedimentos de lagos a grandes altitudes, sedimentos marinos y nieve y/o hielo del ártico (Ravindra y cols., 2008).

Todos ellos, con sus diferencias físico-químicas, son considerados lipofílicos (Douben, 2003), característica que propicia su entrada descontrolada a diferentes compartimentos, donde son capaces de persistir durante periodos muy largos de tiempo, motivo por el cual (pese a no cumplir con todas sus características), se les considera contaminantes tóxicos persistentes. Además, su naturaleza lipofílica los hace disponibles para la ingesta y acumulación en los seres vivos, aunque a diferencia de los COP descritos anteriormente, los PAH son transformados por el metabolismo de los organismos y por lo tanto no se acumulan en la cadena trófica, es decir, **no se biomagnifican**. Sin embargo, existen diferencias en cuanto al grado de metabolización y su bioacumulación será diferente en función de los diferentes organismos, dependiendo por ejemplo de la biodisponibilidad, es decir, la susceptibilidad de un compuesto para ser incorporado por un organismo y la fisiología del mismo.

Tabla 8. Propiedades fisicoquímicas de los PAH prioritarios según la EPA.

Compuesto	PM[a]	Punto Fusión (ºC)	Punto Ebull. (ºC)	Solubilidad Agua (mg^{-1})[b]	log k_{ow}[c]	log k_{oc}[d]
Naftaleno	128	80	218	30,0	3,37	3,1
Acenaftileno	152	92	265	16,1	4,07	ND[e]
Acenafteno	154	96	279	3,47	4,33	3,8
Fluoreno	166	116	298	1,8	4,18	3,9
Fenantreno	178	101	340	1,29	4,46	4,1
Antraceno	178	218	342	0,073	4,45	4,3
Flouranteno	202	110	375	0,260	5,33	4,3
Pireno	202	150	404	0,135	5,32	4,8
Benzo(a)antraceno	228	159	435	0,014	5,61	4,8
Criseno	228	256	448	0,0006	5,86	4,9
Benzo(b)fluoranteno	252	168	ND	0,0012	6,57	6,2
Benzo(k)fluoranteno	252	217	480	0,00055	6,84	5,6
Benzo(a)pireno	252	179	495	0,0038	6,04	5,3
Dibenzo(a,h)antraceno	278	267	524	0,0005	6,75	6,3
Benzo(g,h,i)perileno	276	278	ND	0,00026	7,23	ND
Indeno(1,2,3-cd)pireno	276	162	ND	0,062	7,66	6,2

[a]PM: peso molecular. [b]20ºC. [c]k_{ow}: equivale a la proporción del número de moléculas que existirían en la fase octanol respecto a la fase agua si utilizáramos una mezcla octanol:agua 1:1 como solvente. [d]k_{oc} es la proporción de moléculas de PAH que se encontrarían en la materia orgánica del suelo respecto a las que encontraríamos en la fase agua, en un suelo saturado de agua. [e]ND: no determinado.

Estos contaminantes también han adquirido una notable importancia debido a su persistencia en el medio y a que muchos son potentes tóxicos, mutágenos y teratógenos para los animales e incluso para el hombre (IARC, 1987), lo que los llevó a ser considerados contaminantes prioritarios por la Agencia Americana de la Protección del Medio Ambiente (EPA) y la Unión Europea mediante la **Directiva 2000/60/EC** (CE, 2000b). La EPA incluye en su lista de contaminantes prioritarios a un grupo de 16 de estos compuestos, debido a su mayor toxicidad y prevalencia en el medioambiente, detallándose en la **Tabla 8** sus principales propiedades fisicoquímicas (Kästner, 2000). Además, este grupo de compuestos se presentan habitualmente a concentraciones elevadas en zonas sometidas a mayor actividad humana, por lo que son unos excelentes marcadores de contaminación.

4.1.3.3 Parámetros toxicocinéticos

- **Absorción:** Los PAH se absorben por las vías respiratorias, el aparato digestivo y la piel. Su naturaleza lipofílica los hace disponibles para la ingesta y facilita su paso por las membranas celulares, siendo de esta manera los alimentos la principal vía de entrada en los organismos. Su ingesta está influenciada internamente por factores biológicos, como el tamaño del organismo, la tasa de respiración y de crecimiento, muchos de ellos intrínsecos a las especies, interdependientes entre si y a menudo influenciados por factores ambientales como el pH, la temperatura y la salinidad.

  Por tanto, la absorción de los PAH a través de la dieta se determina por el tamaño y la lipofilicidad de la molécula, la presencia de bilis en el tracto digestivo, la dosis ingerida y el contenido lipídico de la dieta. En animales de experimentación, la absorción gastrointestinal de benzo(a)pireno (**BaP**) tras su exposición dietética, osciló entre un 35-99% en 2-4horas (CE, 2002; Ramesh y cols., 2004). Las concentraciones máximas sanguíneas de fluoranteno, pireno y benzo(a)antraceno se alcanzaron a las 1-2 horas tras su administración, siendo 2 y 5 veces mayor para el fluoranteno que para el pireno y benzo(a)antraceno, respectivamente (Lipniak y Brandys, 1993), hecho que refleja la importancia del peso molecular.

- **Distribución:** Tras la administración por vía intravenosa u oral de PAH individuales radiomarcados o sin marcar, en los ensayos descritos los PAH y/o metabolitos se detectaron en casi todos los órganos, encontrándose los niveles más altos en el tracto gastrointestinal y en todos los tejidos ricos en lípidos (WHO/IPCS, 1998). En el año 2002 se hallaron cantidades significativas de metabolitos de BaP en el cerebro de ratas (Saunders y cols., 2002). Los metabolitos diol predominaban especialmente en estadios tempranos (exposición hasta 12 h), mientras que los metabolitos hidroxilo lo eran en estadios tardíos (24 a 96 h después de la exposición). Estos resultados se correlacionaban con otros datos (Lipniak y Brandys, 1993; Modica y cols., 1983) publicados con otros PAH (benzo[a]antraceno, criseno o pireno), que demuestran la capacidad de estos compuestos o sus metabolitos para cruzar la BHE. Además, estudios en roedores gestantes demostraron que los PAH se distribuyen ampliamente en los tejidos maternales y fetales, y por tanto, su capacidad para cruzar la barrera placentaria (CE, 2002).

- **Metabolización**: Muchos organismos son capaces de metabolizar determinados PAH mediante reacciones enzimáticas que transforman los compuestos hidrofóbicos en metabolitos más polares y solubles, que pueden ser excretados más fácilmente por medios activos o pasivos. La exposición a PAH induce al sistema MFO, que aumenta su actividad en el hígado, pulmones y otros tejidos. Para los PAH con estructuras aromáticas planas, se requiere la activación metabólica para su acción tóxica, mutagénica y carcinogénica (Harvey, 1991; Miller, 1978).

  En total, para los PAH carcinógenos y mutágenos se han descrito tres principales rutas metabólicas que forman reactivos altamente intermedios (Xue y Warshawsky, 2005). La primera en descubrirse fue la activación de la vía epóxido dihidrodiol, considerada la vía pro-mutagénica de aductos del ADN más importante cuantitativamente (Hall y Grover, 1990; Harvey, 1991). Algunos derivados son particularmente electrófilos y pueden reaccionar con nucleófilos y estos dioles epóxidos, destacando su catálisis por la CYP1A1 (Shimada y cols., 1999). Otra vía es la oxidación de un electrón que lleva a la formación de radicales PAH reactivos (Cavalieri

y Rogan, 1992; Cavalieri y Rogan, 1995), jugando un papel relevante en los PAH con potencial de ionización bajo. Normalmente conlleva la formación de aductos inestables de ADN, que dan lugar eventualmente a sitios apurínicos. La tercera vía en ser descrita fue la vía de orto-quinona, resultando también en la formación de metabolitos de PAH electrofílicos reactivos (Penning y cols., 1999; Penning y cols., 1996). Esta vía se produce por oxigenación de las deshidrogenasas dihidrodiol (DD), y las orto-quinonas actúan como aceptores altamente reactivos. Esta vía también puede producir aductos estables e inestables, dando lugar a especies de oxígeno reactivas (ROS) que dañan el ADN.

Además, otras rutas como la sulfatoconjugación, pueden tener un papel relevante en la activación metabólica de los PAH. La mayoría de los metabolitos fenólicos de los PAH, pueden ser considerados productos de la desintoxicación. Asimismo, el metabolismo de los fenoles mediante enzimas incluye la glucuronidación, mientras que los metabolitos electrofílicos pueden someterse a la conjugación de glutatión. La inducción de enzimas CYP por un número de PAH se produce a través de la unión y activación del receptor AhR y se ha descrito en los seres humanos después de la exposición al humo del tabaco o tras el consumo de carnes asadas o al grill (Fontana y cols., 1999).

- **Excreción:** La eliminación pasiva puede ser rápida para los PAH de bajo peso molecular, pero muy lenta para PAH con alto peso molecular y bajas solubilidades (Meador y cols., 1995). Después del metabolismo inicial, la mayor parte de la porción desintoxicada de los PAH se excreta mediante la bilis en forma de metabolitos, y posteriormente se elimina por las heces, siendo inferior el porcentaje excretado en orina (van Schooten y cols., 1997). Además, la reabsorción y circulación enterohepática alarga el tiempo de permanencia de los PAH en el cuerpo y puede conducir a una vida media larga de los metabolitos reactivos.

4.1.3.4 Mecanismos de acción y toxicidad

A día de hoy se consideran los siguientes mecanismos de acción de los PAH:

a) Conversión metabólica a intermediarios reactivos electrofílicos que pueden unir covalentemente blancos nucleofílicos en el ADN, ARN y proteínas, por lo que, además de formar aductos en el ADN e inducir mutaciones y eventualmente tumores, los metabolitos reactivos pueden reaccionar con otras células blanco e interferir con la transcripción, replicación del ADN y la síntesis de proteínas. Asimismo, ciertos metabolitos de los PAH pueden inducir procesos inflamatorios (Bostrom y cols., 2002).

b) Alta afinidad por el *receptor aril hidrocarburo* (AhR), y la subsecuente sobre-regulación transcripcional de una serie de genes involucrados en la biotransformación, crecimiento y diferenciación celular. La estimulación del crecimiento parece ser el principal componente de promoción en la carcinogénesis química mediada por algunos PAH (Bostrom y cols., 2002).

c) Efecto inhibitorio sobre la comunicación intercelular (Bostrom y cols., 2002).

Aunque la toxicidad aguda de muchos PAH en animales de experimentación suele ser baja, los niveles de estos compuestos son habitualmente objeto de estudio en todo tipo de matrices por sus efectos tóxicos a medio y largo plazo. Por ejemplo, como consecuencia de su solubilidad, el comportamiento en el ambiente acuático de los PAH de bajo y alto peso molecular es muy diferente (Law y Klungsoyr, 2000). Los PAH de bajo peso molecular son, en general, moderadamente tóxicos, y muchos PAH de elevado peso molecular son mutagénicos

y teratogénicos (Meador y cols., 1995) y, además, sus metabolitos son potenciales carcinógenos para animales y humanos. Además, muchos de los PAH son reconocidos como **disruptores endocrinos**, como se ha observado para el benzo(a)antraceno, el pireno o el antraceno, y sobretodo el BaP (Irigaray y cols., 2007) y, por tanto, se encuadran en el mecanismo de disrupción endocrina ya explicado.

Algunos autores han sugerido una asociación entre los niveles de exposición a estas sustancias y la alteración del **desarrollo cognitivo en niños**. El riesgo de presentar déficits en el lenguaje, la lectura y las matemáticas en los primeros años escolares es mayor entre los niños expuestos a mayores niveles de PAH (Perera y cols., 2006b). Asimismo, se ha asociado la exposición prenatal a PAH con una disminución significativa del peso de los recién nacidos (Choi y cols., 2006).

A **nivel inmunológico**, se ha sugerido que los PAH ejercen efectos inmunes a través del receptor AhR. En experimentación animal, se ha descrito que la exposición a altas concentraciones atmosféricas de BaP reduce los niveles de las inmunoglobulinas séricas IgG, IgA e IgM y que el CYP1A1 puede proteger contra los efectos inmunotóxicos. En un estudio realizado en Estados Unidos con niños residentes expuestos en la etapa prenatal a diferentes concentraciones de PAH, se asociaron los niveles de exposición a un aumento del riesgo de desarrollo de asma (Perera y cols., 2006a).

Existe poca información sobre la **toxicidad reproductiva** de los PAH a nivel individual. Así, para el BaP no se observó ningún efecto sobre la capacidad reproductiva en una generación de ratones bajo una exposición dietética a este compuesto. Sin embargo, era evidente la alteración de la fertilidad en crías de ratones hembras que recibieron BaP a dosis>10µg/kg p.c./día por sonda, y se observó toxicidad en el desarrollo en ratones de un genotipo susceptible, después de la administración de 120 µg/kg/p.c./día de este compuesto a través de la dieta (JECFA, 2005).

4.1.3.5 Genotoxicidad, mutagenicidad y carcinogenicidad de los PAH

Como ya se ha dicho, los PAH presentan ciertos efectos genotóxicos, esto es, incremento de mutaciones en linfocitos periféricos y formación de aductos en el ADN, que se considera como uno de los primeros pasos en la carcinogenicidad de los PAH mutagénicos (Schoket, 1999; Tang y cols., 2006). Algunos PAH son carcinógenos transplacentarios en bioensayos experimentales, por lo que producen tumores en hígado, pulmón, tejido linfático y sistema nervioso. El número de aductos encontrados en madres e hijos expuestos es mayor a altas concentraciones de PAH; y el peso al nacimiento, así como el diámetro de la cabeza de los niños, es significativamente menor a mayores niveles de exposición (Choi y cols., 2006; Tang y cols., 2006). También se han descrito asociaciones entre el daño al ADN y la reducción del crecimiento fetal (Choi y cols., 2006). Según la IARC, existen evidencias suficientes de que algunos PAH son agentes que pueden relacionarse con cáncer de pulmón, vejiga y piel (Armstrong y cols., 2004; Ruan y cols., 2007).

Además, muchos de los PAH estudiados en esta Tesis son considerados carcinogénicos por distintas organizaciones gubernamentales e independientes. Si nos referimos a la IARC, dicha organización clasificó a 48 PAH según su probable carcinogenicidad en humanos (IARC, 1987). De acuerdo a la lista de agentes evaluados por la IARC, **el BaP** se encuentra clasificado dentro del grupo 1 (carcinógeno para el hombre) desde el año 2012 (IARC, 2012b). Esta categoría se aplica cuando existen pruebas suficientes de carcinogenicidad en humanos o excepcionalmente, como es en este caso, si las pruebas en humanos no son suficientes, pero sí

lo son en animales de experimentación y existen pruebas contundentes en humanos expuestos de que el agente actúa mediante mecanismos relevantes para la carcinogenicidad.

Por otra parte, recopilando y sustentándose en todos los estudios disponibles, el IPCS identificó a 8 PAH con potencial cancerígeno (ICPS, 1998). Por ello, el potencial tóxico de los PAH se evalúa a menudo en base a factores de equivalencia tóxico (TEF) (Pikkarainen, 2004; Yang y cols., 2014; Yu y cols., 2014). Pueden encontrarse distintos grupos de TEF en la literatura, que difieren en el modo en que han sido calculados, aunque todos ellos están basados en el valor unitario del BaP, ya que los datos toxicológicos disponibles para este compuesto son mucho mayores que para cualquier otro.

Si nos centramos en los ensayos realizados en experimentación animal, anteriormente se evaluaron varios estudios sobre PAH individuales por el SCF (CE, 2002) y el JECFA (FAO/OMS, 2006). Así, la carcinogenicidad de los PAH se ha evaluado en un gran número de estudios tras administrarse por vía cutánea, subcutánea, inhalatoria u oral. En la mayoría de los estudios, el lugar de desarrollo del tumor estaba asociado con la vía de administración, por ejemplo, tumores gástricos después de la administración oral o tumores de piel después de la aplicación dérmica. Sin embargo, también se observaron tumores en sitios distintos del lugar de aplicación. De conformidad con el SCF y el JECFA de la CONTAM, se ha descrito que el BaP cuando se administra por vía oral produce tumores del tracto gastrointestinal y, si los ratones se alimentaban además con alquitrán de hulla, se relacionaban también con tumores de hígado, pulmones y glándulas mamarias. Además, se observaba un aumento significativo en la incidencia de adenomas y carcinomas alveolares y bronquiales a medida que la dosis administrada era mayor, así como de tumores del tracto alimentario, especialmente en estómago e intestino.

Por otro lado, en su evaluación de 2002, el SFC concluyó que 15 de los PAH estudiados mostraban claras evidencias de mutagenicidad y genotoxicidad en células somáticas mediante estudios experimentales in vivo y además, excepto el benzo (g,h,i)perileno, mostraban efectos carcinogénicos en varios tipos de bioensayos con animales. Una observación importante era la unión de los metabolitos activos de PAH al ADN, predominantemente a grupos amino de guanina y adenina, siendo los aductos más estables los localizados en la posición N2 de la desoxiguanosina. Sin embargo, existía una débil relación cuantitativa entre los niveles de aductos en el tejido y la formación de tumores (SCF, 2002). Además, basándose en el examen de los perfiles de PAH en los alimentos, el SCF estableció el uso del BaP como marcador de incidencia y efectos carcinogénicos de los PAH en alimentos. No obstante, se consideró que era necesario disponer de nuevos datos y ampliar los estudios sobre este tipo de sustancias.

Posteriormente, el **JECFA** en el año 2005 reevaluó los PAH tomando como punto de partida las evaluaciones del IPCS y del SCF (ICPS, 1998; SCF, 2002), teniendo en cuenta además nuevos estudios. En la citada evaluación, el JECFA estimó los márgenes de exposición (MOE por su siglas en inglés) y concluyó que 13 de los PAH evaluados por el SCF eran claramente genotóxicos (tanto *in vitro* como *in vivo*) y carcinogénicos (FAO/OMS, 2006). Adicionalmente, se recomendó la inclusión del benzo(c)fluoreno en futuros análisis, dado que a pesar de la escasa información sobre su presencia en alimentos, estudios en ratas indicaban que podría contribuir a la formación de tumores pulmonares (Goldstein, 2001). Por esta razón, al conjunto formado por los quince PAH identificados por el SCF en 2002 y el benzo(c) fluoreno, se les ha denominado los 15+1 PAH prioritarios en la UE (Gómez-Ruiz y Wenzl, 2009). En la **Tabla 9**, se citan dichos PAH (JECFA, 2005), clasificados según los grupos de la IARC.

Tabla 9. Clasificación de los PAH prioritarios del JECFA acorde a la IARC.

Hidrocarburo aromático policíclico	Carcinogenicidad IARC (Grupo)
Benzo(a)pireno	1
Ciclopenta(cd)pireno	2A
5-metilcriseno **Benzo(a)antraceno** **Benzo(b)fluoranteno** **Benzo(j) fluoranteno** **Benzo(k)fluoranteno** **Criseno** **Dibenzo(a,h)antraceno** **Dibenzo(a,h)pireno** **Dibenzo (a,i)pireno** **dibenzo(a, l)pireno** **Indeno(1,2,3-c,d)pireno**	2B
Benzo(c)fluoreno **Benzo(g,h,i)perileno** **Dibenzo(a,e)pireno**	3

En el año 2007, una evaluación posterior de la **EFSA** a partir de unos 10.000 datos sobre el contenido de PAH en diversos alimentos, permitió demostrar que el BaP estaba presente en el 50% de las muestras; aunque también se pudo comprobar que un 30% de las muestras contenían otros PAH carcinogénicos y genotóxicos, a pesar de que en éstas no se detectaba el BaP. El criseno era el PAH detectado con mayor frecuencia en las muestras negativas para el BaP, con concentraciones relativamente altas (EFSA, 2007b).

A tal efecto, la EFSA llevó a cabo una revisión en base a los datos disponibles sobre la toxicidad y presencia de los PAH (EFSA, 2007b; EFSA, 2008b), teniendo en cuenta los 15+1 PAH propuestos por el JECFA. Tras descartar, por falta de datos, la utilización de un factor de equivalencia tóxica, la EFSA concluyó que los únicos indicadores del potencial carcinogénico de los PAH en alimentos, tanto de forma individual como conjunta son un grupo de 8 PAH (**PAH8**) formado por: benzo(a)pireno, benzo(a)antraceno, benzo(b)fluoranteno, benzo(k)fluoranteno, benzo(g,h,i)perileno, criseno, dibenzo(a,h)antraceno e indeno(1,2,3-cd)pireno, para los que se dispone de datos que demuestran su carcinogenicidad por vía oral. La exclusión de la utilización de TEF para la evaluación del riesgo de los PAH, se sustenta en que no hay datos suficientes sobre la carcinogenicidad por vía oral de algunos PAH y porque no hay evidencia científica de que todos los PAH actúen por el mismo mecanismo de acción. Así, no se puede generalizar que todos los PAH sean activados por la misma vía metabólica, se unan al ADN en las mismas posiciones e induzcan idéntica genotoxicidad en los mismos órganos y tejidos.

Además del grupo PAH8, la EFSA evaluó la información disponible para un grupo formado por 4 PAH (**PAH4**: benzo(a)pireno, criseno, benzo(a)antraceno y benzo(b)fluoranteno) y otro formado por 2 PAH (PAH2: benzo(a)pireno y criseno). Los cálculos de los márgenes de exposición (MOE) para cada uno de estos grupos y para el BaP en solitario, permitieron concluir que PAH8, PAH4 y PAH2 pueden utilizarse como marcadores de carcinogenicidad, constituyendo alternativas al BaP, el cual ya no se considera un marcador adecuado (EFSA, 2007b; EFSA, 2008b). De ellos, PAH8 y PAH4 son los marcadores más adecuados, no encontrándose diferencias significativas entre la utilización de uno u otro.

4.1.3.6 Factores que influyen en los niveles de PAH en los alimentos

El nivel de PAH en los alimentos puede variar debido a las técnicas de procesado del cocinado de los alimentos con fines comerciales o a nivel doméstico. Debemos tener en cuenta que las muestras de alimentos incluidas en esta Tesis Doctoral, se procesaron directamente tal y como son adquiridas en el mercado, por lo que se excluyó la contaminación adicional debido al método de cocinado. Los factores a destacar son:

a) Técnicas de procesamiento comerciales: Las técnicas de **ahumado tradicional** pueden llevar a una considerable contaminación por PAH si el proceso no está controlado adecuadamente. Los parámetros críticos son la temperatura, el tiempo, la humedad, los tipos de humo utilizado (natural o generado) y el tipo de horno. Así, las **técnicas de ahumado modernas** permiten controlar el flujo de aire mecánica o eléctricamente y los parámetros de forma más estricta. El humo de leña puede generarse por la quema de madera o, más comúnmente, por serrín de calefacción o pequeñas astillas de madera; utilizándose una amplia variedad de maderas para ahumar y conseguir diversos sabores y colores, incluyendo roble, nogal, pino, haya, etc. En consecuencia, dependiendo de la madera utilizada, pueden existir concentraciones hasta 6 veces superiores en el alimento (Jahncke y Herman, 2001).

En las dos últimas décadas, se ha sustituido progresivamente el ahumado tradicional en la producción comercial por el uso de aromatizantes de humo líquido, dando lugar a unos niveles inferiores de PAH, una mejora en sus características organolépticas, una reducción de costos y una menor contaminación ambiental. Diferentes estudios han demostrado este hecho, observándose mayores niveles de BaP y PAH carcinogénicos en muestras de productos de la pesca ahumados bajo el método tradicional frente a aquellas sometidas a un horno que genera un ahumado externo (Karl y Leinemann, 1996). Además, en 1997 se estudió la formación de PAH en filetes de pavo según el método de cocinado, concluyendo que el ahumado, seguido de la parrilla, eran los métodos que favorecían en mayor medida la formación de PAH cancerígenos (Chen y Lin, 1997).

b) Cocina casera y otras prácticas culinarias a pequeña escala: Se pueden detectar diferencias considerables de PAH en los alimentos a la parrilla, en función de la fuente de calor y el tipo de parrilla, hecho especialmente importante en la cocina doméstica. En este caso, los PAH derivan de la pirólisis de la grasa al gotear con la fuente de calor. La cantidad de PAH formados es mayor a superior contenido graso, tiempo de exposición del alimento a las llamas y cercanía a la fuente de calor. Por el contrario, se ha descrito la ausencia total de PAH adicionales en la carne de pollos asada mediante gas o electricidad, ya que la fuente de calor está por encima de la comida; o por el cocinado con ventilación sobre carbón vegetal, que atrapa la grasa fundida y evita el contacto con las llamas. Así, Larsson y cols. (1983) demostraron que niveles tan altos de BaP como 212 µg/kg en salchichas a la parrilla con fuego de leña, disminuyó a un nivel promedio de 7,7 µg/k, al evitarse el contacto con las llamas (Larsson y cols., 1983).

Por otra parte, White y cols. (2008) investigaron los efectos de los diferentes métodos de cocinado en la formación de PAH en los alimentos preparados en el hogar y en los de catering en los puntos de venta. El estudio concluyó que en las prácticas culinarias caseras, en general, existen pocas evidencias de la formación de PAH durante los diferentes métodos de preparación (parrilla, frito, asado y tostado), con escasas diferencias en las concentraciones de PAH en las muestras bajo diferentes condiciones (White y cols., 2008). Sin embargo, los niveles más altos de BaP se detectaron en cada alimento mediante su preparación a la barbacoa con alta proporción de astillas de carbón de madera, niveles que aumentaban a medida que la fuente de calor era más cercana (White y cols., 2008). Por tanto, la reducción de la exposición a estos compuestos parece factible si se aplican técnicas que eviten la pirólisis de la grasa en ciertas prácticas culinarias.

4.1.3.7 Nivel de exposición alimentaria de los ciudadanos de la UE a PAH

Esta evaluación de la exposición alimentaria se basa en los datos sobre las concentraciones de PAH y el consumo de las categorías de alimentos correspondientes, publicado en la **Base de Datos Concisa de Consumo de Alimentos de la EFSA**. Como primer paso, las categorías de alimentos utilizadas para el estudio se relacionaron lo mejor posible con las correspondientes categorías de consumo de alimentos, debido a que éstas eran más amplias que las empleadas para exponer los resultados de los análisis de los contaminantes. Por tanto, se realizó un muestreo específico de los alimentos sospechosos, utilizando los datos de consumo del mayor percentil sin ajustar, para dar lugar a una sobreestimación conservadora de la situación real de exposición. Por último, el pescado y sus productos se representaron por las concentraciones de PAH recogidos para "todo el pescado procesado", aunque los datos recopilados se basaron en la presencia de PAH en pescados enlatados y curados, que comprenden alrededor de un tercio del consumo total de pescado en todo el mundo (FAO, 2004).

En cuanto al nivel de contaminación, debido a la alta proporción de muestras por debajo del LD, el uso de la mediana no permitía el cálculo de la exposición dietética. Por lo tanto, los cálculos se basaron en las concentraciones medias que representan la tendencia central de la distribución, aunque ello represente una sobreestimación de la exposición alimentaria a largo plazo. La exposición se calculó para cuatro grupos distintos de PAH, incluyendo al BaP de forma independiente, PAH2, PAH4 y PAH8.

Debido a la alta sensibilidad de los métodos analíticos y a pesar de la relativa alta proporción de muestras por debajo del LD/LC, todos los escenarios de exposición detallados se basan en el límite superior de las concentraciones medias de los cuatro grupos de PAH considerados. La información sobre el consumo de alimentos utilizada en el modelo, incluye el consumo medio y el percentil 97,5 de la distribución. En la **Tabla 10,** se recoge la exposición dietética media a los PAH (ng/día) para 10 categorías de alimentos (EFSA, 2008b).

Como se observa, los dos mayores contribuyentes a la exposición dietética fueron los **cereales y productos de cereales** y los **mariscos y productos del mar,** grupos que se utilizaron para estimar la exposición en consumidores altos. Se puede concluir que la exposición dietética media general en Europa, asumiendo un consumidor con un peso corporal estándar de 60 kg, es de 3,9 ng/kg p.c./día para el BaP y de 28,8 ng/kg p.c./día para PAH8 y una exposición dietética superior podría representar 5.1 y 45,2 ng/kg p.c./día para el BaP y PAH8, respectivamente. Además, se destaca que un **alto consumo de ciertos alimentos a la parrilla**, si no se usan técnicas que eviten la pirólisis de la grasa, puede dar lugar a una exposición a PAH que excede considerablemente la ingesta dietética recomendada. En relación a la exposición media alimentaria a PAH en consumidores de alto riesgo, sumando los percentiles 95 de

consumo de los grupos alimentarios, el valor medio era de 6,5 y 51,3 ng/kg p.c./día para el BaP y PAH8, respectivamente (EFSA, 2008b).

Tabla 10. Exposición del consumidor (ng/día) a BaP, PAH2, PAH4 y PAH8 por categoría de alimento según estudio de la EFSA.

Categoría	BaP	PAH2	PAH4	PAH8
Cereales y productos a base de cereales	67	129	257	393
Azúcar y productos, incluido el chocolate	5	13	25	39
Grasas (vegetales y animales)	26	112	177	293
Vegetales, frutos secos y legumbres	50	124	221	378
Frutas	5	40	75	87
Café, té, cacao	21	55	106	156
Carne, productos cárnicos y sustitutos	42	107	195	279
Marisco y subproductos	36	140	289	421
Pescado y productos a base de pescado	21	84	170	210
Queso	6	12	20	30

Además, el JECFA examinó las estimaciones de ingesta de los 13 PAH que considera como carcinógenos y genotóxicos. Con el fin de proporcionar una ingesta probable de BaP que cubra los principales grupos de alimentos de la dieta y caracterizar dicho riesgo, se realizó una determinación utilizando los estudios para los grupos alimentarios de mayor contribución, incluyendo alimentos que eran "listos para comer" (por ejemplo, carne cocinada), y por lo tanto, las concentraciones de PAH debido a la cocción de los alimentos. Con estos datos, el JECFA asignó una ingesta media de **4 ng/kg p.c./día** como valor representante para caracterizar el riesgo al **BaP**, asumiendo que un alto consumo podría superar de 2 a 2,5 veces este valor (FAO/OMS, 2006).

4.1.4 Normativa legal sobre residuos de COP en alimentos

Todos los alimentos destinados al consumo humano o animal están sujetos en la UE a un **LMR** (Límite Máximo de Residuo) en su composición, con el fin de proteger la salud humana y animal. El LMR es el límite legal superior de concentración de un residuo de plaguicida en alimentos o piensos, basado en las buenas prácticas agrícolas y la menor exposición del consumidor, necesaria para proteger a todos los consumidores vulnerables.

Los LMR están basados en la **IDA** (Ingesta Diaria Admisible) de las sustancias reguladas. La IDA es la estimación de la cantidad de sustancia presente en los alimentos, expresada en función del peso corporal, que puede ingerirse diariamente a lo largo de toda la vida sin provocar un riesgo apreciable para el consumidor, según todos los hechos conocidos en el momento de la evaluación, teniendo en cuenta los grupos vulnerables de población (por ejemplo, los niños y los no nacidos).

El **Reglamento CE 396/2005** del Parlamento Europeo y del Consejo reúne y armoniza en un solo texto las cantidades máximas autorizadas de residuos de plaguicidas que pueden encontrarse en los productos de origen animal o vegetal destinados al consumo humano o animal (CE, 2005). Dichos LMR comprenden, por una parte, **LMR específicos** para ciertos alimentos destinados a las personas o los animales y, por otra, un límite general aplicable cuando no se haya fijado ningún LMR. El LMR de plaguicidas en los alimentos se sitúa en 0,01

mg/kg y es aplicable «por defecto», es decir, en todos los casos en que no se haya fijado un LMR de forma específica para un producto. En el caso de los **Plaguicidas Organoclorados,** se han fijado LMR en todos los casos. En la **Tabla 11** se indican los LMR para los alimentos de origen animal en la UE, conjuntamente con los valores de IDA. Dichos LMR de un alimento no sólo deben garantizar la protección directa del consumidor, sino también asegurar que no ocurra una acumulación o concentración indeseable en los eslabones de la cadena alimentaria, especialmente en los más críticos, como la leche materna y el niño, para evitar un riesgo en la salud del niño (Campoy y cols., 2001).

Tabla 11. LMR e IDA de los POC en los alimentos de origen animal incluidos en esta Tesis Doctoral según el Reglamento CE 396/2005.

POC	IDA (mg/kg)	LMR (mg/kg)
Aldrina y dieldrina (aldrina y dieldrina sumados expresados como dieldrina)	0.0001	0.006
Clordano (suma de *cis*- y *trans*- clordano)	0.0005	0.002
DDT (suma de 4,4'-DDT, 2,4'-DDT, 4,4'-DDE y 4,4'-DDD, expresado como DDT)	0.01	0.04
Endosulfán (suma de los isómeros α- y β- endosulfán y endosulfán sulfato, expresados como endosulfán)	0.006	0.05
Endrina	0.0002	0.0008
Heptacloro	0.0001	0.004
HCB	No disponible	0.01
HCH (isómero α)	No disponible	0.004
HCH (isómero β)	No disponible	0.003
HCH (isómero γ) – lindano	0.005	0.001
Metoxicloro	0.1	0.01

Además, a nivel comunitario, el **Reglamento 2015/595** (CE, 2015d) se refiere a un programa plurianual coordinado de control de la UE, destinado a garantizar el respeto de los LMR de los plaguicidas y evaluar el grado de exposición de los consumidores a estos residuos, estableciendo los alimentos y productos a analizar, además de la correcta aplicación de la legislación (CE, 2015d). Este programa está en curso y finalizará en el año 2018.

En relación a las **dioxinas y compuestos análogos,** el Comité Científico sobre Alimentos (SCF) de la UE informó en el año 2000 y 2001 sobre los riesgos para la salud y el Comité Científico sobre Nutrición Animal informó sobre la relevancia de la contaminación por dioxinas en los alimentos para animales y su contribución a la contaminación en alimentos de origen animal (SCAN, 2000). Estos informes proporcionaron la base científica para las medidas establecidas en la UE, con la finalidad de limitar la presencia de dioxinas en alimentos como parte de la **estrategia de llegar a la exposición cero a estos compuestos.**

Por otra parte, la estrategia comunitaria para dioxinas, furanos y PCB fue adoptada por la Comisión en octubre de 2001 y contiene medidas específicas para limitar o eliminar la emisión al medioambiente y su presencia en los alimentos. La Comisión adoptó esta estrategia principalmente teniendo en cuenta que los resultados del SCF estiman que **una parte muy**

considerable de los habitantes de la UE exceden ampliamente la Ingesta Semanal Tolerable de TEQ fijada por la OMS (14 pg TEQ_{OMS}/kg p.v).

La **estrategia comunitaria** para la eliminación de dioxinas y compuestos análogos se apoya en el establecimiento de LMR estrictos pero factibles en los productos alimenticios de origen animal, en el establecimiento de un plan de alerta para desencadenar protocolos de actuación cuando se detecte que los niveles en alimentos o piensos se encuentran claramente por encima de los niveles base y establece objetivos con la finalidad de que la exposición media europea sea inferior al valor fijado por la OMS.

Con el fin de poder determinar la toxicidad de los diferentes congéneres de mayor preocupación (las 17 dioxinas y los 12 DL-PCB), el **Reglamento 1881/2006/CE** define que los resultados analíticos de todas las dioxinas y compuestos análogos deben expresarse en términos de equivalentes tóxicos, utilizando la revisión de los valores TEF de 2005 propuestos por la OMS, para facilitar la evaluación de riesgos (CE, 2006).

Tabla 12. Contenidos máximos de $TEQ_{DL\text{-}PCB\text{-}OMS}$ y M-PCB en los productos alimenticios de origen animal según el Reglamento (UE) 1259/2011.

Producto	ΣTEQ $_{DL\text{-}PCB\text{-}OMS}$ (pg/g)	ΣMPCB (ng/g)
*Carne y productos cárnicos de bovino y ovino	1.5	40
*Carne y productos cárnicos de aves de corral	1.25	40
*Carne y productos cárnicos de cerdo	0.25	40
*Hígado y productos derivados procedentes de los animales terrestres mencionados	5.5	40
**Hígado de pescado y sus productos derivados	20	200
**Carne de pescado y productos de la pesca y productos derivados	3	75
*Leche y productos lácteos, incluida la grasa láctea	3	40
*Huevos de gallina y ovoproductos	2.5	40
*Grasas animales procedentes de bovinos y ovinos	1.5	40
*Grasas animales procedentes de aves de corral	1.25	40
*Grasas animales procedentes de cerdos	0.25	40
*Mezcla de grasas de origen animal	1	40

** Se muestra el valor en pg/g o de mg/g de producto fresco corregido por el* porcentaje *de materia grasa promedio en ese grupo alimentario.*
*** Para la carne de pescado y derivados los resultados se expresar siempre referidos al peso de producto fresco.*

En la actualidad, **el Reglamento 1259/2011** regula el contenido máximo de dioxinas, DL-PCB y M-PCB en los productos alimenticios (CE, 2011a). En la **Tabla 12,** se muestran los contenidos máximos de $TEQ_{DL\text{-}PCB\text{-}OMS}$ y M-PCB (sustancias incluidas en esta Tesis) según la

legislación vigente. Además, para estimular un enfoque proactivo en la reducción de los niveles de dioxinas y compuestos análogos en alimentos, la **Recomendación 2013/711/UE** de la Comisión, establece umbrales de intervención, que constituyen un instrumento para que las autoridades competentes y los operadores señalen los casos en los que conviene determinar la fuente de contaminación y adoptar medidas para su reducción o eliminación (CE, 2013). Por último, el **Reglamento 589/2014 de la Comisión**, establece los métodos de muestreo y análisis para el control oficial de los niveles de dioxinas y PCB en productos alimenticios (CE, 2014b).

En relación a los **hidrocarburos aromáticos policíclicos**, el **Reglamento 835/2011 de la Comisión**, establece los contenidos máximos para la suma de PAH4 [benzo(a)pireno, benzo(a)antraceno, benzo(b)fluoranteno y criseno] y para el benzo(a)pireno de forma independiente (CE, 2011b). Quedan incluidos las aceites y grasas, los granos de cacao, las carnes y productos cárnicos ahumados, la carne de pescado y productos pesqueros ahumados (incluyendo a los moluscos bivalvos frescos), los alimentos elaborados a base de cereales y los alimentos y preparados para lactantes y niños de corta edad. En la **Tabla 13**, se muestran los contenidos máximos de PAH establecidos para los alimentos de origen animal, considerando las modificaciones por el **Reglamento (UE) 1327/2014** de la Comisión, en relación al contenido máximo de PAH en la carne y los productos cárnicos y pescado y productos de la pesca ahumados de modo tradicional (CE, 2014c). Además, el **Reglamento (UE) 836/2011**, con una modificación por el Reglamento 2016/582 de la Comisión, establece los métodos de muestreo y análisis para el control oficial de los niveles de PAH en los productos alimenticios (CE, 2011c).

Tabla 13. Contenidos máximos de PAH en los productos alimenticios de origen animal comercializados en la UE según el Reglamento 835/2011 y modificaciones por el Reglamento 1327/2014.

Producto	Contenido máximo Benzo (a) pireno (µg/kg)	Contenido máximo ΣPAH4 (µg/kg)
Carnes ahumadas y productos cárnicos ahumados	5	30
Carne de pescado ahumado y productos pesqueros ahumados	5	30
Espadines ahumados y espadines ahumados en conserva, moluscos bivalvos (frescos, refrigerados o congelados), carnes y productos cárnicos tratados térmicamente	5	30
Moluscos bivalvos ahumados	6	35
Preparados para lactantes y preparados de continuación, incluidas la leche para lactantes y la leche de continuación	1	1

4.2 Contaminantes inorgánicos persistentes

A diferencia de otros contaminantes ambientales, los metales y metaloides son elementos químicos que el hombre no crea ni destruye. El papel que el hombre desempeña en su presencia ambiental es, por una parte, introducir en el medioambiente estos elementos como consecuencia de las distintas actividades humanas y, por otra, alterar la forma química o bioquímica en que se encuentran. Los metales están sujetos de forma natural a ciclos biogeoquímicos que determinan su presencia y concentración en los compartimentos ambientales y los seres vivos. La intervención humana puede modificar considerablemente la concentración de metales en estos compartimentos y facilitar su distribución a partir de las reservas minerales en donde se encuentran naturalmente confinados.

Desde el punto de vista toxicológico, los metales suelen presentar una acusada multiplicidad de efectos tóxicos. La especie química concreta del metal influye poderosamente en sus efectos tóxicos y sus parámetros toxicocinéticos. La trascendencia toxicológica de los metales es enorme, teniendo en cuenta su ubicuidad, la extensión de sus usos industriales y domésticos y su persistencia medioambiental, resultado directo de su condición de elementos químicos. Estas características son las que hacen que varios de ellos se consideren como Contaminantes Tóxicos Persistentes. En esta Tesis Doctoral nos limitamos a seis Contaminantes Inorgánicos Persistentes (**CIP)**, que son: aluminio, arsénico, cadmio, mercurio, níquel y plomo, para los cuales se estimó el nivel de exposición mediante el consumo de productos de la pesca.

4.2.1 ALUMINIO

El aluminio (**Al**) es tercer elemento químico común encontrado en la corteza terrestre, constituyendo el 8% de la misma y se encuentra presente en la mayoría de las rocas, de la vegetación y de los animales. En estado natural se encuentra en muchos silicatos, mientras que como metal se extrae únicamente del mineral bauxita. El Al posee una combinación de propiedades (baja densidad, resistente a corrosión, conductor eléctrico, bajo coste, etc.), que hacen que sea el metal más utilizado tras el acero. Por tanto, presenta una gran variedad de **aplicaciones y usos**, como fabricación de espejos, latas y tetrabriks de uso alimentario, papel de Al, medicamentos y cosméticos, ingeniería de materiales (aeronáutica, tendidos eléctricos, soldaduras, piezas de vehículos, instrumentos de cocina, pulido de metales, etc.). Aunque, tradicionalmente, se ha considerado un agente no excesivamente tóxico, las observaciones relativas a su asociación con ciertos daños neurológicos y la sospecha de un aumento a su exposición de forma progresiva, introducen la incertidumbre suficiente para establecer la necesidad de evaluar el riesgo alimentario actual.

En relación a sus **parámetros toxicocinéticos**, se estima que la biodisponibilidad oral por el consumo de agua es del 0,3% y del 0,1% en el caso de alimentos y bebidas. La absorción del Al dietético depende de las formas químicas presentes en el tracto intestinal, siendo elevada la del bromuro de Al. El grado de solubilidad en agua de los compuestos de Al incrementa la biodisponibilidad del ión aluminio. La presencia o ausencia de ligandos en la dieta, puede incrementar (ej: citrato, lactato) o disminuir (ej: fosfato, polifenoles) su absorción intestinal. Tras su absorción, el ión Al se distribuye a todos los tejidos en animales y humanos y se acumula, preferentemente, en el hueso. En el plasma, el Al^{+3} se transporta principalmente unido a la proteína transferrina y puede penetrar en el cerebro y llegar al feto a través de la placenta y excretarse, asimismo, a través de la leche materna. Su permanencia en distintos órganos y tejidos antes de su excreción por orina puede ser elevada, variando los tiempos de eliminación media desde horas hasta meses y años. El Al no absorbido se elimina por las heces, siendo la excreción biliar una ruta minoritaria.

En cuanto a su **toxicidad**, se ha descrito que a niveles de exposición elevados algunos compuestos de Al pueden producir, *in vivo* e *in vitro*, daño en el ADN por mecanismos indirectos, efectos improbables mediante dosis dietéticas. Diversos estudios en animales de experimentación han demostrado que algunos compuestos, como cloruro o nitrato de Al, inducen toxicidad testicular y disminución de la calidad del semen, no observándose efectos sobre la fertilidad en hembras. Además, dosis elevadas administradas por sonda a roedores producen disminución del peso fetal y retraso en la osificación. Como elemento neurotóxico, el Al se asocia a encefalopatía en paciente expuestos de forma crónica por diálisis, observándose alteraciones neurológicas y óseas en niños con función renal alterada. También se ha descrito que diversos compuestos con Al tienen potencial neurotóxico en roedores, con preferencia por el sistema nervioso, afectando a su desarrollo en la descendencia.

En relación a su **evaluación toxicológica**, el JECFA estableció una ISTP para el aluminio de 1 mg/kg p.c./semana, posteriormente aceptada por la EFSA (EFSA, 2008a; EFSA, 2008c). Así, la EFSA elaboró un informe sobre la seguridad de la ingesta de Al mediante la dieta. La **exposición dietética** al Al en adultos era muy variable entre países y alimentos, oscilando entre 0,2-1,5 mg/kg p.c./semana, estimándose que en niños y jóvenes de algunos países (Reino Unido y Francia), la exposición dietética usando el percentil 97,5 era 0,7-2,3 mg/kg p.c./semana. Por tanto, se estima que una parte importante de la población europea puede superar el nivel seguro establecido. Los estudios disponibles no permiten conocer las fuentes dietéticas concretas de Al, pero se detectaron altos contenidos de Al en formulaciones para lactantes; ni tampoco la diferenciación entre los distintos orígenes del Al, aunque los aditivos pueden contribuir de forma significativa a la ingesta (EFSA, 2008a; EFSA, 2008c).

4.2.2 ARSÉNICO

4.2.2.1 Características y fuentes actuales de contaminación

El arsénico (**As**) es un no metal o metaloide del Grupo V de la tabla periódica, aunque con frecuencia se conoce y clasifica como un metal para muchos propósitos toxicológicos. Es muy difícil de caracterizar debido a su estructura química compleja, y a que rara vez se encuentra en estado elemental. Su movilidad y disponibilidad en el medioambiente depende del estado de oxidación y de las propiedades del medio en el que se encuentra. En la naturaleza, constituye multitud de compuestos, como el trióxido de As y el arsenito sódico (**formas de As trivalente**) y el pentóxido de As o el óxido de trimetilarsina (**formas de As pentavalente**).

Las **fuentes de As** que existen en el medioambiente son tanto naturales (como las erupciones volcánicas o erosión de rocas y minerales) como antropogénicas. Entre estas últimas, destaca el uso de compuestos de As como agentes conservantes de la madera y plaguicidas, formulaciones de herbicidas, la incineración de productos de madera, la fundición de metales, la fabricación de aleaciones y semiconductores y la combustión del carbón y combustibles fósiles. Sus emisiones a la atmósfera consisten fundamentalmente en trióxido de As en forma de partículas, cuya deposición depende de su tamaño y densidad, pudiendo experimentar oxidación en el aire (Khan y cols., 2009).

La exposición de la población general al As ocurre principalmente a través de la ingesta de alimentos y aguas contaminadas. Debido a que el As puede ser absorbido por algunas plantas como el arroz, una concentración elevada de As en el suelo puede resultar en elevados niveles en piensos y alimentos.

4.2.2.2 Parámetros toxicocinéticos

La **absorción del As** depende de la forma en que se encuentre, del tamaño de las partículas, la pureza, la solubilidad, la especie afectada y la condición física del organismo expuesto. Los compuestos pueden ver disminuida su toxicidad si presentan tamaños de partícula muy grandes, ya que se dificulta su absorción (Tseng, 2007). La susceptibilidad al As inorgánico varía entre las especies, siendo la más alta en los seres humanos. El As pentavalente orgánico es mejor absorbido que el trivalente, especialmente a través del tracto gastrointestinal. Pequeñas cantidades de cualquiera de las formas pueden ser absorbidas a través de piel, aunque esta forma de exposición no es común. El As se **distribuye** mediante la sangre a todos los órganos del cuerpo, acumulándose en el hígado y lentamente en otros tejidos. El bazo, los riñones y los pulmones son capaces de acumular grandes cantidades de As y se ha descrito que es capaz de atravesar la barrera placentaria (Tseng, 2007).

La **biotransformación** del As inorgánico implica la reducción de la forma pentavalente a la trivalente, seguida por la metilación de ambos estados de oxidación, obteniéndose como metabolitos ácidos monometilarsénico (MMA) y dimetilarsénico (DMA), que se excretan rápida y eficazmente. Los riñones pueden reducir una pequeña cantidad de As pentavalente al trivalente más tóxico. El tiempo de vida media del As inorgánico en el cuerpo humano es de unas 10 horas, sin embargo, la exposición continuada puede dar lugar a un desequilibrio entre la absorción y la excreción, proceso que resulta en su acumulación en tejidos como la piel y los tegumentos, donde tiende a concentrarse (Orloff y cols., 2009; Tseng, 2007).

En la mayoría de las especies, entre el 40% y el 70% de la cantidad de As pentavalente absorbido, se **excreta** por la orina en un periodo de 48 horas y en cantidades mucho más pequeñas a través del sudor. Las formas trivalentes se excretan más lentamente mediante la bilis. Una parte pequeña, pero muy relevante para la biomonitorización de la exposición crónica al As, se excreta por el pelo y las uñas (Orloff y cols., 2009).

4.2.2.3 Mecanismos de acción y toxicidad

La mayor parte de los efectos tóxicos son atribuibles a la forma trivalente. La intoxicación aguda por As produce náuseas, vómitos, diarrea, psicosis, neuropatía periférica y erupción cutánea (Ratnaike, 2003). La exposición crónica resulta en la aparición de síntomas orgánicos y sistémicos, tales como hiperpigmentación y queratosis palmar, episodios recurrentes de diarrea, vómitos, arritmia, hipertensión y neuropatía periférica sensitiva (Ratnaike, 2003). Además, la exposición al As se asocia con la aparición de cáncer de piel, pulmones, riñones, hígado y vejiga (Bernstam y Nriagu, 2000).

Desde el año 2012, la IARC especifica que pertenecen al grupo I el Arsénico y sus compuestos inorgánicos, por lo que es fundamental la especiación de este elemento y establecer métodos de determinación específicos para una correcta evaluación a su exposición y valorar sus aspectos toxicológicos (IARC, 2012a). Por otra parte, ensayos experimentales demuestran que el As produce toxicidad para el desarrollo, incluyendo malformaciones, muerte y retraso del crecimiento. También, se ha sugerido que la exposición ambiental al As es un riesgo importante para el feto durante el desarrollo (Golub y cols., 1998).

Además, los resultados de muchos estudios en los últimos años sugieren que gran parte de los efectos adversos se deben a que dosis bajas, compatibles con las dosis a las que está expuesta la población general, el As es capaz de actuar como disruptor endocrino (Watson y Yager, 2007), pudiendo actuar en la modulación de la transcripción nuclear de genes fisiológicamente regulados por hormonas (Nasreddine y Parent-Massin, 2002). Posiblemente, están implicados **mecanismos no genómicos** (Kaltreider y cols., 2001), cuyos efectos pueden ser claramente estimulatorios o inhibitorios de la expresión génica, respuesta bifásica dependiente de la concentración. Esta respuesta también se ha descrito para otros receptores como el RE o el receptor de mineralcorticoides (RM) (Davey y cols., 2007). No obstante, un **mecanismo genómico clásico** parece mediar las alteraciones del sistema reproductor masculino descritas, incluyendo disminución del recuento espermático, de la movilidad espermática y del peso testicular (Sarkar y cols., 2003).

4.2.2.4 Normativa legal sobre la presencia de As en alimentos

Es muy difícil el establecimiento de LMR para el As en alimentos, ya que para el As inorgánico no existe aún una metodología normalizada de análisis y cuantificación. El Comité Europeo de Normalización desarrolla protocolos para la determinación de elementos traza en los alimentos, por lo que una vez establecidos los métodos de análisis pertinentes para el As inorgánico, se espera que se promueva la Directiva que regule los contenidos máximos de este contaminante en los alimentos. Hasta la fecha, se han fijado exclusivamente límites máximos de As inorgánico para el arroz y productos derivados, recogidos en el **Reglamento (UE) 2015/1006** de la Comisión (CE, 2015c). Además, con el objeto de recopilar más información de las formas químicas de As que contribuyen más a su exposición alimentaria, existe un programa de control establecido mediante la **Recomendación (UE) 2015/1381** de la Comisión (CE, 2015a).

4.2.2.5 Niveles de exposición alimentaria de los ciudadanos de la UE al As

La última IST de As establecida por el JECFA era 15 µg/kg/semana, pero ésta se limitaba solo al As inorgánico, lo cual representaba un problema, ya que la mayor parte de los estudios determinan el As total. Por tanto, debido a que en las evaluaciones científicas no se disponía de suficiente información sobre la exposición total al As inorgánico mediante la dieta, no se ha establecido un punto de referencia toxicológico para el As. Tanto la EFSA como el JECFA han adoptado el enfoque del margen de exposición (MOE), que es el ratio entre la dosis a la cual no se observan efectos adversos (BMD, NOAEL) dividido entre el nivel de exposición real a esa sustancia mediante la dieta. Por ello, es necesario recopilar datos de As y sus especies químicas en alimentos para evaluar su exposición (EFSA, 2009b).

La UE evaluó el riesgo por exposición al As mediante la dieta por primera vez en el año 2004, identificándose al pescado como principal fuente de exposición dietética en la población adulta, aunque este estudio se centraba en el As total. La EFSA fue el primer organismo que elaboró un informe sobre la presencia de As inorgánico en los alimentos comercializados en la UE, estimando su exposición alimentaria (EFSA, 2014a), cuyos valores medios se presentan en la **Tabla 14.**

Considerando todos los escenarios, la exposición media dietética en los lactantes y niños oscilaba entre 0.20 y 1.37 µg/kg p.c./día, alcanzando 2,09 µg/kg /p.c./día para el P95 (datos no mostrados). En la población adulta, la exposición media osciló entre 0,09 y 0,38 µg/kg p.c./día, alcanzando 0,64 µg/kg p.c./día para el percentil 95. En todas las clases de edad, excepto en bebés y niños pequeños, el principal contribuyente a la exposición eran los productos transformados a base de cereales (no basados en arroz) y, en particular, el pan de

trigo y los panecillos (EFSA, 2014a). Otros contribuyentes importantes eran el arroz, la leche y productos lácteos (principalmente en lactantes y niños pequeños) y el agua potable.

Tabla 14. Exposición dietética media al As inorgánico (µg/kg p.c./día) en Europa.

Grupo de edad	LB[a]			MB[b]			UB[c]		
	Mín	Mediana	Máx	Mín	Mediana	Máx	Mín	Mediana	Máx
Bebés	0.24	[d]	0.43	0.56	[d]	0.87	0.88	[d]	1.37
Niños pequeños	0.32	0.39	0.45	0.59	0.68	0.81	0.91	1.00	1.17
Otros niños	0.20	0.30	0.36	0.33	0.51	0.61	0.47	0.71	0.87
Adolescentes	0.12	0.18	0.23	0.22	0.29	0.36	0.31	0.42	0.48
Adultos	0.11	0.13	0.17	0.18	0.23	0.28	0.24	0.33	0.38
Ancianos	0.09	0.11	0.15	0.16	0.20	0.24	0.24	0.29	0.34
Muy ancianos	0.09	0.12	0.16	0.17	0.20	0.26	0.25	0.28	0.36

[a] LB: Lower Bound. [b] MB:Medium Bound. [c] UB: Upper Bound.

[d] No se calcularon debido a que las estimaciones estaban disponibles solo en dos encuestas.

4.2.3 CADMIO

4.2.3.1 Características y fuentes actuales de contaminación

El cadmio (**Cd**) es una impureza habitual distribuida de forma natural en el medio, presente en las menas de zinc. Así, utilizando grandes cantidades de zinc, el hombre ha esparcido el cadmio en el entorno, ya que el zinc comercial puede contener hasta el 1% de este metal. El Cd puro es un metal suave de color plata-blanco, de transición divalente, con propiedades químicas similares a las del zinc y se encuentra generalmente como un mineral en combinación con otros elementos para formar óxido, cloruro o sulfato de Cd (ATSDR, 2008). Su presencia en la atmósfera puede ser consecuencia de la polución natural, producida por la capacidad de las plantas de concentrar el Cd de origen geoquímico y dispersarlo en el medioambiente tras su descomposición. Como aplicaciones industriales, se utiliza en baterías, soldaduras, semiconductores, células solares, reactores nucleares y estabilizadores de plásticos y placas de hierro y acero. Además, puede entrar en el entorno por la fundición y refinado de zinc, la combustión de carbón, desechos de minas, producción de hierro y acero y el uso de lodos de fosfato y aguas residuales como fertilizantes.

Cuando se emite a partir de procesos de combustión, se produce habitualmente en forma de partículas de pequeño diámetro sujetas a transportes de larga distancia, mientras que mediante las fundiciones suelen ser partículas de mayor tamaño, más susceptibles a depositarse por gravedad cerca del punto de emisión. Es un elemento común en los suelos de cultivo, donde puede aparecer de manera soluble e insoluble formando complejos con constituyentes orgánicos e inorgánicos y absorberse por las plantas, incorporándose así a la cadena trófica. Por tanto, en la población general no fumadora las principales fuentes son la ingesta de alimentos o aguas contaminadas, particularmente productos vegetales cultivados en terrenos contaminados por Cd y sus compuestos, especialmente en terrenos ácidos (Jarup y cols., 1998).

4.2.3.2 Parámetros toxicocinéticos

La **absorción** del Cd es más eficaz por vía inhalatoria (alrededor del 15%). En comparación con otros cationes divalentes como el zinc y el hierro, la absorción intestinal del Cd es baja, aproximadamente del 1 al 5% en la mayoría de las especies, llegando al 16% en el ganado, dependiendo de la dosis (Jarup y cols., 1998). Dicha absorción gastrointestinal se ve favorecida

por deficiencias dietéticas en calcio, zinc, hierro y proteínas. Una vez alcanzado el torrente sanguíneo, **se transporta** ligado a proteínas plasmáticas de alto peso molecular, como la albúmina, así como a los hematíes. Parte de este transporte ocurre mediante la formación de complejos con la metalotioneína, una proteína de bajo peso molecular que interviene en el transporte y detoxificación de metales gracias a la formación de complejos estables entre sus grupos sulfhidrilo y los iones. La metalotioneína se encuentra en la placenta, donde ejerce un papel protector impidiendo el acceso de metales, como el Cd, a la sangre fetal. El Cd **se distribuye** en todo el cuerpo, alcanzando las mayores concentraciones en el hígado y los riñones, órganos que acumulan aproximadamente la mitad del total de Cd en el cuerpo.

La **excreción** del Cd tiene lugar principalmente por la orina (0,1%), la mayor parte ligada con metalotioneína, y un 0,007% por las heces mediante la bilis. A nivel renal se excretan complejos de Cd-proteínas, pero se reabsorben desde el filtrado en los túbulos proximales, lo que favorece su bioacumulación a lo largo de la vida. Así, su vida media el hombre varía entre 10 y 40 años aproximadamente (Jarup y cols., 1998).

4.2.3.3 Mecanismos de acción y toxicidad

Los mecanismos de acción implicados en la toxicidad aguda del Cd son bien conocidos. Una vez dentro de la célula, el Cd libre se une a los grupos sulfhidrilo de proteínas, alterando el ciclo redox en las células, agotando el glutatión y provocando daños oxidativos intracelulares. Además, su similitud con otros cationes divalentes como el calcio o el zinc, hace que interfiera con su normal funcionamiento (Liu y cols., 2009).

Por otra parte, la unión del Cd a la metalotioneína es parcialmente responsable de su retención intracelular y su larga vida media. En los riñones el complejo Cd-metalotioneína es nefrotóxico y puede desempeñar un papel en la intoxicación crónica en humanos. Así, se produce daño tubular que puede progresar en la reducción del filtrado glomerular y consecuentemente, fallo renal (Klaassen y cols., 2009). Consecuencia de este fallo renal, o por acción directa, el Cd puede provocar desmineralización de los huesos. Además, desde el año 2012, el Cd y sus compuestos se clasifican como carcinógenos para el ser humano por la IARC (IARC, 2012a), basado fundamentalmente en la asociación entre su exposición vía inhalatoria en trabajadores expuestos y la aparición de cáncer de pulmón.

Al igual que el resto de los CIP considerados, el Cd tiene la capacidad de ejercer claros efectos sobre la acción hormonal, incluso a dosis de exposición medioambiental a este metal (Iavicoli y cols., 2009). Así, se ha demostrado que dosis pequeñas de Cd son capaces de afectar el patrón de secreción de hormonas como la prolactina, la hormona adrenocorticotropa (ACTH), o la progesterona, entre otras, siendo estimulada su secreción a las dosis más bajas ensayadas e inhibida a dosis más altas (Lafuente y cols., 2003). Además, existe una clara asociación entre la exposición al Cd durante la gestación con la reducción en el peso del neonato y un incremento en la tasa de abortos espontáneos y nacimientos pretérmino (Frery y cols., 1993; Wier y cols., 1990). Otro efecto muy relevante del Cd es su actividad estrogénica, demostrada tanto *in vivo* como *in vitro*, pudiendo inducir genes regulados por estrógenos (Garcia-Morales y cols., 1994). En otros estudios, se demostró su capacidad para disminuir los índices reproductivos en adultos tras la exposición fetal (Leoni y cols., 2002).

4.2.3.4 Normativa legal sobre la presencia de Cd en alimentos

Actualmente, el contenido máximo de Cd en los productos alimenticios está regulado por el **Reglamento (UE) 488/2014** (CE, 2014a), indicándose los niveles permitidos para los productos de la pesca en la **Tabla 15**. Además, el **Reglamento (UE) 836/2011**, establece los métodos de muestreo y análisis para el control oficial de los niveles de Cd en los productos alimenticios (CE, 2011c).

Tabla 15. Contenidos máximos permitidos de Cd (mg/kg peso fresco) en los productos de la pesca comercializados en la UE según el Reglamento 488/2014.

Producto	Contenido máximo
Carne de pescado de caballa, atún y bichique	0.10
Carne de pescado de melva	0.15
Carne de pescado de anchoa, pez espada y sardina	0.25
Carne de pescado, excluidas las mencionadas anteriormente	0.050
Crustáceos	0.5
Moluscos bivalvos	1.0
Cefalópodos	1.0

4.2.3.5 Niveles de exposición alimentaria de los ciudadanos de la UE al Cd

Tras una evaluación toxicológica para valorar los riesgos para la salud relacionados con la presencia de Cd en los productos alimenticios, la EFSA ha establecido una IST para el Cd de 2,5 µg/Kg p.c./semana, que es la cantidad máxima de Cd que puede ingerir una persona semanalmente durante toda su vida sin manifestar efectos adversos, incluidos los subgrupos poblacionales vulnerables (EFSA, 2009a).

Posteriormente, la EFSA elaboró un nuevo informe utilizando el patrón de consumo de determinados subgrupos poblacionales, como los vegetarianos y los niños. En un informe anterior destaca que debido al elevado consumo de cereales, frutos secos, semillas oleaginosas y legumbres, los vegetarianos tienen una mayor exposición dietética al Cd, llegando hasta los 5,4 µg/kg/semana (EFSA, 2009a). Los consumidores habituales de moluscos bivalvos y setas silvestres podrían exponerse también a altos niveles de Cd. En la **Tabla 16**, se muestran las contribuciones de las categorías de alimentos de origen animal a la exposición dietética al Cd por grupos de edad, recogidas en el último informe de la EFSA (EFSA, 2012a).

Según los resultados de este estudio, los grupos de alimentos de mayor contribución dietética al Cd (datos no mostrados) eran los cereales y productos a base de cereales (26,9%), hortalizas y productos vegetales (16,0%) y raíces y tubérculos (13.2%). La exposición dietética media al Cd en la UE se estimó en 2,04 µg/kg p.c./semana. Los valores más elevados se alcanzaron en niños pequeños y los más bajos en ancianos, con un promedio de 4,85 y 1,56 µg/kg p.c./semana, respectivamente. Si se utilizaba el P95 de consumo, el valor medio de ingesta ascendía a 3,66 µg/kg p.c./semana, con el valor más elevado de 8,19 y el más bajo de 2,82 µg/kg p.c./semana, en niños y ancianos, respectivamente. Por tanto, acorde a esta estimación, se podría exceder el valor de referencia establecido, considerando el consumo medio en niños y el percentil 95 en adultos (EFSA, 2012a). Si bien el panel concluyó que es poco probable que se produzcan efectos adversos en la población mediante la exposición dietética actual al Cd, hay una necesidad de reducirla debido al limitado margen de seguridad.

Tabla 16. Consumo estimado (%) de Cd a través del consumo de alimentos de origen animal por un consumidor adulto estándar de la UE.

Categoría de alimento	Bebés	Niño pequeños	Otros niños	Adolescentes	Adultos	Ancianos	Muy ancianos
Carne y despojos comestibles	4.8	5.3	5.9	6.5	8.7	9.0	7.9
Pescado y otros productos de la pesca	0.4	2.4	5.0	8.2	8.9	7.5	8.1
Leche y productos lácteos	8.4	6.5	6.9	3.2	2.3	2.4	2.0
Huevos y ovoproductos	0.2	0.1	0.1	0.1	0.1	0.1	0.1

4.2.4 MERCURIO

4.2.4.1 Características y fuentes actuales de contaminación

El mercurio (Hg) es un elemento natural que se encuentra en el medioambiente y existe en forma elemental, orgánica e inorgánica. Aproximadamente el 80% del Hg liberado al medio ambiente es Hg metálico y proviene de las actividades humanas, como la combustión de fósiles, minería, fundición e incineración de residuos sólidos. El Hg metálico puro a temperatura ambiente es una sustancia líquida blanca y brillante y es capaz de mantenerse líquido a 0ºC. Forma sales cuando se combina con otros elementos, como el cloro, azufre y oxígeno (ATSDR, 1999). Tanto el Hg, como sus derivados orgánicos e inorgánicos, son tóxicos, ya que reaccionan con enzimas que contienen sulfuro y las desactivan, y tiene especial afinidad por el riñón y por el sistema nervioso.

El vapor de Hg elemental constituye el 95% del Hg presente en la atmósfera. Puede transportarse a grandes distancias en el medio ambiente y su vida media atmosférica oscila entre 6 días y 2 años. El resto de Hg atmosférico se encuentra asociado a materia particulada y se elimina más rápidamente por deposición húmeda o seca.

Las principales fuentes medioambientales de Hg elemental son los procesos naturales de volatilización del Hg a partir de depósitos minerales, los volcanes y las fuentes termales. Los usos del Hg han disminuido o se han modificado en las últimas décadas debido a la preocupación por sus efectos tóxicos. Entre las aplicaciones que han disminuido podemos nombrar la industria extractiva, bien del propio Hg, como la del oro y la plata y las distintas aplicaciones industriales de las amalgamas. Otros usos del Hg tienen fines medicinales, por ejemplo, laxantes, antiparasitarios, antisépticos y desinfectantes. Actualmente, los usos más importantes del Hg en la UE se basan en el proceso de producción cloro-sosa (sobre un 41%), el uso del cátodo de Hg para el depósito electrolítico de sodio y otros como la fabricación de pilas, productos químicos, lámparas fluorescentes, barómetros y material para empastes dentales (COWI, 2008).

Así, en el medioambiente las diversas formas de Hg son intercambiables entre sí por la acción de bacterias, hongos y mamíferos. Por ejemplo, el Hg inorgánico puede ser metilado a metilmercurio (**MeHg)** y a su vez, el MeHg puede cambiar al Hg inorgánico o elemental. Los animales situados en la parte superior de la cadena alimentaria tienden a bioacumular MeHg, tanto en forma inorgánica como, especialmente, en forma orgánica como los derivados del metil y fenilmercurio, siendo el **dimetilmercurio** el componente más común en la cadena alimentaria, tendencia constatada en numerosas especies acuáticas de agua dulce y marina (Tchounwou y cols., 2003). Por tanto, la población general está expuesta al Hg principalmente por la ingesta

de alimentos contaminados, en particular el pescado (en el cual se acumula en forma de MeHg), la carne, los cereales y otros alimentos como los vegetales o los lácteos (Bhan y Sarkar, 2005).

4.2.4.2 Parámetros toxicocinéticos

En cuanto a su **absorción**, el Hg metálico o elemental es el más que se absorbe (80%) desde los pulmones, mientras que se absorbe muy poco desde el tracto gastrointestinal (< 0.5%). En este caso, el 60% está en forma de Hg vapor y el resto es Hg orgánico e inorgánico. Se estima que el vapor de mercurio se absorbe casi en un 100% a través del alveolo pulmonar. Sin embargo, el Hg orgánico (por ejemplo, el MeHg) se absorbe en un 95 % desde el tracto gastrointestinal (Holmes y cols., 2009), mientras que los inorgánicos lo hacen en un 75%. El Hg se **distribuye** rápidamente a otros tejidos y se acumula en hígado, riñón, cerebro y ganglios linfáticos, por lo que el sistema linfático puede desempeñar un papel importante en el transporte de Hg a los órganos diana (Holmes y cols., 2009). El Hg metálico puede permanecer en el cuerpo entre semanas y meses y debido a su alta lipofilicidad, puede cruzar fácilmente las barreras hematoencefálica y placentaria. En los tejidos, el Hg elemental se oxida rápidamente a Hg divalente, forma en la que atraviesa con más dificultad las membranas celulares, en particular las citadas BHE y placentaria. Sin embargo, puede permanecer en la sangre durante unos minutos en estado de oxidación cero, tiempo suficiente para entrar en el sistema nervioso. Por su parte, las formas orgánicas se fijan a los glóbulos rojos con alta afinidad en los grupos –SH de la hemoglobina y atraviesan las barreras con mucha facilidad, por lo que se acumulan en el SNC y los tejidos fetales.

Todas las formas de Hg presentan una **metabolización** similar en el ser humano y en los animales, que se basa en un ciclo de oxidación-reducción en la microflora intestinal. Las formas orgánicas sufren desmetilación en los tejidos. La vida media del Hg en sangre es variable, dependiendo de la forma química, con un promedio de 50 días (Holmes y cols., 2009). La mayor parte del Hg metálico se **excreta** en la orina y las heces y cantidades relativamente altas pasan también a la leche. El Hg orgánico se defeca en forma de Hg inorgánico en las heces durante varios meses (Holmes y cols., 2009).

4.2.4.3 Mecanismos de acción y toxicidad

El **vapor de mercurio,** que se forma ya a temperatura ambiente, es altamente tóxico y a una concentración superior al límite umbral, causa un envenenamiento crónico tras una aspiración prolongada de 5 a 8 horas diarias. En términos generales, se considera que el Hg orgánico es más tóxico y produce más intoxicaciones que el inorgánico. El **dimetilmercurio** es una forma extremadamente tóxica de mercurio orgánico, ya que una exposición muy breve puede producir una neurotoxicidad retardada grave e irreversible, que incluso puede provocar la muerte. La inhalación de concentraciones elevadas de **Hg elemental** puede provocar bronquitis corrosiva y neumonitis aguda, que puede causar la muerte.

Sin embargo, la exposición ocupacional a dosis relativamente altas, tiene sus efectos tóxicos fundamentales en el sistema nervioso central. Los síntomas de intoxicación crónica por Hg incluyen temblores, hipertrofia tiroidea, taquicardia, gingivitis, cambios en la personalidad, eretismo, pérdida de memoria, depresión, delirios y alucinaciones. También se han descrito casos

de afección renal causados por la exposición crónica al Hg elemental, incluyendo proteinuria y enzimuria (Bhan y Sarkar, 2005).

La exposición al MeHg, su forma más tóxica, incluye efectos teratogénicos. La exposición del feto provoca una alteración de las pautas normales de migración de las neuronas, causando la disrupción de la arquitectura del cerebro, posiblemente mediada por un mecanismo de toxicidad sobre la formación de los microtúbulos de las neuronas, lo que repercute en la estructura celular, la alteración de la comunicación intercelular cerebral y la migración de las neuronas (Tchounwou y cols., 2003). Por ello, las mujeres embarazadas o que puedan llegar a estarlo y las lactantes, así como los niños más pequeños, constituyen la población más sensible al mercurio.

El **mecanismo de acción** que subyace a los efectos tóxicos de las formas de Hg orgánico e inorgánico es similar y se relaciona con su acumulación en tejidos delicados. El principal mecanismo de acción en las intoxicaciones, tanto agudas como crónicas, se relaciona con la alta afinidad de unión del Hg catiónico divalente a los grupos sulfhidrilo (SH) de las proteínas, que resulta en la inactivación de diferentes enzimas, proteínas estructurales y de transporte y una alteración de la permeabilidad de las membranas celulares. Otros mecanismos involucrados son: aumento del estrés oxidativo, peroxidación lipídica, disfunción mitocondrial, depleción del glutatión, aumento de la permeabilidad de la BHE, síntesis de proteínas, replicación de ADN, actividad de la ADN polimerasa, homeostasis del calcio, transmisión sináptica y respuesta inmune (Tchounwou y cols., 2003).

Por lo que respecta a su comportamiento como agente cancerígeno, los compuestos de MeHg se encuentran clasificados en el grupo 2B (posiblemente cancerígenos) por la IARC (IARC, 1993), mientras que el Hg elemental y los compuestos de Hg inorgánico están clasificados en el grupo 3, es decir, no son clasificables en cuanto a su actividad cancerígena.

Además, cada vez existen más evidencias que remarcan la importancia de los efectos subclínicos de la exposición a dosis ambientales de Hg, vehiculadas por los alimentos y el agua (Iavicoli y cols., 2009). Así, el Hg parece también tener cierta influencia sobre los niveles fisiológicos de las hormonas reproductivas, ya que en animales de experimentación, la administración de dosis bajas de MeHg en la dieta disminuyó los niveles plasmáticos de 17-β estradiol (Drevnick y Sandheinrich, 2003). Efectos similares se observaron para la testosterona y la corticosterona. En otros experimentos, pequeñas dosis interfirieron en el proceso reproductivo, alterando la espermatogénesis (Chowdhury y cols., 1989); y en hembras abortos, malformaciones congénitas y trastornos de la ovulación (Schuurs, 1999). En estudios poblacionales en humanos, algunos estudios indican una correlación positiva entre los niveles de Hg en el pelo y los niveles de estrona y estradiol (Agusa y cols., 2007), o de TSH (Abdelouahab y cols., 2008), entre otras.

4.2.4.4 Normativa legal sobre la presencia de Hg en alimentos

En la actualidad, el **Reglamento (CE) 629/2008** fija el contenido máximo de determinados contaminantes, incluido el mercurio, en los productos alimenticios (CE, 2008). Este límite queda fijado en 0.5 o 1 mg/kg peso fresco según la especie de producto de la pesca considerada. Además, el **Reglamento (UE) 836/2011** establece los métodos de muestreo y análisis para el control oficial de los niveles de Hg en los productos alimenticios (CE, 2011c).

4.2.4.5 Nivel de exposición alimentaria de los ciudadanos de la UE al Hg

Tanto el Hg inorgánico como el orgánico aparecen frecuentemente en los alimentos a niveles muy variables, reflejando los niveles de contaminación local donde son producidos. Los organismos marinos son particularmente preocupantes, ya que tienen la capacidad de

biotransformar el Hg inorgánico a formas orgánicas de este metal (principalmente MeHg), con lo cual aumenta mucho su biodisponibilidad. Por tanto, se tiende a considerar que el consumo de pescado, particularmente de grandes especies de peces depredadores, constituye la principal fuente de exposición humana al Hg.

Dada la importancia de la toxicidad de este elemento, la OMS ha establecido una guía para identificar poblaciones de riesgo a la exposición del mercurio. Este organismo, junto a los diversos Ministerios de Salud Pública, ha jugado un papel importante en la implementación del **Convenio de Minamata** en el ámbito del PNUMA, cuya finalidad es emprender medidas legales para controlar la producción, emisión, usos y gestión de los residuos del mercurio para proteger el medio ambiente y la salud humana. Además, destacan también la **Estrategia comunitaria sobre el Mercurio** (adoptada por Comisión Europea en 2005) y la **Asociación Mundial del Mercurio** (*Global Mercury Partnership*, GMP), que establecen medidas enfocadas a reducir los niveles de mercurio y su exposición.

La última IST quedó fijada en 1,3 y 4 µg/kg/p.c para el MeHg y el Hg inorgánico, lo que supone una ingesta semanal de 9,1 y 28 µg/kg/p.c. de MeHg y Hg inorgánico, respectivamente (EFSA, 2012c). Acorde a los niveles de exposición alimentaria de mercurio inorgánico y MeHg evaluados por la EFSA en 2012, el Hg inorgánico en los alimentos no supone ningún riesgo para el consumidor, ya que la exposición no excede la IST, mientras que con el MeHg no sucede lo mismo. Así, más del 60% de los datos se encontraban por debajo del LD o LC en 11 de los grupos alimentarios, pero el 12% de los resultados eran superiores al LC para el "pescado y otros productos del mar", que además presentaban los valores más altos, especialmente los peces depredadores. Por tanto, el escenario que ha empleado la EFSA para el cálculo de exposición al metilmercurio utiliza los productos de la pesca como única fuente de exposición. De esta forma, hemos decidido indicar en la **Tabla 17** la contribución del pescado y otros productos del mar a la exposición media dietética al MeHg, mostrando el rango de contribución media para cada grupo de edad (EFSA, 2012c).

Tabla 17. Contribución media de los productos de la pesca (%) a la exposición dietética al MeHg.

Categoría de alimento	Bebés	Otros niños	Adolescentes	Adultos	Ancianos	Muy ancianos
Carne de pescado	59-100	69-100	74-97	81-100	92-100	90-100
Productos de pescado	0-40	0-29	0-22	0-13	0-2.2	0-1.5
Moluscos	0-5.3	0-8.2	0-9.7	0-7.2	0-6.3	0-6.9
Crustáceos	0-5.1	0-3.2	0-12	0.0-6.4	0-3.5	0-2.8
Despojos de pescado	0	0-19.9	0-0.9	0-1.0	0-0.6	0-0.7

La exposición media dietética al MeHg mediante los productos de la pesca osciló entre 0,06 en personas mayores a 1,57 µg/kg p.c./semana en niños pequeños. Mediante el P95, los valores oscilaban de 0,14 a 5,05 µg/kg p.c./semana en muy ancianos y adolescentes, respectivamente. En particular, el atún, el pez espada, el bacalao, la pescadilla y el lucio eran los principales contribuyentes a la exposición alimentaria al MeHg en la población adulta, y éstas especies más la merluza eran los principales contribuyentes en la población infantil. Al considerarse los mayores percentiles de consumo, la exposición era cercana o excedía la IST en todos los grupos de edad. En el caso de los consumidores extremos, donde podrían estar las mujeres embarazadas, la exposición puede ser hasta 6 veces la IST (EFSA, 2012c). Los niveles de exposición más altos se encontraron en las dietas de países mediterráneos, ya que la exposición estaba más relacionada con el tipo de pescado que con las cantidades consumidas.

Otra opinión científica de la EFSA destaca que el pescado y el marisco constituyen una fuente de energía y proteínas de alto valor biológico y contribuyen a la ingesta de nutrientes esenciales como el yodo, el selenio, el calcio y las vitaminas A y D, además de ácidos grasos poliinsaturados de cadena larga omega-3, componente que se asocia a mejores resultados funcionales del neurodesarrollo en niños y a un menor riesgo de mortalidad por enfermedad cardiaca coronaria en adultos (EFSA, 2014b). En el último informe, que compara riesgos y beneficios, se enfatiza que la limitación del consumo de especies con un alto contenido de MeHg es la manera más eficaz de alcanzar los beneficios para la salud por el consumo de pescado (EFSA, 2015b).

4.2.5 NÍQUEL

El níquel (**Ni**) en su forma pura es un metal duro blanco plateado, que junto con sus compuestos, ocurre naturalmente en la corteza terrestre. Existe en diversas formas minerales y está presente en todos los compartimentos del medio ambiente y omnipresente en la biosfera. El Ni se encuentra en combinación con Fe y otros metales como Co, Cu y Mg. La descarga de Ni en el medio ambiente es resultado de **actividades tanto naturales como antropogénicas** (erupciones volcánicas, el polvo transportado por el viento, los incendios forestales, la minería, la galvanoplastia, los procesos metalúrgicos, la combustión de combustibles fósiles, la incineración de residuos, etc.). El Ni es resistente a la corrosión y al calor, por lo que se utiliza a menudo en aleaciones y, más comúnmente, en el acero inoxidable. También está presente en baterías, joyas, pigmentos, etc. Además, puede existir en diferentes estados de oxidación, pero el más estable (Ni^{+2}), se presenta generalmente en aguas y comidas. Así, la exposición al Ni en la población general no fumadora se debe principalmente a los alimentos, y en menor medida, al agua potable.

En relación a sus **parámetros toxicocinéticos** en humanos, la absorción gastrointestinal puede variar significativamente (entre el 1 y el 40%) en función de su forma química, composición de la dieta y el estado de ayuno, siendo mucho mayor su biodisponibilidad mediante el agua de bebida. Una vez entra en la circulación, puede unirse a proteínas séricas (en particular la albúmina) y se distribuye por todos los tejidos, incluyendo el pulmón, el hígado, la tiroides, las glándulas adrenales o los riñones. Es capaz de atravesar la BHE y placentaria. Se excreta principalmente mediante la orina y, en menor medida, por la leche materna.

Los compuestos de Ni se clasifican como carcinógenos (Grupo 1) por la IARC (IARC, 2012a), ya que después de su inhalación causan cáncer de pulmón, cavidad nasal y senos paranasales; mientras que las aleaciones y el níquel metálico se clasifican como posible carcinógeno para humanos (Grupo 2B) (IARC, 1990). El Panel CONTAM, según diferentes estudios de carcinogenicidad oral, considera poco probable que la exposición alimentaria se asocie con cáncer.

La dermatitis de contacto alérgica es el **efecto tóxico** más frecuente del Ni en la población general, ya que el consumo de alimentos ricos en Ni puede provocar brotes de reacciones eccematosas en la piel en individuos sensibilizados. En cuanto a su toxicidad en el desarrollo, en ratones se ha observado una disminución de la espermatogénesis y la fertilidad a dosis>2.2 mg/kg, aumento de la mortalidad de las crías, disminución de peso al nacer y anomalías esqueléticas. Su toxicidad ha sido estudiada *in vivo* e *in vitro*, teniendo en cuenta su capacidad para formar ROS, inhibir la reparación de ADN o desregular la señalización celular, concluyendo que es probable la genotoxicidad mediante efectos indirectos (EFSA, 2015a). También se ha descrito hepatoxicidad y nefrotoxicidad.

En la actualidad, no existe una regulación de la UE con respecto a los niveles máximos de níquel en los alimentos, pero en las aguas de consumo existe un valor paramétrico de 20 µg/L, establecida por la **Directiva 98/83/CE** (CE, 1998). Además, el panel científico de la EFSA estableció una IDT para el Ni de 2,8 µg/Kg p.c. (EFSA, 2015a).

Con respecto al riesgo para la salud pública relacionado a la presencia de Ni en alimentos y aguas, la EFSA publicó un informe en 2015 a partir de muestras recolectadas entre 2003 y 2012 por 15 países de la UE. La e**xposición dietética media** osciló entre 2 (usando el MB) y 13,1 (usando el UB) µg/Kg p.c. en ancianos y niños, respectivamente (EFSA, 2015a). Por tanto, estos valores son cercanos o superan la IDT establecida (especialmente en niños y adolescentes). Los principales contribuyentes a dicha exposición eran los granos y productos a base de cereales, las bebidas no alcohólicas, el azúcar y confitería, las legumbres, los frutos secos y semillas oleaginosas y los vegetales y sus productos. Por tanto, la exposición de la población vegetariana es mayor. Con estos resultados, se concluyó que es necesario realizar más estudios para evaluar sus efectos en el desarrollo y la reproducción.

4.2.6 PLOMO

4.2.6.1 Características y fuentes actuales de contaminación

El plomo (**Pb**) es un metal pesado de color blanco azulado grisáceo que fue probablemente el primer elemento tóxico reconocido por el hombre y que aún hoy tiene gran relevancia. La principal fuente de Pb es el mineral llamado galena, que contiene sulfuro de Pb. Este metal plantea un grave problema para la salud de los ecosistemas, sobre todo de las aves acuáticas. Su forma inorgánica es más común en el medioambiente.

El uso histórico de Pb como aditivo en la gasolina, en pinturas, materiales de construcción y muchos otros productos se ha traducido en que sea uno de los contaminantes ambientales más importantes en el mundo, aunque en la gasolina está prohibido en la UE desde el año 2000 (CE, 2000a). Actualmente, se usa principalmente en baterías, soldaduras, productos electrónicos, vidrio, esmaltes y munición de armas de fuego. Por tanto, las principales fuentes de contaminación ambiental por Pb son la fundición y la minería, el procesado industrial de hierro y acero, las refinerías, la eliminación de basuras y las industrias de reciclaje de Pb (OMS, 1995), incluyendo el tratamiento incorrecto de pilas y baterías.

La población general está expuesta al Pb a través de la ingestión de alimentos y agua contaminados y mediante la inhalación de aire con partículas de Pb (OMS, 1995), ya que en la atmósfera el Pb forma parte de la materia particulada, normalmente como óxidos o carbonatos, que en función del tamaño y de la densidad de partícula se depositan por gravedad o se transportan a grandes distancias. Una vez depositado en el suelo, no se lixivia fácilmente hacia las capas profundas del subsuelo, excepto en medios muy ácidos y con mucha materia orgánica, lo que favorece su biodisponibilidad para las plantas.

4.2.6.2 Parámetros toxicocinéticos

La principal vía de **absorción** de Pb en el cuerpo es el tracto digestivo. En adultos, su tasa de absorción por ingestión es del 5-15%, siendo más eficaz en niños. Además, su absorción se incrementa de 7-20 veces por dietas pobres en grasa y minerales (O'Flaherty, 1998). Los compuestos orgánicos generalmente se absorben mejor que las sales inorgánicas o el Pb elemental. La absorción dérmica es buena para los compuestos orgánicos, pero nula para las

inorgánicas. La absorción inhalatoria es eficaz y completa solo para partículas con diámetro inferior a 0,05 µm.

El Pb se transporta mayoritariamente unido a la membrana de los eritrocitos y gran parte del resto se une a proteínas como la hemoglobina y se **distribuye** muy desigualmente entre los distintos tejidos. La máxima concentración se alcanza en los huesos, donde es capaz de almacenarse mucho tiempo (vida media entre 10 y 30 años). Este depósito puede movilizarse durante la lactancia, el embarazo y agentes quelantes. De lo contrario, la tasa de eliminación ósea es muy lenta y se retiene y moviliza en los huesos por el mismo mecanismo que regula el calcio. Así, el Pb depositado en los huesos se intercambia fácilmente con la sangre y llega especialmente al hígado, los riñones y el sistema nervioso central. El Pb cruza la barrera placentaria y se puede detectar en cantidades significativas en la sangre y órganos del feto. Normalmente el Pb se **excreta** lentamente a través de la bilis y una pequeña proporción en la orina. También aparece en el sudor y en la leche materna (O'Flaherty, 1998).

4.2.6.3 Mecanismos de acción y toxicidad

El Pb interfiere con varios procesos bioquímicos en el cuerpo mediante el establecimiento de enlaces con los grupos sulfhidrilo de las proteínas y otros grupos funcionales nucleófilos. Esto produce la inhibición de varias enzimas y cambios en el metabolismo de calcio y vitamina D. Posee también un papel inhibidor de la síntesis de hemoglobina, hecho asociado a la anemia observada en la intoxicación crónica por Pb (Palis, 2008). Además, la exposición a niveles relativamente bajos se asocia también con presión arterial elevada, nefropatía y cólicos abdominales en adultos (OMS, 1995). Con respecto a su potencial carcinógeno, la IARC clasificó en 2006 a los compuestos inorgánicos como probablemente carcinógenos (2A), mientras que los compuestos orgánicos se encuadraron en el Grupo 3 (IARC, 2006).

Diversos estudios experimentales sugieren que el Pb es neurotóxico, alterando significativamente ciertas funciones y estructuras cerebrales. Además de un efecto tóxico directo en las células del endotelio, puede alterar indirectamente la microvascularización, al dañar los astrocitos encargados de mantener la integridad de la BHE. Consecuentemente, moléculas normalmente excluidas de los territorios nerviosos centrales, como la albúmina, entran al cerebro de animales inmaduros expuestos a diversas concentraciones de Pb. A la entrada de proteínas le sigue la de iones y agua, dando lugar a edema cerebral, que puede progresar a isquemia cerebral (Marchetti, 2003). Muchos de los efectos neurotóxicos del Pb se relacionan también con su capacidad para imitar o inhibir la acción del calcio como regulador del funcionamiento celular. A nivel neuronal, se altera la liberación de neurotransmisores como la dopamina o la acetilcolina y, por ello, pueden aparecer trastornos psicológicos y neuropatía periférica. Se ha descrito que niveles sanguíneos de Pb entre 5 y 10 mg/dl se asocian con trastornos neuroconductuales y una reducción significativa en la capacidad cognitiva en niños (Tellez-Rojo y cols., 2006).

Hay que destacar también las alteraciones en el embarazo y en los espermatozoides descritas con la exposición a dosis ambientales de Pb (Iavicoli y cols., 2009), efectos que encajan en el fenómeno de disrupción endocrina. Dosis muy pequeñas de exposición al Pb se han asociado con alteraciones en la afinidad del RE y el receptor para la hormona luteinizante

(Wiebe y Barr, 1988; Wiebe y cols., 1988); o una reducción en los niveles séricos de IGF-1, LH, testosterona y estradiol (Srivastava y cols., 2004), entre otros.

4.2.6.4 Normativa legal sobre la presencia de Pb en alimentos

Actualmente, el contenido máximo de Pb en los productos alimenticios comercializados en la UE está regulado por el **Reglamento (UE) 2015/1005** (CE, 2015b), indicándose los niveles permitidos para los productos de la pesca en la **Tabla 18**. Además, el **Reglamento (UE) 836/2011**, establece los métodos de muestreo y análisis para el control oficial de los niveles de Pb en los productos alimenticios (CE, 2011c).

Tabla 18. Contenido máximo de Pb (mg/kg peso fresco) en los productos de la pesca según el Reglamento 2005/1015.

Producto	Contenido máximo
Carne de pescado	0.30
Cefalópodos	0.30
Crustáceos	0.50
Moluscos bivalvos	1.50

4.2.6.5 Nivel de exposición alimentaria de los ciudadanos de la UE al Pb

Se estima que aproximadamente la mitad de la exposición de los seres humanos al Pb es alimentaria. En las regiones en las que siguen utilizándose tuberías de Pb, se encuentran concentraciones elevadas de este metal en el agua potable, lo cual aumenta significativamente la ingesta a través de las bebidas y de los alimentos cocinados con agua o al vapor.

Ante la falta de evidencia de umbrales de toxicidad para una serie de efectos críticos en la salud por el plomo (teniendo en cuenta sus efectos neurotóxicos en niños y nefrotóxicos y cardiovasculares en adultos), la EFSA y el JECFA concluyeron que no era posible establecer una ingesta tolerable recomendada para el Pb. Como medida alternativa, el dictamen de la EFSA de 2010 estableció un $BMDL_{01}$ de 0,50 µg/kg p.c./día para el desarrollo de neurotoxicidad en niños pequeños (EFSA, 2010c). Sin embargo, las agencias han subrayado su preocupación sobre el posible efecto negativo del Pb en el desarrollo neuronal de fetos, bebés y niños (especialmente de 1 a 7 años) mediante los niveles actuales de exposición dietética, por lo que recomiendan tomar medidas para identificar las principales fuentes dietéticas y establecer métodos para reducir esta exposición (EFSA, 2012b).

En el año 2012, la EFSA publicó un informe sobre la exposición dietética al Pb en la UE a partir de resultados analíticos de un periodo de 9 años. Los resultados de este informe se representan para cada grupo de edad en la **Tabla 19**, considerando además el consumo medio y el P95. Según los resultados evaluados, más del 50% de los alimentos presentaban valores inferiores al LD o LC, cuantificándose las mayores concentraciones en la carne de caza y las algas marinas (EFSA, 2012b).

La exposición alimentaria al plomo en la población general europea se estimó en 0,68 µg/kg p.c/ día, basada en el MB. La exposición era mayor en niños pequeños y otros niños con 1,32 y 1,03 µg/kg p.c./día, respectivamente; mientras que en adultos se estimó en 0,50 µg/kg p.c./día, perfil muy similar a la población anciana. Las categorías más importante en contribución eran el pan y bollos (8.5%), té (6.2%), agua del grifo (6.1%), papas y productos

derivados (4.9%), productos lácteos fermentados (4.2%) y cerveza o bebidas similares (4.1%), aunque ello puede variar mucho entre grupos de edad y encuestas (EFSA, 2012b).

Tabla 19. Exposición dietética al Pb (µg/kg p.c./día) en consumidores de la UE según el consumo medio y el P95 en los diferentes grupos de edad.

Grupo de edad	Media			P95		
	LB[a]	MB[b]	UB[c]	LB	MB	UB
Bebés	0.73	0.81	1.09	1.39	1.80	2.22
Niños pequeños	1.10	1.32	1.54	1.95	2.28	2.56
Otros niños	0.87	1.03	1.18	1.46	1.68	1.92
Adolescentes	0.46	0.55	0.63	0.84	0.97	1.11
Adultos	0.43	0.50	0.57	0.74	0.85	0.97
Ancianos	0.42	0.48	0.55	0.72	0.82	0.92
Muy ancianos	0.40	0.47	0.53	0.71	0.79	0.89
Media ajustada	0.58	0.68	0.78	1.02	1.17	1.33

LB[a]: a los resultados inferiores al LC se consideran 0. MB[b]: a los resultados inferiores al LC se les asigna la mitad de dicho límite. UB[c]: a los resultados inferiores al LC se les asigna el valor de dicho límite.

Justificación y Objetivos

JUSTIFICACIÓN Y OBJETIVOS

Como hemos visto, los COP constituyen un conjunto de sustancias químicas que comparten cuatro características básicas: (1) son tóxicos para la salud animal y humana y contaminan el medio ambiente; (2) son "orgánicos" por tener carbono en su estructura química, lo que los hace ser solubles en grasas y permite que se bioacumulen y biomagnifiquen a lo largo de la cadena alimentaria; (3) son persistentes ya que duran años o décadas en degradarse; y (4) pueden desplazarse a grandes distancias. Además de los COP, también preocupan, por comportarse de forma muy similar, algunos compuestos inorgánicos presentes de forma natural en el ambiente, pero que debido a las actividades humanas han sido concentrados cientos de miles de veces en determinadas regiones del planeta. Compuestos de arsénico, cadmio o mercurio se comportan en este sentido como los COP, ya que también resultan tóxicos para los seres vivos a dosis muy bajas, pueden comportarse como disruptores endocrinos y son capaces de distribuirse por el medioambiente e incorporarse a los seres vivos a través de la cadena alimentaria. Es por esto que se habla de Contaminantes Toxicos Persistentes (CTP) cuando se incluyen tanto a los compuestos orgánicos como a los inorgánicos. Así, los efectos crónicos de estos compuestos incluyen cáncer, problemas reproductivos, alteración del sistema inmunológico y neurológico, etc.

Por tanto, estos contaminantes aparecen en forma de residuos en los alimentos, que constituyen la principal fuente de exposición de la población general a CTP. Debido a su lipofilicidad, destaca su concentración en los alimentos de origen animal, como lácteos, carnes, huevos y pescados. Asimismo, existe una gran variabilidad de los niveles de estos contaminantes en cada región, hecho que depende de numerosos factores. En consecuencia, es fundamental la monitorización de estas sustancias en los alimentos para conocer sus niveles y la exposición de los consumidores de los diferentes grupos de edad mediante su ingesta en los diferentes territorios. Por ello, para una correcta evaluación del riesgo, dicha estimación se realiza en el contexto de una encuesta laboral, de nutrición o de salud. En nuestro caso, hemos utilizado las encuestas ENCA (ENCA, 1998) y ENIDE (AECOSAN, 2011) y el Modelo de dieta española para la determinación de la exposición del consumidor a sustancias químicas (AECOSAN, 2006).

Para el cálculo de estimaciones fiables de las cantidades de un compuesto específico ingerido a través de la dieta, hay que tener en cuenta tres elementos: (1) cuantificación de los niveles del compuesto en los alimentos, (2) patrón de consumo de ese alimento o grupo alimentario y (3) la integración de estos elementos para determinar la exposición.

Por todo lo expuesto, en esta Tesis Doctoral se plantean los siguientes **OBJETIVOS:**

1) **Determinación de los niveles de los contaminantes tóxicos persistentes objeto de estudio en los grupos de alimentos de origen animal seleccionados, incluyendo muestras de productos lácteos, huevos, carne y productos cárnicos y productos de la pesca, considerando diferentes métodos de producción.** Los contaminantes se clasificaron en cuatro grupos según su origen, uso y características. Así, dentro los Contaminantes Orgánicos Persistentes se clasificaron en plaguicidas organoclorados (POC), bifenilos policlorados (PCB) e hidrocarburos aromáticos policicíclicos (PAH); y en el caso de los productos de la pesca, se estudiaron también seis Contaminantes Inorgánicos Persistentes: aluminio (Al), arsénico (As), cadmio (Cd), mercurio (Hg), níquel (Ni) y plomo (Pb).

- Mediante esta cuantificación, se puede estimar el nivel actual de contaminación química en los alimentos de origen animal consumidos en Canarias y España.

2) **Estimación del nivel de exposición de la población general adulta e infantil canaria y española a contaminantes tóxicos persistentes mediante el consumo de los alimentos de origen animal de mayor consumo, utilizando para ello los datos de consumo de alimentos de la Encuesta Nutricional de Canarias (ENCA) y la Agencia Española de Consumo, Seguridad Alimentaria y Nutrición (AECOSAN).** Para ello, se realizó el cálculo de la ingesta media diaria de cada uno de los contaminantes aportados por los alimentos de origen animal seleccionados, incluyendo productos lácteos (leche, queso, yogur), huevos, carne y productos cárnicos y productos de la pesca (pescado blanco y azul, cefalópodos y marisco), considerando en la medida de lo posible, las potenciales diferencias entre métodos de producción. Con ello se puede establecer:

- Media de contaminantes aportados por los alimentos de origen animal e ingesta media de un contaminante por grupo alimentario o alimento seleccionado en la población canaria/española.

- Alimentos que contribuyen de forma más significativa a la ingesta de contaminantes.

- Determinación de los contaminantes que aparecen con mayor frecuencia y mayor concentración, diferenciándose además según los distintos métodos de producción de alimentos.

- Comparación de los resultados, valores de ingesta y los límites legales establecidos con estudios similares de dieta total, o de aporte dietético de contaminantes a partir de alimentos de origen animal en otras poblaciones, considerando los diferentes modos de producción. Algunos ejemplos pueden ser los estudios desarrollados por la Autoridad Europea de Seguridad Alimentaria (EFSA) o estudios de nuestro país (estudio de Dieta Total de País Vasco y Cataluña) o de la Unión Europea.

3) **Estimación del riesgo asociado a la ingesta de estos contaminantes mediante los alimentos de origen animal seleccionados.**

- En el caso de los POC, relación de los niveles de ingesta de contaminantes por la población española con los valores establecidos como límites máximos de residuos (LMR), ingestas diarias admisibles (IDA) e ingestas diarias tolerables (IDT) para cada uno de los contaminantes y grupos de contaminantes considerados.

- Para los PCB, determinación de sus valores de ingesta y la consideración de sus límites legales. De forma específica para los DL-PCB, cálculo de la ingesta en forma de TEQ, valor establecido por la OMS que expresa el potencial carcinogénico de estos contaminantes, incluyendo la comparación de dichos valores con el nivel máximo recomendado por dicha organización.

- Valoración de los PAH con reconocida carcinogenicidad según la EFSA (ΣPAH8). Acorde a otros estudios de toxicidad de los PAH y para establecer si la población puede estar expuesta a riesgo carcinogénico mediante la ingesta de alimentos, se expresa la totalidad de la concentración de 7 PAH considerados como carcinógenos (c-PAH) según la USEPA. Para ello, se usa la equivalencia de toxicidad al benzo (a) pireno,

expresada como B[a]Peq, referencia utilizada en los artículos de la carne y productos cárnicos y los productos de la pesca.

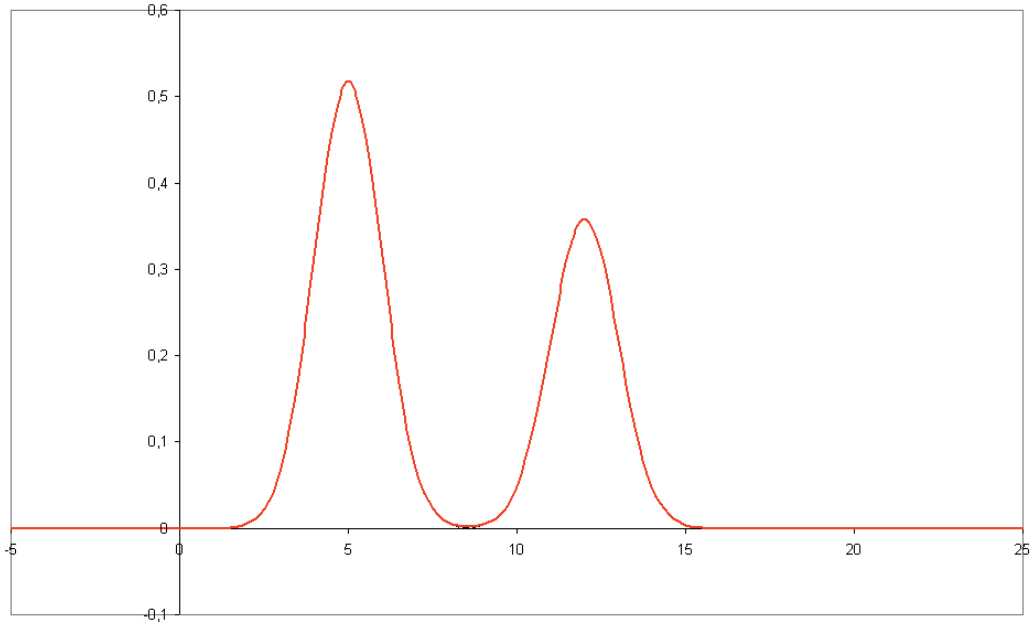
- Para la carne, los productos cárnicos y los productos de la pesca, estimación del riesgo carcinogénico mediante la exposición dietética a contaminantes con potencial carcinógeno reconocido, usando un cociente de riesgo (RQ) basado en el consumo actual y la Ingesta Máxima Tolerable (IMT) de estos alimentos. Además, para los productos de la pesca, estimación del riesgo de sufrir efectos tóxicos agudos, considerando los efectos aditivos de múltiples contaminantes. Con estas estimaciones, cálculo del consumo máximo permisible para establecer recomendaciones dietéticas que eviten superar el valor recomendado, en base al número de raciones mensuales.

- Estimación de exposición alimentaria al Al, As, Cd, Hg, Ni y Pb mediante el consumo de productos de la pesca y valoración del riesgo asociado a dicha ingesta, especialmente considerando la Ingesta Semana Tolerable (IST).

Mediante la consecución de estos objetivos se puede:

- Publicar los datos a nivel científico, estando disponibles para la comunidad científica y las Autoridades Sanitarias, así como para el conocimiento general, pudiendo ser valiosos para postular recomendaciones que minimicen la exposición de la población y permitan comprobar las medidas instaladas por los organismos internacionales en cuanto a la prohibición, restricción y eliminación de estos compuestos. Además, constituyen resultados útiles para estudios poblacionales de enfermedades relacionadas a la carga corporal de estos contaminantes, como el cáncer, la obesidad, la diabetes, trastornos reproductivos, alteraciones del sistema inmune o enfermedades neurológicas.

- Establecer medidas dietéticas para la reducción de la ingesta de contaminantes tóxicos persistentes en los seres humanos. Así, mediante los métodos analíticos actuales, se puede detectar la carga corporal de estos contaminantes y la probabilidad de una alta exposición exógena. Es decir, la exposición exógena puede ser reducida con mayor probabilidad a través de un menor consumo de los alimentos que presentan los niveles más altos de contaminantes (alimentos de riesgo), disminuyendo en consecuencia la fuente exógena para el desarrollo de enfermedades asociadas positivamente a estas sustancias, especialmente en las etapas tempranas de la vida.


0,6
0,5
0,4
0,3
0,2
0,1
0
-0,1
-5
0
5
10
15
20
25

BLOQUES

Dado que el trabajo realizado durante estos años ha dado lugar a la preparación de siete manuscritos para su publicación en revistas científicas internacionales, hemos decidido presentar los resultados de esta Tesis Doctoral manteniendo la estructura de dichas publicaciones. No obstante, para facilitar su lectura y contextualización, hemos preparado para cada bloque específico un resumen individual que recoge los principales resultados.

De esta manera, las publicaciones de esta Tesis Doctoral se dividen en cuatro bloques diferentes: productos lácteos (con un total de 2 publicaciones), huevos (1 publicación), carnes y productos cárnicos (2 publicaciones) y productos de la pesca (2 publicaciones).

La publicación dedicada al estudio de la exposición a contaminantes organoclorados mediante el consumo de marcas de leche de producción convencional y ecológica (que se encuadraría dentro de los productos lácteos), se recoge en el Anexo I, ya que el doctorando de esta Tesis Doctoral no se incluye como autor de la misma, pero se ha realizado por nuestro grupo de investigación y guarda relación directa con el contenido de esta Tesis Doctoral. Pasamos a describir cada uno de los bloques y a la presentación de cada uno de los artículos.

Bloque A Productos Lácteos

RESUMEN

En este bloque se presentan las publicaciones destinadas a la evaluación de contaminantes tóxicos ubicuos en el grupo alimentario de mayor consumo después de las bebidas (sin incluir la leche) en la población española, los productos lácteos. Este consumo se cifra en 304 mL-g/persona/día (AECOSAN, 2011), el cual queda principalmente representado por la leche y sus dos derivados principales, el queso y el yogur. En el año 2014, el último informe elaborado por el Ministerio de Agricultura, Alimentación y Medio Ambiente (MAGRAMA), cifró el consumo doméstico de productos lácteos en una cifra casi idéntica, unos 307 g-mL/persona/año (MAGRAMA, 2015).

El principal objetivo de estas publicaciones era estimar la ingesta diaria de estos contaminantes por la población canaria/española a través del consumo de lácteos y considerar si pueden ser una fuente relevante de exposición y tener impacto en dicha población.

En el artículo dedicado a la leche y presentado en el **Anexo I**, se monitorizaron los niveles de 21 pesticidas organoclorados y 18 bifenilos policlorados de importancia toxicológica en 16 marcas de leche de producción convencional y en 10 de producción ecológica comercializadas en Canarias, y se estimó el nivel de exposición en niños y adultos de las Islas Canarias a través del consumo de leche de ambos tipos de producción. Nuestros resultados muestran que el hexaclorobenceno, el trans-clordano y el PCB153 estaban presentes en todas las muestras, independientemente del método de producción considerado. Para ambos tipos de leche, la concentración de POC era muy baja, mostrando las marcas de leche ecológica valores más bajos que las de leche convencional. Con respecto a los PCB, los niveles detectados eran bajos, pero en este caso eran mayores en la leche de producción ecológica. De forma inesperada, los niveles de PCB similares a las dioxinas (DL-PCB) llegaron a alcanzar valores de 25 pg OMS-TEQ/g grasa en el percentil 75 en ambos tipos de productivos, indicando la existencia de un número de marcas de leche altamente contaminadas por estas sustancias.

En el estudio centrado en el queso se evaluaron los niveles de los mismos analitos (POC y PCB) que en el caso de la leche, en un total de 61 marcas comerciales de queso (54 convencionales y 7 ecológicas), para estimar su relevancia como fuente de compuestos organoclorados. Los resultados muestran que el HCB, α-HCH, dieldrín, p,p'-DDE y los PCB 153 y 180 se detectaban en la mayoría de las muestras, independientemente del tipo de producción. Al igual que para la leche, los valores de concentración para los POC y PCB eran bajos y se repetía el mismo patrón de contaminación, donde los niveles de POC eran mayores en quesos de producción convencional y los de PCB en las de producción ecológica. Al igual que para la leche, considerando el percentil 75, se superó con creces el límite de 3 pg OMS-TEQ/g grasa establecido por la Unión Europea.

En el caso del yogur, además de los POC y PCB, también se evaluaron los niveles de 16 hidrocarburos aromáticos policíclicos (PAH) en 17 marcas comerciales de yogur convencionales y 15 ecológicas. Por una parte, con respecto a los contaminantes organoclorados (POC y PCB), nuestros resultados muestran que el HCB, el p,p'-DDE y todos los M-PCB se detectaron en casi todas las muestras de yogur. En relación a los POC, los valores de concentración cuantificados eran menores en yogures ecológicos, y en cambio para los PCB eran muy similares. Es destacable que en un 35% de las muestras de producción convencional y en un 20% de producción ecológica, se sobrepasó el nivel máximo actual para los DL-PCB (2.5 pg OMS-TEQ/g grasa). Por otra parte, con respecto a los PAH, aunque los valores detectados eran bajos, los compuestos carcinogénicos y mutagénicos benzo[k]fluoranteno, benzo[b]fluoranteno y criseno se detectaron a alta frecuencia.

A partir de estos resultados, hemos estimado que la ingesta diaria de estos contaminantes es en general baja, presentando las ingestas diarias estimadas (IDE) valores inferiores a las ingestas diarias tolerables (IDT) y límites establecidos por las autoridades correspondientes, aunque se demuestra que existe una fuente de exposición activa. Sin embargo, se debe reseñar que si los consumidores eligen inadvertidamente ciertas marcas de leche, queso y yogur, podrían estar expuestos a altas cantidades de DL-PCB, lo que conlleva que las IDE pueden ser incluso más altas que la IDT recomendada por las Autoridades Europeas (2 pg OMS-TEQ/Kg p.c./día). Estos resultados son preocupantes si consideramos los afectos adversos en la salud relacionados a los compuestos análogos a las dioxinas.

No obstante, estos resultados deben tratarse con precaución, ya que existen marcas altamente contaminadas por organoclorados o con niveles indetectables de estos tóxicos. Además, en las encuestas y modelos de exposición alimentaria utilizados no se especifica el consumo de lácteos ecológicos, por lo que no se puede asumir el mismo patrón de consumo entre los consumidores ecológicos. Los resultados se pueden extrapolar a toda la población española, ya que las marcas adquiridas, exceptuando algunas de producción local, provienen en su mayoría de la península u otros países europeos y, por lo tanto, pueden ser considerados por las Autoridades pertinentes en Salud Pública y Seguridad Alimentaria.

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Levels of organochlorine contaminants in organic and conventional cheeses and their impact on the health of consumers: An independent study in the Canary Islands (Spain)

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ABSTRACT

In the present work we have evaluated the levels of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) in 61 commercially available brands of cheese (54 conventional and 7 organic) to estimate their relevance as a source of organochlorines. Our results showed that hexachlorobenzene, α-HCH, dieldrin, *p,p′*-DDE, and PCBs 153 and 180 were present in most of the samples independent of the cheese type. The concentration of OCPs was low for both types of cheese, although organic had lower concentrations than conventional. The estimated daily intake (EDI) of OCPs was lower than the tolerable daily intake (TDI). The levels of PCBs in cheese were also low; however, there were higher levels of PCBs in organic than in conventional brands. Levels of dioxin- like PCBs (DL-PCBs) in both types of cheese reached concentrations in the 75th percentile higher than 3 pg WHO–TEQ/g fat, and above 100% of the levels established by the EU. People consuming the most contaminated brands could have an EDI well above the recommended TDI (2 pg WHO–TEQ/kg bw/day). These results are of concern as the adverse health effects exerted by dioxin-like compounds are well known.

1. Introduction

Organochlorine contaminants include a large variety of toxic substances, such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and dioxins (PCDDs), and are characterized by the presence of halogenated atoms in their structure and their high lipophilicity which results in its ability to persist in the environment, ultimately accumulating and biomagnifying up the food chains.

Different sources of human exposure have been identified (Linares et al., 2000); however more than 90% of the human exposures to these environmental contaminants can be attributed to the consumption of contaminated food, where animal and fish products are the main sources of exposure (Liem et al., 2000). In fact, it has been reported that residues of these chemicals are found mainly in meat, fish, dairy products, and eggs (Domingo and Bocio, 2007; Llobet et al., 2003). Dairy products, because of the high fat content, are a dietary route of exposure for organochlorine compounds (Focant et al., 2002) and they supply approximately 30% of the total dietary intake with chlorinated contaminants in Western populations (Bordajandi et al., 2004; Focant et al., 2002). To monitor the presence of these substances in food, international agencies have developed food contaminant monitoring programs and total diet studies. The control of contaminants in food identified for human consumption and in animal feed are regulated through the EU Council Regulation 315/93/EEC, 1993 and Maximum Residue Levels (MRLs) have been established for the regulation of pesticides and other toxic contaminants in food (Regulation 396/2005/EC). Additionally, a tolerable daily intake (TDI) has been established by international agencies for these compounds.

The population of the Canary Islands has been studied in depth regarding its levels of contamination by OCPs and PCBs; the results show that this population has been exposed to a relatively high levels as a result of the chronic exposure to OCPs contamination by OCPs indicating the existence of a that persisted throughout the 1990's (Luzardo et al., 2006, 2009; Zumbado et al., 2005); and has been exposed to PCBs at low concentrations (Henriquez-Hernandez et al., 2011), in spite of the fact that such substances were banned in Spain in the late 1970's. As mentioned previously, the main route of exposure to organochlorines appears to be the intake of fish and dairy products (Agudo et al., 2009), and the population of the Canary Islands has been known to consume high quantities of dairy products (Serra Majem et al., 2000a,b). Additionally, we have

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recently reported that, among the consumers of dairy products from these islands, only milk consumption could account for exposures greater than the TDI for dioxin-like compounds established by the World Health Organization (WHO) (Luzardo et al., 2012). Under this scenario, and to complete the evaluation of the significance of dairy products as a source of organohalogenated contaminants exposure, the objectives of this study were to investigate the concentration levels and the patterns of organochlorine contaminants (OCPs and PCBs) in 54 of the top-selling, commercially available brands of cheese conventionally produced and 7 commercially available brands of cheese organically produced that are sold from various supermarkets in the Canary Islands (Spain), and to estimate the dietary exposure in humans of this archipelago.

2. Materials and methods

2.1. Study area

The Canary Islands are located in the Atlantic Ocean, near the African coast (southwest of Morocco). Geographically, the Islands are part of the African continent; however, from an historical, economic, political and socio-cultural point of view, the Canaries are entirely European. In the Canary Islands, as in the rest of Spain, there is a long tradition of production and consumption of the local cheeses (made from milk produced on the islands), but there is also wide distribution and consumption of cheeses from mainland Spain and other European countries.

The Canary Islands' economy relies primarily on a few economic sectors: tourism and, to a lesser extent, fishing and agriculture (where, in recent decades, agriculture has become more aggressive with an increased use in greenhouses). Other economic sectors, such as industries who contributed to pollutant emissions as traditional polluting have relatively limited presence in the Islands.

2.2. Sampling and collection

In this study, 54 commercial brands of conventionally produced cheeses were randomly selected from high delivery rate supermarkets of the Canary Islands. To investigate other possibilities, we also included 7 brands of organic cheese in the study. Samples were collected between December 2006 and April 2008. Whereas all the organic cheeses collected were produced in mainland Spain or in north European countries, 38 (70%) of the 54 conventionally produced cheeses, were locally produced.

Each of the 61 selected brands (six samples for any brand) was sampled monthly during this period of time to obtain a representative estimation for each brand and to study potential fluctuations in concentrations between different batches. The lipid content of the samples was determined in triplicate by the Gerber method with a butyrometer (with a graduation scale of 0 to 40%) to obtain the final lipid-corrected values. All samples were collected and frozen at −80 °C until analysis. The average fat content the in conventional cheese was 21%, whereas the average fat content in organic cheese was 29%.

2.3. Sample preparation and analytical procedure

The cheese stored at −80 °C was acclimated to room temperature and homogenized with 5 ml of water (previously cleaned with hexane) per gram of cheese. A total of 20 g of this homogenate was lyophilized for 72 h before removing 2 g of the lyophilized cheese for the extraction of fat, OCPs, and PCBs according to a Soxlet extraction method (FOSS Soxtec Avanti 2055) and EU recommendations (EN 1528-2, 1996). The extracts (extracted in 3 ml of dichloromethane; DCM) were purified using gel permeation chromatography (GPC), as recommended by the European Standard EN 1528-3:1996 for the determination of pesticides and polychlorinated biphenyls in fatty foods. To achieve maximum sensitivity in our analysis, two sequential cleanup steps were performed; the 3 ml fatty extract in DCM obtained by Soxlet was divided into three 1 ml aliquots that were then individually purified using GPC with a 100% Fluorinated divinylbenzene GPC column (50 cm × 10 mm i.d. EPA 3640a Pesticide Cleanup GPC Jordi column, Sorbtech Technologies, Atlanta, USA), and using DCM as the eluting solvent at a flow rate of 1.6 ml/min. This GPC system was operated using an automated apparatus (GPC-CL1, Cromlab S.L., Barcelona, Spain). The first fraction (22 ml) eluted, contained the lipids and was discarded. The second fraction (14 ml), contained the organochlorine compounds and was collected. The three organochlorines-containing fractions per sample were combined and evaporated to near dryness leaving a small amount of oily residue. The oily residue was dissolved in 1 ml DCM and subjected to GPC purification, thereby obtaining a new 14 ml organochlorine-containing fraction that was dried and diluted with cyclohexane up to a volume of 200 μl. This diluted sample was used for GC–MS analysis using the appropriate internal standards.

2.4. Analytes of interest

The following were the analytes of interest in this study: the diphenyl-aliphatic pesticides and metabolites (methoxychlor, *p,p′*-DDT, *o,p′*-DDT *p,p′*-DDE, *o,p′*-DDE, *p,p′*-DDD, and *o,p′*-DDD); the persistent and bioaccumulative contaminant hexachlorobenzene (HCB); the four isomers of hexachlorocyclohexane (α-, β-, γ-, and δ-HCH); the cyclodienes dieldrin, aldrin, endrin, heptachlor epoxide (cis- and trans-isomers), chlordane (cis- and trans-isomers) and mirex; and endosulfan (α- and β-isomers). In this study, we also included the measurement of the most relevant PCB congeners (IUPAC numbers# 28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180, and 189).

2.5. Procedure of chemical analyses

Chromatographic analysis was performed using 4a Thermo-Finnigan TRACE DSQ GC/MS instrument as previously reported (Dmitrovic and Chan, 2002; Luzardo et al., 2009). A fused silica capillary column BPX5 (Crosslinked 5% phenyl methylpolysiloxane, SGE Inc., USA) with a length of 30 m, an i.d. of 0.25 mm and a film thickness of 0.25 μm was used as the stationary phase. Helium, at a flow rate of 1 ml/min, was used as the carrier gas. The oven temperature was programmed as follows: an initial oven temperature of 80 °C held constant for 1 min, increased to 300 °C at 10 °C/min increments and then held at 300 °C for 9 min. The Injector and transfer line temperatures were set at 200 °C and 310 °C, respectively. Standards and samples were injected (2 μl) in splitless mode. Two chromatographic analyses were performed for each sample to obtain mass spectra in two different ionization modes. For DDT and metabolites, methoxychlor, endrin, and PCB congeners 28, 52, 101, and 118, mass spectra were obtained in electronic impact mode (GC/EIMS) at 70 eV, with an ion source temperature of 200 °C. For the remaining analytes included in this study, mass spectra were obtained in negative chemical ionization mode (GC/NCIMS) using methane as the buffer gas at a flow rate of 2.5 ml/min. Both GC/EIMS and GC/NCIMS analyses were conducted using selected ion monitoring (SIM). Tetrachloro-*m*-xylene was used as the nternal standard (IS) in the GC/EIMS mode, and PCB 202 as the IS in the GC/NCIMS mode. We determined the limit of quantification (LOQ) to be 10-fold the standard deviation of the blank and the limit of detection (LOD) as half of the LOQ. Nevertheless, only LOQ has been employed throughout this study (values below the LOQ have not been considered).

The MS system was routinely programmed in SIM using one target and two qualifier ions. Confirmation of the organochlorine pollutants was determined by the retention time of the target ion and the two qualifier-to-target ion ratios. The abundance of the target and qualifier ions were determined by injecting individual pollutant standards and using full scan mode (50–500 *m/z*) under the same chromatographic conditions. The qualifier-to-target ion ratio was then determined by dividing the abundance of the selected qualifier ion by that of the target ion (almost consistently the base peak) and multiplying by 100. Determination of the relative percent of the theoretical relative abundance uncertainty of the qualifier ions was conducted using the criteria described in the EU recommendations (SANCO/2007/3131). Quantification was based on the pollutant target ion/IS peak area ratio and was achieved by using linear regression: a six-point calibration curve was generated from the standard solutions ranging from LOQ of each pollutant to 10 ng/ml and by using the GC–MS Xcalibur 2.0.7 software. The standard analytes were purchased from Dr. Ehrenstorfer (Riedel-de Haën, Sigma–Aldrich Laborchemikalien GmbH, Germany).

The daily variation in the method was evaluated over 5 days using duplicates of these two different pools of spiked samples. The coefficient of variation was <20% for every case and was therefore considered acceptable.

In this study, we expressed the total value of OCP residues as the sum of the quantified 22 OCPs and its metabolites; the total value of HCH residues (∑HCHs) as the sum of the 4 HCH isomers quantified (α-, β-, γ-, and δ-HCH); and the total value of cyclodienes quantified (∑cyclodienes) as the sum of aldrin, dieldrin, endrin, cis-chlordane, trans-chlordane, and heptachlor epoxide (cis- and trans-isomers). Because the cyclodiene endosulfan was banned recently (December 2005, 2005/864/EC), we have considered this pesticide separately, expressing the total value of endosulfan residues (∑endosulfan) as the sum of the 2 quantified endosulfan isomers (α-, and β-endosulfan).

Similarly, we expressed the total value of PCB residues (∑PCBs) as the sum of the 18 PCBs quantified. Additionally, the congeners considered as markers of environmental contamination for PCBs (IUPAC congeners #28, 52, 101, 118, 138, 153, and 180) were considered as a group (∑Marker-PCBs; ∑M-PCBs), and total value of dioxin-like-PCB residues (DL-PCBs) were also expressed as the sum of the 12 DL-PCBs quantified (∑DL-PCBs; IUPAC congeners #77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189).

We also estimated the potential toxicity (in terms of toxic equivalence to dioxins; TEQs) of the DL-PCBs quantified using the toxicity equivalent factors (TEF), as revised by the World Health Organization (WHO) in 2005 (Van den Berg et al., 2006). We also expressed the total TEQ (∑TEQs) as the sum of the TEQs obtained from the quantified DL-PCBs.

2.6. Exposure assessment

Dietary intake was calculated by multiplying the respective concentration (median) by the amount of cheese consumed by an average adult from Canary

Islands (18 years and above, average weight 70.1 kg) per day. Exposures for small children (6–10 years; average weight, 30.4 kg) were also estimated.

Exposures were assessed for both OCPs and DL-PCBs. For calculations, when a congener concentration was under the limit of quantification (LOQ), the value was assumed to be 0 (lower bound approach).

Consumption data of cheese by the Islands population were obtained from the Canary Islands Nutritional Survey (Serra Majem et al., 2000a,b).

The daily intake of dioxins (pg WHO–TEQ kg^{-1} b.w d^{-1}) is equal to the occurrence (pg WHO–TEQ g^{-1} w.w.) multiplied by the consumption (g kg^{-1} b.w. d^{-1}).

2.7. Statistical analyses

Database management and statistical analyses were performed using PASW Statistics v 17.0 (SPSS Inc., Chicago, IL, USA). The OCP and PCB concentrations did not follow a normal distribution; therefore, the results are expressed in terms of the median, the 25th and 75th percentiles (p25 and p75, respectively), and range (values maximum and minimum). Differences in the OCP and PCB levels between three groups were tested with the non-parametric Mann–Whitney U-test and Kruskal Wallis test. The categorical variables are presented as percentages and were compared between variables with the Chi-square test. A *P* value of less than 0.05 (two-tail) was considered to be statistically significant.

3. Results and discussion

The concentrations of OCPs and PCBs did not show significant differences among the six collected samples from each cheese brand during the period of collection (data not shown). As a consequence, we have used the median, maximum and minimum values (range), and 25th–75th percentiles of the distribution obtained for each chemical in all samples analyzed (Tables 1a, 1b, 2a, and 2b).

3.1. Occurrence of selected OCP residues in cheese samples

The results showed that all analyzed samples (100% of the cheeses) had a quantifiable amount of OCP residues, HCB and α-HCH being the most frequently observed residues (Tables 1a and 1b). The number of OCP residues was similar in both types of cheeses; therefore, an average of 10 OCP residues per sample was measured in non-organic cheese samples (within a range of 3–14), whereas organic cheese samples had an average of 8 OCP residues per sample (within a range of 6–11).

Only the cyclodiene trans-chlordane was observed more frequently in conventional cheese brands than in the organically produced cheese brands (89% vs. 43%, respectively; $p = 0.002$). Furthermore, all the analyzed samples (both, from conventional and organic cheeses) showed, to some extent, the presence of HCH-isomer residues, although the total HCH residue level (∑HCHs) was higher in conventional than in organic cheese samples (6.64 *vs.* 1.35 ng/g fat, respectively; $p = 0.007$) (Table 1a). This result may be attributed to lindane's (γ-HCH, currently banned in Spain) recent use as an ectoparasitic agent in livestock by non-organic farmers (Botella et al., 2004) and, was, in fact found at higher levels in conventional cheeses brands than in organic cheese brands (median values of 3.24 *vs.* 0.51 ng/g fat, respectively; $p = 0.005$). Similarly, the levels of α-HCH were also higher in conventional cheeses than in samples from organically produced brands of cheese (median values of 1 *vs.* 0.22 ng/g fat, respectively; $p = 0.008$) and higher levels of cyclodiene pesticides (Σcyclodienes) in conventional cheese brands than in organic cheese brands (median value of 10.52 and 2.73 ng/g fat, respectively; $p = 0.014$). Specifically, the cyclodienes dieldrin and trans-chlordane were found at higher levels in conventionally produced cheeses than in organic cheeses (median values 6.61 *vs.* 2.64 ng/g fat, respectively; $p = 0.031$ and 2.08 *vs.* 0.00 ng/g; $p = 0.002$, respectively); however, the pesticide dieldrin was found in all the organic brands cheese samples, and in 87% of the samples from conventionally produced cheeses.

There were no differences in the levels of endosulfan residues (Σendosulfan) between both types of cheeses. Similarly, the residue levels of DDT-derivatives (ΣDDTs) in the organic cheese brands were similar compared with the levels in the conventional brands of cheese. Nevertheless, the most ubiquitous DDT-derivative, *p*, *p*′-DDE, was quantified in most cheese samples (83% in conventional and 100% in organic brands) and was the pesticide found in the highest concentration in both types of cheese.

As a consequence of the aforementioned results, the total burden of OCPs (∑OCPs), was higher in conventional than in organic brands of cheese (median values of 42.73 *vs.* 14.44 ng/g fat, respectively; $p = 0{,}001$).

Nevertheless, as shown in Tables 1a, 1b, and 3, the median levels of OCPs in both types of cheeses were found to be relatively low and consistently below the maximum residue limit (MRL) established by the European Legislation (OJEC, 1993 and 1994).

The results obtained for OCP residues in cheese are consistent with those found for milk samples (Luzardo et al., 2012), and

Table 1a
Frequency of detection (%), and average concentrations (ng/g fat) of organochlorine pesticides found in conventional and organic cheese samples from the Canary Islands market (Spain).

OC–compound	Conventional cheese ($n = 54$)				Organic cheese ($n = 7$)				p^a	p^b
	Mean ± SD	Median (p25–p75)	Range	%	Mean ± SD	Median (p25–p75)	Range	%		
HCB	6.95 ± 7.77	4.62 (1.39–10.01)	n.d.–34.63	98.0	2.27 ± 1.46	1.77 (1.38–2.45)	1.13–5.38	100.0		
HCH										
α-HCH	1.65 ± 2.64	1.00 (0.32–1.80)	n.d.–17.35	98.0	0.23 ± 0.10	0.22 (0.19–0.23)	0.11–0.45	100.0	0.008	
β-HCH	21.07 ± 62.54	1.69 (0.43–5.82)	n.d.–335.98	85.7	0.65 ± 0.58	0.35 (0.23–1.16)	n.d.–1.60	85.7	0.054	
δ-HCH	0.03 ± 0.23	0.00 (0.00–0.00)	n.d.–1.73	1.9	n.d.	n.d.	n.d.	0.0		
Lindane (γ-HCH)	8.46 ± 21.01	3.24 (1.01–6.26)	n.d.–115.25	87.0	0.53 ± 0.51	0.51 (0.00–0.89)	n.d.–1.39	71.4	0.005	
∑HCH	31.22 ± 78.99	6.64 (3.09–18.20)	0.25–349.79	100.0	1.41 ± 0.50	1.35 (1.08–1.78)	0.62–2.18	100.0	0.007	
Cyclodienes										
Aldrin	1.19 ± 3.32	0.00 (0.00–0.97)	n.d.–22.06	38.9	0.08 ± 0.14	0.00 (0.00–0.25)	n.d.–0.31	28.6		
Dieldrin	10.47 ± 13.23	6.61 (2.83–11.96)	n.d.–68.24	87.0	3.01 ± 1.32	2.64 (2.06–4.02)	1.67–5.52	100	0.031	
Endrin	n.d.	n.d.	n.d.	0.0	n.d.	n.d.	n.d.	0.0		
cis-Chlordane	2.44 ± 5.26	0.99 (0.00–2.13)	n.d.–32.41	70.4	0.62 ± 0.61	0.76 (0.00–1.12)	n.d.–1.42	57.1		
trans-Chlordane	2.39 ± 2.02	2.08 (0.46–3.27)	n.d.–7.47	88.9	0.16 ± 0.21	0.00 (0.00–0.38)	n.d.–0.47	42.9	0.002	0.002
∑Chlordanes	4.83 ± 6.72	2.89 (0.97–5.42)	n.d.–39.88	88.9	0.77 ± 0.70	1.01 (0.00–1.24)	n.d.–1.80	71.4	0.012	
Heptachlor	2.09 ± 2.97	0.18 (0.00–4.09)	n.d.–12.31	57.4	0.18 ± 0.23	0.00 (0.00–0.43)	n.d.–0.53	42.9		
∑Cyclodienes	13.75 ± 15.69	10.52 (3.35–16.61)	n.d.–81.40	88.9	3.27 ± 1.37	2.73 (2.26–4.56)	2.06–5.77	100.0	0.014	

SD: standard deviation; p25: 25th percentile; p75: 75th percentile; %: percentage of detectable samples; HCB: hexachlorobenzene; HCH: hexachlorocyclohexanes; n.d.: non-detectable.

[a] values result from the comparison between the medians (Mann–Whitney test).

[b] values result from the comparison between the frequencies of detection (Chi-square test).

Table 1b
Frequency of detection (%), and average concentrations (ng/g fat) of organochlorine pesticides found in conventional and organic cheese samples from the Canary Islands market (Spain).

OC-compound	Conventional cheese (n = 54)				Organic cheese (n = 7)				p^a	p^b
	Mean ± SD	Median (p25–p75)	Range	%	Mean ± SD	Median (p25–p75)	Range	%		
Endosulfans										
α-Endosulfan	1.18 ± 2.30	0.00 (0.00–1.94)	n.d.–14.49	46.6	0.10 ± 0.16	0.00 (0.00–0.30)	n.d.–0.37	28.6		
β-Endosulfan	0.55 ± 1.15	0.00 (0.00–0.61)	n.d.–5.19	33.3	n.d.	n.d.	n.d.	0.0		
∑Endosulfans	1.73 ± 2.68	0.22 (0.00–3.00)	n.d.–14.49	51.9	0.10 ± 0.16	0.00 (0.00–0.30)	n.d.–0.37	28.6		
Diphenyl-alyphatics										
p,p′-DDE	26.21 ± 50.65	9.99 (2.28–21.38)	n.d.–303.15	83.3	4.81 ± 3.59	5.80 (1.31–8.44)	0.81–9.05	100.0		
p,p′-DDT	0.66 ± 1.26	0.04 (0.00–0.48)	n.d.–4.80	51.9	0.10 ± 0.17	0.00 (0.00–0.33)	n.d.–0.37	28.6		
p,p′-DDD	0.04 ± 0.10	0.00 (0.00–0.00)	n.d.–0.52	18.5	0.02 ± 0.04	0.00 (0.00–0.08)	n.d.–0.09	28.6		
∑DDTs	26.91 ± 51.35	10.05 (2.36–22.03)	n.d.–306.48	83.3	4.93 ± 3.72	5.80 (1.31–8.44)	0.81–9.51	100.0		
Methoxychlor	0.32 ± 1.20	0.00 (0.00–0.00)	n.d.–7.66	11.1	0.13 ± 0.25	0.00 (0.00–0.27)	n.d.–0.65	28.6		
∑OCP residues	85.16 ± 111.7	42.73 (25.91–96.88)	3.31–502.30	100.0	12.88 ± 4.44	14.44 (6.89–15.81)	6.49–17.99	100.0	0.001	

OCP: organochlorine pesticide; SD: standard deviation; p25: 25th percentile; p75: 75th percentile; %: percentage of detectable samples; HCB: hexachlorobenzene; HCH: hexachlorocyclohexanes; n.d.: non-determined.
[a] values result from the comparison between the medians (Mann–Whitney test.
[b] values result from the comparison between the frequencies of detection (Chi-square test).

Table 2a
Frequency of detection (%) and average concentrations (ng/g fat) of PCBs residues found in conventional and organic cheese samples from the Canary Islands market (Spain).

Congeners	Conventional cheese (n = 54)				Organic cheese (n = 7)				p^a	p^b
	Mean ± SD	Median (p25–p75)	Range	%	Mean ± SD	Median (p25–p75)	Range	%		
Marker PCBs										
PCB 28	0.36 ± 1.42	0.00 (0.00–0.00)	n.d.–8.74	11.1	n.d.	n.d.	n.d.	0.0		
PCB 52	0.46 ± 1.96	0.00 (0.00–0.00)	n.d.–11.69	18.5	n.d.	n.d.	n.d.	0.0		
PCB 101	0.11 ± 0.43	0.00 (0.00–0.00)	n.d.–2.53	9.3	n.d.	n.d.	n.d.	0.0		
PCB 118	0.34 ± 1.23	0.00 (0.00–0.00)	n.d.–6.73	9.3	n.d.	n.d.	n.d.	0.0		
PCB 138	5.07 ± 8.23	0.00 (0.00–7.39)	n.d.–33.24	46.3	4.94 ± 6.26	0.00 (0.00–11.21)	n.d.–13.55	42.9		
PCB 153	5.08 ± 6.89	1.98 (0.00–7.22)	n.d.–27.16	66.7	11.15 ± 4.53	10.46 (8.81–13.01)	5.61–20.10	100.0	0.004	
PCB 180	1.40 ± 1.53	1.00 (0.00–1.95)	n.d.–7.39	74.1	2.25 ± 0.93	2.19 (1.68–2.58)	0.87–3.89	100.0	0.032	
DL-PCB (Non-ortho)										
PCB 77	0.03 ± 0.06	0.00 (0.00–0.00)	n.d.–0.22	22.2	n.d.	n.d.	n.d.	0.0		
PCB 81	0.71 ± 2.11	0.00 (0.00–0.00)	n.d.–8.27	11.1	n.d.	n.d.	n.d.	0.0		
PCB 126	0.16 ± 0.30	0.00 (0.00–0.24)	n.d.–1.38	33.3	0.39 ± 0.68	0.00 (0.00–1.16)	n.d.–1.57	28.6		
PCB 169	0.02 ± 0.05	0.00 (0.00–0.00)	n.d.–0.27	9.3	n.d.	n.d.	n.d.	0.0		
DL-PCB (Mono-ortho)										
PCB 105	n.d.	n.d.	n.d.	0.0	n.d.	n.d.	n.d.	0.0		
PCB 114	n.d.	n.d.	n.d.	0.0	n.d.	n.d.	n.d.	0.0		
PCB 118	0.34 ± 1.23	0.00 (0.00–0.00)	n.d.–6.73	9.3	n.d.	n.d.	n.d.	0.0		
PCB 123	n.d.	n.d.	n.d.	0.0	n.d.	n.d.	n.d.	0.0		
PCB 156	0.44 ± 0.53	0.24 (0.00–0.82)	n.d.–1.91	51.9	0.12 ± 0.13	0.11 (0.00–0.19)	n.d.–0.37	57.1		
PCB 157	n.d.	n.d.	n.d.	0.0	n.d.	n.d.	n.d.	0.0		
PCB 167	0.24 ± 0.39	0.00 (0.00–0.34)	n.d.–1.28	40.7	0.03 ± 0.07	0.00 (0.00–0.00)	n.d.–0.18	14.3		
PCB 189	n.d.	n.d.	n.d.	0.0	n.d.	n.d.	n.d.	0.0		

SD: standard deviation; p25: represents the 25th percentile; p75: represents the 75th percentile; %: percentage of detectable samples; n.d.: non-detectable.
[a] values result from the comparison between the medians (Mann–Whitney test).
[b] values result from the comparison between frequencies of detection (Chi-square test).

Table 2b
Frequency of detection (%) and distribution of concentrations (ng/g fat) of PCBs residues, and TEQs (pg WHO–TEQ/g fat) found in conventional and organic cheese samples from the Canary Islands market (Spain).

Congeners	Conventional cheese (n = 54)				Organic cheese (n = 7)				p^a	p^b
	Mean ± SD	Median (p25–p75)	Range	%	Mean ± SD	Median (p25–p75)	Range	%		
∑PCBs	14.42 ± 16.90	9.57 (2.38–19.33)	n.d.–77.70	98.1	18.87 ± 7.25	22.55 (13.23–24.42)	6.48–25.89	100.0	0.074	
∑M-PCBs	12.81 ± 17.27	5.83 (0.64–19.11)	n.d.–77.40	81.5	18.34 ± 7.33	20.84 (11.51–24.30)	6.48–25.70	100.0	0.049	
∑DL-PCBs	1.94 ± 2.84	1.02 (0.18–2.26)	n.d.–11.07	77.8	0.53 ± 0.81	0.11 (0.00–1.71)	0.00–1.72	57.1		
∑TEQs	10.37 ± 28.45	0.91 (0.01–2.94)	n.d.–137.68		23.33 ± 39.96	0.00 (0.00–76.19)	0.00–87.09			

SD: standard deviation; p25: represents the 25th percentile; p75: represents the 75th percentile; %: percentage of detectable samples; ΣPCBs: Sum of all PCB congeners; ΣM-PCB: Sum of Marker PCBs (IUPAC numbers 28, 52, 101, 118, 138, 153 and 180); ΣDL-PCB: Sum of Dioxin Like PCBs in pg/g fat (IUPAC numbers 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189); ΣTEQs: Sum of TEQs for DL-PCBs in pg/g fat; n.d.: non-detectable.
[a] values result from the comparison between the medians (Mann–Whitney test).
[b] values result from the comparison between frequencies of detection (Chi-square test).

indicate that although in recent years there has been an evident decline in residue levels of these pollutants world-wide, these persistent pesticides remain present in the environment and are therefore present in the food chain; several decades may pass

Table 3
Tolerable daily intake (TDI) and maximum residue levels (MRL) of organochlorine pesticides (OCPs) and dioxin-like PCBs (DL-PCBs) in dairy products established by the European Authorities.

Contaminants	TDI (mg/kg b.w.)	MRL (mg/kg fat)
Aldrin and Dieldrin	0.0001	0.006
∑Chlordanes	0.0005	0.002
∑DDT	0.01	0.04
∑Endosulfans	0.006	0.05
Endrina	0.0002	0.0008
Heptachlor	0.0001	0.004
HCB	NA	0.01
α-HCH	NA	0.004
β-HCH	NA	0.003
γ-HCH (lindane)	0.005	0.001
Methoxychlor	0.1	0.01
∑DL-PCBs	2×10^{-9}	3×10^{-6}

TDI: Tolerable daily intake; MRL: maximum residue level; NA: not available; DL-PCBs: dioxin-like PCBs; Data related to OCPs were obtained from Regulation (EC) No 299/2008 (EU), and data related to DL-PCBs were obtained from Regulation (EC) No 466/2001 (EU).

before the residue levels are undetectable in food items. Nonetheless, geographical and regulatory variations in the use and restriction of OCPs around the world may explain the differences in residual levels of these chemicals, based on of different patterns of use and exposure routes.

Although OCPs are currently banned in Spain for use on agricultural practices, they have been used in vast quantities on vegetable crops in this archipelago over the last decades (as the result of the introduction of intensive agriculture in the Canary Islands) and even they have been used as ectoparasitic agents on domestic animals and livestock (Botella et al., 2004). This misuse of OCPs and the geological features of islands, may have caused heavy contamination of the soil and water (Allen et al., 1997; Díaz-Díaz et al., 1999). Additionally, a number of OCPs are currently used in the neighboring State of Morocco; therefore, some deposition of OCPs onto Archipelago soils via volatilization and atmospheric transport from Morocco may occur (Rapaport et al., 1985; Fries, 1995). In this context, milk producing livestock may be environmentally exposed to OCPs. Because 70% of the conventionally produced cheeses analyzed throughout this study are locally produced, the existence of active environmental sources of OCPs may explain the higher levels of OCPs in conventional cheese compared with organic brands. Further studies are required to evaluate this possibility.

3.2. Occurrence of selected PCB residues in cheese samples

We found that 100% of the cheese samples (both conventionally and organically produced brands) showed quantifiable levels of PCBs (Tables 2a and 2b). The number of PCB residues in both types of cheese was similar thus, an average of 4 PCB congeners per sample (range 1–8) were found in conventional brands, while an average of 3 PCB congeners (range 2–6) were detected in organic brands. Five congeners (105, 114, 123, 157 and 189) were not detected in any brand of cheese, and the most frequently detected congeners in our samples were PCBs 153 and 180. PCBs 28, 52, 101, and 118 were detected only in samples from conventional brands of cheese. All analyzed cheeses showed residue concentrations above the LOQ of some of the congeners which are considered to be indicators of environmental contamination by PCBs, (marker PCBs; M-PCBs) (Table 2a).

The total concentration of PCB residues (Σ-PCBs; Table 2b) was higher in organic than in conventional cheeses (although such differences were not statistically significant; 22.55 *vs.* 9.57 ng/g fat, respectively; $p = 0.07$). This result appear to be a consequence of M-PCB levels (ΣM-PCBs) being higher in organic than in conventional cheeses (median values of 20.84 *vs.* 5.83 ng/g fat, respectively; $p = 0.049$; Table 2b). The M-PCBs most frequently detected were congeners 153 and 180, (detected in 100% of the analyzed organic cheese samples, and 67% in the case of PCB 153 and 74% in the case of PCB 180 in conventional cheeses). Additionally, concentrations of congeners 153 and 180 were higher in organic than in conventional brands of cheese (p values of 0.004 and 0.032 respectively).

There were not significant differences between the both types of cheeses in the DL-PCB levels (Table 2a). Among the 12 DL-PCBs analyzed for in the samples, only congeners 105, 114, 123, 157 and 189 were not detected in any sample. In contrast, the most frequently DL-PCBs detected were the congeners 156 (52 and 57% in conventional and organic cheese samples, respectively), 167 (14 and 41% in organic and conventional cheese samples, respectively), and 126 (29 and 33% in organic and conventional cheese samples, respectively). Nevertheless, as shown in Tables 2a, 2b, and 3, the median levels of DL-PCBs in both types of cheeses were observed to be relatively low and were consistently below the maximum residue limit (MRL) established by the European Legislation (OJEC, 1993 and 1994).

Our results demonstrate that commercially available brands of cheese present in the Canary Islands market showed measurable levels of various PCBs and the results are consistent with the presence of these organochlorine contaminants found in milk brands available in the Canary Islands market (Luzardo et al., 2012) and with those data published by other authors (Durand et al., 2008; Focant et al., 2002; Marin et al., 2011; Windal et al., 2010) who detected measurable levels of PCBs in milk and dairy samples (including cheese) from France, Belgium, and mainland Spain. We, however, found a different profile of contamination by PCBs as compared with data describing dairy products from mainland Spain (Marin et al., 2011). For example, Marin et al. (2011) reported that PCB 118 was the most abundant congener followed by PCBs 105 and 156; however, in our study we found that PCBs 180 and 153 were the congeners most frequently measured (present in 100% of the organic brands and 67 and 74% of the conventional brands), whereas that PCB 105 was not measured in any sample, and PCB 118 was measured only in 9% of the conventional brands. The congener 156, however, was measured in more than 50% of both types of cheese (conventional and organic). Approximately 63% of the analyzed samples were from cheeses locally produced, which may explain such differences in levels of PCB residues among both studies. The environmental PCB contamination level in these islands (where the presence of traditional pollution via industries is scarce) is assumed to be relatively low.

As shown in Table 2b, median values of TEQ–PCBs in conventionally and organically-produced cheeses were very low (0.9 and 0.0 pg WHO–TEQ/g fat, for conventional and organic cheeses, respectively), and well below the levels established by International Agencies; therefore, a maximum concentration of 6 pg WHO–TEQ/g fat has been defined for the sum of PCDDs, PCDFs and DL-PCBs in food, and the action level for DL-PCBs is 3 pg WHO–TEQ/g fat for milk and dairy products (Fattore et al., 2006; Recommendation 2006/88/EC). Most contaminated cheeses (those included in the 75th percentile), however, were observed to have concentrations as high as 76 pg WHO–TEQ/g fat in organically produced cheese and 3 pg WHO–TEQ/g fat in conventionally produced cheese.

Our data suggesting that cheese could be a significant source of dioxin-like toxicants for the population under study (and to a greater extent with those cheeses organically produced), agree with the results described in commercially available brands milk from the Canary Islands (Luzardo et al., 2012). The origin of the organic cheese may explain these results: most brands of organic cheese available in the Canary Islands market are produced mainly

in industrialized European countries (Holland, Belgium, Germany), where the level of environmental contamination by PCBs is potentially high (Covaci et al., 2002a,b); however, as cited previously, most of the conventional brands of cheese analyzed throughout this study were locally produced.

3.3. Assessment of cheese-related dietary exposure of the population of the Canary Islands to OCP residues and dioxin-like PCBs

As previously published (Luzardo et al., 2012) the population of the Canary Islands may have milk-related estimated daily intakes (EDI) of certain organochlorine contaminants (specifically, DL-PCBs) well above the recommended Tolerable Daily Intake (TDI) established by European Union Authorities (2 pg WHO–TEQ kg^{-1} - b.w. d^{-1}). Additionally, this population shows the highest consumption rates of milk and dairy products in Europe (ENCA, 1998; Serra Majem et al., 2000a,b) thus, a mean value of 26.1 g and 20.6 g of cheese are consumed daily by the adult population and children of the archipelago (respectively). Considering the mean body weight of adults (18–75 years) as 70.1 kg, and that of children (6–10 years) as 30.4 kg (ENCA, 1998), we have calculated the cheese-related EDI for the organohalogenated contaminants measured throughout this study using the deterministic method for chronic exposure as previously published (Dorne, 2010).

As expected, the cheese-related EDI of OCPs for adults and children living in the Canary Islands are low in comparison with the TDI established by the European Food Safety Authority (EFSA) (2002) (Tables 3, 4a, and 4b). Nevertheless we have to consider that the population of the Canary Islands has been subjected to a chronic exposure to OCPs that persisted in the late 1990's (Henriquez-Hernandez et al., 2011; Luzardo et al., 2006; Zumbado et al., 2005) up to the early 2000's current century (Luzardo et al., 2009). It is well known that the main route of exposure to organochorinated contaminants is through the dietary intake diet (Bordajandi et al., 2004; Darnerud et al., 2006; Hanaoka et al., 2002), and that milk and dairy products could be a relevant source of these environmental contaminants in the general population (Focant et al., 2002). Under this premise and considering the concentrations (and the intake) of OCPs present in cheese in addition to those concentrations reported previously from milk (Luzardo et al., 2012), the possibility exists that this population may be subject to a high dietary exposure of OCPs. Because many OCPs have endocrine- and metabolic-disrupting properties (mainly, DDT, aldrin or dieldrin, and its metabolites), they have been linked to environmentally induced diseases, such as obesity, diabetes and cancer (Everett et al., 2007; Soto et al., 1995; Snedeker, 2001; Wolff et al., 2000; Holtcamp, 2012). Consequently, the existence of active sources of OCPs for this population, especially children and pregnant women, should not be overlooked.

As shown in Table 2b, TEQ levels were similar in organic and in conventional cheese samples. In any case it should be highlighted the existence of huge differences in the Total TEQ levels among the cheese samples analyzed, thus, while the lowest contaminated samples showed low levels of DL-PCBs (nearly 0 or undetected pg WHO–TEQ/g fat for organic and conventional cheeses), the most contaminated brands of cheese (those included in the 75th percentile) reached levels as high as 76 (organically produced brands of cheese) or 3 pg WHO–TEQ/g fat (conventionally produced brands of cheese), (Table 2b). Ultimately, only the daily consumption of cheese could account for approximately half of the TDI established by International Agencies (Table 5) for the adult population consuming the most contaminated brands of conventionally produced cheese, while for adults consuming organically produced brands the percentage could reach as high as 125% of the TDI. Children who consume the most contaminated brands of cheese are in a worse situation (Table 5).

These results are extremely worrisome and further studies required to clarify the origin of cheese contamination, especially if we consider that the average contribution of DL-PCBs from dairy products to the total TEQ, account for no more than 60% (Durand et al., 2008), and that, fish and fishery products are the main contributors of PCBs to the diet (Fattore et al., 2006; Llobet et al., 2008; Marin et al., 2011). The toxicity of dioxins and dioxin-like compounds is related to the amount accumulated in the body during a lifetime. Toxicological properties of DL-PCBs are similar to those characteristic of polychlorodibenzodioxins (PCDDs) and polychlorodibenzofurans (PCDFs) (Van den Berg et al., 2006) and certain evidence suggests that even low doses of DL-PCBs, similar to those found in the background contamination of food, can cause subtle effects during prolonged exposure, especially in children's

Table 4a
Cheese-related estimated daily intakes (EDI) of organochlorine pesticides in adults (26.10 g cheese/day) and children (20.60 g cheese/day) from the Canary Islands, and percentage (%) that it means in relation to the tolerable daily intakes (TDI) established by International Agencies.

	Adults (10–75 years)						Children (>10 years)					
	Conventional cheese			Organic cheese			Conventional cheese			Organic cheese		
	Mean ng/g fat	EDI ng/kg bw	%TDI[a]	Mean ng/g fat	EDI ng/kg bw	%TDI[a]	Mean ng/g fat	EDI ng/kg bw	%TDI[a]	Mean ng/g fat	EDI ng/kg bw	%TDI[a]
HCB	6.95	0.55		2.27	0.25		6.95	0.99		2.27	0.45	
HCH												
α-HCH	1.65	0.13		0.23	0.02		1.65	0.23		0.23	0.05	
β-HCH	21.07	1.66		0.65	0.05		21.07	3.00		0.65	0.13	
δ-HCH	0.03	0.00		n.d.	n.d.		0.03	0.00		n.d.	n.d.	
Lindane	8.46	0.66	0.00	0.53	0.04	0.00	8.46	1.20	0.02	0.53	0.10	0.00
∑HCH	31.22	2.45		1.41	0.11		31.22	4.44		1.41	0.28	
Cyclodienes												
Aldrin	1.19	0.09	0.01	0.08	0.01	0.00	1.19	0.17	0.17	0.08	0.02	0.02
Dieldrin	10.47	0.82	0.95	3.01	0.24	0.27	10.47	1.49	1.49	3.01	0.59	0.59
Endrin	n.d.	n.d.		n.d.	n.d.		n.d.	n.d.		n.d.	n.d.	
cis-Chlordane	2.44	0.19		0.62	0.05		2.44	0.35		0.62	0.12	
trans-Chlordane	2.39	0.19	0.06	0.16	0.01	0.00	2.39	0.34	0.07	0.16	0.03	0.01
∑Chlordanes	4.83	0.38	0.02	0.77	0.06	0.00	4.83	0.69	0.14	0.77	0.15	0.03
Heptachlor	2.09	0.16	0.16	0.18	0.01	0.01	2.09	0.30	0.30	0.18	0.04	0.04
∑Cyclodienes	13.75	1.08	0.27	3.27	0.26	0.06	13.75	1.96	0.39	3.27	0.64	0.13

n.d.: non-detectable.
[a] Percentage of TDI provided by the cheese-associated EDI for these populations.

Table 4b
Cheese-associated estimated daily intakes (EDI) of organochlorine pesticides in adults (26.10 g cheese/day) and children (20.60 g cheese/day) from the Canary Islands, and percentage (%) that it means in relation to the tolerable daily intakes (TDI) established by International Agencies.

	Adults (10–75 years)						Children (>10 years)					
	Conventional cheese			Organic cheese			Conventional cheese			Organic cheese		
	Mean ng/g fat	EDI ng/kg bw	%TDI[a]	Mean ng/g fat	EDI ng/kg bw	%TDI[a]	Mean ng/g fat	EDI ng/kg bw	%TDI[a]	Mean ng/g fat	EDI ng/kg bw	%TDI[a]
Endosulfans												
α-Endosulfan	1.18	0.09	0.01	0.10	0.01	0.00	1.18	0.17	0.00	0.10	0.02	0.00
β-Endosulfan	0.55	0.04		n.d.	n.d.		0.55	0.08		n.d.	n.d.	
∑Endosulfans	1.73	0.14	0.03	0.10	0.01	0.00	1.73	0.25	0.00	0.10	0.02	0.00
Diphenyl-alyphatics												
4,4-DDE	26.21	2.06	2.10	4.81	0.38	0.39	26.21	3.73	0.04	4.81	0.95	0.01
4,4-DDT	0.66	0.05		0.10	0.01		0.66	0.09		0.10	0.02	
4,4-DDD	0.04	0.00		0.02	0.00		0.04	0.01		0.02	0.00	
Methoxychlor	0.32	0.03	0.01	0.13	0.01	0.00	0.32	0.05	0.00	0.13	0.03	0.00
∑DDTs	26.91	2.11	2.11	4.93	0.39	0.39	26.91	3.83	0.04	4.93	0.97	0.01
∑OC-compounds	85.16	6.69		12.88	1.01		85.16	12.12		12.88	2.53	

n.d.: non-detectable.
[a] Percentage of TDI provided by the cheese-associated EDI for these populations.

Table 5
Cheese-associated estimated daily intakes (EDI) of TEQ–PCBs (pg WHO–TEQ/kg bw/day) in adults (26.10 g cheese/day) and children (20.60 g cheese/day) from the Canary Islands and percentage (%) that it means in relation to the tolerable daily intakes (TDI) established by International Agencies.

	Adults (10–75 years)						Children (>10 years)					
	Conventional cheese			Organic cheese			Conventional cheese			Organic cheese		
	Mean pg/g fat	EDI pg/kg bw	%TDI[a]	Mean pg/g fat	EDI pg/kg bw	%TDI[a]	Mean pg/g fat	EDI pg/kg bw	%TDI[a]	Mean pg/g fat	EDI pg/kg bw	%TDI[a]
TEQ–PCB 77	0.03	0.00	0.12	n.d.			0.03	0.00	0.21	n.d.		
TEQ–PCB 81	0.61	0.05	2.38	n.d.			0.61	0.09	4.34	n.d.		
TEQ–PCB 105	n.d.			n.d.			n.d.			n.d.		
TEQ–PCB 114	n.d.			n.d.			n.d.			n.d.		
TEQ–PCB 118	0.12	0.01	0.47	n.d.			0.12	0.02	0.85	n.d.		
TEQ–PCB 123	n.d.			n.d.			n.d.			n.d.		
TEQ–PCB 126	8.87	0.69	34.68	23.32	2.52	125.90	8.87	1.26	63.11	23.32	4.58	229.13
TEQ–PCB 156	0.38	0.03	1.49	0.00	0.00	0.02	0.38	0.05	2.70	0.0	0.00	0.00
TEQ–PCB 157	n.d.			n.d.			n.d.			n.d.	0.00	0.00
TEQ–PCB 167	0.22	0.02	0.86	0.00	0.00	0.00	0.22	0.03	1.57	0.00	0.00	0.00
TEQ–PCB 169	0.13	0.01	0.51	n.d.			0.13	0.02	0.92	n.d.		
TEQ–PCB 189	n.d.			n.d.			n.d.			n.d.		
∑TEQ–PCBs	10.37	0.81	40.54	23.33	2.52	125.95	10.37	1.48	73.78	23.33	4.58	229.23

n.d.: non-detectable.
[a] Percentage of TDI provided by the cheese-associated EDI for these populations.

neurological development (Park et al., 2010). For these reasons, the European Commission's Scientific Committee on Food (2001), established a TDI of 2 pg WHO–TEQ/kg bw/day for WHO–TEQ, including all food items containing PCDDs, PCDFs, and DL-PCBs (Fattore et al., 2006). The European Union, through the Scientific Committee on Food (SCF), has implemented a strategy (SCF, 2001) to reduce human exposure to toxicants (mainly dioxin-like compounds) present in food items of animal origin.

In conclusion, we have developed an independent survey in the Spanish archipelago of the Canary Islands to analyze the relevance of cheese (a dairy product frequently consumed by the population of these islands) as an active source of organochlorine contaminants and to evaluate its impact on consumers. We observed that cheese consumption could be a major exposure route for DL-PCBs and also, on a much smaller scale, for OCPs. Our results are worrisome because to the deleterious health effects have been attributed to organochlorine contaminants exposure (ATSDR, 2001; Bilau et al., 2008; Park et al., 2010; Holtcamp, 2012; Everett et al., 2007; Wolff et al., 2000); however, our results should be taken with caution because, (a) as showed in Table 1a, 1b, and 3, there are cheese brands that are highly contaminated by organochlorine contaminants, but there were a number of cheese brands that showed undetectable levels of these toxicants; and (b) in the Nutritional Survey of the Canary Islands the consumption of organic cheese was not specifically recorded, and it cannot be assumed a high consumption of cheese among consumers of organic products. Nevertheless, because all organically produced cheeses are from European countries and approximately 30% of the conventionally produced cheeses are from mainland Spain or for other European countries, our results can be extrapolated to the rest of the Spanish population, and consequently, should be considered by the Spanish Public Health Authorities.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Daily intake of anthropogenic pollutants through yogurt consumption in the Spanish population

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In the present study we have quantified the levels of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in commercially available brands of yogurt (17 conventional and 15 organic) present in the Spanish market, with the goal of estimating the daily intake of these contaminants by the Spanish population through the yogurt consumption. On the one hand, with respect to organochlorine contaminants (OCPs and PCBs) our results showed that hexachlorobenzene, *p,p′*-DDE, and all the PCBs congeners that are considered markers of environmental contamination were present in almost all the yogurt samples. The concentrations of these pollutants were found to be very low [well below the toxicological standard limits established by the European Union (http://ec.europa.eu/sanco_pesticides/public/?event=homepage)] in almost all the samples, but were even lower in organic than in conventionally produced yogurts. It is remarkable that in some of the samples (six conventional and three organic yogurts) the current maximum level for dioxin-like PCBs (2.5pg WHO-TEQ/g^{-1} fat) was exceeded. On the other hand, with respect to PAHs, the mutagenic and carcinogenic compounds benzo[k]fluoranthene, benzo[b]fluoranthene and chrysene were frequently detected in yogurt. From these results we have estimated that the daily intake of these pollutants is in general low. However, it should be highlighted that if the consumer inadvertently choose certain yogurts they could be exposed to high amounts of certain pollutants, that could be even higher than the tolerated daily intakes established by the European Union.

Keywords: organochlorine pesticides; polychlorinated biphenyls; polycyclic aromatic hydrocarbons; yogurt; organic yogurt; exposure assessment

1. Introduction

Anthropogenic contaminants include a high number of chemicals that have been recognized as important toxic environmental pollutants. Among them persistent organic pollutants (POPs) are organic compounds characterized by their stable structures and lipophilicity that are resistant to the degradation in the environment and biota. Over the last 30 years a number of these substances have been highlighted as a cause for concern (Dorgan et al. 1999; Ribas-Fito et al. 2001; Samanta et al. 2002; Knerr & Schrenk 2006; Valerón et al. 2009; Casals-Casas & Desvergne 2011; Dickerson et al. 2011; Boada et al. 2012) and have been the subject of extensive study and international regulation. Among them the halogenated hydrocarbons such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are especially relevant. Besides, although polycyclic aromatic hydrocarbons (PAHs) cannot be strictly considered POPs because they are efficiently metabolized, due to their lipophilicity and continuous emission to the environment, they are frequently classified as POPs and studied together with other pollutants of this group.

These substances often accumulate and magnify up to the food chain, particularly in fat sources, and it is well accepted that food consumption is the main source of non-occupational human exposures to these contaminants. Thus, the ingestion of food contributes more than 90% of total human exposure (Baars et al. 2004; Darnerud et al. 2006; Polder et al. 2010), and several studies have reported that dairy products, because of their high-fat content, are a dietary route of POPs and supply around 30% of the total dietary intake of these contaminants in Western populations (Focant et al. 2002; Almeida-González et al. 2012; Luzardo et al. 2012; Luzardo, Rodriguez-Hernandez, et al. 2013).

In the Spanish population, milk and dairy products are the second group of food consumption after non-alcoholic drinks (AECOSAN 2006, 2011). Thus, according to the most recent nutritional survey in Spain, 79.1% of the population consumes dairy products every day, with an average consumption of 304 g/day, which is one of the highest of the Western countries. In relation to yogurt, the average consumption is estimated in 53 g/b.w./day, representing 17.43% of the dairy products' intake (AECOSAN 2011).

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Because of the previous reasons the monitoring of these anthropogenic pollutants in yogurt is justified. Thus, in the present study, 32 brands of yogurt [17 conventionally produced and 15 from organic production (EC 2007)] from the Spanish market were purchased during 2013 and screened for the presence of 57 anthropogenic pollutants, including 23 OCPs, 18 PCBs and 16 PAHs. The double goal of this study was to determine the possible differences in POPs' concentrations between the two types of production of yogurt (conventional *vs.* organic), and to estimate the daily dietary exposure of the Spanish population to these substances through the yogurt consumption. To our knowledge, this is the first study to compare the intake of contaminants depending on the mode of production of yogurt. We believe that the results of such studies are very useful for health authorities and consumers, allowing the consumer to choose those products containing a lower burden of environmental pollutants.

2. Materials and methods

2.1. Sampling and collection

In this study, 32 commercial brands of yogurt (17 from conventional production and 15 from organic production) were randomly purchased between April and November 2013 from supermarkets and also stores specialized in organic food of the Canary Islands (Spain). In the Canary Islands, in spite of the fact that there some brands of yogurt which are locally produced, the great majority of the available brands are also marketed throughout the country, and therefore the sampling of this study can be considered as representative of the Spanish market. Each of the 32 selected brands was sampled by triplicate during this period of time. The three samples of each brand were pooled to homogenize the potential fluctuations in the concentrations of pollutants. The fat contents given in the label by the manufacturer were used to obtain the final lipid-corrected values (average fat content 4.63% for conventional yogurt and 4.45% for organic yogurt). All the yogurt samples were frozen at −80°C until analysis.

2.2. Chemicals and reagents

Dichloromethane, n-hexane, ethyl acetate and cyclohexane were of the highest purity available (>99.9%) and purchased from Fisher Scientific (Leicestershire, UK). Ultrapure water was produced from a Milli-Q Gradient A10 (Millipore, Molsheim, France). Diatomaceous earth was purchased from Sigma-Aldrich (St. Louis, USA). Bio-Beads SX-3 were purchased from BioRad Laboratories (Hercules, USA). Standards of OCPs, PCB congeners and internal standards (ISs, PCB 202, *p,p′*-DDE-d8 phenanthrene-d10, tetrachloro-m-xylene, and heptachlor epoxide cis) were purchased from Dr Ehrenstorfer, Reference Materials (Augsburg, Germany). Standards of PAHs were purchased from Absolute Standards, Inc (Connecticut, USA). All standards were neat compounds (purity from 97% to 99.5%). Stock solutions of each compound at 1 mg/mL were prepared in cyclohexane and stored at −20°C. Diluted solutions from 0.05 ng/mL to 100 ng/mL were used for calibration curves.

2.3. Analytes of interest

A total of 57 analytes belonging to three relevant groups of POPs were selected for this study. The 23 OCPs and metabolites included were the diphenyl-aliphatics (*p,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD, *o,p′*-DDE, *o,p′*-DDT, *o,p′*-DDD, dicofol and methoxychlor); the persistent and bioaccumulative contaminant hexachlorobenzene (HCB); the four isomers of hexachlorocyclohexane (*α*-, *β*-, *δ*- and *γ*-HCH); the cyclodienes heptachlor, dieldrin, aldrin and endrin, chlordane (cis- and trans-isomers) and mirex; endosulfan (*α*- and *β*-isomers) and endosulfan sulphate. With respect to PCBs we decided to include a total of 18 congeners: the dioxin-like congeners (DL-PCBs, IUPAC numbers# 77, 81, 105, 114, 118,123, 126, 156, 157, 167, 169 and 189), and the six congeners that are considered markers of environmental contamination (M-PCBs, IUPAC numbers# 28, 52, 101, 138, 153 and 180). Finally, we also included in the suite of analytes the list of the 16 US-EPA priority PAHs that is often targeted for measurement in environmental samples (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene and benzo[ghi]perylene).

2.4. Sample preparation and analytical procedure

The yogurt samples, stored at −80°C, were defrozen at room temperature and homogenized by brands. A total of 50 g of this homogenate was lyophilized for 72 h. Six grams of lyophilized yogurt were spiked with the ISs mixture and used for the extraction OCPs, PCBs and PAHs, following the method of extraction and clean-up recommended by the European Standard for the determination of pesticides and PCBs in fatty foods (EN 1996a, 1996b). The validity of this method has been previously proven in our laboratory for fatty samples (Almeida-González et al. 2012; Luzardo et al. 2012; Luzardo, Rodriguez-Hernandez, et al. 2013, 2014; García-Álvarez et al. 2014). This method gives acceptable recoveries that ranged between 78.5% and 103.2%. Briefly, for the fat extraction we used a Soxtec™ 2055 Auto Fat Extraction (Foss® Analytical, Hillerød, Denmark) apparatus, which consisted of an extraction unit, a

control unit and a drive unit. The samples were placed into the extraction unit, 20 ml of dichloromethane was added to each of the extraction cups in a closed system and the cups were heated using an electric heating plate. The three-step extraction consisted of boiling, rinsing and solvent recovery. The solvent was evaporated in a rotary evaporator (Hei-VAP Advantage™, Heidolph Instruments®, Schwabach, Germany) at 40ºC to prevent analyte losses. The results were calculated as the total amount of fat (g) per 100 g yogurt. Using a precision balance, the fat obtained was carefully weighted into a zeroed glass tube. The weighted fat was dissolved in 2 ml of cyclohexane/ethyl acetate (1:1) and subjected to purification by gel permeation chromatography (GPC; Bio-Beads) using cyclohexane/ethyl acetate (1:1) at a constant flow of 2 ml/min as the eluent. The first 25-minute elution volume, which contained the great majority of the lipids (>98%), was discarded. The 25–85 minute elution volume (120 ml), which contained all the analytes that were co-extracted with the fat, was collected. The sample was concentrated using a rotary evaporator, and finally, the solvent was evaporated to dryness under a gentle nitrogen stream. The residue was then reconstituted in 1 mL cyclohexane the sample was transferred to a gas chromatography (GC) vial that was used for the chromatographic analysis. The amount of pollutants per gram of fat was obtained multiplying by the correspondent correction factor. No additional clean-up steps were needed, and thus the 1 mL-extracts in cyclohexane obtained after the GPC were used for the detection and quantification by GC/triple quadrupole mass spectrometry analysis.

2.5. Procedure of chemical analysis

GC analyses of 57 compounds, plus ISs were performed in a single run on a Thermo Trace GC Ultra equipped with a TriPlus Autosampler and coupled to a Triple Quadrupole Mass Spectrometer Quantum XL (Thermo Fisher Scientific Inc., Waltham, MA, USA), using appropriate ISs as previously described (Camacho et al. 2012; Luzardo, Rodriguez-Hernandez, et al. 2013, Luzardo, Ruiz-Suarez, 2013, 2014; Luzardo, Ruiz-Suarez, et al. 2013). Briefly, for the chromatographic separation we used a 30 m × 0.25-mm i.d., 0.25 µm film thickness column (BPX5, SGE Inc., Austin, TX, USA) as the stationary phase. Helium (99.999%) was used as the carrier gas at a constant flow of 1 ml/min. The 61-min oven temperature programme was: 60°C held for 1 min, ramped to 210°C at 12°C/min and then to 320°C at 8°C/min and held for 6 min. The injector temperature was set at 270ºC and the transfer line was heated to 310°C. The injection volume was 1 µl in the splitless mode.

The GC was tandem-coupled to a TSQ XLS QqQ mass spectrometer, which was used for the detection and quantification of the 57 pollutants plus ISs. An electron ionization (EI)-MS/MS library was specially created for the target analytes under our experimental conditions. We calibrated the mass spectrometer scale with perfluorotributylamine on a weekly basis to ensure an optimal response over time and proper mass assignments. The instrument control, data acquisition and data analysis was performed using the Thermo Fisher Xcalibur software (Ver. 2.0.1). We constructed a timed SRM method for the simultaneous analysis of 57 pollutants plus ISs in a single run. Calibration curves contained all of the target compounds except for the ISs at each level (0.5–500 ng/mL). The operation conditions of the mass spectrometer were: electron impact ionization (70 eV) in SRM; emission current, 50 µA; ionization source temperature, 220°C; electron multiplier voltage, 1500 V; scan width, 0.15; scan time, 0.05 s; peak width, *m/z* 0.7 Da. Argon (99.99%) was used as the collision gas at 0.2 Pa.

2.6. Dietary intake estimates and calculations (exposure assessment)

The exposure assessment was calculated by multiplying the respective concentrations of contaminants in yogurts (mean values) by the amount of fat contained in the average daily yogurt consumption by adults (17–70 years old, average weight 68.5 kg, mean daily yogurt consumption 52.6 g) and children (7–12 years old, average weight 34.5 kg, mean daily yogurt consumption 64.8 g). Consumption data of yogurt by the Spanish population were obtained from the Spanish Diet Model for the Determination of the Consumer's Exposure to Chemicals (AECOSAN 2006, 2011). Exposures were assessed for all the contaminants, individually considered and also grouped in different forms. For calculations, we estimated on the one hand the lower bound approach, which assigned the zero value to all those results that were below the limit of detection (LOD) of the method. On the other hand, as recommended by the European Agency for Food Safety (EFSA), the upper bound approach was also calculated. In this approach a value equal to the LOD is assigned to all the non-detected results.

In this research we expressed the total value of OCPs residues (ΣOCPs) as the sum of the 23 measured OCPs and metabolites; the total value of DDTs (∑DDTs) as the sum of the measured values of *p,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD, *o,p′*-DDE, *o,p′*-DDT, *o,p′*-DDD; the total value of HCH residues (ΣHCH) as the sum of the four HCH isomers (*α*-, *β*-, *δ*-, and *γ*-HCH); and the total value of cyclodiene residues (∑cyclodienes) as the sum of aldrin, dieldrin, endrin, cis-chlordane, trans-chlordane and heptachlor. Similarly we expressed the total value of PCB residues (∑PCBs) as the sum of the 18 PCB congeners measured. In addition the six congeners considered as

markers of environmental contamination by PCBs (#28, 52, 101, 138, 153 and 180) were also considered as a group (∑M-PCBs), and total value of dioxin-like PCBs (∑DL-PCBs) was also considered as the sum of the measurements of the 12 individual dioxin-like congeners (#77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189). Additionally, we estimated the potential toxicity (in terms of toxic equivalence to dioxins; dioxin toxic equivalents [TEQs]) for the DL-PCBs using the toxic equivalency factors as revised by the World Health Organization (WHO) in 2005 (Van den Berg et al. 2006). We expressed the total TEQs (∑TEQs) as the sum of TEQs individually obtained from the DL-PCBs. With regard to PAHs we considered the sum of the values of the 16 US-EPA compounds included in this study as the total content of PAHs (∑PAHs). Finally, according to the EFSA recommendations (EFSA 2008), we also considered as a group the sum of the eight compounds for which there are evidences of carcinogenicity (∑PAH8): benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, chrysene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene.

2.7. Statistical analyses

Database management and statistical analyses were performed using PASW Statistics v. 19.0 (SPSS Inc., Chicago, USA). The concentration of the contaminants included in this study did not follow a normal distribution; therefore, the results are expressed in terms of the median, and range (values minimum and maximum). Differences of contaminants among groups were tested with the non-parametric Mann–Whitney *U*-test and Kruskal Wallis test. The categorical variables are presented as percentages and were compared between variables with the Chi-square test. A P value of less than 0.05 (two-tailed) was considered to be statistically significant.

2.8. Quality control

The recoveries of the 57 analytes and surrogates were acceptable with this method since in all the cases were above of 72%. All the individual measurements were corrected by the recovery efficiency for each analyte. All the measurements were done in triplicate and the values used for calculations were the mean of the three data. In each batch of samples, two controls were included every 12 samples: a reagent blank consisting on a vial containing only cyclohexane; and an internal laboratory quality control (QC) consisting on melted butter spiked at 20 ng g^{-1} of each of the analytes, which was processed with the same method than the samples. The batch analyses were considered valid when the values of the analytes in the QC were within a 10% of deviation of the theoretical value.

3. Results and discussion

3.1. Occurrence of OCP residues in yogurt samples

Our results showed that 100% of the samples presented quantifiable amounts of OCP residues. The average number of residues per sample from this group of pollutants was four OCPs, regardless the production method (within a range 2–7 residues in conventional and 2–6 residues in organic yogurts).

Fourteen of the analyzed OCPs (α-HCH, δ-HCH, *o,p′*-DDE, methoxychlor, heptachlor, endrin, aldrin, heptachlor epoxide, trans-chlordane, alpha endosulfan, beta endosulfan, sulfate endosulfan and mirex) were not detected in any of the samples.

The most frequently detected compound of this group of pollutants was *p,p′*-DDE, which was found in 100% of the samples (median = 6.17 ng g^{-1} fat in conventional samples *vs.* 2.43 ng g^{-1} fat in organic yogurts, $P < 0.05$), whereas its parent compound *p,p′*-DDT (banned in 1978) was also frequently detected: six samples of conventional yogurt (35.3%), and seven samples from organic production (46.7%; Table 1). These results are in agreement with other studies performed in dairy products and yogurt in China, Jordan or USA, where also *p,p′*-DDE was the most predominant OCP (Zhang et al. 2006; Salem et al. 2009; Schecter et al. 2010). The ∑DDT was higher in the group of conventional yogurts, although no statistical significance difference was reached.

With regard to the rest of OCP residues that were detected in yogurt samples it is remarkable that HCB was also frequently detected in yogurt samples of both methods of production (88.2% of conventional and 93.3% of organic yogurts). This is not a surprising result since HCB is a known by-product of many industrial processes (Nasir et al. 1998) that is frequently detected in foodstuffs. The high frequency of this compound in yogurt samples was also in accordance with our previous studies in other dairy products (Almeida-González et al. 2012; Luzardo et al. 2012). Hexachlorocyclohexane (β- and γ-HCH) isomers were also detected with a similar distribution between organic and conventional yogurts (median values of 0.59 *vs.* 0.74 ng g^{-1} fat, respectively). These concentrations were lower than those reported in other studies in dairy products, where the average values ranged between 1.2 ng g^{-1} and 12.8 ng g^{-1} (Zhang et al. 2006; Salem et al. 2009; Polder et al. 2010; Tornkvist et al. 2011; Gutiérrez et al. 2012; Weiss et al. 2013). We also found residues of dicofol in some of the samples (29.4% of conventional brands and 13.3% of organics), as also described for bovine or human breast milk from Brazil, China, Korea and Japan (Fujii et al. 2011; Avancini et al. 2013). Finally, with

Table 1. Levels of OCPs detected in conventional and organic yogurt samples (ng g^{-1} fat) from the Spanish market.

	CONVENTIONAL YOGURT			ORGANIC YOGURT			
No. of OCPs residues per sample	Mean ± SD 4.47 ± 1.46			Mean ± SD 4.13 ± 1.25			*P* NS
Compound	Median	Detection range	Frequency	Median	Detection range	Frequency	P^a/P^b
HCB	1.07	n.d.–4.01	88.2%	0.94	n.d–4.25	93.3%	NS
p,p′-DDE	6.17	1.49–26.65	100%	2.43	1.27–20.05	100%	0.047/NS
p,p′-DDT	0	n.d.–33.19	35.3%	0	0.00–33.79	46.7	NS
p,p′-DDD	0	n.d.–1.18	11.8%	n.d.	n.d.	n.d.	NS
o,p′-DDE	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	NS
o,p′-DDT	0	n.d.–0.8	11.8%	0	n.d.–0.65	20%	NS
o,p′-DDD	n.d.	n.d.	n.d.	0	n.d.–0.19	13.3%	NS
∑DDTs	7.63	1.66–53.01	100%	5.65	1.33–38.66	100%	NS
Dicofol	0	n.d.–5.11	29.4%	0	n.d.–4.65	13.3%	NS
Mirex	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	NS
Metoxychlor	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	NS
α-HCH	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	NS
B + *γ*-HCH	0.59	n.d.–16.41	82.4%	0.74	n.d.–1.82	80%	NS
δ-HCH	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	NS
∑HCH	0.59	n.d.–16.41	82.4%	0.74	n.d.–1.82	80%	NS
Aldrin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	NS
Dieldrin	7.98	n.d.–30.58	70.6%	0	n.d.–16.28	40%	0.083/0.079
Endrin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chlordane (trans)	0	0.00–4.32	17.6%	0	0.00–40.52	6.7%	NS
Chlordane (cis)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	NS
Heptachlor	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	NS
∑cyclodienes	7.98	n.d.–30.58	70.6%	0	n.d.–56.79	40%	0.083/0.079
∑endosulfan	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	NS
∑OCPs	23.85	3.66–85.59	100%	13.62	2.16–69.70	100%	0.062/NS

[a]Values resulting from the comparison between the medians, referred to the figures with an asteric (Mann-Whitney test).
[b]Values resulting from the comparison between the frequencies, referred to the figures with an asteric (Chi-square test).
n.d., non-detectable.

respect to cyclodienes, the only detected compound was dieldrin. The presence of this contaminant in dairy products has been also reported by other authors (Darko & Acquaah 2008; Schecter et al. 2010; Almeida-González et al. 2012; Luzardo et al. 2012). However it is remarkable that both, dieldrin frequency and median concentration were statistically different between conventional and organic yogurts (Table 1). Finally, it should be noted that, unlike other reports for dairy products (Darko & Acquaah 2008; Weiss et al. 2013), none of the isomers of endosulfan were detected.

From these results we can conclude that the total burden of OCPs (∑OCPs) was higher in conventionally produced yogurts than in organically produced yogurts (median values 23.85 *vs.* 13.62 ng g^{-1} fat, respectively). However, the median levels of the OCPs quantified in this series of samples were relatively low and all the quantified amounts were well below the maximum residue levels (MRLs) established by the European legislation (Table 2). However, our results indicate that, although in recent years there has been an evident decline in these residue levels, these pesticides remain present in the environment and therefore present in the food chain. It is a known fact that the levels of contamination by POPs of the different areas may greatly vary, depending on factors, such as the geographical and regulatory variations in their use and restrictions. This fact explains the differences of levels and patterns between the different studies.

3.2. Occurrence of PCB residues in yogurt samples

We found that all the yogurt samples showed quantifiable levels of PCBs. The number of PCB residues in both production methods was similar (conventional yogurt, average = 9 PCB congeners per sample, range 8–12; organic yogurt, average = 8 PCB congeners per sample, range 7–11). As shown in Table 3 the total content of PCBs of these samples was also very similar between both groups (organic *vs.* conventional), but some differences were found when we studied the content of M-PCBs and DL-PCBs separately.

Table 2. Comparison between the median concentrations determined in the yogurt samples and the legal limits established by the European Authorities.

Compound	MRL (mg/kg fat)	Conventional yogurt	Organic yogurt
OCPs[a]			
Aldrin and dieldrin	0.006	0.00037	0
∑Chlordanes	0.002	0	0
∑DDT	0.04	0.00035	0.00025
∑Endosulfans	0.05	n.d.	n.d.
Endrin	0.0008	n.d.	n.d.
Heptachlor	0.004	n.d.	n.d.
HCB	0.01	0.000049	0.000042
α-HCH	0.004	n.d.	n.d.
γ-HCH (lindane)	0.001	0.000027	0.000033
Methoxychlor	0.01	n.d.	n.d.
PCBs[b]			
EQT DL-PCBs-OMS	2.5 (pg/g fat)	0.16	0.08
∑M-PCBs	40 (ng/g fat)	1255	13.21
PAHs[c]			
Benzo(a)pyrene	1.0 (µg/kg fresh product)	0	0
∑PAH4	1.0 (µg/kg fresh product)	0.05	0

[a]Maximum residue levels (MRL) related to OCPs were obtained from Commission Regulation (EC) No 299/2008 (EU).
[b]Maximum levels related to DL-PCBs and M-PCBs were obtained from Commission Regulation (EC) No 1259/2011 (EU).
[c]Maximum levels related PAHs: benzo[a]pyrene and PAH4 (sum of benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene and chrysene) were obtained from Commission Regulation (EC) No 835/2011 (EU).

Firstly, in relation to the six M-PCBs, all the congeners were detected in all the samples, with the exception of PCB 101 that was below the LOD in two samples. M_PCBs accounted for more than 90% of the total content of PCBs in these samples. The similarity between groups of yogurt was maintained in M-PCBs, being the concentrations and frequencies similar in conventional and organic yogurts (around 12 ng g^{-1}, Table 3). These values were also similar to those values reported by EFSA and other authors in European dairy products (EFSA 2008; Polder et al. 2010; Schecter et al. 2010).

However, in relation to the more worrying congeners, the dioxin-like PCBs, we found that the median values of concentration were higher in conventional yogurts than in organic ones (2.73 ng g^{-1} fat *vs.* 1.64 ng g^{-1} fat). When we used the TEQ approach as defined by the WHO in 2006 (Van den Berg et al. 2006) the median values obtained were 0.16 pg TEQ g^{-1} fat and 0.08 pg TEQ g^{-1} fat in conventional and organic brands, respectively. These values can be considered as low, or at least much lower that those described by EFSA (2012) or other independent studies (Bordajandi et al. 2004; Malisch & Dilara 2007; Esposito et al. 2009). Although the median values of PCBs reported in our study are well below the established limits (40 ng g^{-1} fat for M-PCBs and 2.5 pg WHO-TEQ/g^{-1} fat for DL-PCBs, Table 2), it should be highlighted that the DL-PCB content of several samples widely overpassed these limits. Thus, in six samples of conventional yogurt (35.3%) we found a range from 6.7 to 116.40 pg WHO-TEQ/g^{-1} fat, and in three samples of organic yogurt (20.1%) a range from 6.7 to 36.7 WHO-TEQ/g^{-1} fat, whereas in a previous study on 1415 samples of dairy products it was reported that only 0.5% of the samples overpassed the legal limits (EFSA 2012). This is a worrisome result which suggests that yogurt could be a significant source of dioxins for the Spanish population if some of these brands are unintentionally consumed, and are in agreement with previous results of our research group in milk and cheese samples (Almeida-González et al. 2012; Luzardo et al. 2012).

3.3. Occurrence of PAH residues in yogurt samples

According to our results 100% of the yogurt samples presented contamination by any of the PAHs included in this research, without relevant differences in the number of detected compounds between conventionally and organically produced yogurts. The average number of residues was seven (range 8–12 and 7–11, in conventional and organic yogurts, respectively). Only three PAHs were not detected in any of the samples: dibenzo-[a,h]anthracene, anthracene and benzo[a]anthracene. At the opposite end phenanthrene, fluoranthene and pyrene were detected in 100% of the samples, and these compounds were also found at the highest concentrations in yogurts of both types of production, with similar median values (Table 4). Our results were in agreement with the scarce data that are currently available for PAHs

Table 3. Levels of PCBs (ng g^{-1} fat) and 2005 WHO TEQs (pg g^{-1} fat) detected in conventional and organic yogurt samples from the Spanish market.

Compound	CONVENTIONAL YOGURT			ORGANIC YOGURT			
	Mean ± SD 9.35 ± 1.37			Mean ± SD 8.87 ± 1.25			*p* NS
No. of PCBs residues per sample	Median	Detection range	Frequency	Median	Detection range	Frequency	P^a/P^b
Marker PCBs							
PCB 28	5.87	3.89–16.77	100%	5.48	2.55–12	100%	NS
PCB 52	2.03	1.60–6.13	100%	1.92	0.7–4.1	100%	NS
PCB 101	0.79	n.d.–2.82	94.1%	0.53	n.d–2.31	93.3%	NS
PCB 138	2.58	1.07–8.92	100%	2.33	0.34–9.39	100%	NS
PCB 153	1.3	0.70–4.94	100%	1.12	0.38–5.32	100%	NS
PCB 180	0.65	0.11–4.42	100%	1.03	0.19–4.08	100%	NS
∑M-PCBs	12.55	10.71–38.88	100%	13.21	4.99–36.99	100%	NS
Dioxin-like PCBs							
PCB 77	n.d.	n.d.	n.d.	0	n.d.–0.14	6.7%	NS
PCB 81	0	n.d.–0.91	41.2%	0	n.d.–0.52	33.3%	NS
PCB 105	0.76	n.d.–2.35	94.1%	0.41	n.d.–2.19	80%	NS
PCB 114	n.d.	n.d.	n.d.	0	n.d.–0.24	6.7%	NS
PCB 118	1.32	0.31–4.27	100%	0.97	0.18–4.52	100%	NS
PCB 123	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	NS
PCB 126	0	n.d.–0.63	17.6%	0	n.d.–0.36	6.7%	NS
PCB 156	0	n.d.–0.43	17.6%	0	n.d.–1.21	33.3%	NS
PCB 157	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	NS
PCB 167	0	0.00–1.1	29.4%	n.d.	n.d.	n.d.	0.030/0.022
PCB 169	0	n.d.–1.98	29.4%	0	n.d.–0.37	13.3%	NS
PCB 189	0	n.d.–2.52	11.8%	0	n.d.–0.18	6.7%	NS
∑DL-PCBs	2.73	0.66–11.12º	100%	1.64	0.24–7.13	100%	0.074/NS
$\sum TEQs_{DL\text{-}PCBs}$ (pg/g)	0.16	0.19–116.38	100%	0.08	0.007–36.32	100%	0.074/NS
∑PCBs	14.72	11.43–47.38	100%	14.86	5.64–43.91	100%	NS

[a]Values resulting from the comparison between the medians, referred to the figures with an asteric (Mann-Whitney test).
[b]Values resulting from the comparison between the frequencies, referred to the figures with an asteric (Chi-square test).
n.d., non-detectable.

content in dairy products (Bordajandi et al. 2004; Falco et al. 2003; Kim et al. 2008; Çok et al. 2012; Veyrand et al. 2013) or in similar matrices such as human breast milk (Kim et al. 2008; Çok et al. 2012).

We considered very interesting to focus our study on the group of eight PAHs that have been signalled by the EFSA as the most worrying (PAH8; (benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, chrysene, dibenzo[a,h]anthracene and indeno[1,2,3-c,d]pyrene) due to their mutagenic and carcinogenic properties (EFSA 2008). Thus, we found that benzo[a]pyrene (a group I-carcinogen according the International Agency of Research of Cancer) was detected in 5.9% of conventional yogurts and in 13.3% of organic samples. Other contaminants from this subgroup were also detected with a relatively high frequency: benzo[k]fluoranthene, benzo[b]fluoranthene and chrysene (Table 4), which appeared with similar frequencies between samples of both production methods. High concentrations of chrysene and benzo[b]fluoranthene were also detected in the foodstuffs that were analyzed in the second French Total Diet Study (Veyrand et al. 2013). However, the results that we report in this study (Table 4) are higher than those from the above-mentioned study (Veyrand et al. 2013), and also than those reported by the EFSA (2008). Nevertheless, it should be noted that the median levels of PAHs in yogurt did not exceed the legal limits (Table 2), as also occurred with the other groups of pollutants of this study.

3.4. Assessment of yogurt-related dietary intakes of OCPs, PCBs and PAHs residues

Dietary exposure calculations are done by combining data on consumption habits with the concentrations of contaminants in food samples. In Table 5 we have summarized the dietary intakes of all the substances included in this study arranged by two groups of age: children (6–10 years) and adults (18–64 years), and considering two possible scenarios: (1) the consumers choose yogurts from conventional production, and (2)

Table 4. Levels of PAHs (ng g^{-1} fat) detected in conventional and organic yogurt samples from the Spanish market.

Compound	CONVENTIONAL YOGURT			ORGANIC YOGURT			
	Mean ± SD 7.12 ± 1.83			Mean ± SD 7.20 ± 2.01			*p* NS
No. of PAHs residues per sample	Median	Detection range	Frequency	Median	Detection range	Frequency	P^a/P^b
Naphthalene	0	n.d.–9.69	23.5%	0	n.d.–3.59	46.7%	NS
Acenaphthylene	5.54	n.d.–85.28	52.9%	5.71	n.d.–40.42	53.3%	NS
Acenaphthene	6.40	n.d.–55.05	94.1%	0.99	n.d.–14.72	66.7%	NS/0.047
Fluorene	2.47	n.d.–14.25	70.6%	3.66	n.d.–7.40	93.3%	NS
Phenanthrene	37.82	16.60–85.99	100%	35.65	13.47–88.87	100%	NS
Anthracene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	NS
Fluoranthene	11.69	6.48–18.60	100%	9.65	3.52–35.15	100%	NS
Pyrene	27.48	10.72–56.65	100%	30.78	8.11–144.70	100%	NS
Benzo[a]anthracene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chrysene	0	n.d.–17.52	47%	0	n.d.–20.04	33.3%	NS
Benzo[b]fluoranthene	0	n.d.–5.00	41.2%	0	n.d.–6.13	40%	NS
Benzo[k]fluoranthene	0	n.d.–1.64	47%	0	n.d.–1.62	46.7%	NS
Benzo[a]pyrene	0	n.d.–0.70	5.9%	0	n.d.–1.73	13.3%	NS
Indeno[1,2,3-cd] pyrene	0	n.d.–0.26	11.8%	0	n.d.–0.39	6.7%	NS
Dibenz[a,h]anthracene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	NS
Benzo[ghi]perylene	0	n.d.–1.46	17.6%	0	n.d.–1.67	20%	NS
∑PAH8	1.03	n.d.–24.15	64.7%	0	n.d.–29.47	46.7%	NS
∑PAHs	110.05	60.33–306.59	100%	102.21	38.28–294.69	100%	NS

[a]Values resulting from the comparison between the medians, referred to the figures with an asteric (Mann-Whitney test).
[b]Values resulting from the comparison between the frequencies, referred to the figures with an asteric (Chi-square test).
n.d., non-detectable.

the consumers choose yogurts from organic production. It is important to note that, to our knowledge, this is the first study estimating the intake of pollutants through the consumption of organic yogurt.

As regards to the intake of OCPs through yogurt, this can be considered very low in both groups of age, but there are relevant differences depending of the type of yogurt chosen. Thus, those consumer that choose organic

Table 5. Median values of dietary intakes of POPs (ng kg^{-1} b.w. day^{-1}) in relation to yogurt consumption for children and adults living in Spain and depending on the production method chosen.

	CONVENTIONAL				ORGANIC			
	Children		Adults		Children		Adults	
	Lba	Uba	Lba	Uba	Lba	Uba	Lba	Uba
OCPs								
∑HCH	0.051	0.086	0.021	0.035	0.062	0.095	0.025	0.039
∑Cyclodienes	0.692	0.818	0.284	0.336	0	0.128	0	0.053
∑DDTs	0.662	0.681	0.272	0.280	0.490	0.510	0.201	0.209
∑OCPs	2.068	2.242	0.850	0.921	1.181	1.363	0.485	0.560
PCBs								
∑M-PCBs	1.230	1.489	0.506	0.402	1.240	1.500	0.509	0.405
∑DL-PCBs	0.236	0.273	0.097	0.112	0.142	0.184	0.059	0.075
∑PCBs	1.277	1.316	0.525	0.541	1.288	1.329	0.529	0.546
$\sum TEQs_{PCBs}$[a]	0.014	0.514	0.006	0.211	0.007	0.507	0.208	0.166
PAHs								
∑PAH8	0.089	0.132	0.037	0.054	0	0.046	0	0.019
∑PAHs	9.544	9.594	3.921	3.942	8.863	8.922	3.642	3.666

[a]Values expressed in pg kg^{-1}b.w. day.
Lba, lower bound approach; Uba, upper bound approach.

yogurts are even less exposed to these contaminants (especially to DDTs and cyclodienes). This also occurred with the intake of equivalents of dioxins: the consumers of organic yogurts would consume the half of DL-PCBs than the consumers of conventionally produced ones. However, it is interesting to note that the results greatly vary depending on the approach chosen for the calculations. Thus, if we had chosen the lower bound approach we may say that the consumption of yogurt do not represent an important source of these contaminants, as our estimates of $\sum TEQs_{PCBs}$ indicate that in the highest mean value of intake (children that consume conventional yogurts= 0.017 ng kg^{-1} b.w. day^{-1}) the amount ingested would represent only 0.85% of the tolerable daily intake (TDI) of dioxins (2 pg kg^{-1} b.w.d^{-1}) recommended by the WHO (Fattore et al. 2008). However, according the recommendation of the European Commission (EFSA 2012), it is necessary to apply the upper bound approach in the calculations for the comparison with the TDI. Using this approach the intake of DL-PCBs through yogurt would represent as much as 31% of the TDI in children. Moreover, it is important to note that, independently of the approach used for the calculations, if 5 out of 32 samples (15.6%, four conventional and 1 organic yogurts) had been consumed, the TDI would be greatly overpassed, because of their high content of the most toxic congeners: PCB 126 and PCB 169 (Table 3). The intake of two of these yogurts would represent in children as much as the 608% and 503% of the TDI for dioxins. These findings are a matter of concern and further studies are needed that clarify which is the origin of this high contamination in certain yogurt samples, because some consumers could be highly exposed to dioxins through the intake of this food.

Finally, as regards to the intake of PAHs through the yogurt consumption, this can be considered as very low in the Spanish population. However, again it is remarkable that when consumers choose yogurts from organic production the intake of these pollutants is two times lower than if conventionally produced yogurts are consumed (Table 4).

Although according our estimates the intake of these anthropogenic pollutants through yogurt consumption is below the TDIs, it is necessary to emphasize that the daily intakes are based on 'acceptable' risk to human health and does not mean zero risk. If we take into account that many of these contaminants are endocrine disruptors and/or carcinogens, a completely 'safe' level cannot be established and therefore the efforts for diminishing their presence in the environment should continue until their complete elimination.

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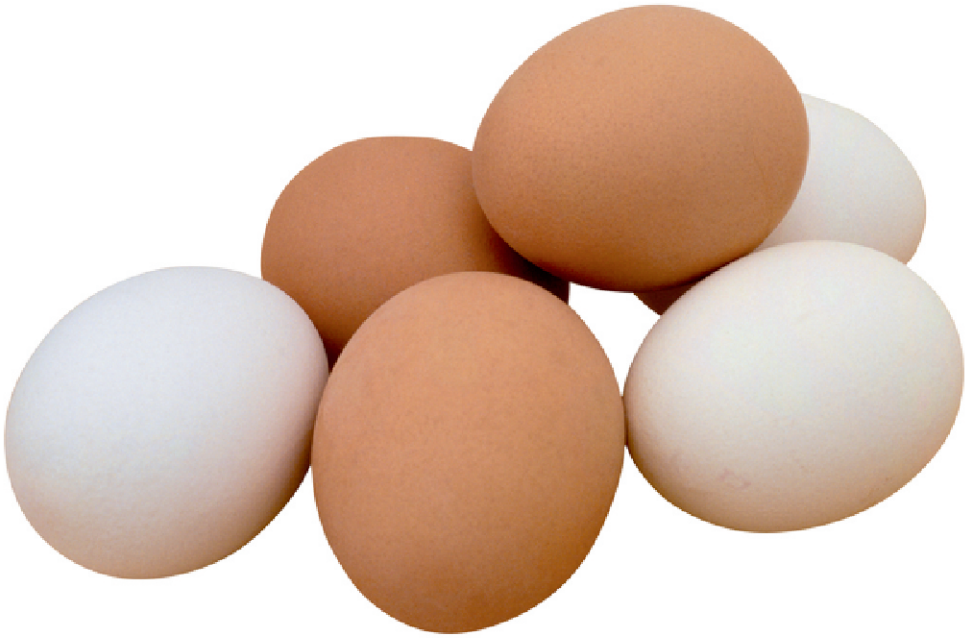
Bloque B

Huevos



RESUMEN

En este bloque se presenta el artículo destinado a la evaluación de contaminantes orgánicos persistentes mediante la ingesta de huevos. Debido al exponencial crecimiento del consumo de huevos de gallinas ponedoras producidos bajo métodos alternativos, además de huevos de producción convencional, también se incluyeron huevos de producción campera y ecológica. El consumo medio de huevos en España es de 31,4 g/persona/día y los consumidores altos (3.7% de la población) pueden llegar a consumir 120 g/día (AECOSAN, 2011). El consumo doméstico por persona en 2014 fue de 135 huevos anuales, lo que representa un consumo semanal de 2,6 huevos (MAGRAMA, 2015).

En este artículo se llevó a cabo el análisis de 16 hidrocarburos aromáticos policíclicos (PAH), 20 pesticidas organoclorados (POC) y 18 bifenilos policlorados (PCB) en 36 muestras de huevos de los tres tipos de producción (convencional, campera y ecológica) adquiridas en supermercados y tiendas de las Islas Canarias. A diferencia de otros estudios llevados a cabo en otros países europeos o en Canadá, no encontramos diferencias en el contenido de PCB o POC en los huevos en relación al tipo de producción. Los valores cuantificados fueron extremadamente bajos y se situaban muy por debajo de los límites máximos de residuos establecidos. El contenido en POC era de 3,87 ng/g grasa (valor de la mediana), siendo el dieldrín, el dicofol, el HCB, el p,p´-DDE y el p,p´-DDT los compuestos detectados con mayor frecuencia. El valor de la mediana de los PCB era 3,93 ng/g grasa, en el cual los M-PCB aportaban un 79.9%. Dos muestras, una campera y otra ecológica, sobrepasaban ampliamente el actual límite de la Comisión Europea (CE) de 2.5 pg TEQ/PCDD/F g grasa, pero el resto de muestras presentaba valores bastante inferiores a este límite. La concentración de PAH en los huevos producidos convencionalmente era casi 4 veces más alta que en huevos camperos o ecológicos, siendo además la primera vez que dicha comparativa era descrita científicamente.

La estimación de la ingesta media dietética de contaminantes organoclorados basada en el consumo de huevos, en cualquiera de los tipos de producción, es insignificante para la población canaria y podríamos considerar que la contribución de los huevos a la ingesta diaria es mínima. Sin embargo, es posible que los consumidores estén involuntariamente expuestos a altos niveles de compuestos similares a las dioxinas (DL-PCB) a través del consumo de huevos, ya que nuestros resultados muestran que la IDT recomendada se superaba en gran medida en el 5% de las muestras (con altas concentraciones de PCB 126).

Por último, es muy relevante el hallazgo de que la mediana de las estimaciones de ingesta dietética de PAH depende enormemente del tipo de producción elegido. Por tanto, los consumidores que optan por huevos de gallinas producidas en libertad, tienen un consumo significativamente menor de PAH, incluidos los considerados carcinógenos, especialmente en los niños. Por tanto, el consumo de huevos orgánicos o camperos puede ser una buena alternativa para reducir la exposición dietética a estos contaminantes cancerígenos.

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Influence of the method of production of eggs on the daily intake of polycyclic aromatic hydrocarbons and organochlorine contaminants: An independent study in the Canary Islands (Spain)

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ABSTRACT

Analysis of 16 polycyclic aromatic hydrocarbons (PAHs), 20 organochlorine pesticides (OCPs) and 18 polychlorinated biphenyls (PCBs) were performed on eggs from three different production types (conventional, free-run and organic) collected from the markets of the Canary Islands (Spain). Unlike other studies we did not found differences in the content of PCBs or OCPs of eggs in relation to its production type. Median ∑OCPs content was 3.87 ng g^{-1} fat, being dieldrin, dicofol, hexachlorobenzene, *p,p′*-DDE and *p,p′*-DDT the most frequently detected. Median ∑PCBs value was 3.93 ng g^{-1} fat, with 79.9% of this amount coming from the marker PCBs. Two samples, one free-run and one organic, greatly exceeded the current European Commission (EC) limit of 2.5 pg $TEQ_{PCDD/F}$ g^{-1} lipid, but the rest were well below of this limit. The concentrations of PAHs in conventionally produced eggs were almost 4 times higher than in free-run or organic eggs. Mean dietary intake estimates of the organochlorine contaminants based on consumption of eggs, regardless of the type chosen, is negligible for the Canary Islands' population. However, the median dietary intake estimates of PAHs greatly depend on the type of eggs chosen, being much lower when free-run and organic eggs are consumed.

1. Introduction

Persistent organic pollutants (POPs) are toxic chemicals that are resistant to degradation in the environment and biota. Over the last 30 years a number of these substances have been highlighted as a cause for concern (Boada et al., 2012; Casals-Casas and Desvergne, 2011; Dickerson et al., 2011; Dorgan et al., 1999; Knerr and Schrenk, 2006; Ribas-Fito et al., 2001; Samanta et al., 2002) and have been the subject of extensive study and international regulation. Due to their stable structure and lipophilic character POPs such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) tend to concentrate and magnify in the food chain particularly associated with fat. It is well accepted that food consumption is the main way of non-occupational human exposure to these contaminants compared to other ways such as inhalation and dermal contact. The ingestion of contaminated food contributes more than 90% to the total exposure and foodstuffs of animal origin are recognized as one of the main contributors (Almeida-Gonzalez et al., 2012; Luzardo et al., 2012; Marti-Cid et al., 2008; Mezzetta et al., 2011).

The laying hen eggs are one of the main sources of protein in human food all over the world. During the last decades in most of the countries the major production method of this food item has been by means of the housing of the hens in battery cages, without outdoor access. In parallel, in the last years the demand of eggs from alternative production methods, such as free-run production where animals have access to the barn floors, or organic production with strict regulations regarding the feed and welfare of the animals, have steadily increased in Europe (Hsu et al., 2010). The eggs produced by these alternative methods are perceived by many consumers as a better choice because of their healthier nature, better nutritional qualities and also because these production methods are more respectful with the animal wellness (Van Overmeire et al., 2006). Independently of the production method, the transfer of POPs into hen eggs, especially into yolk bound to its lipidic fraction, has been widely documented for OCPs, PCBs and PAHs (Bargar et al., 2001; Fournier et al., 2010, 2012). For this reason securing the quality of hen eggs is an important issue for human food safety, and specific studies are regularly performed in different regions of the planet to determine concentrations of POPs in this and other foods of primary interest.

Several studies have demonstrated that the production method of the eggs could influence their content in organochlorine

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contaminants. Paradoxically, some of these studies point to the possibility that organic and free-run eggs could accumulate higher quantities of dioxins (Hsu et al., 2010; Pussemier et al., 2004), PCBs, and OCPs (Van Overmeire et al., 2006; Windal et al., 2009) than conventionally produced ones, mainly linked to a higher degree of consumption of soil and worms or other insects by the hens that have outdoor access (Kijlstra et al., 2007), whereas other studies do not find such differences (Rawn et al., 2012). However, to our knowledge, comparative studies have not been performed regarding the PAHs content of eggs in relation to the production method, and how this fact could influence the consumer's intake of these contaminants.

In the present study, 12 egg composites of each of three production methods (conventionally caged laying hens, free-run, and organic) were collected in 2012 from supermarkets and stores in the Canary Islands (Spain). The goal was to determine the concentrations of OCPs, PCBs and PAHs and to establish whether relative differences in POPs concentrations occurred between the different types of eggs marketed, with the objective of estimating the exposure in humans to these contaminants through the consumption of eggs depending on the production type chosen.

2. Materials and methods

2.1. Sampling

In this study, a total of 36 composites of egg samples were processed from the same number of packages of 6 units of medium size eggs (53–63 g). The packages were randomly acquired from supermarkets and also stores specialized in organic food of the Canary Islands (Spain). We bought 12 packages of six different brands of each of the three production methods included in this study: conventionally produced eggs (battery caged hens), free-run eggs, and organic eggs. All the samples were acquired within the last week of November and the first of December 2012. 100% of the samples were locally produced. At the arrival to the laboratory the samples were immediately prepared. Yolks were separated from the whites and each sample was made up by combining the yolks of the eggs of the same package in a single composite. The whites were discarded since their fat content was unappreciable. The 36 yolk composites were frozen at −18 °C until ready for analysis.

The lipid content of the samples was determined in triplicate by the Gerber method with a butyrometer (with a graduation scale of 0–40%) to obtain the final lipid-corrected values. There were no statistically significant differences in the fat content of the composites being the average fat content of 11.6% (referred to the whole egg, 58 g average weight).

2.2. Chemicals and reagents

Dichlorometane, hexane, ethyl acetate and cyclohexane were of the highest purity available (>99.9%) and purchased from Fisher Scientific (Leicestershire, United Kingdom). Ultrapure (UP) water was produced from a Milli-Q Gradient A10 (Millipore, Molsheim, France). Diatomaceous earth was purchased from Sigma–Aldrich (St. Louis, USA). Bio-Beads SX-3 were purchased from BioRad Laboratories (Hercules, USA). Standards of OCPs, PCB congeners, the surrogates (PCB 202, *p,p′*-DDE-d8 and phenanthrene-d10) and internal standards (ISs, tetrachloro-m-xylene and heptachloro epoxide cis), were purchased from Dr Ehrenstorfer, Reference Materials (Augsburg, Germany). Standards of PAHs were purchased from Absolute Standards, Inc. (Connecticut, USA). All standards were neat compounds (purity from 97% to 99.5%). Stock solutions of each compound at 1 mg/mL were prepared in cyclohexane and stored at −20 °C. Diluted solutions from 0.05 ng/mL to 100 ng/mL were used for calibration curves.

2.3. Analytes of interest

A total of 54 analytes belonging to three relevant groups of POPs were selected for this study. The 20 OCPs and metabolites included were the diphenyl-aliphatics (methoxychlor, *p,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD and dicofol); the persistent and bioaccumulative contaminant hexachlorobenzene (HCB); the four isomers of hexachlorocyclohexane (α-, β-, δ-, and γ-HCH); the cyclodienes heptachlor, dieldrin, aldrin and endrin, chlordane (cis- and trans-isomers) and mirex; endosulfan (α- and β-isomers) and endosulfan sulfate. With respect to the PCBs we decided to include a total of 18 congeners: the dioxin-like congeners (IUPAC numbers# 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189), and the six marker congeners of non-dioxin-like PCBs (IUPAC numbers# 28, 52, 101, 138, 153 and 180). Finally, we also included in the suite of analytes the list of the 16 EPA priority PAHs that is often targeted for measurement in environmental samples (naphthalene, acenaphtylene, acenaphtene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]-anthracene, benzo[*b*]fluoranthene, benzo[k]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*c,d*]pyrene, dibenz[*a,h*]anthracene and benzo[*g,h,i*]perylene).

2.4. Extraction and clean-up procedure

Because of the known fact that the contaminants included in this study are totally lipid-soluble and therefore found in the lipid fraction of food items, we only processed the yolks of the eggs since the lipid content of the whites is extremely low, next to 0. 10 mL of the composite of yolks formed from the eggs of each package were spiked with the 10 ppm surrogates mix in acetone to yield a final concentration of 100 ppb and mixed with 30 g of diatomaceous earth to absorb all the humidity. The method of extraction and clean up followed that recommended by the European Standard for the determination of pesticides and PCBs in fatty food (EN, 1996a,b), and whose validity has been previously proven in our laboratory for fatty foods (Almeida-Gonzalez et al., 2012; Luzardo et al., 2012). This method combines an automated Soxhlet extraction method (FOSS Soxtec Avanti 2055) with a purification step using gel permeation chromatography (GPC). This method gives acceptable recoveries that ranged between 74.5% and 104.7%. There were no need of additional clean-up steps and the 1 mL-extracts in cyclohexane obtained at the end of the GPC were used for the gas chromatography/triple quadrupole mass spectrometry (GC-MS/MS) analysis.

2.5. Procedure of chemical analysis

Gas chromatography analyses of 54 contaminants, 3 surrogates and 2 ISs were performed in a single run on a Thermo Trace GC Ultra equipped with a TriPlus Autosampler and coupled to a Triple Quadrupole Mass Spectrometer Quantum XLS (Thermo Fisher Scientific Inc., Waltham, MA, USA), using appropriate internal standards (ISs) as previously described (Camacho et al., 2012, 2013). We have used as ISs a mixture of tetrachloroxylene and heptachloro epoxide cis that was prepared at 1 ppm in cyclohexane, and 20 μL were added to each 1 mL-extract, just before the GC-MS/MS analysis.

2.6. Dietary intake estimates and calculations

The exposure assessment was calculated by multiplying the respective concentrations of contaminants in eggs (median values) by the amount of fat contained in the average daily egg consumption by adults (18 years old and above, average weight 70.1 kg, mean daily egg consumption 25.1 g), youngsters (11–17 years old, average weight 54.5 kg, mean daily egg consumption 30.8 g), or children (6–10 years old, average weight 30.4 kg, mean daily egg consumption 22.6 g) from the Canary Islands. Exposures were assessed for all the contaminants, individually considered and also grouped in different forms. For calculations, when the concentration of a given contaminant was below the limit of detection (LOD), the value was assumed to be that LOD (upper bound approach). Consumption data of eggs by the population of the Canary Islands were obtained from the Canary Islands Nutritional Survey (Serra Majem et al., 2000).

In this work we expressed the total value of OCPs residues (∑OCPs) as the sum of the 20 OCPs and metabolites measured; the total value of DDTs (∑DDT) as the sum of the measured values of *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD; the total value of HCH residues (∑HCH) as the sum of the 4 HCH isomers measured (α-, β-, δ-, and γ-HCH); and the total value of cyclodienes residues (∑cyclodienes) as the sum of aldrin, dieldrin, endrin, cis-chlordane, trans-chlordane, and heptachlor. Similarly we expressed the total value of PCB residues (∑PCBs) as the sum of the 18 PCB congeners measured; in addition the six congeners considered as markers of environmental contamination by PCBs (#28, 52, 101, 138, 153 and 180) were also considered as a group (∑M-PCBs); and total value of dioxin-like PCBs (∑DL-PCBs) was also considered as the sum of the measurements of the 12 individual dioxin-like congeners (#77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189). Additionally, we estimated the potential toxicity (in terms of toxic equivalence to dioxins; TEQs) for the DL-PCBs using the toxic equivalency factors (TEF) as revised by the World Health Organization (WHO) in 2005 (Van den Berg et al., 2006). We expressed the total TEQs (∑TEQs) as the sum of TEQs individually obtained from the DL-PCBs. Finally we considered the total content of PAHs (∑PAHs) as the sum of the values of the 16 US-EPA compounds included in this study and also, following the EFSA recommendations (EFSA, 2008), the sum of the 8 compounds for which there are evidences of carcinogenicity (∑PAH8): benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*g,h,i*]perylene, chrysene; dibenz[*a,h*]anthracene; and indeno[1,2,3-*c,d*]pyrene.

2.7. Statistical analyses

Database management and statistical analyses were performed using PASW Statistics v. 20.0 (SPSS Inc., Chicago, USA). The distribution of the variables included in this study was evaluated through Kolmogorov–Smirnov test. The concentration of the contaminants included in this study did not follow a normal distribution; therefore, the results are expressed in terms of the median, and range (values minimum and maximum). Differences of contaminants among groups were tested

with the non-parametric Mann–Whitney U-test and Kruskal Wallis test. The categorical variables are presented as percentages and were compared between variables with the Chi-square test. A P value of less than 0.05 (two-tail) was considered to be statistically significant.

2.8. Quality control

The recoveries of the 54 analytes and surrogates were acceptable with this method since in all the cases were above of 72%. All the individual measurements were corrected by the recovery efficiency for each analyte. All the measurements were done in triplicate and the values used for calculations were the mean of the three data. In each batch of samples two controls were included every 12 samples: a reagent blank consisting on a vial containing only cyclohexane; and an internal laboratory quality control (QC) consisting on melted butter spiked at 20 ng g^{-1} of each of the analytes that was processed with the same method than the samples. The batch analyses were considered valid when the values of the analytes in the QC were within a 10% of deviation of the theoretical value.

3. Results and discussion

3.1. Occurrence of OCP residues in egg yolk samples

Researchers of other European countries have reported that the eggs that are produced from hens that have outdoor access (free-range, free-run, home-produced, and organic) usually exhibit higher levels of OCPs than those produced from caged hens, (Van Overmeire et al., 2006; Windal et al., 2009), and also reported a high degree of contamination by these substances, surpassing in many cases the maximum residue levels (MRLs) established for these contaminants. This situation have not been confirmed in our study, where we have found similar results on the number of residues detected, frequency and concentrations of these contaminants, with only small differences among groups. Besides, the total amount of OCPs found in our samples is much lower than those found recently in other countries in Europe (Polder et al., 2010; Windal et al., 2009), being as low as 0.95 ng g^{-1} in free-run eggs (median value, Table 1). Nevertheless, it should be taken into account that the Canary Islands, still being part of Europe, are an archipelago that is geographically located very far from the European continent, and that eggs consumed in this territory, unlike other primary food supplies, are almost 100% locally produced. For this reason our results could be reflecting a lower degree of environmental contamination by these organochlorine contaminants of this region.

In any case, as expected, our results showed that all analyzed samples (100% of the eggs) had small but quantifiable amounts of OCP residues. In all the cases the concentrations found in our study were well below the MRLs (EC, 2005) and also below of the levels published in recent studies in Europe. The number of OCP residues detected was almost the same in all three types of eggs, with an average of 7 residues per composite, but the frequency of detection was different depending on the production type. As shown in Table 1 the more frequent OCP residues in conventionally produced and free-run eggs were dieldrin, followed by dicofol and the HCH isomers β and γ, whereas in organic eggs the most frequently detected residue was *p,p′*-DDE, that was present in 83.33% of the composites of this form of producing eggs. Also the concentrations of this contaminant and its parent product, *p,p′*-DDT, were slightly higher in organic egg composites (median ∑DDT values = 0.62 ng g^{-1} fat vs. 0.38 ng g^{-1} fat and 0.22 ng g^{-1} fat), reaching in some of the samples values of around 10 ng g^{-1} fat. These higher concentrations of persistent contaminants in organic eggs is not a surprising result since some authors have pointed to the possibility that laying hens under organic production system, that spend many hours a day ranging outdoors, eating soil and soil's creatures (worms and other insects), are more exposed to environmental pollutants than those that are caged or in outdoor facilities with barn floor, where the possibility of eating soil does not exist (Van Overmeire et al., 2006; Windal et al., 2009). However, it should be highlighted that the highest values found in our study are still very far from the maximum level of 500 ng g^{-1}

Table 1
Levels of organochlorine pesticides detected in composite samples of hen egg yolks from three production methods (n = 12/each).

Compound	Conventional production			Free-run production			Organic production			P^a	P^b
	Median (ng g^{-1})	Frequency (%)	Detection range (ng g^{-1})	Median (ng g^{-1})	Frequency (%)	Detection range (ng g^{-1})	Median (ng g^{-1})	Frequency (%)	Detection range (ng g^{-1})		
Hexachlorobenzene	0.03	33.33	n.d. – 0.06	0.02	25.00	n.d. – 0.02	0.02	33.33	n.d. – 0.03		
α-HCH	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
β-HCH	0.03	50.00	n.d. – 0.07	0.03	33.33	n.d. – 0.05	0.03	33.33	n.d. – 0.09		
γ-HCH	0.06	41.67	n.d. – 0.08	0.08	16.67	n.d. – 0.09	0.04	33.33	n.d. – 0.07		
δ-HCH	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
∑HCH	0.03	66.67	n.d. – 0.13	0.04	50.00	n.d. – 0.09	0.05	50.00	n.d. – 0.12		
Heptachlor	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
Aldrin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
Dieldrin	2.46	91.67*	n.d. – 5.25	0.77*	58.33	n.d. – 1.64	2.95	58.33	n.d. – 6.39	0.0048	
Endrin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
Chlordane (trans)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
Chlordane (cis)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
∑cyclodienes	2.46	91.67*	n.d. – 5.25	0.77*	58.33	n.d. – 1.64	2.95	58.33	n.d. – 6.39	0.0048	
α-Endosulfan	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
β-Endosulfan	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
Endosulfan sulfate	n.d.	n.d.	n.d.	0.59			n.d.	n.d.	n.d.		
p.p′-DDE	0.31	25.00	n.d. – 0.38	0.38	8.33	n.d. – 0.38	0.55*	83.33*	n.d. – 9.87	0.0008	0.0013/ 0.0009
p.p′-DDD	n.d.	n.d.	n.d.	0.37	8.33	n.d. – 0.37	0.21	8.33	n.d. – 0.21		
p.p′-DDT	0.26	25	n.d. – 0.26	0.21	25	n.d. – 0.22	0.34*	58.33*	n.d. – 0.56	0.0260	0.02
∑DDT	0.38	25.00	n.d. – 0.54	0.22	25.00	n.d. – 0.96	0.62*	91.67*	n.d. – 10.22	0.0012	0.0013
Dicofol	0.93	75.00	n.d. – 8.42	0.57	41.67	n.d. – 1.08	1.07	58.33	n.d. – 2.31		
Mirex	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
Metoxychlor	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
∑OCPs	3.87	100.00	0.03 – 11.75	0.95*	100.00	0.02 – 3.29	4.97	100.00	0.28 – 10.76	0.0021	0.049

n.d.: Non-detectable.
[a] Values resulting from the comparison between the medians, referred to the figures with an asterisc (Mann–Whitney test).
[b] Values resulting from the comparison between the frequencies, referred to the figures with an asterisc (Chi-square test).

fat established by the European authorities that has been surpassed in up to 17% of the samples analyzed in other recent studies in Europe (Van Overmeire et al., 2006, 2009).

With respect to the rest of OCPs residues detected in our study, dieldrin was the only cyclodiene found, being aldrin, endrin, heptachlor and chlordanes below the detection level (LOD) of our method. Dieldrin was also the OCP residue that we found at the highest concentrations, which could be explained because in the Canary Islands this pesticide was extensively used in the past until it was banned at the beginning of the 80s. Dieldrin has been also the pesticide found at the highest concentrations in other studies performed in this archipelago (Luzardo et al., 2012, 2006). We found that the median values of this pesticide were lower in free-run eggs than in conventionally or organically produced ones (0.77 ng g^{-1} fat vs. 2.46 ng g^{-1} fat and 2.95 ng g^{-1} fat; $p = 0.0048$), but its concentrations were much lower than those found in other recent studies (Polder et al., 2010; Van Overmeire et al., 2006). A similar situation was observed for HCB residues in egg yolk composites and also the HCH isomers (Polder et al., 2010), but in these cases there were no statistically significant differences regarding the production type. Dicofol was also present in our set of samples with high frequency, and at a concentration around 1 ng g^{-1} fat, with no influence of the production mode on it. This is a residue that is not usually included in the set of analytes measured in food, at least in the studies in eggs, so we cannot compare our results with previous studies, but this pesticide has been found in wild bird eggs with similar frequency but at higher concentrations (Malik et al., 2011). The presence of this residue with high frequency maybe could be explained by the fact that it was thoroughly used in the cultivation of tropical fruits in the Canary Islands in the past, and also because this pesticide, important since it is a source of DTT, has been produced in Spain until late 2008.

3.2. Occurrence of PCB residues in egg yolk samples

Our results demonstrate that commercially available brands of eggs in the markets of the Canary Islands showed quantifiable levels of various PCBs (mean = 8), independently of the form of production employed. Total PCBs (∑18 congeners) concentrations ranged from 0.18 to 14.23 ng g^{-1} fat (median 4.26 ng g^{-1} fat) in eggs from conventional production. Unlike other studies, we did not found higher values of these contaminants in eggs from hens with outdoor access, and thus the values found in conventionally-produced eggs were similar to those found in eggs from organic production (range from 0.21 to 21.39 ng g^{-1} fat, median 3.93 ng g^{-1} fat), or even higher than those found in eggs from free-run production (range from 0.0 to 7.91 ng g^{-1} fat, median 2.07 ng g^{-1} fat) (Table 2). As also shown in Table 2, a similar pattern of contamination was observed with the ∑M-PCBs: the free-run produced eggs had the lowest levels, and no differences were found between organic and conventional production. But when we observed the individual congeners by type of production we found that the organically produced had a higher frequency of PCB congeners 52 and 101, which were not detected in none of the composites of conventionally produced or free-run eggs. This difference could be in relation with the consumption by free ranging hens with access to soil of additional feed sources, such as non-commercial feeds, worms and other insects, and soil (Rawn et al., 2012). In none of the samples levels surpassed the EC level of 40 ng g^{-1} fat for M-PCBs (EC, 2011).

It is difficult to compare the results of ∑PCBs with other studies because a different number of congeners have been measured in each of them. More comparable are the results of M-PCBs that have been homogeneously determined in different studies all over the world. When compared with the most recent series, our results

Table 2
Levels of polychlorinated biphenyls (ng g^{-1} fat) and 2005 WHO dioxin toxic equivalents (pg g^{-1} fat) detected in composite samples of hen egg yolks from three production methods ($n = 12$/each).

Compound	Conventional production			Free-run production			Organic production			P^a	P^b
	Median (ng g^{-1})	Frequency (%)	Detection range (ng g^{-1})	Median (ng g^{-1})	Frequency (%)	Detection range (ng g^{-1})	Median (ng g^{-1})	Frequency (%)	Detection range (ng g^{-1})		
Marker PCBs											
PCB 28	1.04	83.33	n.d. – 1.72	0.59*	33.33*	n.d. – 0.80	0.78	66.67	n.d. – 1.75	0.0012	0.038
PCB 52	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.64	8.33	n.d. – 0.64		
PCB 101	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.96	16.67	n.d. – 1.17		
PCB 138	0.87	33.33	n.d. – 1.04	0.90	16.67	n.d. – 0.93	0.91	25.00	n.d. – 1.12		
PCB 153	0.75	58.33	0.12 – 1.40	0.89	41.67	n.d. – 2.35	0.64	66.67	0.16 – 2.67		
PCB 180	0.72	41.67	0.14 – 1.46	0.62	33.33	n.d. – 2.19	1.16	33.33	0.11 – 1.16		
∑M-PCBs	1.85	100.00	0.18 – 5.87	0.56	91.67	n.d. – 6.01	1.22	100.00	0.21 – 6.50	0.0009	
Dioxin-like PCBs											
PCB 77	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
PCB 81	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
PCB 105	1.51	58.33	n.d. – 3.51	1.05	33.33	n.d. – 1.36	1.31	50.00	n.d. – 4.05		
PCB 114	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
PCB 118	1.65	83.33	n.d. – 5.02	1.25	66.67	n.d. – 2.28	2.01	83.33	n.d. – 10.39		
PCB 123	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
PCB 126	n.d.	n.d.	n.d.	0.56	8.33	n.d. – 0.56	0.67	8.33	n.d. – 0.67		
PCB 156	0.74	33.33	n.d. – 1.08	n.d.	n.d.	n.d.	0.53	25.00	n.d. - 0.53		
PCB 157	0.73	16.67	n.d. – 0.80	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
PCB 167	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
PCB 169	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
PCB 180	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
∑DL-PCBs	2.45	83.33	n.d. – 9.62	1.40*	75.00	n.d. – 3.50	2.40	91.67	n.d. – 14.89	0.032	
∑PCBs	4.26	100.00	0.18 – 14.23	2.07*	91.67	n.d. – 7.91	3.93	100.00	0.21 – 21.39	0.045	
$\sum TEQ_{DIOXIN\text{-}LIKE\ PCBs}$	0.07	83.33	n.d. – 0.29	0.04	75.00	n.d. – 56.58	0.09	91.67	n.d. – 67.94		

n.d.: Non-detectable.
[a] Values resulting from the comparison between the medians, referred to the figures with an asterisc (Mann–Whitney test).
[b] Values resulting from the comparison between the frequencies, referred to the figures with an asterisc (Chi-square test).

were slightly higher than those found in Canada in 2012 (Rawn et al., 2012), but slightly lower than those found in other studies done in Spanish regions: Catalonia in 2002 and 2003 (Eljarrat et al., 2002; Llobet et al., 2003), and Andalusia in 2004 (Bordajandi et al., 2004). Much more important were the differences found with studies from Central and East Europe, where the values found for these contaminants were 30-fold or higher than in our samples (Polder et al., 2010; Van Overmeire et al., 2006; Windal et al., 2009). It is a known fact that the levels of contamination by POPs of the different territories can be enormously different depending on many factors, and we have to take into account that the Canary Islands, where this study was performed, is a relatively non-industrialized region were a low level of contamination for these compounds is expected.

According to literature, background toxic equivalent quantity (TEQ) levels for dioxin-like PCBs in commercially available eggs calculated using the toxic equivalency factors (TEFs) determined in 2005 (Van den Berg et al., 2006), ranged from 0.089 to 12.8 pg TEQ g^{-1} fat (Bordajandi et al., 2004; Darnerud et al., 2006; Eljarrat et al., 2002; Llobet et al., 2003; Polder et al., 2010; Rawn et al., 2012). In our study, the results have been found to be in the lower bound of this range, being the median values of 0.07 pg TEQ g^{-1} fat, 0.04 pg TEQ g^{-1} fat and 0.09 pg TEQ g^{-1} fat for conventionally-produced, free-run and organic eggs, respectively. Nevertheless, we have to remark the worrying result that two yolk composites, one free-run and one organic, greatly exceeded the current European Commission limit of 5 pg $TEQ_{PCDD/F+DL-PCB}$ g^{-1} fat (EC, 2011) because of their high content in PCB 126, being the rest well below of this limit. This represents about a 5% of the analyzed samples in our study, which is in agreement with the reports of European researchers who have found that between 5% and 25% of the eggs produced under free-range or other alternative production types have PCDD/F and DL-PCBs that exceed the EC limits (Darnerud et al., 2006; De Vries et al., 2006).

3.3. Occurrence of PAH residues in egg yolk samples

In spite of the fact that the recent evaluation of the European Food Safety Agency (EFSA) of PAHs presence in food commodities did not included eggs (EFSA, 2008), we decided to investigate their presence in this important food supply since it has been proven that cereals, the main ingredient of the feed of laying hens, can be contaminated with important levels of these hydrocarbons (EFSA, 2008), and that an efficient transfer exist of these compounds from the food to the lipidic fraction of the eggs (Fournier et al., 2010). The same than other previous studies (Falco et al., 2003), we have included those 16 PAHs initially considered by the Environmental Protection Agency (EPA) as priority contaminants due to their potential toxicity.

Our results show that in conventionally produced egg composites 10 of 16 PAHs were present, being naphthalene, phenanthrene, fluoranthene and pyrene detectable in 100% of the samples (Table 3), reaching these compounds also the highest concentrations. In free-run and organic eggs these were also the most frequently detected compounds and also found at the highest levels, but regarding the concentrations it is interesting to note that in general the levels found in organic eggs were approximately the half or the third part than those found for the same contaminants in conventionally produced eggs, and that these levels were even lower for most of them in free-run eggs (Table 3). Nevertheless, as also can be seen in Table 3, in a high percentage of samples from these two alternative methods of producing eggs the contaminants acenaphthene and the highly carcinogenic benzo[*a*]pyrene were detected, whereas none of these two were found in conventionally produced eggs. We did not detect acenaphtylene, anthracene, indeno[1,2,3-*c*,*d*]pyrene and dibenzo[*a*,*h*]anthracene in none of the samples. In other studies these compounds have been found in egg samples but with low frequency and at the lowest concentrations of all the 16 compounds measured (Falco et al., 2003; Veyrand et al., 2013). Differences in extraction and/or analytical methods may explain such differences.

We compared the median values of ∑16PAHs obtained for the three groups and the differences found for individual compounds as regards to the production type were maintained, being more than double in conventionally produced eggs than in free-run and organic eggs (496.26 ng g^{-1} fat vs. 172.14 ng g^{-1} fat and 238.98 ng g^{-1} fat, respectively). Following the EFSA recommendations we also compared the median values of the sum of those 8 compounds for which there are evidences of carcinogenicity and genotoxicity (EFSA, 2008) and the pattern was repeated, being

Table 3
Levels of polycyclic aromatic hydrocarbons (ng g^{-1} fat) detected in composite samples of hen egg yolks from three production methods (n = 12/each).

Compound	Conventional production			Free-run production			Organic production			P^a
	Median (ng g^{-1})	Frequency (%)	Detection range (ng g^{-1})	Median (ng g^{-1})	Frequency (%)	Detection range (ng g^{-1})	Median (ng g^{-1})	Frequency (%)	Detection range (ng g^{-1})	
Naphtalene	147.49*	100.00	50.01 – 286.31	23.69	33.33	n.d. – 23.69	59.24	50.00	n.d. – 132.76	0.0008
Acenaphtylene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Acenaphthene	n.d.	n.d.	n.d.	15.95*	66.67	n.d. – 24.32	20.82*	16.67	n.d. – 21.79	0.0012
Fluorene	12.64*	50.00	n.d. – 23.87	7.96	8.33	n.d. – 7.96	6.54	8.33	n.d. – 6.54	0.018
Phenanthrene	200.80*	100.00	71.78 – 549.00	63.02	100.00	28.71 – 121.65	115.26	100.00	68.96 – 319.20	0.0009
Anthracene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Fluoranthene	79.16*	100.00	33.26 – 195.96	28.54	83.33	n.d. – 46.29	34.09	83.33	n.d. – 108.67	0.0022
Pyrene	82.05*	100.00	25.74 – 141.49	42.24	91.67	n.d. – 80.30	59.92	100.00	15.30 – 152.18	0.050
Benz[a]anthracene	36.75*	41.67	n.d. – 127.23	8.68	8.33	n.d. – 8.68	7.70	25.00	n.d. – 21.27	0.036
Chrysene	21.81	50.00	n.d. – 154.34	19.41	25.00	n.d. – 25.89	17.97	25.00	n.d. – 30.57	
Benzo[b]fluoranthene	12.51	75.00	n.d. – 32.30	9.67*	33.33	n.d. – 14.61	13.29	16.67	n.d. – 15.55	
Benzo[k]fluoranthene	12.86	58.33	n.d. – 30.08	8.42	33.33	n.d. – 11.87	12.19	16.67	n.d. – 13.42	
Benzo[a]pyrene	n.d	n.d.	n.d.	11.03*	66.67	n.d. – 13.33	10.51*	58.33	n.d. – 58.33	0.0011
Indeno[1.2.3-c.d]pyrene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Dibenzo[a.h]anthracene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Benzo[g.h.i]perylene	24.91	8.33	n.d. – 24.91	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
∑PAHs	496.29	100.00	88.91–1521.69	172.14	100.00	50.70–315.80	238.98	100.00	95.66–620.22	0.0009
∑PAH8	65.95	75.00	n.d. – 368.86	18.47	75.00	n.d. – 65.71	31.29	66.67	n.d. – 65.63	0.0007

n.d.: Non-detectable.

[a] Values resulting from the comparison between the medians, referred to the figures with an asterisc (Mann–Whitney test), in comparison with the other two groups, from left ro right.

the conventionally produced eggs those with the highest levels (65.95 ng g^{-1} fat vs. 18.47 ng g^{-1} fat and 31.29 ng g^{-1} fat for conventional, free-run and organic eggs respectively). It is necessary to take into account that in high egg consumers these estimates would be doubled. Our results are similar to others recently found (Veyrand et al., 2013) but we have to say that we can only compare our results obtained for conventionally produced eggs since no studies have been done in eggs from other alternative production methods. To our knowledge this is the first time in which the incidence of the production method of the eggs on the PAHs content is described. The factors that could explain why the conventionally produced eggs have a significantly higher level of PAHs are not known, but probably have to do with the content of the contaminants in the feed used in this production type as compared to that used in the alternative ones. Anyway, we can only speculate with this and more research should be done in order to clarify this aspect.

3.4. Dietary intakes

Dietary exposure calculations are done by combining data on consumption habits with the concentrations of contaminants found in food samples. In this study the consumption habits data used were from the Canary Islands Nutrition Survey 1998–1999 (Serra Majem et al., 2000) and the dietary intake was calculated by multiplying the concentration value of each contaminant in each of the three classes of eggs by the daily consumption of this food (in g egg fat/day), and dividing by the average weight of the people in each segment of age. In Table 4 we have summarized the dietary intakes of all the contaminants included in this study arranged by groups, for children (6–10 years), youngsters (11–17 years), and adults (>18 years), and considering three potential scenarios: (a) consumers that choose conventionally produced eggs; (b) consumers that choose eggs from free-run eggs; and (c) consumers that choose organic eggs.

Our results show that the egg-related estimated daily intake (EDI) of OCPs for people living in the Canary Islands is extremely low in all the segments of age, representing in all the cases less than 0.01% of the tolerable daily intakes (TDIs) established by the World Health Organization for these contaminants (JMPR, 2000), and the level of exposure of this population through this food is lower than estimates recently published for other European populations (Darnerud et al., 2006; Polder et al., 2010; Van Overmeire et al., 2006). Except for the case of ∑DDT no relevant differences were found as regards to the production method. Unlike other studies we have not found higher levels of exposure for most OCPs in those consumers that choose free-range eggs, and even these types of egg showed a tendency of lower intake levels in our study. In the case of DDT and its metabolites a higher level of exposure from organic eggs consumption was observed, but the highest level of exposure calculated (children = 0.054 ng kg^{-1} b.w. d^{-1}) was very far from the TDI of 1000 ng kg^{-1} b.w. d^{-1} established for this pesticide (JMPR, 2000; Lu, 1995).

As shown in Table 4, PCBs intake estimates were very similar in conventionally produced, free-run, and organic eggs. Unlike the rest of the contaminants included in this study, when we consider the intake of the most toxic compounds, the DL-PCBs, expressed in terms of equivalency to dioxin toxicity, our estimations would vary dramatically depending on the approach chosen for the calculations. If we had chosen the lower bound approach we may say that the eggs do not represent an important source for these contaminants, independently of the type of production nor the age segment. Using this approach, our estimates of $\sum TEQs_{PCBs}$ in the highest mean value of intake (children that consume organic eggs = 0.008 pg kg^{-1} b.w. d^{-1}) would represent only 0.4% of the TDI of 2 pg kg^{-1} b.w. d^{-1} (SCF, 2000). Nevertheless, as recommended by the European Commission, in the dietary intake calculations it is necessary to apply the upper bound method in order to compare with the established TDIs. In this case our estimates would be 60 times higher, and therefore the exposure through egg consumption would represent as much as 24% of TDI. This enormous difference depending on the upper/lower bound approaches represents an important limitation of this methodology in this case, and the interpretation of these estimates should be taken with caution. Therefore we have not introduced intake estimation of $\sum TEQs_{DL\text{-}PCBs}$ in Table 4. In any case, regardless of the approach used, we would like to highlight the fact that about a 5% of the samples (2 out 36 composites analyzed), if were inadvertently consumed, would have represented an important intake of dioxin-like compounds, because of their high content in PCB 126, which is in accordance to other studies where authors have estimated that eggs are an important source of dioxins and dioxin-like compounds in more of 10% of the samples analyzed (Van Overmeire et al., 2009). These results are extremely worrisome and further studies are required to clarify the origin of egg contamination, because the possibility exists that certain consumers may be subjected to a high dietary exposure to dioxins.

Table 4
Median values of dietary intakes of POPs (ng kg^{-1} b.w. day^{-1}) by means of egg consumption for adults. youngsters and children living in the Canary Islands (Spain) depending on the egg's production method chosen.

Compound	Conventional production			Free-run production			Organic production		
	Adults	Youngsters	Children	Adults	Youngsters	Children	Adults	Youngsters	Children
OCPs									
∑HCH	0.001	0.002	0.002	0.002	0.003	0.003	0.002	0.004	0.005
∑Cyclodienes	0.105	0.177	0.233	0.036	0.055	0.068	0.139	0.219	0.288
∑DDTs	0.017	0.027	0.036	0.011	0.017	0.021	0.028	0.046	0.060
∑OCPs	0.180	0.284	0.364	0.044	0.068	0.092	0.201	0.309	0.407
PCBs									
∑M-PCBs	0.127	0.200	0.262	0.037	0.060	0.081	0.083	0.130	0.173
∑DL-PCBs	0.160	0.252	0.332	0.092	0.139	0.188	0.123	0.188	0.258
∑PCBs	0.211	0.357	0.451	0.138	0.214	0.275	0.195	0.303	0.389
$\sum TEQs_{PCBs}$ [a,b]	0.003	0.005	0.006	0.002	0.003	0.004	0.004	0.006	0.008
PAHs									
∑PAH8	2.418	4.539	5.971	0.757	1.371	1.842	1.223	2.312	2.832
∑PAHs	22.672	34.81	47.074	7.506	12.073	15.734	10.719	17.075	22.255

[a] Values expressed in pg kg^{-1} b.w. day^{-1}.
[b] For the calculation of TEQs we have used the lower bound approach, because due to the high TEF values for PCB126 and PCB169, the upper bound approach would introduce an overestimation higher than 60X.

Finally, as also shown in Table 4, the daily intake of PAHs greatly depended on the type of eggs chosen. When the consumers choose eggs from free-run hens their intake of ∑PAHs is near three times lower than if conventionally produced eggs are consumed. Also the consumption of organic eggs implies an evident lower intake of these contaminants. These differences are even greater when only the carcinogenic PAHs (∑PAH8) are considered. When compared with the most recently published data, the second French total diet study (Veyrand et al., 2013), our estimations of intake for PAHs from conventional eggs were very similar to that study in adults (∑PAH8 = 2.739 vs. 2.281 ng kg^{-1} b.w. d^{-1}) but almost double in children (∑PAH8 = 5.687 vs. 3.493 ng kg^{-1} b.w. d^{-1}). This is due to the different pattern of consumption of eggs of children from both populations. Nevertheless, until this moment no estimates have been done regarding the intake of PAHs coming from eggs of alternative production methods. Therefore, this study represents the first evidence that consumption of organic eggs or eggs from hens with outdoor access can be a way to reduce the daily intake of these carcinogenic contaminants of food.

4. Conclusion

Unlike the observations in European studies and in accordance to the measurements done in Canada, OCPs and PCBs residues were not significantly higher in free-range eggs yolks. The levels of contamination by these organochlorine compounds in the 100% of the eggs of this study, all of them locally produced, were extremely low and none of the samples surpassed the MRLs established in the EU. Therefore the level of exposure of the inhabitants of the archipelago is well below the established TDIs and we could consider that the contribution of eggs to the total daily intake is negligible. However, it is possible that consumers are unwittingly exposed to high levels of dioxin-like compounds through consumption of eggs, since our results show that the TDI had been largely overcome if 5% of the samples, with high concentrations PCB 126, had been consumed. Finally, it is very interesting the finding that the consumers that choose eggs from free-ranging hens have a significantly lower intake of carcinogenic PAHs, what is very relevant, especially in children. Therefore, the consumption of organic or free-run eggs can be a good option for reducing the exposure to these carcinogenic contaminants.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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RESUMEN

En este bloque se presentan dos artículos con la finalidad de estimar la exposición a contaminantes ambientales a través del consumo de carne y productos cárnicos, cuya media de consumo por la población española es de 164 g/persona/día, siendo la más consumida la carne de ave (48 g/persona/día) (AECOSAN, 2011). El consumo de carne en los hogares en el año 2014 se cifró en 89 g/persona/día, con un consumo de carne de pollo de 40 g/persona/día, y el consumo de jamón se cifró en 10 g/persona/día (MAGRAMA, 2015).

Cabe recalcar que numerosos estudios epidemiológicos han demostrado un vínculo entre el consumo excesivo de carne y la incidencia de varios tipos de cáncer, especialmente el colorrectal, y se ha sugerido que los contaminantes tóxicos ambientales presentes en la carne pueden relacionarse a un incremento del riesgo de padecer cáncer. Sin embargo, no hay estudios que evalúen el potencial carcinogénico de la carne en relación a su contenido en carcinógenos. Además, tal y como se tuvo en cuenta con el resto de alimentos considerados, se llevó a cabo una comparación con las carnes de producción ecológica presentes en el mercado (cordero, pollo y ternera), debido al incremento de consumo de este tipo de alimentos en los últimos años, con el objetivo de comprobar si el consumo de carne de producción ecológica podría ser un medio para disminuir la ingesta de estos contaminantes persistentes frente a la de producción convencional.

Por tanto, en este bloque, además de estimar de forma general la exposición a estos contaminantes antropogénicos, nos centramos en la relevancia de estos carcinógenos ambientales presentes en estos alimentos como un factor determinante entre su consumo y el cáncer. Debido a que en Europa, España muestra un alto consumo de carne y productos cárnicos, este estudio se centró en la población española. En base a las preferencias de los consumidores, se adquirieron muestras de carne de cabra, cerdo, conejo, cordero, ternera y pollo y de productos cárnicos (incluyendo beicon, chorizo, jamón cocido, jamón serrano, mortadela y salchichón). En estas muestras, se cuantificó la concentración de 33 químicos con calculado potencial carcinogénico (PAH, POC y DL-PCB).

Se evaluó el riesgo carcinogénico mediante la exposición dietética a estos contaminantes, usando para ello un cociente de riesgo basado en el consumo actual de carne y productos cárnicos y la ingesta máxima tolerable de estos alimentos, dependiendo del nivel de carcinógenos que contienen. Se calculó también el consumo máximo permisible para poder establecer recomendaciones dietéticas en base al número de raciones mensuales, que permiten establecer niveles mediantes los cuales la población bajo estudio pueda consumir carne sin que ello conlleve riesgo carcinogénico asociado a estos compuestos.

Como se esperaba, ninguna muestra estaba completamente libre de las sustancias incluidas (detectándose una media de 19 contaminantes por alimento), y las diferencias entre carnes de ambos tipos de producción eran mínimas, no superando en ningún caso los LMR establecidos. De acuerdo a estos resultados, el patrón de consumo de carne excedía el cociente de riesgo (RQ>1), hecho que se asociaba con un relevante riesgo carcinogénico en los dos tipos de producción, excepto mediante el consumo de cabra y conejo. Este cociente también era superior al recomendado mediante el consumo de chorizo. Sorprendentemente, el consumo de carne de producción ecológica no disminuye dicho riesgo, sino que parece ser incluso superior, especialmente ligado al consumo de cordero.

Por tanto, los resultados indican que el consumo de carne de vacuno, cerdo,cordero, pollo y del producto cárnico chorizo representa un riesgo carcinogénico considerable para

los consumidores (RQ entre 1,33 y 13,98). Para reducir la continua exposición a estos carcinógenos y por tanto disminuir el riesgo carcinogénico, en adultos se debería reducir al 50-80% el consumo mensual actual de estos alimentos y en los niños no deberían sobrepasarse las 5 raciones de ternera/cerdo/pollo (consideradas juntas). Sin embargo, en ambos grupos de población se aconseja aumentar el consumo de carne de conejo y de cabra. Con respecto a los productos cárnicos, el consumo actual podría mantenerse para adultos y niños, excepto para el chorizo, cuyo consumo debería disminuirse en adultos y, especialmente, en niños.

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An estimation of the carcinogenic risk associated with the intake of multiple relevant carcinogens found in meat and charcuterie products ☆

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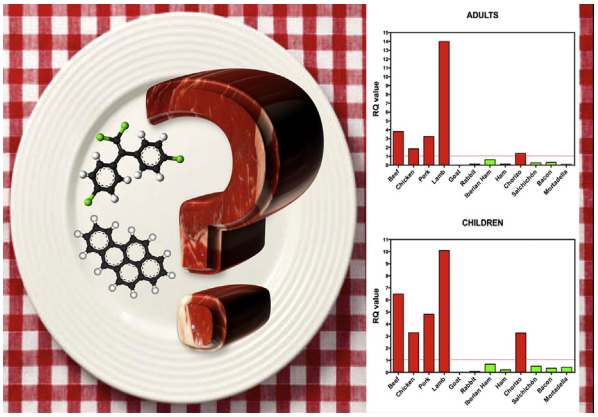
HIGHLIGHTS

- Quantification of 33 potential carcinogens in 58 meat and 42 charcuterie samples.
- Dietary intake of meat carcinogens in Spanish population ranged 0.5–293% of TDIs.
- Consumption of lamb, beef, pork, chicken, and Spanish chorizo poses carcinogenic risk.
- Recommendations of the maximum number of servings/month of each food are provided.

GRAPHICAL ABSTRACT



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ABSTRACT

Numerous epidemiological studies have demonstrated a link between excessive meat consumption and the incidence of various cancers, especially colorectal cancer, and it has been suggested that environmental carcinogens present in meat might be related to the increased risk of cancer associated with this food. However, there are no studies evaluating the carcinogenic potential of meat in relation to its content of carcinogens. Our purpose was to emphasize the relevance of environmental carcinogens existing in meat as a determinant of the association between cancer and meat consumption. Because within Europe, Spain shows high consumption of meat and charcuterie, we performed this study focusing on Spanish population. Based on the preferences of consumers we acquired 100 samples of meat and charcuterie that reflect the variety available in the European market. We quantified in these samples the concentration of 33 chemicals with calculated carcinogenic potential (PAHs, organochlorine pesticides, and dioxin-like PCBs). The carcinogenic risk of these contaminants was assessed for each food using a risk ratio based on the current consumption of meat and charcuterie and the maximum tolerable intake of these foods depending on the level of contamination by the carcinogens they contain. Our results indicate that the current consumption of beef, pork, lamb, chicken, and "chorizo", represents a relevant carcinogenic risk for

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Organochlorine pesticides

consumers (carcinogenic risk quotient between 1.33 and 13.98). In order to reduce carcinogenic risk, the study population should halve the monthly consumption of these foods, and also not to surpass the number of 5 servings of beef/pork/chicken (considered together).

1. Introduction

The consumption of certain foods of animal origin has been associated with the increased incidence of different types of cancer (Abid et al., 2014). Among them, the most clear epidemiological associations have been established with the consumption of red meat and processed meats (Abid et al., 2014; Kim et al., 2013). Thus, the consumption of red meat has been linked to an increase in total cancer mortality (Larsson and Orsini, 2014) as well as the increased incidence of colorectal cancer (Kim et al., 2013) and cancers of the esophagus (Zhu et al., 2014), liver (Freedman et al., 2010), pancreas (Pericleous et al., 2014), kidney (Alexander and Cushing, 2009), prostate (Abid et al., 2014), lung (Abid et al., 2014) and breast (Abid et al., 2014). The consumption of processed meat has also been strongly associated with an increased incidence of colorectal cancer (Aune et al., 2013; Kim et al., 2013) as well as kidney (Alexander and Cushing, 2009) and prostate (Alexander et al., 2010) cancers. According to the European cancer registries, in Spain, where this study is based, both the incidence and mortality of some of these cancers are above the average of the European Union, especially in men. This is the case for cancers of the colon and rectum, liver and lungs (Ferlay et al., 2013).

Although the food culture of Spain is contextualized within the framework of the Mediterranean diet, which is considered to be a pattern of consumption of healthy foods that protect against the development of the most common chronic diseases, including cancer (Giacosa et al., 2013), the most recent nutritional surveys show a decrease in adherence to the traditional Mediterranean diet in this country (Varela-Moreiras et al., 2013). Thus, overall meat consumption in Spain has steadily increased over the last few decades (Kanerva, 2013; Leon-Munoz et al., 2012), and currently, the meat industry is ranked in fifth position in the industrial sector of the Spanish economy and is ranked first among the agro-food industries (Chamorro et al., 2012). Thus, meat consumption in Spain has gone from being one of the lowest in the EU to reaching an average per capita consumption of 52.7 kg/year, which is even higher than the European average (51.2 kg/year) (Chamorro et al., 2012). More relevant is the consumption of charcuterie products, and Spain is at the head of the production and consumption of such meat products in Europe, behind Germany, France and Italy (Leon-Munoz et al., 2012).

Different studies have linked an increased risk of cancer from meat consumption with the presence of carcinogenic chemical substances in meat (Trafialek and Kolanowski, 2014), and according to the literature, the content of certain pollutants in meat is particularly relevant (Gasull et al., 2011). This is the case of organochlorine pesticides (OCPs) (Letta and Attah, 2013; Pardio et al., 2012; Schecter et al., 2010; Wang et al., 2011), dioxin-like polychlorinated biphenyls (PCB) (Costabeber et al., 2006; Malisch and Kotz, 2014; Schecter et al., 2010; Schwarz et al., 2014), and especially polycyclic aromatic hydrocarbons (PAHs) (Gilsing et al., 2012; Liao et al., 2014).

During the past 30 years, many of these substances have been highlighted as a concern (Boada et al., 2007, 2012, 2014; Casals-Casas and Desvergne, 2011; Dorgan et al., 1999; Knerr and Schrenk, 2006; Valeron et al., 2009) and have been the subject of extensive study and international regulation in part because of their carcinogenic potential (Dorgan et al., 1999; Knerr and Schrenk, 2006; Liao et al., 2014). A variety of the most common pollutants in meat from the abovementioned chemical groups have been classified in group B of carcinogenicity (WHO, 2014). Although cancer slope factors (CSF) have been calculated for all of these probable carcinogens (EPA, 2014) and this would allow an estimate of the risk of cancer associated with continuous exposure to them through foodstuff, very few studies have attempted to estimate the carcinogenic risks that are associated with the current pattern of consumption of the pollutants associated with meat and meat products (Trafialek and Kolanowski, 2014).

In this study, we first determined the concentrations of 7 PAHs, 18 PCBs and 8 OCPs for which the CSFs have been calculated in a total of 100 samples of meat and charcuterie products that are most commonly consumed by the studied population. Because it is well known that continued exposure to carcinogens, even at very low doses, is not without risk, the main objective of this study was to use these data to estimate the carcinogenic risk associated with the current level of meat consumption by these consumers. For this purpose, we used the data of food consumption (AECOSAN, 2011) and applied the methodology that has been recently used to estimate the carcinogenic risk associated with food intake (Yu et al., 2014). Finally, we calculated the number of monthly servings of meat and charcuterie products that would be exempt from carcinogenic risk to provide a recommendation for consumption.

2. Materials and methods

2.1. Sampling

From January to March 2014, we randomly acquired samples of meat and charcuterie products from multinational retailers settled in the Canary Islands (Spain). Therefore, all the products sampled came from large suppliers who serve the entire European territory, and as a consequence our results could be extrapolated to the entire European population, only considering their differentiated dietary habits. According the most appreciated choices of Spanish consumers (AECOSAN, 2011) we bought meat samples: beef (n = 14), chicken (n = 16), pork (n = 8), lamb (n = 8), goat (n = 6), and rabbit (n = 6); and also traditional charcuterie products: Iberian cured ham ("serrano ham") (n = 8), ham (n = 7), Spanish chorizo (n = 8), Spanish dry-cured sausage "salchichón" (n = 7), bacon (n = 6), and Italian mortadella (n = 6).

The samples were processed immediately upon arrival at the laboratory. Each individual sample was finely chopped with a knife and was then ground using a stainless steel domestic food processor. The lipid content of the samples was determined in triplicate by the modified Gerber method as previously described (de Langen, 1963), to obtain the final lipid-corrected values. Then, all of the samples were frozen at −18 °C until analysis.

2.2. Chemical analyses

Organic solvents (>99.9%) were purchased from Fisher Scientific (Leicestershire, United Kingdom). Diatomaceous earth was purchased from Sigma-Aldrich (St. Louis, USA). Bio-Beads SX-3 were purchased from BioRad Laboratories (Hercules, USA). Standards of OCPs, PCBs, and the internal standards (ISs, PCB 202, p,p′-DDE-d8, phenanthene-d10, tetrachloro-m-xylene, and heptachloro epoxide cis) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Standards of PAHs were purchased from Absolute Standards, Inc. (Connecticut, USA). All standards were neat compounds (purity ranged from 97% to 99.5%). Solutions diluted from 0.05 ng/mL to 100 ng/mL in cyclohexane were used for calibration curves.

We quantified the levels of 8 OCPs: p,p′-DDT, p,p′-DDE, p,p′-DDD, hexachlorobencene (HCB), and the four isomers of hexachlorocyclohexane (α-, β-, γ-, δ-HCH). We also determined 18 PCB congeners, including marker-PCBs (M-PCBs) and dioxin-like PCBs (DL-PCBs): IUPAC

numbers 28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180, and 189. We also included 7 PAHs listed as carcinogens (EPA, 2001): benzo(a)anthracene, benzo(a)phenanthrene (chrysene), benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene, and indeno(1,2,3-cd)pyrene.

We first extracted the fat from the meat and charcuterie samples because all of the contaminants included in this study are completely lipid-soluble and therefore are found in the lipid fraction of tissues. Thus, the samples (5 g) were homogenized in 5 ml of ultrapure water with a disperser (Ultra-turrax, IKA, China). This homogenate was spiked with a 10 ppm-ISs mix in acetone (100 ppb, final concentration) and was mixed with 30 g of diatomaceous earth to absorb any moisture. The extraction and clean-up method followed the procedures recommended by the European Standard for the determination of pesticides and PCBs in fatty foods (EN, 1996a,b), which had been previously validated in our laboratory for different fatty samples of animal origin (Luzardo et al., 2014). This method achieved acceptable recoveries that ranged between 71.5% and 103.2%. All of the individual measurements were corrected by the recovery efficiency for each analyte. Briefly, for the fat extraction, we used a Soxtec™ 2055 Auto Fat Extraction (Foss® Analytical, Hilleroed, Denmark) apparatus, which consisted of an extraction unit, a control unit and a drive unit. The samples were placed into the extraction unit, and 20 ml of dichloromethane were added to each of the extraction cups in a closed system, and the cups were heated using an electric heating plate. The three-step extraction consisted of boiling, rinsing, and solvent recovery. The solvent was evaporated in a rotary evaporator (Hei-VAP Advantage™, Heidolph Instruments®, Schwabach, Germany) at 40 °C to prevent analyte losses. The results were calculated as the total amount of fat (g) per 100 g of tissue. Using a precision balance, the fat obtained was carefully weighted into a zeroed glass tube. The weighted fat was dissolved in 2 ml of cyclohexane/ethyl acetate (1:1) and subjected to purification by gel permeation chromatography (BioBeads SX-3) using cyclohexane/ethyl acetate (1:1) at a constant flow of 2 ml/min as the eluent. The first 25-minute elution volume, which contained the great majority of lipids (>98%), was discarded. The 25–85 min elution volume (120 ml), which contained all of the analytes that were co-extracted with the fat, was collected. The sample was concentrated using a rotary evaporator, and finally, the solvent was evaporated to dryness under a gentle nitrogen stream. The residue was then reconstituted in 1 mL of cyclohexane and the sample was transferred to a GC vial that was used for the chromatographic analysis. The amount of pollutants per gram of fat was obtained by multiplying by the corresponding correction factor.

Gas chromatography–mass spectrometry analyses of 33 carcinogenic compounds, plus ISs, were performed in a single run on a Thermo Trace GC Ultra coupled to a Triple Quadrupole Mass Spectrometer Quantum XL (Thermo Fisher Scientific Inc., Waltham, MA, USA) as previously described (Camacho et al., 2013, 2014), and identifications were done using an electron ionization (EI)–MS/MS library that was specially created for the target analytes under our experimental conditions. All of the measurements were performed in triplicate, and we used the means for the calculations. In each batch of samples, three controls were included for every 18 vials (6 samples): a reagent blank consisting of a vial containing only cyclohexane; a vial containing 2 ng/mL of each of the pollutants in cyclohexane; and an internal laboratory quality control (QC) consisting of melted meat fat spiked with a mixture of all of the pesticides (20 μg/kg), and processed using the same method that was used for the samples. The results were considered to be acceptable when the quantification of the analytes in the QC was within 15% of the deviation of the theoretical value.

2.3. Dietary intake estimates and calculations

The estimated total daily intake per body weight of each of the contaminants was calculated by multiplying the respective concentrations of contaminants in each food type (median values expressed in ng/g fresh product) by the average daily consumption of these foodstuffs by the study population. Two groups were considered: adults (average weight 70.1 kg) and children (average weight 30.4 kg). For the calculations, when the concentration of a given contaminant was below the limit of quantification (LOQ) but above the limit of detection (LOD) of the technique, the value was assumed to be ½ LOQ. Otherwise the value was considered to be 0. Food consumption data were obtained from the Spanish Agency for Consumer Food Safety and Nutrition (AECOSAN) (AECOSAN, 2011).

We expressed the total value of OCP residues ($\sum$OCPs) as the sum of the 8 OCPs (and metabolites) measured; the total value of DDTs ($\sum$DDT) as the sum of the measured values of p,p′-DDT, p,p′-DDE and p,p′-DDD; and the total value of HCH residues ($\sum$HCH) as the sum of the 4 HCH isomers measured. Similarly, we expressed the total value of PCB residues ($\sum$PCBs) as the sum of the 18 PCB congeners measured. In addition, we expressed the total value of the marker PCB residues ($\sum$M-PCBs) as the sum of the congeners #28, 52, 101, 118, 138, 153 and 180, and the total value of dioxin-like PCBs ($\sum$DL-PCBs) as the sum of the measurements of the 12 individual congeners #77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189. For the risk estimation, we calculated the potential toxicity for the DL-PCBs (in terms of toxic equivalence to dioxins; TEQs) using the toxic equivalency factors (Van den Berg et al., 2006). Finally, we also considered the total content of carcinogenic PAHs ($\sum$c-PAHs) following the EFSA recommendations (EFSA, 2008). For the risk estimation, we additionally used toxic equivalency factors (TEFs), which are established for the carcinogenic PAHs (Nisbet and LaGoy, 1992), to express the results in the form of benzo[a]pyrene toxic equivalents ($B[a]P_{eq}$).

The CSF values of the carcinogens included in this study were taken from the EPA's IRIS and Yu et al. (2014) and were as follows: 1 per mg/kg day^{-1} for marker PCBs (based on Aroclors 1260, 1254, 1242 and 1061); 1.1×10^5 per mg/kg day^{-1} for DL-PCBs (based on 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8,-TCDD)); 0.34 per mg/kg day^{-1} for DDTs; 1.8 per mg/kg day^{-1} for HCHs (as there are no CSF values listed for total HCHs we used the values listed for β- and γ-HCH); 1.6 per mg/kg day^{-1} for HCB; and 7.3 per mg/kg day^{-1} for PAHs (based on benzo[a]pyrene).

2.4. Carcinogenic risk calculation

To estimate whether chemical contamination by carcinogens of meat and charcuterie products endangers the Spanish population, we applied the risk assessment index, known as the risk quotient (RQ), using the methodology that has been used for other food groups, such as fish (Yu et al., 2014). Thus, in this work, the RQ is defined as the ratio between the current consumption of meat and charcuterie products (R_{meat}) and the maximum tolerable consumption of these products, which is calculated taking into account the concentrations of carcinogens in these foods (CR_{lim}) as follows:

$$CR_{lim} = \frac{ARL \cdot BW}{\sum_{m=1}^{X} C_m \cdot CSF_m}$$

where CR_{lim} is the maximum allowable consumption rate (kg/day) for a particular meat or charcuterie product; ARL is the maximum acceptable individual lifetime risk level (dimensionless), and a value of 10^{-5} was used in this study (Yu et al., 2014); BW is the body weight (kg); C_m is the median concentration of contaminant m in a particular meat or charcuterie product (mg/kg) as determined in this study; and CSF_m is the cancer slope factor of a contaminant m with carcinogenic potential. In the case of multiple contaminants with the same CSF, their concentrations in a particular meat or charcuterie product were summed (from $m = 1$ to $m = x$).

Then, the RQ for each food item and contaminant was calculated as follows:

$$RQ = \frac{R_{meat}}{CR_{lim}} \text{ (for a single contaminant)}$$

$$RQ = R_{meat} \cdot \sum_{m=1}^{x} \frac{1}{CR_{lim}} \text{(for multiple contaminants).}$$

Thus, if the value of RQ is equal to or less than 1, it is assumed that there is no carcinogenic risk associated with the ingestion of contaminants through the consumption of a particular type of meat or charcuterie product. Otherwise, the population is considered to be at carcinogenic risk when RQ is greater than 1.

2.5. Calculation of the recommended consumption of meat and charcuterie products

The current and recommended consumption patterns (expressed in servings/month following EPA's recommendations (Yu et al., 2014)) were calculated according to the formulas:

$$C_{mm} = \frac{R_{meat} \cdot TP}{MS}$$

$$RC_{mm} = \frac{RQ}{C_{mm}}$$

where C_{mm} is the current number of meals per month for each type of meat or charcuterie product; *MS* is the meal size (0.15 kg meat/meal, and 0.07 kg charcuterie product/meal); *TP* is the averaged time period (month = 30.44 days); and RC_{mm} is the recommended maximum number of servings of each food per month.

2.6. Statistical analysis

We used PASW Statistics v 19.0 (SPSS Inc., Chicago, IL, USA) to manage the database of the study and to perform the statistical analyses. Normality was examined using the Kolmogorov–Smirnov test. The distributions of carcinogens in the meat and charcuterie products lacked normality and homoscedasticity; therefore, we used non-parametric tests (the Mann–Whitney and Kruskal–Wallis tests). The results are reported as the medians and interquartile ranges. Probability levels of less than 0.05 (two tailed) were considered statistically significant.

3. Results and discussion

3.1. Occurrence of persistent organic pollutants with carcinogenic potential in meat and charcuterie products

An average of 19 residues per sample were found (range 12–24), independent of the food item considered, varying only in the concentration and frequency of detection among the different food types. We present a summary of the obtained results (median and percentiles 25th–75th; p25-75) for each contaminant (or group of contaminants) in each food type in Table 1, either as individual compounds or as the sum of individual compounds according to their carcinogenic potential.

When we consider the results obtained directly from the fat of the meat or charcuterie product, measured in ng/g lipid weight (l.w.), it is interesting to note that, in general, for each carcinogen or group of carcinogens, the results were very similar between the different types of food, with little variation. Thus, for ∑DDTs no differences were found between meat and charcuterie products. The median value for the total sample was 80.1 ng/g l.w. (P25-75 = 53.2–98.6 ng/g l.w.). Only the values in the fat of lamb and goat departed considerably and were significantly higher than the rest ($p < 0.001$ for lamb fat, and $p < 0.01$ for goat fat). In the case of ∑HCHs, we found significant differences between meat (median 17.4 ng/g l.w.; P25-75 = 11.5–30.1 ng/g l.w.) and charcuterie products (4.1 ng/g l.w., P25-75 = 3.1–5.7 ng/g l.w.) ($p < 0.01$). Again, in this case, the highest values were found in samples of lamb fat ($p < 0.005$) and goat fat ($p < 0.05$). In the case of HCB it is interesting to note that lamb meat fat values departed considerably from the median value, being as much as 35 times higher than the median of the whole group of samples (median 27.2 ng/g l.w.; P25-75 = 16.9–47.1 ng/g l.w.), ($p < 0.001$). Additionally we found higher levels of this pollutant in the fat of goat meat than in the rest of the foods ($p < 0.05$, Table 1). The values of the total amount of PCBs (∑PCBs) were also

Table 1
Concentrations of contaminants with carcinogenic potential (ng g^{-1} fat) in samples of meat and charcuterie products most consumed by Spanish population.

	∑DDTs		∑HCHs		HCB		∑M-PCBs		∑DL-PCBs		$\sum TEQ_{DL\text{-}PCBs}$		$\sum B[a]P_{eq}$	
	Median	P25-75	Median	P25-75	Median	P25-75	Median	P25-75	Median	P25-75	Median	P25-75	Median	P25-75
Meat														
Beef	87.5	33.1	6.4	1.8	13.2	3.7	102.3	56.1	8.9	2.3	0.4	0.1	1.4	1.2
		320.1		75.9		52.8		240.9		23.1		1.3		6.7
Chicken	74.3	16.7	13.2	3.2	31.3	11.2	155.1	23.1	2.4	0.5	0.2	0.1	3.9	0.7
		231.0		69.3		132.3		300.3		10.3		0.8		35.8
Pork	85.8	75.9	21.5	3.3	16.5	2.1	173.3	128.7	3.3	1.1	0.4	0.1	4.9	1.2
		399.3		174.9		75.9		330.2		11.3		0.8		9.6
Lamb	603.9	85.8	31.4	6.3	985.1	287.1	153.5	102.3	7.0	0.8	0.5	0.2	1.5	0.1
		3039.1		1168.8		1439.6		247.5		26.4		1.3		5.1
Goat	188.1	151.8	29.7	23.1	85.8	79.2	283.8	198.1	4.9	0.4	1.2	0.5	16.8	0.7
		224.4		36.3		92.4		369.6		9.9		1.9		32.9
Rabbit	52.8	36.3	13.2	6.6	47.4	39.6	137.0	59.4	0.6	0.9	0.2	0.1	0.5	0.1
		99.0		16.5		237.6		148.5		6.2		0.3		1.3
Meat products														
Iberian cured ham	100.7	29.7	3.3	1.2	28.1	22.1	82.5	61.2	1.7	1.1	0.1	0.1	0.7	0.2
		42.9		11.4		39.5		92.4		5.4		0.3		1.2
Ham	36.3	82.5	6.5	1.8	11.2	1.8	133.7	53.2	4.9	2.3	0.3	0.1	1.3	0.4
		108.9		16.5		22.9		204.6		7.6		0.4		2.6
Spanish chorizo	54.4	29.7	5.4	2.1	18.2	5.3	110.6	29.5	2.7	0.8	0.4	0.2	38.8	12.8
		85.8		18.3		19.8		151.8		9.6		0.7		96.7
Spanish salchichón	74.2	2.3	3.1	1.1	26.4	32.1	105.6	12.1	0.8	0.2	0.2	0.1	0.2	0.1
		115.5		7.2		46.8		145.6		3.4		0.7		2.3
Bacon	42.9	36.3	2.8	0.8	46.2	29.3	123.8	75.1	0.6	0.3	0.1	0.1	0.4	0.1
		49.5		4.3		52.8		232.4		1.3		0.6		1.7
Italian mortadella	92.4	36.3	4.9	2.8	26.4	13.2	145.2	97.6	2.5	0.4	0.2	0.1	0.5	0.1
		191.4		7.4		51.9		214.7		7.6		0.6		1.5

quite homogeneous among the different types of food (median 138.1 ng/g l.w.; P25-75 = 111.8–159.8 ng/g l.w.). Again, the values found in the fat of meat of one of the small ruminant species considered (goat) were above average ($p < 0.01$, Table 1). The PCB concentrations were completely dominated by congeners 118, 138, 153 and 180, and thus, the ∑M-PCBs contributed 92.3%–99.7% to the ∑PCBs. Therefore, the $TEQ_{DL\text{-}PCBs}$ levels were low (median from 0.1 to 1.2 pg/g l.w.) (Table 1). Finally, with respect to the c-PAHs (expressed as $B[a]P_{eq}$), two types of fat samples had higher values than the rest (median 1.4 ng/g l.w.; IqQ = 0.5–4.7 ng/g l.w.), namely, goat meat fat ($p < 0.01$) and Spanish chorizo fat ($p < 0.001$).

We have considered it also interesting to show our findings graphically by expressing the concentrations of the pollutants relative to the food wet weight (as they are finally consumed), as this more clearly shows the real differences in contamination among different products (Fig. 1). In this figure it can be appreciated that enormous differences exist among foods. The biggest burden of OCPs and PCBs is exhibited by lamb meat and bacon, and for c-PAHs the most contaminated foods are lamb meat and Spanish chorizo.

3.2. Daily intake of carcinogenic pollutants through the consumption of meat and charcuterie products in the Spanish population

In Table 2, we summarized the dietary intake of all of the contaminants by food item, calculated for children and adults on the basis of their different consumption habits and average weights.

First, with regard to the intake of OCPs, our results show that the meat-related estimated daily intake (EDI) of ∑DDTs for people living in Spain is 88.8 ng/kg b.w. day in adults and 142.1 ng/kg b.w. day in children, and the consumption of meat is the main contributor of this exposure (mainly lamb, followed by beef, pork, and chicken). The contribution of charcuterie products to this exposure can be considered comparatively minimal (5.95% in adults and 9.62% in children). The EDI of ∑HCHs through meat and charcuterie products is 24.3 ng/kg b.w. day in adults

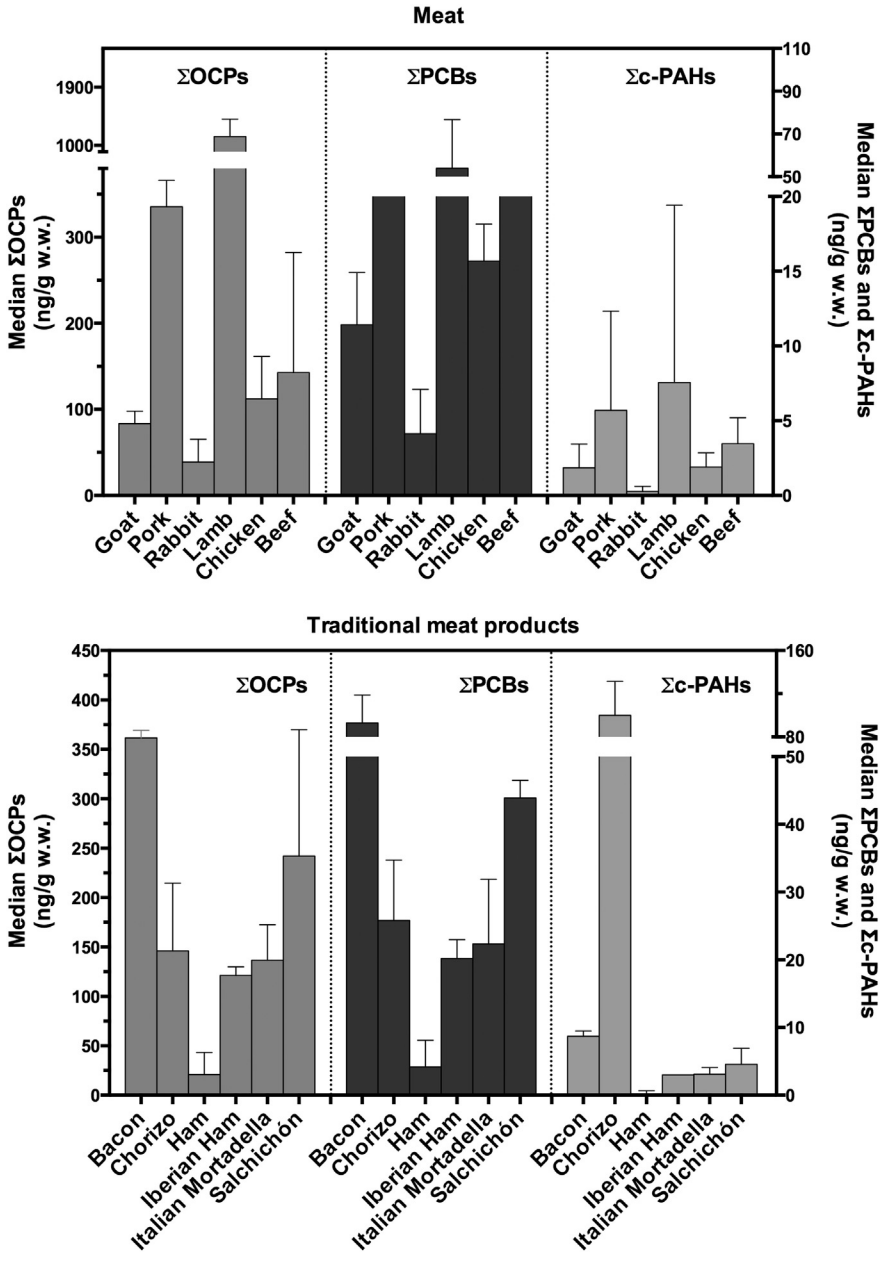

Fig. 1. Levels of ∑OCPs, ∑PCBs, and ∑c-PAHs in meat (fresh products). Upper panel, meats. Lower panel, processed meats (traditional charcuterie products).

Table 2
Median values of dietary intakes of carcinogenic pollutants (ng/kg b.w. day) by means of the consumption of meat and meat products for Spanish adults and children.

	Adults								Children							
	Food consumption (g/day)	∑ DDT	∑ HCH	HCB	∑ M-PCB	∑ DL-PCB	∑ TEQ [a]	∑ B[a]P$_{eq}$	Food consumption (g/day)	∑ DDT	∑ HCH	HCB	∑ M-PCB	∑ DL-PCB	∑ TEQ [a]	∑ B[a]P$_{eq}$
Meat																
Beef	50.4	15.09	3.24	2.95	18.83	1.35	0.08	3.17	47.9	33.07	7.10	6.47	41.27	2.96	0.18	6.94
Chicken	42.7	4.87	1.39	2.14	8.56	0.15	0.01	1.72	43.1	11.35	3.25	4.97	19.92	0.35	0.03	4.01
Pork	24.1	11.42	3.52	2.05	14.81	0.29	0.03	2.71	19.5	21.30	6.57	3.83	27.63	0.55	0.05	5.05
Lamb	11.5	51.92	15.52	49.90	9.27	0.38	0.03	1.79	6.1	62.47	18.67	60.04	11.15	0.46	0.04	2.15
Goat	0.4	0.04	0.01	0.02	0.06	0.00	0.00	0.01	0.07	0.02	0.00	0.01	0.03	0.00	0.00	0.00
Rabbit	4.4	0.19	0.04	0.35	0.29	0.01	0.00	0.02	2.5	0.25	0.05	0.46	0.38	0.01	0.00	0.02
Meat products																
Iberian cured ham	11.2	1.39	0.13	1.08	3.16	0.06	0.01	0.51	7.6	2.18	0.20	1.68	4.95	0.10	0.02	0.79
Ham	11.7	0.42	0.05	0.02	0.71	0.02	0.00	0.05	12.9	1.07	0.12	0.06	1.81	0.06	0.00	0.14
Spanish chorizo	8.9	1.64	0.22	0.31	3.08	0.10	0.01	12.55	11.5	4.88	0.65	0.93	0.19	0.29	0.03	37.40
Spanish salchichón	2.9	1.04	0.05	0.36	1.41	0.01	0.00	0.05	3.4	2.81	0.14	0.98	3.82	0.04	0.01	0.14
Bacon	1.6	0.57	0.04	0.61	1.64	0.00	0.00	0.11	1.1	0.90	0.07	0.97	2.60	0.00	0.00	0.17
Italian mortadella	1.3	0.24	0.02	0.10	0.43	0.00	0.00	0.02	4.4	1.85	0.12	0.80	3.33	0.03	0.01	0.15

[a] Expressed in pg/kg b.w. day.

and 36.9 ng/kg b.w. day in children. As for ∑ DDTs, the main contributors of these pollutants are lamb, pork and beef meats, among the foods studied (approximately 92%). With regard to the intake of HCB, it is important to note that the highest contribution of this contaminant is related to the consumption of lamb meat (83% of the total). In this case, as in the above, the contribution of the consumption of charcuterie products to the intake of this pesticide is relatively low, with the exception of Iberian ham (Table 2). With regard to exposure to PCBs, charcuterie products pose a relevant contribution, particularly Iberian ham and Spanish chorizo, although it is considerably less than the contribution of meats. Thus, the main dietary intake of these contaminants within this food group consumption is from beef, followed by pork. The contribution of charcuterie product to PCB intake is relatively low, especially for DL-PCBs. Finally, the daily intake of ∑ c-PAHs, in terms of B[a]P$_{eq}$, greatly depended on the type of meat or charcuterie product consumed. In our study the highest exposure to these carcinogens is through the consumption of chorizo, which contributes over 55% of the total.

3.3. The carcinogenicity RQ of the current consumption of meat and charcuterie products

The CR_{lim} values, calculated for each one of the food items studied are presented in Table 3. As it can be observed the current pattern of consumption of this population implies that some of these limits are exceeded through the consumption of meat and charcuterie products. Thus, the CR_{lim} of ∑ M-PCBs is clearly exceeded by the consumption of beef and pork in adults and children, and in the latter, also by the consumption of chicken meat. The pork consumption also appears to exceed the ∑ HCHs CR_{lim} in children. Finally, the current consumption of lamb exceeds the CR_{lim} for ∑ DDTs, ∑ HCHs and ∑ HCB in both children and adults.

In Fig. 2, we present the calculated RQ for all of the carcinogens and foods for both, adults and children. Thus, the RQ for the carcinogenic effects of multiple pollutants ranges between 0.02 and 13.98 for adults and between 0.01 and 10.98 for children. The lowest risk is associated with the consumption of goat meat and the highest is associated with the consumption of lamb meat. As noted in Fig. 2, in both age groups, the current consumption of beef, chicken, pork, lamb, and Spanish chorizo has a RQ > 1.

3.4. Meal suggestions for the consumption of meat and meat products

On the light of the above results we calculated the recommended monthly maximum number of servings of each food (RC_{mm}) as defined by the USEPA (USEPA, 2000) (Table 4). Thus, in adults the total consumption of meat could be maintained with a slight decrease (from 27.1 to 22.4 servings/month) but a significant reduction in the consumption of monthly rations of beef and pork meat (approximately one third), and chicken meat (approximately the half) should be recommended. The consumption of lamb meat should be limited to one serving every 4 or 5 months. In children, however, it would be advisable to reduce the total meat consumption (from 24.2 to 14.1 servings/month), and also to limit consumption of beef, pork and chicken so that children should consume at most five servings of these meats each month (considered together). Lamb consumption in children should be severely limited.

The current consumption of charcuterie products does not seem to be a problem in general and in almost all cases can be maintained and even increased if desired. The only exception within this group would be Spanish chorizo, for which consumption should be limited, especially in children.

4. Discussion

As expected, the pollutant values found in our sample of foods were similar to, but not fully comparable with, those described in the literature

Table 3
Maximum allowable meat or meat product consumption rate (CR_{lim}) expressed in kg/day. As a reference also current values of consumption of these products by the study population (kg/day) are included.

	Current food consumption (kg/day)	CR_{lim} adults (kg/day)						Current food consumption (kg/day)	CR_{lim} children (kg/day)					
		∑ DDT	∑ HCH	HCB	∑ M-PCB	∑ DL-PCB	∑ B[a]P_{eq}		∑ DDT	∑ HCH	HCB	∑ M-PCB	∑ DL-PCB	∑ B[a]P_{eq}
Meat														
Beef	0.0504	0.0953	0.0839	0.1034	0.0260*	0.5475	0.3327	0.0479	0.0490	0.0432*	0.1034	0.0134*	0.5475	0.1712
Chicken	0.0427	0.2499	0.1650	0.1212	0.0484	2.6306	0.2451	0.0431	0.1286	0.0849	0.1212	0.0249*	2.6306	0.1262
Pork	0.0241	0.0602	0.0369	0.0711	0.0158*	0.7436	0.0835	0.0195	0.0310	0.0190*	0.0711	0.0081*	0.7436	0.0430
Lamb	0.0115	0.0063*	0.0040*	0.0014*	0.0120	0.3434	0.2008	0.0060	0.0033*	0.0021*	0.0014*	0.0062	0.3434	0.1033
Goat	0.0004	0.2658	0.3180	0.1238	0.0599	1.3014	0.1386	0.00007	0.1368	0.1637	0.1238	0.0308	1.3014	0.0713
Rabbit	0.0044	0.6642	0.6105	0.0752	0.1459	8.2424	0.9315	0.0025	0.3419	0.3142	0.0752	0.0751	8.2424	1.8440
Meat products														
Iberian cured ham	0.0112	0.2296	0.4770	0.0631	0.0343	0.7359	0.5680	0.0076	0.1182	0.2455	0.0631	0.0177	0.7359	0.2923
Ham	0.0117	0.7949	1.3874	3.0292	0.1594	7.2727	0.9315	0.0129	0.4091	0.7141	3.0292	0.0821	7.2727	3.1963
Spanish chorizo	0.0089	0.1550	0.2212	0.1723	0.0280	0.8270	0.0104	0.0115	0.0798	0.1138	0.1723	0.0144	0.8270	0.0054*
Spanish salchichón	0.0029	0.0797	0.3013	0.0484	0.0199	1.3014	1.8630	0.0034	0.0410	0.1551	0.0484	0.0102	1.3014	0.9589
Bacon	0.0016	0.0804	0.1974	0.0159	0.0095	2.1317	0.4273	0.0011	0.0414	0.1016	0.0159	0.0049	2.1317	0.2199
Italian mortadella	0.0013	0.1569	0.4625	0.0770	0.0295	0.9366	0.9315	0.0044	0.0807	0.2381	0.0770	0.0152	0.9366	1.0654

* The CR_{lim} for these carcinogens is exceeded with the current pattern of food consumption.

in other regions of the world, which is logical because it is common to find regional variations (sometimes very significant) in the reported pollutant concentrations (Costabeber et al., 2006; Letta and Attah, 2013; Malisch and Kotz, 2014; Pardio et al., 2012; Polder et al., 2010; Schecter et al., 2010; Tornkvist et al., 2011; Wang et al., 2011). It is also important to note that for some of the foods included in this study, we found no reference data in the literature. Therefore, to make a realistic estimate, in this study we preferred to directly quantify the contaminants in a representative sample of the main types of meat and charcuterie products in the consumers' market basket, as the main goal of this study was not to perform a monitoring study and establish comparisons with other available studies but to provide an estimate of carcinogenic risk associated to relevant carcinogens through meat consumption in the Spanish population.

Although our results showed that 100% of the food samples investigated had quantifiable amounts of the majority of the carcinogens included in the study, and that we found very similar concentrations in the extracted fats among type of meats and charcuterie products, the quantification referred to fresh products was fairly different among food items. Thus, in relation to the types of meats, we observed that, by far, lamb meat was the most contaminated by all types of pollutants (∑ OCPs, ∑ PCBs, and ∑ c-PAHs), followed by pork and beef, and that the less contaminated meats were rabbit and goat meats. This is mainly in relation to the percentage of fat presented by each type of meat. The case of the traditional charcuterie products is most striking, as the differences in contaminant levels in fat were in general minimal; however, in the fresh product, the differences became very relevant. Thus, the charcuterie product most contaminated by ∑ PCBs and ∑ OCPs was bacon, and the more contaminated by ∑ c-PAHS was by far Spanish chorizo. The least contaminated of the charcuterie products (by all contaminants) was ham in its two varieties.

Dietary exposure calculations are performed by combining data on consumption habits with the concentrations of contaminants found in food samples. The estimation of food contaminant exposure is a topic of growing interest in the field of public health as a means to inform and guide the actions on Food Security and Nutrition as a predictive method for determining the state of health of populations. For this reason, the European Food Safety Authority (EFSA) performs a collection of food consumption data from the different Member States, which must develop nutritional surveys in their territories. For this study we have used the data provided by the AECOSAN (AECOSAN, 2011). From our estimates, the meat-driven EDIs of some of the carcinogens considered were well below the tolerable daily intakes (TDIs) established by the World Health Organization for these contaminants (JECFA, 2000). Thus, the intake of ∑ DDTs and ∑ HCHs represented less that 1.2% of those TDIs. However for other pollutants the exposure is considerably higher. Thus, for HCB the EDI reaches 16% of its TDI (mainly related to lamb consumption). This was also the case for the exposure to ∑ $TEQ_{DL\text{-}PCBs}$ since the estimated EDI would represent 9% or 19% (in adults and children, respectively) of the TDI of 2 pg/kg b.w. day (SCF, 2000). Moreover if we used the upper bound approach for calculations (as recommended by the European Commission (SCF, 2000)) our estimates would be almost triple, and therefore, the exposure through the consumption of meat and charcuterie products would represent as much as 44% of the TDI in children. These results are worrisome because the possibility exists that certain consumers may be subject to high dietary exposures to dioxins, having into consideration that it has been established that intake of DL-PCBs would represent almost 70% of total dioxin dietary exposure (SCF, 2000; Tornkvist et al., 2011). Finally, with respect to exposure to ∑ c-PAHs, the WHO has not yet established TDI values for c-PAHs. However other references may be used and thus, using the Contaminated Land Exposure Assessment model of UK, which has established a TDI for B[a]P_{eq} of 20 ng/kg b.w. day (CLEA-UK, 2008), the current meat consumption in the evaluated population would represent between 113 and 284% of these values, particularly due to the consumption of Spanish chorizo (a smoke cured type of sausage) and lamb. It is logical that Spanish chorizo is one of the major contributors to PAHs exposure because the manufacturing of Spanish chorizo implies the smoking of the pieces during the first 5 days of ripening using smoke from wood (Lorenzo et al., 2011), and among the more than 400 volatile components recorded in biomass smoke composition, the PAHs are present at high concentrations (Lorenzo et al., 2011). One should also keep in mind that dietary exposure to these contaminants could be even higher than those reported in this study, since it is well known that the way in which food is cooked, processed, and packaged may introduce chemicals (such as bisphenol A, phthalates, acrylamide) that are not present in the raw food, or increase their content in those naturally occurring pollutants (such as PAHs) (Pieters and Focant, 2014; Vogt et al., 2012).

It has been established that the RQ evaluation is a good method to estimate the risk of a population and to establish exposure limits to chemicals (USEPA, 2000). It is well known that chemical contaminants in foods, which are usually present in mixtures of various compounds belonging to different chemical classes, may exert their adverse effects on consumers interacting with each other in synergistic, additive or

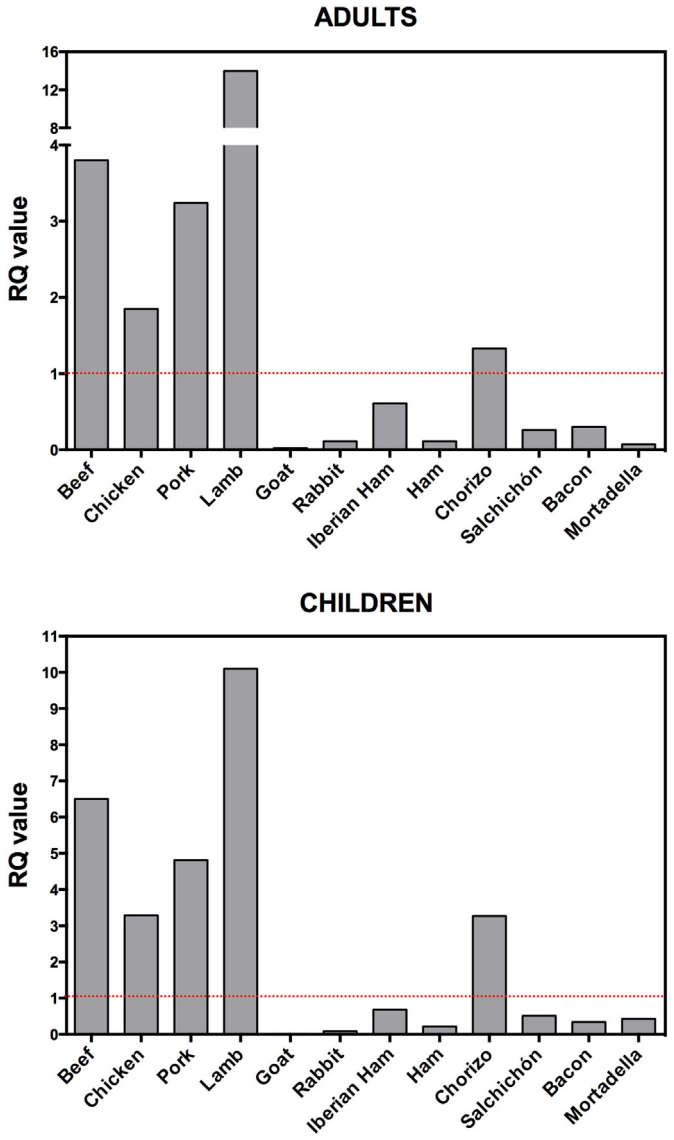

Fig. 2. Hazard ratios of the contaminants for carcinogenic effects in adults (upper panel) and children (lower panel) via consumption of meat and charcuterie products. The red line indicates the threshold for carcinogenic risk (RQ = 1). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

even in antagonistic manners. However, the calculation model of the RQ implies the assumption that all pollutants cause similar toxicological effects and that the combined effect is the sum of the individual effects (USEPA, 2000). As recommended by the USEPA for calculating the RQ of some other food items (such as fish) (USEPA, 2000), we have considered that all carcinogens studied are similar and therefore we have used the additive model. To calculate RQ first it is necessary to calculate the CR_{lim}, which is based on the load of each pollutant for each one of the food items. According to our results the maximum allowable consumption rates are currently being exceeded in Spanish population for various carcinogens. According the USEPA (USEPA, 2000) it can be assumed that a RQ ≦ 1 means that there is not an appreciable risk of carcinogenicity associated to the consumption of a particular food. Thus, in this study we found that the current consumption rate of several meat and charcuterie products by the Spanish population (considering all carcinogens together), has a RQ > 1 for pork, chicken, Spanish chorizo, and especially high for lamb (RQ > 10), and for beef in children (RQ > 6), and therefore the consumption of these foods is associated to carcinogenic risk.

Table 4
Recommended maximum number of meals per month of each food item.

	Adults		Children	
	Current pattern of consumption [a] (meals/month) C_{mm}	Maximum recommended consumption (meals/month) RC_{mm}	Current pattern of consumption [a] (meals/month) C_{mm}	Maximum recommended consumption (meals/month) RC_{mm}
Beef	10.2	2.7	9.7	1.5
Pork	4.9	1.5	4.0	0,8
Chicken	8.7	4.7	8.7	2.7
Lamb	2.3	0.2	1.2	0.1
Goat	0.1	5.1	0.0	3.0
Rabbit	0.9	8.3	0.5	6.1
Total meat	27.1	22.4	24.2	14.1
Iberian ham	4.9	8.0	3.3	4.8
Ham	5.1	44.4	5.6	25.9
Spanish chorizo	3.9	2.9	5.0	1.5
Spanish salchichón	1.3	4.9	1.5	2.9
Bacon	0.7	2.3	0.5	1.4
Italian mortadella	0.6	7.6	1.9	4.5
Total charcuterie products	16.4	70.1	17.8	41.0

[a] Data obtained from AECOSAN, 2011.

Based on the levels of contaminants found in the foods of this study, on the calculations done, and also assuming that consumers want to maintain their monthly intake of protein from meat and charcuterie products (i.e., the number of servings per month would not change substantially), we calculated the maximum number of meals of each food item that would not pose a carcinogenic risk to make consumption recommendations (this is, consumption which that would allow a RQ ≦ 1 for all products). In general, if the adult consumer wants to maintain a high intake of meat, it would be advisable to increment the consumption of rabbit and goat meat, and considerably reduce (50–80%) the consumption of the rest. The recommendation for children would be a reduction in both, the total intake of servings per month of meat to almost 50%, and also not to surpass the number of 5 servings of beef/pork/chicken (considered together). With respect to charcuterie products, the current consumption could be maintained or even incremented for both, adults and children, except for Spanish chorizo, whose consumption should be reduced in adults and especially in children.

We conclude that the consumption of beef, pork, chicken, Spanish chorizo, and lamb all have a high carcinogenic risk for consumers. To diminish the continued exposure at very low doses of carcinogens, the monthly consumption of these products should be considerably reduced.

Conflict of interest

The authors declare no conflict of interest.

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Consumption of organic meat does not diminish the carcinogenic potential associated with the intake of persistent organic pollutants (POPs)

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Abstract Numerous studies have shown an epidemiological link between meat consumption and the incidence of cancer, and it has been suggested that this relationship may be motivated by the presence of carcinogenic contaminants on it. Among the most frequently detected contaminants in meat are several types of persistent organic pollutants (POPs), and it is well known that many of them are carcinogenic. On the other hand, an increasing number of consumers choose to feed on what are perceived as healthier foods. Thus, the number of consumers of organic food is growing. However, environmental contamination by POPs is ubiquitous, and it is therefore unlikely that the practices of organic food production are able to prevent this contamination. To test this hypothesis, we acquired 76 samples of meat (beef, chicken, and lamb) of two modes of production (organic and conventional) and quantified their levels of 33 carcinogenic POPs. On this basis, we determined the human meat-related daily dietary exposure to these carcinogens using as a model a population with a high consumption of meat, such as the Spanish population. The maximum allowable meat consumption for this population and the carcinogenic risk quotients associated with the current pattern of consumption were calculated. As expected, no sample was completely free of carcinogenic contaminants, and the differences between organically and conventionally produced meats were minimal. According to these results, the current pattern of meat consumption exceeded the maximum limits, which are set according to the levels of contaminations, and this is associated with a relevant carcinogenic risk. Strikingly, the consumption of organically produced meat does not diminish this carcinogenic risk, but on the contrary, it seems to be even higher, especially that associated with lamb consumption.

Keywords Meat · Organic meat · Carcinogens · Persistent organic pollutants · Carcinogenic risk · PCBs · PAHs · Organochlorine pesticides

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Introduction

It is well known that the food is the primary route of exposure to pollutants from numerous chemical classes (Vogt et al. 2012). Among the multitude of different chemical compounds that food may contain, persistent organic pollutants (POPs) are especially worrisome, and during the last decades, many POPs have been highlighted as a cause of concern and have been the subject of extensive study and international regulation in part because of their carcinogenic potential (Boada et al. 2012; Casals-Casas and Desvergne 2011; Dickerson et al. 2011; Dorgan et al. 1999; Knerr and Schrenk 2006; Ribas-Fito et al. 2001; Valeron et al. 2009). Since these compounds are highly resistant to degradation and are highly distributed in the environment, their presence in food is very difficult to avoid (Li et al. 2006; Rychen et al. 2014). Thus, it has been established that the ingestion of food contributes more than 90 % to the total current exposure to these compounds,

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especially those food from animal origin such as fish, dairy products, or meat (Li et al. 2006). According to the literature, the content of POPs in meat is particularly relevant, and several studies have reported high levels of organochlorine pesticides (OCPs) (Letta and Attah 2013; Pardio et al. 2012; Schecter et al. 2010; Wang et al. 2011), polychlorinated biphenyls (PCBs) (Costabeber et al. 2006; Malisch and Kotz 2014; Schecter et al. 2010; Schwarz et al. 2014), and especially polycyclic aromatic hydrocarbons (PAHs), which although they are not strictly considered as POPs, they are usually included in this group due to their high environmental prevalence and lipophilicity (Helmus et al. 2013; Lammel et al. 2013; Martorell et al. 2012; Veyrand et al. 2013).

It is known that dietary practices may influence exposure to chemical contaminants such as POPs, through the food consumption pattern, the forms of cooking or processing the food, the production modes, packaging types, etc. (Luzardo et al. 2013a; Oates and Cohen 2011; Vogt et al. 2012). For example, a growing number of people choose organic food as a healthier choice, although the study of the profile of these consumers has indicated that they are also motivated by concern for environmental health, the animal welfare, or by the perception that organic food has a higher nutritional value than conventional products (Oates et al. 2012; Smith-Spangler et al. 2012; Vogt et al. 2012). In fact, in the USA, organic food production increased by 50 % during the last decade, and in Europe, this increase has been even higher (in some countries like Spain, the land surface devoted to organic production has tripled during this period) (FIBL-IFOAM 2012).

Numerous studies have compared organic and conventional food production, both in relation to their nutritional value and in relation to its content of chemical residues (Smith-Spangler et al. 2012). With respect to nutrient content, most studies indicate that organic food production does not have a higher nutritional value than conventional production (Smith-Spangler et al. 2012). However, studies have shown that organically produced foods have much less risk of being contaminated by residues of pesticides or other chemical pollutants than conventional foods (Smith-Spangler et al. 2012). Organic livestock are fed with organically produced feed that is free of pesticides and animal by-products (Beane 2013), and therefore it is supposed that there should be lower accumulation of chemical residues. However, practically, there are no studies on the chemical residues' content in organic meat, although some authors have studied the presence of residues of veterinary drugs, heavy metals, microorganisms, and antibiotic resistance in organically and conventionally produced pigs (Hoogenboom et al. 2008). Therefore, the comparison with conventional production meat has also been scarcely studied.

The study of chemical contamination of meat is relevant because the consumption of meat has been associated with the increased incidence of different types of cancer (Abid et al. 2014), and different studies have linked this increased risk of cancer with the presence of carcinogenic chemical substances in meat (Trafialek and Kolanowski 2014). Meat consumption in Europe is high (51.2 kg/year/person) (Chamorro et al. 2012), and according to the Integrated Risk Information System, a variety of the most common pollutants in meat, such as PCBs, hexachlorocyclohexane (HCH), dichlorodiphenyltrichloroethane (DDT) and its metabolites, and some congeners of PAHs, have been classified in group B of carcinogenicity (probable human carcinogens) (WHO 2014). Although cancer slope factors (CSFs) have been calculated for all these probable carcinogens (EPA 2014) and this would allow an estimate of the risk of cancer associated with continuous exposure to them through foodstuff, very few studies have attempted to estimate the carcinogenic risks that are associated with the current pattern of consumption of meat and meat products (Trafialek and Kolanowski 2014). To our knowledge no study to date has considered studying whether organic meat production could be an option to reduce the carcinogenic potential of meat consumption in relation to their content of chemical carcinogens, especially POPs.

This study was designed to test this hypothesis, where the concentrations of 7 PAHs, 18 PCBs, and 8 OCPs for which the CSFs have been calculated were determined in samples of meats (chicken, beef, and lamb) from organic and conventional production. The samples were acquired in large suppliers who serve the entire European territory. The main objective of this study was to use these data to estimate the carcinogenic risk associated with the current level of meat consumption by the European population considering two possible scenarios: consumers that choose organic meats and consumers that choose conventional meats. The methodology that has been recently used to estimate the carcinogenic risk in other food groups, such as fish (Yu et al. 2014), was applied, using the data of food consumption of the Spanish population.

Materials and methods

Sampling

Two purchases of meat samples of the two modes of production (organic and conventional) were made in the last quarter of 2013 and the first of 2014. These purchases were made in supermarkets belonging to large European retail chains located in the Canary Islands (Spain), which have common suppliers, and can therefore be considered representative of the products available to consumers throughout the continent. A total of 76 samples of meat were acquired, which were distributed as follows: 16 samples of lamb (8 from conventional production and 8 from organic production), 32 samples of chicken (20 conventional and 12 organic), and 28 samples of beef (16 conventional and 12 organic). The samples were

processed immediately after arrival at the laboratory. Each meat sample was finely chopped with a knife and milled using a stainless steel food processor. Then, all the samples were frozen at −18 °C until analysis.

Chemicals, reagents, and analytes of interest

Dichloromethane, hexane, ethyl acetate, and cyclohexane (purity >99.9 %) were purchased from Fisher Scientific (Leicestershire, UK). Ultrapure water was produced using a Milli-Q Gradient A10 (Millipore, Molsheim, France). Diatomaceous earth was purchased from Sigma-Aldrich (St. Louis, USA). Bio-Beads SX-3 were purchased from BioRad Laboratories (Hercules, USA). Standards of OCPs, PCB congeners, and the internal standards (ISs, PCB 202, p,p′-DDE-d8, phenanthene-d10, tetrachloro-m-xylene, and heptachloro epoxide cis) were purchased from DrEhrenstorfer, Reference Materials (Augsburg, Germany). Standards of PAHs were purchased from Absolute Standards, Inc. (CT, USA). Stock solutions of each compound at 1 mg/mL were prepared in cyclohexane and stored at −20 °C. Solutions diluted from 0.05 to 100 ng/mL were used for calibration curves.

The analytes selected for this study were 8 OCPs (p,p′-DDT, p,p′-DDE, p,p′-DDD, hexachlorobencene, and the four isomers of hexachlorocyclohexane (α-, β-, γ-, δ-HCH)); 18 PCB congeners, including marker-PCBs (M-PCBs) and dioxin-like PCBs (DL-PCBs) (IUPAC numbers # 28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180, and 189); and 7 PAHs listed as carcinogens in the Toxics Release Inventory Program of the USA and the EPA's Priority Chemical list (EPA 2001) (benzo(a)anthracene, benzo(a)phenanthrene (chrysene), benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene, and indeno(1,2,3-cd)pyrene).

Extraction and cleanup procedure

All the contaminants included in this study are completely lipid-soluble and therefore are found in the lipid fraction of tissues. For this reason, the fat from the meat was firstly extracted. Thus, the samples (5 g) were homogenized in 5 mL of ultrapure water with a disperser (Ultra-turrax, IKA, China). This homogenate was spiked with the ISs mix in acetone (10 μg/mL) to yield a final concentration of 100 ng/mL and was mixed with 30 g of diatomaceous earth to absorb any moisture. The extraction and cleanup method followed the procedures recommended by the European Standard for the determination of pesticides and PCBs in fatty foods (EN 1996a, b), which had been previously validated in our laboratory for different fatty samples of animal origin (Almeida-Gonzalez et al. 2012; Garcia-Alvarez et al. 2014; Luzardo et al. 2014). This method achieves acceptable recoveries that ranged between 71.5 and 103.2 %. Briefly, the fat was extracted using a Soxtec™ 2055 Auto Fat Extraction (Foss® Analytical, Hilleroed, Denmark) apparatus, which consisted of an extraction unit, a control unit, and a drive unit. The samples were placed into the extraction unit, and 20 mL of dichloromethane was added to each of the extraction cups in a closed system, and the cups were heated using an electric heating plate. The three-step extraction consisted of boiling, rinsing, and solvent recovery. The solvent was evaporated in a rotary evaporator (Hei-VAP Advantage™, Heidolph Instruments®, Schwabach, Germany) at 40 °C to prevent analyte losses. Using a precision balance, the fat obtained was carefully weighted into a zeroed glass tube to determine the fat content of each meat sample (percentage). The weighted fat was dissolved in 2 mL of cyclohexane/ethyl acetate (1:1) and subjected to purification by gel permeation chromatography (BioBeads SX-3) using cyclohexane/ethyl acetate (1:1) at a constant flow of 2 mL/min as the eluent. The first 25-min elution volume, which contained the great majority of lipids (>98 %), was discarded. The 25–85-min elution volume (120 mL), which contained all the analytes that were co-extracted with the fat, was collected. The sample was concentrated using a rotary evaporator, and finally, the solvent was evaporated to dryness under a gentle nitrogen stream. The residue was then reconstituted in 1 mL of cyclohexane, and the sample was transferred to a GC vial that was used for the chromatographic analysis. The amount of pollutants per gram of fat was obtained by multiplying by the corresponding correction factor. The amount of contaminants in fresh meat was obtained by correcting for the fat percentage of each sample.

Chemical analysis procedure

All the compounds, plus ISs, were analyzed by gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) (Quantum XL, Thermo Fisher Scientific Inc., Waltham, MA, USA) as previously described (Camacho et al. 2013, 2014; Luzardo et al. 2013b). Briefly, a 30 m×0.25 mm i.d., 0.25-μm film thickness column (BPX5, SGE Inc., Austin, TX, USA) was used as the stationary phase. Helium (99.999 %) was used as the carrier gas at a constant flow of 1 mL/min. The 61-min oven temperature program was as follows: 60 °C held for 1 min, ramped to 210 °C at 12 °C/min and then to 320 °C at 8 °C/min and held for 6 min. The injector temperature was set at 270 °C, and the transfer line was heated to 310 °C. The injection volume was 1 μl in the splitless mode. A timed selected reaction monitoring (SRM) method for the simultaneous analysis of all the compounds in a single run was constructed. The operation conditions of the mass spectrometer were as follows: electron impact ionization (70 eV) in SRM; emission current, 50 μA; ionization source temperature, 220 °C; electron multiplier voltage, 1500 V; scan width, 0.15; scan time, 0.05 s; and peak width, *m/z* 0.7, and Da. Argon (99.99 %) was used as the collision gas at 0.2 Pa.

Quality control

All the recoveries were above 71 %, and it was thus considered acceptable to use this method for all the pollutants. All the individual measurements were corrected by the recovery efficiency for each analyte. All the samples were injected three times, and the values used for the calculations were the mean of the three values. In each batch of samples, three controls were included for every nine vials (three samples): a reagent blank consisting of a vial containing only cyclohexane, a vial containing 2 ng/mL of each of the pollutants in cyclohexane, and an internal laboratory quality control (QC) consisting of melted meat fat spiked with a mixture of all the pesticides (20 μg/kg), and processed using the same method that was used for the samples. The results were considered to be acceptable when the quantification of the analytes in the QC was within 15 % of the deviation of the theoretical value, which occurred in all the injections. The limit of quantification (LOQ) was set to 0.1 ng/g for all the analytes. A zero value was assigned to all the compounds below the limit of detection (LOD), and those compounds below the LOQ were assigned half of the LOQ.

Dietary intake estimates and calculations

To estimate the daily intake of pollutants through the consumption of a certain type of meat, it is necessary to know the concentration of pollutants in that meat (median and mean values expressed in ng/g fresh product) and multiply that value by the daily average consumption of that meat in a given population. Data on food consumption in Europe are published by the European Food Safety Authority (EFSA) from data provided by the Member States of the EU (EFSA 2011). However, the data for the whole EU are available by food groups (meat) rather than for individual foods (pork, beef, and chicken). Given that Spain is one of the EU countries with the highest meat consumption per capita (the third after the Czech Republic and Hungary in the adult population, and the first in child and adolescent population), the values of consumption of individual foods by the Spanish population were used in this study, which have been published by the Spanish Agency for Consumer Food Safety and Nutrition (AECOSAN 2006, 2011). While it has been established that regular organic food consumers tend to consume less amount of meat (up to 33 %) than non-consumers (Kesse-Guyot et al. 2013), in this paper, we have assumed that consumption is identical, for comparison purposes. For the risk assessment, two groups were considered: adults (18 years old and above, average weight 70.1 kg) and children (6 to 10 years old, average weight 30.4 kg).

For calculations of this paper, analytical values have been considered separately and grouped as follows: the total value of OCP residues (∑OCPs) as the sum of the 8 OCPs and metabolites measured; the total value of DDTs (∑DDT) as the sum of the measured values of p,p′-DDT, p,p′-DDE, and p,p′-DDD; and the total value of HCH residues (∑HCH) as the sum of the 4 HCH isomers measured (α-, β-, δ-, and γ-HCH); the total value of PCB residues (∑PCBs) as the sum of the 18 PCB congeners measured; the total value of the marker PCB residues (∑M-PCBs) as the sum of the 7 congeners considered as markers of environmental contamination by PCBs (#28, 52, 101, 118, 138, 153, and 180); the total value of dioxin-like PCBs (∑DL-PCBs) considered as the sum of the measurements of the 12 individual congeners (#77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189); and the total content of carcinogenic PAHs (∑c-PAHs) as the sum of the values of the 7 US-EPA compounds following the EFSA recommendations (EFSA 2008). Additionally, for the risk estimation, we calculated the potential toxicity (in terms of toxic equivalence to dioxins, TEQs) for the DL-PCBs using the toxic equivalency factors (TEFs) as revised by the World Health Organization (WHO) in 2005 (Van den Berg et al. 2006), and the potential toxicity in terms of benzo[a]pyrene toxic equivalents ($B[a]P_{eq}$) using the TEFs, which are established for the carcinogenic PAHs (Nisbet and LaGoy 1992).

The CFSs of the carcinogens included in this study were taken from the EPA's IRIS (EPA 2014) and were as follows: 1 per mg of substance/kg body weight-day (mg/kg-day) for marker PCBs (based on Aroclors 1260, 1254, 1242, and 1061), 1.1×10^5 per mg/kg-day for dioxin-like PCBs (based on 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8,-TCDD)), 0.34 per mg/kg-day for DDTs, 1.8 per mg/kg-day for HCHs (as there are not CFS values listed for total HCHs, the values listed for β- and γ-HCH were used), 1.6 per mg/kg-day for hexachlorobencene, and 7.3 per mg/kg-day for PAHs (based on benzo[a]pyrene).

Carcinogenic risk calculation

To estimate whether chemical contamination by carcinogens of meat endangers the consumers, we applied the risk assessment index, known as the risk quotient (RQ), using the methodology that has been used for other food groups, such as fish (Yu et al. 2014). RQ is defined as the ratio between the current consumption of meat (R_{meat}) and the maximum tolerable consumption of these products, which is calculated taking into account the concentrations of carcinogens in these foods (CR_{lim}) as follows:

$$CR_{\mathrm{lim}} = \frac{ARL \cdot BW}{\sum_{m=1}^{X} C_{\mathrm{m}} \cdot CSF_{\mathrm{m}}}$$

where CR_{lim} is the maximum allowable consumption rate (kg/day) for a particular meat; ARL is the maximum

acceptable individual lifetime risk level (dimensionless), and a value of 10^{-5} was used in this study (Yu et al. 2014); BW is the body weight (kg); C_m is the median concentration of contaminant m in a particular meat (mg/kg) as determined in this study; and CSF_m is the cancer slope factor of a contaminant m (mg/kg/day) with carcinogenic potential. In the case of multiple contaminants with the same CSF, their concentrations in a particular type of meat were summed (from $m=1$ to $m=x$).

Then, the RQ for each food item and contaminant was calculated as follows:

$$RQ = \frac{R_{meat}}{CR_{lim}} \text{ (for a single contaminant)}$$

$$RQ = R_{meat} \cdot \sum_{m=1}^{x} \frac{1}{CR_{lim}} \text{ (for multiple contaminants)}$$

Thus, if the value of RQ is equal to or less than 1, there is no carcinogenic risk associated with the ingestion of contaminants through the consumption of a particular type of meat. Otherwise, the population is considered to be at carcinogenic risk when RQ is greater than 1, indicating that the current consumption of that foodstuff is greater than its CR_{lim} value.

Statistical analysis

The PASW Statistics v 19.0 software package (SPSS Inc., Chicago, IL, USA) was used to manage the database of the study and to perform the statistical analyses. Normality was examined using the Kolmogorov-Smirnov test. The POP distributions in the meat samples lacked normality and homoscedasticity; therefore, we used non-parametric tests (the Mann-Whitney and Kruskal-Wallis tests). The results are reported as the medians and percentiles 25th–75th ranges. Probability levels of less than 0.05 (two-tailed) were considered statistically significant.

Results and discussion

Distribution of persistent organic pollutants with carcinogenic potential in organically and conventionally produced beef, chicken, and lamb

The main objective of this paper is to provide an estimate of the level of exposure to carcinogenic POPs through consumption of beef, chicken, or lamb, depending on their mode of production (conventional production or organic production). While the levels of many of these substances have been identified in previous studies carried out in different parts of the world, all these works have been performed in conventionally produced meat. As far as we know, no work has been done specifically on organic meats. Also, as one might expect, the levels of carcinogenic POPs in meats published to date are highly variable (sometimes very significantly) (Costabeber et al. 2006; Letta and Attah 2013; Malisch and Kotz 2014; Pardio et al. 2012; Polder et al. 2010; Schecter et al. 2010; Tornkvist et al. 2011; Wang et al. 2011), which is logical because it is very common to find regional variations in contaminant levels. Since the objective of this paper is not to compare our results with previous works but to make a comparison of exposure depending on the product chosen by consumers, to make a realistic estimate, we preferred to directly quantify the contaminants in a representative sample of the main types of meat that any European consumer can find in supermarkets of the continent, and directly determine over them carcinogenic contaminant levels. Table 1 presents a summary of the data obtained directly from these samples, expressed as median and percentiles 25th and 75th.

As it would be expected in foods of animal origin, none of the samples was free of all the contaminants investigated. Both, meat samples from organic production and from conventional production, presented an average of 19 residues (ranging from 11 to 24 residues out of 33). In any case, the levels found in all samples were below the levels legally established in Europe (maximum residue levels, MRLs) (EC 2006a, b). The highest levels of contaminants found in this study were those of the M-PCBs in lamb, both organically and conventionally produced, and those of DDTs, also in organic and conventional lamb. In fact, these two sets of pollutants, the M-PCBs and DDTs, were the most abundant in all types of meat, as shown in Table 1. With regard to the PCB content of meats, it is remarkable that these were completely dominated by congeners 118, 138, 153, and 180 in all the cases, and thus, the ∑M-PCBs contributed with 94.3–99.8 % to the ∑PCBs. In fact, DL-PCBs were the contaminants that reached the lowest levels in all meat types, and therefore the toxic equivalent quantity (TEQ) levels for dioxin-like PCBs in the meat samples analyzed had low median values (range from 0.01 to 0.41 pg/g w.w.) (Table 1).

Two facts attracted attention of our results. First, the fact that pollution levels are quite different between distinct types of meats. Thus, lamb is by far the one with the highest levels of all pollutants studied. At the other extreme, we find the chicken (skinless) having the lowest levels in all cases. The beef meat shows intermediate values (Table 1). These differences are probably attributable to the very different percentages of fat of each type of meat, because when we compare the data expressed as nanograms of carcinogen per gram of fat rather than per gram of fresh product, the differences are much smaller (data not shown).Second, it is interesting to note that the differences between the two modes of production, organic and conventional, can be considered minimal, generally speaking. Table 1 shows the values of statistical significance found for each of the pollutants and meats. As seen above, the highest differences were found between organic and

Table 1 Concentrations of contaminants with carcinogenic potential (ng g^{-1} w.w.) in samples of organic and conventional meat available in the European market

	∑DDTs		∑HCHs		HCB		∑M-PCBs		∑DL-PCBs		$\sum TEQ_{DL\text{-}PCBs}$[a]		$\sum B[a]P_{eq}$	
	Mean	Median (P25–75)	Mean	Median (P25–75)	Mean	Median (P25–75)	Mean	Median (P25–75)	Mean	Median (P25–75)	Mean	Median (P25–75)	Mean	Median (P25–75)
Lamb														
Conventional	196.0	247.4 (34.6–345.2)	9.3	9.8 (2.7–15.2)	314.4	325.1 (99.3–452.1)	51.7	50.6 (23.9–70.4)	3.5	2.7 (0.0–7.9)	0.29	0.27 (0.19–0.41)	3.7	3.3 (1.4–6.3)
Organic	436.9	300.5 (34.1–661.2)	179.9	164.4 (5.4–369.9)	294.0	302.3 (96.2–472.6)	61.3	64.8 (37.3–81.7)	1.1	1.1 (0.0–2.2)	0.07	0.06 (0.01–0.13)	3.4	2.5 (0.9–6.7)
Ratio[b]	0.82		0.06		1.01		0.78		2.45		4.5		1.32	
P value[c]	*0.8918*		*0.0123*		*0.9842*		*0.2709*		*0.1324*		*0.0004* ***		*0.7590*	
Chicken														
Conventional	8.51	7.5 (4.6–10.7)	2.9	2.6 (0.8–4.5)	2.3	3.0 (0.3–3.3)	13.9	14.0 (5.6–17.2)	0.3	0.0 (0.0–0.7)	0.02	0.01 (0.01–0.03)	0.3	0.2 (0.1–0.3)
Organic	7.2	7.6 (3.8–12.4)	1.3	0.9 (0.3–2.1)	5.5	4.3 (1.3–9.2)	14.3	16.8 (7.9–18.2)	0.2	0.2 (0.0–0.4)	0.02	0.02 (0.01–0.03)	0.2	0.2 (0.2–0.3)
Ratio[b]	0.99		2.88		0.70		0.83		0.5		0.5		1	
p value	*0.9796*		*0.0275**		*0.0105* *		*0.8676*		*0.6927*		*0.7386*		*0.9527*	
Beef														
Conventional	25.6	20.4 (10.6–37.1)	1.1	1.1 (0.1–1.4)	2.1	2.4 (1.1–2.7)	28.2	25.6 (17.2–37.6)	2.4	2.1 (1.4–3.3)	0.15	0.12 (0.08–0.21)	1.2	0.9 (0.6–1.5)
Organic	14.9	14.2 (8.7–20.8)	9.0	11.4 (0.1–15.4)	6.8	7.9 (2.1–10.4)	23.6	17.7 (13.6–34.5)	1.2	1.1 (0.7–1.4)	0.07	0.06 (0.04–0.11)	0.6	0.6 (0.4–0.7)
Ratio[b]	1.43		0.09		0.30		1.44		1.91		2		1.5	
p value	*0.3626*		*0.0604*		*0.0131**		*0.3482*		*0.0014* **		*0.0009* ***		*0.0054* **	

[a] $\sum TEQ_{DL\text{-}PCBs}$ are expressed in pg g^{-1} w.w.

[b] Ratio of the median levels (conventional to organic)

[c] *P* value results from the comparison between the medians (Mann-Whitney test), where:

*=p<0.05

**=p<0.01

***=p<0.001

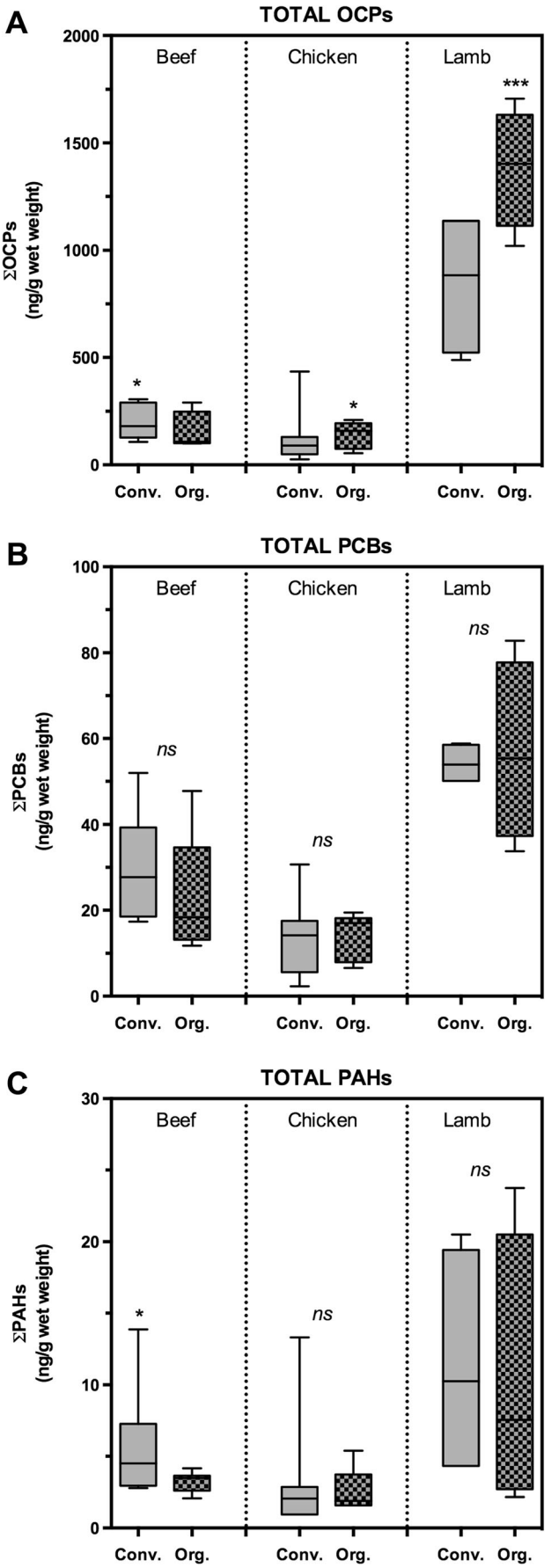

Fig. 1 Box plots of the levels of ∑OCPs (**a**), ∑PCBs (**b**), and ∑c-PAHs (**c**) in the three types of meat studied, and comparison between the two modes of production of these meats (conventional vs. organic). The *line* inside the *boxes* represents the median, the *bottom* and *top* of the *boxes* are the first and third quartiles of the distribution, and the *lines* extending vertically from the *boxes* indicate the variability outside the upper and lower quartiles

conventional beef. However, contrary to what one might think, not in all cases the values were lower in meat from organic production. Thus, as it can be observed in Table 1, in the case of hexachlorobenzene (HCB), levels found in samples of organic beef and organic chicken were significantly higher than those found in the same meats from conventional production. Also noteworthy is the case of HCH isomers (∑HCH) since, although not statistically significant, a trend is observed that the levels are higher in lamb and beef from organic production. However, in relation to these contaminants, the trend was reversed in chicken meat (in this case with statistical significance, $p>0.05$, Table 1).

As the differences were not very relevant when analyzed by pollutants, we also performed the comparison by grouping them by chemical classes: OCPs, PCBs, and PAHs. The results are shown in Fig. 1. The only significant differences in the level of pollutants according to the mode of production were found for OCPs (in the three types of meat) and PAHs (in beef). The most striking results were that organic lamb meat contained much higher levels of OCPs than conventionally produced lamb ($p<0.001$, Fig. 1). These higher levels were also found in chicken meat, but the differences were much smaller ($p<0.05$). However, for beef meat, the situation was reverse: conventional production beef showed higher levels of OCPs than beef from organic production, and the same was obtained for the levels of PAHs ($p<0.05$ in both cases).

Dietary intake of carcinogenic POPs through the consumption of organic and conventional meats by adults and children

Dietary exposure calculations are performed by combining data on consumption habits with the concentrations of contaminants found in food samples. The estimation of food consumption, nutrient intake, and contaminant exposure is a topic of growing interest in the field of public health as a means to inform and guide the actions on food security and nutrition, and as a predictive method for determining the state of health of populations. For this reason, the European Food Safety Authority (EFSA) performs a collection of food consumption data from the different Member States (MS), which must develop nutritional surveys in their territories. On this basis, the Authority prepares an European database of Food Consumption (http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm). For the estimates in this paper, the data provided by the authorities on food security of our country,

Spain (AECOSAN 2006, 2011), were used. The reason for this is that Spain overall meat consumption has steadily increased over the last few decades (Kanerva 2013; Leon-Munoz et al. 2012), and currently, the meat industry is ranked in fifth position in the industrial sector of the Spanish economy and is ranked first among the agro-food industries (Chamorro et al. 2012). Thus, meat consumption in Spain increased from one of the lowest ones in the EU reaching an average per capita consumption of 52.7 kg/year/person, which is even higher than the European average (51.2 kg/year/person) (Chamorro et al. 2012). In any case, using food consumption data from any EU country, and the concentrations of pollutants reported in this paper, daily intake levels of these pollutants for the different European populations can be easily obtained. It should be noted that in our estimates, we have assumed that all consumers (strict consumers of organic products, occasional consumers of organic products, and non-consumers of organic products) equally fit the pattern of consumption defined in nutrition surveys. This assumption has been made for comparison purposes, although it has been recently described that those strict consumers of organic products tend to consume up to 30 % less meat than consumers of conventional products (Kesse-Guyot et al. 2013), and therefore their actual exposure would be lower than the estimates presented here.

Table 2 summarizes the dietary intake of all the contaminants included in this study arranged by groups (on the basis of their similar carcinogenic potential), for children (6–10 years) and adults (>18 years).

First, with regards to the OCPs, our results show that the estimated daily intake (EDI) of ∑DDTs through the consumption of these three types of meat for adults living in Spain is 55.71 ng/kg body weight (b.w.)/day if they choose to consume conventional products, and 86.75 ng/kg b.w./day if they choose to consume organic meats, mainly due to the contribution of lamb meat in both cases. In children, the exposure to ∑DDTs through meat consumption is even higher, being 91.02 ng/kg b.w./day for consumers of conventionally produced meat, and up to 119.86 ng/kg b.w./day for consumers of organic meats. In any case, it should be noted that these consumptions represent only between 0.5 and 1.2 % (depending on the consumer and their choice) of the provisional tolerable daily intake (TDI) for humans established by the World Health Organization for these contaminants (0.01 mg/kg b.w./day)(JECFA 2000). With regard to the exposure to ∑HCHs through meat consumption, the EDIs were much higher in adults and children consuming organic products (36.8 and 51.59 ng/kg b.w./day, respectively) than in consumers of conventionally produced meats (4.08 and 7.67 ng/kg b.w./day, respectively) ($p<0.005$). However, it is remarkable that, again, in this case, the exposure to ∑HCHs is also far from the established TDIs (5000 ng/kg b.w./day) (JECFA 2000), representing less than 1 % of this value even in the worst scenario. The main contributors of this exposure to ∑HCHs were organic lamb and organic beef. Finally, within the group of OCPs, with regard to the intake of HCB, we found that once again the major contributor was by far lamb meat. However, in this case, organically and conventionally produced lamb contributed almost equally to the exposure to this contaminant. Thus, the HCB EDIs for adults and children who consume organic meat were 56.49 and 76.57 ng/kg b.w./day, respectively, and were 54.49 and 68.62 ng/kg b.w./day for

Table 2 Median values of dietary intakes of carcinogenic POPs (ng/kg b.w./day) by means of the consumption of organic and conventional meats for Spanish adults and children

	Food consumption (g/day)	∑DDT	∑HCH	HCB	∑M-PCB	∑DL-PCB	∑TEQ[a]	∑B[a]P_{eq}
Adults								
Lamb (conventional)	11.5	32.16	1.52	51.59	8.49	0.58	0.05	0.61
Lamb (organic)	11.5	71.68	29.52	48.24	10.05	0.18	0.01	0.55
Chicken (conventional)	42.7	5.18	1.75	1.41	8.46	0.16	0.01	0.17
Chicken (organic)	42.7	4.36	0.80	3.35	8.71	0.13	0.01	0.13
Beef (conventional)	50.4	18.37	0.81	1.49	20.24	1.74	0.11	0.86
Beef (organic)	50.4	10.71	6.48	4.90	16.94	0.83	0.05	0.40
Children								
Lamb (conventional)	6.0	38.69	1.83	62.06	10.21	0.70	0.06	0.73
Lamb (organic)	6.0	86.24	35.52	58.03	12.09	0.21	0.01	0.66
Chicken (conventional)	43.1	12.07	4.07	3.28	19.70	0.37	0.03	0.41
Chicken (organic)	43.1	10.14	1.87	7.80	20.27	0.31	0.03	0.31
Beef (conventional)	47.9	40.26	1.77	3.28	44.36	3.82	0.23	1.89
Beef (organic)	47.9	23.48	14.20	10.74	37.13	1.82	0.10	0.88

[a] Expressed in pg/kg b.w./day

Table 3 Values of maximum allowable consumption rate of conventional or organic meat (CR_{lim}), expressed in g/day

	Food consumption (g/day)	∑DDT	∑HCH	HCB	∑M-PCB	∑DL-PCB	∑B[a]P_{eq}
CR_{lim} for adults (g/day)							
Lamb (conventional)	11.5	10.2	40.8	1.4	13.1	210.5	25.1
Lamb (organic)	11.5	4.6	2.1	1.4	11.1	936.6	27.8
Chicken (conventional)	42.5	234.9	131.6	184.0	48.9	2512.9	325.8
Chicken (organic)	42.5	279.7	286.2	77.3	47.6	2853.1	427.4
Beef (conventional)	50.4	78.3	335.5	204.4	24.2	418.3	77.6
Beef (organic)	50.4	134.2	41.9	62.4	28.9	929.6	167.0
CR_{lim} for children (g/day)							
Lamb (conventional)	6.0	5.3	21.0	0.7	6.8	108.3	12.9
Lamb (organic)	6.0	2.4	1.1	0.7	5.7	482.1	14.3
Chicken (conventional)	43.1	120.9	67.7	94.7	25.2	1293.4	167.7
Chicken (organic)	43.1	144.0	147.3	39.8	24.5	1468.5	220.0
Beef (conventional)	47.9	40.3	172.7	105.2	12.4	215.3	39.9
Beef (organic)	47.9	69.1	21.6	32.1	14.9	478.5	85.9

The current values of consumption of these products by the Spanish population (g/day) are included for reference

those adults and children consuming conventionally produced meats. In the case of this contaminant, these EDIs represent between 6.8 and 9.57 % of the TDI set by WHO for HCB (JECFA 2000).

With regard to the group of M-PCBs, the exposure through meat consumption was very similar between consumers of organic and conventional meats, as seen in Table 2. The main dietary intake of these contaminants is from beef. Thus, adult population would be exposed to 37.19 ng/kg b.w./day if they consume conventionally produced meat, and to 35.71 ng/kg b.w./day if they consume organic meats. In children, the PCB EDIs are almost double than in adults (74.27 and 69.49 ng/kg b.w./day for conventional and organic meat consumers, respectively). As, for the total PCBs, a TDI value has not been established, it is necessary to consider the intake of the most toxic compounds, the DL-PCBs, expressed in terms of the equivalency to dioxin toxicity, to assess the level and exposure. Using this approach, our estimates of $\sum TEQ_{DL\text{-}PCBs}$ would represent 3.5 to 16 % of the TDI of 2 pg/kg b.w./day (SCF 2000). In this case, the highest levels of exposure were found for children who consume conventionally produced meats. Our results are in accordance to other studies where authors have estimated that meat is an important source of dioxins and dioxin-like compounds in a high percentage of the samples analyzed (Costabeber et al. 2006; Malisch and Kotz 2014; Schecter et al. 2010; Schwarz et al. 2014; Tornkvist et al. 2011). These results are worrisome because the possibility exists that certain consumers may be subject to high dietary exposures to dioxins, even though they choose to consume organic food.

Finally, with regard to the last chemical group studied, the c-PAHs, we found that conventionally produced beef meat was the major contributor. As shown in Table 2, the EDIs (expressed as equivalents of B[a]P) for the adult population is 1.6 ng/kg b.w./day if they consume conventionally produced meat, and a little bit lower if they choose to consume organic meats (1.08 ng/kg b.w./day). In children, the EDIs are 3.03 and 1.85 ng/kg b.w./day for consumers of conventional and organic meats, respectively. To date, the WHO has not yet established TDI values for c-PAHs or benzo[a]pyrene (JECFA 2000). However, other references may be used. Thus, using the Contaminated Land Exposure Assessment (CLEA) model of the UK, which has established a TDI for B[a]P_{eq} of 20 ng/kg b.w./day (CLEA-UK 2008), the current meat consumption in Spain would represent up to 15 % of these values (in children consuming conventionally produced meats).

POP-associated carcinogenic potential of the current consumption of organically or conventionally produced meats

As shown in the previous section, in all cases, the intake of contaminants through the consumption of meat represents a relatively discrete percentage of the TDI established for each of them. However, the main objective of our research is focused on the study of the carcinogenic potential associated with the consumption of carcinogenic POPs with meat. It has been established that the RQ evaluation is a good method to estimate the risk of a population (in this case the carcinogenic risk), and to establish exposure limits to chemicals (USEPA

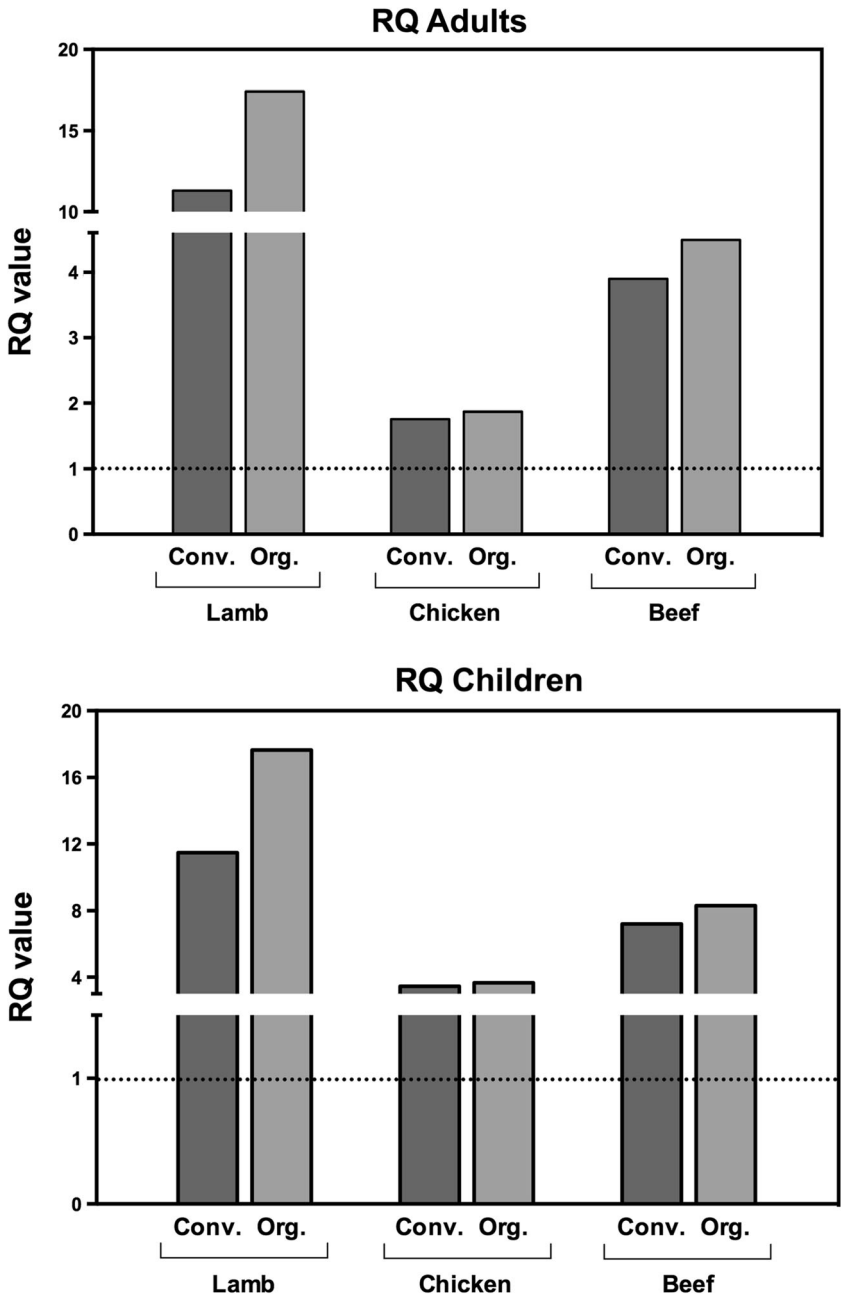

Fig. 2 Risk quotients of the contaminants for carcinogenic effects in adults (*upper panel*) and children (*lower panel*) via consumption of conventionally or organically produced meats. The *discontinuous horizontal line* indicates the threshold for carcinogenic risk ($RQ=1$)

2000). However, the application of this method to multiple chemical contaminants present in food has some weaknesses because assumptions must be made. Thus, it is well known that chemicals in foods are usually present in mixtures of various compounds belonging to different chemical classes, and thus they may exert their adverse effects on consumers interacting with each other in synergistic, additive, or even in antagonistic manners. However, the calculation model of the RQ implies the assumption that all pollutants cause similar toxicological effects and that the combined effect is the sum of the individual effects (USEPA 2000). Nevertheless, as recommended by the USEPA, we have considered that all carcinogens studied are similar and, therefore, we have used the additive model for calculating the RQs.

To do this, the CR_{lim} values (which represent the maximum allowable consumption of each meat type on the basis of their load of pollutants) were used for both adults and children (Table 3). According to these results, the current pattern of consumption of meat in the Spanish population implies that some of these limits are exceeded. Thus, the CR_{lim} of ∑M-PCBs is exceeded by the consumption of all three types of meat, either organic or conventional, both in adults and

especially in children. The CR_{lim} of HCB and ∑DDTs are also exceeded by lamb consumption in both age groups, regardless the type of meat production and consumers' choice. Finally, current consumption of conventionally produced beef also implies that the CR_{lim} values of ∑DDTs and $B[a]P_{eq}$ are overpassed in children.

According to the CR_{lim}, we calculated *RQs* associated with the current consumption of each meat type for both adults and children (Fig. 2). From our results, two facts powerfully attract the attention. First, the current pattern of consumption of meat implies a carcinogenic risk ($RQ>1$) in all cases. The calculated carcinogenic risk ranges between 1.76 and 17.41 in adults, and between 3.45 and 17.65 in children. On the other hand, it is striking that the POP-associated carcinogenic risk tends to be higher in organic meats that in those which are conventionally produced. This is especially relevant in lamb meat, where the consumption of organic product implies a carcinogenic risk up to 1.5 times higher than the consumption of the conventionally produced option, both in adults and children.

Conclusions

In this research, the concentrations of 33 persistent organic pollutants with carcinogenic potential were determined in a large sample of meats available in the European supermarkets from two modes of production: conventional and organic. A mean of 19 of these 33 contaminants in all the samples tested (11–24) were found, but in no case the established MRLs were exceeded. Some significant differences in the levels of pollutants between organically and conventionally produced meats were found, but these differences can be considered of minor entity. As it is well known that continued exposure to carcinogens is not without risk (even at very low doses), the daily intake of these contaminants from the meat were estimated, taking a high meat consumer population such as the Spanish population as a model (adults and children). According to these estimates, exposure is similar in children than in adults, and also very similar if these consumers choose conventional or organic meats, generally speaking. The approximation of the risk ratio was used to evaluate the carcinogenic risk of the current pattern of consumption of these meats in the studied population, and a relevant risk was found in all the cases. Surprisingly, the risk seems to be even higher if the consumer chooses to consume organic meats (especially lamb). This work demonstrates once again that environmental contamination by POPs is ubiquitous and human exposure is very difficult to avoid. Even those consumers who choose to consume organic food, which is theoretically healthier, have exposure rates to these legacy pollutants that can become even higher than those of consumers who eat conventional food. This shows that efforts to minimize the environmental presence of these toxic pollutants should be continued and even strengthened.

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Bloque D Productos de la Pesca

RESUMEN

El consumo medio de productos de la pesca en España se cifra en 89 g/persona/día, donde el consumo de pescado representa aproximadamente el 84% y los otros productos de la pesca un 16% (AECOSAN, 2011). Al menos el 80% de la población lo consume una vez en semana. Según los datos de consumo de pescado recopilados en hogares en el año 2014, cada habitante en España consumió unos 72 g de pescado diariamente (MAGRAMA, 2015).

En este bloque se incluyen dos artículos desarrollados para esta Tesis Doctoral. En el primero de ellos, al igual que en el caso de la carne y los productos cárnicos, la estimación general de exposición a contaminantes tóxicos persistentes se realizó con la premisa de evaluar el riesgo carcinogénico mediante la ingesta de carcinógenos ambientales presentes en los productos de la pesca. Además del riesgo carcinogénico, para este grupo alimentario también se evaluó el riesgo no carcinogénico mediante la ingesta de 8 POC, 18 PCB y 7 PAH (expresados como benzo(a)pireno equivalentes, B[a]Peq) a través del consumo de productos de la pesca. Se incluyeron también tres elementos tóxicos inorgánicos: arsénico (As), cadmio (Cd) y mercurio (Hg). Se adquirieron muestras de las especies más consumidas en España y en el territorio canario, incluyendo pescado blanco (cherne, gallo, lenguado, lubina, merluza, sama, vieja, dorada y panga), pescado azul (atún, salmón, sardina y trucha), cefalópodos (calamar y pulpo) y marisco (gambas, langostino y mejillón).

Así, teniendo en cuenta las ingestas, en este grupo alimentario se calcularon los cocientes de riesgo de carcinogenicidad y toxicidad aguda para niños y adultos. Nuestros resultados indican una mayor ingesta de POC, PCB y B[a]Peq mediante el consumo de pescado azul. Dichas ingestas estimadas (IDE) pueden considerarse bajas o muy bajas para los contaminantes a nivel individual si las comparamos con los valores de referencia, excepto en el caso del hexaclorobenceno (HCB) y el Arsénico (As), y en todos los casos estaban por debajo de las ingestas diarias tolerables (IDT) establecidas.

Considerando los efectos aditivos de múltiples contaminantes, el riesgo de sufrir efectos tóxicos agudos puede considerarse bajo o muy bajo. Sin embargo, nuestros resultados reflejan que el consumo actual de pescado blanco y marisco en adultos y niños, y también de pescado azul en el caso de los adultos, representa un riesgo carcinogénico moderado (RQ>1) para los consumidores españoles, fundamentalmente relacionado con su contenido en As.

En el caso del segundo artículo, su finalidad era determinar los niveles de POC, PCB, PAH y 6 elementos tóxicos inorgánicos (Pb, Cd, Ni, Al, As y Hg) en muestras de productos de la pesca procedentes de pesca extractiva y de acuicultura y estimar la ingesta de dichos contaminantes, considerando consumidores que optan exclusivamente por un método de producción u otro, para estudiar las posibles diferencias a la exposición entre ambos. Para ello, se seleccionaron las mismas especies que para el artículo anterior, a excepción de los cefalópodos, que no se incluyeron al no existir producción acuícola de los mismos.

Se detectaron 30 de los COP estudiados, de los cuales 21 se cuantificaron a mayores concentraciones en los productos de acuicultura, mientras que solo en el caso de los PAH carcinogénicos existían mayores concentraciones en los productos extractivos. Así, los niveles de ingesta de PAH y POC eran significativamente mayores mediante el consumo de productos de acuicultura y, en el caso de la población infantil, también se alcanzó la significación para la ingesta de PCB. Para los elementos inorgánicos, la ingesta de Al, As, Ni y Pb podría ser significativamente mayor en ambos grupos de población mediante el consumo de productos de acuicultura, mientras que para el Cd y el Hg el patrón era inverso (mayor ingesta mediante los productos extractivos).

Se concluye que se debería disminuir el consumo de productos de la pesca para reducir el potencial carcinogénico asociado a los contaminantes presentes en los mismos, especialmente el de pescado blanco, cuyo consumo debería reducirse a la tercera parte en adultos. El consumo de cefalópodos podría mantenerse en ambos grupos de edad, al igual que el del pescado azul en niños, teniendo siempre en cuenta que estas recomendaciones se centran exclusivamente en el potencial carcinogénico asociado a la ingesta de estos contaminantes persistentes. Además, es importante destacar que la implementación de algunas prácticas de descontaminación en la acuicultura podría no solo igualar los niveles de contaminación a los de la pesca extractiva, sino también aportar productos con menores niveles de contaminantes que los capturados en estado salvaje. Este hecho podría representar un incremento del consumo actual recomendado en una ración semanal, beneficiando al consumidor de los efectos positivos para la salud de sus nutrientes.

Basándonos en los resultados de estos artículos, nuevamente parece ser necesario mantener programas de vigilancia que monitoricen la tendencia de la presencia de contaminantes persistentes en los grupos alimentarios y, especialmente, de la concentración de elementos tóxicos, como el arsénico. Los resultados de estos estudios pueden ser útiles para las agencias y organismos en el diseño de campañas de comunicación adecuadas, dirigidas a disminuir el riesgo por el consumo de determinados productos de la pesca, con el objetivo de alcanzar un balance óptimo entre los beneficios y riesgos asociados al consumo de pescado.

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Assessment of human health hazards associated with the dietary exposure to organic and inorganic contaminants through the consumption of fishery products in Spain

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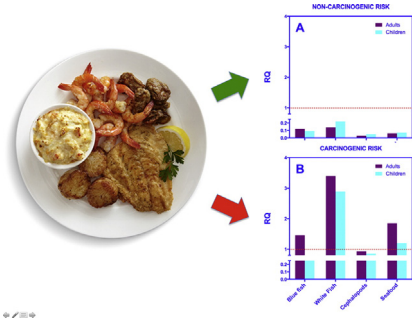
HIGHLIGHTS

- The daily intake of persistent pollutants through fish consumption is estimated
- Dietary intake of individual pollutants did not exceed the Tolerable Daily Intakes.
- Consumption of fishery products does not pose risk of acute toxicity for the Spaniards
- These results may be useful for the design of proper risk communication campaigns.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work we have evaluated the potential carcinogenic and acutely toxic risks associated to the exposure to highly prevalent organic and inorganic contaminants through the consumption of fishery products by the Spanish population. The concentrations of 8 organochlorine pesticides (OCPs), 18 polychlorinated biphenils (PCBs), 7 polycyclic aromatic hydrocarbons (expressed as benzo[*a*]pyrene toxic equivalents ($B[a]P_{eq}$)), and three inorganic toxic elements [arsenic (As), cadmium (Cd), and mercury (Hg)] were determined in 93 samples of the most consumed species of white fish, blue fish, cephalopods and seafood species, which were acquired directly in markets and supermarkets in the Canary Islands, Spain. The chemical concentration data were combined with the pattern of consumption of these foodstuffs in order to calculate the daily intake of these contaminants, and on this basis the risk quotients for carcinogenicity and acute toxicity were determined for Spanish adults and children. Our results showed that the daily intake of OCPs, PCBs and $B[a]P_{eq}$, which is associated to blue fish consumption was the highest within the fish group. The estimated intake of pollutants can be considered low or very low for the individual contaminants, when compared to reference values, except in the case of HCB and As. All the estimated intakes were below the reported Tolerable Daily Intakes. Considering the additive effects of multiple contaminants, the risk of acute toxic effects can also be considered as low or very low. However, our results reflect

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that the current consumption of white fish in adults and children, and also the blue fish in the case of adults, poses a moderate carcinogenic risk to Spanish consumers, mainly related to their concentrations of As. The conclusions of this research may be useful for the design of appropriate risk communication campaigns.

1. Introduction

Organic and inorganic contaminants, such as legacy pesticides, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), mercury (Hg), arsenic (As), or cadmium (Cd) are commonly targeted contaminants for research and in monitoring programs. In the last decades, efforts have been made to raise knowledge about the adverse effects on humans and animals, worldwide distribution pattern, and new methods are developed to analyze these compounds in very different matrices and various environmental media (Luzardo et al., 2013b; Sharma et al., 2014). Thus, numerous studies have revealed that these toxic compounds, individually and in combination, may contribute to the development of severe health problems such as cancer, immune suppression or genotoxic effects in humans, even with long-term low-dose exposure (Bergman et al., 2012; Jarvis et al., 2014; WHO, 2003), and many of them have demonstrated endocrine disrupting effects in both animals and humans (Camacho et al., 2014; Kortenkamp et al., 2011). In fact, the use of organochlorine pesticides (OCPs) and PCBs is now banned in most developed countries, but they are still widespread in the environment (Almeida-González et al., 2012; Kakuschke et al., 2010; Luzardo et al., 2014).

Although there are different routes of exposure for humans to these pollutants, it has been established that ingestion of food contributes more than 90% of total human exposure, and that the fatty fraction of food represents the main entrance to the human body (Darnerud et al., 2006; Vazquez et al., 2015). In the last decade, studies on human dietary exposure to persistent pollutants have been carried out in various countries over the world and it has been reported that the dietary intakes vary considerably between countries. The dietary intakes are mainly influenced by the specific dietary habits of each country (Domingo and Bocio, 2007; Storelli et al., 2011). The daily intake of contaminants needs to be calculated on the basis of the typical food basket consumed in the country obtained from surveys on consumers. The dietary exposure to a wide range of persistent organic and inorganic pollutants of Spanish consumers has been investigated by several authors in the past years for different food groups, such as milk and cheese (Almeida-González et al., 2012; Luzardo et al., 2012), eggs (Luzardo et al., 2013a), yogurt (Rodríguez-Hernández et al., 2015c), meat and processed meat (Rodríguez-Hernández et al., 2015a; Rodríguez-Hernández et al., 2015b), and seafood (Bocio et al., 2007; Domingo and Bocio, 2007; Falcó et al., 2006). Also several basket market studies have been performed in Spain including the major food groups (Bocio and Domingo, 2005; Bocio et al., 2005; Falco et al., 2003; Llobet et al., 2003a; Llobet et al., 2003b; Llobet et al., 2003c), and even the consumption of foods of animal origin has been investigated as a determinant of contamination by OCPs and PCBs (Boada et al., 2014). However, to date only few studies have estimated the carcinogenic risk associated to the exposure to contaminants associated to certain food groups in the Spanish population (Rodríguez-Hernández et al., 2015a; Rodríguez-Hernández et al., 2015b), and to our knowledge none has been developed for the seafood group.

Fish is an important supplier of high quality nutrients such as omega 3 fatty acids, which have been proven reduce the risk of stroke, lower blood pressure and improve arterial integrity, and even decrease the risks of certain cancers (Kris-Etherton et al., 2002). However, fish is also one of the main contributors of the total dietary intake of environmental pollutants (Bocio et al., 2005; Falco et al., 2003; Llobet et al., 2003b; Llobet et al., 2003c). Thus, on the one hand, the health benefits of sea foodstuff consumption have been proven but on the other hand there also exist an increasing concern of the potential risk arising from exposure to toxic pollutants through the intake of fishery products. Because of the growing public concern about the health effects of food borne diseases related to chemical pollutants, there exists the need carrying out studies on particular food groups (such as fish), based on their current pattern of consumption by a given population. In some guidance documents for environmental risk assessment, a reference point from toxicity testing is divided by a default assessment factor and the result compared to the predicted exposure by computing their ratio, which is known as the risk quotient (RQ) (EFSA, 2015; USEPA, 2000). It has been proposed that RQ is a good method to estimate the risk to carcinogenic and acutely toxic effects associated to food contaminants in a population and that is useful to establish exposure limits to those chemicals.

As fish is a staple food of the Spanish diet, with an average consumption of 26.4 kg/person/year (MAGRAMA, 2015) we have designed this study in which we assess the toxic potential of the current pattern of consumption of this food group by the Spanish population. We have acquired seafood samples directly at points of sale to the consumer, and the sampling was designed to follow the Spanish consumers' preferences. We have assessed two types of health risks associated with the consumption of seafood: the carcinogenic risk, and the acute toxicity potential. In this research we have calculated the RQs considering multiple contaminants in fishery products for both carcinogenic and acutely toxic effects, and on this basis we calculated the number of healthy meals per month for a safe consumption in the Spanish population. Obviously, the results of this study need to be considered in the context of the proven health benefits of the nutrients of fish, but may serve for the design of appropriate risk communication campaigns in order to reduce the consumption of certain types of seafood with the aim of optimizing the risk-to-benefit balance.

2. Material and methods

2.1. Sampling

We selected for this study the most consumed species of seafood: fish (white fish and blue fish), cephalopods, crustaceans and bivalve mollusks in Spain, according to the data available (AECOSAN, 2006; AECOSAN, 2011). A total of 93 samples from the main commercial species (MAGRAMA, 2015; Martín Cerdeño, 2010) were randomly acquired from multinational retailers settled in the Canary Islands (Spain) between September and November of 2014. The samples purchased were transported to the Laboratory of Toxicology of the University of Las Palmas de Gran Canaria (ULPGC) and processed immediately upon arrival at the laboratory. We processed and analyzed only the edible parts of seafood (muscle + skin, depending on how the species are consumed). Each sample was constituted by five individual subsamples for each species of fish and cephalopods (fillets, small fishes, or parts of octopus and squids), and six subsamples of each species of crustaceans and mollusks to give pooled samples (using a stainless steel domestic food processor). Thus, 5 to 6 of these composites were used to obtain the data of each species. After that, all samples were frozen at −80 °C (until analysis).

The species of white fish included in this study were: wreckfish (*Polyprion americanus*), megrim (*Stephanolepis hispidus*), sole (*Solea vulgaris*), seabass (*Dicentrarchus labrax*), hake (*Merluccius merluccius*), toothed sparus (*Dentex dentex*), parrot fish (*Sparisoma cretense*), gilt head fish (*Sparus aurata*) and iridescent shark (*Pangasius hypophthalmus*). The selected species of blue fish were: tuna (*Thunnus thynnus*), salmon (*Salmo salar*), sardine (*Sardina pilchardus*), and trout (*Salmo trutta*). Additionally, we included those most consumed species of other seafood

(crustaceans and mollusks) and, cephalopods: shrimp (*Parapenaeus* spp.), prawn (*Penaeus* spp.), mussel (*Mytilus galloprovincialis*), octopus (*Octopus vulgaris*), and squid (*Teuthida* spp.).

2.2. Chemicals, reagents and analytes of interest

All the organic solvents (dichloromethane, hexane, ethyl acetate, and cyclohexane) were of mass spectrometry grade (VWR International, PA, USA). Ultrapure (UP) water was produced in the laboratory using a Milli-Q Gradient A10 apparatus (Millipore, Molsheim, France). The inert desiccant (Celite® 545) was purchased from Sigma-Aldrich (St. Louis, USA). Bio-Beads SX-3 was purchased from BioRad Laboratories (Hercules, USA). Standards of OCPs, PCB congeners, and internal standards (ISs, PCB 202, tetrachloro-*m*-xylene, p,p′-DDE-d8, heptachlor epoxide cis, and phenanthrene-d10), were purchased from Dr Ehrenstorfer, Reference Materials (Augsburg, Germany). Standards of PAHs were purchased from Absolute Standards, Inc. (Connecticut, USA). All standards were neat compounds. Stock solutions of each compound at 1 mg/mL were prepared in cyclohexane and stored at −20 °C. Diluted solutions from 0.05 ng/mL to 40 ng/mL were used for calibration curves (9 points).

All samples were screened for the presence of the following anthropogenic contaminants: (a) 8 OCPs: the four isomers of hexachlorocyclohexane (α-, β and γ-, and δ-HCH), p,p′-DDT and its metabolites (p,p′-DDE, and p,p′-DDD) and hexachlorobenzene (HCB); (b) a total of 18 congeners of PCBs: the six marker PCBs (M-PCBs), and the 12 dioxin-like PCBs (DL-PCBs), which were numbered according to the International Union of Pure and Applied Chemistry (IUPAC): IUPAC numbers 28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 180, 189; (c) the 7 PAHs listed as carcinogens by the United States Environmental Protection Agency: benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a*,*h*]anthracene and indeno[1,2,3,-*cd*]pyrene. Finally, we also included the analysis of 3 inorganic toxic elements, which have been reported to be very abundant in fish: arsenic (As), cadmium (Cd), and mercury (Hg).

2.3. Extraction and clean-up

Prior to the extraction procedure, samples were lyophilized for 72 h. For the extraction of organic pollutants from fishery products samples, we firstly extracted the fat because all these chemicals are completely lipid-soluble and therefore found bound to the lipid fraction. 5 g of each lyophilized sample was spiked with the ISs mix (10 μg/mL) in acetone to yield a final concentration of 20 ng/g and mixed with 30 g of Celite® to absorb all humidity. The method of extraction and purification followed that recommended by the European Standard for the determination of pesticides and PCBs in fatty food (EN, 1996a; EN, 1996b), whose validity has been previously proven in our laboratory for fatty samples (Camacho et al., 2014; Camacho et al., 2013a; García-Álvarez et al., 2014a). This method combines an automated Soxhlet extraction method (FOSS Soxtec Avanti 2055) with a purification step using gel permeation chromatography (GPC), and gives acceptable recoveries that range between 74.5% and 104.7%. Briefly, the Soxtec™ 2055 Auto Fat Extraction (Foss® Analytical, Hilleroed, Denmark) apparatus consisted of an extraction unit, a control unit and a drive unit. The samples, prepared as described above, were inserted into the extraction unit, 40 mL of solvent (dichloromethane) were added to the extraction cups in a closed system and the cups were heated with an electric heating plate. The three-step extraction consisted of boiling, rinsing and solvent recovery. The recovered solvent was evaporated in a rotary evaporator (Hei-VAP Advantage™, Heidolph Instruments®, Schwabach, Germany) at 40 °C to prevent analytes loses. Using a precision balance, the fat obtained was carefully weighted into a zeroed glass tube in order to be able of correcting the results and express them against fresh weight of product. 100 mg of the Soxhlet extracted fat were dissolved in 2 mL of cyclohexane/ethyl acetate (1:1) and subjected to purification by gel permeation chromatography (BioBeads SX-3) using cyclohexane/ethyl acetate (1:1) at a constant flow of 2 mL/min as the eluent. The first 25 min of elution, containing the great majority of lipids (>98%), were discarded. The 25–90 min elution volume (130 mL), containing all of the analytes that were co-extracted with the fat, was collected. The sample was concentrated using a rotary evaporator, and finally the solvent was evaporated to dryness under a gentle nitrogen stream. The analytes were re-dissolved in 1 mL of cyclohexane without any further purification and these extracts in cyclohexane were used for the gas chromatography/triple quadrupole mass spectrometry (GC–MS/MS) analysis.

For the analyses of inorganic contaminants, 0.5 g aliquots of lyophilized samples were mineralized with 6 mL of nitric acid (HNO_3) and 50 μL of yttrium (Y) was added as an internal standard. Vessels were then placed inside a microwave oven (Milestone ETHOS ONE) and heated up to 190 °C for 50 min. All of the reagents used were of high quality, for analysis of trace elements (Suprapur, Merck, Darmstadt, Germany). After cooling, digested samples were filtered with 1 μm strainer and diluted to a final volume of 50 mL with distilled water into a conical polypropylene tube.

2.4. Procedure of chemical analysis, quality assurance (QA) and quality control (QC)

Gas chromatography analyses of organic contaminants were performed in a single run on a Thermo Trace GC Ultra equipped with a TriPlus Autosampler and coupled to a Triple Quadrupole Mass Spectrometer Quantum XLS (Thermo Fisher Scientific Inc., Waltham, MA, USA), as previously described (Bucchia et al., 2015; Formigaro et al., 2014), and identifications were done using an electron ionization (EI)-MS/MS based on the retention time and the relative ion ratios of each of the analytes. Quantifications were performed against calibration curves as mentioned above. The LOQs of organic pollutants ranged from 0.008 to 0.028 ng/g wet weight, as previously described (García-Álvarez et al., 2014b) (Supplementary Table 1).

All the measurements were performed in triplicate, and we used the means for the calculations. In each batch of samples, four controls were included for every 18 vials (6 samples): a reagent blank consisting of a vial containing only cyclohexane; a vial containing 2 ng/mL of each of the pollutants in cyclohexane; and an internal laboratory quality control sample (QCs) consisting of fish oil spiked at 20 ng/mL of each of the analytes, which was processed using the same method as the seafood samples. The results were considered to be acceptable when the concentration of the analytes determined in the QC sample was within 15% of the deviation of the theoretical value.

Inorganic elements (As, Cd, and Hg) were quantified with inductively coupled plasma-optic emission spectrometry technique (ICP-OES) using a PerkinElmer Optima 2100 DV instrument coupled with a CETAC U5000AT + ultrasound nebulizer for mercury. A calibration curve and two blanks were run during each set of analyses to check purity of the chemicals, and the blank reading was subtracted from all of the experimental readings. The sample readings (two replicates for each sample and three readings for each replicate) were performed using axial plasma, which provides increased sensitivity, lower background, and improved the limits of detection (LODs) compared to traditional radial plasma. This sensitivity enhancement results in a 5- to 10-fold improvement in the detection limits compared with radially viewed plasma. The concentration values were obtained from the mean of each three readings. The accuracy of the method was verified using reference materials (CRM 278: lyophilized mussel, Community Bureau of Reference, BCR, Brussels). All values of reference materials were within the certified limits. LODs, expressed by wet weight (w.w.), were 0.1 ng/g for As; 1.8 ng/g for Cd; and 0.061 ng/g for Hg. The LODs were determined following the protocol described by Perkin Elmer ICP application study number 57.

2.5. Dietary intake estimates and calculations

For the assessment of the contaminants' exposure through the consumption of fishery products, we first grouped the results of contaminants in food as white fish, blue fish, cephalopods, and seafood (mean values, expressed in ng/g fresh product), and then multiplied these values by the average daily consumption rate of each one of these types of food (expressed in grams/day). Following the recommendations of the EFSA we have used also the percentile 97.5th of consumption to calculate the estimated daily intakes (EDIs) using the upper-bound approach. These assessments (middle-bound (MB) and upper bound (UB)) were done for both adults and children (average body weight: 68.48 and 34.48 kg, respectively). A zero value was assigned to all the compounds below the LOD, and for those compounds below the limit of quantification (LOQ) but above the LOD, the value was assumed to be 1/2 LOQ (Camacho et al., 2013b; Luzardo et al., 2013b). Food consumption data of the Spanish population were obtained from the Spanish Diet Model for the Determination of the Consumer's Exposure to Chemicals of the Spanish Agency for Consumer Food Safety and Nutrition (AECOSAN, 2006; AECOSAN, 2011).

In this research, for the calculations we considered the total value of DDTs (ΣDDTs) as the sum of the measured values of p,p′-DDT, p,p′-DDE and p,p′-DDD; the total value of HCH residues (ΣHCH) as the sum of the 4 HCH isomers (α-, β-, γ- and δ-HCH); the HCB as an independent contaminant; the value of the PCB congeners that are considered markers of exposition (ΣM-PCB: # 28, 52, 101, 138, 153 and 180); the value of the PCB congeners that are similar to dioxins (ΣDL-PCBs: # 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189). For the risk estimation, we calculated the potential toxicity for the DL-PCBs (in terms of toxic equivalence to dioxins; $\sum TEQ_{DL\text{-}PCBs}$) using the OMS 2005 TEQs (Van den Berg et al., 2006). Finally, we also considered the total content of carcinogenic PAHs ($\sum$ c-PAHs) following the EFSA recommendations (EFSA, 2008). Benzo[*a*]pyrene is the most widely known and studied compound of this group due to its importance as one of the most potent carcinogenic hazards. Thus, the carcinogenic risk of a PAH mixture is often expressed by its BaP equivalent concentration ($B[a]P_{eq}$). Thus, for the risk estimation, we used toxic equivalency factors (TEFs), which are established for the carcinogenic PAHs (Nisbet and LaGoy, 1992), to express the results in the form of benzo[*a*]pyrene toxic equivalents ($B[a]P_{eq}$).

2.6. Risk characterization

We applied a risk quotient (RQ) to estimate whether the intake of contaminated sea foodstuff endangers the Spanish population. We calculated this intake RQ as the ratio between the consumption of a given foodstuff (in this case seafood expressed in grams/day, R_{fish}) and the maximum tolerable consumption of that foodstuff (CR_{lim}), which is calculated taking into account their concentrations of contaminants. We have used this index both, for the calculation of the carcinogenic risk, and also for the risk of acutely toxic effects associated to the consumption of that food.

Thus, in the present study, the carcinogenic effects of multiple contaminants were evaluated using the methodology previously used for different food groups (Rodríguez-Hernández et al., 2015a; Rodríguez-Hernández et al., 2015b; Yu et al., 2014), according to the following formulas:

$$RQ = \frac{R_{fish}}{CR_{lim\ single}} (\text{for a single contaminant}) \quad (1)$$

$$RQ = R_{fish} \cdot \sum_{m=1}^{x} \frac{1}{CR_{lim\ multiple}} (\text{for multiple contaminant groups}) \quad (2)$$

$$CR_{lim(single\ or\ multiple)} = \frac{ARL \cdot BW}{\sum_{m=1}^{X} C_m \cdot CSF_m} \quad (3)$$

where CR_{lim} is the maximum allowable consumption rate for a particular fishery product (kg/day), and may be calculated either for a single contaminant or for various chemicals belonging to the same chemical group, and assuming they share the toxicological properties; ARL is the maximum acceptable individual lifetime risk level, which is dimensionless and a value of 10^{-5} (one-in-100.000) was used in this study, base on the literature (Yu et al., 2014); BW is the body weight (kg); C_m is the median concentration of contaminant m in a particular fishery product (mg/kg); and CSF_m is the cancer slope factor of contaminant m for a carcinogenic hazard (mg/kg/day) − 1. In the case of multiple contaminants with the same CSF, their concentrations in a particular type of seafood were summed (from m = 1 to m = X).

In addition, we evaluated the acutely toxic effects of multiple contaminants using the following equation:

$$CR_{lim} = \frac{BW}{\sum_{m=1}^{X} \frac{C_m}{RfD}} \quad (4)$$

where RfD_m is the reference dose of contaminant *m* for acute toxic effects (mg/kg/day).

The RfD and CSF values of contaminants for carcinogenic and toxic effects were taken from the Integrated Risk Information System (IRIS) of the USEPA (http://www.epa.gov/IRIS/).

According to the previous reports it is considered that if the RQ value is equal or <1 then it can be considered that the risk is low (<10^{-5}) via fishery products consumption. However, the population is considered to be at health risk when RQ is >1. (Yu et al., 2014).

2.7. Meal suggestions for the consumption of seafood

Once we determined the concentrations of pollutants in seafood we considered very useful for the consumer and the health authorities to calculate the maximum intake of these foods that can be considered safe. The USEPA notes that daily fish consumption limits may be more conveniently expressed as the allowable number of fish meals (of a specified meal size) that may be consumed over a given time period (USEPA, 2000; Yu et al., 2014). For the consumer to express this as the number of allowable meals per month is more practical. Therefore, we calculated the number of allowable meals per month considering multiple contaminants for carcinogenic and acute toxic effects according to the following equations:

$$C_{mm} = \frac{R_{fish} \cdot TP}{MS} \quad (5)$$

$$RC_{mm} = \frac{C_{mm}}{RQ} \quad (6)$$

where C_{mm} is the current number of meals per month for each type of fishery product; MS is the meal size (225 g for fish, and 120 g for cephalopods and seafood); TP is the averaged time period (month = 30.44 days); and RC_{mm} is the recommended maximum number of serving of each food per month.

2.8. Statistical analysis

Database management and statistical analysis were performed with PASW Statistics v 20.0 (SPSS Inc., Chicago, IL, USA). Because the data did not follow a normal distribution, the statistical analyses involved the use of non-parametric tests. The differences of contaminants between two independent groups were tested with the Mann–Whitney *U* test and Kruskal Wallis test. *P* values of <0.05 (two-tailed) were considered statistically significant.

Table 1
Concentrations of toxic contaminants associated with carcinogenic and non-carcinogenic effects in fish and seafood most consumed by Spanish population. Results are expressed in ng/g fresh product.

	Blue fish (BF)[a] (n = 20)			White fish (WF)[b] (n = 45)				Cephalopods (CE)[c] (n = 10)			Other Seafood (SE)[d] (n = 18)			
	Mean ± SD	Median	P25 – p75	Mean ± SD	Median	P25 – p75	*P* value (BF *vs.* WF)	Mean ± SD	Median	P25 – p75	Mean ± SD	Median	P25 – p75	*P* value (CE *vs.* SE)
∑DDTs	2.2 ± 1.4	1.5	0.8 – 3.2	0.5 ± 0.7	0.21	0.07 – 0.8	< 0.001	0.3 ± 0.2	0.2	0.0 – 0.5	0.1 ± 0.1	0.1	0.03 – 0.2	n.s.
∑HCHs	0.4 ± 0.2	0.0	0.1 – 0.8	0.1 ± 0.3	0.0	0.0 – 0.5	< 0.01	0.01 ± 0.02	0.01	0.0 – 0.03	0.01 ± 0.02	0.0	0.0 – 0.06	n.s.
HCB	1.0 ± 0.9	0.6	1.1 – 1.7	0.2 ± 0.3	0.1	0.0 – 0.2	< 0.001	0.1 ± 0.09	0.1	0.02 – 0.2	0.02 ± 0.02	0.01	0.0 – 0.02	< 0.01
∑M-PCBs	3.6 ± 3.9	2.6	1.0 – 6.3	0.8 ± 0.9	0.3	0.1 – 1.6	< 0.001	0.4 ± 0.4	0.4	0.02 – 0.8	0.6 ± 1.1	0.12	0.06 – 0.5	n.s.
∑TEQDL-PCBs[e]	0.006 ± 0.005	0.006	0.0003 ± 0.009	0.0015 ± 0.0015	0.0009	0.0003 ± 0.0027	< 0.001	0.0009 ± 0.0008	0.0009	0.0 – 0.0018	0.0009 ± 0.0018	0.0003	0.0 – 0.0006	n.s.
$B[a]P_{eq}$	0.2 ± 0.3	0.2	0.1 – 0.4	0.04 ± 0.05	0.03	0.02 – 0.06	< 0.001	0.2 ± 0.3	0.2	0.01 – 0.4	0.09 ± 0.1	0.04	0.02 – 0.16	n.s.
As	177.7 ± 153.4	126.7	82.0 – 210.2	332.3 ± 271.5	168.1	105.0 – 567.6	< 0.05	514.5 ± 291.0	504.0	239.1 – 801.2	538.7 ± 301.6	597.7	292.2 – 824.6	n.s.
Cd	16.3 ± 7.1	12.3	11.2 – 21.8	14.3 ± 5.8	11.3	11.2 – 20.1	n.s.	28.8 ± 19.9	23.6	11.2 – 48.7	58.1 ± 54.1	22.4	11.7 – 118.0	n.s.
Hg	36.2 ± 16.1	33.6	22.5 – 50.4	45.2 ± 26.8	40.1	33.6 – 44.8	n.s.	27.3 ± 11.8	26.2	17.5 – 38.4	29.3 ± 12.7	22.4	19.2 – 41.05	n.s.

[a] The data were obtained from the individual analysis of 5 samples from each of the following species: Tuna (*Thunnus thynnus*); Salmon (*Salmo salar*); Sardine (*Sardina pilchardus*); and Trout (*Salmo trutta*).
[b] The data were obtained from the individual analysis of 5 samples from each of the following species: Wreckfish (*Polyprion americanus*); Megrim (*Stephanolepis hispidus*): Sole (*Solea vulgaris*); Seabass (*Dicentrarchus labrax*); Hake (*Merluccius merluccius*); Toothed sparus (*Dentex dentex*); Parrot fish (*Sparisoma cretense*); Gilt head fish (*Sparus aurata*); and Iridescent shark (*Pandasius hypophthalmus*).
[c] The data were obtained from the individual analysis of 5 samples from each of the following species: Octopus (*Octopus vulgaris*); Squid (*Theutida* spp.).
[d] The data were obtained from the individual analysis of 6 samples from each of the following species: Shrimp (*Parapenaeus* spp.); Crayfish (*Penaeus* spp.); Mussel (*Mytilus galloprovincialis*).
[e] In the case of $\sum TEQ_{DL\text{-}PCBs}$ data are expressed in pg/g fresh product, as it is usual when considering TEQs.
n.s.: not significant.
Shaded cells indicate the values that are significantly higher in the statistical comparison between groups.

3. Results and discussion

3.1. Occurrence of chemical pollutants in fishery products

Table 1 shows the concentrations of the toxic contaminants included in this study in the different groups of fishery products: blue fish, white fish, cephalopods and other seafood (crustaceans and mollusks). We also present in this table the statistical comparison between the two classes of fish, and also the comparison between cephalopods and seafood. In addition, we also considered interesting to present the comparison between total seafood (including cephalopods) in a graphical manner as supplementary material (Suppl. Fig. 1).

We found great differences in the levels of contaminants among the different groups of fishery products (Table 1), and also among the different species within each group (data not shown). This is logical, because the distribution of the pollutants in the aquatic organisms is highly dependent on the environment that they live, as well as on other many factors, such as the trophic levels, the feeding habits of the species, differences in metabolism due to different abilities of biotransformation, and the excretion rate of these compounds from the body (Liao et al., 2016). Moreover, it is well know that most of the contaminants included in this study are lipid soluble and therefore it is reasonable to find a direct relationship between their concentration and the lipid content of each species. Thus, as seen in Table 1, we found that blue fish (which contains at least 5% of lipids in the edible part) has higher levels of organic pollutants than white fish: ΣDDTs (median: 1.5 vs. 0.21 ng/g); ΣHCHs (median: 0 ng/g in both groups; mean: 0.4 vs. 0.1 ng/g); HCB (median: 0.6 vs. 0.1 ng/g); M-PCBs (median: 2.6 vs. 0.3 ng/g); $\sum TEQ_{DL\text{-}PCBs}$ (0.006 vs. 0.0009 pg/g), and $B[a]P_{eq}$ (0.2 vs. 0.03 ng/g). These findings are consistent with other studies that found that higher levels of contamination occur in blue fish (Mezzetta et al., 2011). We also found that fish in general (blue and white fish) presented higher levels of organic pollutants than cephalopods, mollusks, and crustaceans, which may be also related with the lower percentage of fat of these foods. This is also consistent with the data published previously (Bayarri et al., 2001; Carubelli et al., 2007). The only group in which these differences were not observed was PAHs, (expressed as $B[a]P_{eq}$), as we found that the levels in cephalopods were similar to those in blue fish. Other authors have also previously reported high levels of PAHs in mollusks, even higher than in fish (Martí-Cid et al., 2007), probably due to the fact that most edible sea mollusks are filter feeders.

With regards to inorganic pollutants, we included in this study the determination of As, Cd, and Hg due to the concerns on human health of these elements, and that it has been reported these metals are the most abundant in sea foodstuff (EFSA, 2009a; EFSA, 2009b; EFSA, 2012). There are many studies, which have determined their contents in the edible parts of commercial seafood species, since the monitoring of metal concentrations in fish meat is very important to ensure compliance with food safety regulations and consequent consumers' protection (Bosch et al., 2016). In the present study the pattern of contamination observed for organic pollutants in which blue fish species are the most contaminated is not maintained. Except in the case of Hg, we found that cephalopods, crustaceans, and mollusks exhibited the highest levels of these elements (Table 1), which probably relates with their different feeding habits. White fish also had higher concentrations of As than those detected in blue fish species. We considered this especially of concern since white fish is the most consumed fish by the Spanish population, and several studies have shown that the intake of As, particularly the inorganic forms of this metalloid, is related with an increase incidence of cancer (Carlin et al., 2015; Di Lorenzo et al., 2015). Although we could not perform the speciation of As and only the total content of As was measured, it is accepted that in the edible parts of marine fishes, ~10% of As is generally present in inorganic forms (Rahman et al., 2012). Assuming that this ratio is maintained in the samples of aquatic organisms included in this study, we considered only 10% of the values depicted in Table 1 in the further risk assessment, which is detailed in the following sections.

3.2. Daily intake of toxic contaminants through the consumption of fishery products

The estimation of the daily intake (EDI) of pollutants through the consumption of fishery products was obtained by combining the results of contamination of the samples and the pattern of consumption of these products as reported by the Spanish authorities (median and percentile 97.5th, in ng/day) (AECOSAN, 2006; AECOSAN, 2011). The results of these estimations (MB and UB approaches) for both adults and children are presented in Table 2.

3.2.1. Organic contaminants

According to our results the greater contribution to the EDI of organochlorine compounds (in both adults and children) occurs through the consumption of blue fish (68.4% and 50.8%, respectively) followed by white fish (25.3 and 40.1%, respectively), seafood (4.2% and 5.0%, respectively), and cephalopods (2.3 and 4.0%, respectively). This pattern was observed for all the individual compounds, and this had been also

Table 2
Mean values of the daily intake of fish and seafood by the Spanish population. Data are expressed in ng contaminant/person/day (±SD) for both, adults (average body weight = 68.48 kg) and children (average body weight = 34.32 kg).

Middle-bound approach (percentile 50th of consumption)					
Adults					
Consumption rate	Blue fish (26.79 g/day)[a]	White fish (44.28 g/day)	Cephalopods (7.97 g/day)	Other seafood (15.36 g/day)	Total (94.4 g/day)
∑ DDTs	58.4 ± 39.5	22.1 ± 20.5	2.4 ± 1.6	1.5 ± 1.3	85.0 ± 21.6
∑ HCHs	10.7 ± 4.3	4.4 ± 11.1	0.1 ± 0.2	0.2 ± 0.3	15.4 ± 14.9
HCB	26.8 ± 23.6	8.9 ± 13.3	0.8 ± 0.7	0.3 ± 0.3	36.7 ± 35.6
∑ M-PCBs	96.4 ± 103.9	35.4 ± 40.7	3.2 ± 2.9	9.2 ± 16.6	144.3 ± 153.9
$\sum TEQ_{DL\text{-}PCBs}$[b]	0.4 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.2
$B[a]P_{eq}$	5.4 ± 6.9	1.8 ± 2.2	1.6 ± 2.1	0.6 ± 1.5	9.3 ± 11.8
As	4760.6 ± 4109.6	14,714.2 ± 12,022.0	4100.6 ± 2319.3	8274.4 ± 4632.6	31,849.8 ± 21,675.4
Cd	436.7 ± 190.2	633.2 ± 256.8	229.5 ± 158.6	892.4 ± 831.0	2191.8 ± 1346.1
Hg	969.8 ± 431.3	2001.5 ± 1186.7	193.0 ± 83.4	450.1 ± 195.1	3569.3 ± 1782.7
Children					
Consumption rate	Blue fish (10.23 g/day)	White fish (36.19 g/day)	Cephalopods (7.07 g/day)	Other seafood (9.82 g/day)	Total (63.31 g/day)
∑ DDTs	22.5 ± 15.1	18.1 ± 16.7	2.1 ± 1.4	1.0 ± 0.8	43.7 ± 12.3
∑ HCHs	4.1 ± 1.6	3.6 ± 10.8	0.1 ± 0.1	0.1 ± 0.2	7.9 ± 12.2
HCB	10.2 ± 9.0	7.2 ± 10.9	0.7 ± 0.6	0.2 ± 0.2	18.4 ± 19.4
∑ M-PCBs	36.8 ± 38.7	28.9 ± 31.8	2.8 ± 2.7	5.9 ± 10.5	74.5 ± 78.5
$\sum TEQ_{DL\text{-}PCBs}$[b]	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
$B[a]P_{eq}$	2.1 ± 3.0	1.4 ± 1.8	1.4 ± 1.8	0.4 ± 1.0	5.3 ± 5.4
As	1817.9 ± 1569.3	12,025.9 ± 9825.6	3637.5 ± 2057.4	5290.0 ± 2961.7	22,755.9 ± 12,891.8
Cd	166.8 ± 72.6	517.5 ± 209.9	203.6 ± 140.7	570.5 ± 531.3	1458.4 ± 894.8
Hg	370.3 ± 164.7	1635.8 ± 969.9	193.0 ± 83.4	372.1 ± 124.7	2571.2 ± 1336.2
Upper-bound approach (percentile 97.5th of consumption)					
Adults					
Consumption rate	Blue fish (87.03 g/day)	White fish (108.86 g/day)	Cephalopods (79.23 g/day)	Other seafood (76.54 g/day)	Total (351.66 g/day)
∑ DDTs	189.7 ± 128,4	54.4 ± 50.5	23.7 ± 15.8	7.6 ± 6.3	274.8 ± 200.9
∑ HCHs	34.8 ± 13.9	10.9 ± 27.2	0.8 ± 1.6	0.7 ± 1.6	47.2 ± 44.3
HCB	84.0 ± 76.6	21.8 ± 33.1	7.9 ± 7.1	1.5 ± 1.5	115.2 ± 118.2
∑ M-PCBs	313.3 ± 337.7	87.1 ± 100.2	31.8 ± 29.3	46.7 ± 82.9	478.9 ± 550.1
$\sum TEQ_{DL\text{-}PCBs}$[b]	1.2 ± 0.5	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	1.5 ± 0.8
$B[a]P_{eq}$	17.4 ± 22.7	4.3 ± 5.4	15.8 ± 20.6	3.1 ± 7.4	40.6 ± 56.0
As	15,465.2 ± 13,350.4	36,174.2 ± 29,555.5	40,763.8 ± 23,053.5	41,372.2 ± 23,162.9	133,775.3 ± 88,572.4
Cd	1418.6 ± 617.9	1556.7 ± 631.4	2281.9 ± 1576.5	4462.1 ± 4154.9	9719.3 ± 6980.6
Hg	3150.5 ± 1401.2	4920.5 ± 2917.4	1918.7 ± 829.3	2250.0 ± 975.4	12,239.7 ± 6123.3
Children					
Consumption rate	Blue fish (64.79 g/day)	White fish (61.92 g/day)	Cephalopods (64.79 g/day)	Other seafood (27.48 g/day)	Total (218.98 g/day)
∑ DDTs	142.6 ± 95.5	30.9 ± 28.6	19.4 ± 12.9	2.7 ± 2.3	195.64 ± 139.3
∑ HCHs	25.9 ± 10.4	6.2 ± 18.5	0.6 ± 1.3	0.3 ± 0.4	32.94 ± 30.7
HCB	64.7 ± 57.1	12.4 ± 18.6	6.5 ± 5.7	0.5 ± 0.5	84.17 ± 81.8
∑ M-PCBs	233.1 ± 244.8	49.5 ± 54.5	25.9 ± 24.6	16.5 ± 29.3	325.03 ± 353.2
$\sum TEQ_{DL\text{-}PCBs}$[b]	0.3 ± 0.5	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.5 ± 0.7
$B[a]P_{eq}$	12.9 ± 19.1	2.5 ± 3.1	12.9 ± 16.8	1.1 ± 2.7	29.51 ± 41.7
As	11,507.1 ± 9933.5	20,564.4 ± 16,801.7	33,319.6 ± 18,845.5	14,759.2 ± 8263.2	80,150.24 ± 53,843.9
Cd	1055.5 ± 459.7	884.9 ± 358.9	1865.2 ± 1288.7	1591.8 ± 1482.2	5397.45 ± 3589.7
Hg	2344.2 ± 1042.6	2797.2 ± 1658.5	1767.9 ± 764.2	1038.2 ± 347.9	7947.56 ± 3813.2

[a] Figures between parentheses indicate the consumption rate of each type of seafood.
[b] In the case of $\sum TEQ_{DL\text{-}PCBs}$ data are expressed in pg/g fresh product, as it is usual when considering TEQs.

reported for these pollutants by several authors (Mezzetta et al., 2011; Moon et al., 2009). According to our calculations the EDI of ΣM-PCBs was the highest, followed by ΣDDTs. To adequately evaluate the exposure to contaminants by means of the consumption of a given food group it is necessary to compare the values with the previously calculated reference values, such as the Tolerable Daily Intake (TDI). Regarding this we have to note that none of the OCPs exceeded their respective TDIs (JECFA, 2000), and even did not surpass 1% of those values, nor in the MB nor in the UB approach (TDI ∑ DDTs = 10,000 ng/kg b.w., TDI ∑ HCHs = 5000 ng/kg b.w., TDI HCB = 160 ng/kg/day) (ATSDR, 2002; Luzardo et al., 2013a). To be able of comparing the exposure to PCBs with some reference values it is necessary to use the approximation of toxic equivalence to dioxins as defined by the WHO (Van den Berg et al., 2006), as the TDI for PCBs has been set in the context of dioxin exposure (2 pg WHO-TEQ/kg b.w./day (SCF, 2000)). Once the results were transformed using the corresponding TEFs, our results indicate that the exposure to dioxin-like PCBs through the consumption of fishery products only accounts for 1.08% of that TDI in the worst scenario (adults, UB approach, Table 2).

Regarding to the other group of organic pollutants included in this research – the PAHs – the EDI of $\Sigma B[a]P_{eq}$ was estimated to be 9.34 ng/day and 5.30 ng/day in Spanish adults and children respectively, and fivefold when the UB approach is considered. Similarly to organochlorine pollutants, blue fish species were the main contributors to

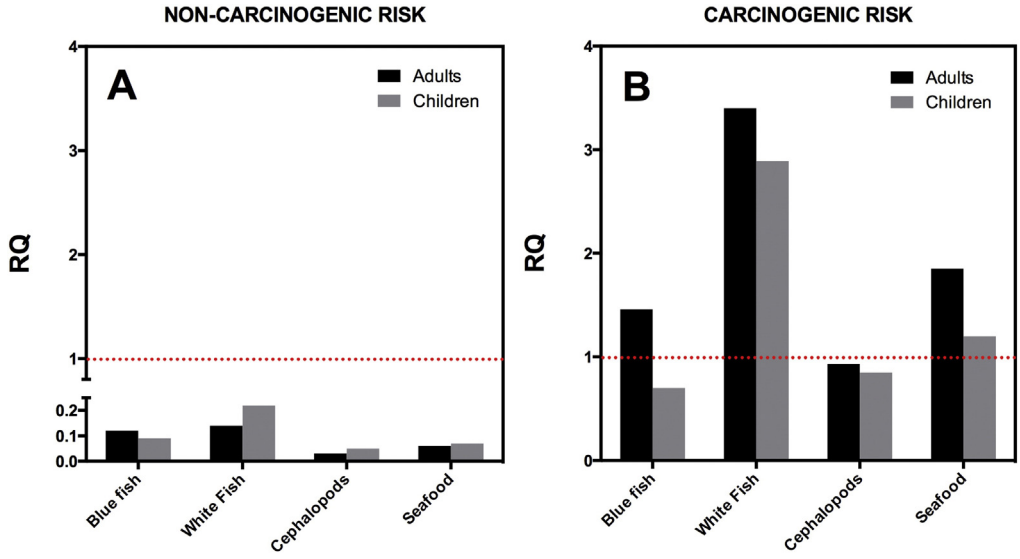

Fig. 1. Hazard ratios of the contaminants for acutely toxic effects (A) and carcinogenic effects (B) in adults and children via consumption of fishery products. The red line indicates the threshold for toxic effect (RQ = 1). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the exposure to these carcinogenic pollutants within this food group (57.4% in adults and 46.9% in children, Table 2). Although the TDI for the carcinogenic PAHs has not yet officially established, we used the TDI for B[*a*]P_{eq} of 20 ng/kg b.w. day, as recommended for the Contaminated Land Exposure Assessment of UK (CLEA-UK, 2008). The EDIs of B[*a*]P_{eq} calculated in this study account for <3% of this reference value in both adults and children, in the worst-case scenario (Table 2).

3.2.2. Inorganic contaminants

When we consider the intake of inorganic contaminants, contrary to what is described above, we found that white fish is the main contributor. Arsenic is considered one of the most dangerous elements for health and all the studies conducted so far show that the foods that are the richest in inorganic arsenic are seaweed, fish, other seafood and cereals (EFSA, 2009b). According to our estimations the daily intake of total As through fishery products could be as much as 1.96 μg/kg/day (adults, worst case scenario (UB approach), Table 2), which would represent almost 94% of the established TDI (2.1 μg/kg/day, (JECFA, 2010)), which is of very much concern. If we take into account that the most dangerous As is that which is in inorganic form, and we assume that 10% of total As in fishery products is inorganic As (Rahman et al., 2012), the average intake would represent around 14%-60% of the estimated average inorganic As exposure from food and water across 19 European countries (0.13 to 0.56 μg/kg b.w./day, (EFSA, 2009b)). Moreover, the EFSA CONTAM Panel has identified a range of values for the 95% lower confidence limit of the benchmark dose of 1% extra risk ($BMDL_{01}$) for each endpoint of a wide range of key epidemiological studies (0.3 to 8 μg/kg/day, (EFSA, 2009b)), and recommended that the overall range is used as reference instead of a single reference value. Thus, the lowest values, which correspond with the risk of lung cancer, are well below the MB-EDI of 0.78 μg/kg/day reported in this study, which would mean that theoretically the current pattern of fish consumption in Spain would not be exempt of risk (even more if the UB approach is taken into account, Table 2).

The Cd has also been extensively studied due to its toxic properties (EFSA, 2009a), being considered primarily toxic to the kidney, where it accumulates over time and may cause renal dysfunction. Besides, the International Agency for Research on Cancer has classified Cd as a probable human carcinogen on the basis of occupational studies, and recently epidemiological studies have revealed an increased risk of lung, endometrium, bladder, and breast cancer in relation with the environmental exposure to this metal (EFSA, 2009a; Menon et al., 2015; Vilahur et al., 2015; Weidemann et al., 2015). However, basically all the carcinogenicity data available are related to inhalation exposure, and there are no studies of orally ingested cadmium suitable for quantitation, so we did not further considered this metal as a carcinogen in the present study. Nevertheless, as many other toxic effects (other than cancer) have been described for Cd, a Provisional Tolerable Weekly Intake (PTWI) of 7 μg/kg has been established. According to our estimations the average intake in Spanish population through the

Table 3
Maximum allowable fish or others fishery products consumption rate (CR_{lim}) expressed in kg/day and hazard ratios (RQ) of contaminants with acutely toxic effects in both adults and children.

	ΣDDTs		ΣHCHs		ΣHCB		ΣM-PCBs		$\Sigma TEQ_{DL\text{-}PCBs}$		B[*a*]P_{eq}		As[a]		Cd		Hg	
	CR_{lim}	RQ	CR_{lim}	RQ	CR_{lim}	RQ	CR_{lim}	RQ	CR_{lim}	RQ	CR_{lim}	RQ	CR_{lim}	RQ	CR_{lim}	RQ	CR_{lim}	RQ
Adults																		
Blue fish	15.45	0.0017	51.00	0.0005	54.40	0.0005	0.38	0.07	6.80	0.004	272.00	0.0001	1.15	0.06	41.71	0.0001	1.86	0.0003
White fish	68.00	0.0007	204.00	0.0002	272.00	0.0002	1.70	0.03	27.20	0.002	1360.00	0.0000	0.61	0.04	47.55	0.00	1.50	0.0001
Cephalopods	113.33	0.0001	2040.00	0.00	544.00	0.0000	3.40	0.002	45.33	0.0002	272.00	0.0000	0.39	0.005	23.61	0.00	2.49	0.0000
Other seafood	340.00	0.0000	2040.00	0.00	2720.00	0.0000	2.27	0.007	45.33	0.0003	1360.00	0.0000	0.37	0.02	11.70	0.00	2.32	0.0000
Children																		
Blue fish	7.95	0.001	26.25	0.0004	28.00	0.0004	0.19	0.05	3.50	0.003	140.00	0.0001	0.59	0.09	21.47	0.0001	0.97	0.0004
White fish	35.00	0.001	105.00	0.0003	140.00	0.0003	0.87	0.04	14.00	0.003	700.00	0.0001	0.32	0.13	24.47	0.0001	0.77	0.0004
Cephalopods	58.33	0.0001	1050.00	0.0000	280.00	0.0000	1.75	0.004	23.33	0.0003	140.00	0.0001	0.20	0.02	12.15	0.0000	1.28	0.0000
Other seafood	175.00	0.0001	1050.00	0.0000	1400.00	0.0000	1.17	0.008	23.33	0.0004	700.00	0.0000	0.19	0.04	6.02	0.0001	1.19	0.0000

[a] Inorganic As form (10% of total As).

consumption of fishery products does not reach 2% of its PTWI (9% when the UB approach is considered). The EFSA has determined from the analyses of more than 140,000 food samples that seafood are the commodities where the highest Cd levels are detected, and besides it has also been determined that only 3–5% of this metal is absorbed after dietary exposure (EFSA, 2009a). Considering this and the estimations done in this research, we can conclude that the dietary exposure to Cd in Spain is currently very low, and very far away from being worrying.

Finally, regarding to the Hg, it has also been established the foods in the group "Fish and other seafood" have the highest values of this highly toxic heavy metal in comparison to all other food groups, although the different surveys available indicate that the total Hg content varies widely among different fish species, and is highest in predatory fish (JECFA, 2004; JECFA, 2006). The toxic properties of Hg are well known, especially for kidney and the developing nervous system. Therefore the EFSA's CONTAM Panel has established a PTWI of 4 μg/kg (EFSA, 2012) for this metal. According to our results, the dietary exposure to total Hg from fishery products of an average Spanish consumer is 0.37 μg/kg/week in adults and 0.53 μg/kg/week in children (Table 2). These values are more than tripled for both age groups when the UB approach is considered. As is estimated that approximately 90% of the total mercury in fish and shellfish is present in the form of methyl mercury (MeHg) (EFSA, 2005), our results would indicate that Spanish adults would be exposed to 0.33 μg/kg/week, and Spanish children to 0.48 μg/kg/week of this extremely toxic form of Hg, in the Mb approach (1.13 μg/kg/week and 1.47 μg/kg/week, respectively in the UB approach). However, it should be also noted that one of the major risks that have been associated to Hg, and in particular to MeHg, is developmental toxicity, where a brief exposure to the foetus can lead to permanent damage. Various organizations have estimated the daily intake of mercury (as MeHg) that is unlikely to be harmful. The World Health Organization has estimated that 0.22 μg/kg/day is unlikely to be harmful, with pregnant women identified for concern (Wise, 2004). Considering this, our estimates indicate that in the upper bound approach a Spanish pregnant woman could be exposed to 73% of this reference value (0.16 μg/kg/day), only via seafood consumption, and the children, which are high consumers of seafood would almost reach this threshold (96%). These results can be considered of very much concern.

The estimates of this study regarding Hg are consistent with the exposure estimates in the European Union (EU) as calculated by the EFSA using the middle bound approach, which range from the lowest minimum of 0.14 μg/kg/week in very elderly to the highest maximum of 5.05 μg/kg/week in adolescents. If we additionally consider that it has been estimated that Hg in fish would represent approximately 37% of total dietary intake (36.8% of food product coverage) (EFSA, 2012), a bulk calculation indicate that Spanish adults would be exposed to 25% of the PTWI through their total diet (9.2% from fishery products), and that this exposure would reach 35.7% of PTWI in the case of children (13.2% from fishery products). Therefore, the estimated exposure to total Hg in Spain from the diet alone would not exceed the PTWI, as it has also been reported for the rest of EU's countries (EFSA, 2012).

3.3. Health risk assessment via multiple contaminants associated to the consumption of fishery products in Spain

Although according to the above calculations none of the individual TDIs is exceeded for any of the contaminants, the consumption of fish implies the exposure of the consumer to multiple contaminants, and antagonistic, synergistic, and additive interactions among the contaminants can occur. For the adequate human health risk assessment the USEPA recommends that the additive model be used for multiple contaminants that cause similar toxicological effects (USEPA, 2000; Yu et al., 2014). Using the calculated acute reference doses (RfDs) and cancer slope factors (CSFs) for the contaminants included in this study (USEPA, 2014) we have considered two types of health risks: acute toxicity and carcinogenic (genotoxic) potential of fish consumption. For each of these endpoints, we first calculated the individual CR_{lim} to estimate the exposure limits to these chemicals through the consumption of fishery products, as previously reported (Yu et al., 2014). Secondly, from the calculated CR_{lims} we calculated the individual RQs. The RQ evaluation has been proposed as a convenient method of estimating population risk and to provide a plausible worst-case scenario for initial screening of potential risk (USEPA, 2000; Yu et al., 2014). Finally, the RQs of each type of pollutant were summed and presented as the overall risk associated to each subgroup of food (blue fish, white fish, cephalopods, and other seafood) (Fig. 1).

3.3.1. Acute toxicity potential of the consumption of fishery products

When the acute toxic effects of the contaminants were considered, the maximum allowable consumption rates (CR_{lims}) (Table 3) of blue fish in children were from 350 times higher (for $\sum TEQ_{DL\text{-}PCBs}$) to 5185 times higher (for B[*a*]P_{eq}) than the current consumption rate of this type of food (Table 2), and these values were more than double in adults. For white fish the CR_{lims} were from 7 times higher (for As) to 15,801 times higher (for B[*a*]P_{eq}); for cephalopods the CR_{lims} ranged from 25 (As) to 127,000 times higher (ΣHCHs); and for seafood from 12 (As) to 91,145 times higher (HCB) than the current consumption rates of these food subgroups by Spanish children. Again, in all the cases the estimations of maximum allowable consumption for Spanish adults were more than double than in children (Table 3). Therefore, the individual RQs ranged from 0 to 0.06 in adults and 0 to 0.13 in children for the individual contaminants (Table 3), and at most 0.2 for all contaminants (white fish, children) (Fig. 1A). Thus, as all the RQ values were much lower than 1 we can conclude that the consumption of the

Table 4
Maximum allowable fish or others fishery products consumption rate (CR_{lim}) expressed in kg/day and hazard ratios (RQ) of contaminants with carcinogenic effects in both adults and children.

	ΣDDTs		ΣHCHs		ΣHCB		ΣM-PCBs		$\Sigma TEQ_{DL\text{-}PCBs}$		B[*a*]Peq		As[a]	
	CRlim	RQ	CRlim	RQ	CRlim	RQ	CRlim	RQ	CRlim	RQ	CRlim	RQ	CRlim	RQ
Adults														
Blue fish	0.909	0.0295	0.944	0.0284	0.425	0.0630	0.189	0.1418	0.309	0.0867	0.465	0.0575	0.025	5.5595
White fish	4.000	0.0111	3.777	0.0117	2.125	0.0208	0.850	0.0521	1.236	0.0358	2.328	0.0190	0.013	3.8186
Cephalopods	6.666	0.0012	37.777	0.0002	4.250	0.0019	1.700	0.0047	2.060	0.0039	0.465	0.0171	0.008	0.5321
Other seafood	20.000	0.0008	37.777	0.0004	21.250	0.0007	1.133	0.0136	2.060	0.0075	2.328	0.0066	0.008	1.6105
Children														
Blue fish	0.468	0.0219	0.486	0.0210	0.219	0.0468	0.097	0.1052	0.159	0.0643	0.239	0.0427	0.025	4.1246
White fish	2.058	0.0176	1.944	0.0186	1.094	0.0331	0.437	0.0827	0.636	0.0569	1.198	0.0302	0.013	6.0635
Cephalopods	3.431	0.0021	19.444	0.0004	2.187	0.0032	0.875	0.0081	1.060	0.0067	0.239	0.0295	0.008	0.9170
Other seafood	10.294	0.0010	19.444	0.0005	10.937	0.0009	0.583	0.0168	1.060	0.0093	1.198	0.0082	0.008	2.0004

[a] Inorganic As form (10% of total As) (Rahman et al., 2012).

Table 5
Recommended maximum number of meals per month of each grouped food item (considering the calculated carcinogenic potential).

	Adults		Children	
	Current pattern of consumption[a] (meals/month) C_{mm}	Maximum recommended consumption (meals/month) RC_{mm}	Current pattern of consumption[a] (meals/month) C_{mm}	Maximum recommended consumption (meals/month) RC_{mm}
Blue fish	3.6	2.5	1.4	2.0
White fish	6.0	1.8	4.9	1.7
Cephalopods	2.0	2.2	1.8	2.1
Seafood	3.9	2.1	2.5	2.1

[a] Data obtained from AECOSAN, 2011.

fishery products would not pose risk of producing acute toxicity associated to their content in chemical contaminants.

3.3.2. Carcinogenic potential of the consumption of fishery products

In a similar manner we also calculated the maximum allowable consumption limits and the RQs associated to the current consumption of this group, but considering the carcinogenic potential (Table 4).

Based on the contamination and the consumption data of fishery products, our calculations indicate that again all the CR_{lim} of the individual pollutants were higher than the pattern of current consumption (which would not indicate obvious health risks due to the intake or uptake of contaminants via fish consumption would be experienced) except in the case of inorganic As (using the current CSF value of 1.5 mg/kg/day on IRIS, (USEPA, 2014)), for which the current consumption of all the subgroups of fishery products would exceed the maximum allowable rate. When we considered the additive effect of all contaminants by food subgroups (Fig. 1B) the RQs were higher than 1 for blue fish, white fish, and seafood in Spanish adults, and for white fish and seafood in Spanish children, mainly due to the contribution of As. This means that the current dietary intake of fishery products would represent a risk of carcinogenicity, especially associated to the consumption of white fish. These results are consistent with those recently reported in the Mediterranean region, where the highest risk of carcinogenicity of the fish consumption pattern was associated with the content in As of these foods (Copat et al., 2013). In that study Copat et al. (2013) suggested a modification of the pattern of consumption of these foods, as we also do in the following section.

3.4. Meal recommendation for consuming fishery products

The USEPA has suggested that the CR_{lim} for carcinogenic and acute toxic effects (whichever value is lower) should be used to calculate the maximum number of meals of fishery products per month, and thus be able of giving advice to consumers to protect the human health (USEPA, 2000; Yu et al., 2014). As in this study we found that the CR_{lims} for carcinogenic effects were lower than those of acute toxicity, we used these values to calculate the maximum number of meals of each food subgroup that would no pose obvious health risks due to the intake or uptake of contaminants via fish consumption (this is, consumption which that would allow a RQ ≦1 for all products). In Table 5 we summarize these recommendations for adults and children (RC_{mm}), and compare these recommended maximum numbers of meals with the current pattern of consumption (C_{mm}). According to our calculations, and strictly considering the results of our study, the Spanish population should reduce the consumption of fishery products in general terms, but more importantly in adults. Since the white fish involves greater risk, as detailed in this research, its consumption should be further reduced, to around one-third of the current consumption rate (that is, no more than one meal every two weeks). Adults should also slightly reduce consumption of blue fish and cephalopods, crustaceans and mollusks (Table 5). However, it is also necessary to consider that the health benefits of the high value nutrients from seafood have been deeply studied (PUFa as well as vitamin D_3, iodine, vitamin B12, etc.), and therefore, it is not advisable to recommend abruptly reducing fish consumption (EFSA, 2014). Nevertheless, the results of this study should be taken into account for the design of appropriate risk communication campaigns aimed to reduce the consumption of certain types of seafood; the aim should be an optimal risk-to-benefit balance.

4. Conclusions

In this research we have estimated the daily intake of contaminants through the consumption of fishery products. When these intakes are individually considered we found that none of the reference values (tolerated daily intakes) were exceeded, although the case of As, HCB, and B[*a*]P_{eq} could be somewhat of concern. However, when we estimated the risk associated to multiple contaminants acting together we found a moderate risk of carcinogenicity. Therefore, a decrease in the consumption of fish and seafood is recommended to avoid the carcinogenic risk associated to these pollutants, especially in the case of white fish, whose consumption should be reduced to one-third of the current level. It seems necessary to maintain surveillance programs that monitor the trend of persistent pollutants in sea foodstuffs, and especially of the concentrations of toxic elements, such as arsenic. The results of this study may be taken of utility for risk managers in the design of appropriate risk communication campaigns aimed to reduce the consumption of certain types of seafood with the aim of obtaining an optimal risk-to-benefit balance of fish consumption.

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Conflict of interest

The authors declare no conflict of interest.

Competing financial interest declaration

There are no actual or potential conflicts of interest to declare for any author.

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Comparative study of the intake of toxic persistent and semi persistent pollutants through the consumption of fish and seafood from two modes of production (wild-caught and farmed)

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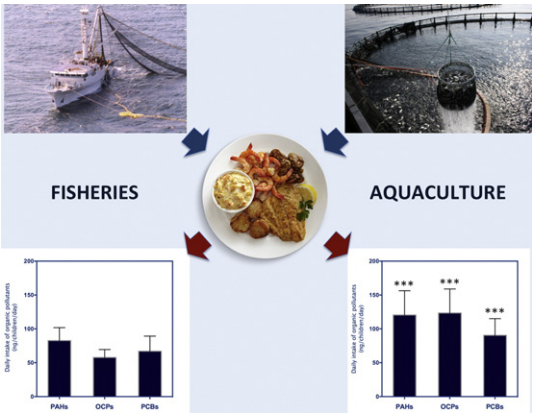
HIGHLIGHTS

- Comparative assessment of intake of pollutants through farmed and wild seafood
- Higher levels of total PAHs, total OCPs, and total PCBs in aquaculture products
- Higher levels of carcinogenic PAHs in wild-caught seafood
- Concentrations of elements had a variable pattern between farmed and wild specimens
- Intake of most of these pollutants is higher through consumption of farmed seafood

GRAPHICAL ABSTRACT



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ABSTRACT

Adverse effects of chemical contaminants associated with seafood counteract the undoubted benefits for the health of its valuable nutrients. So much so that many dietary guidelines recommend no more than one serving a week of fish and seafood. Although is estimated that aquaculture provides more than 50% of the fish and seafood consumed globally, few research studies have focused in the assessment of the intake of pollutants through aquaculture products. In this study we determined the levels of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and toxic elements (Pb, Cd, Ni, Al, As, and Hg) in a large sample of farmed and wild-caught seafood, and we estimated the intake of these contaminants in two hypothetical models of consumers: those consuming only farmed fish, and those consuming only wild fish. Measured levels of most organic and many inorganic pollutants were higher in aquaculture products, and consequently intake levels if only such products were consumed would be also significantly higher. Thus, the intake of ∑PAHs in adults consuming aquaculture seafood would be 3.30 ng/kg-bw/day, and consuming

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Heavy metals
Aquaculture

seafood from extractive fishing 2.41 ng/kg-bw/day ($p < 0.05$); $\sum$ OCPs, 3.36 vs. 1.85 ng/kg-bw/day, respectively ($p < 0.05$); $\sum$ PCBs, 2.35 vs. 2.11 ng/kg bw/day, respectively; and the intake of Pb, Ni, As, and Al would be also significantly higher consuming farmed seafood. For children the estimations were very similar, but the difference of intake of PCBs reached statistical significance. The implementation of several decontamination practices in aquaculture would allow not only match the levels of pollution from wild-caught seafood, but also could provide products with much lower levels of pollutants than those, which in turn would allow to increase consumption over the "one serving per week", and so benefit the consumer of the enormous positive health effects of the valuable nutrients of seafood.

1. Introduction

Regular consumption of fish is widely recommended worldwide by government and health organizations because it represents a valuable source of the very long-chain polyunsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid (PUFAs) and essential nutrients such as vitamin D, iodine and selenium (Kromhout et al., 2016; Mozaffarian and Ludwig, 2010). Numerous studies have established beneficial associations between moderate consumption of fish and risk factors for several diseases, such as obesity and metabolic syndrome (Torris et al., 2014), cardiovascular disease and stroke (Ndanuko et al., 2016; Shen et al., 2015), hypertension (Ndanuko et al., 2016), kidney disease (Ndanuko et al., 2016), neurodegenerative diseases (Pan et al., 2015), and even cancer (Eltweri et al., 2016; Lovegrove et al., 2015; Vaughan et al., 2013). The contribution of PUFAs in fish has also proved beneficial to all stages of human development, from the moment of conception to maturity and aging (Gil and Gil, 2015).

Despite all the benefits attributable to the PUFAs from fish, usually dietary guidelines recommend that consumption of this food is limited to one serving per week (Kromhout et al., 2016; Mozaffarian and Ludwig, 2010). This is mainly due to the potential adverse effects of chemical contaminants that are usually present in fish (e.g. heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), dioxins, furans and chlorinated pesticides (OCPs)) (Rodriguez-Hernandez et al., 2016; Tang et al., 2016; Zhang et al., 2016). Paradoxically, many adverse effects attributable to chemical contaminants in fish are opposed to the benefits of PUFAs, such as degenerative effects on nervous system, alteration of the immune system, and increased risk of cardiovascular disease or cancer, among others (Gil and Gil, 2015; Rodriguez-Hernandez et al., 2016). Thus, the dietary recommendations have to take into account the chemical load that is present in the commercial fish species. Therefore, the monitoring of chemical contamination in fish is crucial to establish the risk-benefit ratio from fish consumption. The majority of dietary guidelines conclude that with the current level of chemical contamination of fish at a consumption level of one serving per week there is no evidence for toxicological risks if a variety of different types of fish are eaten (Kromhout et al., 2016; Mozaffarian and Ludwig, 2010).

During the last decades, the consumption of seafood from aquaculture has been steadily increasing, as farmed fish represent an affordable alternative to wild-caught fish for many consumers worldwide. Thus, since 2012 available data indicate that aquaculture already provides more food to people than extractive fishing (57 vs. 43%) (APROMAR, 2014; FAO, 2014). The European Commission intends to further boost the growth of the aquaculture industry as a means to meet future demands seafood, and as an important potential source of employment and economic development (Tornero and Hanke, 2016). While one of the negative highlights of aquaculture production is related precisely with chemical pollution that this activity produces in the environment (Cole et al., 2009; Grigorakis and Rigos, 2011; Leung et al., 2015), it is also true that the controlled conditions in which this activity is carried would enable the control of pollution levels, both in the environmental water as in the final product. This is, fish farming (unlike extractive fishing) theoretically would allow the management of pollution levels in fish (mainly through the control of the contamination in feed), and therefore producing safer products. Subsequently, by diminishing the risks associated to chemicals in farmed fish, the consumption of this beneficial food could be higher. For all these reasons, as a preliminary step, it is necessary to know the levels of contamination of fish taking into account their mode of production. In the case of aquaculture, this information is needed to further verify the effectiveness of possible measures aimed to reduce the levels of these contaminants.

The aquaculture industries are required to verify that the levels of chemical contaminants in products comply with current legislation, and that the chemicals in their products are below the maximum residue levels (MRLs) before being put on the market (EC, 2005; EC, 2011). However, this kind of routine controls usually seeks only the compliance of current regulations and are made in the products taken individually (each individual fish or seafood species farmed). With regard to the levels of exposure to chemicals (such as organochlorine pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, or heavy metals) in the human population through the consumption of the variety of fish and shellfish that are part of the diet, there are also many available scientific studies worldwide (Groth, 2016; Naji et al., 2016; Rodriguez-Hernandez et al., 2016). There are also many studies comparing levels of contamination found in aquaculture species compared to individuals of the same species obtained from extractive fishing at sea (Airaksinen et al., 2015; Kelly et al., 2011). However, relatively little scientific research has made a comparative study of human exposure to chemicals considering the mode in which the fish is obtained (Cirillo et al., 2010; Cirillo et al., 2009), and as far as we know, none in the Spanish population. This gap in knowledge is what has motivated the design of this study, which is aimed to (i) determine the level of contamination by PCBs, OCPs, PAHs, and heavy metals of the wild-caught and farmed seafood species most consumed in Spain, and (ii) estimate the daily dietary exposure of seafood consumers to these pollutants considering two theoretical groups of consumers: a) those who would consume only wild-caught seafood, and b) those who would consume only farmed seafood. The goal was to determine whether relative differences in pollutants occurred between the consumption of wild or farmed seafood in Spain.

2. Material and methods

2.1. Sampling

Using the data of the pattern of food consumption in Spain we sampled the most consumed species of fish (whitefish and bluefish), and other seafood (crustaceans and bivalve molluscs), both from aquaculture production and wild-caught (AECOSAN, 2006; AECOSAN, 2011). Whenever available we sampled the same species from both methods of production. A total of 84 pooled samples from the main commercial seafood species were analyzed (52 pooled samples from fishing species and 32 pooled samples from aquaculture species). Each pooled sample was formed from 4 different pieces of each seafood (complete individuals in the case of species of fish or shellfish small, or fillets in the case

of large fish species), which were acquired within the same month (n = 21 pooled samples per month). This sampling scheme was repeated during four consecutive months (February to May 2015) to obtain the final 84 pooled samples (336 individual samples). Seafood samples were randomly acquired from fish markets and supermarkets located in the Canary Islands (Spain). Although due to logistical reasons the sampling was performed only in this territory within Spain, we acquired the samples of fish and seafood from multinational retailers settled around the whole country. Therefore, all the products sampled came from large suppliers who serve the entire nation, and the local products sold in these retailers represent only a small percentage of the total (<3–5%). Therefore, we consider that our results could be extrapolated to the entire nation. After the purchase the samples were immediately transported to the Laboratory of Toxicology of the University of Las Palmas de Gran Canaria (ULPGC), and processed upon arrival at the laboratory. The pooled samples were obtained using a stainless steel domestic blender, and were immediately frozen at −80 °C until analysis.

The species of wild-caught whitefish were: wreckfish (*Polyprion americanus*), megrim (*Stephanoiepis hispidus*), sole (*Solea vulgaris*), seabass (*Dicentrarchus labrax*), hake (*Merluccius merluccius*), toothed sparus (*Dentex dentex*), and parrot fish (*Sparisoma cretense*). The species of aquaculture whitefish were: gilt head fish (*Sparus aurata*), sole (*Solea vulgaris*), iridiscent shark (*Pangasius hypophthalmus*), and seabass (*Dicentrarchus labrax*). The species of aquaculture bluefish were: salmon (*Salmo salar*), and trout (*Salmo trutta*). The species of wild-caught bluefish were: tuna (*Thunnus thynnus*), salmon (*Salmo salar*), and sardine (*Sardina pilchardus*). In addition, we analyzed different species of seafood, from aquaculture: crayfish (*Penaeus* spp.), and mussel (*Mytilus galloprovincialis*), and from wild origin: shrimp (*Parapenaeus* spp.), crayfish (*Penaeus* spp.), and mussel (*Mytilus galloprovincialis*).

The sampling scheme we designed (the species selected) would represent the main intake of the studied pollutant through this food group, according to the preferences of Spanish consumers (Martín Cerdeño, 2010).

2.2. Reagents and analytes of interest

We focused this study in the screening of the following pollutants in fish: (a) 19 OCPs: hexachlorocyclohexane (α-, β and γ-, and δ- HCH), heptachlor, aldrin, dieldrin, endrin, chlordane (cis- and trans-isomers), endosulfan (α- and β-isomers), endosulfan-sulfate, *p,p′*-DDT and its metabolites (*p,p′*-DDE, and *p,p′*-DDD), dicofol, methoxychlor, mirex and hexachlorobenzene (HCB); (b) 18 congeners of PCBs, including 6 marker PCBs (M-PCBs, IUPAC numbers #28, 52, 101, 138, 153, 180), and 12 dioxin-like PCBs (DL-PCBs, IUPAC numbers #77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189); (c) 16 PAHs: naphthalene, acenaphtene, acenaphtylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a,h*]anthracene, benzo[*ghi*]perylene and indeno[1,2,3,-*cd*]pyrene; and d) 6 elements: Pb, Cd, Ni, As, Al and Hg. Stock solutions of each compound at 1 mg/mL were prepared in cyclohexane and stored at −20 °C. Diluted solutions from 0.05 ng/mL to 40 ng/mL were used for calibration curves in cyclohexane (9 points). PCB 202, tetrachloro-m-xylene, *p,p′*-DDE-d8, heptachloro epoxide cis, and phenanthrene-d10 were employed as internal standards (ISs). All the standards were neat compounds, and were acquired from Dr. Ehrenstorfer Reference Materials (Augsburg, Germany) (OCPs, PCBs, and ISs), Absolute Standards, Inc. (Connecticut, USA) (PAHs), and CPA Chem, Ltd. (Stara Zagora, Bulgaria). All the organic solvents were of mass spectrometry grade (Merck, Darmstadt, Germany), and the ultrapure water was produced in the laboratory using a Milli-Q Gradient A10 apparatus (Millipore, Molshein, France). The rest of the reagents employed in this work were acquired from Sigma-Aldrich (St. Louis, USA), except the neutral styrene divinylbenzene resin Bio-Beads SX-3, which was purchased from Bio-Rad Laboratories (Hercules, USA).

2.3. Extraction, clean-up procedure, and chemical analysis

The procedure of chemical analysis of organic pollutants, including the extraction and purification of extracts was performed according the method optimized in our laboratory for fish and other foods of animal origin (Luzardo et al., 2013; Rodriguez-Hernandez et al., 2016). Briefly, we first extracted the seafood fat from 2 g lyophilized samples (to extract the organic pollutants, all of them lipophilic), using an automated Soxhlet apparatus (Soxtec™ 2055 Auto Fat Extraction, Foss® Analytical, Hilleroed, Denmark). The fat content of each sample was gravimetrically determined and the value was used to correct the results to express them referred to fresh weight of product. Fifty milligrams of the extracted fat were dissolved in 1 mL of cyclohexane:ethyl acetate (1:1) and subjected to gel permeation chromatography (GPC) to remove the bulk of lipids. The contaminants of interest were recovered in a total volume of 124 mL of cyclohexane:ethyl acetate (minutes 23 to 85 of GPC procedure), and concentrated to dryness using a rotary evaporator (Hei-VAP Advantage™, Heidolph Instruments®, Schwabach, Germany). The analytes were re-dissolved in 1 mL of cyclohexane, and used for the chromatographic analysis, without any further purification. This method achieved acceptable recoveries that ranged between 71.5% and 103.2%. Gas chromatography analyses were performed according the previously published method (Camacho et al., 2014) using a Thermo Trace GC Ultra equipped with a TriPlus Autosampler coupled to a Triple Quadrupole Mass Spectrometer Quantum XLS (Thermo Fisher Scientific Inc., Waltham, MA, USA). Identifications were done using electron ionization (EI)-MS/MS, and based on the retention time and the relative ion ratios of each of the analytes. Quantifications were performed against calibration curves as mentioned above. The limits of quantification (LOQ) varied among compounds and ranged from 0.05 to 0.65 ng/mL.

For the analysis of elements (Pb, Cd, Ni, As, Al and Hg) we used 1 g of lyophilized sample, which was microwave-digested in 6 mL of nitric acid using a Milestone MLS 1200 mega oven, as previously described (Rodriguez-Hernandez et al., 2016). The digested material was filtered through 0.45 μm syringe filters and diluted up to 50 mL with ultrapure water. Elements were quantified with Inductively Coupled Plasma-Optic Emission Spectrometry technique (ICP-OES) using a Perkin Elmer Optima 2100 DV instrument coupled with a CETAC U5000AT plus an ultrasound nebulizer for mercury. The sample readings (three readings for each sample, two replicates for each sample) were performed using axial plasma, which provides increased sensitivity, lower background, and improved detection limits compared to traditional radial plasma. This sensitivity enhancement results in a 5- to 10-fold improvement in the detection limits compared with radially viewed plasma. The concentration values were obtained from the mean of each reading. Detection limits, expressed as wet weight (w.w.), were 0.1 ng/g for Al, As, and Pb; 1.8 ng/g for Cd and Ni; and 0.061 ng/g for Hg. The LODs were determined following the protocol described by Perkin Elmer ICP application study number 57. The element concentrations are expressed as μg/g of w.w.

2.4. Quality of analyses and quality control (QA/QC)

All of the measurements were performed in triplicate, and we used the means of all data obtained for each species for the calculations. In the analyses of organic pollutants we included three controls for every 18 vials (6 samples): a reagent blank consisting of a vial containing only cyclohexane; a vial containing 2 ng/mL of each of the pollutants in cyclohexane; and an internal laboratory quality control samples (QCs) consisting of melted fish fat spiked at 20 ng/mL of each of the analytes. This QCs was processed using the same method as for the fish fat samples, respectively. The results were considered to be acceptable when the concentration of the analytes determined in the QC sample was within 15% of the deviation of the theoretical value. In the analyses of elements two blanks were run during each set of analyses

to check purity of the chemicals, and the blank reading was subtracted from all of the experimental readings. As a QC for this method we used a certified reference material (CRM 278: lyophilized mussel, Community Bureau of Reference, BCR, Brussels), which was processed using the same method as for the samples of fish. All values of reference material were within the certified limits.

2.5. Dietary intake estimates and calculations

For the estimation of the daily intake of the studied pollutants through the consumption of seafood we first grouped the results of individual measurements as whitefish, bluefish, and other seafood both, for aquaculture species and for wild-caught species. Using the data of consumption of seafood in the Spanish population (AECOSAN, 2006; AECOSAN, 2011) (Table 1) we calculated the exposure by multiplying the median values of pollutants in each group of food (expressed in ng/g fresh weight (fw)) by the average consumption of each food group (expressed in g/day). These estimations were done for both, adults (average body weight (bw) 68.48 kg) and children (average bw 34.48 kg). Therefore, the results of the estimations are expressed as ng pollutant (or group of pollutants) per kg bw per day (ng/kg bw/day). Since the objective of this study was comparing the intake of contaminants through seafood from aquaculture with consumption of seafood from extractive fishing, to simplify we performed the estimates using only the median values, and we did not consider ranges (lower-bound and upper-bound approaches). For comparison purposes, two theoretical scenarios were considered: (a) consumers would eat only seafood from aquaculture, and (b) consumers would eat only wild-caught seafood.

The exposures were assessed for all the contaminants, both individually considered and also grouped in different forms. Thus, we expressed the total value of the OCP residues (ΣOCPs) as the sum of the 19 OCPs and metabolites measured; the total value of the PCB congeners (ΣPCBs) as the sum of the 18 congeners included; the total value of marker PCBs (ΣM-PCB) as the sum of the six congeners considered indicators of PCB contamination; the total value of dioxin-like PCBs (ΣDL-PCBs) as the sum of the twelve PCB congeners considered more toxic because of their similarity to dioxins (Van den Berg et al., 2006); and the total value of PAHs as the sum of the values of the 16 US-EPA compounds included in this study.

2.6. Statistical analysis

Database management and statistical analysis were performed with PASW Statistics v 20.0 (SPSS Inc., Chicago, IL, USA). Because the data were not normally distributed, the statistical analyses involved the use of non-parametric tests. The differences between two independent groups were tested with the Mann–Whitney *U* test. The results were reported as medians and ranges for concentrations of pollutants, and as means for the daily intakes. P values of <0.05 (two-tailed) were considered statistically significant.

Table 1
Mean values of the daily intake of fish and seafood by the Spanish population. Data are expressed in g/day for both, adults (average weight = 68.48 kg) and children (average weight = 34.32 kg).

Consumption	Adults	Children
White fish	44,28	36.19
Blue fish	26,79	10.23
Seafood	15,36	9.81

3. Results and discussion

3.1. Occurrence of organic and inorganic pollutants in fish and seafood species from the two modes of production: wild and farmed species

The levels of the individual pollutants (PAHs, OCPs, PCBs and elements) determined in the three groups of seafood considered are presented in Tables 2 to 5. Furthermore, contaminant concentrations in the four species (seabass, salmon, crayfish and mussel) in which it was possible to sampling specimens of the two modes of production are shown in Fig. 1 (summations of PAHs, OCPs, and PCBs) and Fig. 2 (individual elements).

As previously mentioned, during the last decade there has been a great awareness about the need to evaluate the negative effects for the consumer of contaminants in aquatic organism. In some research studies around the world the authors have compared the levels of organic pollutants in farmed and wild species and compared them. The majority of those studies have focused on the most commercial species, seabass and salmon (Bustnes et al., 2010; Carubelli et al., 2007; Cirillo et al., 2009; Easton et al., 2002; Fernandes et al., 2009; Fernandes et al., 2008; Ferreira et al., 2008a; Ferreira et al., 2010; Hites et al., 2004). However, scientific studies comparing the levels of toxic elements in seafood species from aquaculture with those of extractive fishing are scarce, although there are some (Cirillo et al., 2010; Ferreira et al., 2008b; Ikem and Egilla, 2008; Szlinder-Richert et al., 2011; Vahabzadeh et al., 2013). In general terms, the levels of contamination reported in the present study are similar or lower than those reported elsewhere, although the majority of studies comparing wild-caught and aquaculture seafood are usually focused in a limited number of species, usually the most commercial ones. However, we have included in this study a higher number of species, in an attempt to obtain an approximate representation of the exposure to pollutants through fish and seafood intake from both modes of production of an average Spanish consumer, as we covered the most consumed species in Spain. In the following subsections we detail the results by chemical groups.

3.1.1. PAH residues

In all the 84 pooled samples at least one of the hydrocarbons investigated were detected. The results from PAHs analyses of whitefish, bluefish, and other seafood are summarized in Table 2. The predominant chemical of this group of organic pollutants was phenanthrene, followed by pyrene and fluoranthene, and this pattern was observed in all the subgroups of marine foodstuff considered, and independently of the mode of production. Therefore, the composition pattern within this chemical group was dominated by PAHs with three rings and four rings, as previously reported in other studies (Cirillo et al., 2009; Perugini et al., 2006). Regarding the concentrations, our study indicates that there are statistically significant differences between farmed and wild species belonging to the whitefish group, being the concentrations higher in farmed fish in the case of phenanthrene, fluoranthene, and fluorene. Mainly due to the differences in these three compounds, also the $\sum$ PAHs (16 compounds) was significantly higher in farmed fish than in wild fish. The same result was observed in three of the four species that were sampled in duplicate (the two modes of production): sea bass, salmon and mussels (Fig. 1A), where levels of PAHs were significantly higher in samples from aquaculture than in wild specimens. This result is in agreement with those reported by other authors in these three species (Cirillo et al., 2009; Easton et al., 2002). Opposedly, when only those PAHs considered as carcinogenic by the USEPA (benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene and indeno[1,2,3,-*cd*]pyrene) (Nisbet and LaGoy, 1992) were used in the statistical comparison, the results showed that the levels were significantly higher in wild fish than in farmed fish (Table 2). Ferreira et al. (2010) have also reported higher levels of carcinogenic PAHs in wild fish than in farmed fish, although they employed bile as an alternative method of assessing PAHs

Table 2
Concentrations of polycyclic aromatic hydrocarbons in fish and seafood from the two modes of production: fisheries and aquaculture. All the results are presented as medians, and expressed in ng/g (fresh product weight).

	White fish			Blue fish			Seafood		
	Fisheries[a] (n = 28)	Aquaculture[b] (n = 16)	p	Fisheries[c] (n = 12)	Aquaculture[d] (n = 8)	p	Fisheries[e] (n = 12)	Aquaculture[f] (n = 8)	p
Naphtalene	0.0 (0.00–0.03)	0.0 (0.00–0.01)	n.s.	0.0 (0.00–1.11)	0.0 (0.00–0.04)	n.s.	0.0 (0.00–0.02)	0.0 (0.00–0.03)	n.s.
Acenaphthene	n.d.	n.d.	–	n.d.	n.d.	–	0.0 (0.00–0.06)	n.d.	–
Acenaphtylene	0.0 (0.00–0.02)	0.0 (0.00–0.03)	n.s.	0.0 (0.00–0.07)	n.d.	–	0.0 (0.0–0.07)	0.01 (0.00–0.13)	n.s.
Fluorene	0.0 (0.00–0.02)	0.02** (0.00–0.27)	0.0026	0.17 (0.03–0.43)	0.14 (0.00–0.48)	n.s.	0.02 (0.00–0.17)	0.02 (0.00–0.07)	n.s.
Phenanthrene	0.19 (0.05–0.52)	0.74*** (0.21–3.05)	<0.0001	2.35 (0.51–3.09)	2.59 (0.28–6.88)	n.s.	0.29 (0.13–1.94)	0.41 (0.04–1.28)	n.s.
Anthracene	0.0 (0.00–0.02)	0.0 (0.00–0.03)	n.s.	0.0 (0.00–0,0.21)	n.d.	–	0.0 (0.00–0.19)	0.09 (0.00–0.27)	n.s.
Fluoranthene	0.08 (0.03–0.21)	0.17** (0.04–0.53)	0.0036	0.57 (0.13–1.09)	0.64 (0.08–1.95)	n.s.	0.12 (0.05–1.50)	0.19 (0.02–0.65)	n.s.
Pyrene	0.16 (0.08–0.54)	0.20 (0.05–0.66)	n.s.	0.88 (0.25–1.96)	0.71 (0.12–2.65)	n.s.	0.25 (0.13–1.66)	0.22 (0.04–0.72)	n.s.
Benzo[*a*]anthracene	n.d.	n.d.	–	0.0 (0.00–0.03)	0.0 (0.00–0.02)	n.s.	0.0 (0.00–0.26)	0.01 (0.00–0.04)	n.s.
Chrysene	0.02 (0.00–0.03)	0.01 (0.00–0.01)	n.s.	0.01 (0.00–0.03)	0.0 (0.00–0.04)	n.s.	0.01 (0.00–0.58)	0.04 (0.00–0.11)	n.s.
Benzo[*b*]fluoranthene	n.d.	n.d.	–	0.0 (0.00–0.02)	n.d.	–	0.0 (0.00–0.59)	0.0 (0.00–0.11)	n.s.
Benzo[*k*]fluoranthene	n.d.	n.d.	–	n.d.	n.d.	–	0.0 (0.00–0.39)	0.01 (0.00–0.08)	n.s.
Benzo[*a*]pyrene	n.d.	n.d.	–	n.d.	n.d.	–	0.0 (0.00–0.11)	n.d.	–
Dibenzo[*a*,*h*]anthracene	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
Benzo[*g*,*h*,*i*]perylene	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
Indeno[1,2,3-*c*,*d*]pyrene	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
∑ c-PAHs	0.02*** (0.00–0.06)	0.0 (0.00–0.01)	<0.0001	0.02 (0.00–0.06)	0.0 (0.00–0.06)	n.s.	0.03 (0.00–1.71)	0.09 (0.00–0.29)	n.s.
∑ PAHs	0.49 (0.19–1.29)	1.18*** (0.33–4.51)	0.0004	4.32 (0.93–7.25)	4.11 (0.47–11.97)	n.s.	0.72 (0.39–6.97)	1.09 (0.11–3.28)	n.s.

n.d. Nondetected.
n.s. Not significant.
[a] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Wreckfish (*Polyprion americanus*); Megrim (*Stephanoiepis hispidus*); Sole (*Solea vulgaris*); Seabass (*Dicentrarchus labrax*); Hake (*Merluccius merluccius*); Toothed sparus (*Dentex dentex*); Parrot fish (*Sparisoma cretense*).
[b] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Gilt head fish (*Sparus aurata*); Sole (*Solea vulgaris*); Iridiscent shark (*Pandasius hypophthalmus*); and Seabass (*Dicentrarchus labrax*).
[c] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Tuna (*Thunnus thynnus*); Salmon (*Salmo salar*); Sardine (*Sardina pilchardus*).
[d] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Salmon (*Salmo salar*); Trout (*Salmo trutta*).
[e] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Shrimp (*Parapenaeus* spp.); Crayfish (*Penaeus* spp.); Mussel (*Mytilus galloprovincialis*).
[f] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Crayfish (*Penaeus* spp.); Mussel (*Mytilus galloprovincialis*).
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.005$.

exposure in fish, as proposed by other authors (Fernandes et al., 2009; Fernandes et al., 2008). At least in the case of benthic organisms such as mussels this difference is logical, as carcinogenic PAHs are heavy compounds (4, 5 and 6 rings), which usually are more concentrated in marine sediments and seabed, including rocky ecosystems (Li et al., 2016), where the wild mussels live. Also in the case of salmon, their feeding habits in the wild could explain the higher levels of carcinogenic PAHs found, since much of their diet, both at sea and in the streams consists of animals associated with benthic ecosystems (euphausiids, amphipods, decapods, or sand lance among others) (Jonsson et al., 2016). In the case of seabass, it has been described that wild juvenile specimens also feed on benthic organisms, but adults are known to have piscivorous habits. However, European seabass is considered as having a trophic level of 3.8 (www.fishbase.org), which means that is a secondary consumer that feed mainly on herbivorous fish (which in turn feed on seabed, and would be subsequently more exposed to heavier PAHs), and only occasionally feed on other carnivores.

3.1.2. OCPs

As seen in Table 3, only 5 organochlorine pesticides were detected in seafood samples (dieldrin, *p*,*p*′-DDT, *p*,*p*′-DDE, *p*,*p*′-DDD, and hexachlorobenzene). Interestingly, the detected pesticides were the same regardless the mode of production (extractive fishing or aquaculture). As expected being lipophilic chemicals, the highest levels of these contaminants were detected in bluefish species, probably due to their higher content in fat. These differences were statistically significant when compared with the group of whitefish species and with the group of other seafood (crayfish and mussels). Focusing on the main objective of this work, which is the comparison of aquaculture species with extractive fishing, the results indicated that the levels of all detected contaminants were higher in the farmed species of whitefish and bluefish, reaching statistical significance for all the individual pollutants detected in whitefish, and for *p*,*p*′-DDT and *p*,*p*′-DDD in bluefish. In both cases also the sum of all organochlorine pesticides was significantly higher in farmed species (∑OCPs in whitefish = 1.56 ng/g vs.

Table 3
Concentrations of organochlorine pesticides in fish and seafood from the two modes of production: fisheries and aquaculture. All the results are presented as medians, and expressed in ng/g (fresh product weight).

	White fish			Blue fish			Seafood		
	Fisheries[a] (n = 28)	Aquaculture[b] (n = 16)	p	Fisheries[c] (n = 12)	Aquaculture[d] (n = 8)	p	Fisheries[e] (n = 12)	Aquaculture[f] (n = 8)	p
Hexachlorocyclohexane (alpha)	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
Hexachlorocyclohexane (beta + gamma)	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
Hexachlorocyclohexane (delta)	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
Heptachlor	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
Aldrin	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
Dieldrin	0.16 (0.08–0.27)	0.34** (0.06–1.39)	0.0098	1.15 (0.32–2.77)	1.42 (0.19–4.78)	n.s.	0.13 (0.09–0.47)	0.14 (0.05–0.38)	n.s.
Endrin	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
Chlordane (cis)	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
Chlordane (trans)	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
Endosulfan (alpha)	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
Endosulfan (beta)	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
Endosulfan sulfate	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
p,p′-DDT	0.0 (0.00–0.02)	0.02*** (0.00–0.11)	0.0004	0.03 (0.00–0.12)	0.18* (0.01–0.53)	0.0112	0.0 (0.00–0.04)	0.0 (0.00–0.02)	n.s.
p,p′-DDE	0.05 (0.02–0.47)	0.85*** (0.01–2.01)	0.0002	1.15 (0.48–2.85)	1.34 (0.47–3.26)	n.s.	0.15* (0.02–0.27)	0.04 (0.02–0.11)	0.0435
p,p′-DDD	0.01 (0.00–0.08)	0.17*** (0.00–0.45)	<0.0001	0.11 (0.05–0.71)	0.54* (0.10–1.60)	0.0266	0.01 (0.00–0.08)	0.01 (0.00–0.03)	n.s.
Dicofol	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
Metoxychlor	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
Mirex	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
Hexachlorobenzene	0.02 (0.00–0.37)	0.13* (0.00–1.19)	0.0225	0.59 (0.31–1.85)	0.91 (0.01–3.24)	n.s.	0.01 (0.00–0.02)	0.02 (0.00–0.06)	n.s.
∑OCPs	0.28 (0.11–1.14)	1.56*** (0.08–4.57)	0.0006	3.33 (1.20–7.29)	4.23* (0.78–12.99)	0.0356	0.32 (0.15–0.87)	0.21 (0.07–0.58)	n.s.

n.d. Nondetected.
n.s. Not significant.
[a] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Wreckfish (*Polyprion americanus*); Megrim (*Stephanoiepis hispidus*): Sole (*Solea vulgaris*); Seabass (*Dicentrarchus labrax*); Hake (*Merluccius merluccius*); Toothed sparus (*Dentex dentex*); Parrot fish (*Sparisoma cretense*).
[b] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Gilt head fish (*Sparus aurata*); Sole (*Solea vulgaris*); Iridiscent shark (*Pandasius hypophthalmus*); and Seabass (*Dicentrarchus labrax*).
[c] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Tuna (*Thunnus thynnus*); Salmon (*Salmo salar*); Sardine (*Sardina pilchardus*).
[d] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Salmon (*Salmo salar*); Trout (*Salmo trutta*).
[e] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Shrimp (*Parapenaeus* spp.); Crayfish (*Penaeus* spp.); Mussel (*Mytilus galloprovincialis*).
[f] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Crayfish (*Penaeus* spp.); Mussel (*Mytilus galloprovincialis*).
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.005$.

0.28 ng/g in farmed vs. wild-caught, $p < 0.005$; ∑OCPs in bluefish = 4.23 ng/g vs. 3.33 ng/g in farmed vs. wild-caught, $p < 0.05$). When comparing the same species obtained by the two methods of production also we observed this pattern in case of fish (sea bass and salmon). Particularly in the farmed seabass the levels of organochlorine pesticides were considerably higher than in wildlife specimens (Fig. 1B), being *p,p′*-DDE the compound found at the highest concentrations (data not shown). Other previous studies have also reported higher levels of DDT contamination (and its metabolites) in farmed seabass (Antunes and Gil, 2004; Cirillo et al., 2009; Ferreira et al., 2008a) and salmon (Easton et al., 2002; Hites et al., 2004). Other authors have suggested several theories to explain these differences, such as the proximity of aquaculture facilities to the coasts with high human activity; the fact that farmed fish generally eat more, and because they are caged they have their long-distance movements restricted, and therefore farmed fish tend to reach larger sizes and accumulate more body fat than wild specimens; or the fact that the level of contamination of the raw materials used for aquaculture feed (especially fish oil) is usually high, and this may also influence significantly the highest levels of these contaminants detected in the final product (Berntssen et al., 2010a; Berntssen et al., 2010b; Sprague et al., 2010). The latter is a factor that could be controlled in aquaculture to reduce the total burden of contaminants in fish and seafood produced.

In the case of other species considered in this paper (invertebrates grouped as "other seafood") we found no significant differences between wild and farmed specimens, except in the case of *p,p′*-DDE, whose levels were higher in wild specimens (Table 3). However, unlike the case of fish, these differences were not significant enough to be reflected in a significant difference in the sum of OCPs. Several authors have indicated that the relatively higher levels of organochlorine pesticides (as well as other persistent pollutants, such as PBDEs and PCBs) in benthic invertebrates could be acquired through indirect release from contaminated sediments or direct releases from various industries (Ali et al., 2015; Tomaszewski et al., 2008).

3.1.3. PCBs

As can be seen in Table 4, we found that most PCB congeners were frequently detected in all the investigated samples. Only PCB congeners # 77, 81, 114, 126 and 169 were not detected in any sample. On the contrary, PCBs 153 and 28 were detected in all the samples, and also exhibited the highest concentrations. As described by other authors for aquatic organisms, the group of M-PCBs was the most abundant, and accounted for more than 90% of ∑PCBs (Bayarri et al., 2001; Carubelli et al., 2007). As described above for PAHs and OCPs, also in the case of PCBs the levels found were significantly higher in farmed fish, especially in farmed whitefish species (∑PCBs = 1.64 vs. 0.19 ng/g, P = 0.0019,

Table 4
Concentrations of polychlorinated biphenyls in fish and seafood from the two modes of production: fisheries and aquaculture. All the results are presented as medians, and expressed in ng/g (fresh product weight).

	White fish			Blue fish			Seafood		
	Fisheries[a] (n = 28)	Aquaculture[b] (n = 16)	p	Fisheries[c] (n = 12)	Aquaculture[d] (n = 8)	p	Fisheries[e] (n = 12)	Aquaculture[f] (n = 8)	p
Marker PCBs									
PCB 28	0.05 (0.01–0.12)	0.22*** (0.03–0.69)	<0.0001	0.51 (0.11–0.98)	0.89 (0.10–2.29)	n.s.	0.05 (0.02–0.17)	0.06 (0.01–0.16)	n.s.
PCB 52	0.02 (0.01–0.08)	0.08*** (0.01–0.31)	<0.0001	0.21 (0.04–0.43)	0.38 (0.04–0.92)	n.s.	0.02 (0.01–0.11)	0.02 (0.00–0.06)	n.s.
PCB 101	0.01 (0.00–0.13)	0.11*** (0.00–0.31)	0.0002	0.18 (0.09–0.45)	0.27 (0.04–0.99)	n.s.	0.02 (0.00–0.37)	0.02 (0.00–0.06)	n.s.
PCB 138	0.02 (0.00–0.36)	0.33** (0.00–0.65)	0.0029	0.38 (0.16–1.76)	0.40 (0.12–1.15)	n.s.	0.01 (0.00–0.76)	0.01 (0.00–0.06)	n.s.
PCB 153	0.03 (0.01–0.74)	0.64** (0.00–1.13)	0.0039	0.78* (0.33–4.48)	0.36 (0.27–1.59)	0.0328	0.02 (0.00–1.75)	0.04 (0.00–0.15)	n.s.
PCB 180	0.01 (0.00–0.62)	0.16 (0.00–0.38)	n.s.	0.15 (0.03–2.50)	0.18 (0.07–0.57)	n.s.	0.0 (0.00–0.06)	0.0 (0.00–0.01)	n.s.
∑ M-PCBs	0.17 (0.04–1.93)	1.56** (0.05–3.42)	0.0019	2.55 (0.83–10.04)	2.47 (0.63–7.51)	n.s.	0.12 (0.06–0.49)	0.15 (0.02–0.49)	n.s.
Dioxin-like PCBs									
PCB 77	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
PCB 81	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
PCB 105	0.01 (0.00–0.04)	0.02** (0.00–0.07)	0.0010	0.04 (0.02–0.13)	0.03 (0.01–0.17)	n.s.	0.0 (0.00–0.04)	n.d.	–
PCB 114	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
PCB 118	0.01 (0.00–0.06)	0.06*** (0.00–0.12)	0.0004	0.11 (0.03–0.21)	0.09 (0.02–0.35)	n.s.	0.0 (0.00–0.08)	0.0 (0.00–0.01)	n.s.
PCB 123	0.0 (0.00–0.02)	0.01* (0.00–0.02)	0.0130	0.01 (0.00–0.02)	n.d.	–	0.0 (0.00–0,02)	n.d.	–
PCB 126	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
PCB 156	0.0 (0.00–0.03)	0.0 (0.00–0.01)	n.s.	0.02* (0.00–0.11)	0.0 (0.00–0.03)	0.0228	0.0 (0.00–0,01)	n.d.	–
PCB 157	n.d.	n.d.	–	0.0 (0.00–0.02)	n.d.	–	n.d.	n.d.	–
PCB 167	0.0 (0.00–0.01)	0.0 (0.00–0.01)	n.s.	0.01 (0.00–0.07)	n.d.	–	0.0 (0.00–0,02)	n.d.	–
PCB 169	n.d.	n.d.	–	n.d.	n.d.	–	n.d.	n.d.	–
PCB 189	n.d.	n.d.	–	0.0 (0.00–0.02)	n.d.	–	n.d.	n.d.	–
∑ DL-PCBs	0.02 (0.00–0.13)	0.09** (0.00–0.21)	0.0100	0.16 (0.07–0.53)	0.13 (0.02–0.55)	n.s.	0.01 (0.00–0.18)	0.01 (0.00–0.02)	n.s.
∑ PCBs	0.19 (0.04–2.01)	1.64** (0.05–3.55)	0.0018	2.71 (0.90 - 10.57)	2.59 (0.66–8.06)	n.s.	0.12 (0.06–3.41)	0.15 (0.02–0.52)	n.s.

n.d. Nondetected.
n.s. Not significant.
[a] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Wreckfish (*Polyprion americanus*); Megrim (*Stephanoiepis hispidus*): Sole (*Solea vulgaris*); Seabass (*Dicentrarchus labrax*); Hake (*Merluccius merluccius*); Toothed sparus (*Dentex dentex*); Parrot fish (*Sparisoma cretense*).
[b] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Gilt head fish (*Sparus aurata*); Sole (*Solea vulgaris*); Iridiscent shark (*Pandasius hypophthalmus*); and Seabass (*Dicentrarchus labrax*).
[c] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Tuna (*Thunnus thynnus*); Salmon (*Salmo salar*); Sardine (*Sardina pilchardus*).
[d] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Salmon (*Salmo salar*); Trout (*Salmo trutta*).
[e] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Shrimp (*Parapenaeus* spp.); Crayfish (*Penaeus* spp.); Mussel (*Mytilus galloprovincialis*).
[f] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Crayfish (*Penaeus* spp.); Mussel (*Mytilus galloprovincialis*).
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.005$

Table 5
Concentrations of toxic metals and metalloids in fish and seafood from the two modes of production: fisheries and aquaculture. All the results are presented as medians, and expressed in μg/g (fresh product weight).

	White fish			Blue fish			Seafood		
	Fisheries[a] (n = 28)	Aquaculture[b] (n = 16)	p	Fisheries[c] (n = 12)	Aquaculture[d] (n = 8)	p	Fisheries[e] (n = 12)	Aquaculture[f] (n = 8)	p
Pb	0.03 (0.00–0.06)	0.03 (0.01–0.04)	n.s.	0.03 (0.02–0.06)	0.02 (0.02–0.03)	n.s.	0.04 (0.01–0.04)	0.09 (0.03–0.24)	n.s.
Cd	0.05*** (0.01–0.18)	0.01 (0.00–0.02)	0.0037	0.01 (0.01–0.03)	0.01 (0.01–0.02)	n.s.	0.02 (0.01–0.15)	0.06 (0.01–0.13)	n.s.
Ni	0.04 (0.01–0.53)	0.08* (0.02–2.63)	0.0120	0.05 (0.01–0.27)	0.21* (0.08–0.37)	0.0116	0.08 (0.01–0.12)	0.31*** (0.12–0.53)	<0.0001
As	0.15 (0.05–0.62)	0.58** (0.07–0.92)	0.0044	0.19 (0.01–0.50)	0.12 (0.08–0.15)	n.s.	0.32 (0.08–0.91)	0.72* (0.54–0.93)	0.0403
Al	0.28 (0.08–5.45)	1.15** (0.11–2.26)	0.0065	1.08* (0.42–1.31)	0.46 (0.22–0.73)	0.0124	5.41 (0.13–6.75)	7.83 (3.61–13.46)	n.s.
Hg	0.04 (0.02–0.13)	0.04 (0.02–0.06)	n.s.	0.04 (0.02–0.07)	0.03 (0.01–0.04)	n.s.	0.04* (0.02–0.05)	0.02 (0.01–0.03)	0.0452

n.d. Nondetected.
n.s. Not significant.
[a] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Wreckfish (*Polyprion americanus*); Megrim (*Stephanoiepis hispidus*): Sole (*Solea vulgaris*); Seabass (*Dicentrarchus labrax*); Hake (*Merluccius merluccius*); Toothed sparus (*Dentex dentex*); Parrot fish (*Sparisoma cretense*).
[b] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Gilt head fish (*Sparus aurata*); Sole (*Solea vulgaris*); Iridiscent shark (*Pandasius hypophthalmus*); and Seabass (*Dicentrarchus labrax*).
[c] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Tuna (*Thunnus thynnus*); Salmon (*Salmo salar*); Sardine (*Sardina pilchardus*).
[d] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Salmon (*Salmo salar*); Trout (*Salmo trutta*).
[e] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Shrimp (*Parapenaeus* spp.); Crayfish (*Penaeus* spp.); Mussel (*Mytilus galloprovincialis*).
[f] The data were obtained from the individual analysis of 4 consecutive month composite samples from each of the following species: Crayfish (*Penaeus* spp.); Mussel (*Mytilus galloprovincialis*).
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.005$.

Table 4). In the comparison between the same species (Fig. 1C) this pattern was maintained in fish, and our findings were consistent with previous reports in seabass (Antunes and Gil, 2004; Carubelli et al., 2007; Ferreira et al., 2010), and salmon (Dewailly et al., 2007; Easton et al., 2002; Hites et al., 2004). With regard to the other marine species included in this study (invertebrates), when we considered all the species as a group (Table 4) we found no significant differences between cultivated and wild species. However, when we compared the same species, we found that in the case of mussels levels of PCBs were significantly higher in wild specimens than in aquaculture ones. This result is contrary to that described by other authors (Cirillo et al., 2009), as they reported the same pattern than in fish: higher levels in farmed mussels. As we discussed

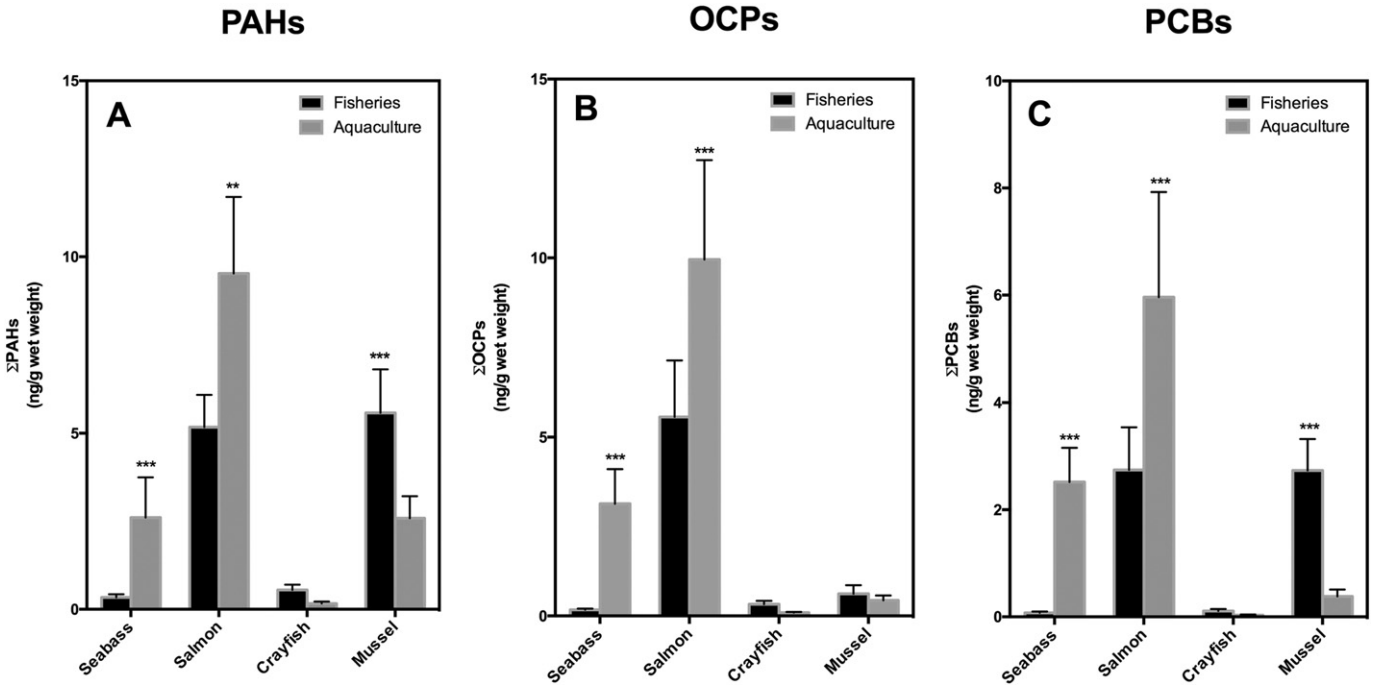

Fig. 1. Comparison of the levels of polycyclic aromatic hydrocarbons (A), organochlorine pesticides (B), and polychlorinated biphenyls (C) in four species of marine products obtained by aquaculture or extractive fishing. **$p < 0.01$, ***$p < 0.005$.

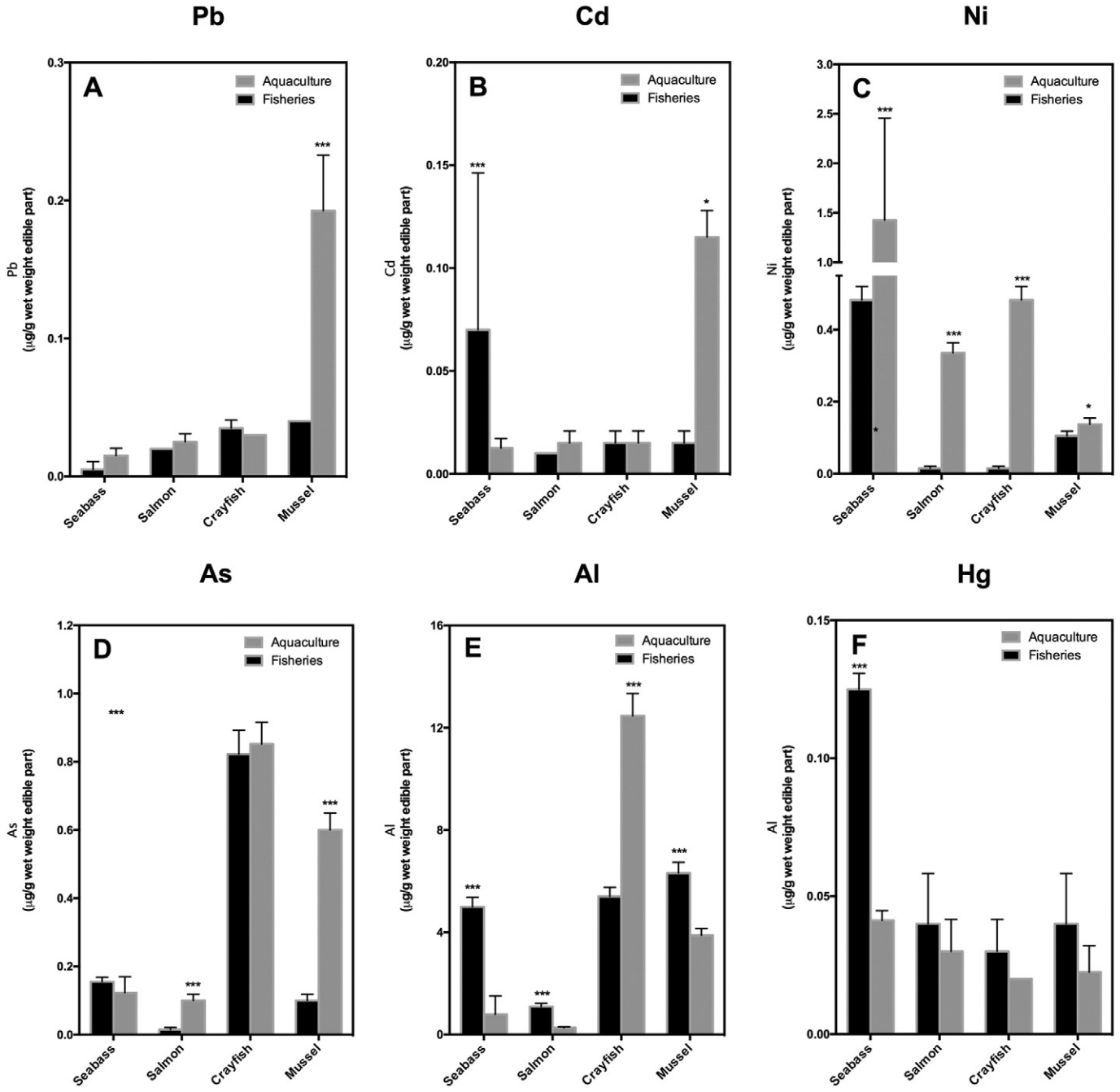

Fig. 2. Comparison of the levels of Pb (A), Cd (B), Ni (C), As (D), Al (E), and Hg (F) in four species of marine products obtained by aquaculture or extractive fishing. *$p < 0.05$, ***$p < 0.005$.

above for organochlorine pesticides, is not surprising to find higher levels of these contaminants in wild benthic organisms, since these are pollutants that tend to accumulate in marine sediments. However, the discrepancy with those data reported by other authors probably has to do with the origin of the mussels sampled in each research work (data not available), because that for persistent organic pollutants it has been reported that there is a heterogeneous distribution north to south, with maximum concentrations in estuarine regions (Robinson et al., 2016).

3.1.4. Toxic metals and metalloids

Finally, we also determined the concentrations of key inorganic elements in fish and seafood species in order assess possible differences among both farmed and wild specimens. The results are summarized in Table 5, and as it can be observed we also found significant differences depending the mode of production. However, in this case not always the concentrations were higher in farmed animals than in wild ones. Whereas the Ni concentrations were significantly higher in farmed than in wild specimens of seafood, the rest of elements exhibited a variable behaviour. Thus, in whitefish the levels of As and Al were also higher in farmed species, but Cd was significantly higher in wild specimens; in bluefish apart from the Ni no one element was detected to be higher in farmed than in wild specimens, but oppositely the levels of Al were higher in wild than in farmed species; and in invertebrate seafood specimens As was higher in farmed, but Hg was higher in wild specimens. In Fig. 2 the results of the comparison of element concentrations in the same species from both modes of production are

shown. Again, the results show statistically significant differences between farmed and wild specimens, but without a definite pattern so it is very difficult to draw conclusions.

It has been described that elements in marine environment have a more complex distribution than organic pollutants, reflecting local anthropogenic inputs, natural sources and hydrological conditions (Robinson et al., 2016). The accumulation of elements in marine organisms depends on factors such as size, age, physiological status, habitat preferences and degree of contamination, feeding behaviour, ecological needs, growth rates of aquatic organisms, metabolic activity, and even the season (Alam et al., 2002; Alasalvar et al., 2002; Kalantzi et al., 2014). Moreover, Ferreira et al. (2010) suggested metal accumulation can be also species-dependent. As shown in Fig. 2 we found higher levels of Cd and Hg in wild than in farmed seabass, contrary to what other authors have reported for this species (Cirillo et al., 2010). However, Cirillo et al. (2010) reported higher levels of Cd and Hg in fresh catch fish (all species considered as a group) compared to farmed fish. As a possible explanation for the lower levels of certain elements found in farmed fish some authors have postulated the effect of biodilution due to the faster growth rates of farmed specimens compared with the wild ones, at least for certain species such as salmon (Kelly et al., 2008). However, the results of this and other studies are not always consistent with this explanation. For instance, the same than Cirillo et al. (2010) we found that the levels of Cd and Ni in farmed mussels were much higher than those found in wild mussels.

As discussed above, the ecological risks of metal contaminants are difficult to document because the responses differ among species, threats differ between metals, and environmental influences are complex (geochemical influences, biological differences, and differences between metals) (Luoma and Rainbow, 2005). Probably the differences found in this research work cannot be attributed solely to the production mode.

3.2. Dietary intake of organic and inorganic pollutants from farmed and wild seafood consumption

In recent years, a number of studies on human dietary exposure to POPs and toxic elements through seafood have been carried out around the world (Domingo and Bocio, 2007; Muralidharan et al., 2009; Su et al., 2012), and specifically in Spain (Bordajandi et al., 2006; Llobet et al., 2008; Marti-Cid et al., 2007), and it has been established that the dietary intakes of these compounds vary considerably between regions and between population groups within countries. However, limited data are available concerning the human exposition of POPs by seafood consumption taking into account whether this food have been farmed or wild-caught (Cirillo et al., 2009). For this reason, in this research work two theoretical groups of consumers were considered: a) consumers who would choose only farmed seafood; and b) consumers who would choose only seafood captured from the wild. In Fig. 3 we represent the estimated daily intake of the total burden of PAHs, OCPs and PCBs for both age groups (adults and children), and in both scenarios. According to our results, and although the levels of POPs compared with the tolerable daily intakes (TDIs) can be considered low in both groups of age, relevant differences depending on the type of production model were observed. Thus, statistically significant higher levels of daily intake of $\sum$ PAHs $\sum$ OCPs and $\sum$ PCBs were detected if only farmed seafood were consumed, compared with the same estimations done for consumers who would choose wild caught seafood. Similarly the estimations in both scenarios and ages were done for elements, and the results of the comparison are shown in Fig. 4. As mentioned above for organic pollutants also the intake of Pb, Ni, As, and Al would be significantly higher if only farmed seafood were consumed. However, in the case of Cd and Hg the exposure would be higher consuming only wild-caught seafood in both, adults and children.

These results were expected considering what we have described above, since the levels of most organic pollutants and many elements were higher in farmed seafood than in seafood from extractive fishing. Therefore, it can be concluded that farmed seafood would represent a more important source of these organic and inorganic pollutants for humans than wild-caught seafood.

It has been reported that the main source of organic and inorganic contaminants to farmed fish is aquafeed (Antunes and Gil, 2004; Hites et al., 2004; Jacobs et al., 2002). It has been also reported that whereas the use of fish oil for the manufacture of aquafeed is the main source of POPs for farmed fish, fish meal is the main source of elements such

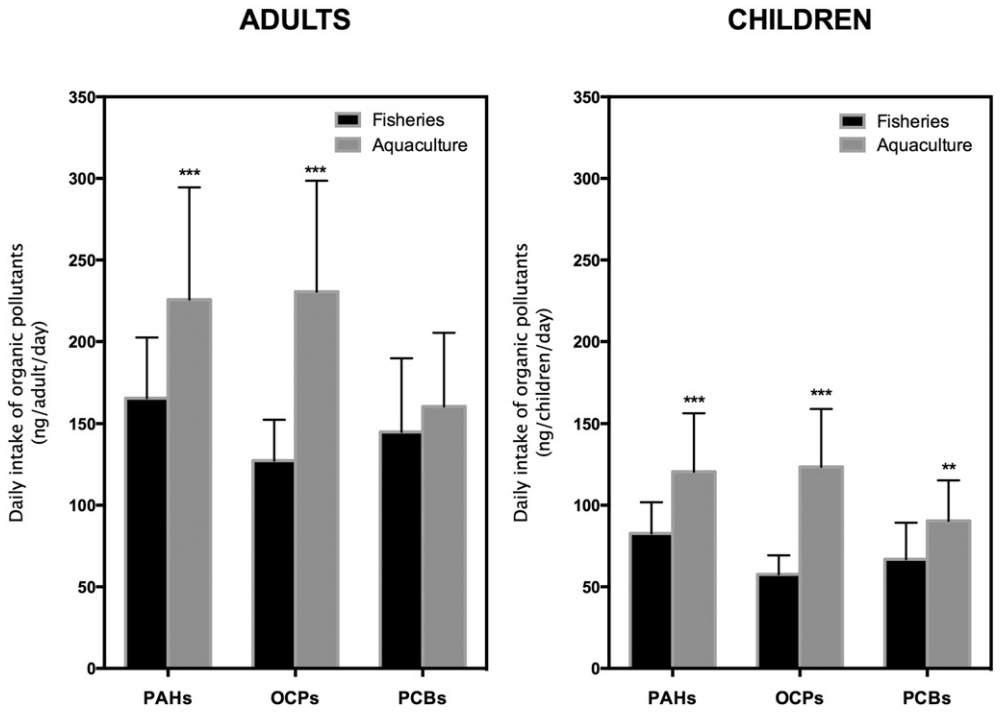

Fig. 3. Comparison of the intake of polycyclic aromatic hydrocarbons, organochlorine pesticides, and polychlorinated biphenyls in Spanish adults (A) and children (B) who theoretically would consume only seafood from aquaculture or only wild-caught seafood. $^{**}p < 0.01$, $^{***}p < 0.005$.

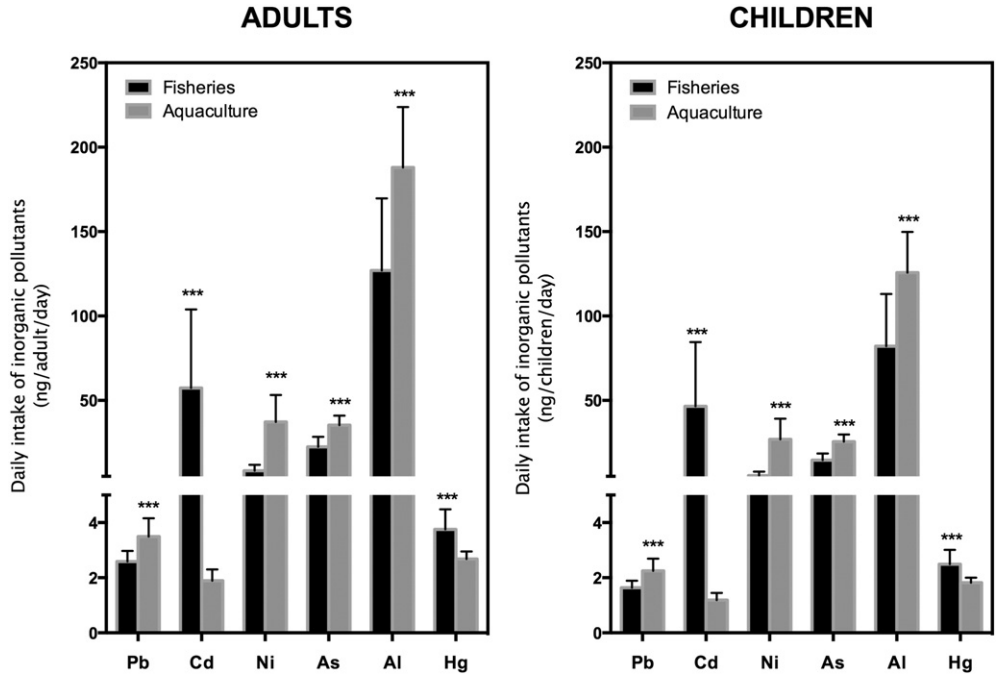

Fig. 4. Comparison of the intake of Pb, Cd, Ni, As, Al, and Hg in Spanish adults (A) and children (B) who theoretically would consume only seafood from aquaculture or only wild-caught seafood. ***$p < 0.005$.

as mercury, cadmium and arsenic. Moreover, as farmed fish tend to have higher lipid levels than their wild counterparts (Cirillo et al., 2009; Ferreira et al., 2008a), this can lead to higher bioaccumulation of lipophilic persistent pollutants through the food chain. In addition, the location of aquaculture facilities in areas with high anthropogenic pressure could add to the higher levels of contamination of farmed seafood. However, one of the great advantages of aquaculture is the control that can be exercised over the production process. It has been reported that the change of formulations of aquafeed, replacing marine ingredients with vegetable ingredients, alone or in combination with animal origin ingredients, is an effective way to reducing the use of fish oil and fish meal in fish feeds (Miller et al., 2008; Torstensen et al., 2011). Thus, several authors have demonstrated how alternative feeds reduced the load of most POPs in farmed salmon (Berntssen et al., 2010a; Berntssen et al., 2010b; Sprague et al., 2010), while maintaining its concentrations of beneficial omega-3 PUFAs. Moreover, it has been demonstrated that seafood produced in decontaminated environments (i.e. treatment of the sediment below the farming cages with activated charcoal) accumulated significantly less total organochlorine residues in their soft tissues, as compared to those raised in facilities with untreated sediments (Tomaszewski et al., 2008).

It is important to note that the seafood contamination and intake of pollutants reported in this study does not represent any unacceptable risk to humans, regardless if farmed or wild-caught fish is consumed, as all the residues found in seafood in both farmed and wild caught, were well below the limits set for commercial species by the European authorities (EC, 2005; EC, 2011), and the estimated intakes were below the tolerable daily intakes established for the different pollutants. However, the use of decontaminated aquafeeds, as well as the decontamination of sediments under aquaculture facilities could be useful measures to lowering the level of pollutants in farmed seafood, while maintaining beneficial nutrients (Berntssen et al., 2010b; Sprague et al., 2010). These practices would allow not only match the levels of pollution from wild-caught seafood, but also could provide products with much lower levels of pollutants than those, which in turn would allow to increase consumption over the "one serving per week" recommended in current dietary guidelines, and so benefit the consumer of the enormous positive health effects of the valuable nutrients of fish.

4. Conclusions

In this research work we have investigated a total of 53 persistent organic pollutants and 6 toxic elements in samples of fish produced in aquaculture as well as in those obtained by extractive fishing. Of pollutants investigated we detected 30 out of 53 organic pollutants, and the 6 elements. Contaminants detected were virtually the same, regardless of the mode of production. However, it is noteworthy that for 21 organic contaminants levels found in aquaculture products were significantly higher than those in samples from extractive fishing. Only in the case of carcinogenic PAHs (PAH7) we found higher levels in wild seafood species. In addition we also found that the levels of Ni were significantly higher in all aquaculture products, and the same occurred with the levels of As, although differences in bluefish species investigated did not reach statistical significance. However, for other elements such as Cd, the ratio was reversed, with much higher levels in wild products (particularly in whitefish species). Taking all these data as a whole, and based on the rates of consumption of fish and seafood of the Spanish population, our results indicate that a theoretical consumer who chose to consume only aquaculture products would be exposed to levels of pollutants investigated about twice higher than if this theoretical consumer had chosen only products from extractive fisheries. Obviously, this is a purely theoretical model, but allows us to highlight the fact that it seems necessary to implement certain measures, which are currently possible technologically, in the practice of aquaculture production in order to reduce these levels and produce seafood with very low levels of contaminants. Although our results do not indicate that neither the maximum residue limits or levels tolerable intake of contaminants are exceeded, we actually believe that there is an opportunity to produce "toxicologically safer food", so this would allow an increase in consumption, which in ultimately would favour both the commercial interests of the aquaculture industry and, above all, the consumers who would benefit from the well-known beneficial effects of nutrients from fish and seafood.

Competing financial interest declaration

There are no actual or potential conflicts of interest to declare for any author.

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Food and Chemical Toxicology

Influence of the method of production of eggs on the daily i of polycyclic aromatic hydrocarbons and organochlorine cor An independent study in the Canary Islands (Spain)

Chemosphere

Polychlorobiphenyls and organochlorine pesticides in conventional and organic brands of milk: Occurrence and dietary intake in the population of the Canary Islands (Spain)

Science of the Total Environment

An estimation of the carcinogenic risk associated wit multiple relevant carcinogens found in meat and ch

Science of the Total Environment

Levels of organochlorine conta and their impact on the health in the Canary Islands (Spain)

Science of the Total Environment

Assessment of human health hazards associate exposure to organic and inorganic contamina consumption of fishery products in Spain

Science of the Total Environment

Comparative study of the intake of toxic persistent and semi per pollutants through the consumption of fish and seafood from two of production (wild-caught and farmed)

Science of the Total Environment

Consumption of organic meat does not diminish the carcinogenic potential associated with the intake of persistent organic pollutants (POPs)

Science of the Total Environment

Daily intake of anthropogenic pollutants through yogurt consumption in the Spanish population

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Discusión general: Estimación de la exposición a CTP mediante el consumo de alimentos de origen animal en la población española.

Este apartado tiene como finalidad realizar un análisis descriptivo del aporte general de los diferentes grupos alimentarios de origen animal y alimentos seleccionados en esta Tesis Doctoral a la exposición dietética a contaminantes tóxicos persistentes, incluyendo plaguicidas organoclorados (POC), bifenilos policlorados (PCB) e hidrocarburos aromáticos policíclicos (PAH); y en el caso de los productos de la pesca también al arsénico, cadmio y mercurio.

Para ello hemos considerado **dos tipos de consumidores** acorde a la variedad de productos disponibles en el mercado nacional. Por una parte, tenemos los **consumidores convencionales**, para los cuales las estimaciones de ingesta se hicieron en base a las concentraciones de contaminantes de alimentos producidos de forma convencional (productos lácteos, huevos, carne, productos cárnicos y productos de la pesca); y por otra, los consumidores que optan por el consumo de alimentos de producción alternativa (**consumidores alternativos**), en cuyas estimaciones se tuvo en cuenta las concentraciones de estos compuestos en productos lácteos ecológicos, huevos camperos y ecológicos y carnes ecológicas. Para este último escenario, se consideró la misma concentración aportada por los productos cárnicos y los productos de la pesca a la estimación de la ingesta total, ya que la disponibilidad de alimentos ecológicos en estos dos grupos es muy limitada. En las **Tablas 1 y 2 del Anexo II**, se representan las IDE de los contaminantes orgánicos persistentes en los dos escenarios considerados.

Además, debido a que no existen datos específicos del consumo de alimentos de producción alternativa en España, se asumió que el patrón de consumo de cada grupo alimentario por la población infantil y adulta era el mismo entre los consumidores que optan por alimentos de producción convencional o alternativa.

1. Plaguicidas organoclorados

Como se esperaba, las ingestas diarias estimadas (IDE) de POC mediante el consumo de los alimentos de origen animal incluidos eran muy bajas en los dos grupos de edad considerados, en comparación con las ingestas diarias tolerables (IDT) establecidas por la EFSA. No obstante, se debe tener en cuenta que la población canaria y española ha sufrido una exposición crónica a contaminantes tóxicos persistentes que persiste actualmente, siendo la ingesta dietética la principal fuente de exposición, incluyéndose todos los alimentos recogidos en esta Tesis Doctoral.

En cuanto a la ingesta de **hexaclorobenceno**, los valores obtenidos fueron similares entre las dos producciones (producción convencional: 60,80 y 80,85 ng/kg p.c./día en adultos y niños, respectivamente; producción alternativa: 62,71 y 88,90 ng/kg p.c./día en adultos y niños, respectivamente), la cual era dominada por la carne. El alimento de mayor contribución a la ingesta total de HCB era con mucha diferencia la carne de cordero, que para los grupos de mayor exposición al HCB (niños que consumen alimentos convencionales y alternativos), representaba el 77 y el 65% a la ingesta total, respectivamente. Además, en el caso de los niños que consumen alimentos alternativos, el aporte de la carne de ternera representaba el 12% de la ingesta total de HCB. El consumo de productos lácteos representaba un valor cercano a un 2% en cada uno de los grupos de edad y métodos productivos, mientras que los productos cárnicos aportaban el 4 y 6% si se optaba por la producción convencional o ecológica, respectivamente. El consumo de productos de la pesca representaba valores inferiores al 1% en todos los casos.

En relación a la **ΣDDT**, los valores de ingesta total obtenidos (consumidores convencionales: 77,00 y 134,23 ng/kg p.c./día en adultos y niños, respectivamente; consumidores alternativos: 106,23 y 160 ng/kg p.c./día en adultos y niños , respectivamente) eran dominados por la carne en todos los casos, con una mayor aportación en consumidores ecológicos. En este caso, la carne de cerdo, cordero y ternera eran los principales contribuyentes a dicha ingesta, destacando nuevamente el aporte de la carne de cordero (hasta un 54% en niños que consumen alimentos de producción alternativa). Es destacable el aporte de la carne de ternera en consumidores de alimentos convencionales, representando un 24 y 30% a la ingesta total en adultos y niños, respectivamente. El segundo grupo de mayor aporte eran los productos cárnicos, con mayor importancia en los consumidores convencionales (hasta un 10% en adultos). El aporte de los lácteos era mayor en consumidores convencionales (4% aproximadamente) y disminuía a un 2% en los alternativos. El consumo de productos de la pesca representaba aproximadamente un 1% en todos los casos.

Con respecto a la ingesta de **ΣHCH**, ésta era significativamente mayor mediante el consumo de alimentos de producción alternativa (producción convencional: 11,25 y 21,51 ng/kg p.c./día en adultos y niños, respectivamente; producción alternativa: 41,45 y 60,69 ng/kg p.c./día, respectivamente). En los consumidores convencionales, el consumo de carne representaba el 67% a la ingesta total de ΣHCH y, en este caso, los productos lácteos era el grupo de segunda mayor contribución (cerca de un 24% en niños). Así, los alimentos de mayor aporte eran la carne de cerdo y el queso, con una contribución conjunta superior al 50% en los dos grupos poblacionales. En los consumidores ecológicos, destaca de forma muy clara el aporte de la carne (superior al 95% en los dos grupos de edad). Así, el aporte conjunto de la carne de cordero y ternera era del 87 y 82 % en adultos y niños, respectivamente. En estos consumidores, el aporte de los lácteos disminuía a un 1%.

Acorde a nuestros resultados, la **ingesta total de POC** por la población adulta española mediante el consumo de alimentos de origen animal es de unos **155** y **212 ng/kg p.c./día** si se opta por la producción convencional o alternativa, respectivamente. El consumo de carne representa un 85% de dicha ingesta si se consideran alimentos convencionales y aumenta a un 93% si se consideran los alimentos ecológicos. Esta diferencia de ingesta se explica por una mayor contaminación de plaguicidas organoclorados en las carnes de producción ecológica, destacando el aporte de la carne de cordero. En el caso de los consumidores convencionales, el segundo grupo de mayor aporte eran los productos lácteos (7.5%) y en tercer lugar los productos cárnicos (5%). En el caso de los consumidores alternativos, se alcanzaban mayores valores de ingesta mediante el consumo de productos cárnicos, mientras que la aportación de los productos lácteos disminuía a un 2%, lo que denota una menor contaminación por POC en dichos alimentos.

En los niños, la **ingesta total de POC** aumenta hasta los **212** y **314 ng/kg p.c./día** mediante los alimentos convencionales y ecológicos, respectivamente. En este caso, la carne representa un 80% a la ingesta total en los consumidores convencionales y dicho aporte aumenta a un 89% en los consumidores alternativos. En este grupo de edad, los productos lácteos representan aproximadamente un 11% al total de dicha ingesta en los consumidores convencionales; mientras que en los alternativos disminuye a un 3%, con una mayor ingesta a través de los productos cárnicos (6.5%).

En relación a los huevos, considerando los dos grupos de población y ambos métodos de producción, su consumo representa un valor de ingesta de POC inferior al 1% en todos los casos. De esta forma, el patrón de ingesta de POC mediante el consumo de alimentos de origen animal se puede representar de la siguiente manera:

Consumidores convencionales: Carne>productos lácteos>productos cárnicos>productos de la pesca> huevos.
Consumidores ecológicos: Carne>productos cárnicos>productos lácteos>productos de la pesca> huevos.

Bajo este contexto, los alimentos de origen animal se contaminan diariamente por POC presentes en el medioambiente. Pese a estar prohibidos en la mayoría de los países a partir de la década de los 70 o más recientemente, las variaciones geográficas y regulatorias en el uso y restricción de POC en el mundo pueden explicar las diferencias de los niveles de estos compuestos, basándonos en los diferentes patrones y rutas de exposición, incluyendo la contaminación de los alimentos. En consecuencia, los niveles de POC varían en función de donde se producen, dependiendo de si existen fuentes activas de contaminación o de un uso excesivo e incontrolado de los mismos en el pasado. Finalmente, ello se traduce en diferentes niveles de estos residuos químicos en aguas, suelos y alimentos a lo largo de todo el mundo.

Por ejemplo, el DDT se sigue utilizando en algunos países para el control de enfermedades tropicales, como la malaria. Otros POC presentan más usos, como el control ambiental o como agente ectoparasitario en ganadería convencional (ej: lindano). El DDT además se ha usado recientemente en países subsaharianos, lo que podría favorecer la deposición en los suelos vía volatilización y el transporte atmosférico desde esta zona a territorios cercanos. Otro ejemplo lo constituye el HCB, usado como pesticida en el pasado, pero emitido actualmente como un subproducto en la manufacturación de numerosos químicos clorados. Mientras que sus niveles de producción han disminuido, su difusión en el medio ambiente como subproducto de procesos industriales es motivo de preocupación.

2. Bifenilos policlorados

En relación a la **ingesta de M-PCB**, compuestos que representan en todos los estudios realizados la principal fuente de exposición alimentaria al total de PCB, el principal aporte a la ingesta dietética mediante los alimentos de origen animal seleccionados procedía de la carne y los productos cárnicos. Mediante el consumo de todos los grupos alimentarios incluidos, la ingesta de estos contaminantes alcanzó valores de **67,84** y **137,55 ng/kg p.c./día** en adultos y niños que consumían alimentos convencionales, respectivamente; y en consumidores alternativos de **68,81** y **139,27 ng/kg p.c./día** en adultos y niños, respectivamente. Por tanto, la ingesta total de M-PCB era muy similar entre ambos tipos de consumidores.

En todos los escenarios considerados, la carne era el grupo alimentario que representaba una mayor exposición dietética a los M-PCB, con un aporte a la ingesta total que oscilaba entre el 70 y el 80%. Dentro de este grupo, el alimento de mayor contribución era la carne de ternera, con un aporte aproximado del 31% al total de la ingesta de M-PCB en la producción convencional, mientras que en la ecológica disminuía al 25%. El segundo alimento de mayor aportación era la carne de cerdo, con un aporte a la ingesta total del 21%; y el tercero la carne de pollo, con una contribución aproximada del 13%, independientemente del modo de producción.

En el caso de los productos cárnicos, su aporte a la ingesta total de M-PCB en la población adulta era de un 15%. En los niños aumentaba al 19% y era reseñable que mediante el consumo de chorizo se alcanzaba el 7% de la ingesta total en este grupo de edad. El tercer grupo alimentario de mayor contribución eran los productos lácteos, destacando el aporte de la leche, especialmente en la población infantil. Así, considerando la producción convencional, los lácteos aportaban un 5% a la ingesta de M-PCB, mientras que mediante la producción

alternativa aumentaba al 9%. Por último, la contribución de los productos de la pesca se estimó en torno al 3% en cada uno de los escenarios.

En relación a la **ingesta de DL-PCB**, es de gran importancia reseñar que la toxicidad de las dioxinas y compuestos análogos se relaciona a la cantidad acumulada en el cuerpo durante el periodo de vida. Las propiedades toxicológicas de los DL-PCB son similares a las de las PCDD y las PCDF y, ciertas evidencias, sugieren que incluso bajas dosis de DL-PCB pueden causar efectos durante una exposición prolongada, especialmente en el desarrollo neurológico de los niños. En este sentido, en el año 2001 la Comisión Europea del Comité Científico en Alimentos, estableció una **IDT recomendada de 2 pg OMS-TEQ/kg p.c./día** para todos los grupos alimentarios (incluyendo las PCDD, PCDF y los DL-PCB).

Acorde a los resultados publicados, el consumo de los alimentos de origen animal incluidos representa un riesgo moderado en relación a la ingesta de TEQ, alcanzándose un 53% de dicha recomendación mediante el consumo de alimentos de producción convencional por la población adulta, mientras que en los niños se alcanza el límite recomendado. El escenario es aún peor si se consideran los alimentos de producción ecológica, ya que el límite se supera en la población adulta e infantil, representando valores de 4.24 y 6.97 pg OMS-TEQ/kg p.c./día, respectivamente. Este hecho se agrava más si consideramos que dicha ingesta recomendada se establece para el total de alimentos (no sólo los de origen animal) y no solo para los DL-PCB, sino también para los grupos de dioxinas PCDD y PCDF.

Según nuestras estimaciones, el grupo que más contribuye a la ingesta de TEQ son los lácteos, teniendo el queso una gran relevancia, seguido por la carne (principalmente debido al consumo de ternera); mientras que el aporte de los productos cárnicos, productos de la pesca y huevos es escaso. Así, mediante el consumo de lácteos de producción convencional se alcanza el 41 y el 77% de dicho límite en adultos y niños, respectivamente, con la mayor contribución por el queso.

Por otra parte, a través del consumo de alimentos ecológicos se supera dicho valor considerando solo el consumo de lácteos, mediante los cuales se alcanzaban valores de 4.11 y 6.7 pg OMS-TEQ/kg p.c./día en adultos y niños, respectivamente. En este escenario, el queso aporta un 59 y un 66% a la ingesta total de TEQ la población adulta e infantil, respectivamente; seguido de la leche, que representa un 38 y un 30% de la ingesta en adultos y niños, respectivamente. Estos resultados diferían bastante a los de la evaluación de la EFSA, cuyo informe concluye que la leche y productos lácteos contribuyen en solo un 7.3-24.6% a la IDT, considerando a los productos de la pesca el grupo de mayor aporte a la ingesta de DL-PCB.

El segundo grupo alimentario con una mayor contribución a la ingesta de TEQ era la carne, con un aporte a la ingesta total en consumidores de alimentos de producción convencional del 10 y el 19% en adultos y niños, respectivamente. Sin embargo, en consumidores de alimentos ecológicos, dicha contribución disminuía hasta el 3% aproximadamente, debido a un aporte significativamente mayor por los productos lácteos.

Además, en todos los grupos alimentarios incluidos en esta Tesis Doctoral, la recomendación establecida por la OMS podía ser superada mediante la ingesta de solo un determinado alimento o subgrupo alimentario, principalmente ligado a la detección de los congéneres de mayor toxicidad. Como ejemplos, podemos nombrar a los huevos o el yogur, que a pesar de ser alimentos de muy poco aporte a la ingesta media global de TEQ (en los huevos inferior al 1% en todos los casos), un 5 y un 16% de las muestras de huevos y yogures analizadas, respectivamente, superaron dicho límite, alcanzándose valores de ingesta de hasta 12 pg OMS-TEQ/kg p.c./día en las muestras de yogur más contaminadas.

En cualquier caso, se debe destacar la existencia de grandes diferencias en los niveles de TEQ entre las muestras analizadas y las ingestas diarias estimadas. Por tanto, mientras que las muestras menos contaminadas mostraban valores de DL-PCB cercanos a 0 o indetectables de TEQ, las más contaminadas (incluidas en el percentil 75) alcanzaron niveles tan altos como 76 pg (en muestras ecológicas de queso), encontrándose los niños siempre en una situación peor. Estos resultados son extremadamente preocupantes debido a los efectos en la salud (neurológicos, reproductivos, inmunológicos y carcinogénicos) que han sido atribuidos a los DL-PCB y se necesitan más estudios para aclarecer el origen de esta contaminación. Sin embargo, debido a que pueden estar involucrados diversos mecanismos en los efectos inducidos por los PCB, los diferentes congéneres de PCB pueden producir diferentes efectos en los humanos, que están expuestos a mezclas complejas de PCB con actividades biológicas diferenciadas.

De nuevo, es importante subrayar que el perfil de distribución de los contaminantes está influenciado ampliamente por las diferencias geográficas de contaminación, detectándose mayores concentraciones en algunos países debido a una mayor actividad industrial o una mayor acumulación debido a las propiedades fisicoquímicas de estas moléculas, dependiendo también de las condiciones de regulación de estas sustancias dentro de cada territorio.

3. Hidrocarburos aromáticos policíclicos

Con respecto a la ingesta total de PAH, se incluyó un total de 16 sustancias (consideradas prioritarias por la EPA) en los estudios desarrollados para el yogur y los huevos. La ingesta total estimada de los mismos era muy superior en los huevos frente al yogur, cuyas ingestas estimadas en el grupo de mayor exposición estudiado (niños que consumen alimentos convencionales) alcanzaron valores de **9,54** y **47,07 ng/kg p.c./día** para el yogur y los huevos, respectivamente. En el yogur, las ingestas eran muy similares considerando ambos tipos de producción, pero la ingesta de PAH era significativamente inferior al consumirse huevos de producción campera o ecológica. Así, al consumirse huevos de producción alternativa, dicha ingesta disminuía hasta **18,99 ng/kg p.c./día** (media entre la producción campera y ecológica).

Además, el patrón anterior se repetía al considerar la ingesta de los 8 PAH con reconocida carcinogenicidad según la EFSA, siendo muy superior su ingesta dietética mediante los huevos que con el yogur (cuya ingesta era inferior a 1 ng/kg p.c./día en todos los casos). Así, la ingesta de PAH8 era significativamente superior mediante el consumo de huevos de producción tradicional (**2,41** y **5,97 ng/kg p.c./día** en adultos y niños, respectivamente) frente a los de producción alternativa (**0,99** y **2,34 ng/kg p.c./día** en adultos y niños, respectivamente).

Para la carne, los productos cárnicos y los productos de la pesca se consideró un total de 7 PAH, expresados como B(a)Peq, al asumirse la equivalencia tóxica de los mismos con el compuesto más tóxico, el benzo(a)pireno. De esta forma, el objetivo era evaluar la exposición de la población adulta e infantil a estas sustancias y considerar si dicha población puede estar expuesta a unos niveles de contaminantes carcinogénicos ambientales superiores al cociente de riesgo recomendado (RQ), de acuerdo al modelo propuesto por la USEPA. El grupo alimentario de mayor aporte a la ingesta de estas sustancias en ambos grupos de edad era el de los productos cárnicos, seguido por la carne y por último los productos de la pesca (cuya aportación era escasa).

Considerando las carnes convencionales, la ingesta total de B(a)Peq mediante estos grupos alimentarios alcanzaba valores de **17,81** y **47,04 ng/kg p.c./día** en adultos y niños**,**

respectivamente. Dicha ingesta se asociaba principalmente al consumo de chorizo, que por sí solo, aportaba aproximadamente el 70 y 80% a la ingesta total de B(a)Peq en adultos y niños, respectivamente. El segundo alimento de mayor contribución al total de dicha ingesta era la carne de cerdo, con un aporte del 15 y el 11% al total de dicha ingesta en adultos y niños, respectivamente. Teniendo en cuenta las carnes de producción ecológica, la ingesta total de B(a)Peq es casi idéntica, con valores de **17,25** y **45,86 ng/kg p.c./día** en adultos y niños, respectivamente, con una aportación semejante de cada alimento en particular. Por lo tanto, se puede concluir que el aporte global de las carnes al total de la ingesta B(a)Peq es muy similar entre ambos tipos de producción, con la excepción de la carne de ternera, cuyo aporte es superior en consumidores convencionales.

4. Contaminantes inorgánicos persistentes en productos de la pesca

Se llevó a cabo el estudio de la exposición a tres elementos tóxicos inorgánicos mediante el consumo de productos de la pesca, incluyendo especies de pescado azul, pescado blanco, cefalópodos y marisco, con la premisa de evaluar el riesgo carcinogénico asociado a la ingesta de estos contaminantes. La ingesta total de estos tres elementos mediante estos productos alimenticios alcanzó valores cercanos a **550** y **781 ng/kg p.c./día** en adultos y niños, respectivamente.

4.1 Arsénico

Este era con diferencia el elemento de mayor aporte dietético, ya que representaba en adultos y niños el 85% de la ingesta total de estos tres elementos inorgánicos. Las especies de pescado blanco consumidas aportaban hasta el 46 y el 53% a la ingesta total de As en adultos y niños, respectivamente. El segundo grupo de mayor aportación era el marisco, pese a tener un consumo inferior al del pescado azul.

4.2 Cadmio

Era el elemento inorgánico de menor aporte dietético, con un 5-6% aproximadamente en adultos y niños. Las especies de marisco seleccionadas (gamba, langostino y mejillón) eran las de mayor contribución dietética, seguidas de las especies de pescado blanco.

4.3 Mercurio

La ingesta de este elemento a través de los productos de la pesca seleccionados, representaba sobre un 10% a la ingesta total en adultos y niños. El alimento de mayor aporte era el pescado blanco, representando un 55 y un 64% a la ingesta total de Hg en adultos y niños, respectivamente. Seguidamente, el alimento de mayor aporte era el pescado azul, contrariamente a muchos estudios, que describen a las especies de pescado azul como la principal fuente de exposición dietética al mercurio.

Como **conclusión general** a este apartado, debemos recalcar que la presencia y cuantificación de CTP en todas las muestras de alimentos analizadas, presentándose incluso mayores niveles de contaminación en determinados alimentos bajo métodos de producción alternativa, sugiere que no puede evitarse la contaminación por compuestos ubicuos persistentes y que las medidas de restricción, eliminación y monitorización de estas sustancias en muestras medioambientales y alimentarias deben incrementarse. Mediante estas estimaciones se confirma la presencia y la exposición dietética continua a CTP mediante los alimentos de origen animal.

Conclusiones

CONCLUSIONES

1. En todas las muestras de alimentos, independientemente del tipo de producción considerado, se cuantifican contaminantes orgánicos persistentes pertenecientes a los tres grupos incluidos en el estudio: POC, PCB y PAH, aunque los PAH no se incluyeron para la leche y el queso. Adicionalmente, se cuantifican residuos de aluminio, arsénico, cadmio, mercurio, níquel y plomo en cada uno de los productos de la pesca seleccionados. Por tanto, tal y como se esperaba, el consumo de los alimentos incluidos representa una fuente exógena activa de exposición a estos contaminantes.

2. Se observa la existencia de una disminución de los niveles de residuos de plaguicidas organoclorados en los alimentos de origen animal, coincidiendo con estudios desarrollados a nivel mundial, como consecuencia de su prohibición o restricción de uso. En todos los casos, los niveles de estos plaguicidas son inferiores a los LMR establecidos en la UE.

3. Es destacable la detección de estos compuestos en los métodos de producción alternativos, lo que demuestra la extremada persistencia medioambiental de los mismos. Así, en los lácteos, el patrón de contaminación por POC es mayor en leches, quesos y yogures de producción convencional; mientras que para los PCB el perfil es dominado por la producción ecológica, con la excepción del yogur, en el cual los niveles son similares en ambos tipos de producción. Estos resultados se extrapolan a las ingestas de dichos contaminantes. Además, en el yogur se detectaron PAH carcinogénicos a muy baja concentración pero con alta frecuencia. No obstante, es destacable la existencia de una enorme variabilidad en los niveles de contaminantes cuantificados entre las diferentes marcas analizadas, tanto de producción convencional como ecológica.

4. En cuanto a las IDE, la mayoría de las muestras presentan valores inferiores a las IDT establecidas, pero con patrones bien diferenciados. La ingesta de POC y PCB es claramente dominada por el consumo de carne, destacando el aporte de la carne de cordero. En cuanto a la ingesta de B(a)Peq, el alimento de mayor contribución dietética es el chorizo.

5. En los huevos se estiman los valores de ingesta más bajos para los POC y los PCB, con niveles similares entre métodos productivos. Sin embargo, la ingesta de PAH es relativamente alta y, mediante el consumo de huevos de producción alternativa (campera y ecológica), la ingesta de dichos compuestos (incluidos aquellos de reconocida carcinogenicidad y genotoxicidad), es significativamente inferior frente a la ingesta estimada a través del consumo de huevos de producción convencional, con mayor relevancia en los niños.

6. En relación a la ingesta de PAH, POC, PCB, Al, As, Ni y Pb mediante el consumo de productos de la pesca, ésta es mayor en el caso hipotético que un consumidor opte exclusivamente por los productos de acuicultura frente a los de pesca extractiva, en los cuales el patrón es inverso para el Cd y el Hg. Sin embargo, si se utilizan técnicas de descontaminación en la acuicultura, los niveles de sus productos podrían ser incluso inferiores a los detectados en la pesca extractiva, por lo que se podría incrementar el consumo actual recomendado.

7. Según nuestros cálculos, el límite recomendado de ingesta de TEQ (tóxicos equivalentes a dioxinas), establecido por la OMS en 2 pg OMS-TEQ/kg p.c./día, se alcanza actualmente mediante el consumo de alimentos de origen animal por la población infantil española, independientemente del tipo de producción considerado. En el caso de la población adulta, este valor también se sobrepasa al consumirse alimentos de producción alternativa, mientras que mediante el consumo de alimentos convencionales se alcanza más del 50%. Es notable el hecho de que los productos lácteos constituyen el grupo de mayor contribución global a la ingesta de TEQ, principalmente ligada al queso. Además, los valores estimados son notablemente mayores mediante el consumo de alimentos ecológicos (hasta 6.97 pg/kg p.c./día en niños que consumen dichos alimentos). Además, algunas muestras al presentar los congéneres más tóxicos de PCB, presentan niveles superiores al límite legal establecido.

8. En los estudios mediante los cuales se evalúa el riesgo carcinogénico a través de la exposición alimentaria a carcinógenos ambientales, se concluye que el consumo actual de carne de vacuno, pollo, cerdo, cordero y además del producto cárnico chorizo, se podría asociar a un riesgo carcinogénico moderado en la población española adulta y especialmente en niños, debido a que el cociente de riesgo estimado presenta valores superiores a 1 (RQ>1), no disminuyendo dicho riesgo si se consumen carnes ecológicas. En los productos de la pesca, este cociente se supera por el consumo de pescado blanco y marisco en adultos y niños, y también para el pescado azul en adultos, siendo el Arsénico el principal elemento ligado a dicho riesgo en todos los casos.

9. En base a estos resultados, podemos establecer las siguientes recomendaciones dietéticas desde un punto de vista exclusivamente toxicológico: moderación del consumo de lácteos, ya que constituyen una fuente importante de exposición ocasional a la ingesta dietética de compuestos similares a las dioxinas; consumo de huevos de producción campera y ecológica, debido a una menor ingesta de PAH frente a los de producción convencional; disminución notable (50-80%) del consumo de carne de vacuno, cerdo, pollo y cordero y aumento del consumo de carne de conejo y cabra; disminución notable del consumo de chorizo, especialmente en niños; disminución moderada del consumo de pescado y marisco, especialmente del pescado blanco en adultos. No obstante, se debe establecer y tener en cuenta una adecuada relación entre los riesgos y los beneficios asociados al consumo de cada alimento, considerando todos los hechos científicos posibles.

10. Estos resultados confirman la presencia en diferentes concentraciones de contaminantes tóxicos persistentes en los alimentos de origen animal y recalcan la importancia de su control por las Autoridades Sanitarias. Por lo tanto, deben mantenerse e incluso aumentarse los esfuerzos para disminuir los niveles de estas sustancias en el medio ambiente y los alimentos, debido fundamentalmente a los efectos negativos en la salud asociados a una exposición crónica a estas sustancias; tales como alteraciones neurológicas, reproductivas, inmunológicas y carcinogénicas, especialmente en la población infantil, considerando su especial gravedad en las etapas de desarrollo embrionario y primera infancia.

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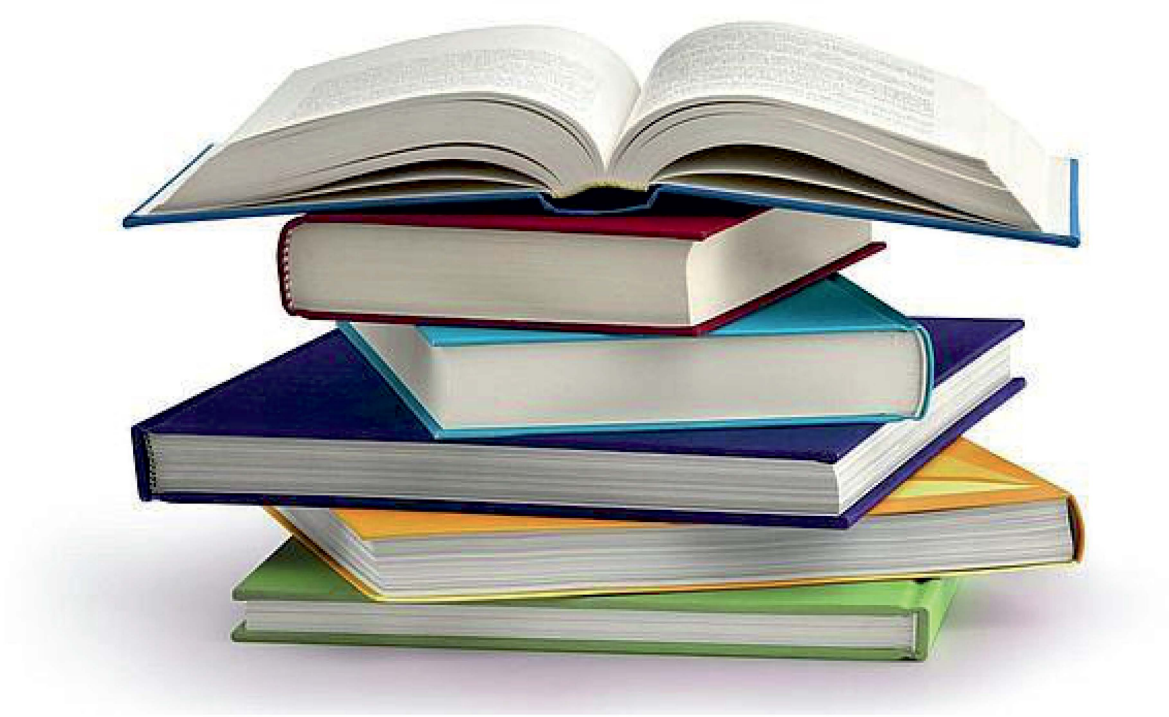

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NOTA: ***Las referencias se refieren a los apartados de Introducción, Justificación y Objetivos y el resumen de cada uno de los bloques.***

Glosario

GLOSARIO

4,4'-DDD*: *Dichlorodiphenyldichloroethane* - Diclorodifenildicloroetano (IUPAC 1,1-dicloro-2,2-bis(p-clorofenil)etano).

4,4'-DDE*: *Dichlorodiphenyldichloroethylene* - Diclorodifenildicloroetileno (IUPAC 1,1-dicloro-2,2-bis(p-metoxifenil)etano).

4,4'-DDT*: *Dichlorodiphenyltrichloroethane* - DiclorodifenilTricloroetano (IUPAC 1,1,1-tricloro-2,2-bis(p-metoxifenil)etano).

ACTH*: *Adrenocorticotropic hormone* - Hormona Adenocorticotropa.

ADN: Ácido desoxirribonucleico.

AECOSAN: Agencia española de Consumo, Seguridad alimentaria y Nutrición.

AEMA: Agencia Europa de Medio Ambiente.

AhR*: *Aryl hidrocarbon Receptor* - Receptor aril hidrocarburo.

AINES: Antinflamatorios no esteroideos.

Al: Aluminio.

ARN: Ácido ribonucleico.

As: Arsénico.

BaP: benzo(a)pireno.

B[a]Peq: benzo(a)pireno equivalentes.

BHE: Barrera hematoencefálica.

BMDL*: *Benchmark dose lower confidence limit* - Límite por debajo del mínimo detectable.

CE: Comisión Europea.

Cd: Cadmio.

CIP: Contaminante Inorgánico Persistente.

CONTAM*: *Panel on Contaminants in the Food Chain* - Comisión Técnica Científica de Contaminantes de la Cadena Alimentaria.
COP: Contaminante Orgánico Persistente.

c-PAH*: *Carcinogenic Polycyclic Aromatic Hydrocarbon* - Hidrocarburo Aromático Policíclico Carcinogénico.

CTP*:* Contaminante Tóxico Persistente.

CYP: *Cytochrome P450* - Citocromo P450.

DD*: *Dihydrodiol dehydrogenase* **-** Di-hidrodiol deshidrogenasa.

DE: Disruptor Endocrino.

DES: Dietilestilbestrol.

DL-PCB*: *Dioxin-like PCB* - PCB análogo a las dioxinas.

DMA: Dimetilarsénico.

DRE*: *Dioxin Responsive Element* - Elemento de respuesta a las dioxinas.

ECHA*: *European Chemicals Agency* - Agencia Europea de Sustancias Químicas.

EFSA*: *European Food Safety Autority* - Autoridad Europea en materia de seguridad alimentaria.

ENCA: Encuesta Nutricional de Canarias.

ENIDE: Encuesta Nacional de Ingesta Dietética Española.

EPA*: *US Environmental Protection Agency* - Agencia de protección del medio ambiente de los Estados Unidos.

E-waste*: *Electronic waste* - Basura electrónica.

FAO*: *Food and Agricultural Organization* - Organización para la Agricultura y la Alimentación.

GABA*: *Gamma-aminobutyric acid* - Ácido γ-aminobutírico.

GH*: *Grown Hormone* - Hormona de crecimiento.

HCB: Hexaclorobenceno.

hCG*: *Human Chorionic Gonadotropin* - Gonadotropina Coriónica Humana .

HCH: Hexaclorociclohexano.

GMP*: *Global Mercury Partnership* **-** Asociación Mundial del Mercurio.

Hg: Mercurio.

IARC: * *International Agency for Research on Cancer* - Agencia Internacional de Investigación sobre el Cáncer.

IDA: Ingesta Diaria Admisible.

IDE: Ingesta Diaria Estimada.

IDMT: Ingesta diaria máxima teórica.

IMT: Ingesta diaria teórica.

IGF-1*: *Insulin-like Growth Factor 1* - Factor de crecimiento tipo insulina.

IPCS*: *International Programme on Chemical Safety* - Programa Internacional de Seguridad Química.

IST: Ingesta Semanal Tolerable.

JECFA*: *Joint FAO/WHO Expert Committee on Food Additives* - Comité Mixto de la FAO/OMS de Expertos en Aditivos Alimentarios.

LC: Límite de cuantificación.

LD: Límite de detección.

LH: Hormona luteinizante.

LHR*: *Luteinizing hormone* Receptor - Receptor de la hormona luteinizante.

LMR: Límite Máximo de Residuos.

MAGRAMA: Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente.

M-PCB*: *Marker Polichlorinated biphenyl* **-** Bifenilo policlorado marcador.

MeHg: Metilmercurio.

MFO*: *Mixed-function oxidase* - Oxidasa de función mixta.

MMA: Ácido monometilarsénico.

MOE*: Margin of Exposure - Margen de exposición.

Ni: Níquel.

NOAEL*: *No-observed-adverse-effect level* - Nivel sin efecto adverso observable.

OC: Organoclorado.

OMS: Organización mundial de la salud.

PAH*: *Polycyclic aromatic hydrocarbon* - Hidrocarburo aromático policíclico.

Pb: Plomo.

PBB*: *Polybrominated Biphenyl* - Bifenilo Polibromado.

PBDE*: *Polybrominated diphenylether* - Difeniléter polibrominado.

PCB*: *Polychlorinated Biphenyl* - Bifenilo policlorado.

PCDD*: *Poly-chlorinated Dibenzo-p-dioxin* - Dibenzo-p-dioxina policlorada.

PCDF*: *Poly-cholrinated Dibenzofuran* - Dibenzofurano policlorado.

PNUMA: Programa de las Naciones Unidas para el Medio Ambiente.

POC: Plaguicida organoclorado.

PPARγ: Receptor gamma proliferador de peroxisomas.

RA: Receptor de andrógenos.

RAR: Receptor de ácido retinoico.

RE: Receptor de estrógenos.

REACH*: *Registration, Evaluation, Authorization and Restriction of Chemicals* - Registro, Evaluación, Autorización y Restricción de las sustancias químicas.

RG: Receptor de Glucocorticoides.

RM: Receptor de Mineralcorticoides.

P50: Percentil 50.

P95: Percentil 95.

ROS*: *Reactive oxygen specie* - Especie reactiva de oxígeno.

RP: Receptor de progesterona.

RQ*: *Risk quotient* - Cociente de riesgo.

RT: Receptor de hormonas Tiroideas.

SCAN*: *Scientific Committee on Animal Nutrition* - Comité Científico de Nutrición Animal.

SCF*: *Scientific Committee on food* - Comité Científico sobre Alimentos.

-SH: Sulfhidrilo.

SNC: Sistema Nervioso Central.

TCDD: *2,3,7,8-Tetrachlorodibenzo-p-dioxin* - 2,*3*,*7*,*8*-tetraclorodibenzo-p-dioxina (IUPAC 2,3,7,8-tetraclorodibenzo[*b,e*][1,4]-dioxina).

TEF*: *Toxic Equivalence Factor* - Factor de equivalencia de toxicidad.

TEQ*: *Toxic Equivalence* - Equivalencia de toxicidad.

TSH*: *Thyrotropin-Stimulating Hormone* - Hormona tireoestimulante.

UE: Unión Europea.

UNEP*: *United Nations for Environmental Protection* - Naciones Unidas para la protección del medio ambiente.

WHO*: *World Health Organization* - Organización Mundial de la Salud.

* Siglas internacionalmente aceptadas. Hemos mantenido, por tanto, su denominación original e indicamos su significado tanto en lengua inglesa como española.

***NOTA: las abreviaciones de este glosario excluyen las incluidas en los artículos publicados*.**

Anexo I

Polychlorobiphenyls and organochlorine pesticides in conventional and organic brands of milk: Occurrence and dietary intake in the population of the Canary Islands (Spain).

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Polychlorobiphenyls and organochlorine pesticides in conventional and organic brands of milk: Occurrence and dietary intake in the population of the Canary Islands (Spain)

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ABSTRACT

The population of the Spanish archipelago of the Canary Islands has been studied in depth regarding its levels of contamination by organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs). Foodstuffs of animal origin, such as milk, are prominent contributors to the body burden of these contaminants. As this population presents one of the highest milk-intake in Spain and Europe, we evaluated the level of OCs and PCBs in 26 commercially available brands of milk (16 conventional and 10 organic brands) present in the market of these Islands, in order to estimate the relevance of milk as a source of these chemicals for the Canary population. Our findings showed that hexachlorobenzene, trans-chlordane, and PCB153 were present in almost all the samples with independence of the type of milk. For both types of milks, the concentration of OCs was very low, showing organic milks lower levels than conventional ones. As a consequence, the estimated daily intake for OCs was lower than the tolerable daily intake (TDI) established by International Agencies. The levels of PCBs in milk were also found to be very low, but, in this case, the situation was the opposite: there were higher levels of PCBs in organic than in conventional brands of milk. Unexpectedly, levels of dioxin-like PCBs (DL-PCBs) reached values higher than 25 pg WHO-TEQ g^{-1} fat in percentile 75 for both types of milk indicating the existence of a number of brands of milk highly contaminated by these toxicants. Consequently, the population who consume the most contaminated milk brands could have estimated daily intakes well above the recommended TDI (2 pg WHO-TEQ kg^{-1} b.w. d^{-1}) established by European Union Authorities. These results are of concern if we consider the well known adverse health effects exerted by dioxin-like compounds.

1. Introduction

Organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs) are a class of persistent chlorinated compounds to which humans are most often non-intentionally exposed. Many sources of these man-made toxicants have been identified and eliminated in last decades; however, their occurrence in our environment is still of major concern. More than 95% of human intake appears to be via the consumption of food (Focant et al., 2003; Darnerud et al., 2006). Due to the high lipophilic nature of these chemicals, they enter human body along with the food fat. Foodstuffs of animal origin are prominent contributors of the OCs and PCBs intake (Fries, 1995; Focant et al., 2002). In this sense, milk and dairy products account around a 30% of the total dietary intake of these chlorinated contaminants in Western populations (Focant et al., 2002; Bordajandi et al., 2004).

Similarly to other foodstuffs, a maximum residue limit (MRL) for these chemicals has been attributed to milk and dairy products. In addition, a tolerable daily intake (TDI) has been established by International Agencies for OCs and PCBs. Despite these regulations, data regarding both, OCs and PCBs (mainly dioxin-like PCBs, DL-PCBs) are required to estimate human exposure to these contaminants. This issue is especially relevant since toxic equivalent factors (TEFs) have been assigned to a number of DL-PCBs (Van den Berg et al., 2006). Thus, by using TEFs we can estimate human exposure in terms of toxic equivalence to dioxins (TEQs).

Our group has previously evaluated the level of chemical contamination by OCs and PCBs showed by the population of the

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Canary Islands (Spain). Despite the fact that most OCs pesticides were banned in Spain in the late 1970s, and PCBs in the late 1980s, our results showed that people living in the Canary Islands in the late 1990s presented a characteristic pattern of chemical contamination as compared with other Western populations. Thus, while they showed a high degree of contamination by OCs, their levels of contamination by PCBs were very low (Zumbado et al., 2005; Luzardo et al., 2006; Henríquez-Hernández et al., 2011). In fact, such results seemed to indicate the existence of a chronic exposure to OCs that persisted in the late 1990s (Zumbado et al., 2005; Luzardo et al., 2006) and even in the first decade of the current century (Luzardo et al., 2009) indicating the existence of active environmental sources of OCs, while, on the contrary, the exposure to PCBs seemed to be low and depending mainly from dietary sources (Henríquez-Hernández et al., 2011).

As cited previously, milk and dairy products could be an important route of exposure to OCs and PCBs in Europe (Focant et al., 2002; Marin et al., 2011). In this sense, it should be highlighted that the population of the Canary Islands shows the highest intake of milk and dairy products among European and Spanish regions (Serra-Majem et al., 2000a,b). Thus, the daily consumption of milk and dairy products have been estimated in 301 and 90 grams (g) per day respectively in the Canary Islands, compared with the 294 and 56 g d^{-1} reported in the Basque Country or the 286 and 74 g d^{-1} reported in Madrid (Serra-Majem, 1999). Furthermore, according to data published by the European Food Safety Authority (EFSA), the mean consumption of milk and dairy products in the European population (257 g d^{-1}) is much lower than in the Canarian population (391 g d^{-1}) (Serra-Majem, 1999).

The present study reports the results obtained from an independent survey of the most of the commercially available brands of milk (16 conventional and 10 organic brands of milk) available in the market of these islands for the occurrence of OCs and PCBs in order to evaluate the potential differences in levels of contamination among organic and non-organic milks and to estimate the milk-related dietary exposure to these chemicals suffered by the people living in this Archipelago.

2. Materials and methods

2.1. Study area

The Canary Islands are located in the Atlantic Ocean, about 100 km away from the nearest point of the North African coast (southwest of Morocco). Geographically, the Islands are part of the African continent; however, from a historical, economic, political and socio-cultural point of view, the Canaries are completely European. Due to its geographical, economic and cultural circumstances, in the Canary Islands, there is a high rate of imports of animal- and vegetable-origin foods from the Spanish mainland and from other countries, mainly from the European Union (EU), but also from Asian or South American countries (where a number of OCs are still in use).

2.2. Sampling

For this study, 16 commercial brands of conventional full-fat milk and 10 brands of organic full-fat milk were randomly collected from high-delivery-rate supermarkets of the Canary Islands. The milk samples were collected between November 2007 and April 2008. Each of the 26 selected brands was sampled monthly during this period of time (six samples for each brand) to obtain a representative estimation for each one and to evaluate potential fluctuations among different batches. The packaging material was Tetra-Brick® in all cases. Tetra-Bricks were gently shaken before an aliquot of 80–100 mL was taken. The aliquots were then frozen at −20 °C until extraction and analyses procedures. Lipid content values given by the producer (around 3.5% fat) were used to obtain the final lipid-corrected values. Some classical liquid–liquid extractions of fat were performed on randomly selected milks to ensure that producer's lipid content values were accurate. A total of 156 samples were collected and stored at −20 °C until analysis. Samples were analyzed individually and then the results grouped to evaluate average levels and ranges. All collection and handling equipments in contact with milk samples were tested for possible OCs and PCBs contamination. No contaminating materials were identified.

2.3. Analytical procedure

The aliquots of whole fat milk were subjected to solid-phase extraction (SPE), gel permeation chromatography (GPC) cleanup, silica-gel SPE cleanup and analyzed by gas chromatography/mass spectrometry (GC/MS), using appropriate internal standards. The analytes included in this study were the diphenyl-aliphatic pesticides and metabolites (methoxychlor, *p,p′*-DDT, *o,p′*-DDT *p,p′*-DDE, o,*p′*-DDE, *p,p′*-DDD, and *o,p′*-DDD); the persistent and bioaccumulative contaminant hexachlorobenzene (HCB); the four isomers of hexachlorocyclohexane (α-, β-, γ-(commonly known as lindane), and δ-HCH); the cyclodienes dieldrin, aldrin, endrin, heptachlor (and cis- and trans-epoxides) and chlordane (cis- and trans-isomers); and endosulfan (α- and β-isomers); we also included the measurement of 19 congeners of PCBs (IUPAC congeners #28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 138, 153, 156, 157, 167, 169, 170, 180 and 189). The standard analytes under study were purchased from Dr. Ehrenstorfer (Riedel-de Haën, Sigma–Aldrich Laborchemikalien GmbH, Germany).

The stored milk (−20 °C) was brought to room temperature and homogenized by vortexing vigorously for 5 min prior to aliquoting. 10 μL of the internal working standard solution (500 ng mL^{-1} each compound in acetone) was added to 2 mL of the milk sample. Column extraction of OCs was performed following a modification of the method suggested by Dmitrovic and Chan (2002). Briefly, the aliquot of 2 mL of milk was mixed with 2 mL of glacial acetic acid and 2 mL of methanol and vortexed. The mixture was then sonicated for 45 min and applied at 1 mL min^{-1} flow rate to a Chromabond C18ec cartridge (3 mL/200 mg, Macherey Nagel, Germany) previously pre-washed with hexane and conditioned with 5 mL each of methanol and de-ionized water by using a RapidTrace SPE Workstation (Caliper Life Sciences, MA, USA). To elute the OCs, 6 mL of hexane were applied at a flow rate of 0.5 mL min^{-1}. The hexane fraction was evaporated to dryness using a RapidVap® N2/48 (Labconco, MIS, USA). The residue was weighed to determine the lipid percent (LeDoux, 2011).

In order to achieve the maximum sensitivity in our analysis, two sequential cleanup steps were performed following a modification of the procedure suggested by Griffitt and Craun (1974). Briefly, the lipid was dissolved in 2 mL of dichloromethane (DCM), and divided in two 1 mL-aliquots that were then individually purified using GPC with a 100% Fluorinated Divinylbenzene GPC column (50 cm × 10 mm i.d. EPA 3640a Pesticide Cleanup GPC Jordi column, Sorbtech Technologies, Atlanta, USA), with DCM as the eluting solvent at a flow rate of 1.6 mL min^{-1}. This GPC system was operated using an automated apparatus (GPC-CL1, Cromlab S.L., Barcelona, Spain). The first 22-mL fraction of the elution, containing the lipids, was discharged. The next 14 mL, containing the organohalogenated contaminants, were collected. The two pesticide-containing fractions per sample were combined and evaporated to near dryness leaving a small amount of an oily residue. This residue was dissolved in 1 mL DCM and subjected to new GPC purification, thereby obtaining a new 14-mL

pesticide-containing fraction that was evaporated to dryness. To remove any remaining trace amount of the lipid, the residue was dissolved with 0.5 mL of hexane, followed by loading onto a silica-gel SPE cartridge, which was pre-washed with 6 mL of 10% methanol/DCM and then 8 mL of 5% DCM/hexane. Organohalogenated chemicals were first eluted from the column with 8 mL of 5% DCM/hexane and then with 8 mL of 10% methanol/DCM. The combined fractions were evaporated almost to dryness and the volume made up to 200 μL with cyclohexane that was used for GC–MS analysis.

Chromatographic analysis was performed using a Thermo-Finnigan TRACE DSQ GC/MS instrument as previously reported (Dmitrovic and Chan, 2002; Syrago-Styliani et al., 2006; Luzardo et al., 2009). A fused silica capillary column BPX5 (Crosslinked 5% phenyl methylpolysiloxane, SGE Inc., USA) with a length of 30 m, an i.d. of 0.25 mm and a film thickness of 0.25 μm was used as the stationary phase. Helium at a flow rate of 1 mL min^{-1} was used as carrier gas. Temperatures were programmed as follows: initial oven temperature of 80 °C held for 1 min, ramped at 10 °C min^{-1} to 300 °C and then held for 9 min. Injector and transfer line were set at 200 °C and 310 °C, respectively. Standards and samples were injected (2 μL) in splitless mode. Two chromatographic analyses were performed for each sample to obtain mass spectra in two different ionization modes. For DDT and metabolites methoxychlor, endrin, and PCB congeners 28, 52, 101, and 118, mass spectra were obtained in electronic impact mode (GC/EIMS) at 70 eV, with an ion source temperature of 200 °C. For the rest of analytes included in this study, mass spectra were obtained in negative chemical ionization (GC/NCIMS) using methane as buffer gas at a flow rate of 2.5 mL min^{-1}. Analyses were carried out in selected ion monitoring (SIM) in both cases. Tetrachloro-m-xylene was used as internal standard (IS) in the GC/EIMS mode, and PCB 202 as the IS in the GC/NCIMS mode. We considered the limit of quantification (LOQ) as 10-fold the standard deviation of the blank, and the limit of detection (LOD) as the half of LOQ. Nevertheless, only LOQ has been employed throughout this study (values below the LOQ have not been considered).

The MS system was routinely programmed in SIM using one target and two qualifier ions. Confirmation was based on the retention time of the target ion and on two qualifier-to-target ion ratios. The target and qualifier ion abundances were determined by injecting individual standards under the same chromatographic conditions, except in full scan mode (50–500 m/z). The qualifier-to-target ion percentage was then determined by dividing the abundance of the selected qualifier ion by that of the target ion (nearly always the base peak) and multiplying by 100. As the identification criteria for the relative percent uncertainty of the theoretical relative abundance of the qualifier ions we followed the EU recommendations (SANCO, 2007; /3131). The sample quantification was based on the pesticide target ion/IS peak area ratio. Quantification was achieved by linear regression against a six-point calibration curve generated from the standard solutions ranging from LOQ of each pesticide to 10 ng mL^{-1} and by using the GC–MS Xcalibur 2.0.7 software.

Each sample batch included two procedural blanks. As procedural blank, 2 mL of water were used instead of milk. The day-to-day variation of the method was evaluated over 5 days using duplicates of these two different pools of spiked samples. The coefficient of variation was <20% for every case and therefore considered as acceptable.

In this work we expressed the total value of OCs residues (∑OCs) as the sum of the 21 OCs and metabolites measured; the total value of HCH residues as the sum of the 4 HCH isomers measured (α-HCH, β-HCH, γ-HCH, and δ-HCH); and the total value of cyclodienes residues (∑cyclodienes) measured as the sum of aldrin, dieldrin, endrin, cis-chlordane, trans-chlordane, and heptachlor. However, due to the fact that the cyclodiene endosulfan was banned recently (December 2005, 2005/864/EC), we have considered this pesticide separately, expressing the total value of endosulfan residues (∑endosulfan) as the sum of the two endosulfan isomers measured (α-, and β-endosulfan). Similarly, we expressed the total value of PCBs residues (∑PCBs) as the sum of the 19 PCBs measured. In addition, those congeners considered as markers of environmental contamination for PCBs (IUPAC congeners #28, 52, 101, 118, 138, 153, and 180) were considered as a group (∑Marker-PCBs; ∑M-PCBs), and total value of DL-PCBs residues were also expressed as the sum of the 12 DL-PCBs measured (IUPAC congeners #77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189).

Additionally, we estimated the potential toxicity (in terms of toxic equivalence to dioxins; TEQs) for the DL-PCBs measured using the toxicity equivalent factors (TEF), as revised by World Health Organization (WHO) in 2005 (Van den Berg et al., 2006). We also expressed the total TEQs (∑TEQs) as the sum of the TEQs obtained from the DL-PCBs measured.

2.4. Exposure assessment

Dietary intake was calculated by multiplying the respective concentration (median) by the volume of the milk consumed by an average adult from Canary Islands (18 years and over, 70.1 kg b.w.) per day (Dorne et al., 2009). Also exposures for small children (6–10 years; average weight, 30.4 kg) were estimated.

Daily intake (pg WHO-TEQ kg^{-1} b.w. d^{-1}) = Occurrence (pg WHO-TEQ g^{-1} w.w.) × Consumption (g kg^{-1} b.w. d^{-1}).

Exposure was calculated for both OCs and DL-PCBs. For calculations, when a congener concentration was under the limit of detection (LOD), the value was assumed to be 0 (lower bound approach).

Consumption data by the general population of milk were obtained from the Canary Islands Nutritional Survey (Serra-Majem et al., 2000a,b).

2.5. Statistical analyses

Database management and statistical analysis were performed with PASW Statistics v 17.0 (SPSS Inc., Chicago, IL, USA). As the presence of organohalogenated contaminants did not follow a normal distribution, the results are expressed with the median, and the 25th and 75th percentiles (p25 and p75, respectively), and range (values maximum and minimum). Differences in the OCs and PCBs levels between two groups or more were tested with the non-parametric Mann–Whitney U-test and Kruskal Wallis test. The categorical variables are presented as percentages and were compared between variables with the Chi-square test. *P* value of less than 0.05 (two-tail) was considered to be statistically significant.

3. Results and discussion

The concentrations of OCs and PCBs did not show significant fluctuations among the six samples collected from each milk brand during the six months-period of collection (data not shown). As a consequence, we have used throughout this work the median, maximum and minimum values (range), and 25th–75th percentiles of the distribution obtained for each chemical in the six samples analyzed (Tables 1 and 2).

3.1. Occurrence of selected OC-pesticide residues in milk samples

Regarding the presence of OC residues, our results showed that all analyzed milk samples (100%) showed some measurable

Table 1
Concentrations of organochlorine pesticides (ng g^{-1} fat) in milk samples from 16 commercial brands of full-fat milk and 10 commercial brands of full-fat organic milk from the Canary Islands market (Spain).

OC-compound	Conventional milk brands (n = 16)			Organic milk brands (n = 10)			[a]P	[b]P
	Median (p25–p75)	Range	%	Median (p25–p75)	Range	%		
HCB	2.22 (1.31–4.60)	0.39–10.06	100	3.67 (1.67–4.80)	0.92–5.64	100		
HCHs								
α-HCH	0.45 (0.29–0.87)	ND-11.21	87.5	0.27 (0.16–0.37)	ND-0.74	80.0		
β-HCH	1.44 (0.76–2.42)	ND-7.37	81.3	0.41 (0.00–0.76)	ND-1.86	60.0	0.009	
δ-HCH	0.00 (0.00–0.00)	ND-4.35	18.8	0.00 (0.00–0.38)	ND-0.51	40.0		
γ-HCH (Lindane)	0.58 (0.00–1.34)	ND-9.28	68.8	0.08 (0.00–1.16)	ND-1.86	50.0		
∑HCHs	2.69 (1.71–4.06)	0.93–28.55	100	1.38 (0.96–2.16)	0.68–2.45	100	0.009	
Cyclodienes								
Aldrin	0.00 (0.00–0.89)	ND-1.26	37.5	0.00 (0.00–0.00)	ND-0.32	10.0		
Dieldrin	3.76 (0.00–6.14)	ND-12.43	68.8	0.66 (0.66–2.46)	ND-7.60	50.0		
Endrin	0.00 (0.00–0.00)	ND-10.08	18.8	ND	ND	0		
cis-Chlordane	0.96 (0.76–1.83)	ND-3.98	87.5	0.71 (0.00–0.92)	ND-1.53	70.0	0.036	
trans-Chlordane	2.45 (1.41–3.86)	0.16–8.71	100	1.50 (1.24–1.62)	ND-4.51	90.0		
∑Chlordanes	3.38 (2.14–5.70)	0.75–12.69	100	2.27 (1.31–2.51)	ND-6.04	90.0		
Heptachlor	1.65 (1.17–2.23)	ND-7.77	87.5	1.14 (0.00–1.18)	ND-1.23	70.0	0.010	
∑Cyclodienes	8.25 (3.82–17.21)	0.75–32.90	100	3.57 (2.36–5.85)	1.23–13.64	100	0.031	
Endosulfans								
α-Endosulfan	0.00 (0.00–2.01)	ND-4.33	43.8	0.00 (0.00–0.00)	ND-1.00	10.0		
β-Endosulfan	0.00 (0.00–0.29)	ND-3.53	37.5	ND	ND	0	0.017	0.035
∑Endosulfans	0.00 (0.00–1.58)	ND-4.61	50	0.00 (0.00–0.00)	ND-1.00	10.0	0.035	0.045
Diphenyl-alyphatics								
o,p′-DDE	ND	ND	0	ND	ND	0		
p,p′-DDE	4.85 (2.80–6.91)	ND-30.22	81.3	4.74 (3.26–9.96)	ND-20.49	80.0		
o,p′-DDD	ND	ND	0	ND	ND	0		
p,p′-DDD	ND	ND	0	ND	ND	0		
o,p′-DDT	ND	ND	0	ND	ND	0		
p,p′-DDT	ND	ND	0	ND	ND	0		
Methoxychlor	ND	ND	0	ND	ND	0		
∑OC-pesticides	27.34 (15.01–36.37)	5.04–54.68	100	14.49 (10.86–22.92)	8.85–28.33	100	0.003	

Abbreviations: OC-compound, organochlorine compounds; HCB, hexachlorobenzene; HCH, hexachlorocyclohexanes; ND, non-detectable. p25 represents the 25th percentile and p75 represents the 75th percentile. %, percentage of detectable samples.
[a] P values result from the comparison between the medians (Mann–Whitney test).
[b] P values result from the comparison between the percentage of detectable samples (χ^2 test).

residue of OCs. HCB was the most frequent residue observed showing values above the LOQ in 100% of the samples from conventional and organic brands of milk (Table 1). An average of 9 OC-pesticide residues per sample were measured in non-organic milk samples (range 4–14), while in organic milk samples, an average of 7 OC-pesticide residues per sample were measured (range 5–9) (p = 0.05). As shown in Table 1, the median levels of OCs in milk were found to be very low and always below the maximum residue limit (MRL) established by the European Legislation (OJEC, 1993 and 1994).

It is noteworthy that, although all the milk samples analyzed (both, conventional and organic) showed some residue of HCH-isomers (∑HCHs), total HCH residue level was significantly higher in conventional than in organic milk samples (2.69 *vs.* 1.38 ng g^{-1} fat, respectively) (Table 1). This result may be due to the fact that lindane (currently banned in Spain) has been used recently as an ectoparisitic agent in livestock by non-organic producers (Botella et al., 2004). Additionally, levels of lindane present in conventional milk samples from the Canary Islands were higher than values described in the US Total Diet Study (2001). Geographical and regulatory variations in the use and restriction of OCs around the World may explain different levels of residue of this chemical on the basis of a different pattern of exposure.

Regarding DDT-derivatives, only the main DDT-metabolite (*p,p′*-DDE) was measured in an important number of conventional and organic milk samples (around 80%) and at similar concentration: median values of 4.85 and 4.74 ng g^{-1} fat, respectively. *p,p′*-DDE was the OC-pesticide residue that we found at the highest concentration in all the analyzed milk samples (median values of 4.85 ng g^{-1} fat) (Table 1). This result agrees with those described in other Western countries (Ghidini et al., 2005; US Total Diet Study, 2001). In our study, there were not differences in *p,p′*-DDE levels nor in the percentage of samples positives among organic and conventional brands, suggesting that the contamination by ubiquitous and persistent pollutants such as OCs-pesticides, could not be avoided by organic production of foodstuffs. On the contrary, technical DDT (*p,p′*-DDT and *o,p′*-DDT) was not present in any milk sample at levels above the LOQ (Table 1) presumably as consequence of its prohibition in the late 1970s.

The environmentally relevant organochlorine HCB was quantified in all the samples. It should be highlighted that the level found in our study for HCB in milk (median: 2.22 ng g^{-1} fat) was lower than those found in the Total Diet Study of Cataluña, 2005 (Spain). HCB has been used as pesticide but it is also produced an as a byproduct during the manufacture of several chlorinated chemicals. While direct production has declined, their diffusion in the environment as by-products of industrial process is of concern (Sala et al., 2001).

As shown in Table 1, other OCs used in the past and prohibited at present, such as the cyclodienes aldrin, cis-chlordane, trans-chlordane, dieldrin, endrin, and heptachlor were also measured in the milk samples analyzed throughout this work. Thus, despite the fact that both types of milks showed residues of some cyclodiene pesticide (∑-cyclodienes), the value for this type of pesticides was lower in organic milk compared with conventional milks (3.57 *vs.* 8.25 ng g^{-1} fat, respectively; Table 1). As in the case of DDT-derivatives, the results obtained with cyclodienes pesticides are not easily explained (they were also banned in Spain in 1970s).

Table 2
Distribution of concentrations of PCBs (ng g^{-1} fat) found in full-fat organic and conventional milk samples from the Canary Islands market (Spain).

Congeners	Conventional milk brands (n = 16)			Organic milk brands (n = 10)			[a]P	[b]P
	Median (p25–p75)	Range	%	Median (p25–p75)	Range	%		
Marker PCBs								
PCB28	ND	ND	0.0	ND	ND	0.0		
PCB52	ND	ND	0.0	ND	ND	0.0		
PCB101	0.09 (0.00–0.59)	ND-1.21	62.5	1.25 (0.75–2.50)	ND-5.63	90.0	0.003	
PCB118	0.00 (0.00–0.32)	ND-0.55	31.3	0.56 (0.28–0.90)	ND-1.37	80.0	0.001	0.041
PCB138	ND	ND	0.0	0.00 (0.00–1.00)	ND-2.44	30.0	0.023	
PCB153	2.28 (1.36–4.69)	ND-15.00	93.8	6.45 (2.90–14.94)	1.99–36.83	100	0.027	
PCB180	0.75 (0.00–1.58)	ND-60.14	68.8	5.43 (1.60–12.29)	0.95–16.71	100	0.003	
DL-PCB (non-ortho)								
PCB77	0.00 (0.00–0.30)	ND-0.60	31.3	0.14 (0.00–0.25)	ND-0.32	70.0		
PCB81	0.00 (0.00–0.00)	ND-0.46	18.8	0.17 (0.00–0.29)	ND-0.47	60.0	0.031	0.046
PCB126	0.00 (0.00–0.15)	ND-0.6	25.0	0.08 (0.00–0.25)	ND-0.32	50.0		
PCB169	0.00 (0.00–0.05)	ND-0.7	25.0	0.00 (0.00–0.10)	ND-0.13	40.0		
DL-PCB (mono-ortho)								
PCB105	ND	ND	0.0	ND	ND	0.0		
PCB114	ND	ND	0.0	ND	ND	0.0		
PCB118	0.00 (0.00–0.32)	ND-0.55	31.3	0.56 (0.28–0.90)	ND-1.37	80.0	0.001	0.041
PCB123	ND	ND	0.0	ND	ND	0.0		
PCB156	0.00 (0.00–0.07)	ND-0.34	25.0	0.11 (0.00–0.23)	ND-0.39	60.0	0.074	
PCB157	0.00 (0.00–0.00)	ND-0.55	12.5	0.00 (0.00–0.00)	ND-0.12	10.0		
PCB167	0.00 (0.00–0.24)	ND-0.98	31.3	0.00 (0.00–0.00)	ND-0.65	10.0		
PCB189	0.00 (0.00–0.04)	ND-0.27	25.0	0.02 (0.00–0.05)	ND-0.09	50.0		
∑PCBs	4.73 (3.71–8.13)	1.64–71.55	100	15.84 (6.16–37.80)	4.80–57.73	100	0.009	
∑Marker-PCBs	3.76 (2.55–7.23)	1.23–71.55	100	14.67 (5.50–35.16)	4.60–56.74	100	0.004	
∑DL-PCBs	0.50 (0.00–1.16)	ND-4.10	78.8	1.19 (0.52–1.93)	0.26–2.78	100		

Abbreviations: ND, non-detectable. p25 represents the 25th percentile and p75 represents the 75th percentile. %, percentage of detectable samples. ΣPCBs: Sum of all PCB congeners. ΣM-PCBs: Sum of Marker PCBs (IUPAC numbers 28, 52, 101, 118, 138, 153 and 180). ΣDL-PCBs: Sum of dioxin like PCBs (IUPAC numbers 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189).

[a] P values result from the comparison between the medians (Mann–Whitney test).

[b] P values result from the comparison between the percentage of detectable samples (χ^2 test).

Nonetheless, geographical variations in the pattern of past use of OCs could have induced geographical differences in the levels of pollution of soils and waters.

Additionally, endosulfan (α- and β-isomers) was found to be present in 50% of the conventional milk samples, and only in 10% of the organic milks samples. Moreover, median values of ∑-endosulfans were lower in organic milk than in conventional milk samples (Table 1). Such results could be due to the fact that this pesticide has been banned recently in the European Union, and therefore it has been used for non-organic producers.

The concentration reached by the sum of all classes of OC residues (∑OC-pesticides) was higher in conventional than in organic milk brands (27.34 *vs.* 14.49 ng g^{-1} fat, respectively; Table 1). Nevertheless, the presence of OC residues reached values as high as 36 ng g^{-1} fat in the most contaminated brands of conventional milk (percentile 75) and around 23 ng g^{-1} fat in the most contaminated brands of organic milk.

In any case, our results agree with those reported by other authors in the sense that there is a steady decline in the levels of OC-residues in milk all over the World as a result of their banning or restriction (Nag and Raikwar, 2008).

3.2. Occurrence of selected PCB congeners residues in milk samples

We found that 100% of milk samples (conventional and organic brands) showed measurable levels of some PCB (Table 2). A mean of five PCB congeners per sample were measured in non-organic milk, while in organic milk a mean of eight PCB congeners per sample were measured (p = 0.021). Only five congeners (28, 52, 105, 114, and 123) were not measured in any milk sample. Congeners 153 and 180 were detected in 100% of the analyzed organic milk samples; while PCB 180 was measured only in around 69% of the conventional milk samples. PCB 138 was detected only in organic milk samples. In any case, all the analyzed milks showed residues above the LOQ of some of the congeners considered as indicators of environmental contamination by PCBs (∑M-PCBs) (Table 2). Our findings demonstrate that commercially available brands of milk present in the Canary Islands market showed measurable levels of a number of PCBs. These results agree with those published by Focant et al. (2003), Durand et al. (2008), and Marin et al. (2011), who detected measurable levels of PCBs in milk samples from France, Belgium, and Spain, respectively. PCB 153 was the most frequently detected congener (94–100% of the samples). In any case, we observed a different profile of PCBs distribution as compared with other studies. Thus, in 19 milk and diary products, Marin et al. (2011) reported that PCB-118 was the most abundant, followed by PCBs-105 and 156. The possibility exists that such differences could be due to a different pattern of environmental contamination (i.e., the absence of contaminant industries in the Canary Islands).

The median levels of PCBs in milk were found to be very low (Table 2). Levels of a number of PCBs (congeners #81, 101, 118, 138, 153, 156, and 180) were higher in organic milk as compared with milk samples from conventional brands. Moreover, ∑PCBs and ∑M-PCBs were significantly higher in organic than in conventional milk samples (Table 2). While ∑PCBs reached levels around 38 ng g^{-1} fat in the most contaminated brands of organic milk (percentile 75), the most PCB-contaminated brands of conventional milk showed values around 8 ng g^{-1} fat. Unexpectedly, these findings demonstrate that milk samples from organic producers showed higher levels of PCBs as compared with the conventional milk ones (especially for M-PCBs). The origin of the organic milk could help to explain such results. These brands are produced mainly in industrialized European countries (Holland, Belgium, Germany), where the level of environmental contamination by PCBs could be very high (Covaci et al., 2002). On the contrary,

conventional brands of milk analyzed throughout this work were locally produced or produced in mainland Spain.

As shown in Table 3, TEQs levels were higher in organic than in conventional milk samples, although such differences reached statistical significance only in the case of TEQ-PCB 118, probably due to the existence of enormous differences between maximum and minimum values described for DL-PCBs (especially in the case of the congeners with the highest TEF value, PCBs 126 and 169). However, the contribution of TEQ-PCB 118 from organic milk to the total TEQ must be considered as modest.

In any case, it should be highlighted the existence of huge differences in Total TEQs level among the milk samples analyzed. Thus, while there were a number of samples that did not show measurable levels of Total TEQs, there were other samples showing levels as high as 81 pg WHO-TEQ g^{-1} fat (data not shown). Such circumstance explains the high median values obtained for Total TEQs in organic brands of milk (around 11 pg WHO-TEQ g^{-1} fat), and the fact that the group of brands of milk (conventional and organic) that showed the highest contamination by DL-PCBs (included in percentile 75), showed levels of Total TEQ higher than 25 pg WHO-TEQ g^{-1} fat (Table 3). Our findings are of concern because the toxicological properties of DL-PCBs are similar to those showed by polychlorodibenzodioxins (PCDDs) and polychlorodibenzofurans (PCDFs) (Van den Berg et al., 2006). Some

Table 3
Distribution of concentrations of DL-PCBs (pg WHO-TEQs g^{-1} fat) found in full-fat organic and conventional milk samples from the Canary Islands market (Spain).

Congeners	Conventional milk brands (n = 16)		Organic milk brands (n = 10)		P
	Median (p25–p75)	Range	Median (p25–p75)	Range	
TEQ-PCB77	0.00 (0.00–0.03)	ND-0.06	0.01 (0.00–0.03)	ND-0.03	
TEQ-PCB81	0.00 (0.00–0.00)	ND-0.14	0.05 (0.00–0.09)	ND-0.14	
TEQ-PCB105	ND	ND	ND	ND	
TEQ-PCB114	ND	ND	ND	ND	
TEQ-PCB118	0.00 (0.00–0.01)	ND-0.02	0.02 (0.01–0.03)	ND-0.04	0.006
TEQ-PCB123	ND	ND	ND	ND	
TEQ-PCB126	0.00 (0.00–22.5)	ND-60.0	8.00 (0.00–24.7)	ND-32.0	
TEQ-PCB156	0.00 (0.00–0.003)	ND-0.01	0.00 (0.00–0.01)	ND-0.01	
TEQ-PCB157	0.00 (0.00–0.00)	ND-0.02	ND	ND	
TEQ-PCB167	0.00 (0.00–0.01)	ND-0.03	0.00 (0.00–0.00)	ND-0.02	
TEQ-PCB169	0.00 (0.00–2.48)	ND-21.0	0.00 (0.00–3.15)	ND-3.90	
TEQ-PCB189	0.00 (0.00–0.002)	ND-0.01	0.00 (0.00–0.002)	ND-0.03	
∑TEQs	0.10 (0.00–25.06)	ND-81.67	10.96 (0.06–28.73)	0.05–32.13	

Abbreviations: ND, non-detectable. p25 represents the 25th percentile and p75 represents the 75th percentile. *P* values result from the comparison between the medians (Mann–Whitney test). ΣTEQs: Sum of TEQs for DL-PCBs.

Table 4
Milk-associated estimated daily intakes (EDI) of organochlorine compounds (pg kg^{-1} b.w.) in adults (286 mL milk d^{-1}) and children (386 mL milk d^{-1}) from the Canary Islands in relation to the tolerable daily intakes (TDI).

	Adults (18–75 years old				Children (6–10 years old)			
	Conventional milk		Organic milk		Conventional milk		Organic milk	
	EDI	%TDI	EDI	%TDI	EDI	%TDI	EDI	%TDI
HCB	0.32	–	0.53	–	0.99	–	1.63	–
HCH								
α-HCH	0.07	–	0.04	–	0.20	–	0.12	–
β-HCH	0.21	–	0.06	–	0.64	–	0.18	–
δ-HCH	0	–	0	–	0	–	0	–
Lindane	0.08	0.08	0.01	0.01	0.26	0.26	0.04	0.04
∑HCH	0.39	–	0.20	–	1.20	–	0.61	–
Cyclodienes								
Aldrin	0	0.00	0	0.00	0	0.00	0	0.00
Dieldrin	0.55	0.19	0.10	0.03	1.67	0.57	0.29	0.10
Endrin	0	0.00	–	0.00	0	0.00	–	0.00
cis-Chlordane	0.14	–	0.10	–	0.43	–	0.32	–
trans-Chlordane	0.36	–	0.22	–	1.09	–	0.67	–
∑Chlordanes	0.49	0.10	0.33	0.07	1.50	0.31	1.01	0.21
Heptachlor	0.24	0.24	0.17	0.17	0.73	0.73	0.51	0.51
∑Cyclodienes	1.20	1.20	0.52	0.52	3.67	3.67	1.59	1.59
Endosulfans								
α-Endosulfan	0	–	0	–	0.46	–	0	–
β-Endosulfan	0	–	–	–	0	–	–	–
∑Endosulfans	0	0.00	0	0.00	0	0.00	0	0.00
Diphenyl-alyphatics								
4,4-DDE	0.71	–	0.69	–	2.16	–	2.11	–
4,4-DDT	–	–	–	–	–	–	–	–
4,4-DDD	–	–	–	–	–	–	–	–
Methoxychlor	–	–	–	–	–	–	–	–
∑DDTs	0.71	0.01	0.69	0.01	2.16	0.02	2.11	0.02
∑OC-compounds	3.98	–	2.11	–	12.15	–	6.44	–

%TDI: Percentage of TDI provided by the milk-associated EDI for these populations.

Table 5
Admissible daily intake and maximum residue levels of persistent organic pollutants in milk and dairy products established by the European Food Safety Authority (EFSA).

POP	ADI (mg kg^{-1} b.w.)	MRL (mg kg^{-1} fat)
Aldrin and dieldrin	0.0001	0.006
∑Chlordanes	0.0005	0.002
∑DDT	0.01	0.04
∑Endosulfans	0.006	0.05
Endrina	0.0002	0.0008
Heptachlor	0.0001	0.004
HCB	NA	0.01
α-HCH	NA	0.004
β-HCH	NA	0.003
γ-HCH (lindane)	0.005	0.001
Methoxychlor	0.1	0.01
∑DL-PCBs	2×10^{-9}	3×10^{-6}

Abbreviations: ADI, admissible daily intake, MRL, maximum residue level; NA, not available; POP, persistent organic pollutant. Data related to OCs were obtained from Regulation (EC) No. 299/2008 (EU); data related to DL-PCBs were obtained from Regulation (EC) No. 466/2001 (EU).

evidences suggest that even low doses of DL-PCBs, similar to those found in the background contamination in food, can cause subtle effects during prolonged exposure, especially if the exposure occurs during prenatal and postnatal development. In this sense, there has been increasing concern regarding the effects on children's neurological development (Chen et al., 2006; Fattore et al., 2008). In fact, a maximum concentration of 6 pg WHO TEQ g^{-1} fat has been defined for the sum of PCDDs, PCDFs and DL-PCBs, and the action level is 2 pg WHO-TEQ g^{-1} fat for milk and dairy products for DL-PCBs (Fattore et al., 2006; Recommendation, 2006/88/EC). Our data suggest that both types of milks could be a relevant source of TEQs for the population under study. Thus, despite the fact that the lowest contaminated samples show low levels of DL-PCBs (0.00 and 0.06 pg WHO-TEQ g^{-1} fat for conventional and organic milks), the most contaminated brands of milk (those included in percentile 75), reached levels as high as 25 and 28 pg WHO-TEQ g^{-1} fat, respectively. This result is extremely worrying and need further studies to clarify the origin of this contamination, even more if we bear in mind that the average contribution of DL-PCBs to the total TEQ account for no more than 60% (Durand et al., 2008), and that, fish and fishery products are the main contributors of PCBs to the diet (Fattore et al., 2006; Llobet et al., 2008; Marin et al., 2011).

3.3. Assessment of milk-related dietary exposure of the population of the Canary Islands to OC pesticides and dioxin-like PCBs

The population of the Canary Islands have been subjected to a chronic exposure to Ocs and PCBs that persisted in the late 1990s (Zumbado et al., 2005; Luzardo et al., 2006; Henríquez-Hernández et al., 2011) and even in the first decade of the current century (Luzardo et al., 2009). It is well known that the main source of organochorinated contaminants is the diet (Hanaoka et al., 2002; Bordajandi et al., 2004; Darnerud et al., 2006), and that milk and dairy products could be a relevant source of these environmental contaminants for the general population (Focant et al., 2002). In addition, European Union, through the Scientific Committee on Food (SCF), has implemented a strategy (SCF, 2001) to reduce human exposure to toxicants present in foodstuffs of animal origin (mainly dioxin-like compounds). The population of the Canary Islands shows the highest intake of milk and dairy products in Europe (Canary Islands Nutritional Survey, 1998; Serra-Majem et al., 2000a,b), Thus, a mean value of 286 mL of milk d^{-1} is consumed by the adult population of the Archipelago, although it must be taken into account that such intake is even higher in children (6–10 years) who reach a daily intake of 386 mL d^{-1}. Taking into consideration the mean body weight of adults (18–75 years) as 70.1 kg, and that of children (6–10 years) as 30.4 (Canary Islands Nutritional Survey, 1998), we have calculated the estimated daily intake (EDI) for the organohalogetated contaminants measured throughout this work using the deterministic method for chronic exposure as previously published (Dorne et al., 2009).

The milk-related mean daily intakes of HCHs, DDTs, heptachlor, chlordanes, dieldrin and HCB were estimated. As expected, the estimated milk-related daily intakes of OCs for adults and children living in the Canary Islands are very low in comparison to the TDI established by the European Food Safety Authority (EFSA) (2010) (Table 4). However, it should be highlighted that, in children consuming conventional brands of milk, only milk-related daily intake provides around 4% of the TDI established for cyclodienes pesticides (Table 4).

The toxicity of dioxins and dioxin-like compounds is related to the amount accumulated in the body during lifetime. In this sense, in the 2001 European Commission's Scientific Committee on Food, established an TDI of 2 pg WHO-TEQ kg^{-1} b.w. d^{-1} for WHO-TEQ (including all foodstuffs and PCDDs, PCDFs, and DL-PCBs) (Fattore et al., 2006). The admissible daily intake and maximum residue levels for milk and dairy products were shown in Table 5. In our results, it has drawn our attention the fact that, in adult population the consumption of organic milk might suppose around 80% of TDI, while in children this value account for more than the 100% of the TDI established by International Agencies (Table 6). On the contrary, the estimated milk-related daily intake of PCBs for adults and children consuming conventional brands of milk is very low in comparison to the TDI established by EU (2001). In any case, our results should be taken with caution because, (a) as showed in Tables 3 and 6, while there are milk brands highly contaminated

Table 6
Milk-associated estimated daily intakes (EDI) of DL-PCBs (pg WHO-TEQ kg^{-1} b.w. d^{-1}) in adults (286 mL milk d^{-1}) and children (386 mL milk d^{-1}) from the Canary Islands in relation to the tolerable daily intakes (TDI).

	Adults (18–75 years old				Children (6–10 years old)			
	Conventional milk		Organic milk		Conventional milk		Organic milk	
	EDI	%TDI	EDI	%TDI	EDI	%TDI	EDI	%TDI
TEQ-PCB 77	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.10
TEQ-PCB 81	0.00	0.00	0.01	0.36	0.00	0.00	0.01	0.48
TEQ-PCB 118	0.00	0.00	0.02	1.24	0.00	0.00	0.03	1.64
TEQ-PCB 126	0.00	0.00	1.18	58.88	0.00	0.00	1.56	77.86
TEQ-PCB 156	0.00	0.00	0.00	0.22	0.00	0.00	0.01	0.29
TEQ-PCB 157	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TEQ-PCB 167	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
TEQ-PCB 169	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TEQ-PCB 189	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.05
∑TEQ-PCBs	0.01	0.50	1.59	79.58	0.04	2.00	2.11	105.61

%TDI: Percentage of TDI provided by the milk-associated EDI for these populations.

by DL-PCBs, it was evident the existence of a number of milk brands where DL-PCBS were not detected; and (b) in the Nutritional Survey of the Canary Islands was not recorded specifically the consume of organic milk, and it could not be hoped a high consume of milk among organic consumers. However, independently of the type of milk consumed, adult people consuming the highest contaminated milk brands (those included in percentile 75) could reach levels closed to 3.7 pg WHO-TEQ kg^{-1} b.w. d^{-1} (around 100% of TDI) and, in the case of children, these data could reach values as high as 11.14 pg WHO-TEQ kg^{-1} b.w. d^{-1} (brands included in percentile 75) (275% of TDI) (data not shown). These findings are of concern due to the deleterious health effects (neurobehavioral, reproductive, immunologic and carcinogenic) that have been attributed to dioxin-like substances (Bilau et al., 2008; ATSDR, 2011). Nonetheless, because multiple mechanisms may be involved in PCB-induced health effects, different PCB congeners may produce effects by different mechanisms, and humans are exposed to complex mixtures of interacting PCBs with differing biological activities, commercial PCB mixtures (e.g., Aroclor 1254) and experimental PCB mixtures (e.g., formulations representing the congeners found in human breast milk) are used to develop health guidance values for environmental mixtures in assessing health risks from exposure (ATSDR, 2000).

4. Conclusions

The present study indicates that organic brands of milk, similarly to conventional ones, show measurable residues of OCs and PCBs, but always below the MRL established by the European Food Safety Authority (EFSA). However, the pattern of contamination varies with the type of production. Thus, conventional milks present higher levels of OCs, while, on the contrary, the level of contamination by PCBs is higher in organic milks. As expected, estimations of milk-related daily intake of OCs and PCBs were below the established TDIs in most cases. However, a number of brands of milk (both, conventional and organic) showed a high level of contamination by DL-PCBs, and as a consequence, people who consume these brands could be subjected to a dietary exposure to dioxins and dioxin-like compounds that exceed by far the limits recommended by EU. These findings should be taken into account by the Public Health Authorities in order to enhance the preventive measures to diminish the presence of these environmental contaminants in milk.

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Anexo II

Tablas de Ingestas Diarias Estimadas

PRODUCCIÓN CONVENCIONAL

Tabla 1. Ingestas diarias estimadas de contaminantes orgánicos persistentes mediante los grupos alimentarios de origen animal (ng/kg p.c./día).

Grupo Alimentario	Lácteos		Huevos		Carne		Productos cárnicos		Productos de la pesca		Total alimentos	
	Adultos	Niños	Adultos	Niños	Adultos	Niños	Adultos	Niños	Adultos	Niños	Adultos	Niños
HCB	0.91	2.07	0.00	0.00	56.91	72.92	2.48	5.42	0.54	0.53	60.80	80.85
Ciclodienos	2.56	6.32	0.11	0.23	-	-	-	-	0.69	0.68	-	-
DDT	3.09	6.65	0.02	0.03	67.36	112.59	5.3	13.69	1.23	1.27	77.0	134.23
HCH	2.86	5.69	0.001	0.002	7.65	14.29	0.51	1.3	0.22	0.23	11.25	21.51
POC	11.52	26.34	0.18	0.36	131.92	199.8	8.29	20.41	2.68	2.72	154.59	249.63
M-PCBs	2.82	7.11	0.13	0.26	52.35	102.31	10.43	25.7	2.11	2.17	67.84	137.55
TEQ[a]	0.825	1.537	0.003	0.006	0.2	0.37	0.02	0.07	0.01	0.01	1.06	1.99
PAH8/B(a)Peq[b]	0.04	0.09	2.41	5.97	4.38	8.1	13.29	38.79	0.14	0.15	17.81	47.04
PAH[c]	3.92	9.54	22.67	47.07	-	-	-	-	-	-	-	-

[a] La ingesta de TEQ se expresa en pg/g grasa/p.c./día.

[b] Para la ingesta de PAH8 por el consumo de lácteos solo se incluye el consumo de yogur.

[c] Para la ingesta total de PAH por el consumo de lácteos solo se incluye el consumo de yogur.

Tabla 2. Ingestas diarias estimadas de contaminantes orgánicos persistentes mediante los grupos alimentarios de origen animal (ng/kg p.c./día).

Grupo Alimentario	Lácteos		Huevos		Carne		Productos cárnicos		Productos de la pesca		Total alimentos	
	Adultos	Niños	Adultos	Niños	Adultos	Niños	Adultos	Niños	Adultos	Niños	Adultos	Niños
HCB	0.81	2.16	0.00	0.00	58.91	80.87	2.48	5.42	0.54	0.53	62.71	88.90
Ciclodienos	0.78	2.23	0.09	0.18	-	-	-	-	0.69	0.68	-	-
DDT	1.28	3.57	0.02	0.04	98.4	141.43	5.3	13.69	1.23	1.27	106.23	160.00
HCH	0.34	0.95	0.002	0.004	40.37	58.21	0.51	1.3	0.22	0.23	41.45	60.69
POC	3.61	10.15	0.12	0.25	197.68	280.51	8.29	20.41	2.68	2.72	212.38	314.04
M-PCB	5.35	13.74	0.06	0.13	50.86	97.53	10.43	25.7	2.11	2.17	68.81	139.27
TEQ[a]	4.112	6.698	0.003	0.006	0.1	0.19	0.02	0.07	0.01	0.01	4.24	6.97
PAH8/B(a)Peq[b]	0.00	0.00	0.99	2.34	52.3	3.82	6.92	38.79	0.14	0.15	17.25	45.86
PAH[c]	3.64	8.86	9.11	18.99	-	-	-	-	-	-	-	-

[a] La ingesta de TEQ se expresa en pg/g grasa/p.c./día.

[b] Para la ingesta de PAH8 por el consumo de lácteos solo se incluye el consumo de yogur.

[c] Para la ingesta total de PAH por el consumo de lácteos solo se incluye el consumo de yogur.

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