Programa de Doctorado en Investigación Aplicada a las Ciencias Sanitarias

Biomonitorización y Diagnóstico de Envenenamientos de Fauna Silvestre Canaria: Desarrollo Metodológico para la Determinación Cuantitativa Simultánea de 360 Sustancias Tóxicas

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Diciembre 2021
Las Palmas de Gran Canaria

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Agradecimientos

Abreviaturas

ABREVIATURA	SIGNIFICADO
ACN	acetonitrilo
AINE	antiinflamatorio no esteroideo
BOC	biocida organoclorado
COP	contaminante orgánico persistente
DDE	difenil dicloroetano
DDT	difenil tricloroetano
DE	disruptor endocrino
FA	ácido fórmico
FGAR	rodenticida anticoagulante de primera generación
GC	cromatografía de gases
HCB	hexaclorobenzeno
IGR	regulador del crecimiento de insectos
LC	cromatografía de líquidos
LMR	límite máximo de residuos
MS/MS	espectrometría de masas de triple cuadrupolo
OPFR	retardante de llama organofosforado
PAH	hidrocarburo aromático policíclico
PBDE	éter de difenilos polibromados
PBT	sustancia persistente, bioacumulativa y tóxica
PCB	bifenilo policlorado
PCDD	policlorodifenil dioxinas
PCDF	policlorodifenil furanos
PFAS	poli- y perfluoralquilos
PPB	parte por billón
PPP	producto de protección para plantas
PPT	parte por trillón
RA	rodenticida anticoagulante
SGAR	rodenticida anticoagulante de segunda generación
UE	Unión Europea

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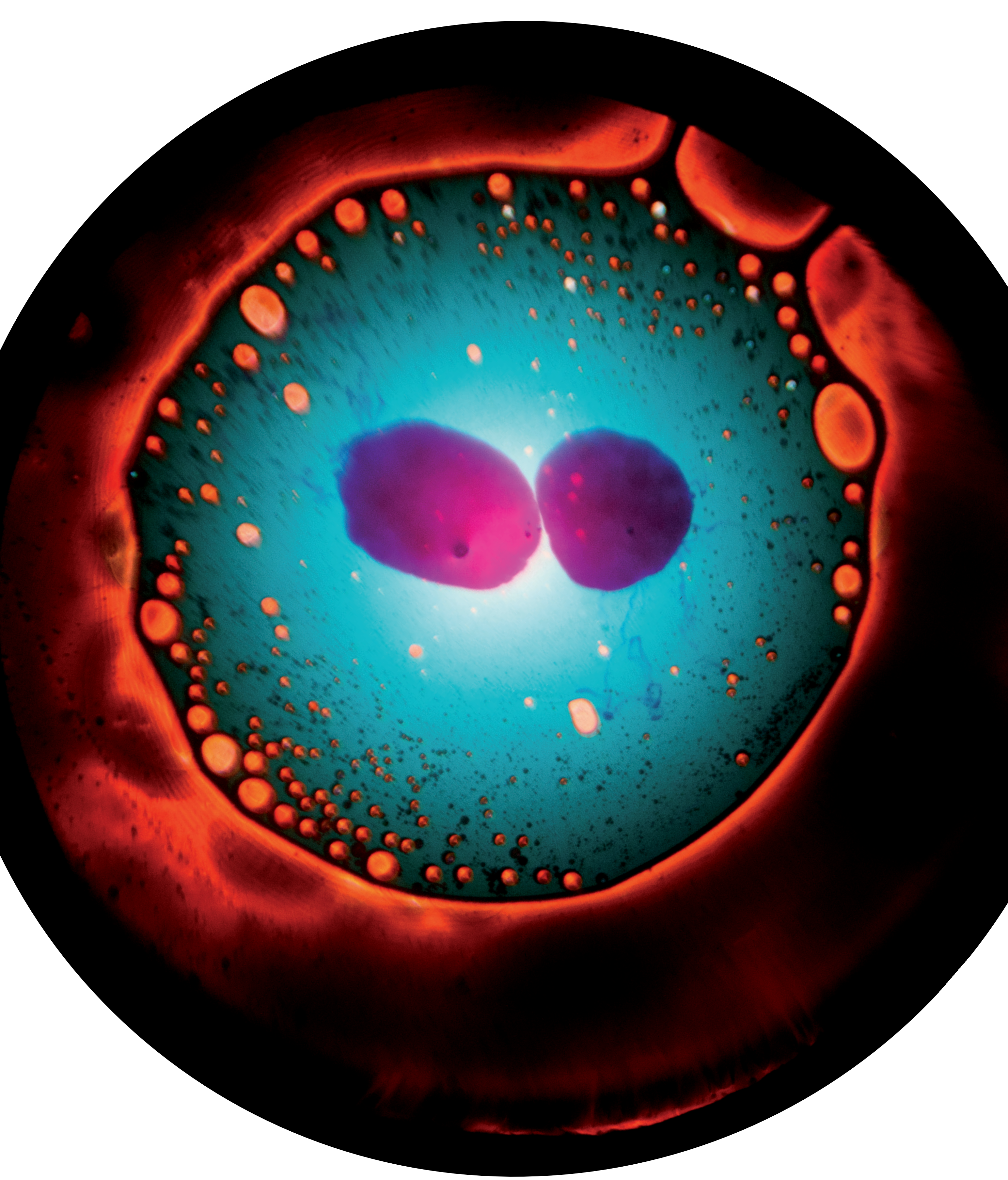

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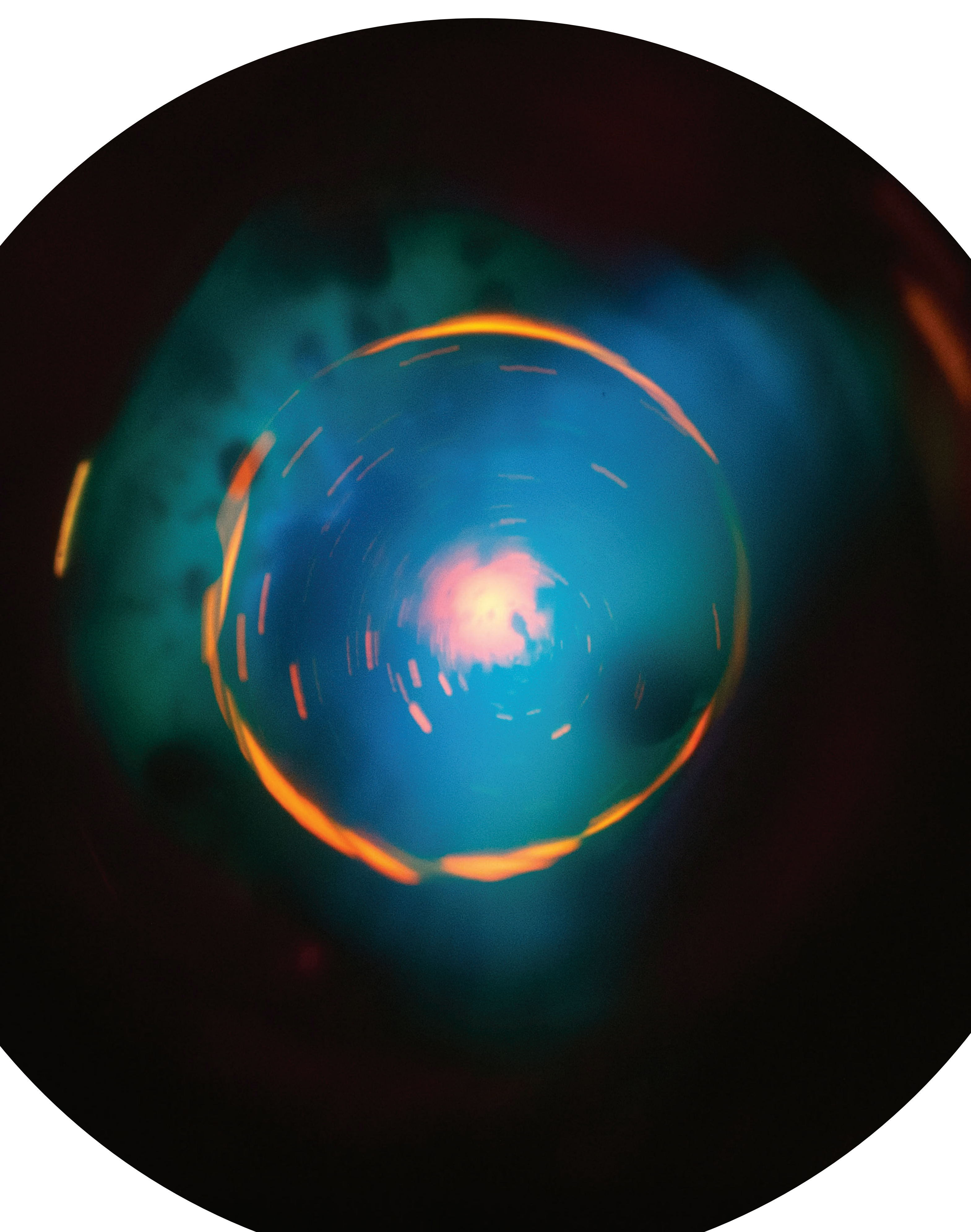
I. RESUMEN/ABSTRACT



Resumen

La biodiversidad mundial está gravemente amenazada, y uno de esos peligros son las sustancias químicas nocivas que provienen del ambiente. Muchas de estas sustancias han sido creadas con numerosos fines, ya sea como como aislantes térmicos y eléctricos, o como biocidas, medicamentos y productos de higiene o con fines industriales, entre otros. Otras, como las dioxinas, los hidrocarburos aromáticos policíclicos o los metales tóxicos son inherentes a la industrialización humana y a la combustión. Los contaminantes orgánicos persistentes comprenden un grupo amplio de tóxicos que son capaces de permanecer durante grandes periodos de tiempo, incluso décadas, en el medio ambiente y los seres vivos, produciendo efectos nocivos a concentraciones muy bajas, como cáncer, malformaciones y alteraciones endocrinas. Los pesticidas y fitosanitarios se usan sobre todo en ganadería y agricultura, aunque también en entornos domésticos. Muchos tienen efectos agudos sobre los artrópodos, y a dosis mayores, pueden producir intoxicaciones agudas en también en vertebrados. La mayoría no suelen persistir más de unas semanas en el ambiente, pero existen otros biocidas con efectos cancerígenos y genotóxicos. Los rodenticidas anticoagulantes se han usado ampliamente en todo el mundo con el fin de eliminar poblaciones de roedores y otros pequeños mamíferos en el campo. Se ha constatado la entrada a la cadena trófica de estos compuestos en aves rapaces, no diana, ya que existen rodenticidas que persisten en el ambiente y se biomagnifican. Por otra parte, los medicamentos como los AINEs pueden ser muy tóxicos para aves de carroña como buitres, que se pueden alimentar de canales de ganado contaminadas, y los antibióticos pueden generar mayores resistencias bacterianas, con lo que se complicaría su uso para infecciones posteriores. Muchas de estas sustancias se usan ilícitamente para producir la muerte por envenenamiento de animales no deseados en entornos rurales y urbanos. La mayor parte de las sustancias usadas son altamente tóxicas, que se llegan a mezclar para producir mayores lesiones.

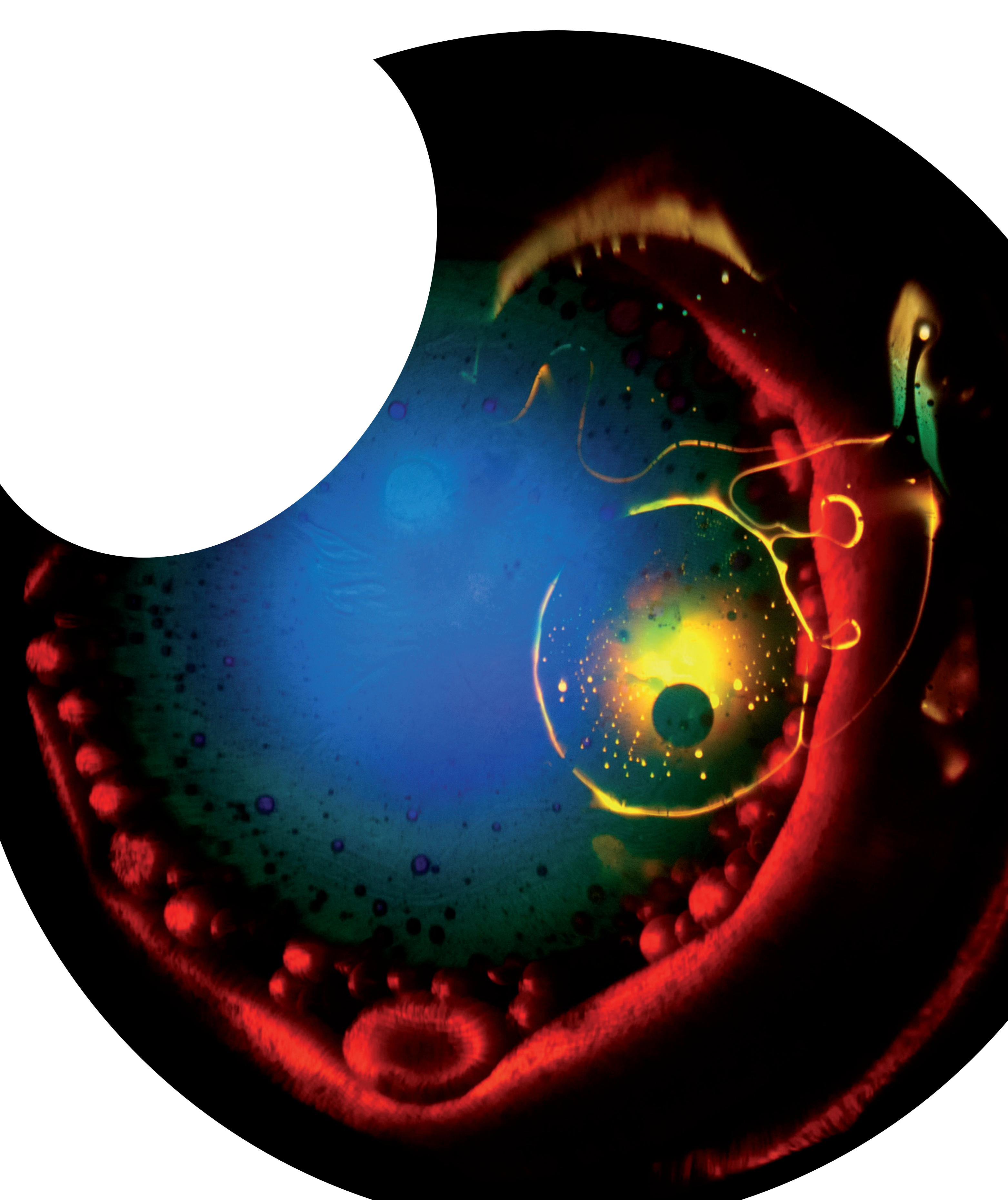
Los estudios de biomonitorización de sustancias químicas en seres vivos necesitan del desarrollo de una metodología que nos pueda brindar información sobre la mayor cantidad de sustancias posible y de su integración en los ecosistemas. A su vez, la seguridad alimentaria y la monitorización de muestras ambientales son cada vez más restrictivas en cuanto a residuos de plaguicidas, medicamentos y contaminantes persistentes. Por lo tanto, el desarrollo de métodos de extracción y cuantificación para un gran número de sustancias, que sean sensibles, de rutina, robustos y económicos, es un requisito indispensable. En este estudio de Tesis Doctoral, hemos desarrollado, validado y aplicado metodología basada en QuEChERS como técnica de extracción, y en cromatografías líquida y de gases acopladas a espectrometría de masas como técnicas de detección y cuantificación. Así, hemos desarrollado técnicas para la detección cuantitativa simultánea de 360 compuestos tóxicos en sangre y de 351 en hígado, la mayoría de ellos a concentraciones ultra traza (< 1 ng/g). Hemos aplicado Los métodos desarrollados los a estudios de biomonitorización y de diagnóstico de venenos de fauna silvestre y doméstica de Canarias.

Abstract

The world's biodiversity is seriously threatened, and one of those dangers is harmful chemicals that come from the environment. Many of these substances have been created for purposes such as thermal and electrical insulators, biocides, pharmaceuticals, and hygiene products or for industrial purposes. Others such as dioxins, polycyclic aromatic hydrocarbons or toxic metals are inherent in human industrialization and combustion. Persistent organic pollutants comprise a wide group of toxics that can remain for long periods of time, even decades, in the environment and living beings, producing harmful effects at very low concentrations, such as cancer, malformations and endocrine alterations. Pesticides and phytosanitary products are used mainly in livestock and agriculture, but also in domestic environments. Many have acute effects on arthropods, and at higher doses, can cause acute poisoning also in vertebrates. Most do not usually persist for more than a few weeks in the environment, but there are other biocides with carcinogenic and genotoxic effects. Anticoagulant rodenticides have been used widely throughout the world to eliminate populations of rodents and other small mammals in the field. The entry into the trophic chain of these compounds has been found in birds of prey, not target, since there are rodenticides that persist in the environment and biomagnify. On the other hand, pharmaceuticals such as NSAIDs can be very toxic to carrion birds such as vultures, which can feed on contaminated livestock carcasses, and therefore, antibiotics can generate greater bacterial resistance, which would complicate their use for subsequent infections. Many of these substances are used illicitly to cause death by poisoning of unwanted animals in rural and urban settings. Most of the substances used are highly toxic, which mix to produce greater injuries.

Biomonitoring studies of chemical substances in living beings require the development of a methodology that can provide information on as many substances as possible and their integration into ecosystems. In turn, food safety and the monitoring of environmental samples are increasingly restrictive in terms of pesticide, pharmaceuticals, and persistent pollutants residues. Therefore, the development of extraction and quantification methods for a large number of substances, which are sensitive, routinely applicable, robust and inexpensive, is an indispensable requirement. In this doctoral thesis study, we have developed, validated, and applied methodology based on QuEChERS as extraction technique, and liquid and gas chromatography coupled to mass spectrometry as detection and quantification techniques. Thus, we have developed techniques for the simultaneous quantitative detection of 360 toxic compounds in blood and 351 in liver, most of them at ultra-trace concentrations (< 1 ng/g). The methods developed have been applied to biomonitoring and diagnostic studies of poisons in wild and domestic fauna of the Canary Islands.

II. INTRODUCCIÓN



INTRODUCCIÓN

Dentro de la Unión Europea, España es uno de los países con mayor diversidad biológica. Las razones para ello tienen que ver con factores tales como su posición geográfica, su diversidad geológica, la gran variabilidad climática, orográfica y edáfica, la historia paleobiogeográfica o la existencia de islas, como el archipiélago oceánico de las Islas Canarias. La preservación de los procesos ecológicos esenciales es fundamental, ya que son imprescindibles para la vida. Entre estos procesos fundamentales podemos citar la producción de oxígeno por los organismos fotosintéticos; la disminución de impactos de meteorología adversa extrema (como riadas, deshielos, olas de calor, etc...); el reciclado de cadáveres y materia orgánica en descomposición por carroñeros e invertebrados; control de vectores de enfermedades; o, el control de poblaciones animales, como roedores e insectos que se alimentan de los cultivos que plantamos. Los insectos y otros artrópodos son reconocidos polinizadores, depredadores y a su vez alimento para otros seres, siendo uno de los pilares de la cadena trófica. La conservación del patrimonio natural y de la biodiversidad, así como articular su uso sostenible, mejora y restauración son obligaciones básicas del Estado español, establecidas por la Ley 42/2007 (BOE, 2007). De forma similar, el resto de los países de nuestro entorno tienen obligaciones similares, ya que han debido trasladar a sus legislaciones nacionales lo establecido en la Directiva Marco de la Unión Europea sobre la a la conservación de los hábitats naturales y de la fauna y flora silvestres (BOE, 1992).

Sin embargo, según el informe Planeta Vivo 2020 de WWF (Worl Wild Fund for Nature (WWF) 2020), las poblaciones de animales a nivel mundial han sufrido un desplome medio del 68% (rango del 62-73%) entre 1970 y 2016. El mayor porcentaje se experimenta en las zonas tropicales y subtropicales, como es el caso de Canarias, que además, son las regiones del planeta en donde mayor biodiversidad existe y, por tanto, se espera que haya una mayor pérdida (Elliott *et al.* 2015), aunque haya especies que puedan adaptarse.

Son varias las causas de este declive, entre los que se incluyen:

- El cambio climático y el calentamiento global: la Agencia Europea del Medioambiente cifró en más de 445.000 millones de euros, las pérdidas económicas entre los años 1980 y 2019 (EEA 2013), sin contar las pérdidas humanas o que favorecen la aparición de vectores y enfermedades y contaminación, los cuales están estrechamente relacionados (Movalli *et al.*, 2018; Encarnação *et al.*, 2019).
- El aumento desproporcional de la población humana: se calcula que 2050 seremos unos 10.000 millones, lo que conlleva una mayor presión de ecosistemas, mayor transmisión de enfermedades, y mayor producción de alimentos, productos y residuos.
- Modificación del uso de suelos y tipo de cultivos: se prioriza la modificación de suelos para la construcción de casas y la explotación agraria en monocultivo (la variedad de cultivos ha disminuido en un 75%), con lo que los cultivos son más propensos a enfermedades y al clima.

- El uso indiscriminado de químicos en el ambiente: para el control de poblaciones, conservación de alimentos y materiales, limpieza y desinfección, o en la industria e investigación.

Este último punto, es el que trataremos en esta Tesis Doctoral, ya que como veremos, los químicos se distribuyen en el ambiente, y terminan regresando a nuestros organismos, ya sea por vía respiratoria o digestiva a través de los alimentos. Las concentraciones que encontramos en muestras biológicas (sangre, orina e hígado), muchas veces llegan a umbrales tóxicos para los seres vivos. Además, al producir la muerte de artrópodos, o pequeñas aves, o incluso de vegetales necesarios para la supervivencia de otros, tenemos una pérdida de hábitat y de biodiversidad.

Canarias es un archipiélago volcánico, región ultraperiférica de la Unión Europea, situado en el océano Atlántico subtropical, a unos 200 km del Sáhara occidental y unos 1.600 km de la península ibérica. Se encuentra entre los paralelos 27 y 29 N y latitud entre 13 y 18 E, en la zona subtropical del Trópico de Cáncer. Está compuesto por 8 islas mayores habitadas y otros islotes (en su mayoría de propiedad privada), y cuenta con unos 7.493 km^2 de superficie total. Esta situación geográfica estratégica, junto con el clima, favorece el tráfico de barcos y personas, donde la población humana permanente incluye casi 2 millones, además de una población flotante cifrada en unos 12 millones de turistas/visitante anuales, lo que se traduce en una densidad humana que ronda las 300 personas/km^2 en las islas capitalinas. Los espacios protegidos de Canarias incluyen (aproximadamente un 40% de la superficie) que incluyen: 4 Parques Nacionales, 11 Parques Naturales, 7 Parques Rurales, 26 Reservas Naturales, (integrales y especiales) 7 Reservas de la Biosfera por la UNESCO, 43 Zona de Especial Protección Terrestre, 11 Zonas Especiales de Protección Marina, y 177 Áreas Especiales de Conservación (https://www.gobiernodecanarias.org/). Así, da cobijo a miles de aves en su migración a África o Europa y es zona de paso de diferentes especies de cetáceos y otra fauna marina. Canarias es considerado un "*hotspot*" o punto caliente de biodiversidad, sobre todo para aves marinas migratorias (Grecian *et al.*, 2016). Existen casi 4.500 especies endémicas, un 28% de todas las especies descubiertas en Canarias (https://www.biodiversidadcanarias.es/biota/), con gran número de aves e invertebrados, así como flora.

1. Contaminación química

Como decíamos, los productos químicos representan una de las mayores amenazas para la biodiversidad. En los últimos 75 años se han inventado y fabricado más de 140.000 nuevos productos químicos, que se incorporan a millones de bienes de consumo y productos industriales que van desde los alimentos y sus envases hasta la ropa, los materiales de construcción, los combustibles para motores, los compuestos de limpieza, los pesticidas, los cosméticos, los juguetes y los biberones. El ritmo de fabricación aumenta un 3,5% al año y va camino de duplicarse en 2045. Más del 60% de la producción actual de productos químicos se lleva a cabo en países de ingresos bajos y medios, donde la protección de la salud y del medio ambiente suele ser escasa y la eliminación de residuos no está bien controlada. Las sustancias químicas manufacturadas se han diseminado ampliamente en el medio ambiente y se

encuentran hoy en día en los lugares más remotos del planeta. Los seres humanos están expuestos a estas sustancias químicas. En los estudios de biomonitorización se detectan habitualmente cantidades medibles de más de 200 sustancias químicas manufacturadas en los tejidos humanos. Hay que destacar que un buen número de los productos químicos fabricados nunca han sido sometidos a pruebas de seguridad o toxicidad. Por tanto, se desconoce su potencial para dañar los ecosistemas o la salud humana (Movalli *et al*., 2018). A veces se descubre tardíamente - después de años o incluso décadas de uso - que han causado daños a los sistemas de apoyo planetarios o daños a la salud. Algunos ejemplos son el DDT, el amianto, el tetraetilo de plomo y los clorofluorocarbonos. Se sabe aún menos sobre los posibles efectos combinados de la exposición a mezclas de productos químicos manufacturados (Lloyd-Smith and Immig, 2018; Landrigan *et al.*, 2020):

Ante este dramático panorama, la contaminación es uno de los retos existenciales de la época actual. Al igual que el cambio climático, la pérdida de biodiversidad y el agotamiento del suministro de agua dulce, la contaminación pone en peligro la estabilidad de los sistemas de equilibrio de la Tierra y amenaza la supervivencia de las sociedades humanas. La contaminación es también una gran y creciente amenaza para la salud humana. Es la mayor causa ambiental de enfermedad en el mundo actual, responsable de aproximadamente 9 millones de muertes prematuras al año. Causa enormes pérdidas económicas, socava las trayectorias nacionales de desarrollo económico e impide la consecución de los Objetivos de Desarrollo Sostenible (ODS) (Fleming *et al.*, 2019).

Por tanto, la contaminación medioambiental de origen antropogénico consiste en una mezcla compleja y cambiante de sustancias químicas y biológicas que incluye residuos plásticos, contaminantes derivados del petróleo, metales tóxicos, productos químicos manufacturados, productos farmacéuticos, biocidas y una mezcla nociva de nitrógeno, fósforo, fertilizantes y aguas residuales. Es decir, que por contaminante químico entendemos que se trata de un elemento o compuesto químico cuyo estado y características fisicoquímicas le permiten entrar en contacto con los individuos, de forma que pueden originar un efecto adverso para su salud. Sus vías principales de penetración en los seres vivos son la inhalatoria, la dérmica y la digestiva, siendo esta última la más relevante en términos cuantitativos, ya que se considera que la exposición general a tóxicos se realiza principalmente a través de la ingesta de alimentos contaminados. Muchos compuestos liposolubles, son transferidos a la leche materna y a través del huevo (Blanco *et al.*, 2018).

Dentro de los contaminantes químicos encontramos tanto compuestos inorgánicos como orgánicos. Con respecto a los primeros, constituyen un grupo heterogéneo de productos, habitualmente disueltos o dispersos en el agua, que provienen de descargas domésticas, agrícolas e industriales o de la erosión del suelo. Los principales son: cloruros, sulfatos, nitratos carbonatos, desechos ácidos, alcalinos, gases tóxicos disueltos en el agua como los óxidos de azufre, de nitrógeno, amoníaco, cloro y sulfuro de nitrógeno (ácido sulfhídrico). El segundo grupo, mucho más amplio, lo constituyen los contaminantes orgánicos. Se trata de compuestos de muy diferente naturaleza, todos ellos basados en la química del carbono, siendo las clases más amplias de contaminantes orgánicos los antibióticos, las hormonas y otros medicamentos utilizados en la producción de animales de granja, los plaguicidas químicos, los residuos líquidos industriales, las emisiones gaseosas industriales; así como algunas sustancias especialmente

tóxicas que merecen ser mencionadas como una clase por sí mismas, los contaminantes tóxicos persistentes (COPs), extremadamente preocupantes porque permanecen en el medio ambiente durante mucho tiempo al ser resistentes a la degradación, son bioacumulables, son altamente tóxicas, y tienen potencial para trasportarse a larga distancia, pudiendo llegar a regiones en las que nunca se han producido o utilizado.

Además, a todo este amplio panorama de sustancias, hay que añadir otro gran grupo, constituido por los metabolitos. Normalmente la metabolización de tóxicos es un proceso beneficioso para los seres vivos, ya que de esta forma las sustancias tóxicas se pueden eliminar del organismo, a través de la orina o las heces. En ocasiones, el compuesto parental es poco tóxico, o no lo es *per se*, pero sí lo son sus metabolitos, como en el caso del etilenglicol, el metanol, el propio DDT o el benfuracarb, por lo que algunos de estos metabolitos, cuando pasan al medioambiente, deben ser tenidos en cuenta también como tóxicos *per se*.

Con respecto a la bioacumulación de tóxicos, mencionada arriba y muy preocupante, dos buenos ejemplos para entenderla son la oruga de la mariposa monarca o la rana dardo. El insecto se alimenta de asclepias (plantas ricas en glucósidos cardiotóxicos, muy tóxicas para pájaros insectívoros pequeños), y el anfibio se alimenta de insectos tóxicos, lo que le confiere una de las toxinas más letales de la naturaleza. Ambos incorporan en su organismo toxinas que ingieren, a las que son inmunes, y que se acumulan a lo largo de su vida, confiriéndole a los adultos una alta toxicidad para sus potenciales depredadores. Al igual que en los ejemplos, muchos otros químicos tóxicos se bioacumulan (aumenta su concentración en el organismo a lo largo de la vida) y se biomagnifican (los depredadores incorporan los tóxicos de sus presas). Así, a mayor grado en la cadena trófica y mayor edad, mayor concentración de contaminantes persistentes se detectarán. Esto conlleva a que se bioconcentren, llegando a presentar efectos negativos para la salud, sobre todo a largo plazo, como malformaciones o tumores. De igual forma, los residuos de biocidas y medicamentos pueden penetrar en las cadenas tróficas, acumulándose en los eslabones más altos, y produciendo pérdida de ecosistemas (Thomas *et al.*, 2011). Las concentraciones tóxicas pueden ser muy bajas, en torno a partes por trillón (ppt), o lo que es lo mismo, nanogramo/kilogramo.

De esta forma, algunos de los factores que modifican la exposición a contaminantes son la dieta, el uso o exposición profesional continuado, la estación (por el uso masivo de calefacción en invierno o un mayor uso de pesticidas en verano), el clima y la región. Los químicos más móviles se adhieren a partículas o seres vivos para ser transportados largas distancias, en lo que se llama efecto saltamontes. Muchas de estas sustancias terminan depositándose en los polos (considerados sumideros de contaminantes) a través de corrientes de aires, o en los fondos oceánicos, formando parte de los sedimentos.

En este trabajo de Tesis Doctoral nos hemos centrado en los contaminantes químicos orgánicos, y de entre ellos, en una amplia selección que incluye la mayoría de los más tóxicos y preocupantes, y pertenecientes a tres grandes grupos: a) los contaminantes orgánicos persistentes (COPs); b) los biocidas y productos de protección de plantas (PPPs); y c) los medicamentos y productos de higiene. En los siguientes subapartados haremos un breve desarrollo de los diferentes grupos de sustancias químicas orgánicas con propiedades tóxicas que han constituido el objetivo de nuestro trabajo.

1.1. Contaminantes Orgánicos Persistentes (COPs)

Como decíamos, los contaminantes orgánicos persistentes (COPs) constituyen un grupo muy preocupante. Se trata de un grupo heterogéneo de sustancias tóxicas, altamente lipofílicas, por lo que se bioacumulan en los tejidos grasos de los seres vivos, volátiles, lo cual los hace muy móviles a nivel planetario, y difícilmente degradables en el medio y/o metabolizables por los seres vivos (Luzardo *et al.*, 2014), por lo que las concentraciones halladas pueden ser aun altas y poseer riesgos para la salud de los animales (Pilar Gómez-Ramírez *et al.*, 2019). En los últimos años se acuña el termino PBT para estas sustancias (acrónimo de Persistentes, Bioacumulativas y Tóxicas), ya que comprenden un mayor rango de sustancias con propiedades comunes y efectos similares, tanto en la salud como en el medio (Movalli *et al.*, 2018). Aunque se han establecido protocolos de eliminación, restricción o reducción de químicos, como el Convenio de Estocolmo (SCE 2004), aún hoy en día son contaminantes prioritarios en los programas de vigilancia y estudios de riesgo.

Nos centraremos en esta tesis en los biocidas organoclorados (BOCs), policlorobifenilos (PCBs), ésteres de polibromodifenilos (PBDEs) e hidrocarburos aromáticos policíclicos (PAHs). Existen otros COPs como las dioxinas y furanos, los compuestos per- y poliflurinados (PFAS), los bisfenoles, los retardantes de llama organofosforados (OPFRs) o los ftalatos (Eljarrat *et al.*, 2019), pero que no han sido objeto de estudio en este trabajo.

1.1.1. Hidrocarburos Aromáticos Policíclicos (PAHs), dioxinas (PCDD) y furanos (PCDF)

Los hidrocarburos aromáticos policíclicos (PAHs) son un grupo de químicos, formados por la unión de mínimo dos anillos aromáticos, producidos principalmente por la combustión fósil, los productos cocinados o el humo del tabaco. Los llamados PAHs similares a dioxinas o planares, son más peligrosos que los coplanares. Son compuestos semipersistentes, ya que algunos organismos tienen la capacidad de metabolizarlos (Ruiz-Suárez *et al.*, 2016), principalmente en el mar, pero altamente prevalentes y ubicuos, ya que se emiten grandes cantidades al medio en todo momento (Camacho *et al.*, 2012), del orden de 90.000 toneladas mensuales a los océanos. Por su parte, las dioxinas y furanos son compuestos químicos similares entre sí, que se forman por la combustión incompleta de materia orgánica con átomos de cloro en su estructura, como madera tratada, papel o PVC en el caso de las dioxinas, o el cocinado de los alimentos, como ejemplo de los furanos. Además, las erupciones volcánicas y los incendios forestales son las fuentes naturales principales de este amplio grupo de compuestos.

La principal fuente de exposición son los alimentos contaminados con alto contenido en grasas, como los yogures, los productos cárnicos o los quesos (Almeida-González *et al.*, 2012; Luzardo *et al.*, 2013; Rodríguez-Hernández *et al.*, 2016). Podemos encontrar residuos de estos contaminantes en el orden de ng/kg de alimento, pero al acumularse en los tejidos grasos, las concentraciones que se acumulan en los seres vivos, en particular en aquellos que nos situamos en lo alto de la cadena trófica, pueden llegar a ser peligrosas. Actualmente la EFSA (*European Food Safety Agency* o Agencia Europea de Seguridad Alimentaria) regula y vigila la presencia de hasta 15, de los más de 100 compuestos que se incluyen, y otras combinaciones con nitrógeno,

los conocidos como nitro-PAHs. El benzo(a)pireno es el más tóxico y estudiado, siendo clasificado como carcinogénico y genotóxico. En la Sección III. Biomonitorización, podemos encontrar una publicación como capítulo de libro, en el cual se abordan los PAHs y la utilidad de biomonitorizar animales domésticos como centinelas de exposición de PAHs.

1.1.2. Biocidas Organoclorados (BOCs)

Los Biocidas Organoclorados (BOCs) son compuestos sintéticos lipofílicos, persistentes, y bioacumulables, con un esqueleto de carbono e hidrógeno, y átomos de cloro (Buck *et al*., 2020), muchos ya prohibidos, pero que fueron usados ampliamente en agricultura, ganadería y entornos domésticos. Después de la segunda guerra mundial, hacia el año 1950, la escasez de alimentos y la invención de nuevos y prometedores pesticidas como el dicloro difenil tricloroetano (DDT) en 1943, hizo que la producción y uso de estos químicos nocivos aumentara. El DDT, por su gran eficacia junto con su bajo costo, han sido (y sigue siendo en algunos países), uno de los principales químicos para el control de mosquitos vectores de la malaria, bajo recomendación de la Organización Mundial de la Salud (OMS) (WHO, 2007). Es importante señalar, que el metabolito principal de DDT, el DDE, es tanto o más tóxico que el compuesto parental, e igual de persistente, por lo que está presente los tejidos y medioambiente, incluso con mayor presencia que el propio DDT (Gómez-Ramírez *et al.*, 2019). Otros BOCs que se comercializaron con gran éxito son el aldrín, dieldrín, endrín clordano, heptacloro, hexaclorociclohexano, lindano, o toxafeno, todos ellos integrantes de la "*docena sucia*" recogida en el Convenio de Estocolmo (SCE, 2004), en el que se establecía la lista de compuestos candidatos prioritarios para su vigilancia y control por las distintas administraciones. Su acción principal es alterar la transmisión nerviosa, pero se ha comprobado la interferencia con el ADN y hormonas a concentraciones de tan solo unos pocos microgramos/kg (ppb).

Ha sido documentado que el abuso de BOCs en la antigüedad en Canarias como consecuencia de la intensificación de cultivos como platanera y tomatera para exportación en los años 70 (Luzardo *et al.*, 2014; Buck *et al.*, 2020), justo cuando se empezaron a prohibir en España, ha ayudado en la extinción de alguna especies en Canarias como el milano real (*Milvus milvus*) y al descenso continuo de poblaciones de guirre (*Neophron percnopterus majorensis*) (Mateo *et al.*, 2000; Buck *et al.*, 2020).

1.1.3. Compuestos halogenados de origen industrial

Incluimos en esta sección diferentes compuestos halogenados sustancias con propiedades interesantes para la industria, pero que se demostraron de forma tardía, como importantes contaminantes crónicos y persistentes, con grandes efectos en la salud de los seres vivos y los ecosistemas. Su estructura se compone principalmente de átomos de carbono e hidrógeno fundamentalmente, pero esta familia se caracteriza por incluir átomos de elementos halogenados como cloro, bromo, flúor o iodo, con múltiples de propiedades interesantes para la industria como aislantes.

Los bifenilos policlorados (PCBs en inglés), son un grupo de sustancias sintéticas cloradas usadas como aislantes térmicos y eléctricos en infinidad de dispositivos electrónicos y de construcción, prohibidos a partir de 1986. La mala gestión de residuos, así como la presencia de PCBs en la vida cotidiana son su fuente principal al medio. Su estructura incorpora cloro, y se incluyen 209 congéneres que se enumeran según las posiciones de los átomos de cloro. Son indicativos de la industrialización de una zona, siendo los niveles bajos en Canarias *(*Luzardo *et al.*, 2014). Se han asociado a la cercanía a carreteras (Buck et al., 2020) y a transformadores eléctricos (Garcia-Heras *et al.*, 2018) en aves nidificantes en la zona cercana.

Los éteres de polibromodifenilos (PBDEs en inglés), son un grupo de 209 congéneres químicos sintéticos usados principalmente como retardantes de llama para componentes electrónicos, plásticos y espumas. Se usaron desde 1970 y se comercializaban como mezclas de congéneres después de la prohibición de los PCBs. A partir de 2004 se prohibió la venta de las mezclas penta y octa-BDE, en EEUU y EU (las mezclas con más compuestos clorados, y por lo tanto, más tóxicas y persistentes) (SCE 2004). Las concentraciones detectadas pueden tener efectos en las poblaciones de rapaces *(*Elliott *et al., 2015;* Eljarrat *et al.*, 2019).

Tras la prohibición de los PCBs y PBDEs, tomaron mayor importancia los retardantes de llama organofosforados (OPFRs). Los decloranos, no regulados pero detectados en muestras ambientales y biológicas, son también nuevas sustancias a vigilar (Blanco *et al.*, 2018). Son compuestos de "*tercera generación*", en una historia que nunca se acaba. Se está investigando profusamente sobre ellos, tanto en métodos de análisis como estudios toxicológicos, ya que se ha comprobado sus perjuicios para la salud, al igual que anteriormente con los PCBs y PBDEs. Actualmente en nuestro grupo de investigación estamos trabajando para implementar nuevos métodos de análisis para estos contaminantes emergentes, y poder así contribuir a la evaluación de los riesgos por la exposición a estos compuestos.

Por último, los per- y polifluorinados (PFAS) son sustancias que contienen átomos de flúor en su estructura, compuesta fundamentalmente por carbono, y que repelen tanto el agua, como los aceites. Se usaban como antiadherentes en utensilios de cocina (el teflón), en materiales impermeables y en espumas ignífugas, por lo que su presencia en el medioambiente es inevitable y constante. Hoy en día encontramos en el supermercado sartenes y calderos con la indicación de estar libres de PFAS.

1.2. Biocidas y productos de protección de plantas (PPPs)

La propagación de enfermedades y vectores se ve afectada, entre otros motivos, por el calentamiento global o el tipo de agricultura, lo que implica a medio y largo plazo el incremento del uso de biocidas y PPPs. Se trata de un grupo amplio de sustancias activas que se destinan para prevenir, destruir o controlar patógenos, animales u otras plantas, para proteger las plantas y animales, o sus productos derivados, durante la producción, almacenamiento y transporte. Comúnmente llamados pesticidas o plaguicidas son un grupo amplio de sustancias usadas tanto en ganadería como agricultura, así como en entornos domésticos. Según el uso al que estén

destinados, se legislan como Productos de protección de plantas (CE 1107/2009; CE, 2009) o como Biocidas (CE 528/2012; CE, 2012).

Estos compuestos se clasifican habitualmente haciendo mención al tipo de plaga contra la que van dirigidos o al tipo de efecto que se pretende conseguir, y así se habla de rodenticidas, avicidas, molusquicidas, antihelmínticos, insecticidas, acaricidas, repelentes o atrayentes. Por su parte, los PPPs, más conocidos como productos fitosanitarios, incluyen fungicidas, algunos biocidas, herbicidas o productos para la regulación del crecimiento de plantas y conservantes para la madera. En esta tesis incluimos la inmensa mayoría de los plaguicidas para los que se regulan los límites máximos de residuos (LMR) en productos de origen animal y vegetal, y piensos (CE, 2020), que sobrepasan ampliamente el número de 200 compuestos. Haremos un breve repaso de los diferentes grupos químicos incluidos en este apartado.

1.2.1. Biocidas

Se trata de un amplio grupo de sustancias usadas para destruir, contrarrestar, neutralizar, impedir la acción o ejercer un control de otro tipo sobre cualquier organismo nocivo o por cualquier medio que no sea una mera acción física o mecánica. Se usan para el control de plagas en el medio doméstico, agrícola, ambiental o industrial. Muchos de estos productos están dotados de elevada toxicidad, aunque actualmente están permitidos muy pocos productos que estén dotados de una toxicidad aguda muy alta para los vertebrados. Sin embargo, muchos de ellos tienen efectos adversos sobre la salud a dosis mucho más bajas, sobre todo cuando la exposición a ellos es prolongada. En Europa, los biocidas están regulados por el Reglamento (UE) 528/2012 del Parlamento Europeo y del Consejo, que los define e incluye cuatro grupos: desinfectantes, conservantes, plaguicidas y otros biocidas. Sin duda, el grupo más numeroso de compuestos lo constituye el de los plaguicidas, y son básicamente los compuestos químicos de este grupo, los que hemos incluido en esta Tesis Doctoral.

Dentro de los plaguicidas hay numerosos grupos de interés. Así, uno de los grupos que más se ha utilizado son los compuestos inhibidores de la colinesterasa (carbamatos y organofosforados). Son compuestos que inhiben la actividad de la acetilcolinesterasa, lo que conlleva a una sobreestimulación del sistema nervioso parasimpático principalmente. Muchos de los compuestos de este grupo están dotados de una toxicidad muy alta, y han sido y son responsables de una gran cantidad de intoxicaciones letales, principalmente por insuficiencia respiratoria. La mayoría de los compuestos de este grupo se han ido prohibiendo durante las últimas dos décadas, y ya sólo van quedando autorizados aquellos de menor toxicidad aguda. Sin embargo, muchos de los compuestos prohibidos han venido empleándose para el envenenamiento intencionado de animales, comprobándose que esto ocurre de forma muy relevante en Canarias (Ruiz-Suárez *et al.*, 2015). Por ello, en nuestro trabajo no sólo hemos incluido los compuestos de uso actual, sino también aquellos que, aun estando ya prohibidos, tuvieron un uso relevante en el pasado.

Otro gran grupo de biocidas, con un número de materias activas que se han aprobado y usado en grandes cantidades, los constituyen las piretrinas y piretroides. Se trata nuevamente

de compuestos que ejercen su toxicidad interfiriendo en la transmisión a nivel del sistema nervioso central, ya que son moduladores de los canales de sodio. Muchos de ellos están dotados de gran toxicidad, como la tetrametrina, la cialotrina o la permetrina, que han sido muy comunes en pipetas, collares y como ectoparasiticidas, además de los usos ambientales y agrícolas. Las intoxicaciones en animales de compañía son relativamente frecuentes, sobre todo por un mal uso o elección del producto ya que, por ejemplo, los gatos son mucho más sensibles a la intoxicación por piretrinas o piretroides que los perros. A fecha de hoy, al igual que ocurre con el grupo anterior, un gran número de las materias activas de este grupo han sido prohibidas o reguladas en la UE.

Por otro lado, un grupo que ha cobrado gran relevancia en los últimos 15 años es el de los neonicotinoides, con compuestos de enorme uso a nivel mundial, como el imidacloprid, acetamiprid, clotianidina o el tiametoxán, son agonistas de los receptores nicotínicos de acetilcolina. Aparentemente, tienen menos potencial tóxico para las aves y mamíferos, pero causan muerte de colmenas de abejas, las cuales son realmente sensibles a este grupo. Es bien conocido el beneficio de las abejas, y las causas de que desaparezcan de nuestro modo de agricultura, pero poco se sabe de su bioacumulación en las redes tróficas. Se han asociado a parámetros reproductivos y en la calidad de la cáscara del huevo (González-Rubio *et al.*, 2021).

Otros biocidas de gran uso, y que merecen mención destacada, son el fipronil (insecticida) y el metaldehído (molusquicida). El fipronil, interfiere el sistema GABA y produce alteraciones nerviosas y la muerte de los insectos. Es un producto muy usado en la clínica de pequeños animales, aunque algunos mamíferos, como los conejos o, las aves, son muy sensibles a este compuesto. El metaldehído es mortal si se ingiere en grandes cantidades, algo no muy difícil ya que los cebos para babosas y caracoles suelen contener grandes cantidades de melaza y cereales, que resultan sumamente atractivos para las especies no diana.

Adicionalmente, existe una nueva gama de productos, basados en la biología del insecto, que no producen directamente su muerte, y que son relativamente inocuos para los vertebrados por no compartir ciertas características fisiológicas. Se trata de los reguladores del crecimiento de insectos (IGRs, en inglés), imitan las hormonas de los invertebrados, impidiendo su crecimiento, muda, eclosión de huevos o reproducción. Por ejemplo, los inhibidores de la síntesis de quitina, como las benzoilureas (lufenurón, diflubenzurón, triflumurón, hexaflumurón, teflubenzurón), o las triazinas (como la ciromazina). Los análogos de la hormona juvenil como el fenoxicarb (carbamato) o el piriproxifeno o, los análogos de la hormona de la muda como la metoxifenocida o la tebufenocida.

1.2.2. Rodenticidas anticoagulantes (RAs)

En realidad, todos los rodenticidas estarían incluidos dentro de la definición de biocidas, por lo que en sentido estricto deberían estar incluidos en el apartado anterior. Su popularidad a nivel mundial, así como la extraordinaria relevancia toxicológica que tienen algunos compuestos raticidas, en particular los denominados anticoagulantes, hacen que los hayamos considerado de forma diferenciada en este texto.

Mención especial merecen las ocho materias activas, usadas como rodenticidas anticoagulantes (RAs) que están actualmente registradas en UE, clasificados en RA de Primera o Segunda Generación (FGAR y SGAR, en inglés, respectivamente). Los SGAR fueron desarrollados en los 70 por la resistencia desarrollada a la Warfarina, de 1ª generación. Los SGAR son más tóxicos y mucho más persistentes, tanto en seres vivos como en el medioambiente que sus predecesores. Intervienen en la síntesis de factores de coagulación dependientes de vitamina K. Algunos ejemplos incluidos en esta tesis son el brodifacoum, bromadiolona, difenacoum, difetialona (SGARs), y la warfarina e indandionas como clorofacinona o difacinona (FGARs). Existen otros rodenticidas no anticoagulantes como la alfa cloralosa, el fosfuro de zinc, de aluminio, de calcio o de magnesio autorizados en la UE (Valverde *et al.*, 2021). Para controlar roedores y otros pequeños mamíferos, como topos, erizos, conejos y liebres, el método más común es el uso de RA, sobre todo en zonas aisladas o archipiélagos como el nuestro, y entornos urbanos (Eason *et al.*, 2002; Lettoof *et al.*, 2020), aunque en ocasiones, estos animales diana pueden resultar como un vector de dispersión del propio veneno (Lohr, 2018; Elmeros *et al.*, 2019).

Afectan a todos los vertebrados, y aunque no se sabe su efecto real en invertebrados (Badry *et al.*, 2020), las concentraciones detectadas en las lombrices, caracoles y entomofauna, son compatibles con una intoxicación aguda en pequeños mamíferos, como musarañas y aves paseriformes insectívoras (Alomar *et al.*, 2018; Elmeros *et al.*, 2019; Walther *et* al., 2021). Asimismo, los reptiles se han estudiado como vector de anticoagulantes. Son más longevos y poseen un metabolismo lento, por lo que pueden excretarlos más lentamente, y por tanto bioacumularlos (Lohr, 2018; González-Mille *et al.*, 2019). Se han detectado concentraciones de hasta 0,8 mg/kg (siendo 0,1 mg/kg tóxico para aves; Thomas *et al.*, 2011), en reptiles sin envenenamiento poblacional, pero sí de aves rapaces (Lettoof *et al.*, 2020). Este año hemos empezado un proyecto a raíz del Proyecto LIFE Lampropeltis, para biomonitorizar los hígados de las culebras capturadas durante las campañas de erradicación de esta especie invasora, y los resultados preliminares indican que están altamente expuestas a los RAs, indicando su enorme penetración en las cadenas tróficas.

1.2.3. Productos de protección de plantas (PPPs)

Entre los productos de protección de plantas o PPPs (EC 1107/2009; CE, 2009) se incluyen herbicidas, reguladores del crecimiento de las plantas o antifúngicos y conservadores de la madera. Son productos destinados a proteger cultivos y plantas antes, durante o después de la cosecha. Como PPPs se incluyen también algunos de los biocidas ya mencionados anteriormente.

Existen herbicidas residuales, que actúan durante meses en las hierbas que están por germinar (como la terbutilazina), o foliares que se centran en la destrucción de la planta, ya sea por contacto directo (como el diquat o paraquat), o absorbidos y transportados por la savia (como el glifosato). Además, existen herbicidas hormonales, como los ácidos fenoxiacéticos como el 2,4-D; las triazinas como la atrazina, simazina y propazina; o nitrosaminas como la trifluralina, que controlan el crecimiento de los vegetales. Después de aplicación con herbicidas,

disminuye la supervivencia de pollos que comen insectos y/o semillas, tanto por falta de alimento como por intoxicación secundaria. Los herbicidas de contacto son cáusticos, como el paraquat, ya prohibido en la UE. Es conocido por su implicación en numerosísimos intentos autolíticos, y las intoxicaciones accidentales que produce, con graves lesiones y secuelas si se supera la intoxicación aguda. Por otro lado, otros herbicidas como el glifosato o la atrazina se han estudiado como disruptores hormonales, o los herbicidas residuales.

Por último, incluimos los compuestos antifúngicos, de enorme uso en Canarias, y los conservadores de la madera, como el vinclozolín y la procimidona (dicarboximidas), el triadimefón o el tolclofós metil (organofosforado) o el penconazol. Son ampliamente usados en agricultura para controlar hongos o prevenirlos durante el almacenamiento principalmente. Su importancia radica en la cantidad de residuos en las verduras y hortalizas, que son monitorizados constantemente.

1.3. Productos farmacéuticos y de cuidado personal

El aumento de población y la mayor longevidad, conlleva a mayor uso de medicamentos, no solo por número de personas si no por mayor número de patologías como la obesidad o problemas crónicos o psicológicos. A su vez, el uso en veterinaria se verá amplificado a medida que la demanda de carne y productos de origen animal, e incluso de mascotas, aumente. En muchas ocasiones acaban en aguas de escorrentía, o productos caducados o desechados (Margalida *et al.*, 2014). Algunos cosméticos son fuente, entre otros de, aluminio o metales pesados, ftalatos y parabenos, no incluidos en esta tesis. A pesar de programas vigilancia y control, el mal uso o eliminación conlleva a que detecten antiinflamatorios, hormonas, antibióticos, analgésicos o barbitúricos, en aguas fecales o vertidos (Blanco *et al.*, 2017), que amenazan la vida salvaje (Richards, 2014; Badry *et al.*, 2020). Los medicamentos veterinarios y humanos con mayor relevancia toxicológica en fauna silvestre y que han sido objeto de estudio en esta Tesis Doctoral son los antibióticos y los antiinflamatorios no esteroideos (AINEs).

1.3.1. Antibióticos

Los grupos más usados son las sulfonamidas, los betalactámicos, los macrólidos y las quinolonas, para uso tópico o sistémico de infecciones bacterianas o parasitarias. Hasta hace unos años, se empleaban como promotores del crecimiento en ganadería (Lopes *et al.*, 2012). Esta práctica se prohibió en la UE ya que la mayor amenaza es la creación de resistencias bacterianas a antibióticos, y con ello una incapacidad para tratar infecciones con estos fármacos. Las aves migratorias pueden exponerse y portar medicamentos o bacterias resistentes, favoreciéndolas en entornos naturales y diseminándolas larga distancia. Las carcasas de animales pueden ser una fuente de exposición para carroñeros, ya que no existen periodos de supresión o LMR para estos residuos. Blanco *et al.*, 2016 encontraron residuos de quinolonas en pollos de buitre, y Blanco *et al.*, 2017 en buitres y alimoches, principalmente enrofloxacino, que pueden causar lesiones orales de tipo levadura con efectos negativos para estas especies

(Arnold *et al.*, 2014). Las formas microscópicas se ven afectadas por estos biocidas, con lo que las cadenas tróficas se pueden ver comprometidas.

Finalmente, los residuos de antibióticos están presentes en gran cantidad de alimentos, por ejemplo nitrofuranos y nitroimidazoles en miel y derivados (Shendy *et al.*, 2016), o en la leche, afectando a la fermentación para la producción de quesos y yogures. Los nitrofuranos, el metronidazol (nitroimidazol) o el cloranfenicol son conocidos carcinogénicos y mutagénicos en animales, por lo que la UE prohíbe residuos de estas sustancias, y regula mediante LMR para el resto en productos de origen animal (Smith, 2015).

1.3.2. Antiinflamatorios no esteroideos (AINEs)

Los antiinflamatorios no esteroideos (AINEs) incluyen una amplia variedad de fármacos para combatir inflamaciones, dolor e hipertermia, tanto en humanos como en fauna. Todos son inhibidores de la ciclooxigenasa disminuyendo la síntesis de prostaglandinas, aunque pertenecen a grupos muy diversos. Una vez más la mala gestión, uso y administración, deriva a que se hallen residuos de ibuprofeno, ácido acetil salicílico (AAS) o paracetamol en aguas residuales que acaban vertidas al mar o ríos. Los animales domésticos en ocasiones han sido envenenados con paracetamol o AAS, o de forma accidental por un mal tratamiento, ya que pueden desarrollar una insuficiencia renal aguda. Las aves, especialmente buitres y otras aves carroñeras son altamente susceptibles a algunos AINEs, causándoles gota visceral y problemas renales (Margalida *et al.*, 2014). Se han encontrado residuos de hasta 4 AINEs (meloxicam, flunixina, diclofenaco y ketoprofeno) en canales o tejidos de aves de carroña en la península ibérica (Zorrilla *et al.*, 2015; Herrero-Villar *et al.*, 2020). El meloxicam, parece ser un sustituto ideal para estos AINEs (Markandya *et al.*, 2008).

En torno al 95% de las poblaciones de buitres, águilas imperiales y milanos europeas residen o permanecen largas temporadas en las península ibérica e itálica. A pesar de la recomendación de la UE de reducir el uso de diclofenaco por sus efectos medioambientales, tanto España como Italia siguen autorizando su uso en ganadería (Eleni *et al.*, 2019; Herrero-Villar *et al.*, 2021). En Nepal, Pakistán e India, fue prohibido, después de una intoxicación masiva de buitres en el que murió hasta el 99% de los individuos (Blanco *et al.*, 2016), y con ello la propagación de enfermedades como la rabia transmitida por el aumento de población de cánidos carroñeros. Igualmente, el ketoprofeno, el carprofeno o el flunixin se han probado muy tóxicos para buitres, a concentraciones que se encuentran en carcasas, con lo que hay que controlar tanto las canales, como los muladares donde se les alimenta (Pain *et al.*, 2008; Naidoo *et al.*, 2010; Margalida *et al.*, 2014; Zorrilla *et al.*, 2015).

2. Efectos en la salud y sanidad de la fauna y los ecosistemas

Los efectos nocivos dependen del tóxico implicado y de la dosis fundamentalmente, aunque también de la especie, edad, o la vía de entrada al organismo, pero todos tienen el

potencial de dañar, mediante mecanismos muy variados, a los organismos y los ecosistemas. Cabe destacar que las sustancias suelen evaluarse de forma individual, y no como "*cócteles químicos tóxicos*", que son a los que estamos habitualmente expuestos, con efectos aditivos, sinergistas o antagonistas, por lo que en ocasiones los perjuicios pueden ser incluso mayores.

La mayoría de los biocidas provocan intoxicaciones agudas evidentes, y muchas veces letales, ya que han sido fabricados con ese propósito. Existen ejemplos bien conocidos, como los potentes carbamatos, organofosforados y organoclorados, que interfieren en la estimulación de la acción de la acetilcolina; los RAs que inhiben la vitamina K, y con ello la síntesis de factores de la coagulación (Lettoof *et al.*, 2020); los AINEs que provocan nefropatías, hepatitis o gota en aves, en aves (Pain *et al.*, 2008; Naidoo *et al.*, 2010) o los organoclorados que interfieren en la transmisión nerviosa. Por otra parte, existe una práctica ilegal, y cuanto menos, inmoral como es el envenenamiento intencionado de animales, lo cual trataremos más adelante en esta introducción.

Además de los efectos agudos, muchas sustancias químicas son capaces de ejercer efecto a dosis muy bajas y, a pesar de que algunas se hayan prohibido hace décadas, seguimos estando expuestos a ellas y continúan siendo un riesgo para la salud (González-Mille *et al.*, 2019). La exposición subletal y/o crónica comprende cambios en la expresión génica, daño celular e interacciones fisiológicas. Estos efectos crónicos perjudiciales se pueden resumir en los siguientes puntos:

- Alteración endocrina (DE)

Los disruptores endocrinos (DE) son químicos que alteran las vías hormonales, siendo agonistas o antagonistas de receptores hormonales que producen desajustes importantes, que interfieren en el normal funcionamiento del organismo. El momento de la exposición es importante, ya que un desajuste en una edad temprana embrionaria puede tener consecuencias graves de adulto (Encarnação *et al.*, 2019), en el desarrollo, el crecimiento o el metabolismo a concentraciones muy bajas. Recordemos que muchas de estas sustancias traspasan el huevo y a la leche materna. Al menos unas 800 sustancias son bien conocidas como DEs, entre las que se encuentran pueden ser los PAHs, los BOCs, los PCBS, PBDEs, PFAS, glifosato y algunos biocidas organofosforados o antimicrobianos, o cosméticos, relacionados con diabetes, síndrome metabólico, entre otros (Pesavento *et al.*, 2018; Encarnação *et al.*, 2019; Ubaid ur Rahman *et al.*, 2021; www.ewg.org).

- Carcinogénesis, teratogénesis, y genotoxicidad

Desde el siglo XX se sabe que algunas sustancias, en un principio "*inocuas*" para los seres humanos o grandes vertebrados, pueden actuar interferir con el ADN, formando aductos, o producir malformaciones o neoplasias. En una revisión reciente se recoge la afectación de algunos cánceres en fauna silvestre y de los procesos oncogénicos, entre los que encontramos los PAHs o los PFAS, que se relacionan con cáncer de mama, vejiga, pulmón o gastrointestinal, piel (Pesavento *et al.*, 2018).

- Defectos del desarrollo

Los efectos tóxicos se exacerban en cartílagos, articulaciones y huesos, produciendo efectos del desarrollo y de malformaciones (Blanco *et al.*, 2016). Estos defectos en el desarrollo alteran la función endocrina como DEs, otros actúan directamente en los tejidos, como cerebro o pulmón, dañándolos o produciendo atrofias. Afectan también a la condición corporal y ganancia de peso. Una vez más, los COPs son los grandes ejemplos, como el DDT o los PCBs, que han sido detectados incluso en el cordón umbilical o leche materna (Luzardo *et al.*, 2009; Cabrera-Rodríguez *et al.*, 2020).

- <u>Efectos reproductivos</u>

Numerosos contaminantes son considerados xenoestrógenos, es decir, moléculas que interaccionan con los receptores de estrógenos, ajenas a la producción endógena. La sobreestimulación de dichos receptores se relaciona con cáncer de mama, la feminización de machos o problemas de criptorquidismo a COPs como es el caso del DDT, DDE y PCBs, o el mercurio. El DDT o el DDE (unos 10 mg/kg, Elliott *et al.*, 2015), se relacionan con la delgadez cáscara de huevo o el número de huevos por puesta, la pérdida de fertilidad y del ratio de sexos, así como el HCB con la pigmentación de la cáscara, la síntesis del grupo hemo, por lo que pueden producir porfirias (Buck *et al.*, 2020).

- <u>Neurotoxicidad</u>

Algunos compuestos inorgánicos como el mercurio alteran el desarrollo del sistema nervioso en etapas tempranas del embrión, que pueden hacerse evidentes tras el paso de los años. Se han vinculado a los COPs y algunos pesticidas organofosforados, con autismo, déficits cognitivos o TDAH. Los rodenticidas anticoagulantes (especialmente SGARs), no solo ejercen una alteración en la hemostasia que muchas veces es letal para el animal, sino que, a concentraciones relativamente bajas, se ha informado que provocan efectos nerviosos y hemorragias no mortales que producen desorientación e incoordinación en aves, o incluso desajustes en la termorregulación (Lettoof *et al.*, 2020). En definitiva, se modifican el comportamiento, vigilia, debilidad, desorientación, siendo más propensos para accidentes o depredación (Berny, 2007; Movalli *et al.*, 2018).

- <u>Inmunotoxicidad</u>

Las intoxicaciones subclínicas, pueden afectar a la susceptibilidad de otras enfermedades, alterando la respuesta inmune, los epitelios o incluso el sistema nervioso en mamíferos y aves (Espín *et al.*, 2018; *Rattner et al.,* 2020), produciendo asma o neumonías, y reacciones dérmicas y alérgicas (Berny, 2007; Koivisto *et al.*, 2018). El incremento de alergias, enfermedades autoinmunes y problemas respiratorios, se sospechan que puedan estar relacionadas con numerosos contaminantes, como los éteres de ftalatos que agravan la dermatitis atópica, o las isotiazolinonas, conservantes de cosméticos y productos de limpieza, se han relacionado también con dermatitis alérgicas en profesionales de la limpieza. Se ha comprobado que los PCBs particularmente, el DDT y DDE, alteran la proporción heterófilos:linfocitos (Garcia-Heras *et al.*, 2018), lo que indica estrés fisiológico y mayor susceptibilidad a enfermedades. De igual modo, se han relacionado las dioxinas y algunos PAHs

similares a dioxinas, junto a otros COPs, con efectos dañinos sobre la inmunidad de los animales más jóvenes, sobre todo en etapas embrionarias.

2.1. Implicaciones de la presencia de sustancias químicas en el medioambiente

Entre los efectos indirectos de los contaminantes, tenemos varios ejemplos que nos ayudan a entender la magnitud del problema. El ejemplo clásico del libro *Silent Spring*, de Rachel Carson, en 1962, en su observación y divulgación de los peligros de la contaminación química en los ecosistemas, especialmente en aves. A raíz de esta publicación comenzaron a prohibirse y regularse diversos biocidas persistentes como el DDT. Si descienden las poblaciones de conejos, por ejemplo, pueden aumentar el número de paseriformes en un área, que acumulan niveles más altos de COPs (Espín *et al.*, 2018), siendo éstos una fuente importante para sus posibles depredadores. Los desajustes en los ecosistemas que se producen pueden ser profundos, afectando o agravando incluso a los programas de reintroducción y conservación de especies (Garcia-Heras *et al.*, 2018).

Los biocidas suelen usarse en amplias áreas, siendo métodos no selectivos con lo que ocurre la muerte de artrópodos polinizadores, y no polinizadores, restringiendo la cantidad de plantas. Así mismo, los rodenticidas como ya hemos visto se introducen en la cadena trófica a todos los niveles, siendo detectados en invertebrados, tanto en moluscos o saltamontes, como en reptiles o anfibios, los cuales no son objetivos, pero pueden comportarse como bombas móviles tóxicas, concentrando RAs a concentraciones letales para sus depredadores (Lohr, 2018; Lettoof *et al.*, 2020). Normalmente, la persistencia de estos compuestos es notoria en las aves rapaces, ápices de la cadena trófica, y que normalmente se alimentan de roedores considerados diana. En definitiva, al contaminar el medio con biocidas, estamos combatiendo contra nosotros mismos, dejando a la naturaleza sin medios de compensación naturales de poblaciones.

Por otro lado, la proliferación de enfermedades y vectores, como en las poblaciones de buitres, que descendieron entre un 97 a 99 % entre 1992-2007, en India, y más tarde Pakistán y Nepal. Al poco, los casos de rabia humana aumentaron (Movalli *et al.*, 2018). Al descender el número de buitres, los perros callejeros, los otros carroñeros de grandes ungulados en la India, aumentaron paralelamente a la disminución de buitres. Los perros son conocidos portadores y transmisores de la rabia en India, mayor que los murciélagos (Markandya *et al.*, 2008). Otras enfermedades como tuberculosis, brucelosis, carbunco y otros vectores, como roedores, podrían ser más prevalentes (Markandya *et al.*, 2008). Poco después, se informó que los buitres estaban muriendo intoxicados por diclofenaco, un fármaco antiinflamatorio muy usado tanto en humanos como veterinaria. Su bajo costo y alta eficacia hace que fuera un medicamento usado ampliamente en el mundo y sobre todo en India, hoy en día prohibido en el país.

Por último, las miles de toneladas de antibióticos, RAs y otros biocidas no solo son un problema para el medio ambiente y los ecosistemas, sino que pueden llegar a la cadena alimentaria humana a través de animales salvajes como el jabalí o el ciervo (López-Perea and Mateo, 2018), produciendo resistencias bacterianas adquiridas por los alimentos, o

intoxicaciones secundarias. Asimismo, los cadáveres o carcasas de animales medicados o intoxicados previamente pueden servir como cebo natural en animales carroñeros. En estos tipos de carnes o productos cárnicos, no existen LMRs, o no se hacen análisis de rutina como en los productos de ganadería como productos lácteos o cárnicos. Recordemos que los COPs, altamente lipofílicos, se encuentran en gran cantidad de los alimentos, constituyendo una amenaza grave para la seguridad alimentaria.

3. Envenenamientos

Los biocidas están destinados a solo un 2% de los vertebrados, pero su efecto en ellos es de gran relevancia (Gibbons *et al.*, 2015). A pesar de ser un delito tipificado en el artículo 336 del Código Penal Español (BOE, 1995), el uso de cebos envenenados sigue siendo un problema serio en Europa, especialmente relevante en Italia (De Roma *et al.*, 2018), Reino Unido (Millins *et al.*, 2014) y España (Valverde *et al.*, 2021), particularmente en Canarias (Ruiz-Suárez *et al.*, 2015). Nos referimos a venenos o envenenamientos cuando haya una intencionalidad de eliminar a alguna población de animales no deseados por alguna persona, aunque a veces pueden ser varias especies e individuos los implicados en un episodio de envenenamiento sin ser el objetivo. En Canarias se ha descrito que se ven afectados una media de 2,5 animales por evento, pudiendo llegar a más de 10 individuos en un solo episodio (Ruiz-Suárez *et al.*, 2015).

Las causas que subyacen a estos envenenamientos intencionados son principalmente la caza, la ganadería y agricultura, así como acciones de venganza (envenenando perros, gatos y animales domésticos y de abasto, denominada violencia vicaria), o la cría de palomas y apicultura (Cano *et al.*, 2020). Normalmente se fabrican cebos artesanales con restos cárnicos frescos, pescados, o algún vegetal que sea atractivo para la especie diana, muchas veces mezclados con melazas y otros productos que aporten palatabilidad a los preparados. En ocasiones, el daño que se busca es doble, con prácticas indeseables como la adición de clavos o chinchetas. El preparado de cebos "*cóctel*", en los que se pueden encontrar varias sustancias, aumenta con la menor disponibilidad de sustancias altamente tóxicas, gracias a la persecución por las autoridades, las campañas de recogida de fitosanitarios o, falta de existencias.

El uso de venenos, aunque parece descender en los últimos años, está estrechamente relacionado con la disponibilidad y la mortalidad que produzcan dichas sustancias (Plaza *et al.*, 2019). En España se usan más de 80 sustancias, y en más del 70% de los casos están implicados tres biocidas (aldicarb, carbofurano y estricnina), que están prohibidos desde hace años e incluso décadas (Cano *et al.*, 2020). En Canarias son el carbofurano y el aldicarb (Ruiz-Suárez *et al.*, 2015), junto con los RAs las sustancias más usadas, seguidas de otros inhibidores de la colinesterasa y algunos piretroides. Otras sustancias comúnmente usadas incluyen los medicamentos, productos de limpieza u otros productos químicos de uso doméstico. El entorno rural sigue siendo donde mayor número de envenenamientos se producen, tanto de domésticos como silvestres. En el entorno urbano es relativamente común el uso de medicamentos, como paracetamol, aspirina o ibuprofeno, (bastante tóxicos para perros, gatos y aves), para el envenenamiento de colonias de gatos, perros callejeros o por venganza. Las administraciones

deberían promover soluciones integradas, vigilando su uso y distribución, e imponiendo mayores penas (Plaza *et al.*, 2019)

En nuestro laboratorio, el Servicio de Toxicología Clínica y Analítica (SERTOX) desde 2010 hemos recibido muestras de animales de todas las islas del archipiélago canario (más de 1.300 ejemplares en 10 años de servicio) en el contexto de la estrategia canaria contra el veneno (BOC, 2014) y la futura ley de biodiversidad. El 26% de los animales y el 72% de los cebos recibidos y analizados en nuestras instalaciones en el período 2014-2021, fueron positivos a alguna de las 77 sustancias detectadas.

4. Monitorización y Biomonitorización

Dada la importancia de estos tóxicos, es necesario lleva a cabo una vigilancia mediante análisis sistemáticos para evaluar la inclusión de estas sustancias en el medio y los organismos, y los riesgos que se generan. Es importante para estudiar el impacto de los químicos en el ambiente y seres vivos, además que nos ofrece información sobre exposición, entrada, biodisponibilidad e impacto de los tóxicos (Gómez-Ramírez *et al.*, 2014; González-Rubio *et al.*, 2021). Esta vigilancia o monitorización, provee de alerta temprana sobre los problemas que generan los contaminantes en el ambiente, a la par que asesora la creación o modificación de leyes y protegiendo los ecosistemas (Movalli *et al.*, 2017). La monitorización analiza muestras ambientales, incluyendo aire, suelo, agua o comida, normalmente como seguimiento de la calidad del ambiente o de los alimentos que tomamos, y en consonancia con los LMRs impuestos por las administraciones de cada país. Así, los alimentos cárnicos y ricos en grasas son los alimentos más contaminados. La biomonitorización es la medida de la carga tóxica de un ser vivo, que se puede realizar directamente sobre muestras de organismos vivos, como sangre, orina o, cordón umbilical para evaluar exposiciones tempranas, o mediante la aplicación de bioindicadores y biomarcadores.

El uso de biomarcadores de exposición o de daño, tiene la ventaja de ser un método indirecto, en el que evaluamos el riesgo de los tóxicos mediante pruebas bioquímicas o moleculares. Sirve de alerta temprana, ya que los daños a nivel celular se manifiestan antes que en el organismo. Algunos ejemplos son la medición de aductos de sustancias químicas con el ADN como biomarcadores de efecto de los PAHs, la inducción del sistema del citocromo P450 tras la exposición a ciertos contaminantes, la proliferación de peroxisomas específicos de PAHs y PCBs, el diagnóstico de gota en aves carroñeras como sospecha de intoxicación por diclofenaco u otros AINEs (P. Gómez-Ramírez *et al.*, 2014), o el método "*imposex*" para la exposición al tributil de estaño en mejillones, potente andrógeno que masculiniza a las hembras. Los tiempos de coagulación se han estudiado como biomarcadores de exposición a RAs en centros de recuperación. Tenemos la intención de llevar a cabo estudios en colaboración con los dos centros de recuperación de las islas (Centro de Recuperación de la Fauna Silvestre de Tafira y Centro de Recuperación de la Fauna Silvestre de La Tahonilla, así como con GREFA - Grupo de Rehabilitación de la Fauna Autóctona y su Hábitat -), con sede en Madrid.

El uso de bioindicadores puede ser útil para generar líneas de evidencia en la evaluación de riesgo ecológico, en la identificación de especies vulnerables a los efectos de la contaminación, así como en el diseño y seguimiento de estrategias de intervención (González-Mille *et al.*, 2019). Animales, plantas y microorganismos se han estudiado como bioindicadores de la contaminación de lagos, mares o incluso suelos. Los huevos de aves y reptiles se informan en numerosos estudios de biomonitorización, como muestras para detectar compuestos lipofílicos que se traspasan a través de la madre (Buck *et al.*, 2020). El recuento de bacterias en agua nos indica la calidad de ésta, estableciendo criterios diferenciados para el agua potable, residual, de riego o marina. Diversos moluscos como los mejillones son buenos indicadores de la contaminación química del área donde se encuentren, ya que son organismos fijos y filtradores capaces de concentrar los contaminantes en sus organismos. Se han analizado como bioindicadores de COPs o elementos inorgánicos como metales pesados u oligoelementos (Rodríguez et al. 2019).

La biomonitorización sobre muestras biológicas, nos da información sobre la carga tóxica real en un momento dado para un individuo, para generar estudios de riesgo a partir de estos datos. Se pueden usar como ventaja, las plumas secreciones de glándula pineal, sangre o huevos (muestras no invasivas) (González-Rubio *et al.*, 2021). De esta forma la biomonitorización promueve la ampliación de conocimiento, optimización de toma de muestras y técnicas analíticas para maximizar la información obtenida e interpretada (Richards, 2014). El programa HBM4EU (*Human Biomonitoring for EU*), de biomonitorización en humanos, nació con el objetivo de esclarecer la evidencia de exposición a químicos por la población europea, así como la identificación de nuevas sustancias de interés y que serán el foco de interés. Los programas como el Apex Predator o la ERBFacility (*European Raptor Biomonitoring Facility*), se centran en el estudio de las rapaces como centinelas de uso y exposición a químicos por la fauna silvestre en Europa. Por otra parte, la biomonitorización de especies centinelas, como las aves (a las que dedicamos la siguiente sección), o los animales domésticos con los que compartimos hábitat, han sido estudiados plenamente. En el caso de los PAHs, nuestro grupo estudió la idoneidad de los perros como centinelas de la exposición a hidrocarburos, como queda reflejado en el capítulo de libro publicado en Springer, y que forma parte de esta tesis en la Sección III. Biomonitorización.

En resumen, la monitorización y biomonitorización son relevantes para estudios de evaluación de exposición y peligros químicos ambientales derivados, evaluación de sustancias nocivas para los ecosistemas y su posterior seguimiento, sistema de alerta temprana y el asesoramiento, vigilancia y verificación de las leyes sobre residuos o límites de exposición a contaminantes, mediante estudios espaciotemporales y de riesgo.

4.1. Biomonitorización en aves rapaces

Las aves son uno de los grupos de animales más investigados en estudios de biomonitorización. Integran los contaminantes a larga escala, tanto espacial como temporal (Elliott *et al.*, 2015). Su posición de ápice en muchas de las cadenas tróficas, junto a su alta longevidad ayuda a la bioacumulación y biomagnificación de los contaminantes (Derlink *et al.*,

2018). Son especies de amplia distribución mundial, es decir, las podemos encontrar en diferentes lugares del mundo, y suelen restringirse a un área concreta, por lo que son buenos indicadores de la contaminación de la zona de estudio, como una foto fiable del estado de la cadena trófica (Richards, 2014). El seguimiento de las aves rapaces tiene un papel importante que desempeñar en el contexto de la política medioambiental de la UE. Existe falta de conocimiento sobre acumulación y exposición en los grandes depredadores (Badry *et al.*, 2020), estudiar y entender las amenazas químicas, las vías de exposición o las sustancias a las que están expuestas, son importantes para su conservación (Badry *et al.*, 2019). Existen en Europa y a nivel nacional, varios programas de vigilancia de contaminantes en rapaces (Gómez-Ramírez *et al.*, 2019; González-Rubio *et al.*, 2021).

El Fondo para la Biomonitorización de Rapaces de Europa (ERBFacility https://erbfacility.eu/, COST Action CA16224) trabaja activamente en la biomonitorización coordinada a través de los países miembros, con el fin de reducir los riesgos químicos derivados en el ambiente y la salud pública, apoyando la implementación de la legislación en cuanto a regulaciones de productos químicos. De igual modo, el proyecto LIFE APEX (https://lifeapex.eu/), trabaja para demostrar la utilidad de biomonitorizar contaminantes emergentes en aves predadoras, favoreciendo el manejo correcto de químicos contaminantes en la UE.

Las aves rapaces comprenden tres grupos principales: las aves de presa, los búhos y los halcones (Espín *et al.*, 2021). Se ha comprobado la validez de analizar a los búhos reales como centinelas para biocidas organoclorados, permitiendo los estudios de uso espaciotemporales (Briels *et al.*, 2019; Gómez-Ramírez *et al.*, 2019). Otras especies más comunes y distribuidas mundialmente, como el búho chico (*Asio otus)* (Gómez-Ramírez *et al.*, 2019) o el cernícalo vulgar (*Falco tinnunculus)*, han demostrado ser buenas indicadoras de la contaminación local, ya que suelen restringirse a un área definida, a la vez que son longevos y relativamente fáciles de capturar. Además, los Sistemas de Información Geográfica, ofrecen información geoespacial, en nuestro caso, de la posición de las aves y la situación de granjas, industrias o minas (Espín *et al.*, 2016).

Proteger la naturaleza, la biodiversidad y los ecosistemas, es autoprotegernos. Es necesario actuar rápido, limitar la producción y uso de sustancias químicas nocivas conocidas, o nuevas emergentes, así como ahondar en conocimiento de otras sustancias usadas comúnmente. Basarnos en políticas "*ONE HEALTH*" puede ser una solución para minimizar la exposición y perjuicio de estas sustancias en el medioambiente y en los seres humanos.

5. Metodología

La biomonitorización de los seres vivos requiere de métodos rápidos, robustos, eficaces y económicos para detectar los contaminantes que corren el riesgo de acumularse en las redes alimentarias y de afectar a la salud de los ecosistemas. Además, con la aplicación de nuevas normativas más restrictivas en cuanto a LMRs se requiere que un método sea capaz de detectar los agentes químicos a concentraciones de ppb o µg/kg, así como la inclusión de nuevas

sustancias en los programas de vigilancia, el desarrollo de métodos analíticos se convierte en un importante desafío. El desarrollo y optimización de un método analítico, o conjunto de técnicas necesarias para realizar los análisis de tóxicos en muestras biológicas complejas, como sangre e hígado, es un tema complejo, que comprende muchas fases, tiempo, trabajo y formación (González-Rubio *et al.*, 2021). En nuestro caso, esta fase experimental de desarrollo metodológico fue la primera etapa, la cual nos llevó más de dos años de trabajo, después de mucho estudio y pruebas. Obtener información de un gran número de sustancias, de grupos químicos tan variados como los que presentamos en esta tesis, partiendo de un volumen pequeño de muestra (Perestrelo *et al.*, 2019) , fue nuestra prioridad. A su vez, otro importante reto que nos planteamos era el de trabajar en consonancia con la denominada "*química verde*", mediante la reducción del material fungible o disolventes y reactivos que son necesarios por cada muestra, intercambiándolos por sustancias menos tóxicas. La simplicidad del método, en términos de tiempo y facilidad de realización, y que admita una gran cantidad de muestras, son otros factores para tener en cuenta, ya que habitualmente trabajamos con series de hasta miles de muestras por proyecto.

La mayoría de los métodos existentes se centran en uno o unos pocos tóxicos, muchas veces pertenecientes a un grupo químico concreto. La aplicabilidad de métodos multirresiduos se ha expandido últimamente, con resultados prometedores, aunque todavía sigue siendo limitado (Lopes *et al.*, 2012). Otros investigadores usan varias extracciones, procedimientos más largos y laboriosos, y/o necesitan varios análisis consecutivos en varios equipos. Nuestro reto principal al plantearnos esta tesis fue el conseguir desarrollar una metodología que incluyera la mayor cantidad de contaminantes químicos ambientales posible, adaptado a pequeñas cantidades de muestras, y que implicara la menor cantidad de pasos y coste posibles, con la finalidad de maximizar la cantidad de información que pudiera obtenerse de las muestras es especímenes de fauna silvestre, habitualmente de escaso volumen y difíciles de conseguir.

A continuación, presentamos un breve resumen de todos los pasos de un procedimiento analítico de este tipo, desde la elección de la muestra ideal, pasando por la técnica de extracción, así como las técnicas de detección y cuantificación y la posterior validación de los métodos presentados. En esta Tesis Doctoral se aborda el desarrollo y optimización de una metodología para la extracción y cuantificación de 360 compuestos en sangre, y 351 en hígado, a niveles de ppb.

5.1. Muestras

En realidad, la muestra ideal depende de los fines del estudio, la disponibilidad de individuos y/o muestras deseadas, y de los compuestos de interés a detectar (González-Rubio *et al.*, 2021). Por ejemplo, la glándula pineal o la grasa visceral se han investigado como muestras ideales para el estudio de contaminantes emergentes y persistentes, ya que en la secreción se excretan gran cantidad de estos contaminantes lipofílicos persistentes, pero no es una muestra ideal para el diagnóstico de venenos. Para la detección de venenos, son útiles varias muestras, como son el hígado, el riñón o la sangre, lo cual verificaría la entrada del tóxico al organismo. Por otra parte, el contenido gástrico, restos regurgitados, o incluso un lavado con disolventes de

extracción de la lengua, cavidad oral o de restos óseos de cadáveres incluso enterrados, pueden ser la única muestra disponible en toxicología forense. Aun así, el hígado y la sangre son las muestras de elección para detección tóxicos en rapaces y se han establecido protocolos unificados para la obtención de muestras para la biomonitorización en fauna silvestre (E*spín et al.*, 2021).

La sangre es una buena muestra, ideal en individuos vivos, ya que los tóxicos se distribuyen y circulan por todo el organismo. La sangre cadavérica, puede ser de utilidad en toxicología forense. Nos da información de una exposición reciente, al contrario que el hígado, el cual tiende a acumular mayores cantidades de tóxicos. Una desventaja en aves es que el volumen obtenido suele ser bajo, sobre todo en aves de pequeño tamaño, donde usualmente solo se pueden extraer unos 500 µL, para varias pruebas (hematología, bioquímica, toxicología, actividad de acetilcolinesterasa, pruebas de coagulación y otras pruebas complementarias). Las vidas medias de las sustancias químicas en la sangre suelen ser cortas, y las concentraciones normalmente menores y más variables que en el hígado (Ruiz-Suárez *et al.*, 2014). Por otra parte con el menor contenido lipídico se esperan menores concentraciones de COPs, aunque se ha probado como muestra válida para estimar la exposición a contaminantes persistentes en tortugas bobas (Camacho *et al.*, 2012).

El hígado por otra parte se puede obtener mediante biopsia de animales vivos, aunque lo habitual es que se obtenga durante una necropsia reglada, como parte de un estudio anatomopatológico. El hígado, al igual que el riñón, es un órgano filtrador y depurador de sustancias, donde muchas son metabolizadas con el fin de eliminarlas. Su composición rica en lípidos y vasos sanguíneos hace que sea una muestra ideal para estudios de biomonitorización, ya que los contaminantes lipofílicos se acumulan en él, donde normalmente son liberados al torrente sanguíneo para ser probablemente excretados.

El hígado suele estar lobulado, por lo que el riesgo de falsos positivo/negativos con submuestras o restos de tejidos, puede ser un problema en estudios de biomonitorización. Por ejemplo, en el caso del brodifacoum, existe diferencia significativa entre los lóbulos hepáticos a concentraciones bajas, aunque en caso de diagnóstico de envenenamiento, donde las concentraciones suelen ser muy altas, la diferencia es despreciable (Taylor *et al.*, 2020).

5.2. Preparación de las muestras y extracción de contaminantes químicos

Las matrices biológicas con las que trabajamos habitualmente en el laboratorio son mezclas complejas, compuestas por azúcares, grasas, proteínas, enzimas, sales y agua. Existen situaciones, como la detección de metabolitos de drogas en orina, en los que no es necesario ninguna preparación o extracción de la muestra, sino que en ocasiones bastaría con diluirla con agua. Sin embargo, para el análisis de metabolitos de organofosforados en la misma matriz, se hace necesaria la derivatización, mediante reacciones químicas controladas de los compuestos, lo cual conlleva mayor trabajo y tiempo de espera que el anterior.

Por otra parte, los tejidos sólidos, como es el caso del hígado, deben de ser homogenizados previo proceso de extracción, ya que la naturaleza solida del órgano no permite que penetre adecuadamente el disolvente en el tejido, con lo que la capacidad de extracción se verá reducida. Para ello, se pueden usar diferentes estrategias, y habitualmente se usan sistemas homogenizadores, que sean capaces de romper el tejido conectivo y los propios hepatocitos, liberando los analitos de su interior.

Para la detección y cuantificación de los analitos, primero debemos aislarlos de la matriz en la que se encuentran. De entre las numerosas técnicas existentes, en los últimos años ha cobrado mucha relevancia la extracción por dispersión de la matriz en medio acuoso salino (Lopes *et al.*, 2012), un tipo de extracción habitualmente denominado QuEChERS por sus siglas en inglés (*Quick, Easy, Cheap, Effective, Robust and Safe*, Rápido, Fácil, Barato, Eficaz, Robusto y Seguro). Se basa en una extracción de tipo dispersiva, en la que el acetonitrilo (ACN) es el disolvente de elección, normalmente acidificado con ácido fórmico o acético. A este extracto, se le añaden sales que ayudan a la partición de los analitos y la separación de fases, en lo que llamamos "*salting-out*" o dispersión de sales en el extracto. Este extracto debe ser libre de lípidos, proteínas y pigmentos que se encuentran en la propia matriz, tras un paso de limpieza que normalmente involucra el uso de cartuchos SPE (*Solid Phase Extraction*), donde pueden quedar retenidos parte de los analitos junto con las impurezas.

El primero en desarrollar este tipo de métodos fue Michelangelo Anastassiades y su equipo en el año 2003, para aplicarlo al análisis de residuos de pesticidas en alimentos de origen vegetal con alto contenido en agua. Posteriormente, en 2007, Steven Lehotay y su equipo modificaron esta extracción con la adición de sales tampón como citrato y acetato, con lo que se mejoró la extracción de compuestos sensibles a altos o bajos pH (Anastassiades *et al.*, 2003; Lehotay *et al.*, 2007; Perestrelo *et al.*, 2019). Desde entonces, se han realizado numerosas modificaciones sobre el método base, con resultados altamente satisfactorios, experimentando con diferentes disolventes de mayor o menor polaridad, como el acetato de etilo, el diclorometano, hexano o el metanol; modificando la composición y cantidad de sales, para deshidratar el extracto o permitir la extracción de ciertos compuestos sensibles; así como ajustar el pH de la extracción mediante adición de tampones, ácidos o bases, según la matriz y los analitos deseados (Perestrelo *et al.*, 2019).

En este trabajo de Tesis Doctoral, desde el principio optamos por utilizar esta metodología, y hemos optimizado dos métodos de extracción basados en QuEChERS, para el análisis de RAs y otros pesticidas, medicamentos y COPs, para las matrices de sangre e hígado. En primer lugar, nos decidimos por el acetonitrilo porque se ha probado como un buen disolvente para extracción de analitos de interés, y que menos impurezas de la matriz extrae, además es menos tóxico que otros como el diclorometano o el acetato de etilo. Además, apostamos por el uso de ácido fórmico, ya que ayuda en la precipitación y desnaturalización de proteínas, eliminándolas con la centrifugación. A pesar de que ciertos compuestos son sensibles al ácido fórmico como los RAs, logramos optimizar la composición para obtener límites de detección y cuantificación bastante bajos. En cuanto a las sales, los experimentos con el método de la Norma Europea, y de la AOAC (*Association of Official Agricultural Chemists*), dieron como mejor resultado la utilización de sulfato magnésico y acetato sódico. El sulfato magnésico deshidrata y elimina el agua residual, lo cual beneficia a la capacidad de los detectores. En

nuestro caso, optamos por no incluir un paso de limpieza, ya que comprobamos que disminuía o incluso interfería completamente con la extracción de diferentes analitos, en particular, con aquellos de mayor polaridad. En lugar de ello, después de los pasos consecutivos de agitación, adición de sales y centrifugación a temperatura de congelación (para coagular y sedimentar los lípidos), el extracto resultante se filtra directamente al vial de cromatografía, por una malla de 0,22 µm para evitar la entrada de suciedad a los cromatógrafos. Estas modificaciones permiten la extracción simultánea de diferentes grupos y tipos de tóxicos.

5.3. Cromatografía líquida y de gases acoplada a espectrometría de masas de triple cuadrupolo (LC-MS/MS y GC-MS/MS)

Antes de optimizar el método de extracción y preparación de muestras, es necesario el desarrollo de las técnicas e instrumentos para la detección y cuantificación de las sustancias. Los instrumentos y técnicas analíticas necesarios son cada vez más sensibles y específicos. Nosotros trabajos con cromatografía de líquidos (LC) y de gases (GC) acoplados a espectrometría de masas de triple cuadrupolo (MS/MS) como detector, ya que suponen una técnica fundamental para la toxicología forense. Proporcionan identificación inequívoca, que permite diferenciar entre isómeros del mismo compuesto. Por ejemplo, algunos anticoagulantes, poseen en su estructura química carbonos asimétricos, lo que les confiere la propiedad de tener varios isómeros (diferentes propiedades químicas, farmacocinética o efectos biológicos y vidas medias diversas) (Alabau *et al.*, 2020). Esto es importante porque normalmente las mezclas comerciales contienen distintas mezclas de estos isómeros, y alguno de estos isómeros puede no tener efectos tóxicos. A nivel analítico, es necesario saber si está implicado uno u otro isómero (Alabau *et al.*, 2020), tanto por las relevancias toxicológicas como legislativas.

Para no saturar el detector inyectando toda la muestra de una vez, es necesaria la separación de los analitos antes de su llegada al espectrómetro de masas. Esta técnica de separación, o cromatografía, se basa en la interacción fisicoquímica de las moléculas con los componentes de una fase estacionaria, mientras que una fase móvil, la atraviesa portando la muestra. Los dos tipos de cromatografía que aquí se abordan son la cromatografía líquida (LC) y de gases (GC), las cuales se diferencia esencialmente, en la fase móvil utilizada para separar los compuestos en una columna específica, lo cual, a su vez, depende de la sustancia que vaya a ser analizada. En GC se detectan sustancias liposolubles, apolares y volátiles, mientras que en LC se detectan sustancias hidrosolubles, más polares y termolábiles. Es la mejor opción para métodos multirresiduo como el que nos ocupa (EC, 2019). En LC las condiciones de la fase móvil, como el pH o la polaridad, se modifican mediante la adición de ácidos o bases, con las que formen aductos los analitos y sean capaces de ionizarse antes de llegar al detector. Esto dependerá en gran medida del tipo de tóxico que queramos analizar, ya que, en función de las polaridades y solubilidades en agua o disolventes orgánicos, obtendremos mejores respuestas. En el GC, es importante la temperatura del horno, ya que en este caso necesitamos que las moléculas se volatilicen por la acción de la temperatura y atraviesen la columna analítica. Estas técnicas nos permiten separar y obtener un tiempo de retención en el detector, que estará relacionado con las propiedades de analito y las condiciones cromatográficas.

Las técnicas de detección se basan en medir una propiedad física o química del analito, como por ejemplo ultravioleta, aunque pueden dar más falsos positivos/negativos. En nuestro caso se trata de interacciones masa carga (m/z). Son detectores muy fiables para nuestro fin, ya que permiten la detección de varios cientos de compuestos en poco tiempo. Para la ionización de las moléculas, se parte de una fuente de ionización de tipo electroespray para LC-MS/MS, que forma microgotas que se ionizan y entran al detector por diferencia de presión, ya que trabajan en condiciones de alto vacío. En el caso de GC-MS/MS, la fuente de ionización es de Impacto Electrónico, ya que la muestra llega en fase de gas y un simple impacto es suficiente para romper e ionizar los compuestos. Una vez en el detector, ya sea LC o GC, a su paso por el primer cuadrupolo se aplican campos electromagnéticos que seleccionan las moléculas por su relación m/z. En el segundo cuadrupolo, o celda de colisión, se le aplica una descarga que permite la fragmentación de la molécula parental en fragmentos conocidos y constantes, y que son posteriormente, seleccionados en el tercer cuadrupolo, detectando dos iones procedentes del primer y el tercer cuadrupolo, en un tiempo de retención definido. A este conjunto de acciones es a lo que se conoce como transición, en la que se define la pareja de ión parental y el ión producto.

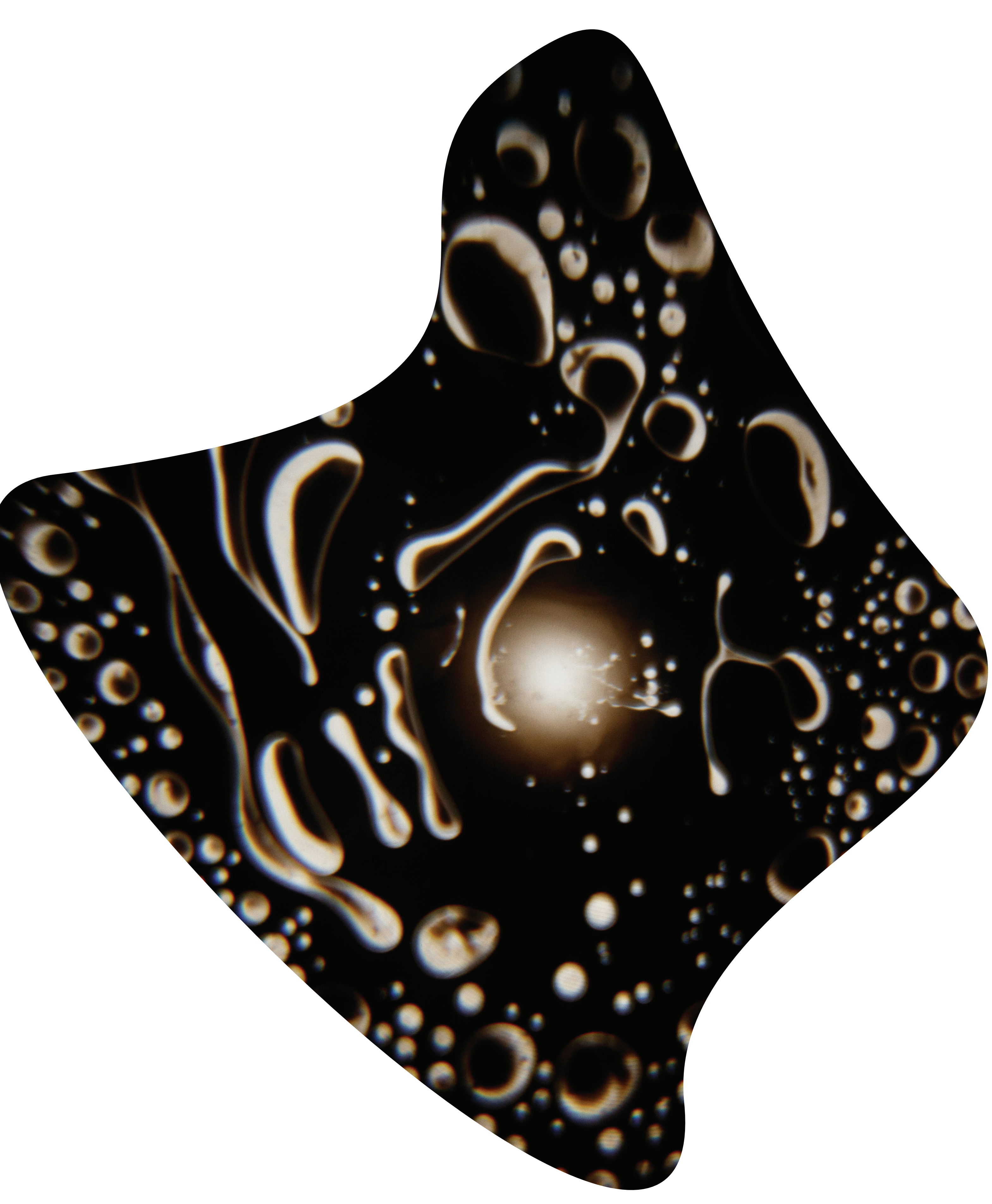
5.4. Validación del método analítico desarrollado

Dados los estándares de calidad de hoy en día, y la necesidad de monitorizar con alta sensibilidad y especificidad la concentración de contaminantes en alimentos, así como la variedad de técnicas disponibles, se hace necesaria una guía de trabajo en química analítica que imponga criterios para la validación interna del método propuesto para la extracción, detección y cuantificación de los analitos. Para este trabajo nos hemos guiado por los criterios de las guías SANTE (EC, 2019) y SWGTOX (*Scientific Working Group for Forensic Toxicology*; SWGTOX, 2013). La primera, es específica para la detección de residuos de plaguicidas en alimentos vegetales, en alimentos y piensos, mientras que la segunda es específica para fines forenses toxicológicos, por lo que se adapta mejor a nuestros fines.

En cuanto a la detección, para que un compuesto cumpla los criterios, hay que definir un tiempo de retención (RT), o el tiempo que tarda el compuesto en atravesar la columna y llegar al detector. Además, son necesarias dos transiciones en un determinado RT para corroborar que se trata de uno u otro compuesto. Se verifican de igual modo que no existan interferencias con compuestos de la matriz o residuos de la extracción. Para la cuantificación, los criterios a seguir son el porcentaje de recuperación, y los coeficientes de variación (CV) *intra* e *interday*. Los porcentajes de recuperación son una medida de lo exitosa que es la técnica de extracción elegida, ya que valora la cantidad de analito detectada frente a la que realmente existe en una muestra. Los coeficientes de variación son una medida de lo robusto que es el método, ya que indica la variabilidad de resultados para una misma muestra con igual concentración, siendo el rango aceptable de entre un 70 y un 120%, así como CV menores del 20% e idealmente menores del 15%. Por último, debemos definir un rango de trabajo, en el que nuestro método sea comprobado que cumple los criterios que acabamos de comentar, en el que se cumplan los criterios de linealidad, medida como coeficiente de regresión o R^2, y los

límites de detección y cuantificación. Estos límites se encuentran en muchas ocasiones por debajo de las 5 ppb, con lo que presentamos técnicas muy sensibles, robustas y validadas.

III. BIBLIOGRAFÍA



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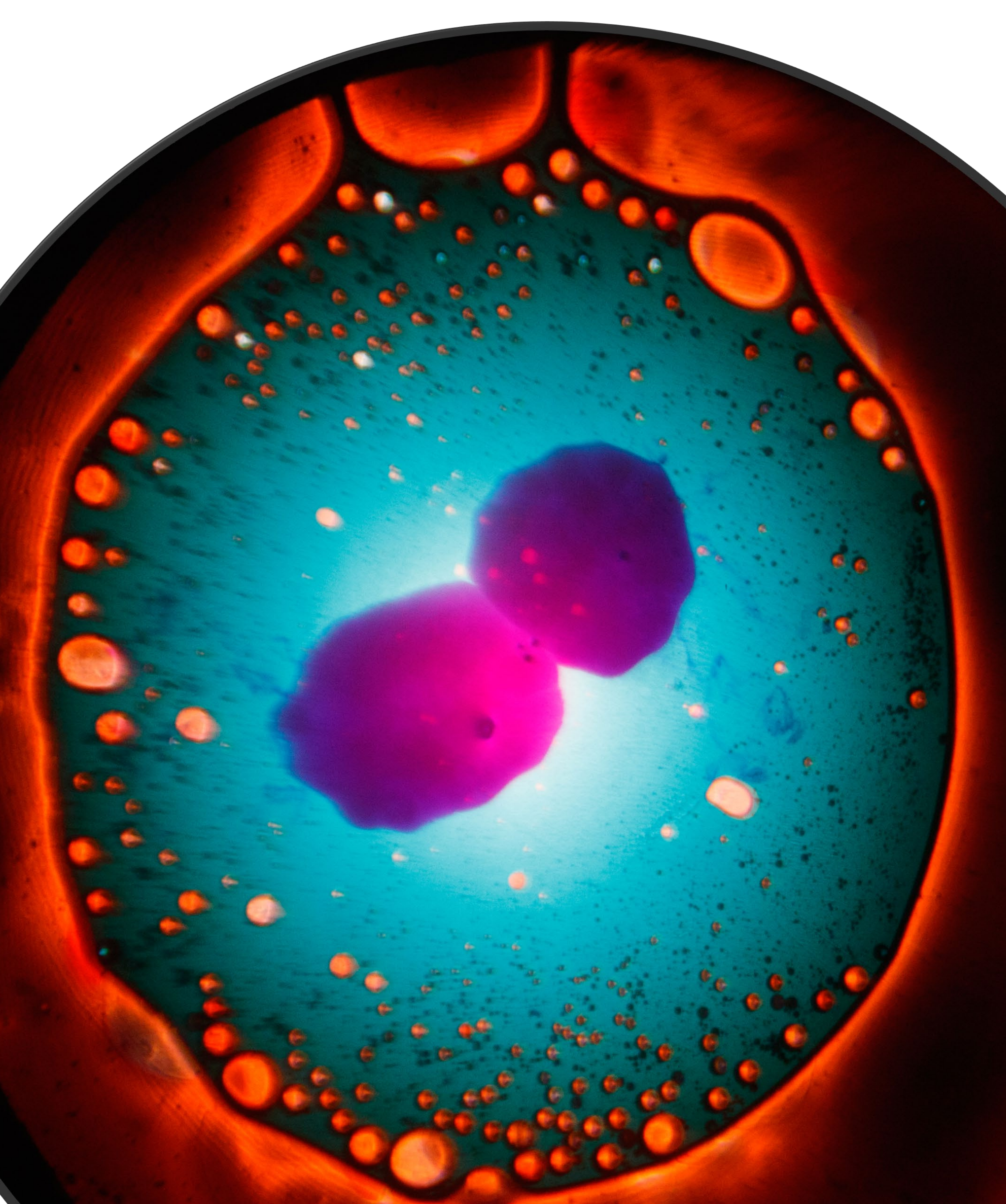
IV. JUSTIFICACIÓN



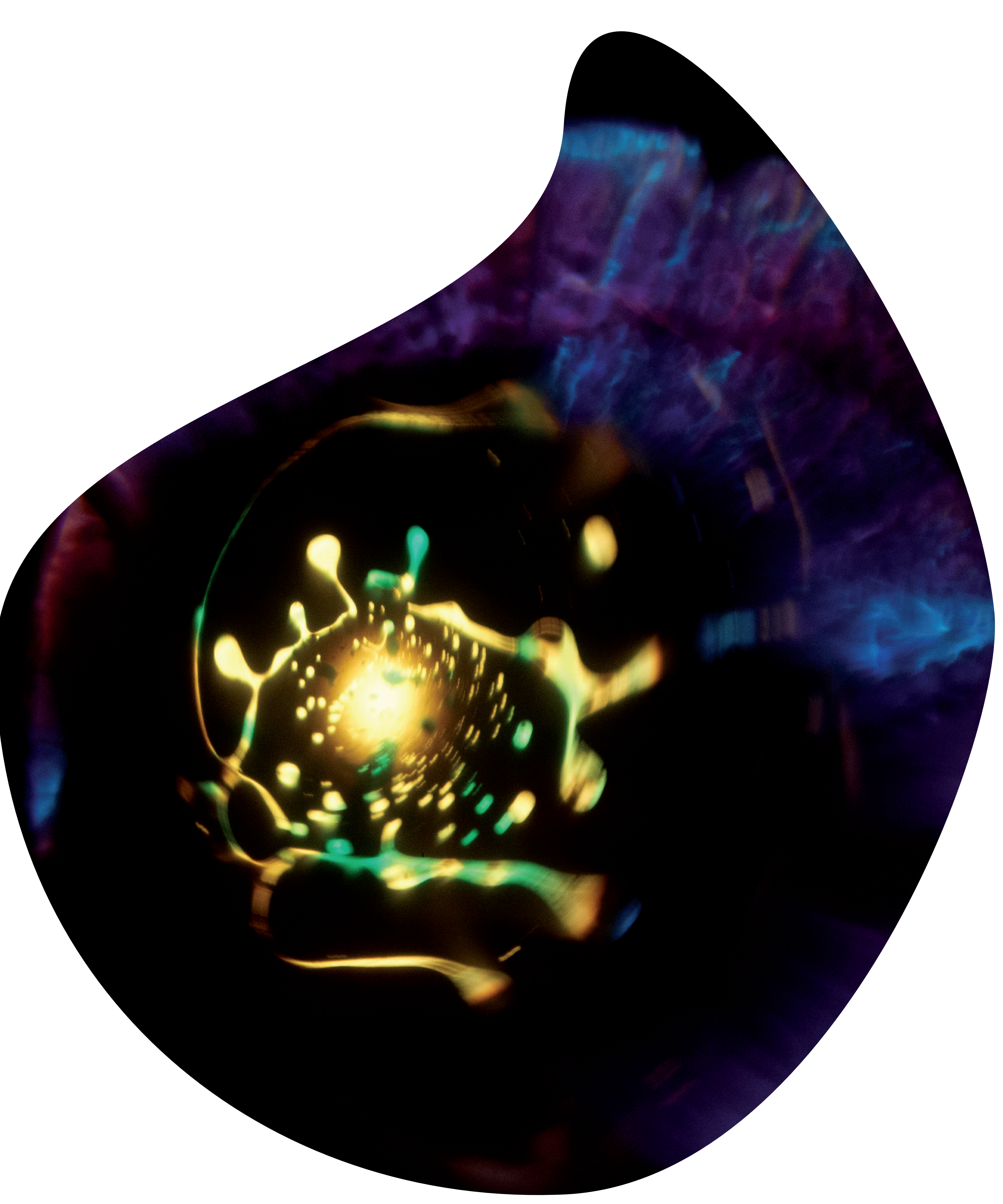
JUSTIFICACIÓN

En la parte introductoria de esta Tesis Doctoral, hemos descrito los contaminantes a los que estamos expuestos constantemente, y cómo pueden interaccionar con los seres vivos para causar efectos nocivos sobre nuestros organismos.

A pesar de los esfuerzos de diferentes organismos internacionales, como la Unión Europea, para minimizar la producción y exposición a químicos como los contaminantes orgánicos persistentes, los plaguicidas incluidos los rodenticidas, o los medicamentos, éstos siguen siendo un riesgo para la salud de los seres vivos. Pueden afectar al sistema hormonal y al genoma, produciendo malformaciones y desajustes metabólicos a dosis muy bajas, además de producir envenenamientos e intoxicaciones agudas y/o letales en fauna silvestre. La biomonitorización de contaminantes en la fauna silvestre es hoy en día un enfoque común para evaluar la exposición química en la fauna silvestre y en los seres humanos. Esta herramienta, junto con los conocimientos existentes en materia de ecología y evolución, permiten mejorar las evaluaciones de peligro y riesgo ecológico de las sustancias químicas. Se hace necesario, por tanto, el desarrollo de métodos analíticos sensibles, robustos y que sean capaz de detectar la mayor cantidad de contaminantes posible, de forma simultánea, rápida, y lo más económica posible.

En esta Tesis hemos desarrollado métodos para la determinación cuantitativa simultánea de más de 350 contaminantes relevantes toxicológicamente, en muestras de fauna silvestre, siendo probablemente la más completa disponible hasta la fecha en este campo. Además, hemos demostrado su aplicabilidad, trabajando con muestras reales en aplicación a muestras reales de fauna silvestre de las Islas Canarias, contribuyendo la evaluación holística de los impactos de las concentraciones de contaminantes relevantes para el medio ambiente del archipiélago canario. Con toda probabilidad, los resultados de esta Tesis Doctoral permitirán implementar estrategias para mejorar el estado de conservación de la biodiversidad única que existe en esta región oceánica.

V. OBJETIVOS/OBJETIVES



OBJETIVOS

1. Desarrollar y optimizar métodos multirresiduo para la extracción simultánea de contaminantes orgánicos persistentes, medicamentos y biocidas, basados en una extracción simple (QuEChERS) para pequeños volúmenes de sangre e hígado, seguidos de la determinación cuantitativa por cromatografía de líquidos y de gases acoplados a espectrometría de masas de triple cuadrupolo (LC-MS/MS y GC-MS/MS).

2. Validar los métodos desarrollados, siguiendo los criterios de guías oficiales para los requerimientos de identificación, sensibilidad, recuperabilidad, linealidad, reproducibilidad y robustez.

3. Aplicar los métodos validados a muestras reales de fauna silvestre para estudios de biomonitorización y aportar datos de exposición a multitud de tóxicos de los ecosistemas canarios.

4. Actualizar la base de datos de envenenamientos de Canarias, en el marco de la Estrategia Canaria contra el Veneno, del Gobierno de Canarias incorporando todos los casos desde el 2014 hasta el 2021.

OBJETIVES

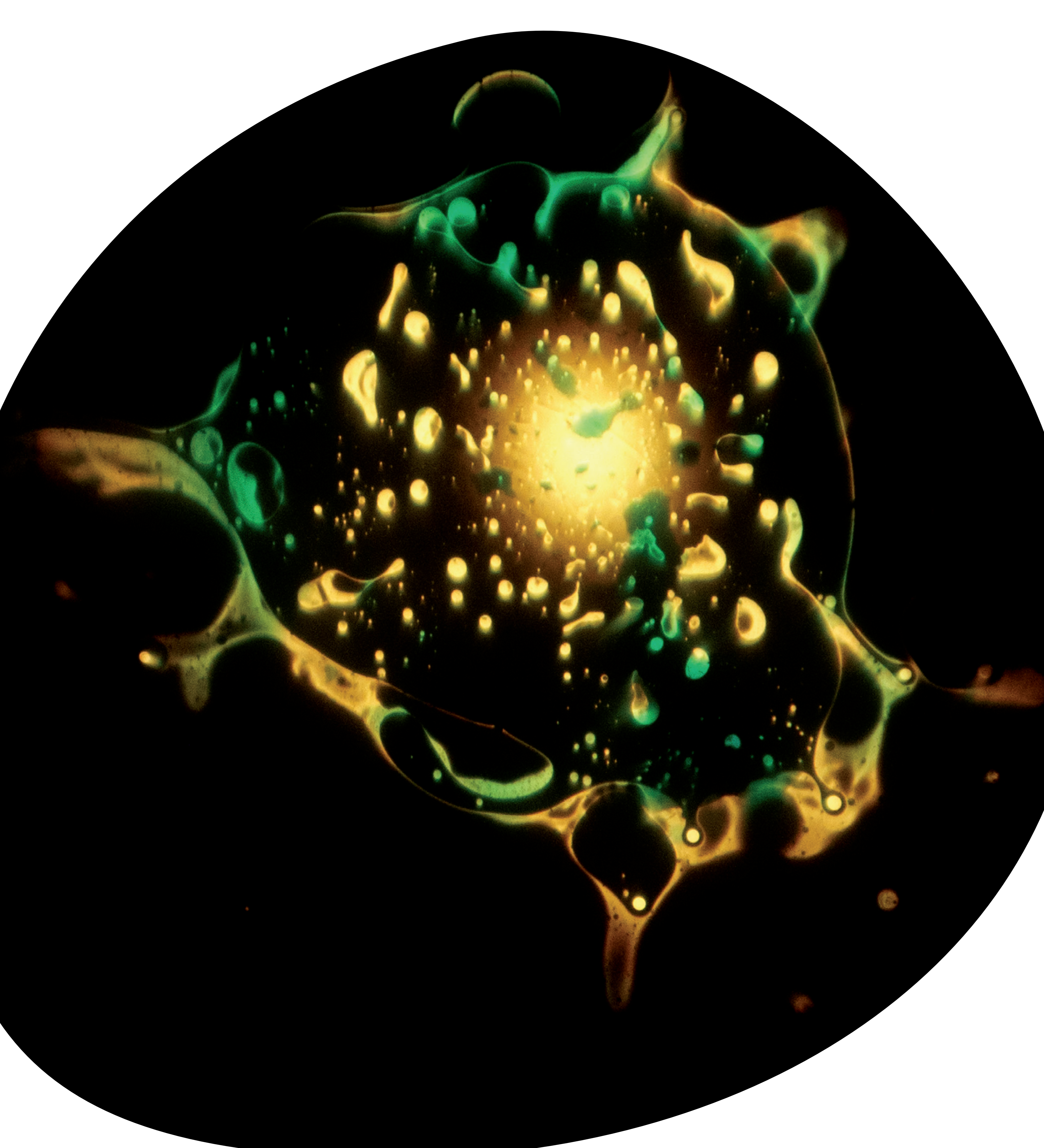
1. Develop and optimize multi-residue methods for the simultaneous extraction of persistent organic pollutants, medicaments, and biocides, based on a simple extraction (QuEChERS) for small volumes of blood and liver, followed by the quantitative determination by liquid and gas chromatography coupled to spectrometry of triple quadrupole masses (LC-MS / MS and GC-MS / MS).

2. Validate the developed methods, following the criteria of official guidelines for the requirements of identification, sensitivity, recoverability, linearity, reproducibility, and robustness.

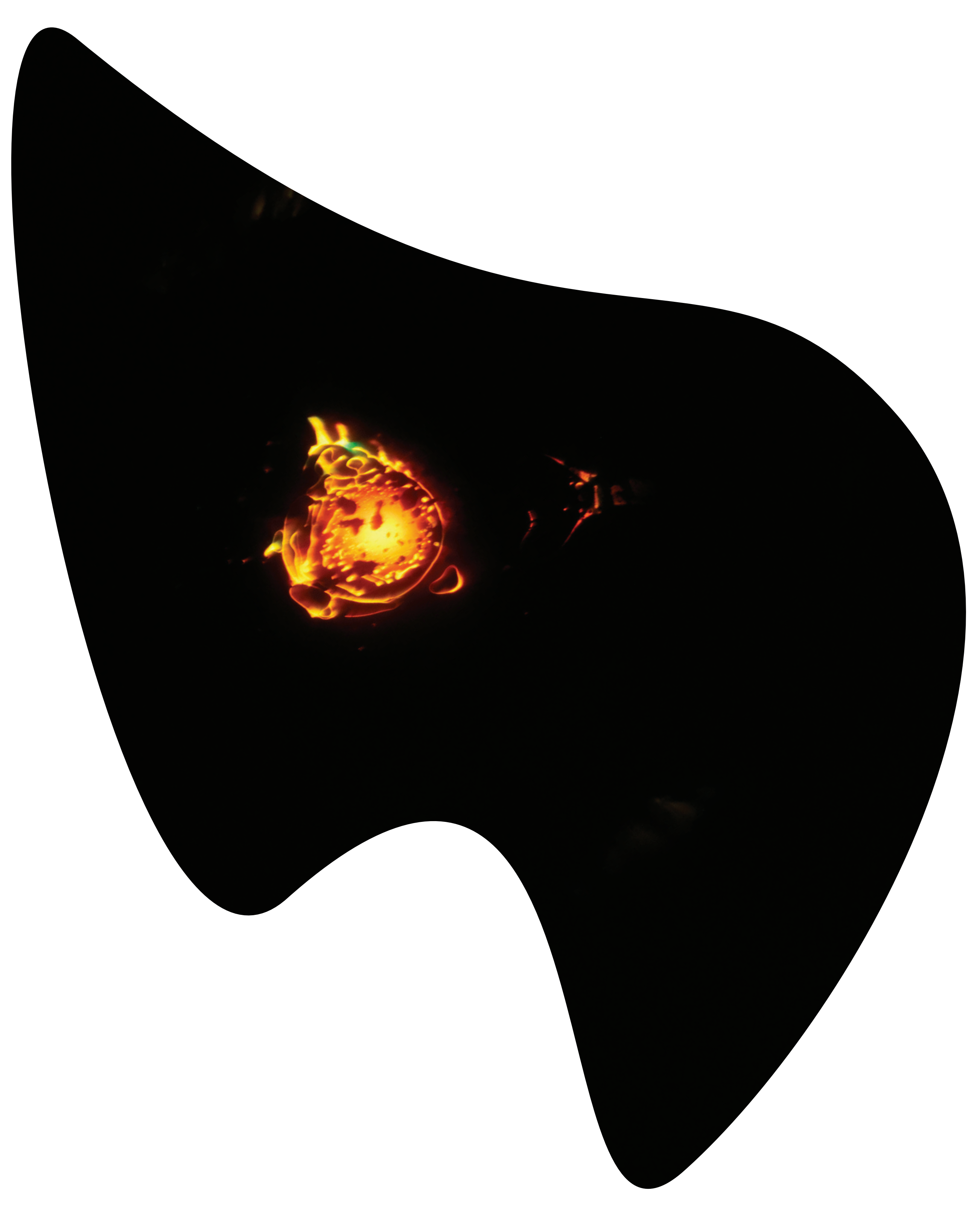
3. Apply validated methods to real wildlife samples for biomonitoring studies and provide data on exposure to a multitude of toxins in canarian ecosystems.

4. Update the database of poisonings in the Canary Islands, within the framework of the Canary Islands Strategy against Poison, of the Government of the Canary Islands, incorporating all cases from 2014 to 2021.

VI. RESULTADOS Y DISCUSIÓN



Bloque A. Metodología

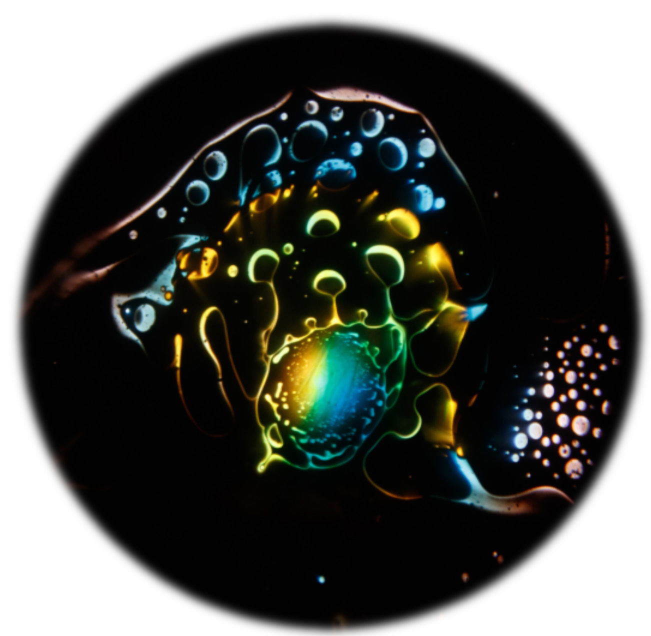


Publicación 1. Micro QuEChERS-based method for the simultaneous biomonitoring in whole blood of 360 toxicologically relevant pollutants for wildlife

Método basado en Micro QuEChERS para la biomonitorización simultánea de 360 contaminantes toxicológicamente relevantes para la vida silvestre en sangre

Science of the Total Environment, 2020, 736: 139444

DOI: https://doi.org/10.1016/j.scitotenv.2020.139444



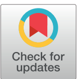
Science of the Total Environment 736 (2020) 139444

Contents lists available at ScienceDirect

Science of the Total Environment

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

Micro QuEChERS-based method for the simultaneous biomonitoring in whole blood of 360 toxicologically relevant pollutants for wildlife



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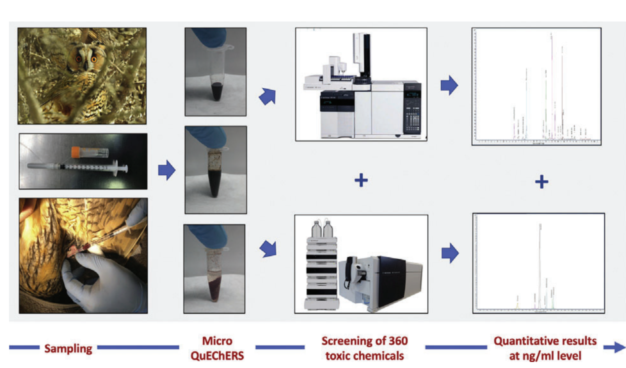
HIGHLIGHTS

- Validation of a method for the simultaneous quantification of 360 toxic chemicals in whole blood
- One-step acetate buffered micro QuEChERS using acidified acetonitrile yielded recoveries >70%.
- Only 250 μl of sample and quantification at the sub-ppb level makes it suitable for biomonitoring.
- Verified in a series of 36 barn owls and 112 common kestrels, which test positive for 3–25 pollutants

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 March 2020
Received in revised form 12 May 2020
Accepted 12 May 2020
Available online 15 May 2020

Editor: Yolanda Picó

Keywords:
Persistent organic pollutants
Pharmaceuticals
Pesticides
Rodenticides
Raptors
Mass spectrometry

ABSTRACT

This work presents the optimization, validation, and verification of a miniaturized method for the determination of 360 environmental pollutants that are of toxicological concern for wildlife. The method implies a one-step QuEChERS-based extraction of 250 μl whole blood using acidified acetonitrile, followed by two complementary analyses by LC–MS/MS and GC–MS/MS. The optimized conditions allow the simultaneous determination of the major persistent organic pollutants, a wide range of plant protection products, rodenticides, pharmaceuticals, and a suite of metabolites that can be used as biomarkers of exposure. The method is very sensitive, and 95% of the pollutants can be detected at concentrations below 1.5 ng/ml. The method was applied to a series of 148 samples of nocturnal and diurnal wild raptors collected during field ecological studies in 2018 and 2019. Fifty-one different contaminants were found in these samples, with a median value of 7 contaminants per sample. As expected, five of the six contaminants that were detected in >50% of the samples were persistent or semi-persistent organic pollutants. However, it is striking the high frequency of detection of some non-persistent pollutants, such as 2-phenylphenol, benalaxyl, metaflumizone, diphenylamine, brodifacoum or levamisole, indicating the penetration of these chemicals into the food chains. The toxicological significance of all these findings should be studied in depth in future research. However, the results clearly demonstrated that the approach developed provides reliable, simple, and rapid determination of a wide range of pollutants in wildlife and makes it very useful to obtain valuable data in biomonitoring studies with only small amounts of sample.
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https://doi.org/10.1016/j.scitotenv.2020.139444
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1. Introduction

In environmental chemistry, biomonitoring is the procedure by which the body burden of toxic or potentially toxic chemicals, in living beings, is assessed as a means of exposure assessment. Blood, its fractions, and urine samples are the most common samples, but tissues and other fluids can also be used, such as hair, feathers, or breast milk, among others (Haines et al., 2017; Ibarluzea et al., 2016).

A recent report, has indicated that since 1974 there has been a 60% decrease in wildlife populations worldwide (WWF, 2018). This alarming decrease responds to multiple causes, among which are the change in land uses, the destruction of the habitat of many species, the climate change, but also the penetration of chemistry in ecosystems (Hernout et al., 2011). A clear and very well documented example of the latter, is found in the drastic decline in populations of Asian vultures due to exposure to diclofenac, widely used as a veterinary anti-inflammatory drug, and that in these birds, produces lethal nephropathy (Sathishkumar et al., 2020). The amount and variety of chemical risks that wildlife faces are enormous, and for many of these pollutants, we have limited knowledge of the potential pressures on wildlife (Hernout et al., 2011).

One of the best-studied chemical groups in wildlife is that of the persistent organic pollutants (POPs, mainly organohalogenated compounds), as these compounds have been linked for decades to population declines, diseases or abnormalities in several species, including certain types of fish, birds, and mammals (Luzardo et al., 2014b; Malarvannan et al., 2020). Besides, their monitoring in wildlife seems to be important because certain animal species can also act as sentinels for human health (Bucchia et al., 2015; Elliott et al., 2018; Fox, 2001; Henriquez-Hernandez et al., 2017; Luzardo et al., 2014a; Reif, 2011), and also for identifying trends in levels that can assess the effectiveness of international control measures (Malarvannan et al., 2020).

In addition to all these legacy pollutants, many other chemicals are of concern for wildlife conservation because of their current or recent extensive use, their high toxicity, their stability, and their relatively high half-life. It is the case of some pesticides, pharmaceuticals, or anticoagulant rodenticides, among others. As regards pesticides, most of them are pollutants from the agricultural sector, where dozens of compounds of multiple chemical classes are used massively (Liao et al., 2019). If we take as a reference the list of substances included in the monitoring programs for residues in food for human consumption, at least 200 chemicals should be considered as worrisome in the European Union (EC, 2019a). Unfortunately, the excessive use of these agricultural pesticides causes their penetration into ecosystems and can compromise the health and even survival of many biological species (Encarnacao et al., 2019; Klich et al., 2020; Krief et al., 2017; Plaza et al., 2019). Also, deliberate abuse of pesticides to poison wildlife occurs throughout the world (Bille et al., 2016; Fajardo et al., 2012; Hernandez and Margalida, 2008; Motas-Guzman et al., 2003; Ntemiri et al., 2018; Ogada, 2014; Ruiz-Suarez et al., 2015). Therefore, in wildlife samples, it is also interesting to monitor not only legal but also frequently employed banned compounds (Luzardo et al., 2014c). A special case of pesticides is that of rodenticides, which are employed extensively around the world, leading to unintended exposure of non-target animals, especially raptors (Nakayama et al., 2019; Ruiz-Suarez et al., 2014).

As well as pesticides, pharmaceuticals have the potential to bioaccumulate and transfer through trophic webs and may threaten wildlife health. Wildlife exposure to pharmaceuticals can occur through contaminated water (Obimakinde et al., 2017), agricultural soils, plants and arthropods (Arnold et al., 2014; Bartikova et al., 2015), and through the excreta and carcasses of medicated livestock (i.e., supplementary feeding of threatened avian scavengers) (Blanco et al., 2017; Cuthbert et al., 2014).

The challenge of detecting such a variety of potentially harmful substances in samples from wild animals, is compounded by the fact that the amount of sample available is often small. Blood can be used as a non-lethal simple sampling matrix, but the sample volume is limited by body size, especially in the case of tiny animals such as songbirds or lizards. Therefore, it is desirable to have robust and sensitive analytical methods, and that these are as miniaturized as possible to maximize the information that can be obtained from a field sampling on a wildlife species. Many authors have published methods for the analysis of pesticides in wildlife samples, and some of them are based on the QuEChERS method. However, most of them have been designed for the analysis of a relatively low number of compounds belonging to the same chemical group (Allender and Keegan, 1992; Brown et al., 1996; Brown et al., 2005; Bucchia et al., 2015; Sage et al., 2010; Taliansky-Chamudis et al., 2017). This makes it necessary to use several of these methods in a complementary manner to biomonitoring all the relevant environmental chemicals, which is often impossible due to sample limitation. For this reason, sensitive and specific multi-residue techniques covering a wide spectrum of toxic or potentially toxic environmental pollutants, can substantially contribute to minimizing the costs and maximizing the chance of assessing the exposure of wildlife to most of the relevant chemicals. Thus, some other authors have developed multi-residue and multi-class methods for the determination of drugs (Qie et al., 2019), or of pesticides (Shin et al., 2018; Srivastava et al., 2017), or of POPs in blood (Vijayasarathy et al., 2019), even some using a very small amount of sample (Shin et al., 2018). However, to our knowledge, none has been developed for the simultaneous detection of contaminants for all of these groups together.

We have developed a multi-class multi-residue method comprising a single-step QuEChERS-based extraction of whole blood and two complementary chromatographic analyses coupled to mass spectrometry. The method allows the simultaneous quantification of 360 toxic or potentially toxic chemicals (POPs, agricultural pesticides, pharmaceuticals, and AR) at the sub-part-per-billion level using only 250 μl of whole blood. Additionally, we present data on environmental exposure to pollutants of 148 chicks belonging to two species of birds of prey from the central region of the Iberian Peninsula (common kestrels and barn owls) and discuss their ecological implications.

2. Materials and methods

2.1. Chemicals, reagents, and biological material

Certified standards of all the individual pollutants and deuterated compounds (P-ISs, procedural internal standards) which were initially tested (purity 93.1 to 99.8%), were obtained from Dr. Ehrestorfer (Augsburg, Germany), CPA Chem (Stara Zagora, Bulgaria), A2S – Analytical Standard Solutions (Staint Jean D'Illac, France), Sigma-Aldrich (Augsburg, Germany), Accustandard (New Haven, USA), and European Pharmacopoeia Reference Standards (Strasbourg, France). Salts for extraction based on the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, and Safe) were purchased in commercial premixes from Agilent Technologies (Palo Alto, USA) in two formats: QuEChERS Extract Pouch, AOAC Method (6 g de magnesium sulfate and 1.5 g sodium acetate) and QuEChERS Extract Pouch, EN Method (4 g magnesium sulfate, 1 g sodium chloride, 1 g sodium citrate dihydrate, and 0,5 g sodium hydrogencitrate sesquihydrate). QuEChERs dSPE Enhanced Matrix Removal-Lipid (EMR-lipid; Agilent, Palo Alto, USA) was used as a clean-up step in the optimization process. Acetonitrile (ACN, 99.9% purity), methanol (MeOH, 99.9% purity), and formic acid (FA, 98.0% purity) were purchased from Honeywell (Charlotte, USA), and were of LC-MS grade. The water for preparing the mobile phase (18.2 MΩ/cm) was obtained using an Elix Advantage 15UV tandem coupled to a MilliQ A10 Gradient system (Millipore, Molsheim, France). Ammonium acetate and ammonium formate were purchased from Fisher (Fisher Scientific UK, Loughborough, UK), and was of Optima LC-MS grade.

For the development, optimization, and validation of the analytical technique, we employed blood samples obtained from chickens and

goats from the animal housing facilities of the Faculty of Veterinary of the University of Las Palmas de Gran Canaria. All the animals were born in this facility, were healthy and had never been exposed to chemicals (no farms or agricultural facilities in the nearby, and no pharmacological treatments in the last two months), to avoid drug interference. Whole blood was obtained by puncturing the brachial vein (chickens, 23G needle) or the jugular vein (goats, 20G needle), using 3.5 ml-vacutainer tubes with heparin as anticoagulant. Upon arrival at the laboratory, aliquots were homogenized, pooled (by species), and stored at −24 °C until use.

To verify the applicability of the validated method to real samples, we studied a series of 148 blood samples. The samples were obtained from a diurnal and a nocturnal species of raptors (*Falco tinnunculus* and *Tyto alba*) and were collected during an ecological field study on the impact on wildlife of the treatment with rodenticides against a common vole (*Microtus arvalis*) plague. The samples were obtained from nest boxes located in the provinces of Palencia, Salamanca, Burgos, Segovia, Valladolid and Zamora (Castilla-León, Spain). All samples were collected after obtaining the corresponding permits and following the animal welfare protocols during the sampling (Espin et al., 2016).

2.2. Stock solutions, calibration standards and quality controls

Stock solutions of all POPs, pesticides, AR, pharmaceuticals, metabolites, and P-ISs were prepared by dissolving an accurately weighed amount in the suitable solvent (ACN, MeOH, water, acetone) to obtain a concentration of either 1 or 0.5 mg/ml. These stock solutions were stored in aliquots at −32 °C until use (maximum 1 year). Three intermediate working solutions were prepared by combining the individual standards (by groups: pesticides, pharmaceuticals and POPs, to avoid interferences between compounds and solvents), to give a concentration of 1 μg/ml/each and stored at −32 °C. Those solutions were renewed every three months. Deuterated standards were prepared separately in the same way in one mixture for both, GC and LC. Calibration standards were made from independent intermediate solutions of the stock solution and spiked in the chicken-, goat-, or combined blood to obtain 12 calibration standards in the range of 0.1 to 20 ng/ml. These matrix-matched calibration standards were freshly prepared for each experiment (daily). Quality controls (QC) samples were made in the same way to obtain three different levels (0.2, 2, and 10 ng/ml) of all the chemicals. Blank matrix samples were prepared to calculate linearity, matrix effect, carryover, interferences, and stability.

2.3. Instrumental analysis

For the detection and quantification of the 360 analytes finally included in this procedure, it is necessary to perform two complementary chromatographic analyses from the blood extract: a liquid chromatography analysis coupled to triple quadrupole mass spectrometry (LC-MS/MS) and an analysis by gas chromatography coupled to triple quadrupole mass spectrometry (GC–MS/MS).

2.3.1. LC-MS/MS

An Agilent 1290 UHPLC tandem coupled to an Agilent 6460 mass spectrometer (Agilent Technologies, Palo Alto, USA) was employed for the analysis of 234 chemicals. The chromatographic separations were performed using an InfinityLab Poroshell 120 (2.1 mm × 100 mm, 2.7 μm). Agilent 1290 Infinity II Inline Filter with 0.3 μm SS frit, and Agilent InfinityLab Poroshell 120 UHPLC Guard column (2.1 mm × 5 mm, 2.7 μm) were used to protect the column. The mobile phase A consisted on 2 mM ammonium acetate and 0.1% FA in ultrapure water, while the mobile phase B consisted on 2 mM ammonium acetate in MeOH. The mobile phase A gradient was: 95% - 0.5 min; 80% - 1 min; 60% - 2.5 min; 15% - 8 min; 0% - 10 to 14 min; 95% - 14.01 min. The flow rate was set at 0.4 ml/min. The injection volume was 8 μl. The column oven temperature was set at 50 °C. Total run time was 18 min. The mass spectrometer was operated in the dynamic multiple reaction monitoring (dMRM) mode. The optimized operating conditions of the mass spectrometer analyses, in positive and negative, electrospray ionization (Agilent Jet Stream Electrospray Ionization Source, AJS-ESI) were the following: gas temperature 190 °C; nebulizer gas flow 11 l/min; nebulizer pressure 26 psi; sheath gas temperature 330 °C; sheath gas flow 12 l/min; capillary voltages 3900 V (positive), 2600 V (negative); cycle time 800 ms; dwell time 8–60 ms. Nitrogen provided by Zefiro 40 nitrogen generator (F-DGSi, Evry, France) was used as drying and desolvation gas. Nitrogen 6.0 (99.9999% purity, Linde, Dublin, Ireland) was used as collision gas.

2.3.2. GC–MS/MS

An Agilent 7890B gas chromatographer equipped with an Agilent 7693 automatic sampler and tandem coupled to an Agilent 7010 mass spectrometer (Agilent Technologies, Palo Alto, USA) was employed for the analysis of 126 chemicals. A 1.5 μl aliquot of the sample extract was injected on an ultra-inert glass wool inlet liner in pulsed splitless mode. Inlet temperature was set at 250 °C. The chromatographic separations were performed using two fused silica ultra-inert capillary columns Agilent J&W HP-5MS (crosslinked 5% phenyl-methyl-polysiloxane, Agilent Technologies), each with a length of 15 m, 0.25 mm i.d., and a film thickness of 0.25 μm. The use of two 15-m columns allowed the use of the backflushing technique. Both columns were connected by a Purged Ultimate Union (PUU; Agilent Technologies). Helium (99.999%) was set in constant flow mode as carrier gas, and the flow was adjusted by the retention time lock feature using chlorpyrifos methyl as a reference (reference time = 9.143 min). Nitrogen 6.0 (99,9999% purity, Linde, Dublin, Ireland) was used as collision gas. The oven temperature program was programmed as follows: (a) 80 °C held for 1.8 min; (b) increase to 170 °C at a rate of 40 °C/min; (c) increase to 310 °C at a rate of 10 °C/min to 310 °C; (d) 3 min hold time at 310 °C. The final run time was 21.05 min. Post-run backflush was set at −5.8 ml/min, 315 °C for 5 min. The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode, using 24-time segments. The optimized operating conditions of the mass spectrometer analyses were the following: electron impact (EI) ionization source temperature 280 °C; collision gas flow 1.5 ml/min; transfer line temperature 280 °C; solvent delay 3.7 min; cycle time between 300 and 600 ms; dwell time between 15 and 40 ms.

2.4. Sample preparation

The optimized extraction protocol consisted of the modification and miniaturization of the QuEChERS method (Anastassiades et al., 2003). For the simultaneous extraction of 360 chemicals, the final extraction protocol was as follows: whole blood samples (250 μl) were placed into a 2 ml Eppendorf tube. At this point, the fortification of blank matrix samples for a matrix-matched 12-point calibration curve, was done using different volumes of intermediate fortification solutions for each calibration point. Ten microliters of the mixture of P-ISs, which included compounds used for both, GC (acenaphthene-d10, chlorpyrifos-d10, chrysene-d12, diazinon-d10, PCB 200, and phenanthrene-d10) and LC (atrazine-d5, carbendazim-d3, cyromazine-d4, diazinon-d10, linuron-d3, and pirimicarb-d6) were added to all samples and calibration points to yield a final concentration of 1 ng/ml. The samples were vortex-mixed for 30 s, and placed in an orbital shaker for 1 h, to ensure the adequate dispersion and homogenization of the analytes with the blood components. After that, 500 μl of acidified acetonitrile (1% FA) were added, and the tubes were well vortexed for 30 s. Then, the tubes were placed in an ultrasonic bath (Selecta, Barcelona, Spain) at room temperature for 20 min. After that, anhydrous magnesium sulfate (150 mg) and sodium acetate (37.5 mg) were added to each tube and thoroughly mixed using vortex for 30 s, and then, vigorous-manually shaken for 1 min. Finally, the samples were microcentrifuged (4200 rpm, 5 min) using an ALC 4214 microcentrifuge (A.L.C.

International SRL, Cologno Monzese, Italy). The supernatant (approximately 400 μl) was collected with a 1-ml syringe, passed through a 0.2 μm Chromafil PET-20/15 MS syringe filter (polyester, HPLC certified, Macherey-Nagel, Düren, Germany), and placed in an amber inserted chromatographic vial. This vial was used directly in two consecutive analyses by GC–MS/MS and LC-MS/MS, without the need for further clean-up, dilution or solvent change steps.

2.5. Assay validation procedures

The main objective of the validation was to demonstrate the reliability and performance of the method, developed and applied to the whole blood matrix. Initially, chicken and goat blood were tested separately. Still, since there were no significant differences in the performance of the extraction procedures, the complete validation was done with a mixture of the two types of blood, as recommended (EC, 2019b; SWGTOX, 2013). The method validation was performed using the following parameters: identity, selectivity, linearity (as a working range), accuracy (as bias and precision), carryover, interferences and LOQ. The assessment of the matrix effect was also carried out. In general, we followed the recommendations contained in the SANTE guide (EC, 2019b). Since this guide is mainly aimed at the analysis of pesticides in food and feed samples, we have also taken into account the recommendations contained in the guide of Standard Practices for Method Validation in Forensic Toxicology (SWGTOX, 2013), mainly with regard to the particularities of working with the whole blood matrix and pharmaceuticals. All validation assays involve adding known concentrations of analytes to the matrix. However, given the enormous amount of substances included in the method, the whole blood was not completely free of 100% of the chemicals, in particular the POPs. Therefore, the response of the white matrix sample was subtracted from calibration standards and QC to calculate the response of the analyte added externally. All the details of the final method validation are generally described in the Results and Discussion section. Still, the data for each compound are summarized in Table 1 of the accompanying Data in Brief article entitled "*Supporting dataset on the method validation of micro QuEChERS-based method for the simultaneous biomonitoring in whole blood of 360 toxicologically relevant pollutants for wildlife and results in 148 real samples (Falco tinnunculus and Tyto alba)*".

2.6. Statistical analysis

Both, within-run and between-run precisions, were calculated using the one-way ANOVA approach with the run number (usually $n = 5$) as the grouping variable. The ANOVA calculations were done using the GraphPad Prism v6.0 (GraphPad Software, CA, USA).

3. Results and discussion

3.1. Optimization of MS/MS conditions and chromatography

The mass spectrometry conditions were optimized for the detection and quantification of 360 compounds (234 by LC-MS/MS and 126 by GC–MS/MS).

3.1.1. LC-MS/MS

For the mass spectrometry optimization of each compound analyzed by LC-MS/MS, individual chromatographic vials with a concentration of around 100–200 ng/ml were prepared. The mobile phase conditions were established based on the literature and methodologies previously developed in our laboratory (Luzardo et al., 2015; Luzardo et al., 2013; Luzardo et al., 2014c; Ruiz-Suarez et al., 2015; Ruiz-Suarez et al., 2014). A stainless steel zero dead volume union was used to replace the chromatographic column. The optimization of the precursor ion signal and the product ion signal was conducted manually, as well as the optimization of the fragmentation and collision energy. The best combination of two MRM transitions was selected for each compound (Table 1). Once the list of transitions for all target compounds and P-ISs was completed, the optimization steps for gas temperature, gas flow, nebulizer gas pressure, sheath gas flow and temperature, capillary voltage (+/−), and nozzle voltage for the AJS-ESI, were performed sequentially using the Mass Hunter Source Optimizer software (Agilent Technologies, Palo Alto, USA).

During the early stage of the chromatographic method development, the suitability and performance of the two different columns were assessed. Both columns are reversed-phased but with slightly different specifications. The first column tested was the ZORBAX Eclipse Plus C18 (2.1 mm × 50 mm, 1.8 μm), that produced a very broad peak with severe peak tailing for many target analytes. The second column, which we routinely use, the Agilent InfinityLab Poroshell 120 (2.1 mm × 100 mm, 2.7 μm), showed an exceptional narrow peak for nearly all target analytes based on the shape of a Gaussian peak. In addition, we tested two different chromatography conditions (mobile phase and gradient) based on our previous experience on the analysis of pesticides and ARs by LC-MS/MS. Condition A: 0.1% FA and 2 mM ammonium formate in both, water and methanol; condition B: 2 mM ammonium acetate in both, water and methanol. We observed that FA was not optimum for ARs, which performed better in the absence of acid. However, FA proved to be necessary for the analysis of many pesticides, so a compromise was adopted, and we continue to use the acid. The different experiments showed that a sufficient degree of ionization for the analysis of all the compounds (positive/negative), was obtained at 2 mM ammonium acetate (water and methanol), with an optimal percentage of 0.1% formic acid in water, nor in methanol. In the last step, a series of experiments were conducted to select the optimal volume of injection, which was finally set in 8 μl.

3.1.2. GC–MS/MS

Acquisition method for GC–MS/MS compounds was initially supplied by Agilent, but it was further optimized in our laboratory. A sequence of injections was programmed to determine the optimal collision energies in increments of 5 eV (range from 5 to 60 eV); the results of this optimization are also reflected in Table 1, where the analytical parameters of the complete list of chemicals (in alphabetical order) has been summarized. Dwell time and cycle time were also optimized. In GC, no optimization was made in relation to the column type or the temperature program, since our group had previous experience on the separation of many of these compounds or very similar combinations (Bucchia et al., 2015; Luzardo et al., 2015; Luzardo et al., 2014c).

However, since ACN extracts are injected in this method, we did carry out a series of experiments to optimize the solvent delay time, the initial temperature of the oven (60 to 90, in increments of 10 °C), the temperature of the injector (range of 230 to 300 °C, in increments of 10 °C), the temperature of the ionization source (range of 250 to 320 °C, in increments of 10 °C), the temperature of the transfer line (from 270 to 320 °C, in increments of 10 °C), and the injection volume (from 0.8 to 1.8 μl, in increments of 0.2 μl). These experiments were performed by injecting a mixture of all the analytes in acetonitrile at two concentrations (1 ng/ml and 50 ng/ml), and the parameters that gave the best shape and peak intensity for most of the compounds were selected.

3.2. Optimization of sample preparation

The previous experience of our laboratory in the development and application of multi-residue methods made us opt for the QuEChERS method. Two widely available modifications of this method were compared (3 replicates at two concentrations (2–20 ng/ml), analyzed in duplicate): the AOAC Official Method 2007.01 (Lehotay et al., 2010); and the UNE-EN 15662:2019 Official Method (EN, 2019). Both methodologies employ acetonitrile as the extraction solvent. We decided not to test others as acetonitrile has proved to be the solvent with the highest

Table 1
List of compounds analyzed in whole blood together with the category of use, legal status, the technique employed, and the instrumental conditions of the optimized methods.

No.	Compound	Category[a]	Legal status in the EU[b]	Subjected to MRL[c]	Technique[d]	Retention time (min)	LOQ (ng/ml)	Polarity	Quantification		Confirmation		Fragmentor voltage (V)
									MRM transition (m/z)	Collision energy (eV)	MRM transition (m/z)	Collision energy (eV)	
1	2-Phenylphenol	F	Approved	Yes	GC	6.28	0.20	Positive	169.0 → 115.0	30	169.0 → 141.0	15	70
2	4,4′-Dichlorobenzophenone (metabolite of dicofol)	Met	–	No	GC	9.99	0.80	Positive	250.0 → 139.0	15	250.0 → 215.0	5	70
3	Abamectine	I, A, AH	Approved	Yes	LC	10.99	4.00	Positive	890.5 → 567.1	10	895.5 → 751.4	45	160
4	Acenaphthene	POP	–	No	GC	6.15	0.20	Positive	153.0 → 152.0	25	153.0 → 151.0	35	70
5	Acenaphtylene	POP	–	No	GC	5.94	0.20	Positive	152.0 → 151.0	25	152.0 → 126.0	30	70
6	Acephate	I	Not approved	Yes	LC	1.64	2.00	Positive	184.0 → 143.0	15	143.0 → 95.0	15	70
7	Acetaminophen (paracetamol)	V, NSAID	Approved	–	LC	2.71	1.20	Positive	152.1 → 65.0	40	152.1 → 93.0	20	150
8	Acetamiprid	I	Approved	Yes	LC	4.43	0.40	Positive	223.1 → 126.0	27	223.1 → 90.0	45	140
9	Acrinathrin	I, A	Approved	Yes	GC	10.70	1.20	Positive	559.0 → 208.0	10	559.0 → 181.0	30	70
10	Albendazole	V, AH	Approved	–	LC	7.14	0.10	Positive	266.1 → 234.1	16	266.1 → 191.0	32	155
11	Aldicarb	I	Not approved	Yes	LC	5.11	0.10	Positive	208.0 → 116.0	10	116.0 → 89.1	4	100
12	Aldicarb-sulfone	Met	–	Yes	LC	3.21	0.40	Positive	240.1 → 76.0	16	223.1 → 86.1	13	75
13	Aldicarb-sulfoxide	Met	–	Yes	LC	2.75	1.60	Positive	207.1 → 131.9	10	207.1 → 89.1	10	86
14	Aldrin	POP	–	Yes	GC	9.90	0.40	Positive	255.0 → 220.0	25	263.0 → 228.0	10	70
15	Anthracene	POP	–	No	GC	8.40	0.80	Positive	178.0 → 176.0	35	178.0 → 152.0	30	70
16	Atrazine	H	Not approved	No	LC	6.73	0.10	Positive	216.0 → 173.9	15	216.0 → 103.8	30	130
17	Azinphos-methyl	I	Not approved	Yes	LC	7.27	0.20	Positive	318.0 → 132.1	8	340.0 → 160.0	10	60
18	Azoxystrobin	F	Approved	Yes	LC	7.59	0.10	Positive	404.1 → 372.1	8	404.1 → 344.1	24	110
19	BDE-28	POP	–	No	GC	12.22	0.20	Positive	406.0 → 246.0	20	406.0 → 167.0	25	70
20	BDE-47	POP	–	No	GC	14.31	0.20	Positive	326.0 → 138.0	45	484.0 → 324.0	25	70
21	BDE-85	POP	–	No	GC	17.08	0.10	Positive	564.0 → 404.0	25	566.0 → 406.0	25	70
22	BDE-99	POP	–	No	GC	16.27	0.10	Positive	566.0 → 406.0	25	564.0 → 404.0	30	70
23	BDE-100	POP	–	No	GC	15.85	0.10	Positive	566.0 → 406.0	25	564.0 → 404.0	25	70
24	BDE-153	POP	–	No	GC	18.04	0.20	Positive	644.0 → 484.0	25	486.0 → 377.0	30	70
25	BDE-154	POP	–	No	GC	17.47	0.10	Positive	644.0 → 484.0	25	486.0 → 377.0	30	70
26	BDE-183	POP	–	No	GC	20.12	0.20	Positive	561.6 → 454.7	40	563.6 → 454.7	40	70
27	Benalaxyl	F	Approved	No	LC	8.96	0.10	Positive	326.2 → 148.0	20	326.2 → 208.0	12	90
28	Bendiocarb	I	Not approved	No	LC	5.88	0.10	Positive	224.1 → 166.9	8	224.2 → 108.9	15	120
29	Bendiocarb metabolite (2, 2-dimethylbenzo-1, 3-dioxol-4-ol)	Met	–	No	GC	4.84	1.20	Positive	166.0 → 151.0	10	166.0 → 126.0	20	70
30	Benfuracarb	I, AH	Not approved	No	LC	9.73	0.10	Positive	411.2 → 190.0	13	411.2 → 252.0	15	110
31	Benzo[a]anthracene	POP	–	No	GC	13.95	0.80	Positive	228.0 → 226.0	40	228.0 → 202.0	35	70
32	Benzo[a]pyrene	POP	–	No	GC	16.89	0.10	Positive	252.0 → 250.0	45	252.0 → 248.0	60	70
33	Benzo[b]fluoranthene	POP	–	No	GC	16.30	0.80	Positive	252.0 → 248.0	60	252.0 → 226.0	35	70
34	Benzo[ghi]perylene	POP	–	No	GC	19.61	0.40	Positive	276.0 → 274.0	50	276.0 → 272.0	60	70
35	Benzo[k]fluoranthene	POP	–	No	GC	16.29	0.40	Positive	252.0 → 250.0	45	252.0 → 224.0	40	70
36	Bifenthrin	I	Not approved	Yes	GC	11.25	0.20	Positive	440.0 → 181.0	5	440.0 → 165.0	60	94
37	Bitertanol	F	Not approved	Yes	LC	9.23	0.40	Positive	338.2 → 70.0	4	338.2 → 269.2	5	100
38	Boscalid (formerly nicobifen)	F	Approved	Yes	GC	7.84	0.10	Positive	3434.0 → 272.0	30	343.0 → 140.0	45	100
39	Brodifacoum	R	Not approved	No	LC	10.78	0.80	Negative	521.3 → 79.0	50	523.3 → 135.0	45	220
40	Bromadiolone	R	Approved	No	LC	9.75	0.40	Negative	525.3 → 250.0	40	527.3 → 250.0	40	200
41	Bromopropylate	A	Not	Yes	GC	13.87	0.20	Positive	341.0 → 183.0	15	341.0 → 157.0	45	70

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Table 1 (*continued*)

No.	Compound	Category[a]	Legal status in the EU[b]	Subjected to MRL[c]	Technique[d]	Retention time (min)	LOQ (ng/ml)	Polarity	Quantification		Confirmation		Fragmentor voltage (V)
									MRM transition (m/z)	Collision energy (eV)	MRM transition (m/z)	Collision energy (eV)	
			approved										
42	Bromuconazole (two isomers)	F	Approved	No	GC	13.81/14.24	0.20	Positive	295.0 → 173.0	10	295.0 → 175.0	10	70
43	Bupirimate	F	Approved	Yes	GC	11.78	0.20	Positive	273.0 → 108.0	15	273.0 → 193.0	5	70
44	Buprofezin	I	Approved	Yes	LC	9.83	0.10	Positive	306.1 → 201.0	12	306.1 → 116.0	12	140
45	Cadusafos (ebufos)	I, AH	Not approved	No	LC	9.39	0.10	Positive	271.1 → 159.0	16	271.1 → 131.0	22	100
46	Carbaryl	I	Not approved	Yes	LC	6.21	0.10	Positive	202.1 → 145.1	4	202.1 → 127.1	28	95
47	Carbendazim (azole)	F	Not approved	Yes	LC	2.90	0.40	Positive	192.1 → 160.1	4	202.1 → 127.1	28	90
48	Carbofuran	I, AH	Not approved	Yes	LC	5.91	0.10	Positive	222.1 → 123.1	20	222.1 → 165.1	30	80
49	Carbofuran-3-hydroxy	Met	–	Yes	LC	4.27	0.40	Positive	238.1 → 163.1	10	238.1 → 181.1	10	110
50	Carbosulfan	I, AH	Not approved	No	LC	11.03	0.40	Positive	381.2 → 160.2	12	381.2 → 76.1	36	120
51	Cefuroxima axetil (two isomers)	V, MB	Not approved	–	LC	5.13	0.80	Positive	533.0 → 447.0	15	533.0 → 386.0	20	160
52	Chloramphenicol	V, MB	Approved	–	LC	4.63	2.00	Negative	321.0 → 152.1	4	323.0 → 152.1	4	113
53	Chlorantraniliprole	I	Approved	Yes	LC	7.32	0.20	Positive	483.9 → 452.9	16	483.9 → 285.9	8	105
54	Chlorfenapyr	I, A	Not approved	Yes	GC	12.01	1.20	Positive	247.0 → 200.0	30	247.0 → 227.0	15	70
55	Chlorfenvinphos	I	Not approved	No	LC	9.09	0.20	Positive	361.1 → 98.9	34	358.9 → 155.1	8	105
56	Chlorobenzilate	A	Not approved	No	GC	12.14	0.40	Positive	251.0 → 111.0	40	251.0 → 139.0	15	70
57	Chlorophacinone	R	Not approved	No	LC	8.88	0.80	Negative	373.2 → 201.0	20	375.2 → 203.0	20	160
58	Chlorpropham	H	Not approved	Yes	GC	7.13	0.20	Positive	213.0 → 127.0	15	153.0 → 90.0	25	70
59	Chlorpyrifos	I	Not approved	Yes	GC	9.93	0.80	Positive	314.0 → 258.0	15	314.0 → 286.0	5	70
60	Chlorpyrifos methyl	I	Not approved	Yes	GC	9.12	0.40	Positive	286.0 → 93.0	25	286.0 → 271.0	15	70
61	Chlorthal dimethyl	H	Not approved	No	GC	10.02	0.20	Positive	300.9 → 166.9	55	300.9 → 222.9	25	70
62	Chrysene	POP	–	No	GC	13.86	0.80	Positive	228.0 → 226.0	40	228.0 → 227.0	25	70
63	Clindamycin	V, MB	Approved	–	LC	5.33	0.40	Positive	425.2 → 126.1	20	425.2 → 377.2	20	150
64	Clofentezine	A	Approved	Yes	LC	9.19	0.40	Positive	303.1 → 138.0	12	303.1 → 102.0	40	120
65	Clothianidin	I	Not approved	Yes	LC	3.91	1.20	Positive	250.0 → 169.0	8	250.0 → 131.9	8	100
66	Cloxacillin	V, MB	Approved	–	LC	6.86	1.60	Positive	436.1 → 160.0	8	436.1 → 277.0	12	126
67	Cortiscosterone	V, GC	Not approved	–	LC	7.89	0.80	Positive	389.1 → 329.0	13	389.1 → 371.0	13	80
68	Coumachlor	R	Not approved	No	LC	8.63	0.20	Positive	343.1 → 162.8	15	342.1 → 285.0	15	120
69	Coumaphos	I, A	Not approved	No	LC	8.98	0.10	Positive	363.0 → 227.0	30	363.0 → 306.9	15	120
70	Coumatetralyl	R	Not approved	No	LC	8.31	0.40	Negative	291.1 → 141.0	30	291.1 → 247.0	20	140
71	Cyazofamid	F	Approved	Yes	LC	8.49	0.80	Positive	325.0 → 108.0	20	325.0 → 261.1	15	90
72	Cyflufenamid	F	Approved	Yes	LC	9.18	0.20	Positive	413.1 → 223.1	33	413.1 → 295.1	23	70
73	Cyfluthrin (sum of four isomers)	I	Not approved[e]	Yes	GC	16.07/16.19/16.25/16.32	1.20	Positive	226.0 → 206.0	25	198.9 → 170.1	25	70

74	Cyhalothrin (lambda isomer)	I	Approved	Yes	LC	10.49	2.00	Positive	467.0 → 225.0	10	467.0 → 141.0	46	66
75	Cymoxanil	F	Approved	Yes	LC	4.67	0.40	Positive	199.1 → 128.0	4	199.1 → 110.9	12	90
76	Cypermethrin (sum of four isomers)	I	Approved[f]	Yes	GC	16.34/16.44/16.52/16.63	4.00	Positive	163.0 → 109.0	20	163.0 → 127.0	5	70
77	Cyproconazole (two isomers)	F	Approved	Yes	LC	8.14	0.40	Positive	292.2 → 70.2	18	292.2 → 125.1	24	100
78	Cyprodinil	F	Approved	Yes	LC	8.46	0.20	Positive	226.0 → 93.0	33	226.0 → 108	25	100
79	Cyromazine	I, A	Not approved	Yes	LC	1.23	2.00	Positive	167.1 → 85.0	16	167.1 → 125.0	20	120
80	Danofloxacin	V, MB	Approved	–	LC	4.04	1.20	Positive	358.2 → 340.1	20	358.2 → 82.1	50	159
81	Dazomet	I, A, AH, F, H	Approved	No	GC	7.80	1.60	Positive	161.9 → 44.0	28	161.9 → 89.0	5	70
82	Deltamethrin	I, A	Approved	Yes	LC	10.65	0.80	Positive	523.0 → 281.0	10	523.0 → 506.0	5	100
83	Demeton-*S*-methyl	I, A	Not approved	No	LC	5.97	0.10	Positive	230.9 → 88.9	5	230.9 → 61.0	30	50
84	Demeton-*S*-methyl-sulfone (Dioxydemeton)	I, A	Not approved	No	LC	3.31	0.40	Positive	263.0 → 169.0	24	263.0 → 109.0	12	120
85	Dexamethasone	V, GC	Approved	–	LC	7.16	0.40	Positive	393.2 → 373.2	2	393.2 → 355.2	6	103
86	Diazinon	I	Not approved	Yes	GC	8.29	0.40	Positive	137.1 → 54.0	20	304.0 → 179.0	15	70
87	Dibenzo[*a*,*h*]anthracene	POP	–	No	GC	19.15	0.40	Positive	278.0 → 276.0	40	278.0 → 250.0	60	70
88	Dichlorodiphenyldichloroethane (p,p′ DDD)	POP	–	Yes	GC	12.31	0.10	Positive	235.0 → 165.0	20	235.0 → 199.0	15	70
89	Dichlorodiphenyldichloroethylene (p,p′ DDE)	POP	–	Yes	GC	11.58	0.10	Positive	318.0 → 176.0	60	318.0 → 248.0	30	70
90	Dichlorodiphenyltrichloroethane (p,p′ DDT)	POP	–	Yes	GC	12.84	1.20	Positive	235.0 → 165.0	40	235.0 → 199.0	15	70
91	Diclofenac	V, NSAID	Approved	–	LC	8.73	0.80	Positive	296.0 → 215.1	16	296.0 → 214.1	48	103
92	Dicloran	F, MB, WP	Not approved	Yes	GC	7.80	0.10	Positive	206.0 → 176.0	10	206.0 → 148.0	25	70
93	Dicloxacillin	V, MB	Not approved	–	LC	7.24	1.20	Positive	470.0 → 160.0	8	470.0 → 310.8	10	106
94	Dieldrin	POP	–	Yes	GC	11.66	1.20	Positive	263.0 → 228.0	15	277.0 → 241.0	15	70
95	Diethathyl ethyl	H	Not approved	No	LC	8.71	0.20	Positive	312.2 → 238.1	15	312.2 → 162.0	30	120
96	Diethofencarb	F, MB, WP	Approved	Yes	LC	7.57	0.10	Positive	268.2 → 226.1	5	268.2 → 152.0	20	110
97	Difenacoum	R	Not approved	No	LC	10.38	0.40	Negative	443.2 → 135.0	40	443.2 → 293.0	35	200
98	Difenoconazole	F, MB, WP	Approved	Yes	LC	9.41	0.40	Positive	406.1 → 250.9	28	406.1 → 337.0	16	176
99	Difethialone	R	Not approved	No	LC	10.93	0.80	Negative	537.3 → 79.0	50	537.3 → 151.0	45	220
100	Difloxacin	V, MB	Not approved	–	LC	3.86	0.80	Positive	400.2 → 382.1	20	400.2 → 356.1	16	149
101	Diflubenzuron	I	Approved	Yes	LC	8.63	1.20	Positive	311.0 → 158.0	8	311.0 → 141.0	32	90
102	Diflufenican	H	Approved	No	LC	9.51	0.10	Positive	395.1 → 266.0	24	395.1 → 246.0	36	150
103	Dimethenamid-P (and its R-isomer)	H	Approved	No	LC	7.68	0.10	Positive	276.1 → 244.1	10	276.1 → 168.1	20	125
104	Dimethoate	I	Not approved	Yes	LC	4.21	0.40	Positive	230.0 → 125.0	16	230.0 → 198.8	20	70
105	Dimethomorph (two isomers)	F, MB, WP	Approved	Yes	LC	7.86	0.40	Positive	388.1 → 301.1	20	388.1 → 165.1	32	180
106	Dimethylphenylsulfamide (DMSA, metabolite of dichlofluanid)	Met[h]	–	No	LC	5.21	0.80	Positive	201.1 → 92.1	15	201.1 → 137.1	5	100
107	Diniconazole-M	F, MB, WP	Not approved	Yes	LC	9.34	0.20	Positive	326.1 → 70.0	28	328.1 → 70.0	28	110
108	Dinocap	F, MB, WP	Not approved	No	LC	10.51	0.80	Negative	295.4 → 208.9	30	295.4 → 193.0	35	150
109	Diphacinone	R	Not approved	No	LC	8.60	1.20	Negative	339.1 → 167.0	25	339.1 → 145.0	20	170
110	Diphenylamine	PHP	Not approved	Yes	GC	6.98	0.20	Positive	168.0 → 167.2	15	169.0 → 66.0	15	70
111	Dodine	F, MB, WP	Approved	Yes	LC	9.02	0.40	Positive	228.3 → 43.0	40	228.3 → 57.0	25	150

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Table 1 (*continued*)

No.	Compound	Category[a]	Legal status in the EU[b]	Subjected to MRL[c]	Technique[d]	Retention time (min)	LOQ (ng/ml)	Polarity	Quantification		Confirmation		Fragmentor voltage (V)
									MRM transition (m/z)	Collision energy (eV)	MRM transition (m/z)	Collision energy (eV)	
112	Endosulfan alfa	POP	–	Yes	GC	11.21	0.80	Positive	241.0 → 206.0	15	195.0 → 160.0	10	70
113	Endosulfan beta	POP	–	Yes	GC	12.21	0.80	Positive	241.0 → 206.0	15	195.0 → 159.0	15	70
114	Endosulfan sulfate	POP	–	No	GC	12.96	0.80	Positive	270.0 → 235.0	15	387.0 → 289.0	5	70
115	Endrin	POP	–	No	GC	12.05	1.60	Positive	263.0 → 193.0	35	245.0 → 173.0	25	70
116	Enrofloxacin	V, MB	Approved	–	LC	3.94	1.20	Positive	360.2 → 316.1	16	360.2 → 245.1	28	144
117	EPN	I, A	Not approved	No	GC	13.90	0.80	Positive	157.0 → 63.0	10	157.0 → 110.0	15	70
118	Epoxiconazole	F	Approved	Yes	LC	8.47	0.20	Positive	330.0 → 120.9	24	330.1 → 100.9	50	120
119	Eprinomectin	V, MB	Approved	–	LC	10.84	0.20	Positive	878.5 → 186.0	15	936.5 → 490.4	60	160
120	Eritromicin	V, MB	Approved	–	LC	6.74	0.20	Positive	734.5 → 158.1	32	734.5 → 576.3	16	172
121	Esfenvalerate	I	Approved	No	GC	17.56	2.00	Positive	167.1 → 125.1	15	167.1 → 89.1	45	70
122	Ethion (diethion)	I, A	Not approved	Yes	LC	10.03	0.10	Positive	385.0 → 199.0	5	385.0 → 171.0	10	100
123	Ethirimol	F, MB, WP	Not approved	Yes	LC	4.80	0.40	Positive	210.2 → 140.1	20	210.2 → 98.1	28	160
124	Ethofumesate	H	Approved	No	GC	9.59	0.80	Positive	286.0 → 207.0	5	286.0 → 161.0	20	70
125	Ethoprophos	I, AH	Not approved	No	LC	8.38	0.20	Positive	243.1 → 97.0	30	243.1 → 130.9	15	90
126	Etofenprox	I, A	Approved	Yes	GC	16.75	0.80	Positive	163.0 → 107.0	20	163.0 → 135.0	10	70
127	Etoxazole	A	Approved	Yes	LC	10.34	0.10	Positive	360.1 → 141.0	26	360.1 → 304.0	16	160
128	Famoxadone	H	Approved	Yes	LC	9.07	1.20	Positive	392.1 → 330.9	5	392.2 → 238.1	12	110
129	Fenamidone	F	Not approved	Yes	LC	9.06	0.10	Positive	392.1 → 330.9	5	392.1 → 238.1	12	110
130	Fenamiphos	I, AH	Approved	Yes	LC	7.72	0.10	Positive	304.1 → 217.1	20	304.1 → 202.0	36	120
131	Fenamiphos sulfone	Met	–	Yes	LC	8.63	0.20	Positive	336.1 → 188.0	31	336.1 → 266.0	23	120
132	Fenamiphos sulfoxide	Met	–	Yes	LC	5.93	0.40	Positive	320.1 → 233.0	20	320.1 → 108.1	44	120
133	Fenarimol	F, MB, WP	Not approved	Yes	GC	15.03	0.20	Positive	139.0 → 75.0	30	139.0 → 111.0	15	70
134	Fenazaquin	A	Approved	Yes	LC	10.73	0.80	Positive	307.2 → 57.1	25	307.2 → 161.1	16	90
135	Fenbendazole	V, AH	Approved	–	LC	8.04	0.10	Positive	300.1 → 268.1	20	300.1 → 159.0	36	156
136	Fenbuconazole	F, V	Approved	Yes	GC	16.17	0.40	Positive	198.0 → 102.0	30	198.0 → 78.0	30	70
137	Fenbutatin oxide	I, A	Not approved	Yes	LC	11.67	0.80	Positive	519.0 → 197.0	55	517.3 → 194.9	60	180
138	Fenhexamid	F	Approved	Yes	LC	8.35	1.60	Positive	302.1 → 97.1	20	302.1 → 55.1	40	130
139	Fenitrothion	I	Not approved	Yes	GC	9.57	0.20	Positive	277.0 → 109.0	15	277.0 → 125.0	15	70
140	Fenoxycarb	I	Approved	Yes	LC	8.69	0.10	Positive	302.1 → 88.0	20	302.1 → 116.1	10	110
141	Fenpropathrin	I, A	Not approved	Yes	LC	10.43	0.40	Positive	367.2 → 125.0	16	350.1 → 125.0	16	72
142	Fenpropidin	F	Approved	Yes	LC	7.13	0.10	Positive	274.3 → 147.0	30	274.3 → 86.0	25	170
143	Fenpropimorph	F	Not approved	Yes	LC	7.37	0.10	Positive	304.3 → 147.1	30	304.3 → 130.0	25	120
144	Fenpyroximate	A	Approved	Yes	LC	10.49	0.40	Positive	422.2 → 366.2	12	422.2 → 135.0	36	160
145	Fenthion	I, A	Not approved	Yes	LC	8.90	0.10	Positive	278.9 → 168.8	18	278.9 → 247.0	8	98
146	Fenthion oxon	Met	–	Yes	LC	7.31	0.10	Positive	263.1 → 231.2	16	263.1 → 216.0	24	120
147	Fenthion oxon sulfone	Met	–	Yes	LC	4.50	0.80	Positive	295.0 → 217.0	15	295.0 → 104.2	24	110
148	Fenthion oxon sulfoxide	Met	–	Yes	LC	4.26	0.20	Positive	279.0 → 264.2	20	279.0 → 104.1	28	110
149	Fenthion sulfone	Met	–	Yes	LC	6.39	0.80	Positive	311.0 → 125.0	22	311.0 → 109.0	28	140
150	Fenthion sulfoxide	Met	–	Yes	LC	6.16	0.40	Positive	295.0 → 108.9	30	295.0 → 280.0	18	140
151	Fenvalerate	I	Not approved	Yes	GC	17.36	2.00	Positive	167.0 → 125.1	22	167.0 → 89.0	30	70
152	Fipronil	I, V	Not	Yes	LC	8.68	0.20	Negative	435.0 → 330.0	12	435.0 → 249.9	26	116

			approved										
153	Fipronil sulfide	Met	–	Yes	GC	10.49	0.80	Positive	351.0 → 255.0	20	420.0 → 351.0	25	70
154	Flocoumafen	R	Not approved	No	LC	10.44	0.20	Negative	541.3 → 382.0	25	541.3 → 161.0	40	230
155	Fluazinam	F	Approved	No	LC	10.01	0.20	Negative	462.9 → 416.0	10	462.9 → 398.0	9	140
156	Flubendiamide	I	Approved	Yes	LC	8.82	2.00	Positive	408.0 → 274.0	15	408.0 → 256.0	30	120
157	Flucythrinate (two isomers)	I, A	Not approved	No	GC	16.67/16.84	0.80	Positive	156.9 → 107.1	15	199.1 → 107.1	25	70
158	Fludioxonil	F	Approved	Yes	GC	11.51	0.20	Positive	248.0 → 127.0	30	248.1 → 182.1	10	70
159	Flufenoxuron	I, A	Not approved	Yes	LC	10.37	0.10	Positive	489.1 → 158.0	20	489.1 → 140.9	56	110
160	Flumequine	V, MB	Approved	–	LC	6.12	0.10	Positive	262.1 → 244.0	16	262.1 → 202.0	32	116
161	Flunixin	V, NSAID	Approved	–	LC	8.09	0.20	Positive	297.1 → 279.1	24	297.1 → 264.1	32	141
162	Fluopyram	F	Approved	Yes	GC	10.61	0.20	Positive	173.0 → 95	35	223.0 → 196.0	40	70
163	Fluoranthene	POP	–	No	GC	10.66	0.20	Positive	202.0 → 201.0	27	202.0 → 152.0	42	70
164	Fluorene	POP	–	No	GC	6.81	0.20	Positive	165.0 → 163.0	40	165.0 → 139.0	30	70
165	Fluquinconazole	F	Approved	Yes	GC	15.81	0.20	Positive	340.0 → 298.0	15	340.0 → 286.0	25	70
166	Flusilazole	F, MB, WP	Not approved	Yes	LC	8.64	0.20	Positive	316.1 → 247.1	15	316.1 → 165.0	20	160
167	Flutolanil	F, MB, WP	Approved	No	LC	7.93	0.10	Positive	324.1 → 262.1	16	324.1 → 242.1	24	130
168	Flutriafol	F	Approved	Yes	GC	11.26	0.20	Positive	219.0 → 95.0	35	219.0 → 123.0	15	70
169	Fluvalinate tau	I, A	Approved	Yes	GC	17.56	4.00	Positive	250.1 → 55.1	30	252.0 → 200.0	20	70
170	Fonofos	I	Not approved	No	GC	8.24	0.40	Positive	246.0 → 109.0	15	246.0 → 237.0	5	70
171	Formetanate	I, A	Approved	Yes	LC	1.76	0.10	Positive	222.1 → 165.1	12	222.1 → 46.2	28	105
172	Fosthiazate	AH, V	Approved	Yes	LC	6.50	0.10	Positive	284.0 → 104.0	20	284.0 → 227.8	8	90
173	Heptachlor	POP	–	Yes	GC	9.31	0.80	Positive	272.0 → 237.0	15	274.0 → 239.0	15	70
174	Hexachlorobencene	POP	–	Yes	GC	7.77	0.20	Positive	284.0 → 214.0	40	284.0 → 249.0	25	70
175	Hexachlorocyclohexane (alpha)	POP	–	Yes	GC	7.64	0.40	Positive	219.0 → 109.0	10	219.0 → 183.0	10	70
176	Hexachlorocyclohexane (beta)	POP	–	Yes	GC	8.02	0.40	Positive	219.0 → 109.0	40	219.0 → 183.0	5	70
177	Hexachlorocyclohexane (delta)	POP	–	No	GC	8.50	0.20	Positive	219.0 → 109.0	45	219.0 → 183.0	5	70
178	Hexaclorocyclohexane (gamma, lindane)	POP	–	Yes	GC	8.13	1.20	Positive	291.0 → 109.0	40	219.0 → 183.0	10	70
179	Hexaconazole (two isomers)	F, MB, WP	Not approved	Yes	LC	8.49	0.80	Positive	314.1 → 70.1	20	316.0 → 70.1	20	95
180	Hexaflumuron	I	Not approved	No	LC	9.58	0.40	Negative	458.8 → 439.0	8	458.8 → 175.0	30	100
181	Hexythiazox	A	Approved	Yes	LC	10.18	0.10	Positive	353.1 → 227.9	8	353.1 → 168.1	24	120
182	Imazalil (enilconazole)	F, MB, WP, V	Approved	Yes	LC	6.53	0.40	Positive	297.1 → 159.0	20	297.1 → 69.1	18	100
183	Imidacloprid	I	Approved	Yes	LC	3.93	0.80	Positive	256.0 → 175.0	12	256.0 → 209.0	12	110
184	Indeno [1,2,3-cd] pyrene	POP	–	No	GC	19.08	0.40	Positive	276.0 → 274.0	50	276.0 → 272.0	60	70
185	Indoxacarb	I	Approved	Yes	LC	9.49	0.20	Positive	528.1 → 293.1	10	528.1 → 202.8	48	140
186	Iprodione	F, MB, WP	Not approved	Yes	GC	13.67	4.00	Positive	314.0 → 56.0	20	314.0 → 245.0	10	70
187	Iprovalicarb	F	Approved	Yes	LC	8.18	0.20	Positive	321.2 → 119.0	15	321.2 → 202.9	20	110
188	Isocarbophos	I	Not approved	Yes	GC	10.04	1.60	Positive	230.0 → 155.0	25	230.0 → 198.0	10	70
189	Isofenphos methyl	I	Not approved	No	GC	10.38	0.40	Positive	199.0 → 121.0	10	241.0 → 121.0	25	70
190	Isoprothiolane	F, MB, WP	Not approved	Yes	LC	7.94	0.10	Positive	291.1 → 189.0	30	291.1 → 145.0	36	100
191	Ivermectin B1a	V, AH, A	Approved	–	LC	11.52	1.60	Positive	897.5 → 753.5	50	897.5 → 329.3	60	160
192	Josamycin	V, MB	Not approved	–	LC	7.40	0.40	Positive	860.5 → 173.9	40	860.5 → 108.9	40	200
193	Ketoprofen	V, NSAID	Approved	–	LC	7.34	0.40	Positive	255.1 → 209.1	8	255.1 → 77.1	48	123
194	Kresoxim methyl	F	Approved	Yes	GC	11.78	1.20	Positive	116.0 → 89.0	15	206.0 → 131.0	10	70
195	Leptophos	I	Not approved	No	GC	14.58	0.80	Positive	171.0 → 77.1	15	377.0 → 362.0	20	70

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Table 1 (*continued*)

No.	Compound	Category[a]	Legal status in the EU[b]	Subjected to MRL[c]	Technique[d]	Retention time (min)	LOQ (ng/ml)	Polarity	Quantification		Confirmation		Fragmentor voltage (V)
									MRM transition (m/z)	Collision energy (eV)	MRM transition (m/z)	Collision energy (eV)	
196	Levamisole	V, AH	Approved	–	LC	3.12	0.20	Positive	205.1 → 178.1	20	205.1 → 123.0	32	141
197	Lincomycin	V, MB	Approved	–	LC	3.50	0.40	Positive	407.2 → 126.1	24	407.2 → 359.2	16	150
198	Linuron	F	Approved	Yes	LC	7.54	0.20	Positive	249.0 → 160.1	20	249.0 → 182.3	8	120
199	Lufenuron	I	Not approved	Yes	LC	10.05	0.40	Negative	509.0 → 339.0	5	509.0 → 326.1	15	90
200	Malaoxon	I	Not approved	No	LC	6.03	0.10	Positive	315.1 → 127.2	12	315.1 → 99.1	36	120
201	Malathion	I	Not approved	Yes	LC	7.93	0.20	Positive	348.0 → 126.7	15	348.0 → 285.0	8	100
202	Mandipropamid	F	Approved	Yes	LC	7.90	0.10	Positive	412.1 → 328.1	8	412.1 → 356.1	4	130
203	Marbofloxacin	V, MB	Approved	–	LC	3.53	2.00	Positive	363.2 → 72.1	25	363.2 → 320.1	15	134
204	Mebendazole	V, AH	Approved	–	LC	6.68	0.10	Positive	296.1 → 264.1	20	296.1 → 77.0	48	151
205	Mefenamic acid	V, NSAID	Not approved	–	LC	9.52	0.40	Positive	242.1 → 209.1	28	242.1 → 180.1	0	108
206	Mefenoxam (metalaxyl-M)	F	Approved	Yes	LC	6.95	0.10	Positive	280.0 → 220.0	10	280.0 → 192.0	15	110
207	Meloxicam	V, NSAID	Approved	–	LC	7.17	0.20	Positive	352.5 → 114.8	20	352.5 → 140.8	20	130
208	Mepanipyrim	F, MB, WP	Approved	Yes	GC	11.13	0.40	Positive	222.0 → 221.0	15	222.0 → 207.0	15	70
209	Mepiquat	H	Approved	Yes	LC	0.64	0.40	Positive	114.0 → 98.0	36	114.0 → 70.0	45	100
210	Metaflumizone	I	Approved	No	LC	9.94	0.20	Negative	505.0 → 302.0	14	541.0 → 302.0	20	90
211	Metaldehyde	M	Approved	No	LC	3.87	4.00	Positive	194.1 → 61.9	5	194.1 → 106.0	5	50
212	Metconazole	F	Approved	No	LC	9.17	0.10	Positive	320.1 → 70.2	33	322.1 → 70.2	24	250
213	Methamidophos (two isomers)	I, A	Not approved	Yes	LC	1.18	1.20	Positive	142.0 → 94.0	12	142.0 → 125.0	12	85
214	Methidathion	I, A	Not approved	Yes	LC	7.12	0.10	Positive	320.1 → 144.8	8	320.1 → 85.0	30	84
215	Methiocarb	I, A, M	Not approved	Yes	LC	7.67	0.10	Positive	226.1 → 169.0	4	226.1 → 121.1	12	90
216	Methiocarb-sulfoxide	Met	–	Yes	LC	4.03	0.80	Positive	242.0 → 185.0	22	242.0 → 122.0	28	90
217	Methomyl	I, A, AH	Nor approved	Yes	LC	3.23	0.40	Positive	163.1 → 88.0	5	163.0 → 106.0	8	80
218	Methomyl oxime	Met	–	Yes	LC	3.25	8.00	Positive	106.2 → 58.1	10	106.2 → 31.2	20	70
219	Methoxyfenozide	I	Approved	Yes	LC	8.00	0.10	Positive	369.2 → 149.0	10	369.2 → 313.1	15	85
220	Metoxychlor	POP	–	No	GC	13.98	0.80	Positive	227.0 → 141.0	20	227.0 → 169.0	15	70
221	Metrafenone	F	Approved	Yes	LC	9.27	0.10	Positive	409.1 → 209.1	8	411.1 → 209.1	12	108
222	Metronidazole	V, MB	Approved	–	LC	2.63	0.80	Positive	172.1 → 128.0	12	172.1 → 82.1	24	98
223	Mevinphos (phosdrin)	I, A	Not approved	No	LC	4.38	0.80	Positive	225.0 → 193.1	15	225.0 → 127.0	12	65
224	Mirex	POP	–	No	GC	5.66	2.00	Positive	237.0 → 143.0	30	274.0 → 237.0	10	70
225	Monocrotophos	I	Not approved	Yes	LC	3.31	0.80	Positive	224.1 → 126.8	12	224.1 → 98.1	15	100
226	Myclobutanil	F, MB, WP	Approved	Yes	LC	8.10	0.10	Positive	289.1 → 70.1	16	289.1 → 125.1	32	110
227	N-(2,4-dimethylphenyl)-*N*′-methylformamidine (DMPF, metabolite of amitraz)	Met[g]	–	No	LC	3.35	0.80	Positive	163.1 → 122.1	15	163.1 → 107.1	15	100
228	*N*,*N*-dimethylformamidine (DMF, metabolite of amitraz)	Met[g]	–	No	LC	5.45	1.20	Positive	150.1 → 77.0	40	150.1 → 105.8	30	100
229	*N*,*N*-dimethyl-N′-p-tolylsulphamide (DMST, metabolite of tolyfluanid)	Met[i]	–	No	LC	6.06	0.20	Positive	215.1 → 106.1	10	215.1 → 151.1	4	90
230	Nafcillin	V, MB	Not approved	–	LC	7.33	0.80	Positive	415.0 → 199.1	8	415.0 → 171.0	36	103
231	Naphtalene	POP	–	No	GC	4.45	0.80	Positive	128.0 → 127.0	15	128.0 → 102.0	25	70
232	Naproxen	V, NSAID	Not approved	–	LC	7.59	1.60	Positive	231.0 → 185.0	10	231.1 → 169.9	21	120

233	Nitenpyram	I	Not approved	No	LC	3.30	2.00	Positive	271.1 → 56.1	36	271.1 → 224.9	12	100
234	Novobiocin	V, MB	Not approved	–	LC	9.69	0.80	Positive	613.2 → 218.1	10	613.2 → 396.1	10	150
235	Nuarimol	F, MB, WP	Approved	No	GC	13.27	0.20	Positive	235.0 → 139.0	15	235.0 → 111.0	40	70
236	Ofurace	F, MB, WP	Approved	No	LC	5.97	0.10	Positive	282.0 → 159.9	20	282.0 → 147.9	30	100
237	Omethoate	I, A	Not approved	Yes	LC	2.80	0.40	Positive	214.1 → 124.8	22	214.1 → 183.0	5	100
238	Oxadixyl	F, MB, WP	Not approved	Yes	LC	5.43	0.20	Positive	279.1 → 219.2	5	279.1 → 132.2	32	110
239	Oxamyl	I, A, AH	Approved	Yes	LC	2.87	0.40	Positive	237.1 → 72.0	12	237.1 → 90.0	5	70
240	Oxfendazole	V, AH	Approved	–	LC	5.61	0.10	Positive	316.1 → 159.0	32	316.1 → 191.1	16	166
241	Oxolinic acid	V, MB	Not approved	–	LC	5.04	0.20	Positive	262.1 → 216.0	32	262.1 → 160.0	36	110
242	Oxydemeton methyl	I	Not approved	Yes	LC	3.01	0.40	Positive	247.0 → 169.0	12	247.0 → 109.0	24	100
243	Oxyfluorfen	H	Approved	No	GC	11.68	0.40	Positive	252.0 → 146.0	40	300.0 → 223.0	15	70
244	Paclobutrazol	H	Approved	Yes	LC	7.89	0.40	Positive	294.1 → 70.1	16	294.1 → 125.2	36	115
245	Paraoxon methyl	I	Not approved	No	GC	9.00	1.60	Positive	230.0 → 106.0	20	230.0 → 136.0	5	70
246	Parathion ethyl	I	Not approved	No	GC	9.95	1.20	Positive	290.9 → 109.0	10	138.9 → 109.0	5	70
247	Parathion methyl	I	Not approved	Yes	GC	9.12	0.80	Positive	263.0 → 109.0	15	263.0 → 79.0	30	70
248	PCB 28	POP	–	Yes	GC	9.01	0.10	Positive	256.0 → 186.0	25	256.0 → 151.0	50	70
249	PCB 52	POP	–	Yes	GC	9.58	0.20	Positive	292.0 → 222.0	25	292.0 → 220.0	25	70
250	PCB 77	POP	–	Yes	GC	11.73	0.20	Positive	292.0 → 220.0	25	292.0 → 222.0	25	70
251	PCB 81	POP	–	Yes	GC	11.56	0.10	Positive	292.0 → 220.0	25	292.0 → 222.0	25	70
252	PCB 101	POP	–	Yes	GC	11.08	0.20	Positive	326.0 → 256.0	30	328.0 → 256.0	30	70
253	PCB 105	POP	–	Yes	GC	12.66	0.10	Positive	326.0 → 256.0	30	328.0 → 256.0	30	70
254	PCB 114	POP	–	Yes	GC	12.38	0.20	Positive	326.0 → 256.0	30	328.0 → 256.0	30	70
255	PCB 118	POP	–	Yes	GC	12.18	0.20	Positive	326.0 → 256.0	30	328.0 → 256.0	30	70
256	PCB 123	POP	–	Yes	GC	12.10	0.40	Positive	326.0 → 256.0	30	328.0 → 256.0	30	70
257	PCB 126	POP	–	Yes	GC	13.23	0.20	Positive	326.0 → 256.0	30	328.0 → 256.0	30	70
258	PCB 138	POP	–	Yes	GC	13.07	0.10	Positive	360.0 → 290.0	25	360.0 → 288.0	25	70
259	PCB 153	POP	–	Yes	GC	12.57	0.10	Positive	360.0 → 290.0	25	360.0 → 288.0	25	70
260	PCB 156	POP	–	Yes	GC	13.96	0.20	Positive	360.0 → 290.0	25	360.0 → 288.0	25	70
261	PCB 157	POP	–	Yes	GC	14.07	0.40	Positive	360.0 → 290.0	25	360.0 → 288.0	25	70
262	PCB 167	POP	–	Yes	GC	13.55	0.10	Positive	360.0 → 290.0	25	360.0 → 288.0	25	70
263	PCB 169	POP	–	Yes	GC	14.61	0.20	Positive	360.0 → 290.0	25	360.0 → 288.0	25	70
264	PCB 180	POP	–	Yes	GC	14.25	0.10	Positive	394.0 → 324.0	30	394.0 → 322.0	30	70
265	PCB 189	POP	–	Yes	GC	15.25	0.10	Positive	394.0 → 324.0	30	394.0 → 322.0	30	70
266	Penconazole	F, MB, WP	Approved	Yes	GC	10.52	0.40	Positive	248.0 → 157.0	30	248.0 → 192.0	15	70
267	Pencycuron	F, MB, WP	Approved	Yes	LC	9.33	0.10	Positive	329.1 → 125.1	24	329.1 → 217.9	12	160
268	Pendimethalin	H	Approved	Yes	GC	10.49	0.80	Positive	252.0 → 162.0	10	252.0 → 191.0	5	70
269	Penicillin G	V, MB	Not approved	–	LC	5.82	2.00	Positive	335.1 → 176.0	10	335.1 → 160.0	4	110
270	Penicillin V	V, MB	Not approved	–	LC	6.47	2.00	Positive	383.2 → 159.9	10	383.2 → 113.9	40	130
271	Permethrin	I, A	Not approved	Yes	GC	15.69	1.20	Positive	183.0 → 128.0	15	183.1 → 153.1	15	70
272	Phenanthrene	POP	–	No	GC	8.40	0.20	Positive	178.0 → 176.0	35	178.0 → 152.0	28	70
273	Phenylbutazone	V, NSAID	Approved	–	LC	8.25	1.60	Positive	309.2 → 160.2	20	309.2 → 77.1	55	140
274	Phosalone	I, A	Not approved	No	LC	9.20	0.20	Positive	385.1 → 182.0	20	385.1 → 110.9	55	80

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Table 1 (*continued*)

No.	Compound	Category[a]	Legal status in the EU[b]	Subjected to MRL[c]	Technique[d]	Retention time (min)	LOQ (ng/ml)	Polarity	Quantification		Confirmation		Fragmentor voltage (V)
									MRM transition (m/z)	Collision energy (eV)	MRM transition (m/z)	Collision energy (eV)	
275	Phosmet	I, A	Approved	Yes	LC	7.34	0.20	Positive	318.0 → 159.9	16	318.0 → 133.0	40	90
276	Phosmet oxon	Met	–	Yes	LC	5.36	0.20	Positive	302.0 → 160.0	10	302.0 → 133.0	38	60
277	Piperacillin	V, MB	Not approved	–	LC	5.68	0.40	Positive	518.2 → 143.0	16	518.2 → 160.0	4	121
278	Pirimicarb	I	Approved	Yes	LC	5.11	0.10	Positive	239.1 → 72.1	20	239.1 → 182.1	12	100
279	Pirimiphos ethyl	I, A	Not approved	No	GC	10.26	0.10	Positive	318.0 → 166.0	15	318.0 → 182.0	15	70
280	Pirimiphos methyl	I, A	Approved	Yes	LC	9.13	0.10	Positive	306.1 → 164.0	20	306.1 → 108.1	32	100
281	Prochloraz	F, MB, WP	Approved	No	LC	9.08	0.10	Positive	376.0 → 308.0	10	376.0 → 70.1	20	100
282	Procymidone	F, MB, WP	Not approved	Yes	GC	10.80	1.60	Positive	283.0 → 67.0	40	283.0 → 68.0	25	70
283	Profenofos	I, A	Not approved	Yes	LC	9.75	0.10	Positive	375.0 → 305.0	20	373.0 → 303.0	20	100
284	Propamocarb	F	Approved	Yes	LC	2.85	0.40	Positive	189.2 → 102.0	12	189.2 → 144.0	8	110
285	Propargite	A	Not approved	Yes	LC	10.37	0.10	Positive	368.2 → 231.1	4	368.2 → 175.0	12	88
286	Propiconazole	A	Not approved	Yes	LC	9.01	0.40	Positive	342.0 → 69.0	21	342.0 → 159.0	39	90
287	Propoxur	I	Not approved	No	LC	5.83	0.10	Positive	210.1 → 168.1	35	210.1 → 65.1	40	70
288	Propyzamide (pronamide)	H	Approved	Yes	LC	7.92	0.10	Positive	256.1 → 190.0	16	256.1 → 173.0	25	90
289	Proquinazid	F	Approved	Yes	GC	13.32	0.20	Positive	288.0 → 245.0	15	288.0 → 217.0	30	70
290	Prothioconazol	F	Approved	Yes	GC	11.85	0.40	Positive	186.0 → 49.0	20	186.0 → 53.0	25	70
291	Prothiophos	F	Not approved	No	GC	11.45	0.40	Positive	266.9 → 221.0	35	162.0 → 63.1	30	70
292	Pymetrozine	I	Not approved	Yes	LC	2.74	0.80	Positive	218.1 → 105.0	20	218.1 → 78.0	52	120
293	Pyraclostrobin	F	Approved	Yes	LC	9.15	0.10	Positive	388.1 → 193.8	8	388.1 → 163.1	28	120
294	Pyrazophos	F, MB, WP	Not approved	No	LC	9.22	0.10	Positive	374.1 → 222.1	23	374.1 → 194.0	32	100
295	Pyrene	POP	–	No	GC	11.13	0.20	Positive	202.0 → 201.0	27	202.0 → 200.0	45	70
296	Pyridaben	I, A	Approved	Yes	LC	10.75	0.10	Positive	365.2 → 309.0	8	309.1 → 147.0	16	168
297	Pyridaphenthion	I, A	Not approved	No	LC	8.11	0.20	Positive	341.0 → 189.0	22	341.0 → 205.0	34	100
298	Pyrimethanil	F	Approved	Yes	GC	8.27	0.20	Positive	198.0 → 118.0	40	198.0 → 158.0	20	70
299	Pyriproxifen	I	Approved	Yes	LC	10.07	0.10	Positive	322.2 → 96.0	12	322.2 → 184.9	24	80
300	Quinalfos	I, A	Not approved	No	LC	8.72	0.20	Positive	299.1 → 96.9	30	299.1 → 147.1	20	130
301	Quinoxyfen	F	Not approved	Yes	LC	10.13	0.10	Positive	308.0 → 197.0	32	308.2 → 161.8	55	120
302	Rifampicin	V, MB	Not approved	–	LC	7.89	0.80	Positive	823.5 → 791.4	15	823.5 → 399.1	25	160
303	Rotenone	I, R	Not approved	No	LC	8.64	0.40	Positive	395.1 → 213.1	20	395.1 → 192.1	25	150
304	Roxithromycin	V, MB	Not approved	–	LC	7.67	0.80	Positive	838.5 → 158.1	40	838.5 → 116.1	55	200
305	Sarafloxacin	V, MB	Not approved	–	LC	4.16	4.00	Positive	386.1 → 342.1	16	386.1 → 299.1	28	144
306	Simazine	I	Not approved	No	LC	5.81	0.20	Positive	202.4 → 68.1	30	202.4 → 68.1	20	120
307	Spinosad (two isomers)	I, V	Approved	Yes	LC	9.10/9.43	0.10	Positive	732.4 → 142.0	22	732.4 → 98.0	60	130
308	Spiramycin (two isomers)	V, MB	Approved	–	LC	4.58/4.90	0.40	Positive	439.1 → 101.1	20	439.1 → 88.0	50	70

309	Spirodiclofen	A	Approved	Yes	LC	10.50	0.80	Positive	411.1 → 71.2	15	411.1 → 313.0	5	110
310	Spiromesifen	I	Approved	Yes	LC	10.27	0.20	Positive	388.0 → 273.0	25	273.0 → 187.0	15	110
311	Spiroxamine	F	Approved	Yes	LC	7.55	0.10	Positive	298.3 → 144.1	16	298.3 → 100.1	32	120
312	Strychnine	R	Not approved	No	LC	3.00/3.61	0.80	Positive	335.1 → 184.0	45	335.1 → 156.0	40	105
313	Sulfacetamide	V, MB	Not approved	–	LC	2.13	0.40	Positive	215.3 → 155.9	10	215.3 → 92.0	20	90
314	Sulfachloropiridacine	V, MB	Not approved	–	LC	3.77	0.80	Positive	285.0 → 156.0	12	285.0 → 92.1	28	101
315	Sulfadiacine	V, MB	Approved	–	LC	2.80	0.80	Positive	251.0 → 92.0	28	251.0 → 156.0	12	111
316	Sulfadimetoxine	V, MB	Approved	–	LC	4.81	0.10	Positive	311.0 → 92.0	32	311.0 → 156.0	16	139
317	Sulfadoxine	V, MB	Approved	–	LC	4.12	0.10	Positive	311.1 → 92.0	32	311.1 → 156.0	16	126
318	Sulfameracine	V, MB	Not approved	–	LC	3.26	0.20	Positive	265.0 → 92.0	28	265.0 → 156.0	12	126
319	Sulfametacine	V, MB	Not approved	–	LC	3.44	0.20	Positive	279.1 → 186.0	12	279.1 → 92.0	32	134
320	Sulfametizole	V, MB	Not approved	–	LC	3.37	0.80	Positive	271.0 → 92.0	28	271.0 → 155.9	8	103
321	Sulfametoxazole	V, MB	Approved	–	LC	3.93	0.40	Positive	254.0 → 92.0	28	254.0 → 156.0	12	111
322	Sulfametoxipiridacine	V, MB	Not approved	–	LC	3.45	0.40	Positive	281.0 → 155.9	12	281.0 → 92.1	28	121
323	Sulfamonomethoxine	V, MB	Not approved	–	LC	4.11	1.20	Positive	281.1 → 156.0	14	281.1 → 92.1	32	120
324	Sulfapyridine	V, MB	Not approved	–	LC	2.82	0.40	Positive	250.0 → 156.0	12	250.0 → 92.0	28	126
325	Sulfaquinoxaline	V, MB	Approved	–	LC	4.99	0.40	Positive	301.0 → 156.0	12	301.0 → 92.1	32	159
326	Sulfatiazole	V, MB	Not approved	–	LC	2.98	0.40	Positive	256.0 → 92.0	28	256.0 → 156.0	12	106
327	Sulfisoxazole	V, MB	Not approved	–	LC	4.12	0.80	Positive	268.0 → 156.0	8	268.0 → 92.1	24	106
328	Tebuconazole	I, A	Approved	Yes	LC	8.92	0.80	Positive	308.2 → 70.2	22	308.2 → 125.1	53	120
329	Tebufenocide	I	Approved	Yes	LC	8.66	0.10	Positive	353.1 → 132.9	22	353.1 → 297.1	20	90
330	Tebufenpyrad	A	Approved	Yes	LC	9.88	0.10	Positive	334.2 → 117.0	47	334.2 → 145.0	37	180
331	Teflubenzuron	I	Not approved	Yes	LC	10.01	1.20	Negative	379.0 → 339.0	15	379.0 → 196.0	25	100
332	Tefluthrin	I	Approved	Yes	GC	8.42	0.10	Positive	177.0 → 127.0	15	177.0 → 87.0	15	70
333	Telodrin (isobenzan)	I	Not approved	No	GC	10.14	0.80	Positive	310.8 → 240.8	25	310.8 → 274.8	5	70
334	Terbufos	I, AH	Not approved	No	GC	8.15	0.20	Positive	231.0 → 97.0	20	231.0 → 129.0	15	70
335	Terbuthylazine	H	Approved	Yes	GC	8.12	0.40	Positive	214.0 → 104.0	20	214.0 → 132.0	10	70
336	Tetrachlorvinphos	I	Not approved	No	LC	8.72	0.40	Positive	367.0 → 127.0	16	365.0 → 127.0	16	110
337	Tetraconazole	F, H	Approved	Yes	GC	10.04	0.20	Positive	336.0 → 204.0	35	336.0 → 218.0	20	70
338	Tetradifon	A	Not approved	No	GC	14.36	0.40	Positive	158.9 → 111.0	20	354.0 → 159.0	10	70
339	Tetramethrin	I	Not approved	No	GC	13.87	1.60	Positive	164.0 → 77.0	30	164.0 → 107.0	15	70
340	Thiabendazole	AH, V	Approved	Yes	LC	3.50	0.20	Positive	202.0 → 175.0	24	202.0 → 131.0	36	170
341	Thiacloprid	I	Approved	No	LC	4.80	0.20	Positive	253.0 → 126.0	16	253.0 → 90.0	40	140
342	Thiamethoxam	I	Not approved	Yes	LC	3.59	0.80	Positive	292.0 → 211.1	8	292.0 → 132.0	22	80
343	Thiophanate methyl	I	Approved	Yes	LC	5.87	0.20	Positive	343.0 → 151.0	20	343.0 → 93.0	46	90
344	Tolclofos methyl	F, MB, WP	Approved	Yes	GC	9.21	0.10	Positive	265.0 → 93.0	30	265.0 → 220.0	25	70
345	Tolfenamic acid	V, NSAID	Not approved	–	LC	9.80	0.40	Negative	260.0 → 216.1	8	260.0 → 35.1	20	108
346	Triadimefon	F, MB, WP	Not approved	Yes	LC	8.03	0.40	Positive	294.1 → 69.3	20	294.1 → 197.2	15	100

(continued on next page)

Table 1 (*continued*)

No.	Compound	Category[a]	Legal status in the EU[b]	Subjected to MRL[c]	Technique[d]	Retention time (min)	LOQ (ng/ml)	Polarity	Quantification MRM transition (m/z)	Quantification Collision energy (eV)	Confirmation MRM transition (m/z)	Confirmation Collision energy (eV)	Fragmentor voltage (V)
347	Triadimenol	F, MB, WP	Not approved	Yes	LC	8.22	0.40	Positive	296.1 → 70.0	10	298.1 → 70.0	10	80
348	Triazophos (hostathion)	I, A	Not approved	Yes	LC	8.18	0.10	Positive	314.1 → 162.0	19	314.1 → 118.9	35	100
349	Trichlorfon	I, AH, V	Not approved	No	LC	4.06	1.20	Positive	256.9 → 109.0	12	258.9 → 109.0	12	170
350	Trifloxystrobin	F	Approved	Yes	LC	9.50	0.10	Positive	409.1 → 186.0	12	409.1 → 145.0	52	110
351	Triflumizole	F	Approved	No	LC	9.53	0.10	Positive	346.1 → 278.0	4	345.9 → 73.0	15	80
352	Triflumuron	I	Approved	Yes	LC	9.19	0.40	Positive	359.0 → 156.0	8	359.0 → 139.0	32	120
353	Trifluralin	H	Not approved	No	GC	7.27	0.20	Positive	264.0 → 160.0	15	306.0 → 264.0	5	70
354	Trimethoprim	V, MB	Approved	–	LC	3.45	0.80	Positive	291.2 → 123.0	24	291.2 → 230.1	20	162
355	Triticonazole	F	Approved	No	LC	8.38	0.40	Positive	318.1 → 70.1	33	320.1 → 70.1	16	110
356	Tylmicosin	V, MB	Approved	–	LC	5.52	1.60	Positive	869.6 → 174.1	48	869.6 → 696.4	44	294
357	Tylosin	V, MB	Approved	–	LC	6.76	0.80	Positive	916.5 → 174.1	40	916.5 → 772.4	28	210
358	Vinclozolin	F, MB, WP	Not approved	Yes	GC	9.10	0.20	Positive	212.0 → 145.0	25	212.0 → 109.0	50	70
359	Warfarin	R	Not approved	No	LC	7.86	0.10	Negative	307.1 → 161.1	20	307.1 → 250.1	20	140
360	Zoxamide	F	Approved	No	LC	9.03	0.40	Positive	336.0 → 187.1	25	187.1 → 88.9	40	200
	Acenaphthene-d10	P-IS	–	–	GC	6.16	–	Positive	164.0 → 162.0	18	164.0 → 160.0	35	70
	Atrazine-d5	P-IS	–	–	GC	7.95	–	Positive	205.1 → 127.1	14	205.1 → 105.0	14	70
	Atrazine-d5	P-IS	–	–	LC	6.74	–	Positive	221.2 → 179.0	15	221.2 → 69.1	50	90
	Carbendazim-d3	P-IS	–	–	LC	2.91	–	Positive	195.1 → 160.1	15	195.1 → 131.9	30	100
	Chorpyrifos-d10	P-IS	–	–	GC	9.94	–	Positive	324.0 → 260.0	35	324.0 → 195.0	55	70
	Chrysene-d12	P-IS	–	–	GC	13.86	–	Positive	240.0 → 238.0	20	240.0 → 236.0	38	70
	Cyromazine-d4	P-IS	–	–	LC	1.24	–	Positive	171.0 → 129.0	15	171.0 → 86.0	15	100
	Diazinon-d10	P-IS	–	–	GC	8.29	–	Positive	314.0 → 199.0	5	314.0 → 183.0	15	70
	Diazinon-d10	P-IS	–	–	LC	8.93	–	Positive	315.2 → 170.1	20	315.2 → 154.3	20	100
	Linuron-d3	P-IS	–	–	LC	7.54	–	Positive	255.1 → 185.0	15	255.1 → 159.8	15	100
	PCB 200	P-IS	–	–	GC	14.51	–	Positive	429.8 → 359.8	30	427.8 → 357.8	30	70
	Phenanthrene-d10	P-IS	–	–	GC	8.40	–	Positive	188.0 → 186.0	20	188.0 → 184.0	35	70
	Pirimicarb-d6	P-IS	–	–	LC	5.12	–	Positive	245.2 → 185.0	5	245.2 → 78.2	30	70

[a] POP – persistent organic pollutant; Non persistent pollutants: A – acaricide, MB – microbiocide, AH – anthelminthic, V – veterinary and human pharmaceuticals, F – fungicide, H – herbicide, I – insecticide, R – plant growth regulator, WP – wood preservative, PHP – post-harvest preservative, M – Molluscicide, Met – metabolite, NSAID – nonsteroidal anti-inflammatory drug, GC – glucocorticoid, P-IS – procedural internal standard.

[b] For pesticides and rodenticides the legal status reflecting the EU Pesticide Database was considered (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN), which is valid for the entire EU. For veterinary drugs, the marketing status in Spain is specified, as shown in the Cima vet search engine of the Spanish agency for drugs and health products (https://cimavet.aemps.es/cimavet/publico/home.html).

[c] Pesticide considered in the coordinated multi-annual plan of the EU for the investigation of residues in food of vegetable or animal origin during the years 2020, 2021 and 2022 (Regulation CE/2019/533).

[d] Gas chromatography (GC) or liquid chromatography (LC), both coupled with tandem triple quadrupole mass spectrometry. Some compounds can be detected by both techniques. However, only that technique for which better performance (lower LOQ, best recovery or lower RSD) has been indicated.

[e] Isomer beta (beta-cyfluthrin) is approved until 31 October 2020.

[f] Isomer beta (beta-cypermethrin) has switch to the "not approved" status since September 2017.

[g] The exposure to the acaricide amitraz is evaluated through the presence of these two major metabolites.

[h] The exposure to dichlofluanide is evaluated through the presence of this metabolite.

[i] The exposure to tolyfluanide is evaluated through the presence of this metabolite.

polarity range, while matrix co-eluents extracts the least (Sell et al., 2018) being at the same time suitable for LC and GC chromatography. Both methods have been developed for the analysis of pesticides in foods of plant origin, using 10 g of sample, and involve a clean-up step with secondary primary amine (PSA) for the removal of organic acids, fatty acids, sugars; and C18 for the elimination of lipids and sterols; and/or graphitized carbon (GC) for pigment removal. However, our previous experience demonstrated that these adsorbents retain various polar compounds (for example, a large number of pharmaceuticals and some POPs) that are of interest to this method. Therefore, we decided not to test clean-up with these adsorbents. However, a novel sorbent, the EMR-Lipid, was recently launched for the clean-up of fatty sample extracts, such as whole blood (Agilent, 2015). Therefore, a clean-up step with EMR-lipid was tested at two levels (2 y 20 ng/ml) in triplicate. As different authors have indicated that, when using the EN method, the extraction efficiency of acetonitrile improves in the acidic condition, this method was tested in the presence or absence of 1% FA (EC, 2015). Ten and one grams of whole blood were tested in these initial experiments (3 replicates at two concentrations (2–20 ng/ml), analyzed in duplicate). We did not find differences between them. Therefore, we continued next experiments with 1 g of whole blood instead of 10 g.

We made the decision on which method to choose based on two criteria. First, we chose the method that extracted more compounds above 60% recovery (all quantifications were performed against matrix-matched calibrators). The second criterion was based on the ion abundance. According to these criteria, the clean-up with EMR-lipid was ruled out since 97 and 113 compounds were lost or poorly recovered with the AOAC 2007.01 method and the UNE-EN 15662: 2019 method, respectively. One-step application of QuEChERS gave excellent results with both protocols, with an ability to adequately extract a similar number of compounds (360 with the AOAC method vs. 354 with the EN method). However, ion abundance was better for the majority of compounds with the AOAC method in the presence of 1% FA, so this was the chosen method. In a further step, the possibility of refining the method by modifying the percentage of FA was tested (0.0, 0.2, 0.5, 1, 2.5 and 5%), but no better results than those of 1% FA were found.

One of the most important objectives of this methodological development was to minimize the amount of sample employed. Therefore, several additional experiments were carried out, in which the amount of sample was progressively decreased (1, 0.5, 0.25, 0.1). Obviously, the amount of salts was proportionally decreased. The minimum amount of sample that did not affect the performance of the extraction method was 0.25 g of whole blood, so all validation of the method was performed using these conditions.

3.3. Validation

This method allows the simultaneous quantification of 360 chemicals, including 56 POPs, 205 agricultural pesticides, 11 rodenticides, 67 pharmaceuticals, and 21 metabolites. The detailed list of compounds, together with the specific category of use, and the technique of instrumental analysis, are presented in Table 1. Besides, for agricultural pesticides, the legal status in the EU and whether or not it is included in the coordinated multi-annual plan of the EU for the investigation of residues in food, is also indicated in Table 1. Data obtained during the validation process meet both, the criteria of the SANTE 2017 guidance document and those of the Scientific Working Group for Forensic Toxicology (EC, 2019b; SWGTOX, 2013).

Identity was evaluated through ion qualifier ratio (±30 average ion qualifier ratio of matrix-matched standards from the same sequence), retention time deviation (±0.1 min), peak shape, and signal/noise ratio (s/n > 3 for all ions, peak-to-peak algorithm). At least two transitions per compound were optimized. Transition with higher response and less noise at the lowest calibration point was used as a quantifier and at least one more as a qualifier (confirmation). Whenever possible, the quasimolecular ion was used in the identification of the compounds. The quantification and confirmation transitions that were selected for each compound are shown in Table 1. On the other hand, selectivity, which is the recommended term in analytical chemistry to express the extent of interferences (EC, 2019b), was evaluated by assessing the absence or presence of interfering or co-eluting chromatographic peaks at the retention time of the target analytes in the blank samples extracted by the optimized micro QuEChERS method.

Linearity was assessed within the range of concentrations that were considered appropriate for the purpose of biomonitoring (0.1 to 20 ng/ml). Within this range, 12 matrix-matched calibration points in quintuplicate were evaluated. All compounds showed acceptable linearity, with the lowest correlation coefficient (R^2) values being those of sarafloxacin, naphthalene, benzo[k]fluoranthene, fluvalinate, and fipronil sulfide (R^2 about 0.93). It is noteworthy, however, that the values of R^2 were within the range 0.97–0.99 for nearly 95% of compounds (n = 342). Detailed R^2 values for all the compounds are shown in Table 1 of the accompanying Data in Brief article.

The influence of the matrix components on the performance of the method was evaluated by applying the extraction method to a sufficient quantity of whole blood to produce a blank matrix extract, which was subsequently fortified at three levels for the mixture of 360 chemicals (0.2, 2, and 20 ng/ml) and quantified against a calibration curve prepared in the solvent. Matrix effect (ME) was observed both for compounds analyzed by LC–MS/MS and GC–MS/MS. Strong or medium signal suppression was demonstrated for 13.88% of compounds, and enhancement for 29.44% of compounds. For 204 pollutants the ME was considered negligible (−20% < ME < 20%). However, as for the rest 156 chemical, there was a significant ME, and it was concluded that matrix-matched calibration had to be used to compensate matrix interferences. All the detailed data of ME for individual compounds in whole blood are graphically shown in Fig. 1 of the accompanying article in Data in Brief.

The average recovery and precision (at least 5 fortification levels, each in quintuplicate) obtained were satisfactory for 356 pollutants, as they ranged from 76.6 to 119.5% with intraday relative standard deviations (RSD) ranging from 0.1 to 19.6%, and interday RSD ranging from 0.08 to 19.2%. Four pollutants did not strictly meet the validation criteria included in the SANTE guide (recoveries in the range 70–120%, and RSD < 20%). However, due to its importance in biomonitoring studies, we consider it important to include in the method compounds whose recoveries were lower or higher than those established in the recommendations, but which were highly reproducible (RSD < 15%). Thus, marbofloxacin (bias 62%, RSD 14.5%), beta hexachlorocyclohexane (bias 132.6%, RSD 14.5%), spirodiclofen (bias 134.4%, RSD 8.8%), and heptachlor (bias 139.4%, RSD 6.7%) were also included. All detailed validation data for the five levels of fortification are shown in Table 1 of the accompanying article in Data in Brief. For some compounds with higher LOQs, fewer levels are displayed, as no data is included in the table for levels < LOQ.

In the validation process, the possibility of carryover was also assessed. For this, blank matrix extracts were analyzed immediately after injecting the highest point of the calibration curve (also prepared in the matrix). According to the guidelines, it is acceptable for validation if carryover after the highest calibrator does not exceed 10% of the signal of the lowest calibrator, and in our case, this condition was met at 20 ng/ml for all analytes, except for fenbutatin oxide. The carryover effect for this pesticide disappeared completely at the second injection of blank matrix. To assess the need for additional clean-up measures for samples with medium to high levels of contaminants levels, we conducted additional experiments in which the signal from the blank matrix was evaluated after the injection of 100 ng/ml of the chemical mixture in whole blood. In this case, in addition to fenbutatin oxide, a low carryover was observed for brodifacoum, chlorophacinone, danofloxacin, difloxacin, enrofloxacin, flocoumafen, marbofloxacin, and sarafloxacin. However, the signal disappeared completely after the second injection of blank also for these compounds.

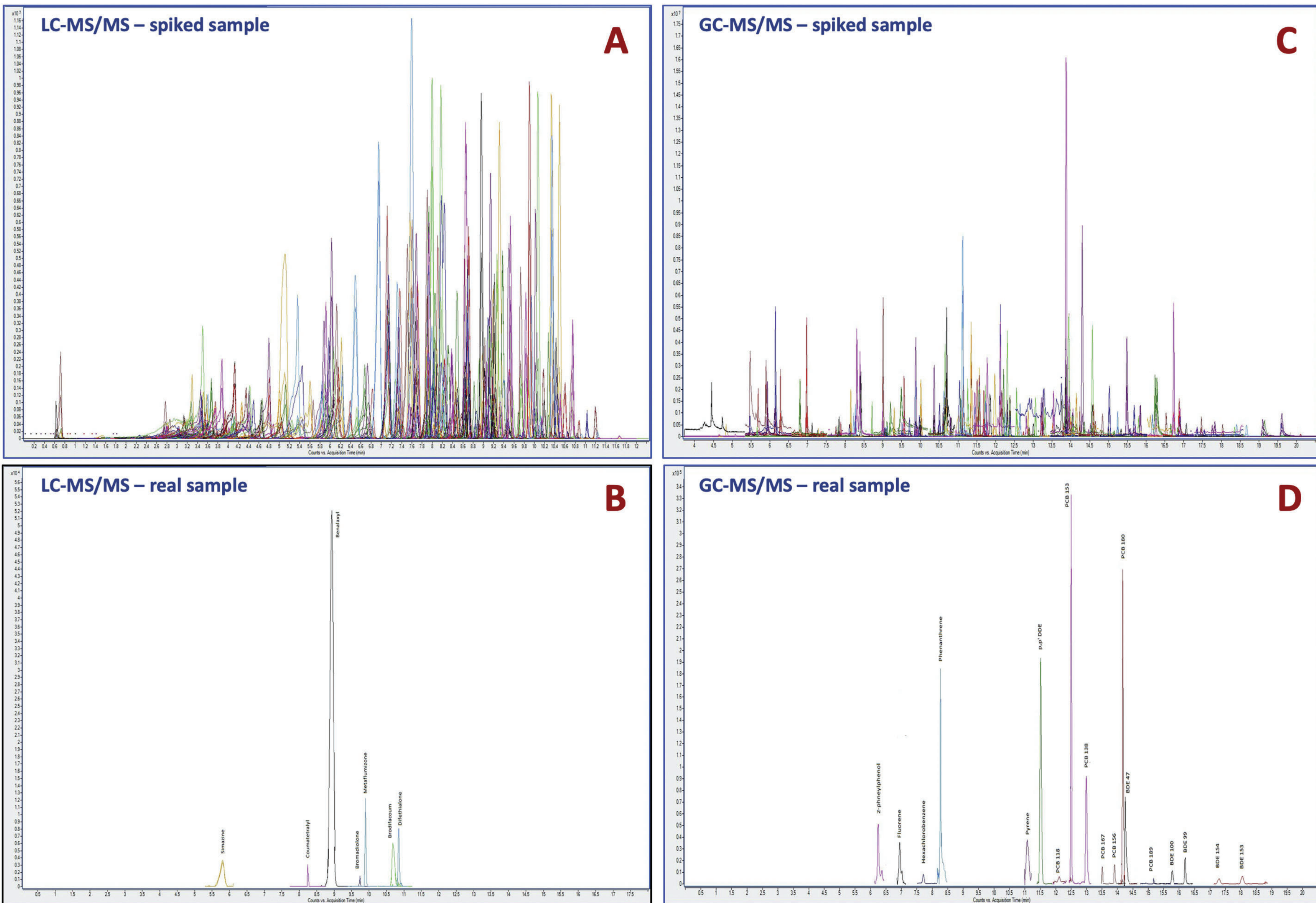

Fig. 1. Top panels. MRM chromatograms of a blank whole blood sample spiked with the mix of 360 chemicals + P-ISs at the level of 20 ng/ml analyzed by LC-MS/MS (A) and GC–MS/MS (C). Bottom panels. MRM chromatograms of the analyses of real samples (barn owl and common kestrel) by LC-MS/MS (B) and by GC–MS/MS (D).

The LOQ of this method was calculated over five runs of five fortified blank matrix samples, within the low working range (below 5 ng/ml), of three different sources (chicken, goat, and a mixture of both), as recommended (SWGTOX, 2013). The lowest non-zero calibrator approximation was employed to calculate de LOQs. This means that the lowest point of the calibration curve that complied identity, bias and precision criteria was set as the LOQ for a given compound. The expanded method uncertainty (MU) was calculated according to the formula specified in the SANTE guide (1st approach). As this is a new method development, no data of proficiency tests or independent reference materials were available. Therefore, reproducibility RSD was employed, as indicated in the SANTE guide, and an expanded covered factor $k = 2$ was chosen (EC, 2019b). The MU was below 58% in all cases. As shown in Table 1, the developed method is very sensitive and allows the quantitative analysis at very low levels. Despite the high number of chemicals included, and the small sample volume employed, 95% compounds can be quantified at LOQs < 1.5 ng/ml. This makes the method very appropriate for the biomonitoring of toxic chemicals in wildlife.

3.4. Application of the method to a series of blood samples of wild birds

This method was applied to a serie of 148 samples of whole blood collected in 2018 and 2019 from several species of nocturnal and diurnal raptors belonging to the Group of Rehabilitation of the Native Fauna and its Habitat (GREFA, Majadahonda, Spain). The whole blood samples do not correspond to a homogeneous series of individuals but include birds from 2 different species (*Falco tinnunculus* and *Tyto alba*), including both chickens and adults, and both males and females. These data are presented for the sole purpose of demonstrating the potential of the method for biomonitoring contaminants in wildlife. The individual data obtained for each of the individuals are presented in Tables 2–5 of the accompanying article in Data in Brief.

Fig. 1 shows the typical chromatograms of the spiked at 20 ng/ml whole blood samples obtained by the LC-MS/MS and GC–MS/MS analyses (top panels), and the chromatograms of two different positive samples for 25 pollutants (*Tyto alba* and *Falco tinnunculus*, bottom panels), as an example of the application of the method. Fig. 2 shows the data on the number of contaminants detected per sample. In all the samples, at least three of the contaminants were detected, with a maximum of 25 contaminants detected in the barn owl shown in Fig. 1. The median value of the number of pollutants per sample was 7.

In total, 51 different compounds were detected, which represents 14% of the chemicals included in the method. Contaminants belonging to 4 of the five groups under study (POPs, agricultural insecticides, rodenticides, and pharmaceuticals) were detected. However, none of the metabolites (nor the parent compounds) used as exposure biomarkers were detected in any of the samples. Tables 2 and 3 show the results of the contaminants found, along with the mean, median, percentiles, and detection frequency values throughout the series. As expected, of the six compounds that were detected in >50% of the samples, five were POPs (phenanthrene, pyrene, fluorene, hexachlorobenzene, and p,p′-DDE) (Table 2). However, it is striking that a contaminant that is neither persistent nor semi-persistent, such as 2-phenylphenol (2PHP), appeared in 96% of raptor blood samples (Table 3). 2PHP is a biocide used as preservative and surface disinfectant on fibers and other materials in households, hospitals, and other places, and is recognized as a potential endocrine disruptor (Scientific Committee On Consumer and Bernauer, 2016). Other authors have also reported that 2PHP is a highly prevalent pollutant in biota samples, such as river fish of different species, in which it is found in up to 100% of the samples (Peng et al., 2018). The rest of the POPs that were detected were the ones that have also been reported most frequently in other series of birds of prey (Espin et al., 2018; Garcia-Heras et al., 2018; Jaspers et al., 2013; Luzardo et al., 2014b; Ortiz-Santaliestra et al., 2015).

Concerning the rest of the agricultural pesticides, the case of benalaxyl is particularly remarkable, since it was detected in 23% of the individuals analyzed, albeit at low concentrations (Table 3). Benalaxyl is a widely used agricultural fungicide, and both, this agrochemical and, in particular, its metabolites, have been classified as endocrine disruptors endowed with potent anti-estrogenic activity (Ji et al., 2020). However, this pesticide is not routinely included in biomonitoring studies, despite studies on its toxicity to wildlife (Wang et al., 2014). The finding of such a high detection frequency of this fungicide in birds of prey samples indicates that this product has an extensive penetration in the food chains and the ecosystem and also, demonstrates the great utility of the method that we have developed for ecotoxicology studies. On the other hand, diphenylamine was found in 11.5% of the samples. Its principal use has been as a post-harvest preservative (mostly apples and pears), although its use is no longer authorized in the EU, and therefore such a high frequency of detection in wildlife samples is shocking. The percentage of raptor blood samples that tested positive for this residue is very similar to that of positives in fresh vegetables produced in countries where it is still authorized (Mutengwe et al., 2016). To our knowledge, this is the first study in which the presence of diphenylamine in the blood of raptors is reported. However, the presence of diphenylamine has been reported in gray partridge eggs from agricultural ecosystems (Bro et al., 2015). Moreover, the fact that it has also been found in herbs growing in agricultural areas (Malinowska and Jankowski, 2015), suggests that, independently from its origin, this compound is likely to easily penetrate the food chain (plants, arthropods, rodents, etc…), thus possibly reaching raptors. Metaflumizone was also found in a similar percentage of raptors (10.8%). Although it is not possible to know the exact origin, it is a permitted insecticide of great use in agriculture, and it has been reported to have a persistence of several days in soil (Chatterjee and Gupta, 2013). Therefore, it also seems possible that this pesticide penetrates the raptors' trophic chain. Five other agricultural pesticides were detected less frequently (Table 3). Among them, it is worth noting simazine, which is another unauthorized pesticide in the EU, and which is of concern because it is a proven endocrine disruptor (Orton et al., 2009). Simazine was detected in 4.73% of the samples ($n = 7$ individuals).

Although the penetration of rodenticides into the trophic chain is a known fact (Plaza et al., 2019; Ruiz-Suarez et al., 2014; Sanchez-Barbudo et al., 2012; Seljetun et al., 2019), it is still surprising that residues of at least one of these compounds have been found in almost 15% of the birds in this series. More than one compound was found in 8 of the individuals (from 2 to 4 compounds). It should be noted that the sample used has been the blood of live animals and not liver samples where these compounds tend to concentrate. These results indicate that these animals are constantly exposed to these rodenticides through feeding, even from the time they are in the nest. In addition, there are

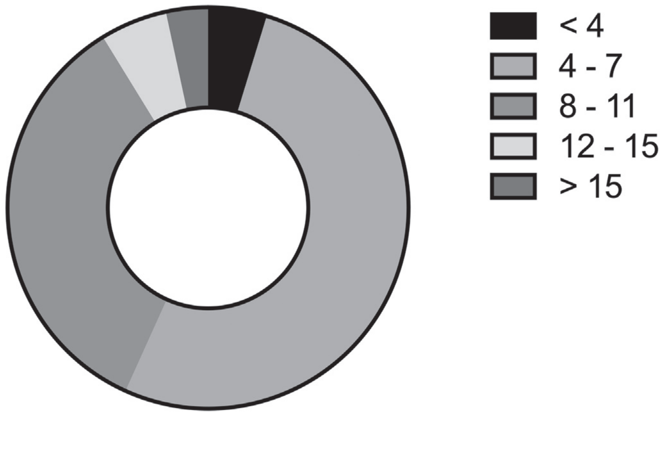

Fig. 2. Occurrence of environmental pollutants in the blood of a series of 148 raptors.

Table 2
Persistent and semi-persistent organic pollutants detected in 148 blood s → amples of raptors.

Pollutant	Frequency	Concentrations in samples with residues			
		Mean ± SD	Median	95th percentile	Max
Phenanthrene	95.95	0.75 ± 0.40	0.61	1.46	2.25
Pyrene	95.95	0.23 ± 0.13	0.21	0.45	0.91
Fluorene	91.22	0.55 ± 0.27	0.47	1.12	1.50
Hexachlorobencene	68.92	0.60 ± 0.72	0.38	1.78	5.49
Dichlorodiphenyldichloroethylene (p,p′ DDE)	51.89	0.52 ± 0.87	0.20	2.90	4.40
PCB 153	35.81	0.55 ± 1.01	0.21	3.02	5.17
PCB 138	31.08	0.33 ± 0.56	0.15	1.68	2.87
PCB 180	30.73	0.76 ± 1.64	0.23	5.66	6.88
Fluoranthene	19.59	0.27 ± 0.06	0.26	0.37	0.43
Acenaphtylene	17.57	0.31 ± 0.08	0.33	0.43	0.45
PCB 189	10.14	0.25 ± 0.17	0.18	0.56	0.58
PCB 118	6.76	0.20 ± 0.16	0.13	0.50	0.50
BDE 99	6.08	0.22 ± 0.20	0.10	0.57	0.68
Acenaphthene	5.41	0.29 ± 0.13	0.30	0.37	0.51
PCB 167	5.41	0.20 ± 0.10	0.22	0.33	0.36
BDE 100	4.73	0.14 ± 0.08	0.10	0.26	0.33
PCB 156	4.05	0.23 ± 0.13	0.21	0.42	0.49
BDE 153	3.38	0.20 ± 0.00	0.20	0.20	0.20
BDE 47	2.03	0.20 ± 0.00	0.20	0.20	0.20
Naphtalene	2.03	1.43 ± 1.07	0.83	2.49	2.67
Dichlorodiphenyldichloroethane (p,p′ DDD)	0.68[a]	0.65	0.65	–	–
Hexachlorocyclohexane (alpha)	0.68[a]	0.40	0.40	–	–
Hexaclorocyclohexane (beta)	0.68[a]	5.95	5.95	–	–
PCB 28	0.68[a]	0.10	0.10	–	–
PCB 101	0.68[a]	0.20	0.20	–	–

[a] These compounds were detected in only one individual each.

Table 3
Non persistent pesticides and veterinary drugs detected in 148 blood samples of raptors.

	Category[a]	Legal status in the EU[b]	Subjected to MRL[c]	Frequency	Concentrations in samples with residues			
					Mean ± SD	Median	95th percentile	Max
Agricultural pesticides								
2-Phenylphenol	F	Approved	Yes	95.95	1.02 ± 1.19	0.59	4.19	5.88
Benalaxyl	F	Approved	No	22.97	0.13 ± 0.04	0.10	0.21	0.26
Diphenylamine	PHP	Not approved	Yes	11.49	0.37 ± 0.20	0.31	0.63	0.82
Metaflumizone	I, V	Approved	No	10.81	0.31 ± 0.21	0.20	0.70	0.92
Simazine	I	Not approved	No	4.73	0.25 ± 0.07	0.23	0.36	0.39
Metrafenone	F	Approved	Yes	2.70	0.10 ± 0.01	0.10	0.10	0.10
Thiacloprid	I	Approved	No	2.70	0.83 ± 1.17	0.31	2.25	2.57
Coumaphos	I, A	Not approved	No	1.35	0.15 ± 0.05	0.15	0.18	0.19
Atrazine	H	Not approved	No	0.68[d]	0.12	0.12	–	–
Rodenticides								
Brodifacoum	R	Not approved	No	7.43	4.38 ± 9.55	0.80	19.55	32.73
Difenacoum	R	Not approved	No	3.38	0.40 ± 0.01	0.40	0.41	0.42
Bromadiolone	R	Approved	No	2.03	0.41 ± 0.01	0.40	0.42	0.42
Coumatetralyl	R	Not approved	No	2.03	41.56 ± 60.78	13.02	101.53	111.36
Coumachlor	R	Not approved	No	0.68[d]	5.84	5.84	–	–
Difethialone	R	Not approved	No	0.68[d]	1.77	1.77	–	–
Flocoumafen	R	Not approved	No	0.68[d]	0.20	0.20	–	–
Pharmaceuticals								
Levamisole	V	Approved	–	8.11	0.29 ± 0.10	0.27	0.34	0.52
Fenbendazole	V	Approved	–	2.70	0.10 ± 0.00	0.10	0.10	0.10
Enrofloxacin	V	Approved	–	1.35	1.20 ± 0.00	1.20	1.20	1.20
Eprinomectin	V	Approved	–	1.35	0.32 ± 0.01	0.32	0.32	0.33
Flumequine	V	Approved	–	1.35	0.10 ± 0.00	0.10	0.10	0.10
Sulfadiacine	V	Approved	–	1.35	7.27 ± 9.28	7.27	9.42	13.69
Albendazole	V	Approved	–	0.68[d]	0.10	0.10	–	–
Dexamethasone	V	Approved	–	0.68[d]	0.40	0.40	–	–
Mebendazole	V	Approved	–	0.68[d]	0.25	0.25	–	–
Sulfacloropiridacine	V	Not approved	–	0.68[d]	0.80	0.80	–	–
Sulfapyridine	V	Not approved	–	0.68[d]	0.40	0.40	–	–

[a] A – acaricide, B – bactericide, AH – anthelminthic, V – veterinary and human pharmaceuticals, F – fungicide, H – herbicide, I – insecticide, R – plant growth regulator, WP – wood preservative, PHP – post-harvest preservative, M – Molluscicide, Met - metabolite.
[b] For pesticides and rodenticides the legal status reflecting the EU Pesticide Database was considered (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN), which is valid for the entire EU. For veterinary drugs, the marketing status in Spain is specified, as shown in the Cima vet search engine of the Spanish agency for drugs and health products (https://cimavet.aemps.es/cimavet/publico/home.html).
[c] Pesticide considered in the coordinated multi-annual plan of the EU for the investigation of residues in food of vegetable or animal origin during the years 2020, 2021 and 2022 (Regulation CE/2019/533).
[d] These compounds were detected in only one individual each.

two other very striking events, such as the fact that six of the seven rodenticides detected in this series are not authorized for agricultural or environmental use in the EU, and also that one of them - brodifacoum - has been the most frequently detected in this series of birds of prey (11 individuals).

Finally, although detection frequencies were low in almost all cases, up to 11 different pharmaceuticals were detected in this series of birds of prey blood samples (Table 3). As in the previous cases, the fact that the sample analyzed is blood implies that the exposure to these contaminants has been recent (and probably regular), possibly only a few hours before sampling. More in-depth studies are needed to assess the toxicological significance of these findings. Still, it is worth noting some data such as the high frequency of detection with which levamisole has appeared in this series (12 individuals with levels > LOQ). To the best of our knowledge, this is the first study that shows data about levamisole in wild raptors. However, some other authors have indicated that this is a prevalent environmental pollutant, and have reported its presence in non-target wild organisms, such as marine mollusks and fish (Moreno-Gonzalez et al., 2016), with frequencies even higher than those reported in this series.

4. Conclusions

The current method, with a one-step miniaturized QuEChERS sample preparation, followed by both LC–MS/MS and GC–MS/MS analyses, allows the simultaneous determination of 360 toxic or potentially toxic environmental pollutants in small amounts of whole blood (250 μl). The analytical scope of this optimized and fully validated method includes a vast number of chemicals of environmental concern for wildlife, and also for humans. Thus, it includes: i) the most relevant POPs (organochlorine pesticides, polychlorinated biphenyls, polybrominated diphenyl ethers, and polycyclic aromatic hydrocarbons); ii) almost 90% of the active substances of the plant protection products included in the coordinated multi-annual plan of the EU for the investigation of residues in food of vegetable or animal origin; iii) the most commonly employed chemicals that are involved in deliberate poisoning of wildlife; iv) the most widely used anticoagulant rodenticides; v) pharmaceuticals, including many of those of major use in veterinary practice; vi) and a suite of metabolites that can be used as biomarkers of exposure. The application of the method to actual raptors samples allows to verify its suitability for biomonitoring studies and to glimpse its potential for obtaining valuable exposure data in ecotoxicological studies.

CRediT authorship contribution statement

Cristian Rial-Berriel: Conceptualization, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Andrea Acosta-Dacal:** Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Manuel Zumbado:** Investigation, Writing - original draft, Writing - review & editing. **Octavio P. Luzardo:** Conceptualization, Investigation, Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare no conflict of interest. This is an independent research. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Acknowledgements

This research was supported by the University of Las Palmas de Gran Canaria via a doctoral grant to the first author Cristian Rial-Berriel (ULPGC-012-2016), and also supported by the Spanish Ministry of Education, Culture and Sports via a doctoral grant to the co-first author Andrea Acosta-Dacal (FPU16-01888). The authors would like to thank people from Grupo de Rehabilitación de la Fauna Autóctona y su Hábitat (GREFA, Madrid, Spain), specially Dr. Fernando González González, and Mrs. Natalia Pastor Tiburón. We would also like to thank Mrs. Ana Macías Montes and Dr. Luis Alberto Henríquez Hernández for their assistance in the Laboratory of Toxicology of University of Las Palmas de Gran Canaria.

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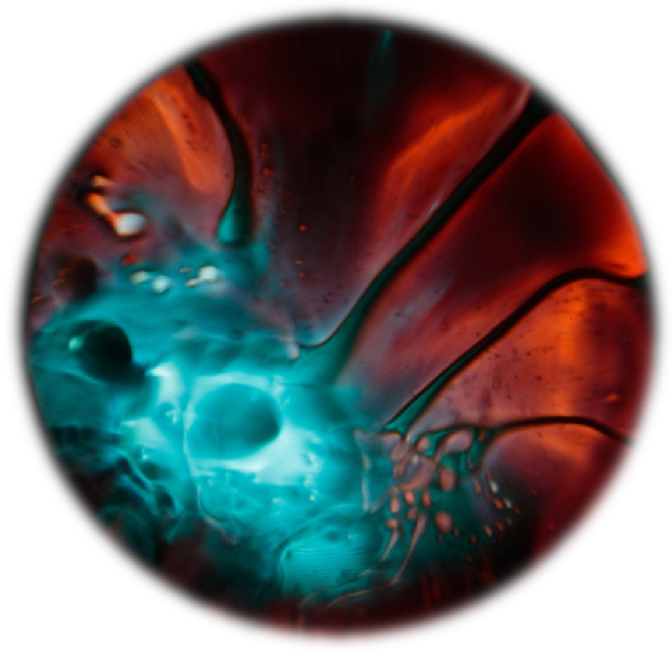
Publicación 2. Supporting dataset on the validation and verification of the analytical method for the biomonitoring of 360 toxicologically relevant pollutants in whole blood

Conjunto de datos de apoyo sobre la validación y verificación del método analítico para la biomonitorización de 360 contaminantes toxicológicamente relevantes en sangre

Data in Brief, 2020, 31: 105878

DOI: https://doi.org/10.1016/j.dib.2020.105878



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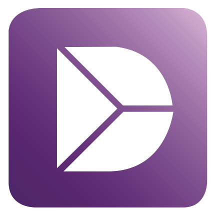

Data Article

Supporting dataset on the validation and verification of the analytical method for the biomonitoring of 360 toxicologically relevant pollutants in whole blood

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A R T I C L E   I N F O

Article history:
Received 19 May 2020
Revised 4 June 2020
Accepted 10 June 2020
Available online 18 June 2020

Keywords:
Persistent organic pollutants
Pesticides
Rodenticides
Veterinary pharmaceuticals
Barn owl
Common kestrel
GC-MS/MS
LC-MS/MS

A B S T R A C T

The dataset presented in this article supports "Micro QuEChERS-based method for the simultaneous biomonitoring in whole blood of 360 toxicologically relevant pollutants for wildlife" (Rial-Berriel et al., 2020). The supplementary data are: (1) Detailed validation data of the LC-MS/MS and GC-MS/MS methods for the quantification of 360 chemicals covering bias and precision (intra- and inter-day variability) for retention times, linearity, and limits of quantification. (2) Graphical data of the matrix effects on the quantification of all of the analytes. (3) Individual data of the 51 chemicals detected in real whole blood samples from two raptor species:

DOI of original article: 10.1016/j.scitotenv.2020.139444

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https://doi.org/10.1016/j.dib.2020.105878
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36 barn owls (*Tyto alba*) and 112 common kestrels (*Falco tinnunculus*).

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Specifications Table

Subject	Environmental Chemistry
Specific subject area	Analytical chemistry applied to biological samples to perform biomonitoring of environmental pollutants
Type of data	Tables and figures
How data were acquired	Ultra-high performance liquid chromatography tandem coupled to triple quadrupole mass spectrometry (LC-MS/MS). Agilent Technologies (Palo Alto, USA) models 1290 (UHPLC) and 6460 (MS/MS). Gas chromatography tandem coupled to triple quadrupole mass spectrometry (GC-MS/MS). Agilent Technologies (Palo Alto, USA) models 7890B (GC) and 7010 (MS/MS).
Data format	Analyzed: • Extracted and analyzed LC-MS/MS and GC-MS/MS data for the validation studies • Quantified chromatogram data of pollutants in the blood of barn owls and common kestrels Raw data: • All raw data corresponding to the 5 replicates of each concentration tested in the validation experiments presented in Table 1. • All individual quantitative data obtained after application of the method on 148 common kestrels and 39 barn owls presented in Tables 2–5. • All raw data used for the elaboration of Fig. 1.
Parameters for data collection	The validation data of the developed method included, limit of quantification (as the lowest calibrator that fulfilled de validation criteria), linearity, accuracy (expressed as % bias and precision (intra- and inter-day RSDs)) for each of the 360 analytes. The matrix effect on the quantification of the analytes is graphically represented as percentages. For clarity the results are expressed as relative percentage. The quantification data were obtained analyzing a series of 148 blood samples, obtained from a field ecology work in nest boxes of barn owls (*Tyto alba*, $n = 36$), and common kestrels (*Falco tinnunculus*, $n = 112$).
Description of data collection	MassHunter Quantitative Analysis was employed to collect and analyze the chromatographic data delivered by the triple quadrupole mass spectrometers coupled to both UHPLC and GC. The linearity was assessed by injecting a 12-point calibration curve prepared in the blank matrix and extracted with the developed micro-QuEChERS method. To test bias and precision, standard solutions of 360 reference standards were employed to spike blank whole blood samples at five concentration levels (0.1, 0.5, 1, 5, and 20 ng/ml) were injected in quintuplicate. The bias and repeatability (intra-day variability) were determined by those quintuplicate analyses of each sample, as these were injected within 24 h. The reproducibility (inter-day variability) was measured on three non-consecutive days within a two-week span. The matrix effect was assessed by extracting enough amount of blank matrix with the developed method and fortifying these extracts with three levels of the mixture of 360 chemicals (0.2, 2, and 10 ng/ml), and quantified against a calibration curve prepared in the solvent (1% FA-acetonitrile). Regarding the quantitative data, the real samples were prepared with the developed methodology and analyzed by UHPLC and GC.

(*continued on next page*)

Data source location	Institution: Toxicology Unit, Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria City/Town/Region: Las Palmas de Gran Canaria Country: Spain
Data accessibility	With the article. Raw data are provided
Related research article	Rial-Berriel, C., Acosta-Dacal, A., Zumbado, M., Luzardo, O.P. Micro QuEChERS-based methodology for the simultaneous biomonitoring in whole blood of 360 toxicologically relevant pollutants for wildlife. Science of the Total Environment 736 (2020) 13944

Value of the Data

- An easy way to consult the validation data of the methodological development detailed in the main article is provided.
- Additionally, the validation data might serve as a reference to other researchers developing methods in complex biological matrices.
- The detail of the matrix effect on each of the 360 compounds analyzed may allow other researchers to decide whether they need to prepare their calibration lines in matrix or in solvent.
- Data on numerous environmental pollutants are presented for the first time for two apex predators: barn owl (*Tyto alba*) and kestrel (*Falco tinnunculus*).
- The biomonitoring data presented can be used by other researchers in ecotoxicology for comparison purposes, and for meta-analyses of chemical contamination in birds of prey.

1. Data Description

Table 1 shows the major validation parameters of each of the 360 chemicals optimized in this methodology. Validation criteria fulfilled those established in the SANTE guidelines [1]. Given the particularities of the matrix - whole blood - the guidelines of the SWGTOX were also considered, especially concerning the preparation of the matrix-matched calibration curve [2]. The data reported in this table complement those included in Table 1 of the article by Rial-Berriel et al. [3], where the parameters of identity and selectivity of each compound are shown. Now, in this table, the data of linearity (as a working range), the LOQ (set as the lowest point of the calibration curve that complied identity, bias and precision criteria), repeatability (as intraday RSD), and reproducibility (as interday RSD) are shown for each of the chemicals. The bias and precision (repeatability and reproducibility) data are presented for five fortification levels (0.1, 0.5, 1, 5, and 20 ng/ml). Raw data of the five replicates for each concentration are provided in the supplementary file 1.

Fig. 1 shows the effect that the components of the matrix have on the quantification of each chemical substance, to demonstrate the need to perform said quantification with calibrators prepared in a white matrix for at least 45% of the compounds. Results are shown as a relative percentage quantified by ACN calibration curve. When the differential is greater 20% or −20%, matrix interference is considered to exist. For clarity, the compounds are identified numerically, from 1 to 360, as they are numbered in Table 1. Raw data of the six charts of this figure are provided in the supplementary file 2.

Tables 2 and 3 show the quantitative results of the contaminants found in 36 barn owls (*Tyto Alba*) and Tables 4 and 5, show those found in 112 common kestrels (*Falco tinnunculus*). The results of persistent and non-persistent organic pollutants are presented separately. For clarity, only contaminants that have tested positive for at least one individual are shown. Raw data of the quantification of the 360 pollutants in these 187 birds are provided in the supplementary file 3.

Table 1
Results of validation process: LOQ, linearity, bias and precision (intraday and interday).

				0.1 ng/ml			0.5 ng/ml			1 ng/ml			5 ng/ml			20 ng/ml		
					Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
N°	Compound	LOQ	Linearity	Bias (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
1	2-Phenylphenol	0.20	0.9946	N/A	N/A	N/A	110.10	8,99	11.18	100.03	8,25	6.86	86.09	6,67	8.32	100.98	13,03	1.62
2	4.4′-Dichlorobenzophenone (metabolite of dicofol)	0.80	0.9905	N/A	N/A	N/A	N/A	N/A	N/A	100.35	8,41	12.21	101.69	6,90	17.71	96.39	13,85	1.72
3	Abamectine	4.00	0.9784	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	107.86	6,77	14.19	102.58	15,24	4.37
4	Acenaphthene	0.20	0.9889	N/A	N/A	N/A	83.21	17,09	8.12	124.25	9,64	7.02	94.92	6,78	10.11	96.71	7,51	8.70
5	Acenaphtylene	0.20	0.9792	N/A	N/A	N/A	108.10	14,25	15.63	99.81	10,39	5.64	94.84	15,98	10.11	102.65	7,26	9.23
6	Acephate	2.00	0.9879	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	119.99	13,67	0.16	103.25	7,44	12.14
7	Acetaminophen (paracetamol)	1.20	0.9548	N/A	N/A	N/A	N/A	N/A	N/A	126.11	7,73	13.96	79.54	17,10	13.04	105.72	14,92	29.30
8	Acetamiprid	0.40	0.9967	N/A	N/A	N/A	113.51	14,76	3.73	97.67	11,75	2.41	104.22	17,94	4.72	95.95	7,89	1.24
9	Acrinathrin	1.20	0.9943	N/A	N/A	N/A	N/A	N/A	N/A	109.46	9,36	10.87	93.81	6,62	5.87	99.70	16,60	9.75
10	Albendazole	0.10	0.9952	121.06	10,61	8.38	96.22	9,08	10.63	99.00	10,54	1.33	108.29	17,77	3.44	96.38	14,56	0.81
11	Aldicarb	0.10	0.9956	118.27	18,28	14.53	98.52	10,06	4.99	92.82	8,85	1.70	102.55	6,62	5.28	95.35	13,66	3.09
12	Aldicarb-sulfone	0.40	0.9966	N/A	N/A	N/A	118.79	12,30	5.58	93.35	10,83	7.71	99.82	6,62	4.56	96.53	7,31	1.87
13	Aldicarb-sulfoxide	1.60	0.9936	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	100.26	6,62	1.11	94.55	7,50	0.14
14	Aldrin	0.40	0.9951	N/A	N/A	N/A	95.34	15,89	4.08	97.07	13,35	9.52	95.19	6,86	0.34	97.66	7,25	1.76
15	Anthracene	0.80	0.9579	N/A	N/A	N/A	N/A	N/A	N/A	114.90	7,72	6.49	92.78	14,13	9.89	95.94	14,59	8.63
16	Atrazine	0.10	0.9960	107.95	8,69	1.74	97.69	16,36	17.22	93.12	13,51	3.34	103.37	15,25	5.44	95.62	8,06	0.71
17	Azinphos-methyl	0.20	0.9961	N/A	N/A	N/A	108.57	8,99	1.85	95.64	10,75	3.62	107.13	15,31	3.49	94.57	13,68	0.15
18	Azoxystrobin	0.10	0.9972	103.13	15,68	1.38	96.51	8,98	17.06	97.09	10,29	4.55	104.23	17,56	7.15	97.49	15,71	4.20
19	BDE-28	0.20	0.9932	N/A	N/A	N/A	103.43	8,92	12.34	101.66	8,32	5.74	90.52	6,73	9.65	90.60	17,40	8.15
20	BDE-47	0.20	0.9924	N/A	N/A	N/A	96.70	15,12	7.67	100.78	11,76	5.69	91.21	17,86	9.72	94.25	8,12	8.48
21	BDE-85	0.10	0.9954	129.76	10,28	5.15	91.22	13,95	11.00	101.98	12,44	5.76	86.00	7,35	9.16	99.65	7,39	8.96
22	BDE-99	0.10	0.9934	91.23	5,37	4.22	105.76	14,85	14.54	103.28	13,00	5.83	94.33	6,64	10.05	94.77	7,85	8.52
23	BDE-100	0.10	0.9873	109.21	8,67	7.98	89.95	10,40	11.20	101.34	10,20	5.72	92.07	6,78	9.81	85.36	16,69	7.68
24	BDE-153	0.20	0.9951	N/A	N/A	N/A	86.36	13,46	10.95	102.38	10,65	5.78	98.11	6,62	10.45	95.42	7,26	8.58
25	BDE-154	0.10	0.9954	111.23	7,50	8.20	109.41	9,31	11.22	101.80	8,92	5.75	94.84	15,34	10.11	91.95	15,43	8.27
26	BDE-183	0.20	0.9815	N/A	N/A	N/A	98.77	10,04	7.64	103.39	8,47	5.84	98.97	14,04	10.55	103.42	15,38	9.30
27	Benalaxyl	0.10	0.9978	97.07	10,61	4.88	91.75	10,74	5.04	96.40	9,17	0.54	102.02	17,93	2.34	98.23	14,16	2.16
28	Bendiocarb	0.10	0.9961	106.43	17,38	11.19	98.54	16,51	11.11	88.59	11,92	0.30	101.12	17,69	7.81	95.22	7,38	1.33
29	Bendiocarb metabolite (2. 2-dimethylbenzo-1. 3-dioxol-4-ol)	1.20	0.9968	N/A	N/A	N/A	N/A	N/A	N/A	100.90	12,67	0.91	82.48	6,69	8.04	99.56	13,52	3.43
30	Benfuracarb	0.10	0.9950	86.06	8,05	10.19	103.65	14,71	15.73	99.20	13,07	2.80	106.49	13,63	4.43	95.72	14,04	5.01
31	Benzo[a]anthracene	0.80	0.9805	N/A	N/A	N/A	N/A	N/A	N/A	111.54	7,78	6.30	94.91	6,69	10.11	89.36	9,08	8.04
32	Benzo[a]pyrene	0.10	0.9948	124.45	14,20	9.32	95.76	14,39	6.53	112.23	10,04	6.34	95.02	6,67	10.13	101.13	12,65	9.09
33	Benzo[b]fluoranthene	0.80	0.9766	N/A	N/A	N/A	N/A	N/A	N/A	105.05	8,54	5.93	92.65	6,62	9.87	98.55	10,27	8.86
34	Benzo[ghi]perylene	0.40	0.9912	N/A	N/A	N/A	119.14	9,12	7.22	111.18	12,61	6.28	99.29	6,67	10.58	103.47	9,06	9.30

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Table 1 (*continued*)

				0.1 ng/ml			0.5 ng/ml			1 ng/ml			5 ng/ml			20 ng/ml		
					Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
N°	Compound	LOQ	Linearity	Bias (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
35	Benzo[k]fluoranthene	0.40	0.9323	N/A	N/A	N/A	95.12	9,17	9.12	95.35	15,14	5.39	100.05	16,54	10.66	94.17	10,62	8.47
36	Bifenthrin	0.20	0.9952	N/A	N/A	N/A	98.48	13,53	12.82	102.14	10,00	1.84	89.51	18,10	3.56	95.27	13,67	5.57
37	Bitertanol	0.40	0.9938	N/A	N/A	N/A	105.62	14,27	15.92	105.68	9,81	13.20	102.68	10,19	5.54	96.23	14,00	7.53
38	Boscalid (formerly nicobifen)	0.10	0.9974	112.80	9,55	7.39	101.63	14,86	4.10	97.54	10,08	6.17	84.80	10,06	7.47	100.58	12,54	5.73
39	Brodifacoum	0.80	0.9806	N/A	N/A	N/A	N/A	N/A	N/A	92.73	13,12	5.11	96.45	15,56	15.43	100.49	9,93	12.33
40	Bromadiolone	0.40	0.9864	N/A	N/A	N/A	116.06	10,17	18.62	89.90	9,56	5.46	112.93	9,46	3.98	100.80	16,46	0.11
41	Bromopropylate	0.20	0.9887	N/A	N/A	N/A	99.38	14,28	11.80	102.94	12,91	14.65	88.10	13,47	2.10	98.29	9,89	17.90
42	Bromuconazole (two isomers)	0.20	0.9821	N/A	N/A	N/A	97.91	12,92	8.33	98.52	13,61	11.16	87.58	14,54	19.50	99.45	9,70	9.08
43	Bupirimate	0.20	0.9940	N/A	N/A	N/A	97.15	13,95	10.65	97.41	12,47	1.67	88.40	9,36	2.98	97.21	10,00	5.20
44	Buprofezin	0.10	0.9930	111.89	12,12	7.55	93.70	10,78	4.48	91.32	8,97	3.26	96.80	11,53	3.61	97.21	15,68	7.98
45	Cadusafos (ebufos)	0.10	0.9956	86.62	9,70	9.96	94.38	9,34	11.50	103.53	9,81	5.16	105.85	13,64	4.40	95.85	15,07	2.52
46	Carbaryl	0.10	0.9978	106.07	9,25	5.31	94.48	9,87	3.35	98.38	10,04	9.69	105.00	13,67	4.09	96.64	14,97	3.48
47	Carbendazim (azole)	0.40	0.9965	N/A	N/A	N/A	118.80	13,28	3.68	94.83	13,87	3.95	101.66	13,34	4.74	97.47	9,11	2.27
48	Carbofuran	0.10	0.9959	118.09	12,71	9.84	93.02	11,68	15.57	90.26	9,61	3.87	98.83	12,60	9.04	96.21	14,66	4.67
49	Carbofuran-3-hydroxy	0.40	0.9963	N/A	N/A	N/A	117.69	12,90	6.37	96.99	13,89	4.38	103.10	13,92	4.90	95.70	9,44	2.86
50	Carbosulfan	0.40	0.9781	N/A	N/A	N/A	132.53	12,51	5.32	84.83	13,15	18.63	110.47	9,64	9.70	105.49	9,25	3.40
51	Cefuroxima axetil (two isomers)	0.80	0.9902	N/A	N/A	N/A	N/A	N/A	N/A	105.25	14,44	3.61	100.04	10,23	5.53	104.99	8,89	2.88
52	Chloramphenicol	2.00	0.9814	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	120.55	9,33	11.01	102.82	12,43	9.86
53	Chlorantraniliprole	0.20	0.9952	N/A	N/A	N/A	107.14	9,74	13.70	91.93	9,32	10.91	104.44	9,61	6.25	95.70	13,94	4.00
54	Chlorfenapyr	1.20	0.9936	N/A	N/A	N/A	N/A	N/A	N/A	97.07	8,89	12.36	85.57	13,88	11.55	106.01	14,26	1.61
55	Chlorfenvinphos	0.20	0.9969	N/A	N/A	N/A	96.71	14,12	11.93	100.05	14,95	5.73	102.06	9,37	0.48	96.97	9,73	0.29
56	Chlorobenzilate	0.40	0.9909	N/A	N/A	N/A	88.96	9,99	9.32	105.63	8,87	5.97	87.66	15,64	9.34	89.59	14,82	8.06
57	Chlorophacinone	0.80	0.9874	N/A	N/A	N/A	N/A	N/A	N/A	96.82	12,73	1.94	103.81	13,52	2.08	100.62	9,72	4.45
58	Chlorpropham	0.20	0.9949	N/A	N/A	N/A	110.12	13,64	10.89	101.16	13,17	9.22	85.27	16,60	9.82	97.24	10,40	9.23
59	Chlorpyrifos	0.80	0.9915	N/A	N/A	N/A	N/A	N/A	N/A	100.64	14,14	10.32	91.52	15,69	7.57	100.79	10,24	7.46
60	Chlorpyrifos methyl	0.40	0.9951	N/A	N/A	N/A	103.73	9,45	7.79	104.81	9,61	13.90	88.99	10,69	10.67	99.45	13,30	4.76
61	Chlorthal dimethyl	0.20	0.9874	N/A	N/A	N/A	91.40	11,74	4.48	107.91	8,89	7.07	90.69	9,27	3.38	96.20	14,69	7.87
62	Chrysene	0.80	0.9789	N/A	N/A	N/A	N/A	N/A	N/A	109.29	9,22	6.17	93.19	9,29	9.93	94.31	13,57	8.48
63	Clindamycin	0.40	0.9970	N/A	N/A	N/A	116.37	11,77	4.52	96.24	12,64	9.31	104.48	10,53	6.42	97.59	9,89	1.83
64	Clofentezine	0.40	0.9944	N/A	N/A	N/A	101.79	9,49	2.24	96.83	9,70	1.32	107.77	13,16	5.27	98.86	14,77	0.82
65	Clothianidin	1.20	0.9941	N/A	N/A	N/A	N/A	N/A	N/A	89.69	13,57	2.55	100.83	9,18	9.29	93.83	9,88	2.69
66	Cloxacillin	1.60	0.9803	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	110.09	14,70	3.00	101.78	9,10	3.38
67	Cortiscosterone 21 acetate	0.80	0.9948	N/A	N/A	N/A	N/A	N/A	N/A	116.41	14,25	25.41	105.76	12,62	1.63	97.81	9,06	4.02
68	Coumachlor	0.20	0.9948	N/A	N/A	N/A	95.39	9,51	2.10	101.51	9,31	2.76	109.44	14,42	6.63	99.44	16,00	6.43
69	Coumaphos	0.10	0.9977	116.47	12,99	9.03	87.29	10,10	2.13	93.40	8,98	5.88	102.10	12,07	4.65	98.22	13,49	5.08
70	Coumatetralyl	0.40	0.9791	N/A	N/A	N/A	104.44	9,23	14.38	104.06	9,03	13.27	91.59	9,96	1.15	98.21	14,37	11.04

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Table 1 (*continued*)

N°	Compound	LOQ	Linearity	0.1 ng/ml			0.5 ng/ml			1 ng/ml			5 ng/ml			20 ng/ml		
					Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
				Bias (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
71	Cyazofamid	0.80	0.9973	N/A	N/A	N/A	N/A	N/A	N/A	106.76	12,52	2.68	103.61	10,04	6.11	98.92	9,10	1.86
72	Cyflufenamid	0.20	0.9936	N/A	N/A	N/A	85.26	11,55	2.59	112.30	9,20	13.66	110.04	9,40	2.12	96.14	14,03	7.41
73	Cyfluthrin (sum of four isomers)	1.20	0.9923	N/A	N/A	N/A	N/A	N/A	N/A	102.22	15,22	2.03	89.47	10,43	0.08	100.93	9,55	0.81
74	Cyhalothrin (lambda isomer)	2.00	0.9938	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	95.52	15,51	10.00	100.53	9,65	1.89
75	Cymoxanil	0.40	0.9975	N/A	N/A	N/A	114.66	12,65	10.00	96.84	14,55	1.40	101.03	9,71	5.35	96.68	10,08	1.83
76	Cypermethrin (sum of four isomers)	4.00	0.9886	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	94.85	13,60	7.83	102.11	14,45	7.39
77	Cyproconazole (two isomers)	0.40	0.9952	N/A	N/A	N/A	102.85	10,03	18.32	98.08	9,04	4.73	104.80	13,46	5.60	96.12	14,27	2.98
78	Cyprodinil	0.20	0.9945	N/A	N/A	N/A	109.65	9,34	10.35	95.04	10,01	19.09	103.39	14,24	5.60	95.75	12,78	3.91
79	Cyromazine	2.00	0.9904	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	97.88	15,22	0.44	101.73	10,16	2.26
80	Danofloxacin	1.20	0.9698	N/A	N/A	N/A	N/A	N/A	N/A	87.87	15,46	4.73	88.06	9,23	1.32	95.42	16,22	21.72
81	Dazomet	1.60	0.9954	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	107.37	10,51	9.72	100.18	9,74	4.16
82	Deltamethrin	0.80	0.9927	N/A	N/A	N/A	N/A	N/A	N/A	104.72	7,30	11.36	118.20	12,06	2.60	98.42	8,92	1.75
83	Demeton-S-methyl	0.10	0.9966	113.64	12,45	4.39	98.56	13,75	7.34	91.34	8,63	1.66	100.20	14,19	7.48	95.83	12,90	0.52
84	Demeton-S-methyl-sulfone (Dioxydemeton)	0.40	0.9962	N/A	N/A	N/A	116.45	10,04	12.58	98.84	15,84	1.65	101.43	12,71	5.48	97.04	9,37	0.23
85	Dexamethasone	0.40	0.9920	N/A	N/A	N/A	129.03	9,03	9.22	100.41	12,98	4.52	102.29	13,81	11.14	94.51	10,33	0.39
86	Diazinon	0.40	0.9923	N/A	N/A	N/A	106.32	10,31	1.65	110.27	15,60	13.64	90.53	9,96	8.70	97.56	10,05	3.30
87	Dibenzo[a.h]anthracene	0.40	0.9884	N/A	N/A	N/A	122.87	13,55	14.78	106.79	7,27	6.03	95.70	13,68	10.20	100.26	14,21	9.02
88	Dichlorodiphenyldichloroethane (p.p' DDD)	0.10	0.9783	93.23	6,40	7.21	95.01	10,77	9.24	115.50	12,04	6.52	88.01	14,59	9.38	86.76	10,58	7.80
89	Dichlorodiphenyldichloroethylene (p.p' DDE)	0.10	0.9848	94.34	8,74	5.45	95.01	15,21	7.23	115.50	7,37	6.52	88.01	13,38	9.38	86.76	15,66	7.80
90	Dichlorodiphenyltrichloroethane (p.p' DDT)	1.20	0.9740	N/A	N/A	N/A	N/A	N/A	N/A	124.05	7,26	4.15	82.09	11,36	19.34	97.14	12,26	17.84
91	Diclofenac	0.80	0.9677	N/A	N/A	N/A	N/A	N/A	N/A	79.69	9,75	18.79	104.25	15,50	6.86	104.76	14,33	0.55
92	Dicloran	0.10	0.9836	123.22	9,55	8.22	108.31	9,22	11.23	114.09	14,60	6.44	107.57	9,01	11.46	99.19	9,49	8.92
93	Dicloxacillin	1.20	0.9832	N/A	N/A	N/A	N/A	N/A	N/A	118.47	13,71	25.92	107.63	9,22	2.05	94.46	11,45	8.19
94	Dieldrin	1.20	0.9916	N/A	N/A	N/A	N/A	N/A	N/A	90.79	16,01	2.75	93.04	10,08	18.65	102.23	10,10	6.68
95	Diethathyl ethyl	0.20	0.9949	N/A	N/A	N/A	101.03	13,95	15.93	97.12	8,14	6.24	109.86	9,80	1.29	97.92	13,74	5.33
96	Diethofencarb	0.10	0.9974	109.28	9,41	9.22	99.69	10,24	14.33	90.50	16,03	5.04	103.94	14,12	11.39	97.29	9,31	3.12
97	Difenacoum	0.20	0.9821	N/A	N/A	N/A	106.25	12,62	0.67	97.13	7,96	1.76	89.59	15,15	10.58	101.79	12,53	13.24
98	Difenoconazole	0.40	0.9962	N/A	N/A	N/A	110.55	14,29	2.18	97.28	7,66	6.95	103.07	15,99	5.52	98.83	15,16	3.65
99	Difethialone	0.80	0.9710	N/A	N/A	N/A	N/A	N/A	N/A	95.73	7,39	17.88	91.34	16,61	15.31	102.52	12,84	22.89
100	Difloxacin	0.80	0.9702	N/A	N/A	N/A	N/A	N/A	N/A	74.73	16,49	11.56	95.04	9,89	3.47	97.27	10,00	24.41
101	Diflubenzuron	1.20	0.9909	N/A	N/A	N/A	N/A	N/A	N/A	82.74	13,96	13.97	104.38	14,08	2.84	97.93	9,13	8.15
102	Diflufenican	0.10	0.9943	104.94	10,18	11.58	95.70	10,05	10.45	85.39	14,12	4.30	110.99	9,10	5.78	99.81	9,32	0.67
103	Dimethenamid-P (and its R-isomer)	0.10	0.9963	127.37	6,66	4.97	101.55	13,43	3.07	91.45	8,20	11.35	91.32	9,36	16.95	96.98	14,60	0.53
104	Dimethoate	0.40	0.9967	N/A	N/A	N/A	122.10	9,22	0.83	98.13	15,13	4.07	102.92	10,29	6.04	97.11	11,18	0.20
105	Dimethomorph (two isomers)	0.40	0.9966	N/A	N/A	N/A	117.73	13,92	4.31	97.14	8,28	2.29	101.37	9,10	8.66	97.41	15,53	2.53

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Table 1 (*continued*)

				0.1 ng/ml			0.5 ng/ml			1 ng/ml			5 ng/ml			20 ng/ml		
					Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
N°	Compound	LOQ	Linearity	Bias (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
106	Dimethylphenylsulfamide (DMSA. metabolite of dichlofluanid)	0.80	0.9971	N/A	N/A	N/A	N/A	N/A	N/A	99.37	8,54	5.30	97.54	13,42	8.62	98.06	12,18	1.27
107	Diniconazole-M	0.20	0.9972	N/A	N/A	N/A	110.11	12,82	18.86	86.32	9,11	8.30	103.99	14,19	10.86	98.08	14,05	0.98
108	Dinocap	0.80	0.9847	N/A	N/A	N/A	**N/A**	N/A	**N/A**	90.93	15,41	5.14	102.73	15,20	10.95	95.70	9,66	8.61
109	Diphacinone	1.20	0.9892	N/A	N/A	N/A	N/A	N/A	N/A	115.88	13,56	4.43	105.36	16,11	8.05	98.59	9,73	5.59
110	Diphenylamine	0.20	0.9938	N/A	N/A	N/A	104.56	10,04	10.33	100.70	16,33	1.21	81.45	9,71	11.19	103.06	9,82	2.82
111	Dodine	0.40	0.9953	N/A	N/A	N/A	98.40	15,62	11.83	102.50	8,23	1.46	101.49	12,47	2.92	95.74	15,22	2.74
112	Endosulfan alfa	0.80	0.9931	N/A	N/A	N/A	N/A	N/A	N/A	101.76	12,52	13.79	86.34	9,89	13.22	100.60	9,22	2.10
113	Endosulfan beta	0.80	0.9859	N/A	N/A	N/A	N/A	N/A	N/A	116.18	8,01	17.11	94.87	8,97	15.61	102.32	12,87	8.66
114	Endosulfan sulfate	0.80	0.9884	N/A	N/A	N/A	N/A	N/A	N/A	111.36	7,97	4.46	99.14	10,28	12.76	101.10	13,93	4.21
115	Endrin	1.60	0.9961	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	105.62	8,92	11.25	83.94	14,41	7.55
116	Enrofloxacin	1.20	0.9569	N/A	N/A	N/A	N/A	N/A	N/A	90.24	14,45	1.61	79.66	13,76	16.02	92.99	11,50	34.07
117	EPN	0.80	0.9873	N/A	N/A	N/A	N/A	N/A	N/A	100.07	16,63	3.78	94.94	15,70	17.91	98.99	9,19	2.59
118	Epoxiconazole	0.20	0.9966	N/A	N/A	N/A	109.95	10,50	2.71	87.76	17,90	15.32	99.14	13,04	5.31	97.64	9,13	1.60
119	Eprinomectin	0.20	0.9878	N/A	N/A	N/A	121.34	13,63	9.72	99.91	7,56	12.53	112.05	8,92	1.98	106.09	11,98	0.52
120	Eritromicin	0.20	0.9967	N/A	N/A	N/A	105.88	10,86	6.29	96.88	16,44	3.65	103.97	9,77	6.11	96.79	10,70	3.68
121	Esfenvalerate	2.00	0.9936	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	96.38	10,37	3.14	96.89	13,24	5.12
122	Ethion (diethion)	0.10	0.9958	103.66	7,72	0.78	89.88	15,17	3.73	95.28	7,88	3.77	108.49	8,87	4.58	100.49	15,28	4.16
123	Ethirimol	0.40	0.9964	N/A	N/A	N/A	122.23	12,67	1.90	96.57	12,01	0.27	104.84	12,91	3.65	98.37	13,21	4.56
124	Ethofumesate	0.80	0.9846	N/A	N/A	N/A	N/A	N/A	N/A	91.55	10,20	4.97	82.64	18,27	14.03	98.52	10,25	11.95
125	Ethoprophos	0.20	0.9953	N/A	N/A	N/A	96.56	9,60	16.85	96.29	10,50	4.92	102.00	14,06	7.17	95.03	11,29	2.62
126	Etofenprox	0.80	0.9963	N/A	N/A	N/A	N/A	N/A	N/A	98.75	10,23	4.30	93.90	15,00	7.98	100.65	9,10	5.37
127	Etoxazole	0.10	0.9972	106.66	6,86	18.17	87.77	16,23	9.12	91.35	9,95	7.34	103.72	9,01	3.18	100.75	13,94	4.65
128	Famoxadone	1.20	0.9942	N/A	N/A	N/A	N/A	N/A	N/A	109.69	9,40	14.42	101.73	12,93	9.40	98.87	10,38	3.93
129	Fenamidone	0.10	0.9976	100.84	9,44	2.17	102.38	12,60	9.41	99.89	9,41	9.34	105.57	9,85	4.55	97.86	13,99	2.26
130	Fenamiphos	0.10	0.9976	92.87	15,36	1.25	94.57	14,70	9.17	99.28	8,99	1.65	107.18	9,85	5.85	95.32	13,62	3.93
131	Fenamiphos sulfone	0.20	0.9971	N/A	N/A	N/A	109.57	13,30	6.60	90.42	10,49	0.60	103.95	8,98	7.13	96.59	15,51	1.85
132	Fenamiphos sulfoxide	0.40	0.9963	N/A	N/A	N/A	120.76	9,74	4.40	96.93	9,25	3.43	99.10	9,42	7.16	97.96	10,91	0.25
133	Fenarimol	0.20	0.9960	N/A	N/A	N/A	107.08	10,55	8.23	100.38	7,97	1.42	91.15	14,84	3.62	98.31	9,95	0.95
134	Fenazaquin	0.80	0.9967	N/A	N/A	N/A	N/A	N/A	N/A	103.00	7,70	4.49	107.95	15,93	4.82	99.17	17,83	1.81
135	Fenbendazole	0.10	0.9959	117.39	10,22	0.85	96.83	15,21	7.43	92.86	10,50	12.54	111.90	15,15	3.55	96.47	6,84	0.78
136	Fenbuconazole	0.40	0.9943	N/A	N/A	N/A	106.97	10,09	11.96	97.78	8,02	4.11	82.91	16,31	2.82	96.67	17,54	4.23
137	Fenbutatin oxide	0.80	0.9705	N/A	N/A	N/A	N/A	N/A	N/A	101.12	10,91	2.09	101.73	10,08	2.23	109.08	6,62	3.83
138	Fenhexamid	1.60	0.9952	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	94.50	12,93	5.93	100.97	6,91	1.63
139	Fenitrothion	0.20	0.9946	N/A	N/A	N/A	96.00	13,47	1.84	104.50	10,63	18.04	89.65	10,40	10.92	98.77	6,63	0.21
140	Fenoxycarb	0.10	0.9950	106.29	15,35	14.48	89.97	9,71	10.85	88.98	8,95	0.61	108.29	14,13	1.92	101.10	17,34	1.20

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Table 1 (*continued*)

				0.1 ng/ml			0.5 ng/ml			1 ng/ml			5 ng/ml			20 ng/ml		
					Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
N°	Compound	LOQ	Linearity	Bias (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
141	Fenpropathrin	0.40	0.9932	N/A	N/A	N/A	96.77	9,48	4.11	108.60	7,36	7.89	114.09	15,57	4.22	98.64	14,32	2.46
142	Fenpropidin	0.10	0.9971	107.57	7,02	6.44	95.01	9,25	14.96	95.57	8,20	6.27	104.76	14,60	3.69	98.78	16,42	2.87
143	Fenpropimorph	0.10	0.9967	112.10	6,69	5.06	91.37	15,36	6.27	98.02	11,73	3.30	105.01	7,29	4.62	98.31	7,08	2.04
144	Fenpyroximate	0.40	0.9972	N/A	N/A	N/A	108.10	9,24	11.74	98.03	9,60	3.42	110.63	7,30	7.96	99.89	14,81	3.51
145	Fenthion	0.10	0.9907	101.39	8,20	9.98	98.48	14,20	6.59	100.84	10,07	1.09	93.69	7,24	3.06	97.74	6,90	2.60
146	Fenthion oxon	0.10	0.9981	102.81	9,49	11.00	93.65	12,81	5.77	95.62	9,66	2.50	103.73	7,29	4.97	97.83	6,63	1.80
147	Fenthion oxon sulfone	0.80	0.9978	N/A	N/A	N/A	N/A	N/A	N/A	99.68	9,78	2.02	101.55	16,22	6.88	98.19	6,93	2.88
148	Fenthion oxon sulfoxide	0.20	0.9977	N/A	N/A	N/A	119.70	9,46	1.24	99.13	9,09	5.16	101.41	7,40	5.89	97.91	18,80	0.84
149	Fenthion sulfone	0.80	0.9980	N/A	N/A	N/A	N/A	N/A	N/A	102.66	8,33	0.50	101.78	13,19	9.01	98.32	17,66	2.07
150	Fenthion sulfoxide	0.40	0.9991	N/A	N/A	N/A	106.77	10,32	6.95	103.30	9,24	8.77	103.57	16,11	4.88	95.47	15,28	1.90
151	Fenvalerate	2.00	0.9939	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	91.05	15,63	10.82	98.09	6,62	1.48
152	Fipronil	0.20	0.9946	N/A	N/A	N/A	104.05	9,16	2.31	90.35	10,22	2.29	101.81	13,15	4.10	100.66	15,46	0.15
153	Fipronil sulfide	0.80	0.9391	N/A	N/A	N/A	N/A	N/A	N/A	98.90	11,00	8.97	84.35	7,92	13.73	95.07	7,23	27.31
154	Flocoumafen	0.20	0.9737	N/A	N/A	N/A	115.03	13,55	2.97	97.42	10,83	10.78	81.77	8,58	6.71	99.46	6,62	19.13
155	Fluazinam	0.20	0.9942	N/A	N/A	N/A	109.04	13,34	9.01	95.48	10,76	4.20	101.92	7,31	1.50	96.92	6,62	2.29
156	Flubendiamide	2.00	0.9829	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	91.98	8,33	10.12	95.45	17,55	9.10
157	Flucythrinate (two isomers)	0.80	0.9951	N/A	N/A	N/A	N/A	N/A	N/A	100.00	9,93	2.37	93.42	16,52	4.71	99.66	14,19	4.20
158	Fludioxonil	0.20	0.9924	N/A	N/A	N/A	99.12	9,56	6.85	96.89	9,31	6.19	88.02	7,25	3.14	98.96	17,89	9.67
159	Flufenoxuron	0.10	0.9942	107.97	6,05	14.68	88.19	14,34	10.25	88.31	12,00	4.19	112.03	7,62	4.34	102.01	6,75	2.92
160	Flumequine	0.10	0.9876	106.13	8,91	23.04	100.69	11,01	10.95	91.44	8,68	9.51	96.97	9,32	10.04	91.89	15,61	17.35
161	Flunixin	0.20	0.9949	N/A	N/A	N/A	96.90	14,66	4.63	91.59	9,37	1.62	110.14	7,43	2.78	98.17	6,63	0.65
162	Fluopyram	0.20	0.9958	N/A	N/A	N/A	110.51	12,65	3.31	97.01	11,93	0.48	94.66	9,95	8.92	96.17	6,77	6.46
163	Fluoranthene	0.20	0.9887	N/A	N/A	N/A	109.74	13,17	6.76	104.20	11,02	5.89	94.00	7,25	10.02	85.54	7,48	7.69
164	Fluorene	0.20	0.9836	N/A	N/A	N/A	148.49	9,00	13.22	111.31	8,80	6.29	92.49	16,86	9.86	93.39	17,74	8.40
165	Fluquinconazole	0.20	0.9869	N/A	N/A	N/A	103.74	10,15	12.58	96.88	8,10	4.65	88.44	17,22	1.18	96.68	17,03	13.29
166	Flusilazole	0.20	0.9946	N/A	N/A	N/A	94.54	9,43	10.00	89.82	10,64	0.48	114.38	15,47	2.02	98.57	14,14	2.32
167	Flutolanil	0.10	0.9975	106.84	7,89	11.91	89.48	13,65	6.56	100.18	11,64	5.67	106.33	16,01	3.74	98.51	6,62	2.19
168	Flutriafol	0.20	0.9953	N/A	N/A	N/A	96.42	10,14	7.37	105.15	9,45	8.63	90.02	8,07	2.90	96.88	17,53	0.89
169	Fluvalinate tau	4.00	0.9356	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	90.97	7,25	5.31	106.96	6,69	10.74
170	Fonofos	0.40	0.9924	N/A	N/A	N/A	104.18	13,29	14.80	101.25	11,79	6.87	87.63	7,29	10.09	97.22	6,69	4.61
171	Formetanate	0.10	0.9967	106.33	13,02	9.84	96.75	14,29	0.48	94.60	10,34	0.85	109.06	7,56	4.34	100.01	6,95	5.73
172	Fosthiazate	0.10	0.9972	106.58	11,43	10.48	97.01	9,22	10.28	95.35	8,80	6.45	101.87	15,78	5.69	96.64	14,66	1.77
173	Heptachlor	0.80	0.9722	N/A	N/A	N/A	N/A	N/A	N/A	87.12	8,39	4.92	139.37	7,28	14.85	92.22	14,83	8.29
174	Hexachlorobencene	0.20	0.9858	N/A	N/A	N/A	99.79	9,37	9.45	105.21	8,36	5.94	93.97	16,62	10.01	93.24	15,11	8.38
175	Hexachlorocyclohexane (alpha)	0.40	0.9818	N/A	N/A	N/A	N/A	N/A	N/A	104.74	10,64	5.92	104.70	13,30	11.16	102.65	6,65	9.23
176	Hexachlorocyclohexane (beta)	0.40	0.9456	N/A	N/A	N/A	N/A	N/A	N/A	113.08	7,52	6.39	132.60	13,78	14.13	109.11	14,55	9.81
177	Hexachlorocyclohexane (delta)	0.20	0.9803	N/A	N/A	N/A	78.98	14,62	7.89	103.75	10,97	5.86	108.30	13,54	11.54	100.42	6,69	9.03
178	Hexaclorocyclohexane (gamma. lindane)	1.20	0.9788	N/A	N/A	N/A	N/A	N/A	N/A	74.44	10,23	4.20	116.82	7,46	12.45	109.76	6,66	9.87
179	Hexaconazole (two isomers)	0.80	0.9956	N/A	N/A	N/A	N/A	N/A	N/A	102.27	13,88	4.57	99.87	7,28	3.77	100.02	6,65	4.07

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Table 1 (*continued*)

				0.1 ng/ml			0.5 ng/ml			1 ng/ml			5 ng/ml			20 ng/ml		
					Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
N°	Compound	LOQ	Linearity	Bias (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
180	Hexaflumuron	0.40	0.9942	N/A	N/A	N/A	108.89	9,42	12.82	90.01	9,26	4.34	105.96	7,25	4.78	101.95	16,19	1.84
181	Hexythiazox	0.10	0.9901	100.56	8,48	12.26	92.79	9,89	0.73	88.30	9,01	12.34	108.98	7,31	4.58	101.78	15,67	2.78
182	Imazalil (enilconazole)	0.40	0.9962	N/A	N/A	N/A	109.11	10,51	1.10	99.07	9,60	0.11	105.22	14,79	1.79	100.07	17,30	5.52
183	Imidacloprid	0.80	0.9952	N/A	N/A	N/A	N/A	N/A	N/A	87.81	15,08	2.99	104.63	7,28	11.64	96.04	6,63	2.58
184	Indeno [1.2.3-cd] pyrene	0.40	0.9919	N/A	N/A	N/A	N/A	N/A	N/A	104.74	9,05	5.92	104.70	13,91	11.16	102.65	15,55	9.23
185	Indoxacarb	0.20	0.9936	N/A	N/A	N/A	107.04	14,47	9.81	90.81	17,33	0.63	104.94	16,20	0.90	100.08	6,69	2.82
186	Iprodione	4.00	0.9910	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	92.28	16,39	18.00	99.85	6,62	5.16
187	Iprovalicarb	0.20	0.9976	N/A	N/A	N/A	104.86	13,80	1.64	96.91	14,24	4.68	104.31	6,64	5.23	99.02	7,01	2.18
188	Isocarbophos	1.60	0.9921	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	97.94	14,54	9.86	101.76	14,94	7.87
189	Isofenphos methyl	0.40	0.9915	N/A	N/A	N/A	109.93	9,31	13.09	103.10	9,03	4.81	90.82	16,19	8.17	97.95	17,33	9.64
190	Isoprothiolane	0.10	0.9964	116.56	8,05	1.22	88.49	11,32	14.49	97.12	9,55	1.20	104.61	17,73	4.03	98.25	16,45	3.16
191	Ivermectin B1a	1.60	0.9537	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	105.65	17,48	10.35	105.44	6,85	17.66
192	Josamycin	0.40	0.9963	N/A	N/A	N/A	116.58	9,84	9.00	103.29	9,03	8.32	100.89	6,75	3.99	97.02	14,41	4.09
193	Ketoprofen	0.40	0.9947	N/A	N/A	N/A	111.09	13,69	9.48	91.37	15,03	5.33	104.97	16,90	2.64	95.12	6,88	4.68
194	Kresoxim methyl	1.20	0.9954	N/A	N/A	N/A	N/A	N/A	N/A	102.98	15,93	5.45	88.44	6,70	7.22	96.75	6,67	3.56
195	Leptophos	0.80	0.9941	N/A	N/A	N/A	N/A	N/A	N/A	101.99	16,06	1.71	98.45	6,98	9.13	98.20	6,83	6.65
196	Levamisole	0.20	0.9928	N/A	N/A	N/A	117.03	9,11	15.55	93.61	9,12	5.55	101.49	6,99	1.47	94.01	15,02	3.40
197	Lincomycin	0.40	0.9954	N/A	N/A	N/A	107.94	10,04	20.27	92.20	9,04	10.96	106.37	6,74	7.35	97.78	16,09	7.36
198	Linuron	0.20	0.9950	N/A	N/A	N/A	111.70	9,18	8.45	96.83	9,31	11.03	104.59	17,99	2.83	97.46	13,85	1.94
199	Lufenuron	0.40	0.9451	N/A	N/A	N/A	124.88	12,48	1.73	104.02	14,96	4.07	76.66	19,41	9.50	108.83	6,75	6.81
200	Malaoxon	0.10	0.9968	105.72	13,29	7.33	98.47	10,73	10.38	93.12	9,21	5.62	103.41	6,91	5.54	96.61	13,88	1.87
201	Malathion	0.20	0.9956	N/A	N/A	N/A	102.68	15,17	2.06	108.09	15,52	6.64	108.81	6,62	6.47	95.13	6,74	1.66
202	Mandipropamid	0.10	0.9965	115.12	12,98	1.19	88.72	12,74	4.12	94.24	16,17	0.83	104.13	6,65	1.48	97.38	6,62	1.67
203	Marbofloxacin	2.00	0.9589	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	58.20	8,81	13.63	95.33	6,67	21.85
204	Mebendazole	0.10	0.9960	139.72	14,34	0.62	93.53	9,81	2.13	93.17	9,63	5.68	104.77	16,51	3.77	97.25	14,46	2.70
205	Mefenamic acid	0.40	0.9938	N/A	N/A	N/A	111.93	10,36	4.50	97.29	9,33	11.22	106.39	15,98	0.91	101.74	16,63	0.41
206	Mefenoxam (metalaxyl-M)	0.10	0.9974	104.72	14,28	6.08	101.99	9,15	10.56	95.87	9,02	4.94	103.12	15,00	7.93	96.94	15,42	2.84
207	Meloxicam	0.20	0.9916	N/A	N/A	N/A	105.76	13,99	8.34	103.10	16,06	8.66	103.87	15,74	0.35	95.21	6,62	7.62
208	Mepanipyrim	0.40	0.9940	N/A	N/A	N/A	112.41	9,22	16.49	100.23	9,21	5.60	86.88	7,31	4.08	98.52	18,16	5.19
209	Mepiquat	0.40	0.9961	N/A	N/A	N/A	116.63	13,85	16.83	102.27	15,30	6.19	109.64	15,10	6.19	98.75	6,68	1.89
210	Metaflumizone	0.20	0.9538	N/A	N/A	N/A	117.95	13,04	7.80	110.07	14,54	14.54	78.67	7,94	1.92	108.66	7,10	15.31
211	Metaldehyde	4.00	0.9883	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	93.85	6,69	11.49	99.36	7,06	5.12
212	Metconazole	0.10	0.9972	112.89	17,30	7.83	90.40	10,89	3.31	94.39	9,51	3.75	106.51	6,73	5.49	99.30	7,31	1.11
213	Methamidophos (two isomers)	1.20	0.9914	N/A	N/A	N/A	N/A	N/A	N/A	106.70	9,02	11.46	118.60	6,73	4.89	100.44	7,26	3.27
214	Methidathion	0.10	0.9971	103.57	9,74	14.60	93.56	11,22	7.71	97.65	9,08	0.47	105.01	15,31	4.15	98.65	7,31	3.48
215	Methiocarb	0.10	0.9988	102.06	8,49	8.48	80.51	15,96	14.62	104.48	15,11	0.95	102.47	15,17	6.24	99.13	15,56	0.40
216	Methiocarb-sulfoxide	0.80	0.9983	N/A	N/A	N/A	N/A	N/A	N/A	106.81	9,41	3.29	100.84	16,38	3.36	99.34	7,92	2.12
217	Methomyl	0.40	0.9957	N/A	N/A	N/A	123.44	12,82	2.09	97.84	14,07	5.96	103.37	18,15	3.88	97.62	16,66	3.36
218	Methomyl oxime	8.00	0.9878	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	104.43	16,22	11.23
219	Methoxyfenozide	0.10	0.9970	101.72	8,47	12.57	94.61	12,59	7.91	92.06	16,58	4.97	107.21	16,10	4.63	98.53	14,79	5.72

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Table 1 (*continued*)

				0.1 ng/ml			0.5 ng/ml			1 ng/ml			5 ng/ml			20 ng/ml		
					Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
N°	Compound	LOQ	Linearity	Bias (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
220	Metoxychlor	0.80	0.9654	N/A	N/A	N/A	N/A	N/A	N/A	103.00	9,01	4.49	107.95	6,76	4.82	99.17	7,25	1.81
221	Metrafenone	0.10	0.9961	105.81	19,69	4.81	96.19	10,87	16.34	92.34	9,58	1.92	104.28	6,62	2.67	97.90	7,25	4.32
222	Metronidazole	0.80	0.9962	N/A	N/A	N/A	N/A	N/A	N/A	98.63	9,76	3.50	102.00	6,68	8.71	95.00	7,37	3.36
223	Mevinphos (phosdrin)	0.80	0.9915	N/A	N/A	N/A	N/A	N/A	N/A	107.53	13,72	1.76	101.38	6,92	3.71	96.27	16,29	4.50
224	Mirex	2.00	0.9549	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	94.53	15,92	10.07	80.16	9,18	7.21
225	Monocrotophos	0.80	0.9965	N/A	N/A	N/A	N/A	N/A	N/A	103.97	8,42	2.26	101.05	18,14	3.31	98.38	16,59	0.70
226	Myclobutanil	0.10	0.9951	124.02	8,55	14.29	90.40	14,35	1.18	93.06	10,29	11.51	104.50	14,50	1.48	97.54	14,00	1.84
227	N-(2.4-dimethylphenyl)-N'-methylformamidine (DMPF. metabolite of amitraz)	0.80	0.9974	N/A	N/A	N/A	N/A	N/A	N/A	97.86	8,07	10.59	103.78	14,78	4.26	97.74	15,81	2.58
228	N.N-dimethylformamidine (DMF. metabolite of amitraz)	1.20	0.9835	N/A	N/A	N/A	N/A	N/A	N/A	100.61	13,54	3.89	89.22	6,62	8.12	94.96	7,43	5.62
229	N.N-Dimethyl-N'-p-tolylsulphamide (DMST. metabolite of tolyfluanid)	0.20	0.9972	N/A	N/A	N/A	103.04	10,88	5.68	93.42	10,84	1.84	103.03	16,30	5.97	96.86	7,99	1.30
230	Nafcillin	0.80	0.9937	N/A	N/A	N/A	N/A	N/A	N/A	88.13	14,49	4.44	108.84	6,82	0.54	103.15	7,68	2.36
231	Naphtalene	0.80	0.9321	N/A	N/A	N/A	N/A	N/A	N/A	101.12	8,54	2.09	101.73	6,83	2.23	109.08	14,76	3.83
232	Naproxen	1.60	0.9915	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	103.39	6,66	13.15	101.78	7,24	7.98
233	Nitenpyram	2.00	0.9983	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	95.74	12,88	1.32	98.39	13,54	2.80
234	Novobiocin	0.80	0.9906	N/A	N/A	N/A	N/A	N/A	N/A	103.08	7,97	18.91	105.94	9,44	19.94	94.20	16,68	2.89
235	Nuarimol	0.20	0.9907	N/A	N/A	N/A	109.73	12,42	0.51	97.78	10,16	3.72	90.15	10,54	2.92	99.33	14,45	13.10
236	Ofurace	0.10	0.9950	116.80	13,54	2.28	101.16	9,23	4.08	92.59	10,84	1.49	103.20	9,09	6.63	96.53	7,41	0.65
237	Omethoate	0.40	0.9955	N/A	N/A	N/A	123.46	9,26	4.50	92.85	14,57	13.56	103.56	14,05	4.38	97.89	7,34	1.03
238	Oxadixyl	0.20	0.9968	N/A	N/A	N/A	104.76	9,57	3.73	95.70	11,92	0.86	103.01	13,24	5.95	95.73	7,27	2.17
239	Oxamyl	0.40	0.9963	N/A	N/A	N/A	121.94	12,38	6.94	100.43	9,24	6.59	100.86	15,07	1.70	97.44	13,92	1.86
240	Oxfendazole	0.10	0.9958	127.49	9,14	3.24	103.94	9,83	7.55	89.18	10,70	3.62	100.67	15,04	7.94	96.27	7,42	1.82
241	Oxolinic acid	0.20	0.9878	N/A	N/A	N/A	115.69	14,38	16.83	83.19	8,62	3.27	93.60	11,38	7.48	91.97	13,85	12.34
242	Oxydemeton methyl	0.40	0.9957	N/A	N/A	N/A	121.11	14,05	6.52	92.99	8,25	1.58	101.52	9,52	5.54	97.61	15,88	2.85
243	Oxyfluorfen	0.40	0.9951	N/A	N/A	N/A	111.90	12,27	10.58	104.83	9,18	6.06	89.81	11,13	8.87	97.95	15,20	6.39
244	Paclobutrazol	0.40	0.9967	N/A	N/A	N/A	116.18	9,54	1.19	93.47	14,62	0.60	101.83	9,58	2.63	98.88	7,40	0.81
245	Paraoxon methyl	1.60	0.9967	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	87.46	14,41	19.55	101.53	7,25	6.01
246	Parathion ethyl	1.20	0.9566	N/A	N/A	N/A	N/A	N/A	N/A	104.49	13,49	4.82	89.57	9,37	14.20	96.19	7,67	3.90
247	Parathion methyl	0.80	0.9976	N/A	N/A	N/A	N/A	N/A	N/A	108.54	9,72	8.23	92.53	12,51	19.31	100.15	13,94	12.02
248	PCB 28	0.10	0.9912	83.22	13,04	15.78	93.92	10,39	11.89	106.19	11,02	6.00	97.60	12,20	10.40	88.62	7,25	7.97
249	PCB 52	0.20	0.9902	N/A	N/A	N/A	104.04	15,11	10.65	98.92	8,87	5.59	92.35	15,12	9.84	93.96	13,69	8.45
250	PCB 77	0.20	0.9923	N/A	N/A	N/A	100.53	14,86	8.65	98.89	9,22	5.59	88.02	16,35	9.38	92.40	14,65	8.31
251	PCB 81	0.10	0.9850	110.87	13,67	12.33	81.87	13,56	7.45	115.62	7,80	6.53	91.34	9,29	9.73	89.92	14,75	8.09
252	PCB 101	0.20	0.9876	N/A	N/A	N/A	107.84	10,20	12.34	110.75	10,27	6.26	93.40	10,87	9.95	92.89	7,29	8.35
253	PCB 105	0.10	0.9789	118.45	12,59	4.12	103.80	9,79	9.54	107.96	10,72	6.10	94.53	9,54	10.07	80.16	7,58	7.21
254	PCB 114	0.20	0.9776	N/A	N/A	N/A	97.14	10,43	6.22	110.95	11,13	6.27	85.53	11,13	9.11	84.13	8,29	7.57
255	PCB 118	0.20	0.9833	N/A	N/A	N/A	93.43	15,84	9.87	116.88	9,41	6.60	88.80	14,30	9.46	86.10	16,14	7.74

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Table 1 (*continued*)

N°	Compound	LOQ	Linearity	0.1 ng/ml			0.5 ng/ml			1 ng/ml			5 ng/ml			20 ng/ml		
					Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
				Bias (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
256	PCB 123	0.40	0.9834	N/A	N/A	N/A	96.14	9,55	11.67	106.30	12,46	6.01	88.58	9,79	9.44	85.35	7,25	7.67
257	PCB 126	0.20	0.9789	N/A	N/A	N/A	108.37	12,34	12.09	106.86	8,54	6.04	90.82	15,17	9.68	79.34	11,78	7.13
258	PCB 138	0.10	0.9798	107.23	9,04	9.56	91.30	13,54	9.07	110.40	8,31	6.24	92.83	14,93	9.89	84.08	9,09	7.56
259	PCB 153	0.10	0.9766	117.78	9,83	8.73	83.70	15,17	4.97	124.77	9,02	7.05	89.77	15,53	9.57	85.65	10,85	7.70
260	PCB 156	0.20	0.9912	N/A	N/A	N/A	98.33	10,47	7.21	107.17	16,36	6.05	91.74	15,28	9.78	77.11	17,71	6.93
261	PCB 157	0.40	0.9789	N/A	N/A	N/A	93.22	10,60	6.54	107.96	15,32	6.10	91.26	9,70	9.72	79.99	15,86	7.19
262	PCB 167	0.10	0.9770	102.89	9,26	6.45	91.43	11,68	8.97	102.06	14,16	5.77	86.31	9,26	9.20	81.14	15,29	7.30
263	PCB 169	0.20	0.9758	N/A	N/A	N/A	109.07	12,62	11.56	107.45	9,51	6.07	86.95	10,36	9.26	90.77	9,14	8.16
264	PCB 180	0.10	0.9807	119.51	8,34	20.01	87.43	12,13	10.76	113.14	15,94	6.39	85.21	11,75	9.08	95.03	13,54	8.55
265	PCB 189	0.10	0.9723	110.12	8,68	12.45	97.91	15,11	6.22	96.96	9,32	5.48	88.62	12,49	9.44	87.18	9,24	7.84
266	Penconazole	0.40	0.9930		N/A		114.19	13,25	14.47	101.18	9,00	3.31	91.43	10,09	1.78	99.02	9,49	10.75
267	Pencycuron	0.10	0.9968	97.11	8,84	9.23	94.99	12,74	13.51	93.82	9,10	3.87	105.44	12,04	6.20	97.74	9,16	0.70
268	Pendimethalin	0.80	0.9902	N/A	N/A	N/A	N/A	N/A	N/A	106.58	16,84	2.61	88.47	15,67	3.62	97.69	16,40	0.06
269	Penicillin G	2.00	0.9903	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	117.02	13,91	3.71	97.77	12,92	3.62
270	Penicillin V	2.00	0.9917	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	105.81	13,39	2.98	97.69	15,53	3.59
271	Permethrin	1.20	0.9889	N/A	N/A	N/A	N/A	N/A	N/A	101.46	9,10	0.12	87.27	10,84	2.23	101.87	9,16	17.84
272	Phenanthrene	0.20	0.9678	N/A	N/A	N/A	109.89	10,49	13.76	106.65	16,96	6.02	92.56	10,39	9.86	95.32	14,63	8.57
273	Phenylbutazone	1.60	0.9721	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	113.13	9,97	6.52	65.14	12,76	6.05
274	Phosalone	0.20	0.9961	N/A	N/A	N/A	98.37	13,12	4.61	92.71	10,27	2.58	107.98	9,30	4.93	101.62	8,94	1.06
275	Phosmet	0.20	0.9972	N/A	N/A	N/A	98.92	13,74	3.15	93.00	9,82	2.84	104.52	14,39	5.06	98.67	9,58	1.56
276	Phosmet oxon	0.20	0.9993	N/A	N/A	N/A	95.79	10,14	7.39	102.23	14,85	5.34	102.16	9,29	5.78	99.08	15,44	1.95
277	Piperacillin	0.40	0.9793	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	96.34	11,70	1.45	98.60	15,17	6.10
278	Pirimicarb	0.10	0.9977	105.98	8,04	2.51	98.18	10,71	6.09	95.11	15,17	5.23	102.97	10,72	4.86	97.54	15,39	1.81
279	Pirimiphos ethyl	0.10	0.9883	104.58	15,84	12.37	98.69	14,95	9.94	97.93	9,00	0.38	104.83	10,74	4.05	98.96	9,62	2.64
280	Pirimiphos methyl	0.10	0.9944	112.73	15,00	0.58	93.49	10,21	1.96	106.42	14,32	3.74	89.01	9,11	3.18	98.28	16,06	5.62
281	Prochloraz	0.10	0.9947	125.26	18,00	18.57	91.55	13,09	1.08	87.91	9,80	7.63	100.57	9,08	13.10	97.13	9,11	2.15
282	Procymidone	1.60	0.9947	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	80.19	15,87	6.54	100.94	9,20	5.65
283	Profenofos	0.10	0.9941	111.06	7,48	4.52	92.80	15,13	3.54	96.49	9,29	1.38	106.87	14,90	3.35	98.58	9,03	3.48
284	Propamocarb	0.40	0.9959	N/A	N/A	N/A	122.72	9,79	9.24	97.58	15,13	4.21	97.03	14,92	10.35	96.69	14,18	1.39
285	Propargite	0.10	0.9936	100.21	8,65	2.94	95.89	9,50	5.23	96.17	15,13	7.55	109.05	11,86	5.19	100.09	15,81	2.82
286	Propiconazole	0.40	0.9932	N/A	N/A	N/A	130.04	9,40	1.49	99.77	14,15	3.82	102.03	10,74	0.34	99.32	14,78	0.40
287	Propoxur	0.10	0.9941	118.68	11,02	14.25	92.88	13,46	13.27	90.94	9,34	1.25	101.89	9,97	6.43	96.39	8,93	1.71
288	Propyzamide (pronamide)	0.10	0.9955	135.09	10,13	25.43	82.62	12,64	4.33	89.32	18,24	8.08	102.57	9,70	4.21	98.22	15,95	2.04
289	Proquinazid	0.20	0.9808	N/A	N/A	N/A	121.75	13,90	4.37	100.38	9,85	10.36	89.96	11,39	7.16	96.95	9,40	16.39

(*continued on next page*)

Table 1 (*continued*)

				0.1 ng/ml			0.5 ng/ml			1 ng/ml			5 ng/ml			20 ng/ml		
					Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
N°	Compound	LOQ	Linearity	Bias (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
290	Prothioconazol	0.40	0.9935	N/A	N/A	N/A	103.16	14,09	9.30	100.90	9,00	8.76	87.83	15,40	1.26	96.35	10,44	0.81
291	Prothiophos	0.40	0.9912	N/A	N/A	N/A	179.86	11,21	33.27	107.75	9,12	5.60	91.21	10,22	8.06	97.75	10,50	11.76
292	Pymetrozine	0.80	0.9952	N/A	N/A	N/A	N/A	N/A	N/A	102.89	14,39	1.86	99.73	12,22	9.64	98.84	12,75	0.26
293	Pyraclostrobin	0.10	0.9970	105.05	8,46	0.95	88.71	11,51	20.65	95.80	15,56	0.93	104.40	13,96	8.09	97.82	13,51	0.60
294	Pyrazophos	0.10	0.9965	111.64	16,39	0.54	98.23	9,29	17.84	95.77	15,41	6.78	107.24	13,69	3.11	99.83	15,58	2.60
295	Pyrene	0.20	0.9880	N/A	N/A	N/A	96.42	13,97	9.56	93.61	9,19	5.29	93.37	13,91	9.95	91.07	9,54	8.19
296	Pyridaben	0.10	0.9973	105.99	9,65	29.54	86.82	10,63	8.41	97.27	11,31	14.36	111.71	10,40	5.14	100.32	13,91	1.83
297	Pyridaphenthion	0.20	0.9970	N/A	N/A	N/A	108.64	13,78	2.34	91.81	11,67	10.38	105.65	10,12	9.67	98.59	8,97	0.24
298	Pyrimethanil	0.20	0.9944	N/A	N/A	N/A	107.96	13,00	6.86	96.98	11,36	3.25	89.29	10,21	11.63	98.03	13,12	0.85
299	Pyriproxifen	0.10	0.9947	104.25	19,77	1.21	99.97	13,33	9.48	94.10	12,89	1.20	107.21	9,99	2.85	100.77	13,76	4.72
300	Quinalfos	0.20	0.9958	N/A	N/A	N/A	97.53	9,47	17.05	90.97	12,09	1.01	106.48	12,86	0.09	100.08	9,35	5.66
301	Quinoxyfen	0.10	0.9874	112.39	6,66	4.49	109.15	9,19	13.38	93.18	11,12	13.57	104.94	9,62	6.76	102.76	9,18	7.44
302	Rifampicin	0.80	0.9826	N/A	N/A	N/A	N/A	N/A	N/A	111.21	10,43	10.64	95.28	14,86	0.19	89.73	11,01	5.48
303	Rotenone	0.40	0.9929	N/A	N/A	N/A	117.98	13,24	31.54	89.20	12,85	5.35	113.57	12,47	3.51	100.90	15,11	3.15
304	Roxithromycin	0.80	0.9971	N/A	N/A	N/A	N/A	N/A	N/A	96.18	9,47	3.69	98.39	9,30	4.00	100.05	9,10	0.03
305	Sarafloxacin	4.00	0.9312	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	80.98	10,55	13.63	97.00	14,62	22.19
306	Simazine	0.20	0.9968	N/A	N/A	N/A	90.28	16,27	2.78	96.46	11,65	1.79	102.84	13,27	6.50	97.85	15,10	1.76
307	Spinosad (two isomers)	0.10	0.9970	119.58	11,44	9.10	84.56	15,73	4.96	94.98	13,13	9.89	100.78	16,05	7.72	96.81	12,98	1.95
308	Spiramycin (two isomers)	0.40	0.9915	N/A	N/A	N/A	131.47	9,05	5.94	95.33	9,51	14.39	106.27	13,21	4.78	97.03	10,02	3.05
309	Spirodiclofen	0.80	0.9869	N/A	N/A	N/A	N/A	N/A	N/A	106.47	10,78	1.93	134.35	14,32	8.81	100.48	10,43	7.70
310	Spiromesifen	0.20	0.9911	N/A	N/A	N/A	96.47	9,96	13.07	101.29	9,33	5.45	115.81	8,86	4.16	100.68	10,51	2.72
311	Spiroxamine	0.10	0.9972	107.65	10,26	2.69	97.02	13,19	5.23	95.80	13,52	6.32	102.89	14,63	3.99	97.41	13,84	4.04
312	Strychnine	0.80	0.9963	N/A	N/A	N/A	N/A	N/A	N/A	85.40	12,44	3.11	111.08	9,35	6.47	96.91	10,99	12.19
313	Sulfacetamide	0.40	0.9952	N/A	N/A	N/A	118.98	12,18	24.55	101.34	12,33	3.97	97.43	9,68	12.30	96.06	12,88	2.45
314	Sulfachloropiridacine	0.80	0.9930	N/A	N/A	N/A	N/A	N/A	N/A	93.69	13,68	3.92	102.35	8,92	8.67	94.47	14,20	0.06
315	Sulfadiacine	0.80	0.9933	N/A	N/A	N/A	N/A	N/A	N/A	97.95	12,68	8.67	95.92	10,00	14.59	98.24	12,94	6.24
316	Sulfadimetoxine	0.10	0.9962	122.34	7,01	0.77	104.34	9,54	10.58	94.70	10,65	12.38	103.45	15,28	9.68	96.93	10,87	1.88
317	Sulfadoxine	0.10	0.9949	129.86	7,48	12.83	92.91	9,62	4.59	92.41	10,95	6.06	104.11	12,45	8.47	95.40	9,22	4.00
318	Sulfameracine	0.20	0.9941	N/A	N/A	N/A	102.32	11,05	16.69	99.10	9,71	8.73	105.86	8,98	9.54	95.49	9,31	1.52
319	Sulfametacine	0.20	0.9939	N/A	N/A	N/A	98.96	12,83	1.51	97.49	11,61	3.97	103.52	8,92	8.64	95.26	13,20	1.45
320	Sulfametizole	0.80	0.9864	N/A	N/A	N/A	N/A	N/A	N/A	100.19	10,28	0.31	103.59	8,94	10.59	91.01	9,92	3.80
321	Sulfametoxazole	0.40	0.9948	N/A	N/A	N/A	112.21	12,92	9.16	94.31	14,06	6.41	103.91	10,23	10.13	94.74	14,50	3.86
322	Sulfametoxipiridacine	0.40	0.9940	N/A	N/A	N/A	104.29	13,02	13.05	98.89	13,20	19.85	105.96	12,95	6.19	95.01	14,99	3.00
323	Sulfamonomethoxine	1.20	0.9913	N/A	N/A	N/A	N/A	N/A	N/A	111.11	11,11	3.29	93.85	13,38	10.90	96.63	14,16	2.94
324	Sulfapyridine	0.40	0.9930	N/A	N/A	N/A	109.50	9,43	3.94	103.66	9,59	8.63	98.18	13,72	7.97	95.32	11,29	0.42
325	Sulfaquinoxaline	0.40	0.9962	N/A	N/A	N/A	115.47	9,35	15.86	92.67	9,83	12.76	101.15	14,97	7.60	96.86	10,75	0.16
326	Sulfatiazole	0.40	0.9922	N/A	N/A	N/A	109.32	10,34	18.92	87.79	11,88	0.71	96.90	9,58	11.07	96.24	9,72	1.08

(*continued on next page*)

Table 1 (*continued*)

N°	Compound	LOQ	Linearity	0.1 ng/ml			0.5 ng/ml			1 ng/ml			5 ng/ml			20 ng/ml		
					Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
				Bias (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
327	Sulfisoxazole	0.80	0.9965	N/A	N/A	N/A	N/A	N/A	N/A	93.73	12,52	5.51	101.82	14,33	5.95	95.32	14,43	1.90
328	Tebuconazole	0.80	0.9949	N/A	N/A	N/A	N/A	N/A	N/A	92.08	10,26	8.58	98.28	9,74	5.09	98.61	9,22	4.53
329	Tebufenocide	0.10	0.9947	92.74	14,93	17.37	89.88	15,24	5.86	96.15	11,17	6.53	105.61	9,47	4.63	101.10	12,57	8.24
330	Tebufenpyrad	0.10	0.9950	111.56	9,92	2.79	86.18	13,25	9.05	95.86	12,29	4.97	106.62	9,27	4.29	100.57	6,66	3.07
331	Teflubenzuron	1.20	0.9860	N/A	N/A	N/A	N/A	N/A	N/A	97.01	11,66	13.94	94.86	9,78	4.53	99.61	6,65	5.63
332	Tefluthrin	0.10	0.9937	97.69	9,68	14.14	104.85	10,00	3.87	107.14	10,41	2.51	88.84	15,18	4.03	95.53	14,71	1.33
333	Telodrin (isobenzan)	0.80	0.9930	N/A	N/A	N/A	N/A	N/A	N/A	93.03	9,80	11.99	92.60	16,28	21.17	101.82	16,65	10.96
334	Terbufos	0.20	0.9942	N/A	N/A	N/A	107.98	9,37	17.24	97.29	9,28	3.06	90.31	13,69	6.42	102.47	17,11	8.75
335	Terbuthylazine	0.40	0.9961	N/A	N/A	N/A	98.38	12,63	4.02	93.37	14,53	2.45	91.01	13,07	11.41	99.87	6,61	3.75
336	Tetrachlorvinphos	0.40	0.9984	N/A	N/A	N/A	105.27	9,21	8.36	93.03	9,91	10.04	104.96	9,12	2.97	101.50	14,60	6.48
337	Tetraconazole	0.20	0.9878	N/A	N/A	N/A	96.23	12,80	9.69	101.26	13,96	5.09	89.84	14,01	3.49	99.52	6,84	8.62
338	Tetradifon	0.40	0.9912	N/A	N/A	N/A	110.62	12,25	10.72	103.75	14,09	8.41	91.10	9,45	2.57	100.16	6,91	8.47
339	Tetramethrin	1.60	0.9871	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	85.18	10,80	2.32	98.04	7,06	1.46
340	Thiabendazole	0.20	0.9953	N/A	N/A	N/A	99.82	9,60	4.72	88.38	9,69	3.77	101.10	9,07	5.74	99.15	18,69	4.46
341	Thiacloprid	0.20	0.9972	N/A	N/A	N/A	107.76	9,74	2.80	94.23	9,09	6.03	102.23	8,91	5.37	97.17	17,95	1.87
342	Thiamethoxam	0.80	0.9968	N/A	N/A	N/A	N/A	N/A	N/A	103.40	9,05	6.94	101.22	14,16	5.43	98.61	16,69	1.97
343	Thiophanate methyl	0.20	0.9968	N/A	N/A	N/A	102.31	13,05	6.57	94.75	16,70	4.03	103.59	14,67	7.43	97.52	7,00	3.77
344	Tolclofos methyl	0.10	0.9922	116.17	11,56	5.73	100.20	9,86	4.71	96.78	9,59	17.32	92.67	16,21	6.45	99.05	14,51	10.76
345	Tolfenamic acid	0.40	0.9917	N/A	N/A	N/A	106.53	12,83	9.72	105.64	15,03	7.99	98.88	14,27	0.15	104.07	6,78	3.23
346	Triadimefon	0.40	0.9967	N/A	N/A	N/A	115.55	12,00	19.61	95.64	14,82	2.87	100.72	9,18	4.89	99.14	6,64	0.92
347	Triadimenol	0.40	0.9949	N/A	N/A	N/A	108.82	13,57	19.22	90.50	14,42	10.27	100.86	14,76	8.82	99.02	6,64	8.06
348	Triazophos (hostathion)	0.10	0.9976	97.70	9,50	9.62	97.52	9,35	2.53	96.68	9,01	10.04	103.87	9,67	4.89	98.73	17,46	3.23
349	Trichlorfon	1.20	0.9981	N/A	N/A	N/A	N/A	N/A	N/A	99.76	9,02	19.77	100.02	8,98	0.34	98.66	16,06	0.59
350	Trifloxystrobin	0.10	0.9950	79.68	8,31	16.36	99.02	9,33	11.67	105.21	9,00	2.26	113.52	9,66	3.24	98.92	16,80	1.91
351	Triflumizole	0.10	0.9946	91.81	7,37	21.56	92.12	16,13	7.68	94.83	16,24	2.17	107.86	10,07	1.06	100.97	6,86	1.32
352	Triflumuron	0.40	0.9954	N/A	N/A	N/A	109.58	9,67	9.83	97.28	9,37	3.38	106.90	13,20	12.75	102.17	15,84	3.65
353	Trifluralin	0.20	0.9928	N/A	N/A	N/A	113.51	14,55	1.27	105.71	15,05	16.83	85.71	15,12	16.24	99.77	6,81	16.19
354	Trimethoprim	0.80	0.9974	N/A	N/A	N/A	N/A	N/A	N/A	98.59	16,81	5.49	108.01	15,11	4.37	96.85	7,03	0.59
355	Triticonazole	0.40	0.9948	N/A	N/A	N/A	119.81	11,96	4.08	97.96	15,07	9.40	99.78	14,17	1.39	98.33	6,91	1.25
356	Tylmicosin	1.60	0.9955	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	103.10	13,68	3.57	95.24	14,57	6.28
357	Tylosin	0.80	0.9975	N/A	N/A	N/A	N/A	N/A	N/A	105.10	9,03	6.25	103.84	14,83	4.75	99.16	18,48	5.44
358	Vinclozolin	0.20	0.9936	N/A	N/A	N/A	112.53	9,07	17.45	95.98	9,13	6.12	85.39	17,83	5.12	97.78	16,65	0.80
359	Warfarin	0.10	0.9962	106.32	9,23	20.28	90.08	13,61	15.11	92.87	16,86	4.58	103.35	13,18	3.78	98.30	6,73	0.08
360	Zoxamide	0.40	0.9960	N/A	N/A	N/A	126.95	9,61	10.37	94.83	9,07	3.13	101.49	12,65	5.65	98.29	15,52	1.08

Table 2
Concentrations of persistent organic pollutants found in whole blood (ng/ml) of a series of barn owls sampled in Castilla–Leon (Spain) during 2018 and 2019.

	Acenaphtylene	BDE-47	BDE-100	BDE-153	BDE-154	BDE-99	Dichlorodiphenyl-dichloroethylene (p,p' DDE)	Fluoranthene	Fluorene	Hexachlorobencene	PCB 118	PCB 138	PCB 153	PCB 156	PCB 167	PCB 180	PCB 189	Phenanthrene	Pyrene
Barn owl 01	0.39						0.14	0.28	0.68	1.51		0.32	0.33			0.42		0.46	0.73
Barn owl 02		0.19	0.33	0.20	0.10	0.68	3.06		0.53	0.83	0.20	2.22	3.21	0.23	0.25	5.81	0.13	0.54	0.20
Barn owl 03	0.32	0.20	0.10	0.20		0.09			0.35			0.10	0.10				0.09	0.49	0.20
Barn owl 04							0.10		0.41			0.10	0.12				0.12	0.29	0.20
Barn owl 05	0.22	0.20	0.12	0.20	0.10	0.40		0.21	0.60									0.75	0.57
Barn owl 06							0.13		0.40	0.38		0.14	0.20				0.22	0.46	0.34
Barn owl 07							0.93	0.32	0.56			0.28	0.42				0.58	0.42	0.29
Barn owl 08																			
Barn owl 09							0.25		0.93	0.44		0.25	0.35				0.41	0.40	0.21
Barn owl 10							0.49		0.52	0.48		0.25	0.32				0.38	0.65	0.60
Barn owl 11							0.53	0.26	1.31	0.90	0.20	0.18	0.20				0.28	1.57	0.45
Barn owl 12							0.10	0.22	1.05	0.43			0.09					1.24	0.27
Barn owl 13									0.55	0.71								1.18	0.19
Barn owl 14									1.13									0.80	0.21
Barn owl 15	0.35								0.78	0.71								0.59	0.20
Barn owl 16							0.16		1.30	0.20		0.10					0.14	1.12	0.21
Barn owl 17	0.38								0.77	0.20								0.39	0.20
Barn owl 18									0.86	0.24								0.50	0.20
Barn owl 19									1.12	0.34								1.88	0.27
Barn owl 20	0.36								0.67									0.58	0.21
Barn owl 21								0.27	1.27									1.06	0.44
Barn owl 22								0.34	0.99								0.10	1.06	0.23
Barn owl 23								0.19	0.72									0.91	0.20
Barn owl 24								0.20	0.64									0.41	0.20
Barn owl 25							0.10	0.28	1.29	0.36							0.10	2.25	0.30
Barn owl 26								0.33	0.26	0.62								1.40	0.26
Barn owl 27							0.14	0.43	0.92	0.61								1.32	0.19
Barn owl 28									0.99									0.57	0.20
Barn owl 29							0.42		0.44	1.00		0.17	0.27				0.39	0.54	0.20
Barn owl 30							1.11		1.27	1.75		0.41	0.48				0.55	0.81	0.20
Barn owl 31									0.81	0.38								0.52	0.20
Barn owl 32								0.30	1.50	0.23								2.23	0.26
Barn owl 33									0.79	0.35								0.85	0.20
Barn owl 34									0.74									0.99	0.23
Barn owl 35									1.05	0.38								0.99	0.26
Barn owl 36									0.80									0.73	0.19

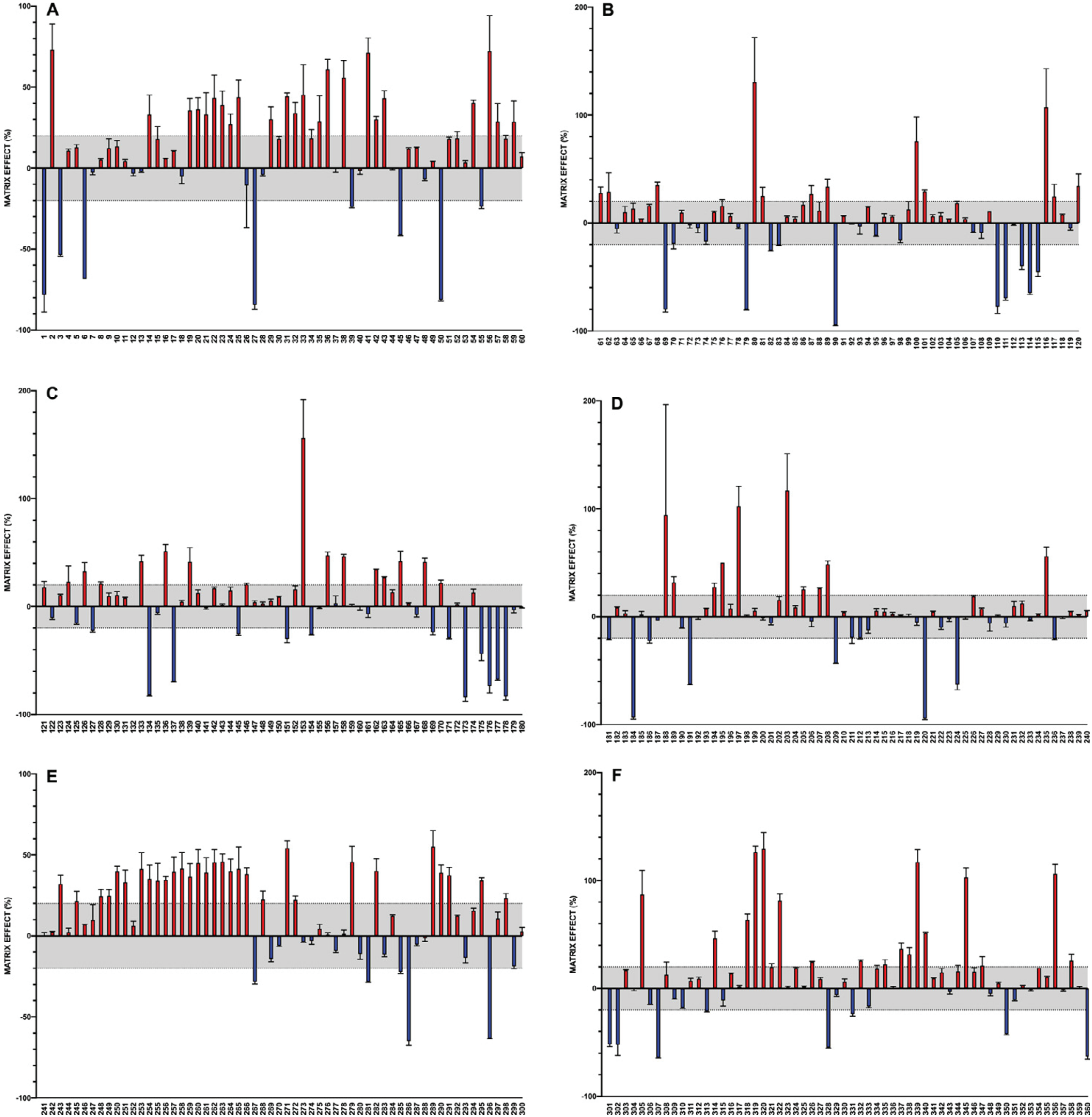

Fig. 1. Matrix effect (%) of 360 compounds included on the method

2. Experimental design, materials, and methods

2.1. Chemicals, reagents, and calibrators

Certified pure standards of the 360 chemicals included in the methodology (Dr Ehrestorfer, Augsburg, Germany; CPA Chem, Stara Zagora, Bulgaria; A2S – Analytical Standard Solutions, Staint Jean D'Illac, France; Sigma-Aldrich, Augsburg, Germany; Accustandard, New Haven, USA; and European Pharmacopoeia Reference Standards, Strasbourg, France) were individually prepared at 1 mg/ml in a suitable solvent. From those, three intermediate working solutions (by groups: pesticides, medicaments and COPs) containing all the analytes at 1 μg/ml was prepared. This working solution was employed to prepare 12-point calibration curve, either in 1% FA-ACN or in whole blood (a 1:1 mixture of chicken and goat blood, obtained from healthy animals in the Veterinary Faculty of the University of Las Palmas de Gran Canaria). The intermediate solution was also employed for preparing quality controls (QC) and all the fortification levels in blank matrix and extracted blank matrix, that were employed in the validation experiments.

Table 3
Concentrations of non-persistent organic pollutants found in whole blood (ng/ml) of a series of barn owls sampled in Castilla–Leon (Spain) during 2018 and 2019.

	2-Phenylphenol	Benalaxyl	Brodifacoum	Bromadiolone	Coumatetralyl	Dexamethasone	Difenacoum	Enrofloxacin	Eprinomectin	Fenbendazole	Flocoumafen	Flumequine	Metaflumizone	Simazine	Sulfadiacine	Thiacloprid
Barn owl 01	0.20		0.80			0.40		1.20			0.20		0.14		13.69	
Barn owl 02	0.21	0.13	1.35	0.41												
Barn owl 03	0.33															
Barn owl 04	0.20	0.10											0.63			
Barn owl 05	0.24															
Barn owl 06	0.20															
Barn owl 07	0.20															
Barn owl 08																
Barn owl 09	0.36								0.31							
Barn owl 10	0.27												0.24			
Barn owl 11	5.29															
Barn owl 12	5.88	0.10														
Barn owl 13	4.19									0.10						2.57
Barn owl 14	1.71															
Barn owl 15	0.34															
Barn owl 16	2.60															
Barn owl 17	0.24															
Barn owl 18	0.34								0.33							
Barn owl 19	4.27															
Barn owl 20	0.29	0.20					0.40									
Barn owl 21	1.35	0.10														
Barn owl 22	0.56															0.21
Barn owl 23	0.32	0.09														
Barn owl 24	0.32													0.39		0.20
Barn owl 25	5.20						0.40									0.43
Barn owl 26	3.43															
Barn owl 27	2.20															
Barn owl 28	0.33	0.10			111.36											
Barn owl 29	0.38		6.37													
Barn owl 30	0.38															
Barn owl 31	0.32															
Barn owl 32	4.25															
Barn owl 33	0.41	0.15										0.10				
Barn owl 34	0.37															
Barn owl 35	0.63						0.40									
Barn owl 36	0.51															

The salts for the QuEChERS extraction according to the AOAC method were acquired in commercial premixes from Agilent Technologies (Palo Alto, USA). Acetonitrile (ACN), methanol (MeOH), and FA were of the maximum purity available and were purchased from Honeywell (Charlotte, USA). Ammonium acetate was from Fisher (Fisher Scientific UK, Loughborough, UK). The water was prepared in the laboratory using an ultra purification system (Millipore, Molsheim, France).

2.2. Sample preparation

Two hundred fifty microliters whole blood from wild birds or blank matrix (goat + chicken whole blood) either fortified with the 360 analytes plus the internal standards (ISs), the ISs alone, or without fortification, were subjected to an AOAC-QuEChERS extraction method (one-

Table 4
Concentrations of persistent organic pollutants found in whole blood (ng/ml) of a series of common kestrels sampled in Castilla–Leon (Spain) during 2018 and 2019.

	Acenaphthene	Acenaphtylene	BDE 99	BDE 100	BDE 153	BDE 154	BDE 183	Dichlorodiphenyl dichloroethane (p,p'DDD)	Dichlorodiphenyl dichloroethylene (p,p'DDE)	Fluoranthene	Fluorene	Hexachlorobencene	Hexachlorocyclohexane (alpha)}	Hexaclorocyclohexane (beta)	Naphtalene	PCB 28	PCB 101	PCB 105	PCB 118	PCB 138	PCB 153	PCB 156	PCB 167	PCB 180	PCB 189	Phenanthrene	Pyrene
Common kestrel 01		0.23	0.21	0.10					4.40	0.29	0.89	0.61					0.20	0.20	0.49	1.83	2.74	0.20	0.25	0.22	0.09	1.16	0.91
Common kestrel 02																					0.10					0.61	0.30
Common kestrel 03																										0.72	0.25
Common kestrel 04											0.31	0.20														0.53	0.20
Common kestrel 05									0.62		0.40	0.36														1.26	0.20
Common kestrel 06		0.35							0.33		0.52									0.22	0.26			0.25		1.08	0.20
Common kestrel 07									0.37			0.34								0.25	0.29			0.28		0.54	0.20
Common kestrel 08									0.54		0.22	0.33								0.30	0.35			0.22		0.63	0.20
Common kestrel 09									0.21		0.28															0.44	0.20
Common kestrel 10																											
Common kestrel 11			0.10	0.10					0.41		0.56	0.27								0.17	0.33			0.37		1.19	0.20
Common kestrel 12											0.31	0.19														0.31	0.20
Common kestrel 13											0.34															0.57	0.20
Common kestrel 14											0.22	0.37														0.37	0.20
Common kestrel 15	0.20										0.40	0.34														0.55	0.20
Common kestrel 16											0.42	0.20														1.36	0.20
Common kestrel 17											0.45															0.85	0.20
Common kestrel 18											0.33															0.79	0.20
Common kestrel 19		0.35																									
Common kestrel 20																											
Common kestrel 21																											
Common kestrel 22											0.33	0.20												0.17		0.26	0.20
Common kestrel 23											0.23	0.19														0.32	0.20
Common kestrel 24											0.47	0.28														0.75	0.20
Common kestrel 25									0.10		0.55	0.20								0.10	0.09			0.16		0.79	0.20
Common kestrel 26									0.10			0.23								0.10	0.10			0.12		0.45	0.20
Common kestrel 27									0.17		0.29	0.36									0.11			0.10		1.30	0.28
Common kestrel 28									0.39		0.54	0.20														0.76	0.20
Common kestrel 29									0.10		0.36	0.47												0.09		0.57	0.20

(continued on next page)

Table 4 (*continued*)

	Acenaphthene	Acenaphtylene	BDE 99	BDE 100	BDE 153	BDE 154	BDE 183	Dichlorodiphenyl dichloroethane (p,p'DDD)	Dichlorodiphenyl dichloroethylene (p,p'DDE)	Fluoranthene	Fluorene	Hexachlorobencene	Hexachlorocyclohexane (alpha)	Hexaclorocyclohexane (beta)	Naphtalene	PCB 28	PCB 101	PCB 105	PCB 118	PCB 138	PCB 153	PCB 156	PCB 167	PCB 180	PCB 189	Phenanthrene	Pyrene
Common kestrel 30									0.10		0.40	0.20												0.09		0.44	0.20
Common kestrel 31											0.27	0.54														0.55	0.20
Common kestrel 32											0.23	0.20														0.31	0.20
Common kestrel 33									0.10		0.20	0.20									0.10			0.11		0.30	0.20
Common kestrel 34		0.27									0.37	0.33														0.40	0.20
Common kestrel 35												0.42									0.10					0.83	0.38
Common kestrel 36		0.24										0.36														0.83	0.25
Common kestrel 37		0.20							0.09	0.25	0.25	0.39			2.67											0.57	0.23
Common kestrel 38									0.36	0.27	0.38	0.66														0.50	0.28
Common kestrel 39	0.25										0.58	0.53														1.55	0.31
Common kestrel 40		0.19							0.15	0.19	0.53	0.99								0.09	0.11			0.09		0.40	0.39
Common kestrel 41		0.28							0.66		0.63	0.95								0.09	0.15			0.10		0.96	0.43
Common kestrel 42		0.27								0.20	0.51	1.10														0.70	0.37
Common kestrel 43	0.31										1.02	0.32														1.97	0.47
Common kestrel 44	0.37								0.35		0.68	0.20														1.41	0.22
Common kestrel 45								0.65	0.91		0.57	0.32								0.10	0.10			0.10		1.01	0.21
Common kestrel 46		0.23									0.28	0.27														0.40	0.20
Common kestrel 47											0.34	0.33														0.73	0.20
Common kestrel 48	0.20								0.10		0.69	0.19														1.04	0.25
Common kestrel 49									0.10		0.31	0.20								0.10	0.09					0.86	0.21
Common kestrel 50									0.09		0.66	0.52												0.10		0.54	0.20
Common kestrel 51											0.54	0.27														0.44	0.21
Common kestrel 52									0.10		0.42	0.44														0.91	0.21
Common kestrel 53											0.51	0.78														0.45	0.20
Common kestrel 54											0.35	0.58														0.81	0.20
Common kestrel 55									0.38	0.38	0.48	1.14								0.09	0.19			0.19		1.27	0.24
Common kestrel 56									0.29		0.41	0.75								0.10	0.19			0.20		0.76	0.20
Common kestrel 57									0.83		0.64	1.02								0.17	0.33			0.35		0.53	0.25

(continued on next page)

Table 4 (*continued*)

	Acenaphthene	Acenaphtylene	BDE 99	BDE 100	BDE 153	BDE 154	BDE 183	Dichlorodiphenyl dichloroethane (p,p'DDD)	Dichlorodiphenyl dichloroethylene (p,p'DDE)	Fluoranthene	Fluorene	Hexachlorobencene	Hexachlorocyclohexane (alpha)}	Hexaclorocyclohexane (beta)	Naphtalene	PCB 28	PCB 101	PCB 105	PCB 118	PCB 138	PCB 153	PCB 156	PCB 167	PCB 180	PCB 189	Phenanthrene	Pyrene
Common kestrel 58											0.46															0.70	0.25
Common kestrel 59											0.65															0.57	0.22
Common kestrel 60											0.54															0.21	0.20
Common kestrel 61											0.35	0.36														0.35	0.20
Common kestrel 62											0.45	0.17														0.35	0.20
Common kestrel 63									0.32	0.23	0.38								0.20	0.13	0.36			0.44		0.51	0.70
Common kestrel 64					0.20				0.10		0.45	2.51	0.40	5.95					0.20	0.64	3.49	0.23	0.27	6.88	0.18	0.52	0.28
Common kestrel 65		0.45							1.40		0.59	5.49							0.26	0.82	2.83	0.20	0.19	2.80		1.45	0.29
Common kestrel 66									0.12		0.63	2.86								0.14	0.23			0.26		0.86	0.20
Common kestrel 67									0.11		0.41	2.72								0.18	0.49		0.10	0.69		0.85	0.20
Common kestrel 68									0.10		0.39	0.38								0.10	0.11			0.11		0.71	0.20
Common kestrel 69											0.48	0.39								0.10	0.20			0.27		0.62	0.20
Common kestrel 70											0.51	0.49														1.08	0.20
Common kestrel 71									2.03		0.32	2.63				0.10			0.20	0.24	0.83		0.09	0.90		0.38	0.20
Common kestrel 72										0.36	0.47	0.24														1.22	0.22
Common kestrel 73											0.31	0.31														0.38	0.20
Common kestrel 74											0.46	0.20														1.15	0.20
Common kestrel 75		0.35									0.46	0.38														0.39	0.20
Common kestrel 76											0.36															0.56	0.20
Common kestrel 77									0.10		0.57	0.56									0.10					0.73	0.24
Common kestrel 78									0.09		0.40									0.10	0.10			0.13		0.39	0.20
Common kestrel 79											0.45	0.39														0.74	0.20
Common kestrel 80											0.45	0.27														0.35	0.20
Common kestrel 81										0.36	0.47															0.79	0.25
Common kestrel 82											0.46	0.28														0.42	0.20
Common kestrel 83										0.26	0.41															0.46	0.34
Common kestrel 84											0.81	0.30														1.04	0.23
Common kestrel 85											0.61	0.35														0.56	0.21
Common kestrel 86											0.54	0.25														0.52	0.20
Common kestrel 87										0.24	0.55															0.99	0.59

(*continued on next page*)

Table 4 (*continued*)

	Acenaphthene	Acenaphtylene	BDE 99	BDE 100	BDE 153	BDE 154	BDE 183	Dichlorodiphenyl dichloroethane (p,p'DDD)	Dichlorodiphenyl dichloroethylene (p,p'DDE)	Fluoranthene	Fluorene	Hexachlorobencene	Hexachlorocyclohexane (alpha)}	Hexaclorocyclohexane (beta)	Naphtalene	PCB 28	PCB 101	PCB 105	PCB 118	PCB 138	PCB 153	PCB 156	PCB 167	PCB 180	PCB 189	Phenanthrene	Pyrene
Common kestrel 88										0.25	0.83															1.65	0.30
Common kestrel 89								0.09			0.46									0.13	0.26			0.26		0.33	0.21
Common kestrel 90								0.37			0.36								0.20	0.53	0.98	0.20	0.12	0.90		0.45	0.20
Common kestrel 91			0.23	0.12	0.20	0.10	0.20	4.29			0.42							0.17	0.50	2.87	5.17	0.49	0.36	6.80		1.05	0.25
Common kestrel 92											0.50	0.57														0.53	0.21
Common kestrel 93		0.21									0.42	0.49														0.44	0.20
Common kestrel 94											0.59	0.73									0.11			0.13		0.55	0.20
Common kestrel 95			0.10					0.11		0.29	0.59										0.10			0.10		1.04	0.36
Common kestrel 96											0.22	0.26								0.10	0.10			0.10		0.59	0.20
Common kestrel 97										0.26	0.51	0.53			0.81									0.10		0.65	0.22
Common kestrel 98								0.37			0.39	0.48			0.83					0.10	0.14			0.11		0.97	0.21
Common kestrel 99		0.37						0.15			0.35	0.44								0.13	0.19			0.24		0.98	0.21
Common kestrel 100		0.42									0.35															0.88	0.30
Common kestrel 101		0.44						0.31			0.40	0.41														0.34	0.20
Common kestrel 102											0.46															1.05	0.20
Common kestrel 103	0.37									0.21	0.23															0.47	0.20
Common kestrel 104		0.31						0.09		0.25	0.41															0.40	0.40
Common kestrel 105	0.29										0.33															0.51	0.27
Common kestrel 106																											
Common kestrel 107			0.09	0.10				0.18			0.39	0.29								0.20	0.26			0.62		0.39	0.20
Common kestrel 108		0.40	0.10					1.44			0.30	0.83								0.41	0.61			0.70		0.37	0.20
Common kestrel 109		0.42						0.22			0.43	0.51								0.10	0.24			0.36		0.25	0.20
Common kestrel 110											0.81	0.50							0.20	0.16	0.46			0.70		0.45	0.20
Common kestrel 111	0.51							0.10			0.57															0.79	0.20
Common kestrel 112		0.26									0.38	1.27								0.10	0.21			0.38		0.45	0.20

step, without cleanup). The mixture of IS (acenaphthene-d10, chlorpyrifos-d10, chrysene-d12, diazinon-d10, PCB 200, and phenanthrene-d10 for the GC method, atrazine-d5, carbendazim-d3, cyromazine-d4, diazinon-d10, linuron-d3, and pirimicarb-d6 for the LC method) was added to all the tubes before the extraction. The samples were orbital shaken for 1 h, and then, 500 µl of 1% FA-ACN were added, before tubes were placed in an ultrasonic bath at room temperature for 20 min. Then, the QuEChERS salts (150 mg anhydrous magnesium sulfate and 37.5 mg sodium acetate) were added and, the tubes were vortexed 30 s and vigorously manually shaken for 60 s. After a 5 min centrifugation, the supernatant was filtered (0.2 µm) and used directly for chromatographic analyses. The optimization of the procedure is detailed in the main article [3].

2.3. Chromatographic analyses

Two complementary analyses were needed for the quantification of 360 environmental pollutants in whole blood. Thus, 234 compounds were analyzed by liquid chromatography and other 126 chemicals were analyzed by gas chromatography. Both techniques were tandem coupled to triple quadrupole mass spectrometry (LC-MS/MS and GC-MS/MS). All the equipment employed was from Agilent Technologies (Palo Alto, USA): UHPLC 1290 coupled to 6460 mass spectrometer for LC, and a 7890B GC coupled to 7010 mass spectrometer for GC. The chromatographic separations in LC were performed using an InfinityLab Poroshell 120 (2.1 mm × 100 mm, 2.7 µm). The stationary phase in GC consisted of two fused silica ultra-inert capillary columns Agilent HP-5MS (15 m × 0.25 mm i.d., 0.25 µm film thickness), that were connected by a purged union, to allow the backflushing. All the conditions in which these apparatus were operated, as well as the optimization procedure, are described in detail in the main article [3].

2.4. Validation

This article provides supporting information on the data of the validation process, which was carried out according to the criteria established in the SANTE and SWGTOX guide [1, 2] and, taking into account our previous experience in developing and validating chromatographic methods in complex biological matrices [4–6]. Although initially the experiments were carried out with chicken and goat blood separately, we found no significant differences. Therefore, we decided to use a 1: 1 mixture of both blood types to complete all validation experiments, following the recommendations of the SWGTOX guide. All the parameters of the validation process are shown in this article, except for the identity and selectivity parameters, which are shown in Table 1 of the main article [3], and carryover, which is also explained in the main article. As the blank matrix was not completely free of all of the contaminants, the signal of the blanks was subtracted from fortified samples in all the experiments.

Linearity was assessed within the range of concentrations that were considered appropriate for the purpose of biomonitoring (0.1 to 20 ng/ml). All the calibrators (12 points) were individually prepared in blank matrix in quintuplicate by adding an appropriate volume of fortification solution. Only compounds that showed a correlation coefficient (R^2) higher than 0.93 were kept in the method.

The accuracy (bias and precision) was calculated for all the 12 fortification levels injected in the chromatographic systems in quintuplicate. The bias is expressed as the percentage of the theoretical level of fortification. As recommended in the guidelines, only compounds with recoveries between 70% and 120% were kept in the method. However, due to their importance for biomonitoring certain exceptions were admitted, including some compounds with recovery percentages below or above these limits, but which were highly reproducible. Precision (repeatability and reproducibility) is expressed in terms of relative standard deviation (RSD) of the different

Table 5
Concentrations of non-persistent organic pollutants in whole blood (ng/ml) of a series of common kestrels sampled in Castilla–Leon (Spain) during 2018 and 2019.

	2-Phenylphenol	Albendazole	Atrazine	Benalaxyl	Brodifacoum	Bromadiolone	Coumachlor	Coumaphos	Coumatetralyl	Difenacoum	Difethialone	Diphenylamine	Enrofloxacin	Fenbendazole	Flumequine	Levamisole	Mebendazole	Metaflumizone	Metrafenone	Simazine	Sulfachloropiridacine	Sulfadiacine	Sulfapyridine
Common kestrel 01	0.20																						
Common kestrel 02	1.23															0.52							
Common kestrel 03	2.25			0.10																			
Common kestrel 04	0.81															0.42							
Common kestrel 05	1.31			0.10								0.51											
Common kestrel 06	0.87											0.23						0.20					
Common kestrel 07	0.52											0.25				0.31		0.20					
Common kestrel 08	2.05											0.31				0.28		0.20					
Common kestrel 09	0.58											0.21				0.33							
Common kestrel 10																		0.30	0.10				
Common kestrel 11	1.73				0.80													0.37					
Common kestrel 12	0.37															0.28							
Common kestrel 13	0.59							0.11				0.28											
Common kestrel 14	0.67															0.21		0.19					
Common kestrel 15	0.73			0.10			5.84																
Common kestrel 16	5.27											0.63		0.10		0.22							
Common kestrel 17	3.90																						
Common kestrel 18	0.98	0.10										0.20											
Common kestrel 19																							
Common kestrel 20				0.10												0.24							
Common kestrel 21				0.17																			
Common kestrel 22	0.64							0.19															
Common kestrel 23	1.07															0.21							
Common kestrel 24	1.09																						
Common kestrel 25	1.20				0.80																		
Common kestrel 26	0.45				0.80																		
Common kestrel 27	1.04																						
Common kestrel 28	0.99																	0.58					
Common kestrel 29	0.52					0.40												0.19					
Common kestrel 30	1.55			0.10											0.10			0.20					
Common kestrel 31	0.78																						
Common kestrel 32	1.07			0.10																			
Common kestrel 33	0.82															0.23							
Common kestrel 34	0.41																	0.92					
Common kestrel 35	1.14											0.20											
Common kestrel 36	0.60																						
Common kestrel 37	0.36																		0.10				

(*continued on next page*)

Table 5 (*continued*)

	2-Phenylphenol	Albendazole	Atrazine	Benalaxyl	Brodifacoum	Bromadiolone	Coumachlor	Coumaphos	Coumatetralyl	Difenacoum	Difethialone	Diphenylamine	Enrofloxacin	Fenbendazole	Flumequine	Levamisole	Mebendazole	Metaflumizone	Metrafenone	Simazine	Sulfachloropiridacine	Sulfadiacine	Sulfapyridine
Common kestrel 38	1.11																						
Common kestrel 39	4.91			0.10								0.50											
Common kestrel 40	0.84																						
Common kestrel 41	1.55																						
Common kestrel 42	0.60																						
Common kestrel 43	2.35											0.33											
Common kestrel 44	2.21				0.98							0.65											
Common kestrel 45	1.80				0.80																		
Common kestrel 46	0.21			0.10																			
Common kestrel 47	0.32																						
Common kestrel 48	2.65			0.10								0.20											
Common kestrel 49	0.91								13.02														
Common kestrel 50	0.34																						
Common kestrel 51	0.60				0.80																		
Common kestrel 52	0.48																						
Common kestrel 53	0.32			0.10																			
Common kestrel 54	0.26			0.10																			
Common kestrel 55	1.56																						
Common kestrel 56	0.29																						
Common kestrel 57	0.20																						
Common kestrel 58	0.21																						
Common kestrel 59	0.60																						
Common kestrel 60	2.01																						
Common kestrel 61	0.70																						
Common kestrel 62	0.78																						
Common kestrel 63	0.21									0.41			1.20										
Common kestrel 64	0.19																0.25						
Common kestrel 65	0.24															0.25							
Common kestrel 66	1.45																						
Common kestrel 67	1.09																						
Common kestrel 68	0.75			0.10																			
Common kestrel 69	1.00																						
Common kestrel 70	0.54																						
Common kestrel 71	0.99				32.73					0.42											0.80		
Common kestrel 72	0.78																						
Common kestrel 73	0.68																						

(continued on next page)

Table 5 (*continued*)

	2-Phenylphenol	Albendazole	Atrazine	Benalaxyl	Brodifacoum	Bromadiolone	Coumachlor	Coumaphos	Coumatetralyl	Difenacoum	Difethialone	Diphenylamine	Enrofloxacin	Fenbendazole	Flumequine	Levamisole	Mebendazole	Metaflumizone	Metrafenone	Simazine	Sulfachloropiridacine	Sulfadiacine	Sulfapyridine
Common kestrel 74	2.50																						
Common kestrel 75	0.20																						
Common kestrel 76	0.66																						
Common kestrel 77	0.59			0.16																0.22			
Common kestrel 78	0.20			0.23	1.93				0.30		1.77											0.85	
Common kestrel 79	1.32			0.20														0.20	0.10	0.29			
Common kestrel 80	0.20		0.1																	0.24			
Common kestrel 81	1.20			0.26																0.23			
Common kestrel 82	0.20			0.14																			0.40
Common kestrel 83	0.20			0.14															0.10				
Common kestrel 84	1.98			0.16																0.19			
Common kestrel 85	0.37			0.16										0.10									
Common kestrel 86	0.29			0.10										0.10									
Common kestrel 87	0.32											0.46											
Common kestrel 88	1.32			0.10																			
Common kestrel 89	0.30																						
Common kestrel 90	0.20																						
Common kestrel 91	2.99																						
Common kestrel 92	0.20																			0.20			
Common kestrel 93	0.20			0.10																			
Common kestrel 94	0.20																						
Common kestrel 95	0.39																						
Common kestrel 96	0.20																						
Common kestrel 97	0.43																	0.20					
Common kestrel 98	0.26			0.10														0.24					
Common kestrel 99	0.20			0.10																			
Common kestrel 100	0.51											0.20											
Common kestrel 101	0.29																						
Common kestrel 102	0.47											0.49											
Common kestrel 103	0.60																						
Common kestrel 104	0.52																						
Common kestrel 105	0.42																						
Common kestrel 106																							
Common kestrel 107	0.20																						
Common kestrel 108	0.20																						
Common kestrel 109	0.45																						
Common kestrel 110	0.80					0.42																	
Common kestrel 111	2.09											0.82											
Common kestrel 112	0.24																						

replicates. For the reproducibility of the method, only 5 levels of fortification (0.1, 0.5, 1, 5, and 20 ng/ml) were prepared in triplicate on three different days, within a period of 2 weeks. Therefore, the RSDs were calculated from 9 values. Only those compounds in which the RSD values were less than 20% remained in the method. In Table 1, we show the data of these 3 parameters only for the five levels of fortification mentioned above, although the rest also met the specified criteria.

The LOQ of this methodology was calculated over five runs of fortified blank matrix samples of three different sources (chicken, goat, and a mixture of both), as recommended (Scientific Working Group for Forensic, 2013). The lowest non-zero calibrator approximation was employed to calculate de LOQs. This means that the lowest point of the calibration curve that complied identity, bias and precision criteria was set as the LOQ for a given compound. All compounds with LOQ> 5 ng/ml were eliminated from the method since these levels are not considered adequate for biomonitoring studies. The calculated LOQs are shown in Table 1.

The influence of the matrix components on the performance of the method was evaluated by applying the extraction method to a sufficient quantity of blank whole blood to produce a blank matrix extract, which was subsequently fortified at three levels for the mixture of 360 chemicals (0.2, 2, and 20 ng/ml), and quantified against a calibration curve prepared in the solvent (1% FA-acetonitrile). The data corresponding to these experiments are shown in Fig. 1.

2.5. Application of the methodology to a series of blood samples of wild birds

The validated methodology was applied to a series of 148 real samples, composed of 36 samples from barn owls (*Tyto alba*) and 112 samples from common kestrels (*Falco tinnunculus*). These raptors were chosen as representative species of nocturnal and diurnal raptors and were sampled in the context of a project aimed to verify the penetration of anticoagulant rodenticides into the trophic chain of these species. Nest boxes located in the provinces of Palencia, Salamanca, Burgos, Segovia, Valladolid, and Zamora (Castilla-León, Spain) were sampled after a campaign with rodenticides against a common vole (*Microtus arvalis*) plague. All samples were collected after obtaining the corresponding permits and following the animal welfare protocols during the sampling [7]. The obtained data are shown in Tables 2 and 3 (barn owls) and Tables 3 and 4 (common kestrels).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

Acknowledgments

This research was supported by the University of Las Palmas de Gran Canaria via a doctoral grant to the first author Cristian Rial-Berriel (ULPGC-012-2016), and also supported by the Spanish Ministry of Education, Culture and Sports via a doctoral grant to the co-first author Andrea Acosta-Dacal (FPU16-01888). The authors would like to thank people from Tafira Wildlife Rehabilitation Center (Gran Canaria, Spain), specially Dr. Alejandro Suárez Pérez. We also thank Mrs. Ana Macías Montes and Dr. Luis Alberto Henríquez Hernández for their assistance in the laboratory of Toxicology of University of Las Palmas de Gran Canaria.

Ethics statement

All samples were collected after obtaining the corresponding permits and following the animal welfare protocols during the sampling [7].

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dib.2020.105878.

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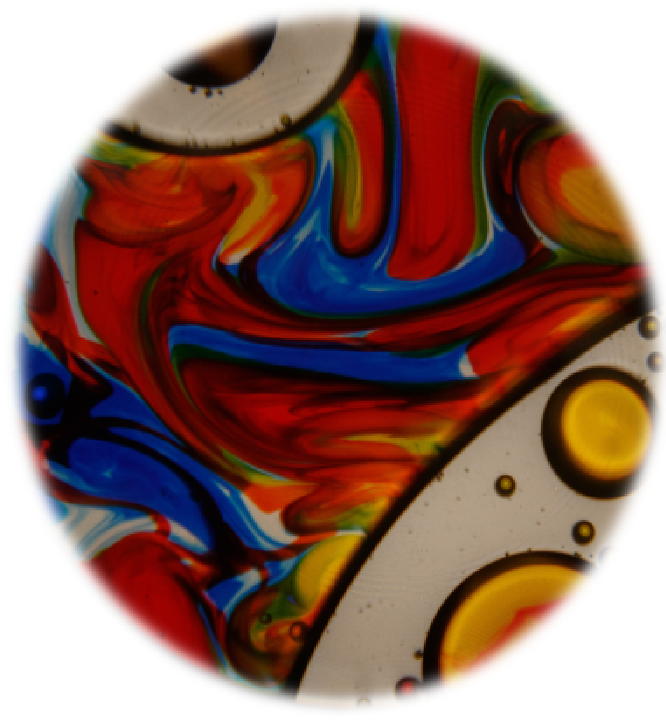
Publicación 3. A method scope extension for the simultaneous analysis of POPs, current-use and banned pesticides, rodenticides, and pharmaceuticals in liver. Application to Food Safety and Biomonitoring

Una extensión del alcance del método para el análisis simultáneo de COPs, pesticidas de uso actual y prohibidos, rodenticidas y productos farmacéuticos en el hígado. Aplicación a la Seguridad Alimentaria y la Biomonitorización

Toxics, 2021, 9: 238

DOI: https://doi.org/10.3390/toxics9100238



Article

A Method Scope Extension for the Simultaneous Analysis of POPs, Current-Use and Banned Pesticides, Rodenticides, and Pharmaceuticals in Liver. Application to Food Safety and Biomonitoring

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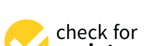

Citation: Rial-Berriel, C.; Acosta-Dacal, A.; Zumbado, M.; Henríquez-Hernández, L.A.; Rodríguez-Hernández, Á.; Macías-Montes, A.; Boada, L.D.; Travieso-Aja, M.d.M.; Martin Cruz, B.; Luzardo, O.P. A Method Scope Extension for the Simultaneous Analysis of POPs, Current-Use and Banned Pesticides, Rodenticides, and Pharmaceuticals in Liver. Application to Food Safety and Biomonitoring. *Toxics* **2021**, *9*, 238. https://doi.org/10.3390/toxics9100238

Academic Editor: Claudio Medana

Received: 5 September 2021
Accepted: 23 September 2021
Published: 27 September 2021

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Abstract: The screening of hundreds of substances belonging to multiple chemical classes in liver is required in areas such as food safety or biomonitoring. We adapted a previous QuEChERS-based method in blood to the liver matrix and applied to these fields of study. The validation of the method allowed the inclusion of 351 contaminants, 80% with a LOQ < 2 ng/g. In the analysis of 42 consumer liver samples, we detected trace levels of 29 different contaminants. The most frequent and concentrated was 4,4'-DDE. POPs accounted for 66% of the compounds detected. In no case was the MRL reached for any of the contaminants detected. We also applied the method to 151 livers of wild birds to perform a biomonitoring pilot study in the Canary Islands. We detected 52 contaminants in 15 bird species. These were also mostly POPs, although high frequencies and concentrations of anticoagulant rodenticides (AR) and some other agricultural pesticides also stand out. POPs and AR contamination levels were significantly higher in terrestrial birds, raptors and particularly in nocturnal birds. Pesticide contamination levels were also higher in terrestrial birds, as well as in non-raptors and diurnal birds. The validated method is simple, robust, and sensitive and performs well in a variety of practical scenarios, where it can be carried out relatively quickly and inexpensively.

Keywords: persistent organic pollutants; agrochemicals; environmental pollution; QuEChERS; LC-MS/MS; GC-MS/MS

1. Introduction

Animal liver is a common matrix for the search of chemical residues for several reasons. On the one hand, the use of veterinary drugs, which is widely accepted in veterinary practice to treat bacterial infections, parasitism (internal or external), inflammation, and other animal diseases or their symptoms in livestock practice [1,2], may condition the appearance of residues of these chemicals in foods of animal origin, especially in the liver [3–5]. This is particularly relevant, since the presence of antimicrobial compounds can induce the spread of drug-resistant pathogenic bacterial strains or produce allergic reactions in humans [3,6]. In addition, pesticide products used in agriculture can leave

residues in the raw materials used in the preparation of animal food, and these residues can in turn generate the appearance of residues in food products of animal origin [5], that can pose a serious risk to the health of consumers [6]. Therefore, to protect consumers from these undesirable effects, as a food safety measure, maximum residue limits (MRLs) have been established for many veterinary medicinal products and pesticides on a range of commodities of animal origin, including meat and meat products, liver, fish, honey, milk, and eggs [5,7]. According to the European regulations, the liver must be investigated for the presence of several hundred compounds, including pesticides commonly used in agriculture, pesticides already banned but of great environmental persistence, and also residues of veterinary drugs [7]. Although there are differences between countries, if we take as a common reference what is established in the Codex Alimentarius, there are at least maximum residue limits (MRLs) established for some 218 pesticides and for another 75 veterinary drugs (also including combinations of drugs and animal feed) [4,5].

On the other hand, wildlife lives in an environment that is increasingly contaminated with chemical substances (pesticides, industrial pollutants, wastewater, urban solid waste, etc.) and therefore can serve as first line indicators of the levels of pollutants and their possible health impacts [8–10]. Wildlife biomonitoring can provide important information about the bioavailability of contaminants in the environment humans share with these species [11–13], which supports the design of appropriate remediation strategies [14]. These data can result in substantial savings of limited remediation resources while maximizing the preservation of important natural areas and supporting effective site remediation [14]. The action of monitoring wildlife exposure to chemical contaminants is usually known as biomonitoring [10,15,16].

Like pesticides and pharmaceuticals, the annual use of anticoagulant rodenticides (ARs) for rodent control is measured in thousands of tons. This extensive use often leads to unintentional exposure of non-target animals, especially birds of prey, to these poisons, so there is a need for these substances to be included in biomonitoring studies. With regard to food safety, it should be noted that ARs are not authorized for use on edible crops within the EU, so the Codex Alimentarius Commission has not set MRLs for any of them, and residues of ARs are not expected to be present in any plant or animal products [17], and therefore they are not usually routinely investigated in food.

Whether it is for residue research in the context of food safety, or in the context of biomonitoring, the liver is an extremely interesting matrix, as it is one of the organs of that concentrates more quantity of chemicals [18]. The range of substances that it is interesting to investigate in one or another circumstance is quite similar, since the substances that are of concern from the point of view of food safety generally also concern from the point of view of the environmental pollution and their effects on wildlife [11,19–21]. The availability of multi-residue methods that are capable of accurately and simultaneously identifying and quantifying the concentration of any of these substances subjected to MRLs that may be present in the liver tissue is extremely interesting [5]. Therefore, it is necessary to develop multi-residue methods belonging to multiple chemical classes. In the case of biomonitoring, moreover, the challenge of detecting such a variety of potentially harmful substances in a complex matrix such as the liver is compounded by the fact that the amount of sample available is usually small [16].

Although there are numerous published multi-residue/multi-class methods for the determination of chemicals in animal liver, most focus either on the analysis of pesticides [22,23] or on the analysis of certain groups of veterinary drugs [24–26]. However, very few of the published methods address the simultaneous analysis of compounds from both classes [27], and are generally limited to a discrete number of compounds. Therefore, to cover the whole spectrum of compounds of interest in any of the fields (food safety and biomonitoring), it is usually necessary to apply several methods in a complementary manner, which consumes time, economic resources, and sample quantity, which may be limited in the case of wildlife.

The first part of this research consists of a validation of a method scope extension. The original method was developed for whole blood [16,28], and now it has been validated for liver matrix. But more interesting, probably, is the second part of our paper, in which we present and discuss the results of the application of this methodology to the two fields described. On the one hand we analyzed the residues of substances subjected to MRLs in 46 samples of liver intended for human consumption sampled, acquired in markets, supermarkets, and slaughterhouses. On the other hand, we applied the method to the biomonitoring of 151 wildlife specimens from the Canary Islands received in our service from mid-2020 to April 2021.

2. Materials and Methods

2.1. Certified Standards and Reagents

Methanol (MeOH, 99.9% purity), acetonitrile (ACN, 99.9% purity), and formic acid (FA, 98.0% purity), all LC-MS grade, were purchased from Honeywell (Charlotte, NC, USA). LC-Grade water (18.2 MΩ/cm) was purified by a MilliQ A10 Gradient system (Millipore, Molsheim, France). Ammonium acetate Optima LC-MS grade was purchased from Fisher (Fisher Scientific UK, Loughborough, UK). QuEChERS Extract Pouch, AOAC Method (6 g de magnesium sulphate and 1.5 g sodium acetate), were purchased in commercial premixes from Agilent Technologies (Palo Alto, CA, USA).

All certified standards (liquid or solid) of all the individual pollutants and deuterated compounds (P-ISs, procedural internal standards) were obtained from A2S—Analytical Standard Solutions (Staint Jean D'Illac, France), Sigma-Aldrich (Augsburg, Germany), CPA Chem (Stara Zagora, Bulgaria), European Pharmacopoeia Reference Standards (Strasbourg, France), Accustandard (New Haven, CT, USA), and Dr. Ehrestorfer (Augsburg, Germany). All standards were from the highest purity available (93.1% to 99.8%). Individual 1 mg/mL stock solutions of each pollutant were prepared either dissolving or diluting certified standards in ACN, MeOH, water, or acetone (according to the solubility of substances), and stored no more than a year at −32 °C. The standard solutions were sorted, grouping by pesticide, pharmaceuticals, COPs, or procedural internal standards (pIS) to get four intermediate solutions at 1 µg/mL/each. Matrix-matched calibration and quality control points were fortified independently, to get 11 points between 0.4 to 40 ng/mL, with 4 quality controls at 1, 4, 10, and 20 ng/mL.

2.2. Liver for Method Validation

For the development, optimization, and validation of the analytical technique, we employed liver samples obtained from chickens of an organic farm. All the chickens were born in this facility, were healthy and had never been exposed to chemicals (no farms or agricultural facilities in the nearby, and no pharmacological treatments, according to the standards of the production mode), to avoid drug interference. The livers were obtained directly from the slaughterhouse, when these animals were slaughtered for consumption, and placed in 50 mL propylene tubes. Upon arrival at the laboratory, these samples were immediately stored at −24 °C until use.

2.3. Sample Preparation and Extraction

The QuEChERS method [29] is a matrix dispersion extraction method, which was initially developed for the analysis of pesticides in fruits and vegetables, but has proven to be versatile, allowing the analysis of many other compounds in complex matrices such as blood, milk, meat, eggs, and even soil [30,31]. We applied it to liver samples, for which it is first necessary to homogenize the liver before applying the QuEChERS extraction. For this purpose, one gram of liver sample was weighed into a tube suitable for homogenization with a Precellys Evolution homogenizer (Bertin Technologies, Rockville, Washington D.C., USA), operated at 6500 rpm, 2 × 30 s. After that, when needed, the fortification was performed, either for validation experiments, for calibration curves, or for the preparation of the quality controls (QC). Then, the homogenate was diluted with 4 mL ultrapure

water, and one milliliter of the diluted homogenate was placed in a 5 mL Eppendorf tube to be processed. Ten µL of pIS mix (acenaphthene-d10, atrazine-d5, carbendazim-d3, chlorpyrifos-d10, chrysene-d12, cyromazine-d4, diazinon-d10, linuron-d3, PCB 200, phenanthrene-d10, and pirimicarb-d6) was added to all the tubes (either fortified or not) to reach a final concentration of 10 ng/mL. Next, anhydrous magnesium sulfate (480 mg) and sodium acetate (120 mg) were added to each sample tube, followed by 30 s of vortexing and 1 min of vertical manual shaking. Finally, the Eppendorf tubes were centrifuged for 5 min, at 4500 rpm and 2 °C. The supernatant was then filtered through a 0.2 µm Chromafil PET-20/15 syringe filter (polyester, certified for HPLC, Macherey-Nagel, Düren, Germany) into an amber vial directly, for sequential LC and GC-MS/MS analysis.

2.4. Instrumental Analysis

We found that two complementary analyses are required to detect and quantify the 351 compounds that finally could be included in this method. Thus, an analysis by gas chromatography coupled to triple quadrupole mass spectrometry (GC-MS/MS) is needed for the analysis of the most volatile compounds (mainly persistent organic pollutants and some less polar pesticides) and an analysis by liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS) for the pharmaceuticals, the rodenticides, and the most polar pesticides.

2.4.1. GC-MS/MS

Gas chromatography was employed for the separation of 126 compounds using an Agilent 7890B gas chromatograph (Agilent Technologies, Palo Alto, CA, USA). Two Agilent J&W HP-5MS (5% cross-linked phenyl-methyl-polysiloxane, Agilent Technologies) ultra-inert fused silica capillary columns, with a total length of 30 m (15 + 15), a film thickness of 0.25 µm and 0.25 mm in diameter, were employed for the separations. The columns were joined by means of a purged joint to allow the application of the back-flushing technique that reduces the background noise and extends the column lifetime. An ultra-inert glass wool inlet liner at 250 °C was used at the injection port, and the injection (1.5 µL) was performed in splitless pulsed mode. The gases used were supplied by Linde (Dublin, Ireland), the carrier gas being helium 5.0 (99.999% purity) at a constant flow 1.5 mL/min, and the collision gas being nitrogen 6.0 (99.9999% purity). The initial oven temperature of 80 °C was maintained for 1.8 min, then increased at a rate of 40 °C/min to 170 °C, then increased at a rate of 10 °C/min to 310 °C, and finally maintained for 3 min at 310 °C. The post-run backflush to clean the column was set at 315 °C for 5 min at −5.8 mL/min for the first column, and the final run time at 21.05 min. For the identification and quantification of the compounds, an Agilent 7010 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) was used. This equipment was operated in the multiple reaction monitoring mode (MRM), with 24-time segments, cycle time between 300 and 600 ms and a dwell time between 15 and 40 ms. The electron impact (EI) and transfer line ionization source temperatures were set at 280 °C, with a solvent delay of 3.7 min.

2.4.2. LC-MS/MS

Liquid chromatography was employed to separate 225 substances using an Agilent 1290 Infinity II UHPLC (Agilent Technologies, Palo Alto, CA, USA). The column was an InfinityLab Poroshell 120 (2.1 mm × 100 mm, 2.7 µm), coupled to an inline filter and an UHPLC guard column with the same characteristics as the analytical column, to protect the column. The gradient of mobile phase A was: 95%—0.5 min; 80%—1 min; 60%–-2.5 min; 15%—8 min; 0%—10 to 14 min; 95%—14.01 min. Mobile phase A contained 0.1% FA and 2 mM ammonium acetate in ultrapure water; mobile phase B consisted of 2 mM ammonium acetate in MeOH. 8 µL were injected at a flow rate set at 0.4 mL/min and an oven column temperature of 50 °C. For identification and quantification, an Agilent 6460 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) was employed. It was operated in the dynamic multiple reaction monitoring mode (dMRM), in both positive

and negative polarities, with a cycle time 800 ms, a dwell time of 8 to 60 ms, and a total run time of 18 min. The Agilent Jet Stream Electrospray Ionization Source (AJS-ESI) was operated under the following conditions: gas temperature 190 °C; nebulizer gas flow and pressure were 11 L/min and 26 psi, respectively; the temperature of the sheath gas and the flow were 330 °C and 12 L/min, respectively; and the positive and negative capillary voltages were 3900 V and 2600 V. The drying and desolvation gas was nitrogen provided by the Zefiro 40 nitrogen generator (F-DGSi, Evry, France). Nitrogen 6.0 (99.9999% purity, Linde, Dublin, Ireland) was used as the collision gas.

2.5. Validation Procedures

Although this is an extension of the analytical scope of a previous method [16]—in this case a change of matrix—it is necessary to undertake a validation process to verify the capacity of the assay to obtain satisfactory results for the analytes in the new matrix. In this research the validation process included the evaluation of linearity, accuracy, precision, calculation of the limit of quantification (LOQ), uncertainty, and the study of the carryover and matrix effect. For most compounds included in this method, there is no specific guide for method validation. For veterinary drugs and considering liver as a food product of animal origin, the requirements for the methods and validation are presented in the UE's Regulation 808/2021 [32]. Therefore, we decided to follow this regulation, and also the guide of Standard Practices for Method Validation in Forensic Toxicology (SWGTOX) [33], and the EU's Directorate-General for Health and Food Safety analytical method validation guide (SANTE) [34].

The linearity of the response was studied by injecting blank liver extract samples spiked with all analytes at 11 levels (range 0.4–40 ng/g) and processed with the method described in Section 2.3 of this section, in quintuplicate. To determine accuracy and precision, % recovery (range 70–120% being acceptable, as specified in the guideline used) and % relative standard deviation (%RSD, values $\leq$ 20% being acceptable) were calculated, respectively. Recovery and RSD experiments were performed with blank liver samples fortified at least in five quintuplicate concentrations within the working range. For the calculation of the LOQ, matrix-matched calibration curves were prepared in quintuplicate (below 20 ng/g). From these, the lowest concentration level of each analyte that met the criteria for identification, accuracy and precision was considered as the LOQ. For confirmation of compound identity and selectivity, 2 MRM transitions were used, one for quantification (Q) and one for confirmation (q). A maximum deviation of $\pm$30% was tolerated for the ion ratio [35]. Similarly, a maximum deviation of $\pm$0.1 min was established for the retention time.

2.6. Samples for the Applicability of the Method

The main objective of this research is to demonstrate the applicability of the validated method in the two fields of application mentioned above: (a) verification of compliance with maximum residue limits in livers intended for human consumption; and (b) biomonitoring of contaminants in wildlife. For this purpose, a set of samples was collected for each of the two independent studies. The samples are described in the following subsections.

2.6.1. Sampling for the Food Safety Study

To verify the applicability of this method for the control of residues subject to MRLs in livers intended for human consumption, 46 liver samples from butcheries, supermarkets, and the general slaughterhouse of Gran Canaria were acquired: 34 samples of beef liver and 12 samples of chicken liver. All the samples, as they were acquired, were transferred to the laboratory and frozen at −20 °C until they were processed.

2.6.2. Sampling for the Biomonitoring Study

The validated method was applied to real samples of wildlife specimens that were received in our laboratory for forensic analyses in the period between September 2020 and

May 2021. Thus, we studied a series of 151 fresh liver samples belonging to 15 different species of birds. All the specimens were sent by environmental agents or by the Tafira Fauna Rehabilitation Centre, within the framework of the Strategy for the Prevention and Control of Poisoning in the Canary Islands [36]. All the birds included in this part of the study died from different classes of trauma, and there was no suspicion that they died of poisoning. The species included in this study were: *Accipiter nisus* (*n* = 5); *Ardea cinerea* (*n* = 12); *Asio otus canariensis* (*n* = 34); *Burrhinus oedicnemus* (*n* = 10); *Buteo buteo insularum* (*n* = 12); *Calonectris diomedea* (*n* = 8); *Ciconia ciconia* (*n* = 2); *Corvus corax canariensis* (*n* = 16); *Egretta garzetta* (*n* = 4); *Falco eleanorae* (*n* = 2); *Falco pelegrinoides* (*n* = 6); *Falco tinnunculus canariensis* (*n* = 14); *Larus michaellis* (*n* = 14); *Turdus merula* (*n* = 4); and *Tyto alba* (*n* = 8). The livers, received or extracted at in situ necropsy, were kept at −24ºC until the moment of their processing for analysis. No animals were sacrificed for the purposes of this work.

2.7. Statistical Analyses

All statistical analyses were performed with GraphPad Prism v9.2 software (GraphPad Software, CA, USA). The distribution of the variables included in this study was evaluated using the Kolmogorov–Smirnov test. The concentration of most of the contaminants detected did not follow a normal distribution, so the results are expressed in terms of median and range. For this same reason nonparametric tests to check for statistical differences between groups were employed, as these evaluate the median rather than the mean, which is appropriate given the relatively high number of undetected values in some groups. Homogeneity of variance (homoscedasticity) was previously tested using Levene's test. The Kruskal–Wallis and Mann–Whitney U tests were used as nonparametric tests for overall and pairwise comparisons, respectively. However, as an additional check, pairwise comparisons were also performed using Student's t-test after logarithmic transformation of the data. A P-value of less than 0.05 (two-tailed) was considered statistically significant. The prevalence of exposure to each contaminant for each species was calculated as the percentage of animals with that residue detected in the liver over the total number of individuals of that species studied. For the study of determinants in the series used for biomonitoring, the response variables considered for comparisons were the amount in the liver of (a) the sum of non-persistent pesticides; (b) the sum of persistent organic pollutants; and (c) the sum of rodenticides.

3. Results and Discussion

3.1. Method Scope Extension Optimization

In our previous research we optimized and validated a multi-residue method for the analysis of 360 substances (pharmaceuticals, pesticides, rodenticides, and POPs) in blood for biomonitoring purposes [16,28]. Therefore, this is not an ex-novo methodological development, but an extension of the scope of our previously published method to include a new matrix, the liver. However, for a better method performance in this more complex matrix, we considered optimizing the previously established chromatographic conditions, including recalculation of RTs, as well as optimization of MRM transitions to allow for higher sensitivity, as well as adjusting qualifiers and qualifier ratios, and identifying possible interferences with matrix components. The compounds are shown in alphabetical order in Appendix A along with their retention time, transitions, and their collision energies. As we did with the original method in blood, we decided to directly inject the extracts obtained in acetonitrile for LC-MS/MS and GC-MS/MS analyses, without using evaporation and solvent change, to avoid the loss of the more volatile compounds. Several authors, including our group [30,37,38], have shown that ACN, although not the most commonly used solvent in GC-MS/MS, is an appropriate solvent for this type of analysis.

The final number of validated compounds in this scope extension counts 351 chemicals and metabolites compared to 360 in the previous work. With respect to the original method, there are 18 compounds that met the validation criteria in whole blood, which do not meet the validation criteria when the method is applied to liver samples: acetaminophen, chlor-

fenapyr, corticosterone 21 acetate, phenbutatin oxide, iprodione, isocarbophos, leptophos, malaoxon, malathion, marbofloxacin, methomyl oxime, N,N,-dimethyl-N-tolylsulfamide, paraoxon ethyl, parathion ethyl, penicillin G, phosmet oxon, piperacillin, and trichlorfon. On the other hand, the opposite occurred with 9 compounds. Dichlorvos, doramectin, metalaxyl, methiocarb-sulfoxide, moxidectin, oxime, pthalimide, pyrimicarb-desmethyl and spirotetramat met the validation criteria in the presence of liver matrix and could therefore be included in the method in liver, whereas in blood they did not and had to be left out.

3.2. Validation Parameters

For confirmation of compound identity and selectivity, 2 MRM transitions were used, one for quantification (Q) and one for confirmation (q). A maximum deviation of ±30% was tolerated for the ion ratio. Similarly, a maximum deviation of ±0.1 min was established for the retention time.

We first studied the linearity of the response by injecting blank liver extract samples spiked with all analytes at 11 levels (range 0.4–40 ng/g) and processed in quintuplicate with the method described in Section 2.3. The linearity study on the response (R^2), indicated that this was higher than 0.98 for all analytes in the range studied.

To determine accuracy and precision, % recovery and % relative standard deviation (RSD) was calculated. A recovery within the range 70–120% and RSD values $\leq$ 20% was considered acceptable, as specified in the guidelines employed [33,34]. Recovery and RSD experiments were performed with blank liver samples fortified at least in four quintuplicate concentrations within the working range. The results of the recovery experiments are presented in Appendix B. Regarding accuracy and precision, most compounds meet the validation criteria for concentrations between their LOQ and the highest level studied (40 ng g^{-1}). There were some exceptions where recoveries were outside the above range, especially at the lower concentrations. However, these cases are covered, both in the SANTE guideline and in the SWGTOX working document [33,34], which also accepts as a good validation criterion obtaining recoveries between 60% and 140% at some of the concentrations tested, provided that the RSD is less than 15%. Likewise, in some cases, the recoveries were within the established limits with an RSD slightly higher than 15%, a scenario that is also contemplated in the methodological guidelines, provided that the result is reproducible. As a rule, this second exception applies for concentrations equal to or lower than 4 ng g^{-1}. As SANTE analytical guide recommends, the expanded measurement uncertainty (U′) was calculated, from precision and bias, and all analytes presented U′ < 50%, that complies with the requirement.

For the calculation of the LOQ, matrix-matched calibration curves were prepared in quintuplicate (0.2–20 ng g^{-1}). From these, the lowest concentration level of each analyte that met the criteria for accuracy and precision was considered as the LOQ. As in the original method, the LOQ for the analytes included in this scope extension was calculated from five replicates of fortified blank matrix, within the working range. The lowest non-zero calibrator approximation was used to calculate LOQs. This means that the lowest point on the calibration curve that met the identity, bias, and precision criteria was established as the LOQ for a given compound. The LOQs for the 351 liver analytes are shown in Appendix B. The LOQ was set at 0.4 ng g^{-1} for 61 compounds, at 0.8 ng g^{-1} for 82 compounds, at 1.2 ng g^{-1} for 40 compounds, at 1.6 ng g^{-1} for 37 compounds, at 2 ng g^{-1} for 50 compounds, at 4 ng g^{-1} for 46 compounds, at 8.0 ng g^{-1} for 24 compounds, at 12 ng g^{-1} for 5 compounds, at 16 ng g^{-1} for 4 compounds, and at 20 ng g^{-1} for 2 compounds. That is, 76.9% of the compounds included in this method can be reliably and accurately quantified at concentrations below 2 ng g^{-1}, making it suitable not only for food safety or poisoning diagnostic studies, but also for biomonitoring studies.

In the original method from which we started it was observed that there was a strong blood matrix effect on about 40% of the analytes. Presumably, a similar situation would occur with the liver matrix. Nevertheless, we decided to include the study of the

matrix effect within the validation strategy of this analytical scope extension to prove it, as recommended in the reference guides. All validation assays involve the addition of known concentrations of analytes to the matrix. For the matrix effect study, we worked with the addition of three known concentrations of all analytes (2 ng g^{-1}, 10 ng g^{-1}, and 20 ng g^{-1}) on blank liver extract, and the quantification was done against calibration curves prepared in solvent (without matrix). Experiments were performed in quintuplicate for each concentration. One difficulty was that, given the enormous number of substances included in the method, the liver was not completely free of 100% of the chemicals, in particular POPs. Therefore, in these cases, the response of the white matrix sample was subtracted from the calibration standards and QC to calculate the response of the externally added analyte. As we expected, matrix effect (ME) was observed for both, compounds analyzed by LC-MS/MS and GC-MS/MS, especially for compounds analyzed by the latter technique. A strong or medium suppression of the signal was demonstrated for 17.66% of the compounds (n = 62), and signal enhancement was verified for 36.47% of the compounds (n = 128). For the remaining 45.87% (n = 161 contaminants, the ME was considered negligible ($-20\% < M < 20\%$). Since for most of the compounds, significant ME was indeed observed, and it was concluded that matrix-matched calibration had to be used to compensate for these interferences. All detailed ME data for individual compounds in liver are shown in Appendix C.

Finally, we also assessed if carryover occurred after injecting a blank matrix fortified at 80 ng g^{-1} and processed with this method, before a blank matrix extract. We were not able to find a clear response in that blank matrix, so we concluded that in our working range, we had not any carryover effect in any of the analyzed compounds.

3.3. Application to Food Safety

In the study of the 34 beef liver samples, the results indicated the presence of a discrete number and concentration of contaminants, which ranged from 0 to 15 residues per sample, with an average of 3.13 residues. Of the 351 contaminants and metabolites included in the method, only 25 were detected in the total of beef liver samples, and of these 19 belong to the group of persistent or semi-persistent contaminants (4,4′-DDE, 4,4′-DDD, Dieldrin, Hexachlorobenzene, beta-hexachlorocyclohexane, BDE 153, PCB congeners #105, 118,126, 138, 155, 156, 157, 180, 189, naphthalene, phenanthrene, and pyrene). It is noteworthy that none of the concentrations in any of the samples exceeded the MRL, or even the value of half the MRL. In general, the concentrations of the contaminants detected were low, with the highest values being those of 4,4′-DDE, which was detected in 65.2% of the samples and with a median value of 92.2 ng g^{-1}. The relatively high levels of DDT derivatives may seem surprising, as this substance was banned in Spain almost 5 decades ago. However, there is abundant literature that has documented that this pesticide was widely used in the Canary archipelago, and how this translates into the levels of this pesticide detected in food for human consumption produced in this region [39–42].

The next in frequency and concentration were PCB 153 (26.1%; 35.2 ng g^{-1}) and PCB 138 (21.8%; 24.5 ng g^{-1}). The other contaminants were detected in frequencies and concentrations much lower than these. Among the non-persistent pesticides detected in this series of consumption livers, very low levels of bifenthrin, fenazaquin, fluquinconazole, flutalonil, flutriafol, and imidacloprid were detected.

If the detection of residues in beef liver was low and of little toxicological relevance, it was even more so in chicken liver. In the 12 samples analyzed, we detected only four contaminants out of the 351 included in the method: fenpropidin, fenpropimorph, levamisole, and 4,4′ DDE. The latter was the more relevant, and it was only detected in three of the 12 livers analyzed and at a much lower concentration than that detected in beef liver (mean = 4.3 ng g^{-1}).

Although it is not the main objective of this study, we made an estimate of the risk of exposure to these contaminants through liver consumption. The calculations were made according to the standard methodology that has been described previously [43], and in no

case were the tolerable daily intake levels for these contaminants exceeded, mainly due to the low consumption of liver by the Spanish population (only 1 g/day for the total offal consumption) [44].

3.4. Application to Biomonitoring

Regarding biomonitoring of chemical substances, this method was applied to fresh livers obtained from 151 carcasses of 15 species of wild birds whose causes of death were not related to poisoning (mainly trauma). Table 1 shows the results for each of the species, limited to show only the 52 contaminants that were detected in the series. This represents that 15% of the contaminants included in the method were detected.

The mean value of the number of contaminants per sample was 17. The species with the greatest variety of residues detected was *Asio otus* (n = 41), followed by *Falco tinnunculus* (n = 27). In contrast, the species with the lowest number of liver contaminants were *Turdus merula* (n = 5) and *Ciconia ciconia* (n = 3). Figure 1 shows the LC-MS/MS and GC-MS/MS chromatograms of one of the birds in the series with the highest number of different contaminants (a long-eared owl).

The most frequently detected contaminant was 4,4′-DDE, which was detected in 138 birds (91.4%), followed by PCB 153, detected in 116 animals (76.8%), brodifacoum in 109 animals (72.2%), bromadiolone in 87 animals (57.6%), and dieldrin in 59 animals (39.1%). With respect to concentrations, the highest concentrations corresponded to enrofloxacin, clindamycin and meloxicam (Table 1). However, these values cannot be considered within the biomonitoring study, since they correspond to drugs used during the hospitalization of many of these animals. Therefore, high concentrations of these substances have been marked with an asterisk. However, other veterinary pharmaceuticals detected in some specimens, such as tetraconazole, metronidazole, or sulfathiazole, are not part of the treatment administered and should be considered contaminants. In general terms, the highest concentrations of contaminants corresponded to 4,4′-DDE in all species. Overall, in quantitative terms, the group of organochlorine pesticides was the most abundant (Figure 2), and the group of persistent and semi-persistent organic pollutants accounted for more than 92% of the total concentration of pollutants detected in the livers of wild birds sampled in the Canary Islands very recently (September 2020–May 2021). This reflects, once again, that contamination by these compounds, in particular organochlorine insecticides, is still very prevalent in the Canary Islands, as has been reported for wildlife in this region [28,45–47]. As indicated in the previous section, there is a large literature body documenting the high levels of contamination by organochlorine pesticides in this region [48–50], which also translates into high levels in the biota that inhabit the archipelago. There is a possibility that the high levels detected could also come from the neighboring African continent [51], but in this biomonitoring study this option is ruled out, since all the birds sampled for this pilot study are residents in the archipelago and not migratory birds.

With respect to non-persistent pollutants, several aspects should be highlighted. First, the high prevalence of second-generation anticoagulant rodenticides in wildlife's liver is noteworthy. It was expected, as it has been described in many parts of the world [52–54] and recently in the Canary Islands [45,55,56]. However, the presence of at least one of these compounds in more than 80% of the birds studied is striking, even in non-predatory birds such as the blackbird (*Turdus merula*) or the common curlew (*Burhinus oedicnemus*), which would point to the fact that these compounds penetrate the trophic chain by several routes, probably including invertebrates, as suggested by other authors [57,58].

Table 1. Median concentrations and frequencies (between parentheses) of organic pollutants detected in wild birds of the Canary Islands. All the results are in ng/g.

Compound	Accipiter Nisus (*n* = 5)	Ardea Cinerea (*n* = 12)	Asio Otus (*n* = 34)	Burrinus Oecdine-mus (*n* = 10)	Buteo Buteo (*n* = 12)	Calonectris Diomedea (*n* = 8)	Ciconia Ciconia (*n* = 2)	Corvus Corax (*n* = 16)	Egretta Garzetta (*n* = 4)	Falco Eleono-rae (*n* = 2)	Falco Pelegri-noides (*n* = 6)	Falco Tin-nunculus (*n* = 14)	Larus Michaelis (*n* = 14)	Turdus Merula (*n* = 4)	Tyto Alba (*n* = 8)
Meloxicam	9.9 (40)	-	47.8 (12)	880.3 * (20)	-	-	-	10.3 (13)	-	-	-	96.1 (29)	-	-	-
Tetraconazole	-	-	0.6 (6)	-	-	-	-	-	-	-	-	-	-	-	-
Clindamycin	1.3 (40)	-	1423 * (18)	1.4 (20)	28.4 (33)	-	-	-	-	-	2.3 (33)	-	-	-	-
Enrofloxacin	5300 * (80)	-	4739 * (29)	4638 * (40)	5453 * (67)	-	-	1970 * (13)	-	20.4 (100)	5144 * (50)	5531 * (57)	10234 * (7)	-	14508 * (50)
Metronidazole	-	-	-	50.8 (10)	-	-	-	-	-	-	-	603.3 (14)	-	-	-
Sulfatiazole	-	-	15.5 (6)	-	-	-	-	-	-	-	-	-	-	-	-
2-Phenylphenol	-	-	16.3 (18)	22.7 (20)	2.4 (17)	-	-	-	-	-	-	-	-	-	-
Boscalid (formerly nicobifen)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2 (25)
Fludioxonil	-	-	0.4 (6)	-	-	-	-	-	-	-	-	-	-	-	-
Fluquinconazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flutriafol	-	-	-	-	4.4 (17)	-	-	-	-	-	-	-	-	-	-
Carbofuran	-	-	10.3 (3)	16.4 (10)	7.2 (26)	-	-	94.5 (38)	-	-	-	-	-	-	-
Carbofuran-3-hydroxy	-	-	-	-	-	-	-	2.1 (38)	-	-	-	-	-	-	-
Fipronil	-	-	1.4 (12)	-	-	-	-	-	-	-	-	-	-	-	-
Fipronil sulfide	-	-	3.0 (6)	-	-	-	-	-	-	-	-	-	-	-	-
Permethrin	-	-	-	-	-	23.4 (13)	-	-	-	-	-	-	12.3 (7)	-	-

Table 1. *Cont.*

Compound	Accipiter Nisus (n = 5)	Ardea Cinerea (n = 12)	Asio Otus (n = 34)	Burrinus Oecdine-mus (n = 10)	Buteo Buteo (n = 12)	Calonectris Diomedea (n = 8)	Ciconia Ciconia (n = 2)	Corvus Corax (n = 16)	Egretta Garzetta (n = 4)	Falco Eleono-rae (n = 2)	Falco Pelegri-noides (n = 6)	Falco Tin-nunculus (n = 14)	Larus Michaelis (n = 14)	Turdus Merula (n = 4)	Tyto Alba (n = 8)
Acenaphthene	2.4 (80)	1.4 (17)	0.8 (18)	-	0.4 (17)	-	-	-	-	-	-	2.4 (14)	-	-	-
Anthracene	1.5 (40)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chrysene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.8 (25)
Fluoranthene	0.4 (40)	-	-	-	-	-	-	-	-	-	-	-	-	-	1.7 (25)
Fluorene	5.9 (80)	-	2.5 (12)	-	-	-	-	-	-	-	-	5.3 (7)	-	-	-
Naphtalene	1.8 (100)	-	2.2 (6)	5.7 (40)	3.9 (26)	16.6 (13)	-	3.4 (26)	0.7 (25)	-	-	1.8 (14)	-	0.9 (50)	8.0 (25)
Phenanthrene	13.3 (100)	-	7.5 (12)	-	-	0.4 (13)	-	2.7 (13)	-	-	-	7.6 (28)	-	-	851.3 (50)
Pyrene	1.8 (40)	-	0.7 (9)	-	-	-	-	-	-	-	-	20.0 (28)	-	-	-
4,4′-Dichlorobenzophenone (metabolite of dicofol)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BDE-100	-	1.9 (33)	0.2 (6)	-	-	-	-	-	-	-	0.4 (33)	-	0.2 (7)	-	-
BDE-153	-	0.3 (33)	4.9 (41)	-	-	1.1 (13)	-	-	-	0.7 (50)	19.0 (67)	0.6 (29)	-	-	0.8 (25)
BDE-154	-	2.1 (17)	2.6 (6)	-	-	-	-	-	-	-	6.9 (33)	-	-	-	-
BDE-183	-	-	0.9 (12)	-	-	-	-	-	-	-	-	-	-	-	-
BDE-47	-	1.5 (17)	2.4 (6)	-	-	-	-	-	-	-	-	0.6 (7)	0.4 (14)	-	-

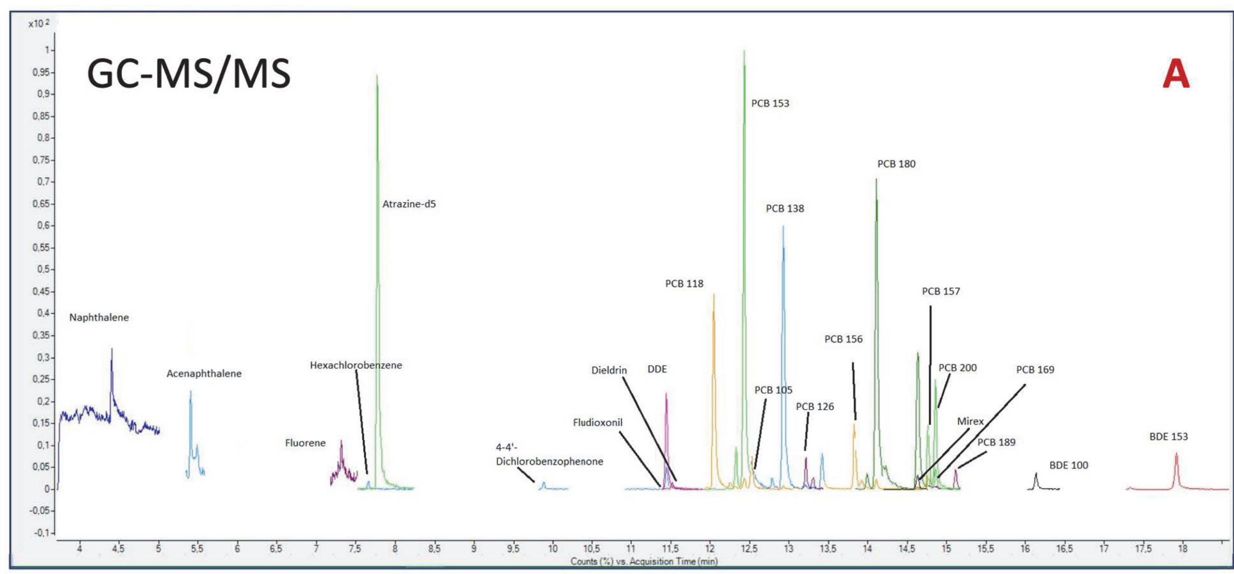
Table 1. *Cont.*

Compound	Accipiter Nisus (*n* = 5)	Ardea Cinerea (*n* = 12)	Asio Otus (*n* = 34)	Burrinus Oecdine-mus (*n* = 10)	Buteo Buteo (*n* = 12)	Calonectris Diomedea (*n* = 8)	Ciconia Ciconia (*n* = 2)	Corvus Corax (*n* = 16)	Egretta Garzetta (*n* = 4)	Falco Eleono-rae (*n* = 2)	Falco Pelegri-noides (*n* = 6)	Falco Tin-nunculus (*n* = 14)	Larus Michaelis (*n* = 14)	Turdus Merula (*n* = 4)	Tyto Alba (*n* = 8)
BDE-99	-	-	1.5 (29)	-	-	-	-	-	-	-	3.6 (33)	0.4 (14)	0.6 (14)	7.5 (100)	0.4 (13)
Dichlorodiphen yldichloroethane (p,p′ DDD)	1.2 (80)	-	-	-	-	-	-	-	-	-	-	2.2 (21)	-	-	-
Dichlorodiphen yldichloroethylene (p,p′ DDE)	211.1 (100)	21.1 (100)	305.6 (100)	25.9 (60)	5.6 (83.3)	16.6 (100)	-	6.7 (75)	4.7 (100)	68.4 (100)	318.6 (100)	45.3 (100)	4.4 (100)	-	24.1 (100)
Dieldrin	7.8 (80)	3.5 (17)	5.6 (41.2)	3.0 (10)	0.9 (33)	3.1 (13)	-	-	2.1 (100)	5.8 (100)	8.5 (100)	11.9 (100)	2.3 (7)	-	1.2 (75)
Hexachlorobencene	-	1.4 (34)	0.6 (24)	-	-	7.1 (13)	-	-	12.9 (50)	0.6 (50)	-	1.1 (7)	1.1 (7)	-	0.8 (25)
Hexachlorocyclohexane (beta)	-	-	31.0 (12)	-	-	-	-	-	-	-	3.4 (50)		-	-	-
Mirex	-	-	3.9 (12)	3.6 (20)	3.1 (17)	25.0 (13)	-	-	-	-	2.3 (33)		-	-	-
PCB 105	-	1.2 (50)	1.3 (35)	-	-	2.0 (26)	-	-	3.6 (25)	0.6 (50)	0.6 (67)	1.1 (14)	0.4 (7)	-	-
PCB 118	0.5 (40)	5.4 (50)	4.8 (35)	-	0.5 (17)	45.1 (13)	-	-	14.9 (50)	5.0 (100)	2.2 (100)	1.3 (28)	1.4 (7)	-	1.2 (37)
PCB 138	1.4 (80)	7.9 (100)	2.9 (76)	2.2 (30)	1.3 (34)	8.8 (75)	-	3.9 (38)	24.1 (100)	15.3 (100)	7.7 (100)	4.2 (71)	2.7 (72)	-	5.3 (75)
PCB 153	3.4 (80)	15.5 (100)	3.6 (94)	0.5 (80)	3.3 (34)	6.8 (100)	-	1.7 (88)	55.3 (100)	115.3 (100)	16.0 (100)	5.7 (71)	-	-	7.8 (100)
PCB 156	-	2.0 (50)	2.0 (33)	-	-	8.5 (13)	-	-	0.8 (25)	8.8 (50)	0.8 (100)	0.9 (7)	-	-	0.5 (25)

Table 1. *Cont.*

Compound	Accipiter Nisus (n = 5)	Ardea Cinerea (n = 12)	Asio Otus (n = 34)	Burrinus Oecdine-mus (n = 10)	Buteo Buteo (n = 12)	Calonectris Diomedea (n = 8)	Ciconia Ciconia (n = 2)	Corvus Corax (n = 16)	Egretta Garzetta (n = 4)	Falco Eleono-rae (n = 2)	Falco Pelegri-noides (n = 6)	Falco Tin-nunculus (n = 14)	Larus Michaelis (n = 14)	Turdus Merula (n = 4)	Tyto Alba (n = 8)
PCB 157	-	0.8 (17)	1.1 (6)	-	-	2.1 (13)	-	-	9.1 (50)	1.1 (50)	-	-	0.4 (7)	-	-
PCB 167	-	1.7 (50)	1.5 (35)	-	-	6.7 (50)	-	-	44.6 (100)	6.7 (50)	1.3 (67)	0.8 (14)	2.2 (72)	-	0.4 (13)
PCB 180	3.9 (80)	24.3 (67)	3.2 (88)	-	2.1 (50)	3.6 (100)	-	2.8 (88)	-	123.6 (100)	19.8 (100)	5.2 (71)	-	-	8.0 (75)
PCB 189	-	-	2.1 (6)	1.0 (80)	-	-	-	-	-	1.8 (50)	-	-	-	-	-
PCB 28	-	-	5.8 (3)	-	-	-	-	-	-	-	-	-	-	-	-
Brodifacoum	1.7 (100)	0.4 (100)	32.9 (100)	2.3 (80)	0.9 (100)	-	-	27.4 (75)	-	-	20.4 (100)	8.8 (50)	1.4 (21)	-	20.31 (100)
Bromadiolone	-	-	1.3 (100)	2.1 (100)	8.5 (100)	-	-	2.25 (38)	-	1.1 (50)	4.6 (100)	2.5 (75)	-	0.34 (25)	2.2 (75)
Difenacoum	-	0.8 (17)	0.6 (24)	-	1.5 (50)	-	-	0.9 (13)	-	-	0.9 (33)	1.2 (57)	-	-	3.6 (25)
Difethialone	-	-	18.9 (18)	-	-	-	-	-	-	-	-	1.9 (29)	-	-	-
Flocoumafen	-	-	0.7 (24)	-	4.1 (17)	-	-	-	-	-	-	2.2 (7)	-	-	-

* These values cannot be considered as biomonitoring, since these pharmaceuticals were employed during the treatment of the animals at the Wildlife Recovery Centers.



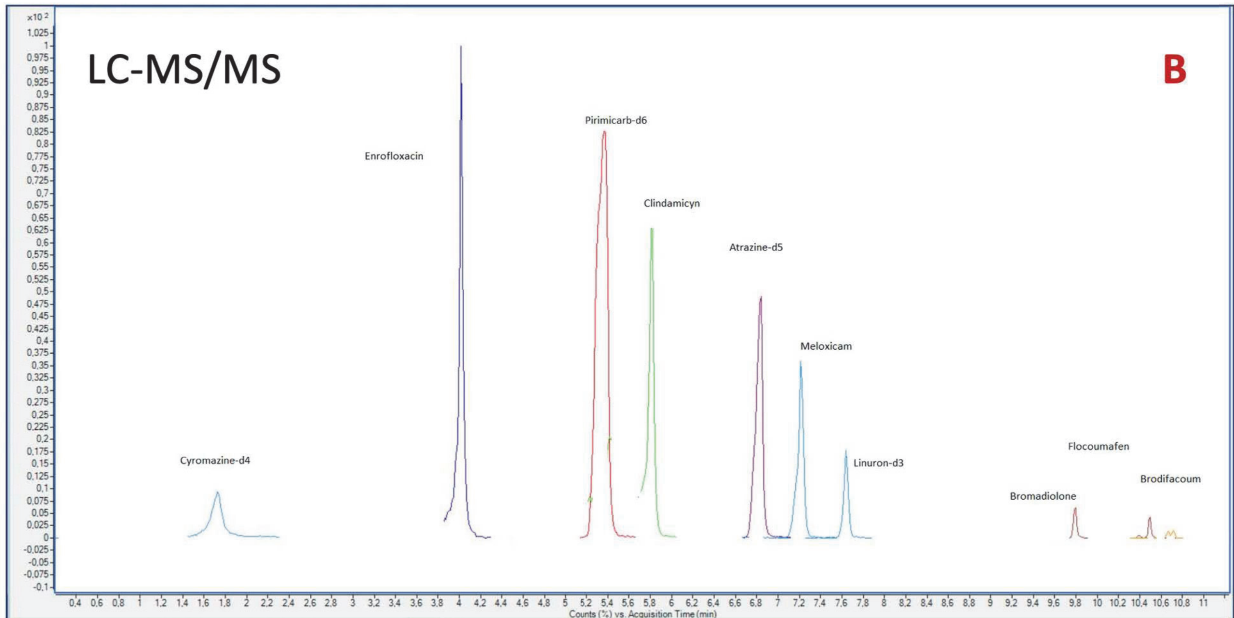


Figure 1. MRM chromatograms of the complementary analyses of a real sample (long-eared owl) by GC-MS/MS (**A**) and by LC–MS/MS (**B**).

The result for carbofuran is also surprising, given that none of the birds studied had any suspicion of intoxication. However, this potent insecticide, banned in the EU since 2007 [59] was detected in small concentrations in the liver of 10 birds of the series, being higher in the case of canary crows (6/12 positives, median = 94.5 ng/g). In all these crows, the main carbofuran metabolite was also detected. This toxicant has widely affected wildlife worldwide [60–63] and in the Canary Islands its use has been extensive and also has affected wildlife in the past [64]. From the results of this study, it still is today, and it can be concluded that it even penetrates the trophic chain. With respect to the rest of the non-persistent compounds detected in this series, 2-phenylphenol (PHP) stands out. PHP was detected in eleven birds, including five common curlews (Table 1). PHP is a biocide used as a preservative and surface disinfectant on fibers and other materials in homes, hospitals, and elsewhere, and is recognized as a potential endocrine disruptor [65]. Other

authors have also reported that PHP is a highly prevalent contaminant in biota samples, such as river fish of different species, where it is found in up to 100% of samples [66].

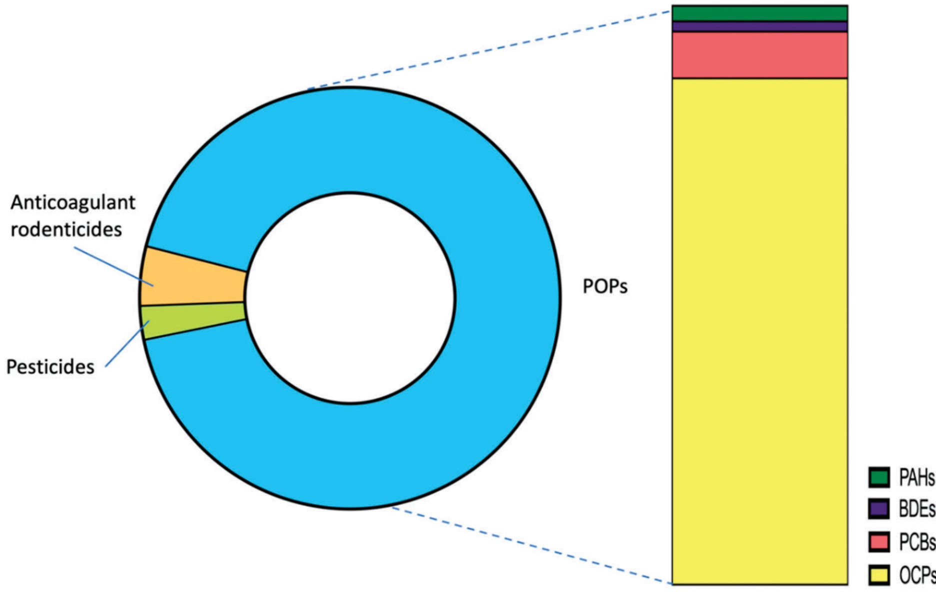


Figure 2. Occurrence of environmental pollutants in the liver of a series of 151 wild birds of the Canary Islands.

Since this was an opportunistic study on carcasses obtained from wildlife recovery centers, we did not have too many quality variables to carry out an in-depth study of the determinants of contamination patterns. Even so, we wanted to explore the influence of the variables inherent to the species studied and found a series of statistically significant differences. Thus, when we compared aquatic versus terrestrial birds, we found that the latter presented significantly higher levels of contamination by the three major chemical groups studied (Figure 3).

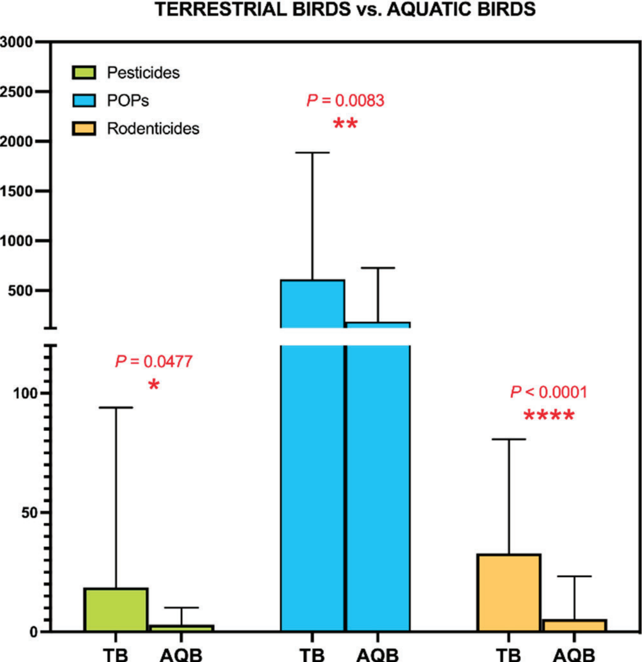


Figure 3. Study of the determinants of environmental contamination detected in the livers of wild birds in the Canary Islands: Habitat type (terrestrial (TB) vs. aquatic birds (AQB)).

This result was expected with regard to rodenticides, since in a previous study by our group focused on these compounds, we had already discarded the group of waterfowl due to their low incidence in these pollutants [56]. Regarding POPs and non-persistent pesticides, although there is not much literature comparing both types of birds from the same region, the available studies usually indicate results similar to ours, with levels in landbirds usually being higher than in waterbirds [67–69].

Another variable that seems to influence the pattern of contamination is the raptor/predator bird status. The raptors in our study presented higher levels of POPs than non-predatory birds (Figure 4), which is logical given that they feed higher in the trophic chain, and has been described in the literature [68,69]. They also presented higher levels of AR, as we expected from having previously observed it in this region [56], and also described by other authors [70]. However, in the case of agricultural pesticides the statistical significance was the opposite, with non-predatory birds presenting the highest levels. There is not much literature to support this finding, but a recent study using the terrestrial pesticide residue exposure (T-REX) model estimated that the highest risk was presented by insectivorous birds, followed by fruit and seed feeders [71].

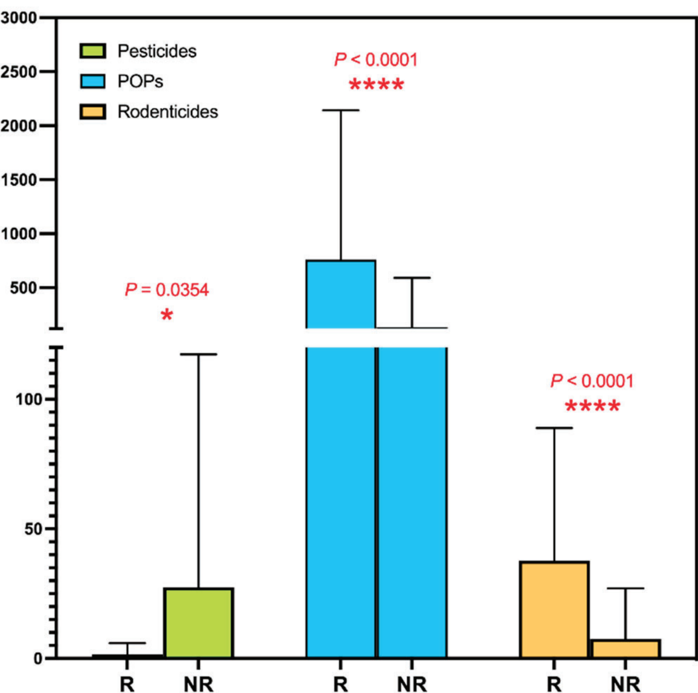


Figure 4. Study of the determinants of environmental contamination detected in the livers of wild birds in the Canary Islands: Diet type (raptors vs. non-raptors).

Finally, we also studied the influence of the diurnal/nocturnal habits of the birds in the study, and found that diurnal species have higher pesticide levels, but lower POPs and ARs than nocturnal species (Figure 5). We believe that the pesticide result has to do with the previous variable, in the sense that, in our study, all insectivorous species, and those that feed on fruits and seeds are diurnal, while the nocturnal birds in our series are both raptors that feed mainly on large and small rodents. For this same reason, and as we had already verified in previous studies, the nocturnal birds of the Canary Islands have higher levels of POPs [72] and AR [45,56].

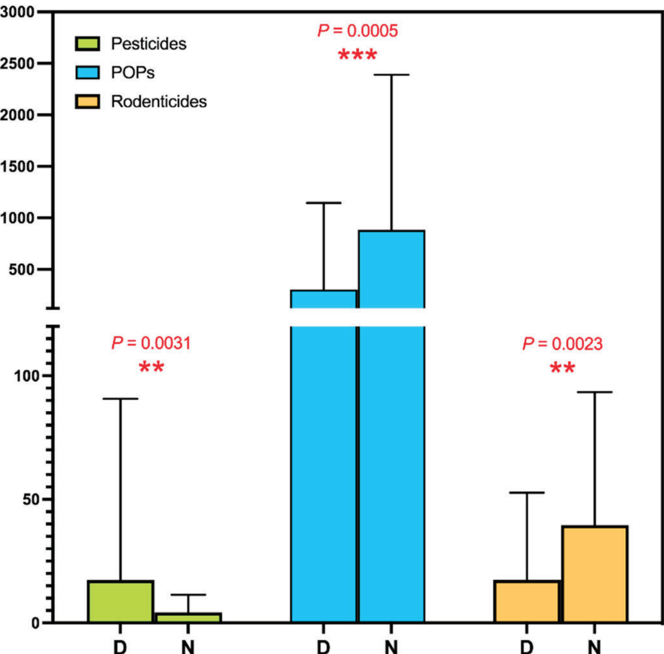


Figure 5. Study of the determinants of environmental contamination detected in the livers of wild birds in the Canary Islands: Habit type (diurnal vs. nocturnal).

4. Conclusions

The validated method allows the simultaneous analysis in liver of 351 substances (POPs, pesticides including rodenticides and drugs), using only 1 gram of sample. This is important, since in veterinary forensic medicine, especially with small animals, the amount of sample available is very limited. The proposed analytical method can detect trace amounts of all chemicals in the liver of multiple species. Therefore, it can be successfully applied and used as a routine method in environmental chemistry and forensic toxicology laboratories. The method we have developed can also be used in residue control studies in food intended for human consumption and for the purpose of food safety assessment.

Author Contributions: Conceptualization, C.R.-B. and O.P.L.; methodology, C.R.-B. and A.A.-D.; software, C.R.-B. and A.A.-D.; validation, C.R.-B., A.A.-D., A.M.-M., and Á.R.-H.; formal analysis, L.A.H.-H., C.R.-B. and O.P.L.; investigation, C.R.-B., A.A.-D., A.M.-M., Á.R.-H. and B.M.C.; resources, L.D.B. and O.P.L.; data curation, C.R.-B. and M.Z.; writing—original draft preparation, O.P.L., M.d.M.T.-A. and C.R.-B.; writing—review and editing, O.P.L., M.d.M.T.-A. and C.R.-B.; visualization, O.P.L.; supervision, O.P.L. and L.D.B.; project administration, O.P.L.; funding acquisition, O.P.L., M.Z., L.A.H.-H. and L.D.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the University of Las Palmas de Gran Canaria, grant number ULPGC-012-2016, to C. Rial-Berriel., and by the Spanish Ministry of Education, Culture and Sports, grant number FPU16-01888, to A. Acosta-Dacal.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: On request to the authors.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Chromatographic and mass spectrometric conditions of the compounds analyzed in liver.

No.	Compound	Technique	Retention Time (min)	Polarity	Quantification		Confirmation		Fragmentor Voltage (V)
					MRM (m/z)	Collision energy (eV)	MRM transition (m/z)	Collision energy (eV)	
1	2-Phenylphenol	GC	6.28	positive	169.0 → 115.0	30	169.0 → 141.0	15	70
2	4,4′-Dichlorobenzophenone (metabolite of dicofol)	GC	9.99	positive	250.0 → 139.0	15	250.0 → 215.0	5	70
3	Abamectine	LC	10.99	positive	890.5 → 567.1	10	895.5 → 751.4	45	160
4	Acenaphthene	GC	6.15	positive	153.0 → 152.0	25	153.0 → 151.0	35	70
5	Acenaphtylene	GC	5.94	positive	152.0 → 151.0	25	152.0 → 126.0	30	70
6	Acephate	LC	1.64	positive	184.0 → 143.0	15	143.0 → 95.0	15	70
7	Acetamiprid	LC	4.43	positive	223.1 → 126.0	27	223.1 → 90.0	45	140
8	Acrinathrin	LC	10.70	positive	559.0 → 208.0	10	559.0 → 181.0	30	70
9	Albendazole	LC	7.14	positive	266.1 → 234.1	16	266.1 → 191.0	32	155
10	Aldicarb	LC	5.11	positive	208.0 → 116.0	10	116.0 → 89.1	4	100
11	Aldicarb-sulfone	LC	3.21	positive	240.1 → 76.0	16	223.1 → 86.1	13	75
12	Aldicarb-sulfoxide	LC	2.75	positive	207.1 → 131.9	10	207.1 → 89.1	10	86
13	Aldrin	GC	9.90	positive	255.0 → 220.0	25	263.0 → 228.0	10	70
14	Anthracene	GC	8.40	positive	178.0 → 176.0	35	178.0 → 152.0	30	70
15	Atrazine	LC	6.73	positive	216.0 → 173.9	15	216.0 → 103.8	30	130
16	Azinphos-methyl	LC	7.27	positive	318.0 → 132.1	8	340.0 → 160.0	10	60
17	Azoxystrobin	LC	7.59	positive	404.1 → 372.1	8	404.1 → 344.1	24	110
18	BDE-28	GC	12.22	positive	406.0 → 246.0	20	406.0 → 167.0	25	70
19	BDE-47	GC	14.31	positive	326.0 → 138.0	45	484.0 →324.0	25	70
20	BDE-85	GC	17.08	positive	564.0 → 404.0	25	566.0 → 406.0	25	70
21	BDE-99	GC	16.27	positive	566.0 → 406.0	25	564.0 → 404.0	30	70

Table A1. *Cont.*

No.	Compound	Technique	Retention Time (min)	Polarity	Quantification		Confirmation		Fragmentor Voltage (V)
22	BDE-100	GC	15.85	positive	566.0 → 406.0	25	564.0 → 404.0	25	70
23	BDE-153	GC	18.04	positive	644.0 → 484.0	25	486.0 → 377.0	30	70
24	BDE-154	GC	17.47	positive	644.0 → 484.0	25	486.0 → 377.0	30	70
25	BDE-183	GC	20.12	positive	561.6 → 454.7	40	563.6 → 454.7	40	70
26	Benalaxyl	LC	8.96	positive	326.2 → 148.0	20	326.2 → 208.0	12	90
27	Bendiocarb	LC	5.88	positive	224.1 → 166.9	8	224.2 → 108.9	15	120
28	Bendiocarb metabolite (2,2-dimethylbenzo-1. 3-dioxol-4-ol)	GC	4.84	positive	166.0 → 151.0	10	166.0 → 126.0	20	70
29	Benfuracarb	LC	9.73	positive	411.2 → 190.0	13	411.2 → 252.0	15	110
30	Benzo[a]anthracene	GC	13.95	positive	228.0 → 226.0	40	228.0 → 202.0	35	70
31	Benzo[a]pyrene	GC	16.89	positive	252.0 → 250.0	45	252.0 → 248.0	60	70
32	Benzo[b]fluoranthene	GC	16.30	positive	252.0 → 248.0	60	252.0 → 226.0	35	70
33	Benzo[ghi]perylene	GC	19.61	positive	276.0 → 274.0	50	276.0 → 272.0	60	70
34	Benzo[k]fluoranthene	GC	16.29	positive	252.0 → 250.0	45	252.0 → 224.0	40	70
35	Bifenthrin	GC	11.25	positive	440.0 → 181.0	5	440.0 → 165.0	60	94
36	Bitertanol	LC	9.23	positive	338.2 → 70.0	4	338.2 → 269.2	5	100
37	Boscalid (formerly nicobifen)	GC	7.84	positive	3434.0 → 272.0	30	343.0 → 140.0	45	100
38	Brodifacoum	LC	10.78	negative	521.3 → 79.0	50	523.3 → 135.0	45	220
39	Bromadiolone	LC	9.75	negative	525.3 → 250.0	40	527.3 → 250.0	40	200
40	Bromopropylate	GC	13.87	positive	341.0 → 183.0	15	341.0 → 157.0	45	70
41	Bromuconazole (two isomers)	GC	13.81/14.24	positive	295.0 → 173.0	10	295.0 → 175.0	10	70
42	Bupirimate	LC	11.78	positive	273.0 → 108.0	15	273.0 → 193.0	5	70

Table A1. *Cont.*

No.	Compound	Technique	Retention Time (min)	Polarity	Quantification		Confirmation		Fragmentor Voltage (V)
43	Buprofezin	LC	9.83	positive	306.1 → 201.0	12	306.1 → 116.0	12	140
44	Cadusafos (ebufos)	LC	9.39	positive	271.1 → 159.0	16	271.1 → 131.0	22	100
45	Carbaryl	LC	6.21	positive	202.1 → 145.1	4	202.1 → 127.1	28	95
46	Carbendazim (azole)	LC	2.90	positive	192.1 → 160.1	4	202.1 → 127.1	28	90
47	Carbofuran	LC	5.91	positive	222.1 → 123.1	20	222.1 → 165.1	30	80
48	Carbofuran-3-hydroxy	LC	4.27	positive	238.1 → 163.1	10	238.1 → 181.1	10	110
49	Carbosulfan	LC	11.03	positive	381.2 → 160.2	12	381.2 → 76.1	36	120
50	Cefuroxima axetil (two isomers)	LC	5.13	positive	533.0 → 447.0	15	533.0 → 386.0	20	160
51	Chloramphenicol	LC	4.63	negative	321.0 → 152.1	4	323.0 → 152.1	4	113
52	Chlorantraniliprole	LC	7.32	positive	483.9 → 452.9	16	483.9 → 285.9	8	105
53	Chlorfenvinphos	LC	9.09	positive	361.1 → 98.9	34	358.9 → 155.1	8	105
54	Chlorobenzilate	GC	12.14	positive	251.0 → 111.0	40	251.0 → 139.0	15	70
55	Chlorophacinone	LC	8.88	negative	373.2 → 201.0	20	375.2 → 203.0	20	160
56	Chlorpropham	GC	7.13	positive	213.0 → 127.0	15	153.0 → 90.0	25	70
57	Chlorpyrifos	GC	9.93	positive	314.0 → 258.0	15	314.0 → 286.0	5	70
58	Chlorpyrifos methyl	GC	9.12	positive	286.0 → 93.0	25	286.0 → 271.0	15	70
59	Chlorthal dimethyl	GC	10.02	positive	300.9 → 166.9	55	300.9 → 222.9	25	70
60	Chrysene	GC	13.86	positive	228.0 → 226.0	40	228.0 → 227.0	25	70
61	Clindamycin	LC	5.33	positive	425.2 → 126.1	20	425.2 → 377.2	20	150
62	Clofentezine	LC	9.19	positive	303.1 → 138.0	12	303.1 → 102.0	40	120
63	Clothianidin	LC	3.91	positive	250.0 → 169.0	8	250.0 → 131.9	8	100
64	Cloxacillin	LC	6.86	positive	436.1 → 160.0	8	436.1 → 277.0	12	126
65	Coumachlor	LC	8.63	positive	343.1 → 162.8	15	342.1 → 285.0	15	120

Table A1. *Cont.*

No.	Compound	Technique	Retention Time (min)	Polarity	Quantification		Confirmation		Fragmentor Voltage (V)
66	Coumaphos	LC	8.98	positive	363.0 → 227.0	30	363.0 → 306.9	15	120
67	Coumatetralyl	LC	8.31	negative	291.1 → 141.0	30	291.1 → 247.0	20	140
68	Cyazofamid	LC	8.49	positive	325.0 → 108.0	20	325.0 → 261.1	15	90
69	Cyflufenamid	LC	9.18	positive	413.1 → 223.1	33	413.1 → 295.1	23	70
70	Cyfluthrin (sum of four isomers)	GC	16.07/16.19/16.25/16.32	positive	226.0 → 206.0	25	198.9 → 170.1	25	70
71	Cyhalothrin (lambda isomer)	GC	10.49	positive	181.1 → 152.1	10	181.1 → 127.1	46	70
72	Cymoxanil	LC	4.67	positive	199.1 → 128.0	4	199.1 → 110.9	12	90
73	Cypermethrin (sum of four isomers)	GC	16.34/16.44/16.52/16.63	positive	163.0 → 109.0	20	163.0 → 127.0	5	70
74	Cyproconazole (two isomers)	GC	11.98	positive	222.0 → 125.0	20	222.0 → 82.0	10	70
75	Cyprodinil	LC	8.46	positive	226.0 → 93.0	33	226.0 → 108	25	100
76	Cyromazine	LC	1.23	positive	167.1 → 85.0	16	167.1 → 125.0	20	120
77	Danofloxacin	LC	4.04	positive	358.2 → 340.1	20	358.2 → 82.1	50	159
78	Dazomet	GC	7.80	positive	161.9 → 44.0	28	161.9 → 89.0	5	70
79	Deltamethrin	LC	10.65	positive	523.0 → 281.0	10	523.0 → 506.0	5	100
80	Demeton-S-methyl	LC	5.97	positive	230.9 → 88.9	5	230.9 → 61.0	30	50
81	Demeton-S-methyl-sulfone (Dioxydemeton)	LC	3.31	positive	263.0 → 169.0	24	263.0 → 109.0	12	120
82	Dexamethasone	LC	7.16	positive	393.2 → 373.2	2	393.2 → 355.2	6	103
83	Diazinon	GC	8.29	positive	137.1 → 54.0	20	304.0 → 179.0	15	70
84	Dibenzo[a,h]anthracene	GC	19.15	positive	278.0 → 276.0	40	278.0 → 250.0	60	70

Table A1. *Cont.*

No.	Compound	Technique	Retention Time (min)	Polarity	Quantification		Confirmation		Fragmentor Voltage (V)
85	Dichlorodiphen yldichloroethane (p,p′ DDD)	GC	12.31	positive	235.0 → 165.0	20	235.0 → 199.0	15	70
86	Dichlorodiphen yldichloroethylene (p,p′ DDE)	GC	11.58	positive	318.0 → 176.0	60	318.0 → 248.0	30	70
87	Dichlorodiphen yltrichloroethane (p,p′ DDT)	GC	12.84	positive	235.0 → 165.0	40	235.0 → 199.0	15	70
88	Diclofenac	LC	8.73	positive	296.0 → 215.1	16	296.0 → 214.1	48	103
89	Dicloran	GC	7.80	positive	206.0 → 176.0	10	206.0 → 148.0	25	70
90	Diclorvos	GC	4.74	positive	184.9 → 93.0	10	185.0 → 109.0	15	70
91	Dicloxacillin	LC	7.24	positive	470.0 → 160.0	8	470.0 → 310.8	10	106
92	Dieldrin	GC	11.66	positive	263.0 → 228.0	15	277.0 → 241.0	15	70
93	Diethathyl ethyl	LC	8.71	positive	312.2 → 238.1	15	312.2 → 162.0	30	120
94	Diethofencarb	LC	7.57	positive	268.2 → 226.1	5	268.2 → 152.0	20	110
95	Difenacoum	LC	10.38	negative	443.2 → 135.0	40	443.2 → 293.0	35	200
96	Difenoconazole	LC	9.41	positive	406.1 → 250.9	28	406.1 → 337.0	16	176
97	Difethialone	LC	10.93	negative	537.3 → 79.0	50	537.3 → 151.0	45	220
98	Difloxacin	LC	3.86	positive	400.2 → 382.1	20	400.2 → 356.1	16	149
99	Diflubenzuron	LC	8.63	positive	311.0 → 158.0	8	311.0 → 141.0	32	90
100	Diflufenican	GC	13.27	positive	394.0 → 266.0	10	266.0 → 246.0	10	70
101	Dimethenamid-P (and its R-isomer)	LC	7.68	positive	276.1 → 244.1	10	276.1 → 168.1	20	125
102	Dimethoate	LC	4.21	positive	230.0 → 125.0	16	230.0 → 198.8	20	70

Table A1. *Cont.*

No.	Compound	Technique	Retention Time (min)	Polarity	Quantification		Confirmation		Fragmentor Voltage (V)
103	Dimethomorph (two isomers)	LC	7.86	positive	388.1 → 301.1	20	388.1 → 165.1	32	180
104	Dimethylphenylsulfamide (DMSA. metabolite of dichlofluanid)	LC	5.21	positive	201.1 → 92.1	15	201.1 → 137.1	5	100
105	Diniconazole-M	LC	9.34	positive	326.1 → 70.0	28	328.1 → 70.0	28	110
106	Dinocap	LC	10.51	negative	295.4 → 208.9	30	295.4 → 193.0	35	150
107	Diphacinone	LC	8.60	negative	339.1 → 167.0	25	339.1 → 145.0	20	170
108	Diphenylamine	GC	6.98	positive	168.0 → 167.2	15	169.0 → 66.0	15	70
109	Dodine	LC	9.02	positive	228.3 → 43.0	40	228.3 → 57.0	25	150
110	Doramectina	LC	11.31	positive	921.5 → 777.4	55	899.5 → 145.1	30	220
111	Endosulfan alfa	GC	11.21	positive	241.0 → 206.0	15	195.0 → 160.0	10	70
112	Endosulfan beta	GC	12.21	positive	241.0 → 206.0	15	195.0 → 159.0	15	70
113	Endosulfan sulfate	GC	12.96	positive	270.0 → 235.0	15	387.0 → 289.0	5	70
114	Endrin	GC	12.05	positive	263.0 → 193.0	35	245.0 → 173.0	25	70
115	Enrofloxacin	LC	3.94	positive	360.2 → 316.1	16	360.2 → 245.1	28	144
116	EPN	GC	13.90	positive	157.0 → 63.0	10	157.0 → 110.0	15	70
117	Epoxiconazole	LC	8.47	positive	330.0 → 120.9	24	330.1 → 100.9	50	120
118	Eprinomectin	LC	10.84	positive	878.5 → 186.0	15	936.5 → 490.4	60	160
119	Eritromicin	LC	6.74	positive	734.5 → 158.1	32	734.5 → 576.3	16	172
120	Esfenvalerate	GC	17.56	positive	167.1 → 125.1	15	167.1 → 89.1	45	70
121	Ethion (diethion)	LC	10.03	positive	385.0 → 199.0	5	385.0 → 171.0	10	100
122	Ethirimol	LC	4.80	positive	210.2 → 140.1	20	210.2 → 98.1	28	160
123	Ethofumesate	GC	9.59	positive	286.0 → 207.0	5	286.0 → 161.0	20	70
124	Ethoprophos	LC	8.38	positive	243.1 → 97.0	30	243.1 → 130.9	15	90

Table A1. *Cont.*

No.	Compound	Technique	Retention Time (min)	Polarity	Quantification		Confirmation		Fragmentor Voltage (V)
125	Etofenprox	GC	16.75	positive	163.0 → 107.0	20	163.0 → 135.0	10	70
126	Etoxazole	LC	10.34	positive	360.1 → 141.0	26	360.1 → 304.0	16	160
127	Famoxadone	LC	9.07	positive	392.1 → 330.9	5	392.2 → 238.1	12	110
128	Fenamidone	LC	9.06	positive	392.1 → 330.9	5	392.1 → 238.1	12	110
129	Fenamiphos	LC	7.72	positive	304.1 → 217.1	20	304.1 → 202.0	36	120
130	Fenamiphos sulfone	LC	8.63	positive	336.1 → 188.0	31	336.1 → 266.0	23	120
131	Fenamiphos sulfoxide	LC	5.93	positive	320.1 → 233.0	20	320.1 → 108.1	44	120
132	Fenarimol	GC	15.03	positive	139.0 → 75.0	30	139.0 → 111.0	15	70
133	Fenazaquin	LC	10.73	positive	307.2 → 57.1	25	307.2 → 161.1	16	90
134	Fenbendazole	LC	8.04	positive	300.1 → 268.1	20	300.1 → 159.0	36	156
135	Fenbuconazole	GC	16.17	positive	198.0 → 102.0	30	198.0 → 78.0	30	70
136	Fenhexamid	LC	8.35	positive	302.1 → 97.1	20	302.1 → 55.1	40	130
137	Fenitrothion	GC	9.57	positive	277.0 → 109.0	15	277.0 → 125.0	15	70
138	Fenoxycarb	LC	8.69	positive	302.1 → 88.0	20	302.1 → 116.1	10	110
139	Fenpropathrin	LC	10.43	positive	367.2 → 125.0	16	350.1 → 125.0	16	72
140	Fenpropidin	LC	7.13	positive	274.3 → 147.0	30	274.3 → 86.0	25	170
141	Fenpropimorph	LC	7.37	positive	304.3 → 147.1	30	304.3 → 130.0	25	120
142	Fenpyroximate	LC	10.49	positive	422.2 → 366.2	12	422.2 → 135.0	36	160
143	Fenthion	GC	8.90	positive	278.0 → 109.0	15	278.0 → 125.0	20	70
144	Fenthion oxon	LC	7.31	positive	263.1 → 231.2	16	263.1 → 216.0	24	120
145	Fenthion oxon sulfone	LC	4.50	positive	295.0 → 217.0	15	295.0 → 104.2	24	110
146	Fenthion oxon sulfoxide	LC	4.26	positive	279.0 → 264.2	20	279.0 → 104.1	28	110
147	Fenthion sulfone	LC	6.39	positive	311.0 → 125.0	22	311.0 → 109.0	28	140
148	Fenthion sulfoxide	LC	6.16	positive	295.0 → 108.9	30	295.0 → 280.0	18	140
149	Fenvalerate	GC	17.36	positive	167.0 → 125.1	22	167.0 → 89.0	30	70

Table A1. *Cont.*

No.	Compound	Technique	Retention Time (min)	Polarity	Quantification		Confirmation		Fragmentor Voltage (V)
150	Fipronil	LC	8.68	negative	435.0 → 330.0	12	435.0 → 249.9	26	116
151	Fipronil sulfide	GC	10.49	positive	351.0 → 255.0	20	420.0 → 351.0	25	70
152	Flocoumafen	LC	10.44	negative	541.3 → 382.0	25	541.3 → 161.0	40	230
153	Fluazinam	LC	10.01	negative	462.9 → 416.0	10	462.9 → 398.0	9	140
154	Flubendiamide	LC	8.82	positive	408.0 → 274.0	15	408.0 → 256.0	30	120
155	Flucythrinate (two isomers)	GC	16.67/16.84	positive	156.9 → 107.1	15	199.1 → 107.1	25	70
156	Fludioxonil	GC	11.51	positive	248.0 → 127.0	30	248.1 → 182.1	10	70
157	Flufenoxuron	LC	10.37	positive	489.1 → 158.0	20	489.1 → 140.9	56	110
158	Flumequine	LC	6.12	positive	262.1 → 244.0	16	262.1 → 202.0	32	116
159	Flunixin	LC	8.09	positive	297.1 → 279.1	24	297.1 → 264.1	32	141
160	Fluopyram	GC	10.61	positive	173.0 → 95.0	35	223.0 → 196.0	40	70
161	Fluoranthene	GC	10.66	positive	202.0 → 201.0	27	202.0 → 152.0	42	70
162	Fluorene	GC	6.81	positive	165.0 → 163.0	40	165.0 → 139.0	30	70
163	Fluquinconazole	GC	15.81	positive	340.0 → 298.0	15	340.0 → 286.0	25	70
164	Flusilazole	LC	8.64	positive	316.1 → 247.1	15	316.1 → 165.0	20	160
165	Flutolanil	LC	7.93	positive	324.1 → 262.1	16	324.1 → 242.1	24	130
166	Flutriafol	GC	11.26	positive	219.0 → 95.0	35	219.0 → 123.0	15	70
167	Fluvalinate tau	GC	17.56	positive	250.1 → 55.1	30	252.0 → 200.0	20	70
168	Fonofos	GC	8.24	positive	246.0 → 109.0	15	246.0 → 237.0	5	70
169	Formetanate	LC	1.76	positive	222.1 → 165.1	12	222.1 → 46.2	28	105
170	Fosthiazate	LC	6.50	positive	284.0 → 104.0	20	284.0 → 227.8	8	90
171	Heptachlor	GC	9.31	positive	272.0 → 237.0	15	274.0 → 239.0	15	70
172	Hexachlorobencene	GC	7.77	positive	284.0 → 214.0	40	284.0 → 249.0	25	70

Table A1. *Cont.*

No.	Compound	Technique	Retention Time (min)	Polarity	Quantification		Confirmation		Fragmentor Voltage (V)
173	Hexachlorocyclohexane (alpha)	GC	7.64	positive	219.0 → 109.0	10	219.0 → 183.0	10	70
174	Hexachlorocyclohexane (beta)	GC	8.02	positive	219.0 → 109.0	40	219.0 → 183.0	5	70
175	Hexachlorocyclohexane (delta)	GC	8.50	positive	219.0 → 109.0	45	219.0 → 183.0	5	70
176	Hexaclorocyclohexane (gamma. lindane)	GC	8.13	positive	291.0 → 109.0	40	219.0 →183.0	10	70
177	Hexaconazole (two isomers)	LC	8.49	positive	314.1 → 70.1	20	316.0 → 70.1	20	95
178	Hexaflumuron	LC	9.58	negative	458.8 → 439.0	8	458.8 → 175.0	30	100
179	Hexythiazox	LC	10.18	positive	353.1 → 227.9	8	353.1 → 168.1	24	120
180	Imazalil (enilconazole)	LC	6.53	positive	297.1 → 159.0	20	297.1 → 69.1	18	100
181	Imidacloprid	LC	3.93	positive	256.0 → 175.0	12	256.0 → 209.0	12	110
182	Indeno [1,2,3-cd] pyrene	GC	19.08	positive	276.0 → 274.0	50	276.0 → 272.0	60	70
183	Indoxacarb	LC	9.49	positive	528.1 → 293.1	10	528.1 → 202.8	48	140
184	Iprovalicarb	LC	8.18	positive	321.2 → 119.0	15	321.2 → 202.9	20	110
185	Isofenphos methyl	GC	10.38	positive	199.0 → 121.0	10	241.0 → 121.0	25	70
186	Isoprothiolane	LC	7.94	positive	291.1 → 189.0	30	291.1 → 145.0	36	100
187	Ivermectin B1a	LC	11.52	positive	897.5 → 753.5	50	897.5 → 329.3	60	160
188	Josamycin	LC	7.40	positive	860.5 → 173.9	40	860.5 → 108.9	40	200
189	Ketoprofen	LC	7.34	positive	255.1 → 209.1	8	255.1 → 77.1	48	123
190	Kresoxim methyl	GC	11.78	positive	116.0 → 89.0	15	206.0 → 131.0	10	70
191	Levamisole	LC	3.12	positive	205.1 → 178.1	20	205.1 → 123.0	32	141
192	Lincomycin	LC	3.50	positive	407.2 → 126.1	24	407.2 → 359.2	16	150
193	Linuron	LC	7.54	positive	249.0 → 160.1	20	249.0 → 182.3	8	120

Table A1. *Cont.*

No.	Compound	Technique	Retention Time (min)	Polarity	Quantification		Confirmation		Fragmentor Voltage (V)
194	Lufenuron	LC	10.05	negative	509.0 → 339.0	5	509.0 → 326.1	15	90
195	Mandipropamid	LC	7.90	positive	412.1 → 328.1	8	412.1 → 356.1	4	130
196	Mebendazole	LC	6.68	positive	296.1 → 264.1	20	296.1 → 77.0	48	151
197	Mefenamic acid	LC	9.52	positive	242.1 → 209.1	28	242.1 → 180.1	0	108
198	Mefenoxam (metalaxyl-M)	LC	6.95	positive	280.0 → 220.0	10	280.0 → 192.0	15	110
199	Meloxicam	LC	7.17	positive	352.5 → 114.8	20	352.5 → 140.8	20	130
200	Mepanipyrim	GC	11.13	positive	222.0 → 221.0	15	222.0 → 207.0	15	70
201	Mepiquat	LC	0.64	positive	114.0 → 98.0	36	114.0 → 70.0	45	100
202	Metaflumizone	LC	9.94	negative	505.0 → 302.0	14	541.0 → 302.0	20	90
203	Metalaxyl	GC	9.31	positive	234.0 → 146.1	20	249.0 → 146.0	20	70
204	Metaldehyde	LC	3.87	positive	194.1 → 61.9	5	194.1 → 106.0	5	50
205	Metconazole	LC	9.17	positive	320.1 → 70.2	33	322.1 → 70.2	24	250
206	Methamidophos (two isomers)	LC	1.18	positive	142.0 → 94.0	12	142.0 → 125.0	12	85
207	Methidathion	LC	7.12	positive	320.1 → 144.8	8	320.1 → 85.0	30	84
208	Methiocarb	LC	7.67	positive	226.1 → 169.0	4	226.1 → 121.1	12	90
209	Methiocarb-sufone	LC	4.52	positive	258.1 → 201.1	8	258.1 → 122.1	22	100
210	Methiocarb-sulfoxide	LC	4.03	positive	242.0 → 185.0	22	242.0 → 122.0	28	90
211	Methomyl	LC	3.23	positive	163.1 → 88.0	5	163.0 → 106.0	8	80
212	Methoxyfenozide	LC	8.00	positive	369.2 → 149.0	10	369.2 → 313.1	15	85
213	Metoxychlor	GC	13.98	positive	227.0 → 141.0	20	227.0 → 169.0	15	70
214	Metrafenone	LC	9.27	positive	409.1 → 209.1	8	411.1 → 209.1	12	108
215	Metronidazole	LC	2.63	positive	172.1 → 128.0	12	172.1 → 82.1	24	98
216	Mevinphos (phosdrin)	LC	4.38	positive	225.0 → 193.1	15	225.0 → 127.0	12	65

Table A1. *Cont.*

No.	Compound	Technique	Retention Time (min)	Polarity	Quantification		Confirmation		Fragmentor Voltage (V)
217	Mirex	GC	5.66	positive	237.0 → 143.0	30	274.0 → 237.0	10	70
218	Monocrotophos	LC	3.31	positive	224.1 → 126.8	12	224.1 → 98.1	15	100
219	Moxidectin	LC	11.24	positive	641.4 → 529.2	5	641.4 → 499.2	5	100
220	Myclobutanil	LC	8.10	positive	289.1 → 70.1	16	289.1 → 125.1	32	110
221	N-(2,4-dimethylphenyl)-N′-methylformamidine (DMPF, metabolite of amitraz)	LC	3.35	positive	163.1 → 122.1	15	163.1 → 107.1	15	100
222	N,N-Dimethyl-N′-p-tolylsulphamide (DMST, metabolite of tolyfluanid)	LC	6.06	positive	215.1 → 106.1	10	215.1 → 151.1	4	90
223	Nafcillin	LC	7.33	positive	415.0 → 199.1	8	415.0 → 171.0	36	103
224	Naphtalene	GC	4.45	positive	128.0 → 127.0	15	128.0 → 102.0	25	70
225	Naproxen	LC	7.59	positive	231.0 → 185.0	10	231.1 → 169.9	21	120
226	Nitenpyram	LC	3.30	positive	271.1 → 56.1	36	271.1 → 224.9	12	100
227	Novobiocin	LC	9.69	positive	613.2 → 218.1	10	613.2 → 396.1	10	150
228	Nuarimol	GC	13.27	positive	235.0 → 139.0	15	235.0 → 111.0	40	70
229	Ofurace	LC	5.97	positive	282.0 → 159.9	20	282.0 → 147.9	30	100
230	Omethoate	LC	2.80	positive	214.1 → 124.8	22	214.1 → 183.0	5	100
231	Oxadixyl	LC	5.43	positive	279.1 → 219.2	5	279.1 → 132.2	32	110
232	Oxamyl	LC	2.87	positive	237.1 → 72.0	12	237.1 → 90.0	5	70
233	Oxamyl-oxime	LC	2.46	positive	163.3 → 115.2	10	163.3 → 72.1	10	70
234	Oxfendazole	LC	5.61	positive	316.1 → 159.0	32	316.1 → 191.1	16	166
235	Oxolinic acid	LC	5.04	positive	262.1 → 216.0	32	262.1 → 160.0	36	110
236	Oxydemeton methyl	LC	3.01	positive	247.0 → 169.0	12	247.0 → 109.0	24	100
237	Oxyfluorfen	GC	11.68	positive	252.0 → 146.0	40	300.0 → 223.0	15	70

Table A1. *Cont.*

No.	Compound	Technique	Retention Time (min)	Polarity	Quantification		Confirmation		Fragmentor Voltage (V)
238	Paclobutrazol	LC	7.89	positive	294.1 → 70.1	16	294.1 → 125.2	36	115
239	Parathion methyl	GC	9.12	positive	263.0 → 109.0	15	263.0 → 79.0	30	70
240	PCB 28	GC	9.01	positive	256.0 → 186.0	25	256.0 → 151.0	50	70
241	PCB 52	GC	9.58	positive	292.0 → 222.0	25	292.0 → 220.0	25	70
242	PCB 77	GC	11.73	positive	292.0 → 220.0	25	292.0 → 222.0	25	70
243	PCB 81	GC	11.56	positive	292.0 → 220.0	25	292.0 → 222.0	25	70
244	PCB 101	GC	11.08	positive	326.0 → 256.0	30	328.0 → 256.0	30	70
245	PCB 105	GC	12.66	positive	326.0 → 256.0	30	328.0 → 256.0	30	70
246	PCB 114	GC	12.38	positive	326.0 → 256.0	30	328.0 → 256.0	30	70
247	PCB 118	GC	12.18	positive	326.0 → 256.0	30	328.0 → 256.0	30	70
248	PCB 123	GC	12.10	positive	326.0 → 256.0	30	328.0 → 256.0	30	70
249	PCB 126	GC	13.23	positive	326.0 → 256.0	30	328.0 → 256.0	30	70
250	PCB 138	GC	13.07	positive	360.0 → 290.0	25	360.0 → 288.0	25	70
251	PCB 153	GC	12.57	positive	360.0 → 290.0	25	360.0 → 288.0	25	70
252	PCB 156	GC	13.96	positive	360.0 → 290.0	25	360.0 → 288.0	25	70
253	PCB 157	GC	14.07	positive	360.0 → 290.0	25	360.0 → 288.0	25	70
254	PCB 167	GC	13.55	positive	360.0 → 290.0	25	360.0 → 288.0	25	70
255	PCB 169	GC	14.61	positive	360.0 → 290.0	25	360.0 → 288.0	25	70
256	PCB 180	GC	14.25	positive	394.0 → 324.0	30	394.0 → 322.0	30	70
257	PCB 189	GC	15.25	positive	394.0 → 324.0	30	394.0 → 322.0	30	70
258	Penconazole	GC	10.52	positive	248.0 → 157.0	30	248.0 → 192.0	15	70
259	Pencycuron	LC	9.33	positive	329.1 → 125.1	24	329.1 → 217.9	12	160
260	Pendimethalin	GC	10.49	positive	252.0 → 162.0	10	252.0 → 191.0	5	70
261	Penicillin V	LC	6.47	positive	383.2 → 159.9	10	383.2 → 113.9	40	130

Table A1. *Cont.*

No.	Compound	Technique	Retention Time (min)	Polarity	Quantification		Confirmation		Fragmentor Voltage (V)
262	Permethrin	GC	15.69	positive	183.0 → 128.0	15	183.1 → 153.1	15	70
263	Phenanthrene	GC	8.40	positive	178.0 → 176.0	35	178.0 → 152.0	28	70
264	Phenylbutazone	LC	8.25	positive	309.2 → 160.2	20	309.2 → 77.1	55	140
265	Phosalone	LC	9.20	positive	385.1 → 182.0	20	385.1 → 110.9	55	80
266	Phosmet	LC	7.34	positive	318.0 → 159.9	16	318.0 → 133.0	40	90
267	Pthalamide (Folpet deg)	GC	5.94	positive	104.0 → 50.0	25	147.0 → 76.0	25	70
268	Pirimicarb	LC	5.11	positive	239.1 → 72.1	20	239.1 → 182.1	12	100
269	Pirimicarb-desmethyl	LC	3.71	positive	225.1 → 168.1	8	225.1 → 72.1	20	100
270	Pirimiphos ethyl	GC	10.26	positive	318.0 → 166.0	15	318.0 → 182.0	15	70
271	Pirimiphos methyl	LC	9.13	positive	306.1 → 164.0	20	306.1 → 108.1	32	100
272	Prochloraz	LC	9.08	positive	376.0 → 308.0	10	376.0 → 70.1	20	100
273	Procymidone	GC	10.80	positive	283.0 → 67.0	40	283.0 → 68.0	25	70
274	Profenofos	LC	9.75	positive	375.0 → 305.0	20	373.0 → 303.0	20	100
275	Propamocarb	LC	2.85	positive	189.2 → 102.0	12	189.2 → 144.0	8	110
276	Propargite	LC	10.37	positive	368.2 → 231.1	4	368.2 → 175.0	12	88
277	Propiconazole	LC	9.01	positive	342.0 → 69.0	21	342.0 → 159.0	39	90
278	Propoxur	LC	5.83	positive	210.1 → 168.1	35	210.1 → 65.1	40	70
279	Propyzamide (pronamide)	LC	7.92	positive	256.1 → 190.0	16	256.1 → 173.0	25	90
280	Proquinazid	GC	13.32	positive	288.0 → 245.0	15	288.0 → 217.0	30	70
281	Prothioconazol	GC	11.85	positive	186.0 → 49.0	20	186.0 → 53.0	25	70
282	Prothiophos	GC	11.45	positive	266.9 → 221.0	35	162.0 → 63.1	30	70
283	Pymetrozine	LC	2.74	positive	218.1 → 105.0	20	218.1 → 78.0	52	120
284	Pyraclostrobin	LC	9.15	positive	388.1 → 193.8	8	388.1 → 163.1	28	120

Table A1. *Cont.*

No.	Compound	Technique	Retention Time (min)	Polarity	Quantification		Confirmation		Fragmentor Voltage (V)
285	Pyrazophos	LC	9.22	positive	374.1 → 222.1	23	374.1 → 194.0	32	100
286	Pyrene	GC	11.13	positive	202.0 → 201.0	27	202.0 → 200.0	45	70
287	Pyridaben	LC	10.75	positive	365.2 → 309.0	8	309.1 → 147.0	16	168
288	Pyridaphenthion	LC	8.11	positive	341.0 → 189.0	22	341.0 → 205.0	34	100
289	Pyrimethanil	GC	8.27	positive	198.0 → 118.0	40	198.0 → 158.0	20	70
290	Pyriproxifen	LC	10.07	positive	322.2 → 96.0	12	322.2 → 184.9	24	80
291	Quinalfos	LC	8.72	positive	299.1 → 96.9	30	299.1 →147.1	20	130
292	Quinoxyfen	LC	10.13	positive	308.0 → 197.0	32	308.2 → 161.8	55	120
293	Rifampicin	LC	7.89	positive	823.5 → 791.4	15	823.5 → 399.1	25	160
294	Rotenone	LC	8.64	positive	395.1 → 213.1	20	395.1 → 192.1	25	150
295	Roxithromycin	LC	7.67	positive	838.5 → 158.1	40	838.5 → 116.1	55	200
296	Sarafloxacin	LC	4.16	positive	386.1 → 342.1	16	386.1 → 299.1	28	144
297	Simazine	LC	5.81	positive	202.4 → 68.1	30	202.4 → 68.1	20	120
298	Spinosad (two isomers)	LC	9.10/9.43	positive	732.4 → 142.0	22	732.4 → 98.0	60	130
299	Spiramycin (two isomers)	LC	4.58/4.90	positive	439.1 → 101.1	20	439.1 → 88.0	50	70
300	Spirodiclofen	LC	10.50	positive	411.1 → 71.2	15	411.1 → 313.0	5	110
301	Spiromesifen	LC	10.27	positive	388.0 → 273.0	25	273.0 → 187.0	15	110
302	Spirotetramat	LC	8.23	positive	374.2 → 302.2	12	374.2 → 216.1	36	150
303	Spiroxamine	LC	7.55	positive	298.3 → 144.1	16	298.3 → 100.1	32	120
304	Strychnine	LC	3.00/3.61	positive	335.1 → 184.0	45	335.1 → 156.0	40	105
305	Sulfacetamide	LC	2.13	positive	215.3 → 155.9	10	215.3 → 92.0	20	90
306	Sulfachloropiridacine	LC	3.77	positive	285.0 → 156.0	12	285.0 → 92.1	28	101
307	Sulfadiacine	LC	2.80	positive	251.0 → 92.0	28	251.0 → 156.0	12	111
308	Sulfadimetoxine	LC	4.81	positive	311.0 → 92.0	32	311.0 → 156.0	16	139

Table A1. *Cont.*

No.	Compound	Technique	Retention Time (min)	Polarity	Quantification		Confirmation		Fragmentor Voltage (V)
309	Sulfadoxine	LC	4.12	positive	311.1 → 92.0	32	311.1 → 156.0	16	126
310	Sulfameracine	LC	3.26	positive	265.0 → 92.0	28	265.0 → 156.0	12	126
311	Sulfametacine	LC	3.44	positive	279.1 → 186.0	12	279.1 → 92.0	32	134
312	Sulfametizole	LC	3.37	positive	271.0 → 92.0	28	271.0 → 155.9	8	103
313	Sulfametoxazole	LC	3.93	positive	254.0 → 92.0	28	254.0 → 156.0	12	111
314	Sulfametoxipiridacine	LC	3.45	positive	281.0 → 155.9	12	281.0 → 92.1	28	121
315	Sulfamonomethoxine	LC	4.11	positive	281.1 → 156.0	14	281.1 → 92.1	32	120
316	Sulfapyridine	LC	2.82	positive	250.0 → 156.0	12	250.0 → 92.0	28	126
317	Sulfaquinoxaline	LC	4.99	positive	301.0 → 156.0	12	301.0 → 92.1	32	159
318	Sulfatiazole	LC	2.98	positive	256.0 → 92.0	28	256.0 → 156.0	12	106
319	Sulfisoxazole	LC	4.12	positive	268.0 → 156.0	8	268.0 → 92.1	24	106
320	Tebuconazole	LC	8.92	positive	308.2 → 70.2	22	308.2 → 125.1	53	120
321	Tebufenocide	LC	8.66	positive	353.1 → 132.9	22	353.1 → 297.1	20	90
322	Tebufenpyrad	LC	9.88	positive	334.2 → 117.0	47	334.2 → 145.0	37	180
323	Teflubenzuron	LC	10.01	negative	379.0 → 339.0	15	379.0 → 196.0	25	100
324	Tefluthrin	GC	8.42	positive	177.0 → 127.0	15	177.0 → 87.0	15	70
325	Telodrin (isobenzan)	GC	10.14	positive	310.8 → 240.8	25	310.8 → 274.8	5	70
326	Terbufos	GC	8.15	positive	231.0 → 97.0	20	231.0 → 129.0	15	70
327	Terbuthylazine	GC	8.12	positive	214.0 → 104.0	20	214.0 → 132.0	10	70
328	Tetrachlorvinphos	LC	8.72	positive	367.0 → 127.0	16	365.0 → 127.0	16	110
329	Tetraconazole	GC	10.04	positive	336.0 → 204.0	35	336.0 → 218.0	20	70
330	Tetradifon	GC	14.36	positive	158.9 → 111.0	20	354.0 → 159.0	10	70
331	Tetramethrin	GC	13.87	positive	164.0 → 77.0	30	164.0 → 107.0	15	70
332	Thiabendazole	GC	5.94	positive	201.0 → 174.0	15	201.0 → 130.0	30	70

Table A1. *Cont.*

No.	Compound	Technique	Retention Time (min)	Polarity	Quantification		Confirmation		Fragmentor Voltage (V)
333	Thiacloprid	LC	4.80	positive	253.0 → 126.0	16	253.0 → 90.0	40	140
334	Thiamethoxam	LC	3.59	positive	292.0 → 211.1	8	292.0 → 132.0	22	80
335	Thiophanate methyl	LC	5.87	positive	343.0 → 151.0	20	343.0 → 93.0	46	90
336	Tolclofos methyl	GC	9.21	positive	265.0 → 93.0	30	265.0 → 220.0	25	70
337	Tolfenamic acid	LC	9.80	negative	260.0 → 216.1	8	260.0 → 35.1	20	108
338	Triadimefon	LC	8.03	positive	294.1 → 69.3	20	294.1 → 197.2	15	100
339	Triadimenol	LC	8.22	positive	296.1 → 70.0	10	298.1 → 70.0	10	80
340	Triazophos (hostathion)	LC	8.18	positive	314.1 → 162.0	19	314.1 → 118.9	35	100
341	Trifloxystrobin	LC	9.50	positive	409.1 → 186.0	12	409.1 → 145.0	52	110
342	Triflumizole	LC	9.53	positive	346.1 → 278.0	4	345.9 → 73.0	15	80
343	Triflumuron	LC	9.19	positive	359.0 → 156.0	8	359.0 → 139.0	32	120
344	Trifluralin	GC	7.27	positive	264.0 → 160.0	15	306.0 → 264.0	5	70
345	Trimethoprim	LC	3.45	positive	291.2 → 123.0	24	291.2 → 230.1	20	162
346	Triticonazole	LC	8.38	positive	318.1 → 70.1	33	320.1 → 70.1	16	110
347	Tylmicosin	LC	5.52	positive	869.6 → 174.1	48	869.6 → 696.4	44	294
348	Tylosin	LC	6.76	positive	916.5 → 174.1	40	916.5 → 772.4	28	210
349	Vinclozolin	GC	9.10	positive	212.0 → 145.0	25	212.0 → 109.0	50	70
350	Warfarin	LC	7.86	negative	307.1 → 161.1	20	307.1 → 250.1	20	140
351	Zoxamide	LC	9.03	positive	336.0 → 187.1	25	187.1 → 88.9	40	200

Appendix B

Table A2. Method validation results: Limits of quantification (LOQ), percentage recoveries and relative standard deviation obtained from intraday and interday studies.

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
No.	Compound	LOQ (ng/mL)	Rec (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
1	2-Phenylphenol	2				101.43	19.69	18.85	107.17	20.66	19.28	106.47	18.24	17.13	96.41	13.81	14.32
2	4,4′-Dichlorobenzophenone (metabolite of dicofol)	2				91.36	13.23	14.48	86.99	13.79	15.85	116.77	11.76	10.07	98.37	15.59	15.85
3	Abamectine	4							106.43	14.53	13.65	93.89	5.21	5.55	99.43	14.50	14.58
4	Acenaphthene	1.2				110.88	19.39	19.29	94.91	6.78	7.14	96.45	12.08	12.52	95.23	4.52	4.75
5	Acenaphtylene	2				107.06	18.90	21.39	95.74	12.53	13.09	100.50	12.26	12.20	98.40	2.93	2.98
6	Acephate	8										90.75	20.15	22.20	93.24	14.96	16.04
7	Acetamiprid	2				122.13	8.03	6.57	101.26	14.21	14.03	104.42	4.70	4.50	104.71	4.76	4.55
8	Acrinathrin	4							116.08	16.73	14.41	103.11	22.19	21.52	104.55	5.45	5.21
9	Albendazole	0.4	121.03	11.39	9.41	92.56	6.35	6.86	86.92	12.48	14.36	99.53	3.66	3.68	105.10	4.30	4.09
10	Aldicarb	0.8	119.20	13.25	10.26	97.22	7.43	7.64	89.92	14.70	16.35	100.15	4.24	4.23	107.89	5.24	4.86
11	Aldicarb-sulfone	2				121.84	18.51	15.19	94.84	16.90	19.91	85.77	12.91	15.05	97.26	10.13	10.42
12	Aldicarb-sulfoxide	4							104.82	18.42	17.11	81.91	5.97	7.29	105.26	12.19	11.58
13	Aldrin	2				123.66	19.11	15.45	91.59	15.25	16.65	106.54	5.58	5.24	97.33	12.12	12.45
14	Anthracene	1.6				99.67	15.35	15.40	91.47	15.47	16.91	105.34	7.32	6.95	94.68	6.50	6.87
15	Atrazine	0.8	124.53	4.21	3.38	93.04	7.01	7.53	87.60	13.23	15.10	102.49	5.46	5.33	110.16	6.62	6.01
16	Azinphos-methyl	2				126.85	18.74	14.77	103.98	13.75	13.22	105.19	3.50	3.33	102.89	5.44	5.29
17	Azoxystrobin	0.4	116.28	7.94	6.83	95.41	3.96	4.15	92.31	6.03	6.53	102.95	4.52	4.39	101.82	2.11	2.07
18	BDE-28	1.2				100.53	14.19	14.12	81.65	9.78	11.98	104.45	6.20	5.94	96.62	11.51	11.91
19	BDE-47	0.8	98.71	20.31	20.58	109.45	21.87	19.98	89.88	8.16	9.08	110.24	8.74	7.93	95.96	8.89	9.26

Table A2. *Cont.*

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
No.	Compound	LOQ (ng/mL)	Rec (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
20	BDE-85	0.8	100.77	15.79	15.59	103.10	16.65	16.15	85.61	12.99	15.17	108.23	16.09	14.87	98.51	17.91	13.96
21	BDE-99	0.4	107.54	18.55	19.69	103.74	15.02	14.48	89.65	12.66	14.12	114.05	18.00	15.78	90.97	11.97	13.16
22	BDE-100	0.8	98.15	21.94	22.35	106.72	17.54	21.81	93.71	10.60	11.31	109.98	12.80	11.64	94.05	19.80	21.05
23	BDE-153	0.8	93.58	16.95	19.48	108.66	17.04	19.88	93.65	15.26	16.29	111.40	15.44	13.86	96.11	17.16	17.85
24	BDE-154	0.4	96.19	15.79	16.42	113.57	19.84	17.47	92.43	10.71	11.59	118.15	16.34	13.83	93.42	13.93	15.62
25	BDE-183	4							78.46	5.92	7.55	104.68	12.90	12.32	95.99	8.73	9.09
26	Benalaxyl	0.4	116.67	15.06	12.91	94.56	3.53	3.73	97.64	6.31	6.46	101.10	3.37	3.33	101.39	3.01	2.97
27	Bendiocarb	0.8	123.80	9.46	7.29	90.11	14.65	16.26	93.23	14.26	15.30	101.72	3.10	3.05	110.76	8.93	8.06
28	Bendiocarb metabolite (2,2-dimethylbenzo-1. 3-dioxol-4-ol)	4							86.86	16.14	21.61	102.82	16.72	16.26	95.65	3.05	3.19
29	Benfuracarb	0.8	121.43	6.42	5.24	88.09	12.05	13.68	85.50	9.48	11.09	99.93	4.45	4.45	102.76	5.96	5.80
30	Benzo[a]anthracene	0.8	98.90	19.38	19.71	97.89	8.00	8.17	85.04	4.75	5.59	109.73	14.44	13.16	101.08	11.10	10.98
31	Benzo[a]pyrene	0.8	118.01	19.78	20.66	94.10	10.74	11.41	90.35	9.53	10.55	106.01	11.75	11.08	98.16	14.17	14.44
32	Benzo[b]fluoranthene	1.2				101.28	11.89	11.61	91.62	10.11	11.03	105.11	8.02	7.63	93.83	8.80	9.38
33	Benzo[ghi]perylene	0.8	80.16	19.32	20.41	100.35	15.43	15.38	93.47	9.37	10.02	114.39	6.35	5.55	95.50	10.80	11.31
34	Benzo[k]fluoranthene	1.2				102.66	13.24	12.90	88.84	14.63	16.47	101.90	7.87	7.72	99.44	17.32	17.42
35	Bifenthrin	2				118.63	18.93	19.10	86.17	14.08	16.34	113.53	16.83	14.82	98.99	10.04	10.14
36	Bitertanol	0.4	121.41	23.34	18.61	84.35	14.31	16.97	80.69	10.74	13.31	99.71	4.76	4.77	105.11	2.05	1.95
37	Boscalid (formerly nicobifen)	0.8	74.96	22.43	22.81	85.76	5.16	6.02	85.67	9.78	11.42	112.15	6.66	5.94	89.33	8.33	9.32
38	Brodifacoum	0.4	102.44	23.57	23.01	88.99	13.67	15.36	85.84	12.99	15.13	90.41	5.61	6.21	107.51	6.87	6.39
39	Bromadiolone	0.4	118.13	11.13	19.82	97.11	20.55	19.19	89.46	13.13	14.68	97.66	8.85	9.06	101.30	8.87	8.76

Table A2. *Cont.*

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
No.	**Compound**	**LOQ (ng/mL)**	**Rec (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**
40	Bromopropylate	0.4	110.83	21.15	22.86	96.37	19.85	20.60	93.29	8.21	8.80	107.25	10.02	9.34	96.53	10.67	11.05
41	Bromuconazole (two isomers)	1.6				106.70	16.63	14.96	89.48	17.19	19.21	112.75	13.08	11.60	94.54	8.05	8.51
42	Bupirimate	1.2				86.77	10.74	12.38	93.94	5.74	6.11	98.53	6.03	6.12	102.43	4.99	4.87
43	Buprofezin	0.4	115.89	11.18	9.65	93.22	1.92	2.06	88.57	10.64	12.01	101.63	4.50	4.43	102.68	3.54	3.45
44	Cadusafos (ebufos)	0.4	126.81	3.48	2.74	94.26	4.04	4.29	96.81	5.74	5.93	101.63	5.00	4.92	100.58	3.04	3.02
45	Carbaryl	0.8	118.84	13.56	10.52	96.23	6.27	6.52	87.81	11.26	12.82	100.43	4.37	4.35	108.80	4.34	3.99
46	Carbendazim (azole)	2				120.17	8.97	6.40	98.20	9.83	10.01	100.39	2.72	2.71	103.56	3.81	3.68
47	Carbofuran	0.4	123.88	10.74	8.67	94.30	6.42	6.81	88.14	13.54	15.36	104.41	6.50	6.23	105.57	3.33	3.15
48	Carbofuran-3-hydroxy	0.8	120.98	10.76	8.89	89.04	8.10	9.10	94.46	15.41	16.31	100.86	3.61	3.58	107.55	4.88	4.54
49	Carbosulfan	1.2				109.16	14.87	19.19	89.29	21.22	20.62	92.19	22.12	23.99	96.57	21.02	21.77
50	Cefuroxima axetil (two isomers)	4							132.33	13.51	10.21	119.80	8.73	7.29	127.08	13.98	11.00
51	Chloramphenicol	16													98.91	7.74	7.83
52	Chlorantraniliprole	16													106.00	2.93	2.76
53	Chlorfenvinphos	0.8	114.28	16.10	14.09	97.14	14.61	15.04	91.56	15.96	17.43	104.40	2.64	2.53	106.32	3.78	3.56
54	Chlorobenzilate	1.6				110.47	17.93	16.23	89.89	4.22	4.69	114.60	12.66	11.05	90.71	2.94	3.24
55	Chlorophacinone	8										98.40	21.84	22.36	99.28	8.31	8.37
56	Chlorpropham	2				119.39	17.61	13.13	93.11	14.39	15.45	112.93	6.10	5.40	100.11	21.56	21.54
57	Chlorpyrifos	1.6				74.81	17.17	19.32	92.21	1.74	1.89	119.54	9.95	8.32	90.50	6.87	7.59
58	Chlorpyrifos methyl	2				115.24	17.63	14.98	88.08	15.56	19.02	90.85	9.97	10.97	106.30	6.98	6.57

Table A2. *Cont.*

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
No.	**Compound**	**LOQ (ng/mL)**	**Rec (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**
59	Chlorthal dimethyl	0.8	104.38	17.25	19.43	96.72	14.55	15.04	91.37	6.53	7.15	113.90	9.82	8.62	90.34	3.66	4.05
60	Chrysene	1.6				109.91	12.42	11.30	91.83	7.03	7.66	106.10	17.06	16.08	100.25	11.34	11.31
61	Clindamycin	4							129.25	10.53	8.15	109.20	7.44	6.81	107.36	6.37	5.93
62	Clofentezine	0.8	116.80	20.59	17.63	94.99	7.99	8.41	83.66	11.18	13.36	96.74	7.45	7.70	105.68	4.56	4.31
63	Clothianidin	12										105.52	8.63	8.18	97.88	11.44	11.69
64	Cloxacillin	8										99.33	18.27	18.39	107.79	10.04	9.31
65	Coumachlor	0.8	120.85	16.85	20.84	99.90	12.14	12.15	85.48	16.06	18.79	101.57	4.20	4.14	102.89	2.20	2.14
66	Coumaphos	0.8	112.59	14.03	12.46	86.24	15.34	17.79	86.49	10.71	12.38	105.36	4.20	3.99	103.38	4.36	4.22
67	Coumatetralyl	1.6				116.12	22.62	19.48	97.65	9.98	10.22	96.87	4.29	4.43	102.71	4.39	4.27
68	Cyazofamid	2				112.26	13.66	12.17	100.29	12.06	12.03	103.98	7.08	6.81	97.87	5.04	5.15
69	Cyflufenamid	1.6				116.75	17.97	23.96	96.02	19.44	20.25	98.16	6.89	7.02	105.14	7.86	7.48
70	Cyfluthrin (sum of four isomers)	8										128.33	19.82	15.44	87.94	19.51	21.60
71	Cyhalothrin (lambda isomer)	4							118.45	17.58	14.84	110.36	18.54	16.80	102.15	14.52	14.21
72	Cymoxanil	2				127.98	23.20	18.13	112.78	13.14	11.65	111.18	3.17	2.85	107.24	7.19	6.70
73	Cypermethrin (sum of four isomers)	20										125.42	15.64	12.47	74.31	10.18	13.70
74	Cyproconazole (two isomers)	4				120.66	19.73	20.01	89.98	15.51	17.24	110.68	5.93	5.36	93.41	7.33	7.85
75	Cyprodinil	1.2				110.91	13.69	12.34	89.72	15.25	17.00	97.14	8.22	8.46	104.22	7.41	7.11
76	Cyromazine	8										94.58	11.29	11.94	96.74	11.32	11.70
77	Danofloxacin	8										113.55	18.30	16.12	89.76	10.39	11.58

Table A2. *Cont.*

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
No.	Compound	LOQ (ng/mL)	Rec (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
78	Dazomet	4							71.53	17.55	21.50	83.03	13.84	16.67	90.82	16.95	18.66
79	Deltamethrin	4							95.89	17.74	19.36	107.28	18.61	17.24	100.61	12.28	12.21
80	Demeton-S-methyl	0.8	123.35	9.93	7.74	95.67	3.32	3.47	87.38	12.64	14.47	101.00	5.18	5.13	108.47	6.18	5.70
81	Demeton-S-methyl-sulfone (Dioxydemeton)	12										80.24	4.83	6.02	96.53	6.34	6.57
82	Dexamethasone	2				123.79	17.67	12.29	104.61	13.93	13.32	106.53	14.48	13.59	102.96	4.32	4.20
83	Diazinon	1.2				101.78	19.05	18.68	97.76	8.04	8.22	110.63	15.03	13.59	92.16	7.39	8.02
84	Dibenzo[a.h]anthracene	0.8	70.39	18.68	16.54	112.50	16.62	14.77	93.06	11.73	12.60	111.29	4.83	4.34	84.41	6.54	7.75
85	Dichlorodiphenyldichloroethane (p,p′ DDD)	0.8	88.27	19.42	17.67	112.25	6.36	5.67	92.57	11.03	11.92	101.42	6.07	5.99	93.46	4.77	5.10
86	Dichlorodiphenyldichloroethylene (p,p′ DDE)	0.8	99.71	18.39	18.44	110.56	17.85	18.67	97.62	7.46	7.64	104.29	0.72	0.69	98.40	8.58	8.72
87	Dichlorodiphenyltrichloroethane (p,p′ DDT)	2				101.87	15.98	14.95	104.65	20.63	19.71	110.43	6.32	5.72	92.27	6.86	7.43
88	Diclofenac	4							111.25	21.71	19.51	91.03	12.48	13.71	94.44	12.42	13.15
89	Dicloran	4							95.68	14.38	15.03	113.17	15.89	14.04	92.28	5.09	5.52
90	Diclorvos	8										113.48	10.38	9.15	98.10	2.98	3.04
91	Dicloxacillin	12										77.54	12.35	15.93	103.35	10.63	10.29
92	Dieldrin	8										80.70	18.40	18.43	95.08	5.85	6.15
93	Diethathyl ethyl	0.4	113.26	12.83	20.16	96.15	13.17	13.70	89.84	15.24	16.96	97.95	6.16	6.29	99.30	7.45	7.50
94	Diethofencarb	0.4	113.98	6.38	5.60	90.65	3.94	4.35	90.80	10.90	12.00	101.24	1.48	1.46	104.26	1.97	1.89
95	Difenacoum	0.8	117.66	13.84	21.51	88.27	10.36	11.74	85.40	7.72	9.04	92.19	6.14	6.66	104.37	3.77	3.61
96	Difenoconazole	0.8	119.78	17.98	13.85	94.45	8.83	9.35	84.08	7.72	9.18	97.08	6.45	6.64	106.47	4.12	3.87
97	Difethialone	1.6				112.44	10.79	9.60	88.10	18.22	20.68	95.86	6.45	6.73	103.22	8.66	8.39

Table A2. *Cont.*

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
No.	**Compound**	**LOQ (ng/mL)**	**Rec (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**
98	Difloxacin	4							123.36	19.34	15.68	102.87	8.83	8.58	99.17	8.54	8.61
99	Diflubenzuron	1.6				121.03	16.61	18.98	99.73	16.54	16.58	102.86	17.01	16.54	105.63	4.13	3.91
100	Diflufenican	0.4	114.39	18.24	19.49	101.56	9.38	9.24	95.89	9.59	10.00	115.49	8.35	7.23	95.62	9.05	9.46
101	Dimethenamid-P (and its R-isomer)	0.4	112.73	15.63	12.74	87.82	4.74	5.40	90.21	8.85	9.81	103.64	3.95	3.81	102.49	5.02	4.90
102	Dimethoate	0.8	121.83	20.22	15.57	93.11	5.47	5.87	87.05	11.89	13.66	100.07	10.40	10.39	108.51	7.39	6.81
103	Dimethomorph (two isomers)	0.4	123.37	18.68	15.14	87.72	6.32	7.20	87.24	11.29	12.94	104.93	4.63	4.41	105.80	5.87	5.55
104	Dimethylphenylsulfamide (DMSA, metabolite of dichlofluanid)	4							114.05	11.55	10.13	109.55	9.65	8.81	102.37	4.33	4.23
105	Diniconazole-M	1.2				97.80	16.52	16.89	88.78	6.85	7.72	102.75	14.54	14.15	100.66	5.86	5.82
106	Dinocap	4							115.19	16.40	14.24	105.54	2.90	2.75	105.88	5.85	5.53
107	Diphacinone	8										96.74	18.11	18.72	103.66	19.59	18.90
108	Diphenylamine	1.6				92.81	20.64	13.01	96.18	5.32	5.53	106.11	10.45	9.85	93.91	2.44	2.60
109	Dodine	0.8	107.19	12.63	11.78	97.64	8.18	8.38	87.26	10.47	12.00	102.27	5.32	5.20	102.46	4.26	4.16
110	Doramectina	8										101.04	19.63	19.43	99.13	9.03	9.11
111	Endosulfan alfa	2				112.45	11.73	10.43	84.22	19.47	23.12	122.57	20.34	20.75	96.35	10.24	10.63
112	Endosulfan beta	4							85.36	23.81	19.45	115.99	3.80	3.28	88.35	9.64	10.91
113	Endosulfan sulfate	4							70.48	20.30	18.80	116.21	19.54	20.32	89.14	17.93	20.11
114	Endrin	4							88.96	17.68	22.54	107.57	12.66	11.77	92.69	12.73	13.73
115	Enrofloxacin	4							118.69	21.02	19.54	98.46	7.06	7.17	104.38	11.64	11.15
116	EPN	2				81.34	17.89	19.10	87.12	22.90	19.18	112.30	12.97	11.55	95.56	5.50	5.76
117	Epoxiconazole	0.8	122.98	16.59	13.49	89.44	11.43	13.96	97.21	19.34	17.51	106.20	12.49	11.76	106.31	5.68	5.34

Table A2. *Cont.*

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
No.	Compound	LOQ (ng/mL)	Rec (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
118	Eprinomectin	1.2				100.03	21.36	21.35	89.96	19.07	21.20	95.57	10.18	10.65	102.43	8.65	8.44
119	Eritromicin	0.8	122.85	10.10	7.96	101.21	7.59	7.50	87.18	8.00	9.18	93.37	6.33	6.78	104.42	3.74	3.58
120	Esfenvalerate	2				127.54	21.58	16.92	105.91	19.49	17.83	101.51	4.20	4.14	90.24	7.45	8.26
121	Ethion (diethion)	0.8	109.21	7.32	6.70	92.19	7.99	8.67	89.52	8.27	9.24	98.50	2.63	2.67	100.66	3.50	3.48
122	Ethirimol	1.2				103.80	13.91	13.40	84.85	8.49	10.01	98.86	6.54	6.62	109.68	5.38	4.91
123	Ethofumesate	2				126.97	15.41	16.48	90.72	15.48	21.11	103.99	13.91	13.38	96.26	8.98	9.33
124	Ethoprophos	0.8	121.58	17.90	22.95	85.95	23.63	17.49	96.79	13.42	13.87	103.33	4.56	4.41	108.44	8.96	8.26
125	Etofenprox	1.2				103.05	20.35	19.75	88.00	12.88	14.64	87.54	4.70	5.37	107.43	8.42	7.84
126	Etoxazole	0.4	116.44	7.26	6.23	95.39	5.73	6.01	88.69	5.00	5.64	98.79	1.96	1.98	99.61	4.04	4.06
127	Famoxadone	1.2				105.50	21.91	19.80	84.93	8.09	9.53	100.06	8.50	8.49	105.60	8.62	8.16
128	Fenamidone	0.4	120.97	11.07	9.15	96.95	7.86	8.11	90.00	12.52	13.91	102.36	3.51	3.43	105.44	5.42	5.14
129	Fenamiphos	0.4	108.30	19.35	26.33	96.23	10.37	10.78	91.06	5.88	6.46	102.96	6.58	6.39	104.25	4.70	4.51
130	Fenamiphos sulfone	1.2				94.25	18.82	19.97	93.80	9.16	9.77	102.18	10.58	10.35	112.34	3.88	3.45
131	Fenamiphos sulfoxide	1.2				89.34	10.67	11.94	98.26	10.61	10.80	99.74	5.76	5.78	112.66	2.19	1.94
132	Fenarimol	1.6				100.34	12.81	12.77	99.76	7.71	7.73	109.44	6.21	5.67	94.11	1.40	1.49
133	Fenazaquin	0.4	120.20	17.57	14.62	91.49	3.55	3.88	82.11	9.35	11.39	100.13	2.89	2.89	101.08	3.40	3.36
134	Fenbendazole	0.4	120.77	22.32	17.07	98.07	9.36	9.54	84.88	12.32	14.51	99.37	8.88	8.94	105.25	5.15	4.89
135	Fenbuconazole	0.8	117.33	16.47	17.97	80.70	15.65	19.39	92.40	23.56	15.50	101.06	9.40	9.30	104.91	5.73	5.46
136	Fenhexamid	4							113.78	16.34	17.33	102.52	7.16	6.98	96.72	9.77	10.10
137	Fenitrothion	4							92.58	17.86	21.77	91.31	11.66	12.77	97.21	6.00	6.17
138	Fenoxycarb	0.4	105.61	17.40	16.48	94.00	12.18	12.96	87.03	6.88	7.91	101.05	3.54	3.50	99.09	6.54	6.60
139	Fenpropathrin	0.8	121.06	18.95	23.91	81.90	11.15	13.61	85.72	10.16	11.85	96.22	3.83	3.98	103.49	5.20	5.02
140	Fenpropidin	0.4	118.57	11.79	9.94	88.81	8.30	9.35	90.98	3.24	3.56	100.27	6.86	6.84	102.94	2.05	1.99

Table A2. *Cont.*

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
No.	Compound	LOQ (ng/mL)	Rec (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
141	Fenpropimorph	0.8	118.79	13.41	11.29	85.42	10.61	12.42	89.45	8.55	9.56	101.39	8.15	8.04	103.06	4.18	4.06
142	Fenpyroximate	0.4	112.81	6.87	6.09	94.83	3.34	3.52	91.94	4.15	4.51	97.90	1.86	1.90	102.42	5.37	5.24
143	Fenthion	0.8	97.17	14.25	26.12	109.28	12.00	10.98	90.09	5.65	6.27	114.72	15.88	13.84	87.33	4.28	4.90
144	Fenthion oxon	0.4	112.27	10.39	9.25	93.67	4.79	5.11	87.28	8.70	9.97	102.19	5.74	5.62	105.28	3.24	3.08
145	Fenthion oxon sulfone	8										119.94	1.82	1.52	105.66	6.01	5.69
146	Fenthion oxon sulfoxide	1.2				104.05	10.41	10.00	88.97	11.85	13.32	101.42	5.20	5.13	107.05	4.90	4.58
147	Fenthion sulfone	1.6				111.17	8.93	8.03	95.30	19.79	20.77	105.00	10.11	9.63	105.57	6.32	5.99
148	Fenthion sulfoxide	1.6				115.59	18.20	15.75	93.36	12.69	13.59	104.11	6.72	6.45	105.76	5.54	5.24
149	Fenvalerate	2				107.17	18.70	19.85	88.71	2.41	2.72	133.74	8.81	6.59	89.16	9.12	10.23
150	Fipronil	0.8	102.09	11.72	11.48	94.23	19.32	19.56	93.24	8.74	9.37	97.93	6.41	6.55	101.30	2.10	2.07
151	Fipronil sulfide	8										99.25	16.95	17.08	101.31	10.48	10.34
152	Flocoumafen	0.4	106.82	17.50	16.38	94.93	7.63	8.04	84.00	8.55	10.18	101.02	3.52	3.48	102.48	3.21	3.13
153	Fluazinam	2				80.64	18.31	22.36	131.43	19.43	14.55	125.26	5.60	14.71	84.12	14.12	16.79
154	Flubendiamide	1.6				114.35	15.21	13.30	90.41	15.13	16.73	100.94	10.26	10.16	97.91	8.97	9.16
155	Flucythrinate (two isomers)	2				94.89	14.70	15.49	103.04	12.90	19.51	126.99	10.32	8.13	89.10	5.70	6.40
156	Fludioxonil	2				108.94	22.52	19.85	88.57	9.19	10.38	112.87	6.35	5.63	98.26	7.70	7.84
157	Flufenoxuron	0.8	118.74	20.77	17.49	90.49	6.24	6.90	83.45	5.81	6.96	100.22	5.17	5.16	102.52	5.23	5.10
158	Flumequine	0.8	127.04	7.72	5.63	91.69	7.33	7.99	90.22	9.12	10.11	95.00	6.52	6.86	104.99	4.54	4.32
159	Flunixin	0.8	123.19	8.48	6.88	99.80	18.40	18.44	86.25	12.61	14.62	93.81	5.62	5.99	103.21	8.75	8.48
160	Fluopyram	0.8	123.13	22.83	18.54	93.37	13.69	15.37	83.31	12.59	15.11	101.20	4.18	4.13	105.16	5.27	5.01
161	Fluoranthene	2				119.58	15.24	17.08	98.38	12.55	12.76	101.89	4.52	4.44	104.26	16.84	16.15
162	Fluorene	1.2				118.54	18.98	21.07	88.32	4.32	4.89	98.39	4.29	4.36	96.27	2.06	2.14

Table A2. *Cont.*

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
No.	Compound	LOQ (ng/mL)	Rec (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
163	Fluquinconazole	1.2				110.55	16.66	18.74	86.38	6.85	7.93	100.01	12.08	12.08	81.40	7.90	9.71
164	Flusilazole	0.8	108.78	10.98	10.09	99.53	10.49	10.54	88.08	8.54	9.70	104.22	12.29	11.79	102.06	4.15	4.07
165	Flutolanil	0.8	117.13	16.40	14.00	99.03	10.07	10.17	90.65	12.51	13.80	100.14	3.12	3.12	102.55	7.51	7.32
166	Flutriafol	1.2				102.18	9.65	9.44	91.70	13.15	14.34	103.28	8.34	8.08	106.17	4.42	4.16
167	Fluvalinate tau	4							78.58	18.54	23.59	99.65	18.40	18.46	105.68	17.54	16.60
168	Fonofos	1.6				96.73	20.46	22.45	82.54	10.97	13.29	115.98	10.45	9.01	90.58	4.37	4.82
169	Formetanate	1.2				105.80	11.33	10.71	91.02	5.72	6.28	90.92	7.25	7.97	102.25	6.83	6.68
170	Fosthiazate	0.4	117.53	12.76	10.86	91.81	5.80	6.32	89.96	11.00	12.23	101.32	5.03	4.96	103.76	2.06	1.99
171	Heptachlor	1.2				105.63	13.94	13.20	95.68	11.66	12.19	109.43	7.97	7.28	91.44	3.65	3.99
172	Hexachlor obencene	0.8	98.45	17.43	18.64	107.10	20.87	20.35	87.60	4.82	5.50	101.47	9.94	9.80	90.60	2.38	2.63
173	Hexachlor ocyclohexane (alpha)	2				117.54	12.12	8.21	88.98	5.41	6.08	103.02	9.89	9.60	92.16	8.25	8.95
174	Hexachlor ocyclohexane (beta)	2				126.04	17.47	13.86	97.43	3.53	3.62	108.18	8.42	7.78	93.18	8.51	9.13
175	Hexachlor ocyclohexane (delta)	4							90.15	19.06	20.45	104.74	16.22	15.49	95.22	11.94	12.54
176	Hexacloro cyclohexane (gamma, lindane)	4							93.75	14.15	23.24	107.48	6.80	6.33	95.82	12.95	13.51
177	Hexaconazole (two isomers)	1.6				95.19	15.08	15.84	91.80	7.62	8.30	102.65	5.70	5.55	103.19	2.94	2.85
178	Hexaflumuron	1.2				99.41	17.91	18.02	80.35	5.75	7.16	98.14	4.07	4.15	98.75	11.10	11.24

Table A2. *Cont.*

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
No.	Compound	LOQ (ng/mL)	Rec (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
179	Hexythiazox	0.4	118.55	17.88	18.46	82.22	8.53	10.37	87.62	9.39	10.72	98.62	2.88	2.92	104.62	8.24	7.88
180	Imazalil (enilconazole)	0.8	130.92	15.49	17.18	95.75	7.74	8.08	89.57	9.74	10.87	100.82	4.01	3.98	104.51	5.19	4.97
181	Imidacloprid	4							117.55	5.49	4.67	107.18	8.37	7.81	102.44	8.24	8.04
182	Indeno [1,2,3-cd] pyrene	1.6				117.89	15.48	13.13	116.35	14.87	12.78	116.45	17.25	14.81	100.18	19.54	19.50
183	Indoxacarb	0.8	124.61	14.87	18.70	99.01	21.65	21.87	88.98	17.78	19.98	93.09	6.87	7.38	109.78	5.44	4.96
184	Iprovalicarb	0.8	115.20	10.97	9.52	93.14	7.55	8.11	89.11	6.56	7.36	103.80	5.49	5.29	102.29	3.92	3.83
185	Isofenphos methyl	2				126.11	13.75	10.90	96.55	9.27	9.60	112.12	5.55	4.95	96.12	9.68	10.07
186	Isoprothiolane	0.4	106.89	3.33	3.12	96.32	18.05	18.74	92.86	12.46	13.42	101.23	2.17	2.14	102.11	4.86	4.76
187	Ivermectin B1a	1.6				113.41	16.89	14.89	98.11	8.63	8.80	91.74	11.91	12.98	101.63	10.59	10.42
188	Josamycin	1.6				127.21	18.85	14.82	103.07	5.89	5.71	94.94	7.07	7.45	108.51	2.46	2.27
189	Ketoprofen	1.6				93.43	17.84	19.09	106.39	15.59	14.05	95.64	10.20	10.66	102.80	3.50	3.40
190	Kresoxim methyl	2				119.34	19.83	18.72	98.02	18.09	18.66	110.88	14.03	12.65	96.62	12.85	13.30
191	Levamisole	1.6				114.26	22.07	19.32	92.62	8.40	9.07	86.67	7.08	8.17	101.93	6.16	6.04
192	Lincomycin	4							120.69	19.64	16.27	112.23	7.04	6.27	96.55	6.39	6.62
193	Linuron	1.6				125.64	15.59	18.33	89.87	13.77	15.32	98.09	5.13	5.23	107.80	5.05	4.68
194	Lufenuron	0.8	112.88	16.42	14.55	97.32	13.82	14.48	78.06	11.50	14.73	102.81	10.91	10.61	102.87	4.15	4.03
195	Mandipropamid	0.4	110.19	14.68	13.32	91.62	7.61	8.31	91.88	8.21	8.94	103.64	4.69	4.53	104.43	3.29	3.15
196	Mebendazole	0.4	128.41	10.28	8.01	92.65	6.20	6.69	86.90	10.23	11.77	95.39	7.83	8.21	104.16	2.75	2.64
197	Mefenamic acid	1.6				123.97	17.95	14.48	102.51	18.77	18.31	92.91	13.78	14.83	102.92	3.83	3.72
198	Mefenoxam (metalaxyl-M)	0.4	119.22	10.17	8.53	93.63	6.26	6.69	91.63	10.08	11.00	101.70	6.18	6.08	103.38	2.08	2.01
199	Meloxicam	1.2				94.27	17.55	18.66	89.51	17.89	16.54	90.45	7.81	8.63	106.55	10.65	10.00

Table A2. *Cont.*

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
No.	**Compound**	**LOQ (ng/mL)**	**Rec (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**
200	Mepanipyrim	2				114.71	20.02	17.45	92.36	6.47	7.01	105.72	8.41	7.95	102.76	2.90	2.82
201	Mepiquat	0.4	88.75	15.72	18.98	96.69	19.96	20.64	84.95	17.75	20.89	92.24	7.48	8.11	101.87	2.68	2.63
202	Metaflumizone	0.4	120.69	6.98	5.78	90.72	10.11	11.14	87.97	8.18	9.30	100.90	6.18	6.12	101.69	3.74	3.68
203	Metalaxyl	1.6				102.24	8.98	8.78	87.83	6.49	7.39	114.56	16.92	14.77	88.61	5.54	6.25
204	Metaldehyde	4							117.18	16.45	16.52	82.85	10.48	12.65	100.36	8.69	8.66
205	Metconazole	0.8	126.92	8.05	6.34	91.62	13.26	14.47	85.87	13.93	16.22	98.86	2.29	2.32	104.83	4.73	4.51
206	Methamidophos (two isomers)	8										90.08	12.33	13.69	95.62	7.09	7.41
207	Methidathion	0.4	121.63	11.90	9.78	94.81	6.86	7.24	92.10	11.60	12.60	101.70	2.15	2.11	105.46	7.13	6.76
208	Methiocarb	0.4	126.57	12.25	9.68	82.56	7.76	9.40	96.74	10.42	10.77	106.92	5.78	5.41	109.28	7.12	6.52
209	Methiocarb-sufone	2				124.44	19.05	20.07	110.41	9.03	8.18	106.55	7.79	7.31	105.17	9.43	8.97
210	Methiocarb-sulfoxide	1.2				97.90	11.90	12.16	97.45	19.13	19.63	102.57	5.80	5.65	107.53	5.47	5.09
211	Methomyl	1.2				105.76	12.28	11.61	116.29	18.70	16.08	114.17	3.66	3.21	106.03	7.48	7.05
212	Methoxyfenozide	0.4							86.63	21.88	23.34	102.29	15.53	15.18	97.40	9.54	9.79
213	Metoxychlor	4	116.96	8.59	7.34	88.75	7.33	8.26	90.85	3.70	4.07	102.21	3.91	3.83	102.30	3.36	3.28
214	Metrafenone	0.4	126.22	20.21	16.01	98.22	13.26	13.50	85.59	7.19	8.40	95.31	6.55	6.87	107.70	5.47	5.08
215	Metronidazole	12										77.85	19.32	22.63	94.46	8.54	9.04
216	Mevinphos (phosdrin)	1.2				107.14	19.43	18.14	87.21	9.70	11.12	99.51	6.93	6.96	112.13	4.49	4.00
217	Mirex	4							87.30	15.87	16.63	99.75	22.33	22.39	104.43	12.55	12.02
218	Monocrotophos	4							113.28	8.74	7.72	99.08	3.05	3.08	103.67	4.46	4.30

Table A2. *Cont.*

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
No.	**Compound**	**LOQ (ng/mL)**	**Rec (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**
219	Moxidectin	4							97.05	17.46	19.81	97.15	19.05	19.61	101.68	7.42	7.30
220	Myclobutanil	0.8	104.83	19.53	19.31	99.69	13.23	13.27	87.82	10.56	12.02	102.16	9.43	9.23	104.53	3.52	3.37
221	N-(2.4-dimethylphenyl)-N′-methylformamidine (DMPF, metabolite of amitraz)	4							112.03	15.22	13.59	98.34	7.94	8.07	102.65	5.61	5.47
222	N.N-Dimethyl-N′-p-tolylsulphamide (DMST, metabolite of tolyfluanid)	4							115.30	9.04	7.84	106.19	4.77	4.49	106.16	3.58	3.37
223	Nafcillin	4							106.61	19.05	17.87	95.48	11.52	12.07	98.70	4.02	4.07
224	Naphtalene	1.6				82.87	15.67	17.47	114.46	17.98	14.45	106.51	18.61	17.47	96.49	6.52	6.76
225	Naproxen	2				128.28	18.41	16.21	112.22	2.15	18.76	113.75	19.60	17.23	102.62	6.86	6.68
226	Nitenpyram	8										109.62	19.55	17.83	100.70	5.77	5.73
227	Novobiocin	1.2				96.87	20.40	19.19	85.98	18.66	21.70	89.43	13.67	15.29	95.39	7.93	8.31
228	Nuarimol	1.2				106.98	17.30	15.52	92.46	7.78	8.41	122.94	10.78	8.77	89.25	5.47	6.13
229	Ofurace	0.8	118.87	20.45	6.62	93.50	18.17	19.43	88.50	9.60	10.85	94.99	4.06	4.27	110.23	4.44	4.03
230	Omethoate	2				106.78	14.85	13.91	97.86	17.25	17.63	87.65	6.69	7.63	105.07	13.17	12.53
231	Oxadixyl	0.8	123.61	18.91	15.30	97.19	4.29	4.41	85.77	7.12	8.30	98.57	5.69	5.77	106.26	2.50	2.35
232	Oxamyl	8										107.50	18.14	16.87	110.16	21.87	19.85
233	Oxamyl-oxime	8							99.19	10.92	11.01	80.81	9.57	11.84	93.86	10.65	11.35
234	Oxfendazole	0.8	132.57	17.25	13.01	100.86	3.40	3.37	90.01	13.08	14.53	95.72	9.30	9.72	105.18	2.02	1.92

Table A2. *Cont.*

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
No.	Compound	LOQ (ng/mL)	Rec (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
235	Oxolinic acid	0.8							110.62	10.56	9.55	93.32	6.53	7.00	101.82	5.02	4.93
236	Oxydemeton methyl	4	125.26	11.57	8.95	103.93	6.92	6.66	90.21	15.13	16.77	94.20	6.15	6.53	103.58	4.77	4.61
237	Oxyfluorfen	4							87.69	18.03	21.96	104.71	11.43	10.92	97.01	14.18	14.62
238	Paclobutrazol	1.6				115.25	5.87	5.09	97.98	10.85	11.07	103.31	5.22	5.05	103.50	2.43	2.35
239	Parathion methyl	8										109.49	18.40	16.81	87.75	3.95	4.50
240	PCB 28	0.4	109.04	17.35	21.35	109.79	10.36	9.44	89.67	5.77	6.43	103.24	6.02	5.83	94.17	7.16	7.60
241	PCB 52	0.8	109.29	10.86	9.94	108.98	14.57	22.55	83.40	13.14	15.76	104.75	10.45	9.98	100.99	8.14	8.06
242	PCB 77	0.8	93.46	20.09	15.79	99.72	20.85	20.91	99.96	10.57	10.57	103.80	7.39	7.12	96.95	10.24	10.56
243	PCB 81	0.8	89.48	15.17	16.95	106.42	16.55	16.19	87.13	11.29	12.96	104.54	2.16	2.07	99.16	8.64	8.71
244	PCB 101	0.4	111.65	12.54	19.14	117.34	14.18	12.08	95.12	2.94	3.09	101.23	5.71	5.64	101.01	10.12	10.02
245	PCB 105	0.4	102.23	14.66	13.90	126.39	14.98	19.76	88.39	7.67	8.68	105.83	8.19	7.74	93.21	5.05	5.42
246	PCB 114	0.8	96.49	20.69	19.42	103.46	13.87	13.41	94.88	11.04	11.64	104.47	5.35	5.12	98.79	7.12	7.21
247	PCB 118	1.2				102.19	11.53	11.28	94.03	8.10	8.61	98.74	4.78	4.84	98.20	8.76	8.92
248	PCB 123	1.2				113.43	22.05	19.44	90.08	7.66	8.50	102.24	8.39	8.21	96.45	5.29	5.48
249	PCB 126	0.8	87.42	18.29	19.43	111.04	14.57	13.12	91.66	7.70	8.40	107.90	9.85	9.13	96.39	7.94	8.24
250	PCB 138	0.4	118.82	20.45	18.79	105.03	11.58	11.03	102.64	10.09	9.83	105.83	6.52	6.16	96.84	6.40	6.61
251	PCB 153	0.8	105.67	17.51	20.11	110.87	8.22	7.41	96.92	7.41	7.65	101.59	4.87	4.79	97.13	3.45	3.55
252	PCB 156	0.8	89.94	17.49	19.45	115.25	23.33	20.24	90.96	7.99	8.78	106.25	7.73	7.28	98.31	9.18	9.34
253	PCB 157	0.8	82.22	19.43	17.63	116.24	20.04	17.24	88.00	7.66	8.70	107.18	9.20	8.58	101.20	10.33	10.21
254	PCB 167	0.8	87.27	18.74	21.47	111.11	16.53	23.88	95.41	9.50	9.96	105.12	7.64	7.27	97.88	10.59	10.82
255	PCB 169	1.2				99.05	18.89	17.17	98.13	9.84	10.03	103.47	10.12	9.78	96.44	8.72	9.04
256	PCB 180	0.4	106.04	19.00	20.25	108.14	15.01	12.37	94.39	9.82	10.40	109.04	9.09	8.34	97.26	7.54	7.75

Table A2. *Cont.*

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
No.	**Compound**	**LOQ (ng/mL)**	**Rec (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**	**Rec. (%)**	**Intraday**	**Interday**
257	PCB 189	0.8	79.22	18.60	10.45	97.14	19.02	19.58	86.39	10.81	12.51	108.00	7.22	6.69	92.10	4.18	4.54
258	Penconazole	0.8	119.47	10.66	18.97	96.80	18.50	19.11	81.78	13.65	16.69	94.22	7.10	7.54	103.41	8.49	8.21
259	Pencycuron	0.4	110.82	13.26	11.97	90.36	11.63	12.87	86.17	10.68	12.39	98.98	3.84	3.88	101.16	6.82	6.74
260	Pendimethalin	2				102.88	19.86	19.30	75.06	16.21	21.60	104.56	5.38	5.15	94.40	11.99	12.70
261	Penicillin V	8										105.22	18.10	17.20	108.53	6.81	6.27
262	Permethrin	4							113.34	17.58	15.51	115.67	16.46	14.23	98.54	12.87	13.06
263	Phenanthrene	1.6				133.74	10.56	5.48	94.00	13.65	14.52	99.67	16.68	16.74	93.74	4.35	4.64
264	Phenylbutazone	16													110.32	17.68	21.84
265	Phosalone	0.4	110.50	17.38	15.73	98.28	8.36	8.51	89.91	5.27	5.86	101.98	2.80	2.75	102.70	2.35	2.29
266	Phosmet	1.2				100.60	6.02	5.98	92.49	12.42	13.43	101.79	5.92	5.82	104.63	5.42	5.18
267	Pthalamide (Folpet deg)	8										103.79	11.71	11.28	100.45	6.35	6.32
268	Pirimicarb	0.4	120.22	8.00	6.65	95.32	4.74	4.97	93.09	11.13	11.96	102.45	4.63	4.52	103.60	2.50	2.41
269	Pirimicarb-desmethyl	2				110.38	7.61	6.89	127.79	12.92	10.11	114.99	8.23	7.16	123.05	4.57	3.71
270	Pirimiphos ethyl	0.8	82.75	17.50	21.15	92.12	9.50	10.31	98.11	12.10	12.33	120.39	11.92	9.90	101.28	6.04	7.51
271	Pirimiphos methyl	0.4	111.79	15.98	14.29	95.45	16.02	16.78	88.67	10.14	11.44	102.62	4.30	4.19	101.90	6.74	6.61
272	Prochloraz	0.4	104.39	17.43	16.70	91.03	5.00	5.49	89.27	11.46	12.84	100.96	5.85	5.79	103.41	5.28	5.11
273	Procymidone	8										92.02	15.09	16.40	106.35	16.63	15.04
274	Profenofos	0.8	115.10	18.63	17.91	93.40	17.40	18.63	88.46	19.43	21.96	84.42	5.94	7.04	108.50	7.24	6.67
275	Propamocarb	2				114.94	19.15	16.66	93.17	2.45	2.63	99.37	9.65	9.71	103.21	7.96	7.71
276	Propargite	0.4	113.51	7.83	6.90	97.28	4.47	4.59	90.04	6.01	6.67	99.27	1.99	2.00	100.59	5.36	5.33
277	Propiconazole	2							103.21	16.19	15.38	98.49	10.53	10.69	103.63	3.30	3.18

Table A2. *Cont.*

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
No.	Compound	LOQ (ng/mL)	Rec (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
278	Propoxur	0.8	124.75	20.23	15.08	92.34	8.00	8.66	93.26	18.90	20.27	101.62	8.07	7.94	111.03	3.68	3.31
279	Propyzamide (pronamide)	0.8	104.03	19.42	19.74	89.58	16.14	19.18	93.02	13.39	14.39	97.32	5.91	6.07	102.92	7.64	7.42
280	Proquinazid	1.6				102.99	15.84	15.38	92.93	3.72	4.00	116.31	13.68	11.76	95.04	8.78	9.24
281	Prothioconazol	1.6				118.94	17.99	15.13	86.94	10.77	12.39	101.86	7.03	6.90	103.49	5.02	4.85
282	Prothiophos	2				127.35	17.52	15.03	91.22	11.43	12.53	110.73	16.36	14.77	90.19	7.42	8.23
283	Pymetrozine	8										95.53	3.63	3.80	99.15	5.84	5.89
284	Pyraclostrobin	0.4	118.10	14.33	12.13	88.90	5.97	6.72	90.62	2.37	2.62	97.96	3.11	3.17	101.93	4.86	4.77
285	Pyrazophos	0.4	116.34	17.02	14.63	87.65	5.37	6.13	95.15	10.11	10.63	103.82	7.88	7.59	104.45	3.19	3.05
286	Pyrene	2				122.26	19.33	15.12	95.97	15.49	16.14	99.99	7.05	7.05	106.97	13.20	12.34
287	Pyridaben	0.4	111.80	8.50	7.60	96.96	11.34	11.70	89.29	7.37	8.25	97.16	3.54	3.64	101.94	5.96	5.85
288	Pyridaphenthion	0.4	125.33	10.87	8.67	88.86	12.18	13.71	90.69	9.17	10.11	103.36	5.75	5.56	104.97	2.29	2.18
289	Pyrimethanil	1.2				107.01	6.57	6.14	83.42	6.56	7.86	99.98	4.42	4.42	106.14	3.93	3.70
290	Pyriproxifen	0.4	108.80	6.84	6.29	93.68	5.81	6.20	84.68	4.33	5.11	99.26	4.88	4.92	97.21	10.36	10.66
291	Quinalfos	1.6				90.14	15.91	17.65	81.56	14.48	17.75	98.78	15.64	15.83	99.05	6.28	6.34
292	Quinoxyfen	0.8	106.79	20.65	20.64	102.29	14.68	14.27	92.06	12.98	14.10	113.55	3.75	3.30	88.93	8.36	9.40
293	Rifampicin	1.2				103.00	18.59	17.76	89.34	13.65	15.28	99.63	7.95	7.98	111.87	15.95	14.26
294	Rotenone	0.8	120.70	16.46	13.64	89.14	17.53	20.88	81.92	14.34	17.50	101.34	12.36	12.20	105.21	3.73	3.55
295	Roxithromycin	1.2				116.45	19.37	16.63	88.89	12.89	14.50	88.85	16.67	18.76	102.53	3.02	2.95
296	Sarafloxacin	20													102.28	16.90	16.52
297	Simazine	0.8	127.26	22.73	17.86	89.60	13.25	14.79	90.53	13.38	14.78	101.60	7.91	7.79	108.74	8.42	7.74
298	Spinosad (two isomers)	1.6	111.72	19.81	17.73	90.14	14.70	16.31	89.32	9.26	10.37	99.29	5.46	5.50	100.87	7.97	7.90

Table A2. *Cont.*

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
No.	Compound	LOQ (ng/mL)	Rec (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
299	Spiramycin (two isomers)	12										105.21	2.31	2.20	98.92	6.55	6.62
300	Spirodiclofen	0.8	114.30	10.24	8.96	98.97	9.73	9.83	86.32	3.99	4.62	96.72	4.66	4.82	96.10	2.65	2.76
301	Spiromesifen	0.4	111.87	16.94	15.14	96.88	10.62	10.96	81.93	9.64	11.77	96.25	5.78	6.01	102.02	6.81	6.68
302	Spirotetramat	0.8	136.54	14.99	18.30	91.58	15.29	17.62	87.65	16.87	19.25	99.19	10.60	10.69	109.75	7.10	6.47
303	Spiroxamine	0.4	115.95	5.12	4.42	92.27	5.97	6.47	93.01	4.62	4.97	100.04	5.41	5.41	101.94	2.10	2.06
304	Strychnine	2				121.82	15.59	16.26	84.19	20.34	20.16	100.68	5.59	5.55	102.95	5.88	5.71
305	Sulfacetamide	16													94.80	9.96	10.51
306	Sulfachloropiridacine	4							109.37	18.24	16.68	98.92	7.84	7.93	102.01	6.91	6.77
307	Sulfadiacine	8										91.59	4.52	4.94	87.73	9.54	10.87
308	Sulfadimetoxine	2				126.54	15.11	10.31	109.89	11.08	10.08	103.69	8.01	7.72	103.97	3.74	3.60
309	Sulfadoxine	2				123.17	11.68	8.48	107.05	13.06	12.20	100.75	7.53	7.47	106.39	7.08	6.65
310	Sulfameracine	4							118.06	8.76	7.42	95.45	3.06	3.21	102.43	3.58	3.50
311	Sulfametacine	2				118.26	12.28	8.28	105.33	12.84	12.19	102.14	7.00	6.85	104.35	4.92	4.71
312	Sulfametizole	4							108.41	7.69	7.09	97.15	4.81	4.95	101.75	12.24	12.03
313	Sulfametoxazole	2				117.53	19.33	21.76	99.36	6.59	6.63	100.10	5.77	5.76	104.08	10.50	10.09
314	Sulfametoxipiridacine	1.6				122.56	10.02	7.23	93.76	15.04	16.04	95.82	4.12	4.30	106.04	6.02	5.68
315	Sulfamonomethoxine	4							115.82	19.10	16.49	109.03	9.00	8.25	102.71	5.89	5.73
No.	Compound	LOQ (ng/mL)	Rec (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
316	Sulfapyridine	4							123.12	5.81	4.72	97.40	5.43	5.57	97.95	5.03	5.14
317	Sulfaquinoxaline	2				137.13	15.86	11.57	107.09	16.46	15.37	103.03	8.49	8.24	105.72	5.77	5.46
318	Sulfatiazole	4							114.09	12.17	10.67	89.17	3.00	3.36	96.75	9.66	9.98
319	Sulfisoxazole	4							123.17	15.55	12.62	109.15	6.85	6.28	102.56	9.83	9.58

Table A2. *Cont.*

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
320	Tebuconazole	2				124.33	10.86	8.73	102.25	9.82	9.60	103.18	10.27	9.95	100.50	6.55	6.52
321	Tebufenocide	0.8	113.40	19.30	17.02	94.01	6.42	6.83	88.38	6.07	6.87	99.16	2.48	2.50	99.92	5.26	5.26
322	Tebufenpyrad	0.8	125.31	18.01	14.37	106.17	5.54	5.22	88.47	11.68	13.20	96.73	4.95	5.12	106.06	2.21	2.08
323	Teflubenzuron	1.6				108.57	19.06	17.56	89.65	5.82	6.49	100.52	12.28	12.22	104.30	4.23	4.06
324	Tefluthrin	0.4	124.36	21.64	16.23	105.00	19.05	18.14	88.44	12.15	13.74	105.91	7.75	7.32	89.59	1.30	1.45
325	Telodrin (isobenzan)	2				121.92	12.85	21.31	88.03	14.49	17.82	108.27	5.89	5.44	99.09	17.36	17.52
326	Terbufos	0.8	104.09	14.21	13.26	88.68	15.21	16.32	81.58	8.12	9.95	110.15	9.52	8.64	95.99	5.50	5.73
327	Terbuthylazine	0.8	120.91	9.86	8.15	93.77	18.06	19.26	85.93	9.78	11.38	106.01	2.62	2.47	104.19	4.52	4.34
328	Tetrachlorvinphos	2				126.81	17.78	13.00	100.05	19.19	19.18	88.06	9.94	11.29	98.20	13.04	13.28
329	Tetraconazole	0.8	116.66	14.53	20.60	90.40	17.43	19.28	89.89	9.78	10.88	115.70	7.74	6.69	88.10	5.70	6.47
330	Tetradifon	1.6				106.92	12.99	19.50	91.97	11.87	12.91	112.56	5.80	5.15	94.92	2.68	2.82
331	Tetramethrin	2							120.30	14.89	12.38	113.61	8.45	7.44	99.35	15.78	15.88
332	Thiabendazole	1.2				96.72	17.21	16.26	86.00	6.75	7.85	109.00	4.57	4.19	95.59	7.21	7.54
333	Thiacloprid	0.8	121.34	7.44	5.66	90.02	6.38	7.09	88.41	14.87	16.82	108.68	3.58	3.29	110.31	5.93	5.38
334	Thiamethoxam	8										121.06	6.35	5.25	92.87	5.21	5.61
335	Thiophanate methyl	2				124.15	8.09	6.03	101.95	12.23	12.00	106.08	6.23	5.87	103.96	7.36	7.08
336	Tolclofos methyl	1.6				123.59	15.07	12.19	84.62	5.68	6.71	109.41	7.30	6.67	96.18	5.78	6.01
337	Tolfenamic acid	1.6				105.31	15.54	20.30	104.76	16.01	15.28	96.52	3.58	3.71	97.33	5.08	5.22
338	Triadimefon	1.2				94.15	15.09	16.33	95.62	7.48	7.82	101.84	6.27	6.16	103.91	2.78	2.68
339	Triadimenol	0.8	114.21	11.55	9.26	106.40	13.80	14.65	89.34	12.93	14.47	103.34	6.45	6.24	106.44	5.74	5.39
340	Triazophos (hostathion)	0.4	112.50	15.63	12.76	91.77	6.14	6.69	91.68	11.49	12.53	104.86	3.62	3.45	102.76	4.43	4.31

Table A2. *Cont.*

			0.4 ng/mL			1 ng/mL			4 ng/mL			20 ng/mL			40 ng/mL		
				Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)			Precision (RSD. %)	
No.	Compound	LOQ (ng/mL)	Rec (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday	Rec. (%)	Intraday	Interday
341	Trifloxystrobin	0.4	112.87	13.12	11.62	92.56	5.89	6.36	93.35	4.78	5.12	100.81	2.86	2.84	102.51	4.76	4.64
342	Triflumizole	0.4	110.08	9.26	8.41	94.01	7.01	7.46	93.26	7.62	8.17	99.91	2.69	2.69	101.62	3.67	3.61
343	Triflumuron	1.2				118.66	19.24	16.21	86.93	7.78	8.95	96.65	3.90	4.04	106.18	6.37	6.00
344	Trifluralin	1.2				93.29	17.78	19.06	92.40	6.37	6.89	105.89	9.83	9.28	93.54	4.17	4.46
345	Trimethoprim	2				116.02	16.54	12.16	109.21	15.05	13.78	94.20	12.51	13.28	97.92	8.70	8.88
346	Triticonazole	1.2				99.19	8.60	8.67	89.71	12.71	14.17	100.84	8.67	8.60	104.04	4.36	4.19
347	Tylmicosin	4							107.14	16.70	15.59	93.58	6.97	7.45	101.18	11.19	11.06
348	Tylosin	8										106.35	4.46	4.19	104.60	2.79	2.67
349	Vinclozolin	1.6				116.52	23.46	17.30	88.94	12.82	14.41	105.60	12.42	11.76	92.86	4.92	5.30
350	Warfarin	0.8	100.47	17.51	19.89	95.53	9.12	9.55	85.61	11.19	14.75	93.09	6.28	6.75	103.65	4.31	4.16
351	Zoxamide	0.8	121.96	19.52	18.47	88.50	11.13	13.88	90.57	19.33	20.34	106.93	5.18	4.84	105.40	6.44	6.11

Appendix C

Table A3. Matrix Effect, expressed as percentage, calculated for all of the analytes. The range form −20% to 20% represent the tolerance range in which it is considered that no significant matrix effect exists.

No.	Compound	Matriz effect	No.	Compound	Matriz effect	Nº	Compound	Matriz Effect
1	2-Phenylphenol	−103.4	118	Eprinomectin	−6.3	235	Oxolinic acid	0.5
2	4,4′-Dichlorobenzophenone (metabolite of dicofol)	96.6	119	Eritromicin	45.4	236	Oxydemeton methyl	3.3
3	Abamectine	−70.6	120	Esfenvalerate	23.4	237	Oxyfluorfen	42.4
4	Acenaphthene	14.3	121	Ethion (diethion)	−13.7	238	Paclobutrazol	3.0
5	Acenaphtylene	17.0	122	Ethirimol	13.9	239	Parathion methyl	12.8
6	Acephate	−90.4	123	Ethofumesate	30.5	240	PCB 28	32.5
7	Acetamiprid	7.0	124	Ethoprophos	−20.5	241	PCB 52	32.7
8	Acrinathrin	16.2	125	Etofenprox	43.0	242	PCB 77	52.5
9	Albendazole	17.7	126	Etoxazole	−29.6	243	PCB 81	43.7
10	Aldicarb	5.8	127	Famoxadone	27.9	244	PCB 101	8.2
11	Aldicarb-sulfone	−4.5	128	Fenamidone	12.7	245	PCB 105	54.6
12	Aldicarb-sulfoxide	−2.9	129	Fenamiphos	14.0	246	PCB 114	46.6
13	Aldrin	43.7	130	Fenamiphos sulfone	10.4	247	PCB 118	45.0
14	Anthracene	23.9	131	Fenamiphos sulfoxide	0.0	248	PCB 123	45.7
15	Atrazine	7.8	132	Fenarimol	55.3	249	PCB 126	52.1
16	Azinphos-methyl	14.3	133	Fenazaquin	−108.8	250	PCB 138	54.9
17	Azoxystrobin	−6.9	134	Fenbendazole	−7.9	251	PCB 153	48.2
18	BDE-28	47.0	135	Fenbuconazole	67.7	252	PCB 156	59.3

Table A3. *Cont.*

No.	Compound	Matriz effect	No.	Compound	Matriz effect	Nº	Compound	Matriz Effect
19	BDE-47	47.8	136	Fenhexamid	5.9	253	PCB 157	51.6
20	BDE-85	43.7	137	Fenitrothion	54.6	254	PCB 167	60.1
21	BDE-99	57.3	138	Fenoxycarb	16.4	255	PCB 169	60.6
22	BDE-100	51.3	139	Fenpropathrin	−2.2	256	PCB 180	52.5
23	BDE-153	35.9	140	Fenpropidin	22.3	257	PCB 189	54.8
24	BDE-154	57.8	141	Fenpropimorph	1.7	258	Penconazole	50.4
25	BDE-183	−14.1	142	Fenpyroximate	19.8	259	Pencycuron	−37.4
26	Benalaxyl	−111.5	143	Fenthion	−33.9	260	Pendimethalin	29.8
27	Bendiocarb	−5.4	144	Fenthion oxon	27.1	261	Penicillin V	−8.1
28	Bendiocarb metabolite (2,2-dimethylbenzo-1,3-dioxol-4-ol)	39.7	145	Fenthion oxon sulfone	5.3	262	Permethrin	71.5
29	Benfuracarb	24.0	146	Fenthion oxon sulfoxide	3.7	263	Phenanthrene	29.6
30	Benzo[a]anthracene	58.5	147	Fenthion sulfone	6.9	264	Phenylbutazone	−4.9
31	Benzo[a]pyrene	45.0	148	Fenthion sulfoxide	11.9	265	Phosalone	−4.0
32	Benzo[b]fluoranthene	59.8	149	Fenvalerate	−39.6	266	Phosmet	5.8
33	Benzo[ghi]perylene	24.4	150	Fipronil	21.0	267	Pthalamide (Folpet deg)	59.3
34	Benzo[k]fluoranthene	38.1	151	Fipronil sulfide	676.5	268	Pirimicarb	1.7
35	Bifenthrin	80.4	152	Flocoumafen	−34.5	269	Pirimicarb-desmethyl	57.7
36	Bitertanol	−0.7	153	Fluazinam	−1.5	270	Pirimiphos ethyl	60.5
37	Boscalid (formerly nicobifen)	73.5	154	Flubendiamide	62.7	271	Pirimiphos methyl	−14.7

Table A3. *Cont.*

No.	Compound	Matriz effect	No.	Compound	Matriz effect	Nº	Compound	Matriz Effect
38	Brodifacoum	−31.7	155	Flucythrinate (two isomers)	4.0	272	Prochloraz	−37.2
39	Bromadiolone	−2.1	156	Fludioxonil	61.2	273	Procymidone	52.9
40	Bromopropylate	94.0	157	Flufenoxuron	1.1	274	Profenofos	−15.0
41	Bromuconazole (two isomers)	39.7	158	Flumequine	−0.8	275	Propamocarb	16.2
42	Bupirimate	57.0	159	Flunixin	−9.4	276	Propargite	−29.3
43	Buprofezin	−0.4	160	Fluopyram	45.4	277	Propiconazole	−85.8
44	Cadusafos (ebufos)	−54.8	161	Fluoranthene	36.0	278	Propoxur	−7.1
45	Carbaryl	15.8	162	Fluorene	17.2	279	Propyzamide (pronamide)	−2.0
46	Carbendazim (azole)	16.8	163	Fluquinconazole	55.3	280	Proquinazid	72.7
47	Carbofuran	−9.0	164	Flusilazole	3.8	281	Prothioconazol	51.5
48	Carbofuran-3-hydroxy	5.5	165	Flutolanil	−10.2	282	Prothiophos	49.5
49	Carbosulfan	−107.2	166	Flutriafol	54.5	283	Pymetrozine	16.2
50	Cefuroxima axetil (two isomers)	24.0	167	Fluvalinate tau	−31.7	284	Pyraclostrobin	−18.0
51	Chloramphenicol	24.4	168	Fonofos	28.9	285	Pyrazophos	20.6
52	Chlorantraniliprole	4.5	169	Formetanate	−38.7	286	Pyrene	45.1
53	Chlorfenvinphos	−31.3	170	Fosthiazate	2.9	287	Pyridaben	−83.6
54	Chlorobenzilate	95.2	171	Heptachlor	−110.9	288	Pyridaphenthion	14.3
55	Chlorophacinone	38.1	172	Hexachlorobencene	16.9	289	Pyrimethanil	30.6
56	Chlorpropham	24.2	173	Hexachlorocyclohexane (alpha)	−58.1	290	Pyriproxifen	−24.8

Table A3. *Cont.*

No.	Compound	Matriz effect	No.	Compound	Matriz effect	Nº	Compound	Matriz Effect
57	Chlorpyrifos	38.0	174	Hexachlorocyclohexane (beta)	−97.4	291	Quinalfos	3.7
58	Chlorpyrifos methyl	9.6	175	Hexachlorocyclohexane (delta)	−89.1	292	Quinoxyfen	−68.5
59	Chlorthal dimethyl	36.6	176	Hexaclorocyclohexane (gamma. lindane)	−109.8	293	Rifampicin	−68.9
60	Chrysene	38.1	177	Hexaconazole (two isomers)	−4.6	294	Rotenone	22.0
61	Clindamycin	−7.5	178	Hexaflumuron	−1.3	295	Roxithromycin	0.0
62	Clofentezine	13.2	179	Hexythiazox	−27.6	296	Sarafloxacin	114.8
63	Clothianidin	17.7	180	Imazalil (enilconazole)	11.5	297	Simazine	−19.7
64	Cloxacillin	4.9	181	Imidacloprid	4.1	298	Spinosad (two isomers)	−84.2
65	Coumachlor	46.2	182	Indeno [1,2,3-cd] pyrene	−132.0	299	Spiramycin (two isomers)	16.8
66	Coumaphos	−105.9	183	Indoxacarb	2.6	300	Spirodiclofen	−12.7
67	Coumatetralyl	−25.6	184	Iprovalicarb	−4.9	301	Spiromesifen	−23.6
68	Cyazofamid	12.7	185	Isofenphos methyl	42.0	302	Spirotetramat	−34.1
69	Cyflufenamid	−3.0	186	Isoprothiolane	−13.5	303	Spiroxamine	9.4
70	Cyfluthrin (sum of four isomers)	−6.1	187	Ivermectin B1a	−82.6	304	Strychnine	11.9
71	Cyhalothrin (lambda isomer)	−22.4	188	Josamycin	−0.4	305	Sulfacetamide	−27.9
72	Cymoxanil	13.6	189	Ketoprofen	10.0	306	Sulfachloropiridacine	61.1
73	Cypermethrin (sum of four isomers)	20.9	190	Kresoxim methyl	36.3	307	Sulfadiacine	−15.0

Table A3. *Cont.*

No.	Compound	Matriz effect	No.	Compound	Matriz effect	Nº	Compound	Matriz Effect
74	Cyproconazole (two isomers)	8.2	191	Levamisole	10.0	308	Sulfadimetoxine	18.2
75	Cyprodinil	−5.7	192	Lincomycin	135.3	309	Sulfadoxine	2.2
76	Cyromazine	−106.0	193	Linuron	0.0	310	Sulfameracine	83.7
77	Danofloxacin	172.1	194	Lufenuron	7.3	311	Sulfametacine	166.8
78	Dazomet	33.1	195	Mandipropamid	20.6	312	Sulfametizole	170.5
79	Deltamethrin	−33.7	196	Mebendazole	11.5	313	Sulfametoxazole	26.0
80	Demeton-S-methyl	−27.1	197	Mefenamic acid	33.5	314	Sulfametoxipiridacine	107.7
81	Demeton-S-methyl-sulfone (Dioxydemeton)	7.5	198	Mefenoxam (metalaxyl-M)	−5.9	315	Sulfamonomethoxine	1.7
82	Dexamethasone	5.4	199	Meloxicam	34.8	316	Sulfapyridine	24.6
83	Diazinon	22.3	200	Mepanipyrim	64.2	317	Sulfaquinoxaline	2.5
84	Dibenzo[a.h]anthracene	35.2	201	Mepiquat	−57.2	318	Sulfatiazole	32.5
85	Dichlorodiphenyldichloroethane (p,p′ DDD)	14.9	202	Metaflumizone	5.4	319	Sulfisoxazole	11.6
86	Dichlorodiphenyldichloroethylene (p,p′ DDE)	44.5	203	Metalaxyl	−47.0	320	Tebuconazole	−72.1
87	Dichlorodiphenyltrichloroethane (p,p′ DDT)	−124.9	204	Metaldehyde	−25.7	321	Tebufenocide	−7.8
88	Diclofenac	8.6	205	Metconazole	−26.1	322	Tebufenpyrad	7.9
89	Dicloran	−0.4	206	Methamidophos (two isomers)	−16.6	323	Teflubenzuron	−31.5
90	Diclorvos	−42.8	207	Methidathion	7.1	324	Tefluthrin	33.9
91	Dicloxacillin	−4.4	208	Methiocarb	6.2	325	Telodrin (isobenzan)	−22.0
92	Dieldrin	19.7	209	Methiocarb-sufone	3.0	326	Terbufos	24.0

Table A3. *Cont.*

No.	Compound	Matriz effect	No.	Compound	Matriz effect	Nº	Compound	Matriz Effect
93	Diethathyl ethyl	−15.6	210	Methiocarb-sulfoxide	3.0	327	Terbuthylazine	29.2
94	Diethofencarb	7.5	211	Methomyl	1.8	328	Tetrachlorvinphos	1.1
95	Difenacoum	7.8	212	Methoxyfenozide	−7.0	329	Tetraconazole	48.4
96	Difenoconazole	−21.3	213	Metoxychlor	−124.6	330	Tetradifon	41.7
97	Difethialone	16.8	214	Metrafenone	6.1	331	Tetramethrin	154.7
98	Difloxacin	99.9	215	Metronidazole	−13.3	332	Thiabendazole	67.7
99	Diflubenzuron	38.5	216	Mevinphos (phosdrin)	−3.3	333	Thiacloprid	12.3
100	Diflufenican	8.1	217	Mirex	−82.6	334	Thiamethoxam	19.7
101	Dimethenamid-P (and its R-isomer)	8.7	218	Monocrotophos	−1.2	335	Thiophanate methyl	−4.2
102	Dimethoate	4.8	219	Moxidectin	20.7	336	Tolclofos methyl	21.0
103	Dimethomorph (two isomers)	24.2	220	Myclobutanil	24.7	337	Tolfenamic acid	136.0
104	Dimethylphenylsulfamide (DMSA, metabolite of dichlofluanid)	4.9	221	N-(2,4-dimethylphenyl)-N′-methylformamidine (DMPF, metabolite of amitraz)	9.6	338	Triadimefon	20.6
105	Diniconazole-M	−11.4	222	N,N-Dimethyl-N′-p-tolylsulphamide (DMST, metabolite of tolyfluanid)	−8.2	339	Triadimenol	27.9
106	Dinocap	−11.9	223	Nafcillin	−8.2	340	Triazophos (hostathion)	−6.3
107	Diphacinone	14.1	224	Naphtalene	12.9	341	Trifloxystrobin	−55.8

Table A3. *Cont.*

No.	Compound	Matriz effect	No.	Compound	Matriz effect	Nº	Compound	Matriz Effect
108	Diphenylamine	−102.4	225	Naproxen	16.5	342	Triflumizole	−14.3
109	Dodine	−92.0	226	Nitenpyram	−4.5	343	Triflumuron	3.6
110	Doramectina	−16.2	227	Novobiocin	2.9	344	Trifluralin	−1.7
111	Endosulfan alfa	−2.5	228	Nuarimol	74.1	345	Trimethoprim	25.2
112	Endosulfan beta	−52.9	229	Ofurace	−27.2	346	Triticonazole	13.7
113	Endosulfan sulfate	−85.8	230	Omethoate	−0.4	347	Tylmicosin	140.3
114	Endrin	−59.9	231	Oxadixyl	6.5	348	Tylosin	−1.7
115	Enrofloxacin	141.8	232	Oxamyl	2.6	349	Vinclozolin	33.9
116	EPN	32.6	233	Oxamyl-oxime	30.1	350	Warfarin	0.0
117	Epoxiconazole	10.0	234	Oxfendazole	7.0	351	Zoxamide	−83.0

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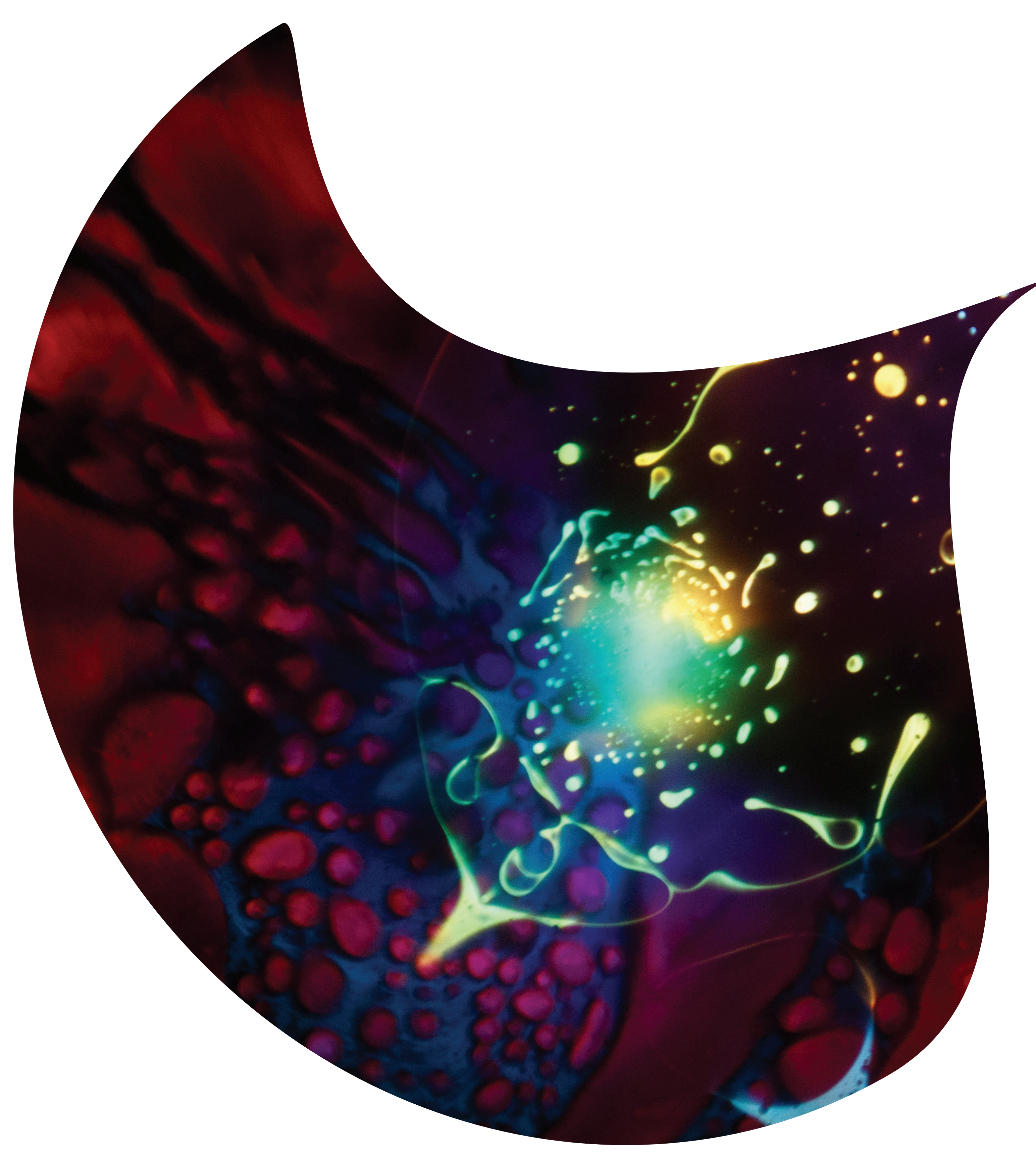
Bloque B. Biomonitorización

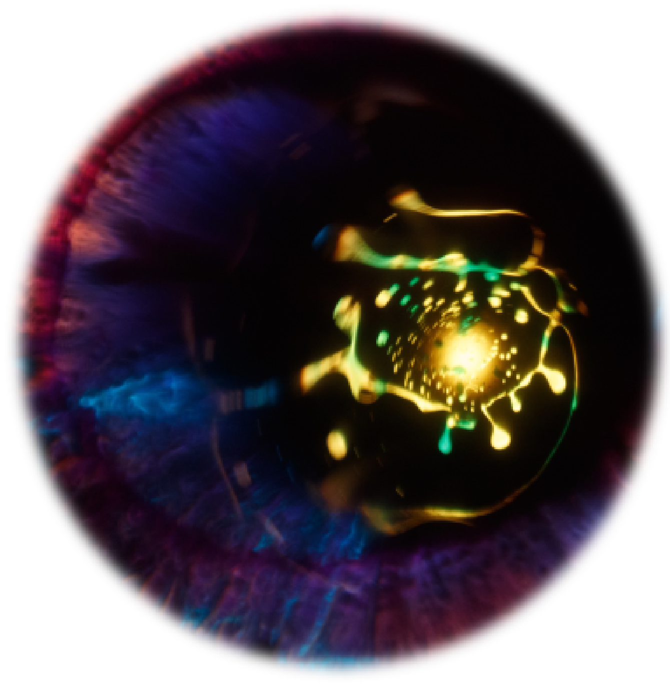


Publicación 4. Role of Pet Dogs and Cats as Sentinels of Human Exposure to Polycyclic Aromatic Hydrocarbons

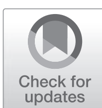
Papel de los perros y gatos domésticos como centinelas de la exposición humana a los hidrocarburos aromáticos policíclicos

Pets as Sentinels, Forecasters and Promoters of Human Health, 2020, Springer, Chapter 4: 65-81

DOI: https://doi.org/10.1007/978-3-030-30734-9_4



Chapter 4
Role of Pet Dogs and Cats as Sentinels of Human Exposure to Polycyclic Aromatic Hydrocarbons



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Abstract Polycyclic aromatic hydrocarbons (PAHs) are a large group of chemical contaminants, predominantly produced via fossil fuel combustion. They spread easily worldwide, so they are considered as semipersistent pollutants. Many of them are considered as carcinogenic or mutagenic compounds, for example, interacting directly with DNA. Benzo(a)pyrene (BaP) is the most important and well-known PAH. Living beings are exposed everyday through air, water, plastic stuff and smoke and almost by food intake, because they are highly lipophilic. In human risk assessments, monitoring these compounds, or their products, in environment, biological or food samples has attracted enormous interest. Pets commonly share habitat and routine life with humans. In this chapter, the possibility that pets were good sentinels of human exposure to PAHs is studied in detail. Concentrations of parental PAHs and some metabolites between human and pets have been compared. In the case of dogs, their concentrations and profiles of PAHs are very different to those of humans when compared. Dogs had lesser concentration of parental compounds and higher concentration of their metabolites than humans. Similarly, cats present different concentrations and detection frequencies than humans. Therefore, the scarce data available indicate that dogs and cats seem to have different sources of exposition to PAHs than humans. Although more studies are needed, pets do not seem to be good sentinels for human exposure to PAHs.

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M. R. Pastorinho, A. C. A. Sousa (eds.), *Pets as Sentinels, Forecasters and Promoters of Human Health*, https://doi.org/10.1007/978-3-030-30734-9_4

Keywords Benzo(a)pyrene · Dogs · Cats · Semipersistent organic pollutants · Sentinels · Carcinogenic chemicals · Polycyclic aromatic hydrocarbons · Pets · Sentinel · Biomonitoring

4.1 Introduction

Polycyclic aromatic hydrocarbons or polynuclear aromatic hydrocarbons (PAHs) are a large class of organic compounds (more than a hundred are known) made from carbon and hydrogen, formed by more than two benzene rings fused and organised on linear, angular or cluster structure. According to their molecular weight, they can be classified as low-molecular-weight PAHs (LMW-PAHs, up to three fused rings) or as high-molecular-weight PAHs (HMW-PAHs, minimum of four rings).

Generally, they are colourless, white or yellowish solid at room temperature; have low vapour pressure, high melting and boiling points and low water solubility; and are hence highly lipophilic (WHO 1998). The most harmful and best-known PAH is benzo(a)pyrene (BaP), but there are many other PAHs of concern (Fig. 4.1) because of their toxicity, human exposure, occurrence in the environment and scope of available information. According to the list of priority pollutants of the United States Environmental Protection Agency (USEPA), there are 16 priority PAHs, because of their occurrence and the fact that they are continuously emitted to the environment (ATSDR 1995). More important are those PAHs that have been identified as mutagenic/teratogenic/carcinogenic by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Fig. 4.1). Based on data from oral bioassays conducted in mice with coal tar mixtures, the JECFA calculated margin of exposure values of 25,000 and 10,000 between the BMDL10 value of 100 μg of benzo[a]pyrene/kg bw/day and mean and 95th-percentile intake levels of 4 and 10 ng/kg bw/d, respectively.

According to their origin, PAHs can be classified as pyrolytic (high temperature), petrogenic (high pressure) or biological (synthesised by microorganisms). Besides, they can be disguised between anthropogenic (combustion of fossil fuels, principally) and natural (forest fires, volcanos, fossil fuel formation, vegetal matter decomposition) sources, although the latter have a minimal contribution to the total environment burden.

Granting that they have no utility per se, PAHs are used as intermediaries in different industries, namely, in the manufacture of pharmaceutical products, polyvinyl chloride (PVC) and plasticisers (naphthalene), pigments (acenaphthene, pyrene), dyes (anthracene, fluoranthene) and pesticides (phenanthrene) (WHO 1998). Nevertheless, production, processing and use of fossil fuels principally coal – and, to a lesser extent, oil and natural gas – for industries, heating or transportation in cities, are the main source of emission of these contaminants to the environment (Cabuk et al. 2014; Villar-Vidal et al. 2014). Concerning traffic, petrol-fuelled vehicles can emit greater amounts of fluoranthene and pyrene, whilst diesel-fuelled

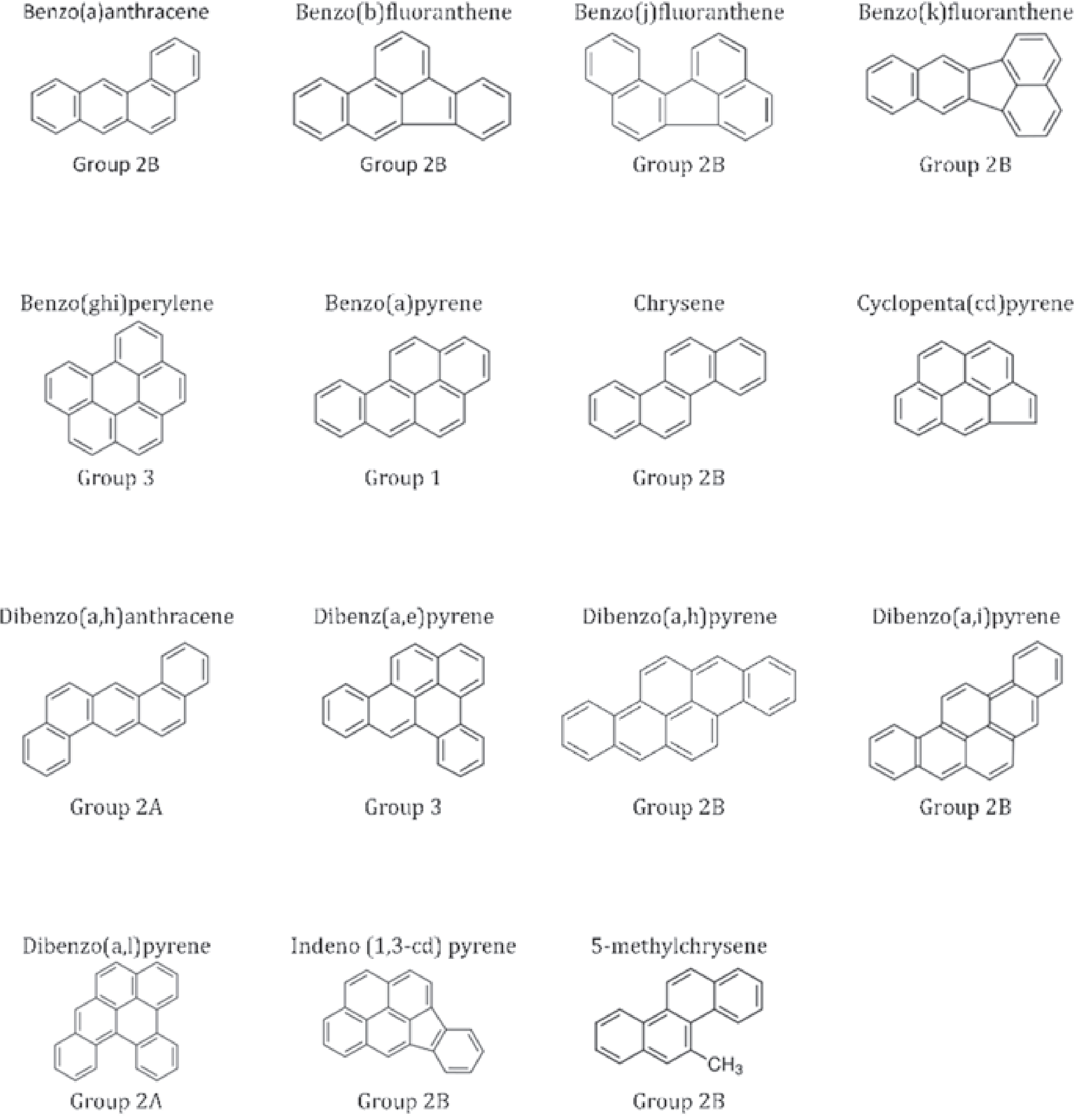

Fig. 4.1 Polycyclic aromatic hydrocarbons for which there is clear evidence of mutagenicity/gen otoxicity in somatic cells in experimental animals in vivo and, with the exception of benzo(ghi) perylene, which have also shown clear carcinogenic effects in various types of bioassays in experimental animals

vehicles emit naphthalene and acenaphthene. In the case of smoking, cooking or burning (of stubble, garbage, tyres or other types of waste), a great variety of different compounds are emitted, including the ones already mentioned.

After being formed, these hydrocarbons are dispersed in the environment according to their molecular weight and climate conditions (Kozak et al. 2003). Thus, the HMW-PAHs can be adsorbed into the organic matter of the soil, water or air, whilst the LMW-PAHs will become a part of the gas phase in the atmosphere (Li et al. 2015). Both can be transported over long distances in several weeks until they are precipitated and/or degraded by solar light or microorganisms in the soil or sediments (Walgraeve et al. 2010). Along the way, they can react with different airborne

compounds, namely, sulphur oxides, nitrogen oxides or ozone, resulting in no less toxic combinations (Li et al. 2015; Walgraeve et al. 2010), like nitro-/oxy-PAHs and radicals formation.

4.2 Sources of Exposure and Health Effects

Humans and other living beings can be exposed to PAHs through inhalation or dermal/mucosa contact or mainly through water and food intake (Boada et al. 2016; Henriquez-Hernandez et al. 2017b; Hernandez et al. 2015, 2017; Luzardo et al. 2013a; Rodríguez-Hernández et al. 2015b, 2016, 2017). Inhalation is an important source in smokers and people who live near or in big cities or industrialised zones, where ten times higher concentrations of PAHs than in rural areas can be found (de la Gala Morales et al. 2015; Srogi 2007). Several authors have described higher concentration of PAHs in winter than in summer because of increased use of domestic heating (de la Gala Morales et al. 2015; Li et al. 2015; Villar-Vidal et al. 2014).

It has also been described that dermal exposure may be relevant, mainly when prolonged or continued contact with products made of petroleum derivatives occurs. Recently, the European Union, through the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation, established new restrictions about PAHs in several day-to-day stuffs made of plastics or rubber, which are in direct, prolonged or short-term repetitive contact with human skin or mucosa. These items should not contain more than 10 mg/kg of the sum of benzo(a)pyrene (BaP), benzo(e)pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene and dibenzo(a,h)anthracene, or no more than 1 mg/kg of BaP alone (ECHA 2017).

Although parental PAHs are generally inert, once in the organism, PAHs can be metabolised to be eliminated, generally via urine (LMW-PAHs) or faeces (HMW-PAHs). By the way, the process may result in active PAH metabolites (m-PAHs) capable of forming adducts with the DNA (Boada et al. 2016; Ramesh et al. 2004; Rodríguez-Hernández et al. 2015a, Hernandez et al. 2017). The biotransformation process is carried out through a series of enzymes like cytochrome P-450, which catalyses mainly oxidation, reduction and hydrolysis reactions. In vertebrates, the liver is the major contributor in the biotransformation process. However, in other organs there are cytochromes, which are able to perform this function according to the entryway (i.e. lungs, intestine or skin) (Ramesh et al. 2004). In addition, conjugation enzymes such as sulphotransferases, epoxide hydrolase, glutathione transferase and UDP-glycosyltransferase can metabolise PAHs, producing a variety of phenols, catechols, quinones and radical cations. Once they are formed, these compounds may produce adverse effects by means of various mechanisms, such as DNA damage diol-epoxides (that give place to formation of adducts), interaction with membranes and oxidative stress (Li et al. 2015; Sikkema et al. 1994; Zhang et al. 2016). Given that, some PAHs are described as carcinogenic (c-PAHs), namely, human carcinogen BaP (Group 1), whilst others are considered as probably (Group 2A) or

possibly carcinogenic (Group 2B) by the International Agency for Research on Cancer (IARC 2005). All these compounds have been described as contributing causes of breast, bladder, lung, skin or gastrointestinal cancers (Alicandro et al. 2016; Boada et al. 2015, 2016; Flesher and Lehner 2016; Korsh et al. 2015).

Often mixtures of hydrocarbons and/or their derivatives (such as nitro-PAH) are more harmful, due in part to synergistic properties. In general terms, the lower the molecular weight, the lower the carcinogenicity potential, but they are more prone to cause acute health effects, such as cardiovascular diseases (thrombosis, haemato-poietic effects), dyspnoea, asthma (Al-Daghri et al. 2014), diarrhoea, vomiting, nausea and eye, dermal or bronchial irritation or inflammation (Ramesh et al. 2004). Moreover, it is well known that some PAHs are endocrine disruptors in animals and humans. Neurological, congenital and development problems in the offspring and mothers (Jedrychowski et al. 2013; Neal et al. 2008; Oliveira et al. 2017) or immunosuppressant effects (Bolden et al. 2017; Ramesh et al. 2004) have been reported.

4.3 Biomonitoring of Polycyclic Aromatic Hydrocarbons

Given the toxicity and environmental prevalence of these compounds, the monitoring of PAHs is a relevant issue, and there is plenty of interest in control and assessment of these substances in food, environmental compartments, living beings and of course humans. Environmental monitoring of these substances is achieved by sampling and analysing samples such as air, water, food or soil (Bucchia et al. 2015; de la Gala Morales et al. 2015; García-Álvarez et al. 2014b; Hernandez et al. 2015; Kakuschke et al. 2010). Specifically, biomonitoring – the monitoring of these compounds in living beings – is usually considered the best approach as it provides a real picture of the exposure of living beings, meaning that it provides an assessment of the whole uptake through all exposure routes (Srogi 2007).

The biomonitoring of human populations may be done either by direct measurement in samples taken from study populations or extrapolating the data from the environmental exposure of other organisms (bioindicators or sentinels). This biomonitoring can be done by directly determining the individual PAHs and/or their metabolites, as well as by determining biomarkers of the effect they produce. In the case of PAHs, it is common to determine the presence of adducts of PAHs with DNA, or the detection of tetrahydroxy-PAHs that can also be measured as an indicator of tissue damage.

For reasons of practicality and ease of collection of samples, it is often considered that urinary metabolites of PAHs are better bioindicators of exposure, being considered the gold standard to determine recent exposure to a single PAH, in particular when multiple routes of exposure have to be taken into account (Jacob and Seidel 2002) or in occupational meaning (Unwin et al. 2006). The main m-PAHs that should be included in biomonitoring studies are 1-hydroxynaphthalene (1-napthol), 2-hydroxynaphthalene (2-naphthol), 1,2-dihydroxynaphthalene, 2-hydroxyfluorene (2-FLUO), 3-hydroxyfluorene (3-FLUO), 9-hydroxyfluorene

(9-FLUO), 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, 4-hydroxyphenanthrene, 9-hydroxyphenanthrene, 1-hydroxypyrene (1-PYR) and 3-hydroxybenzo(a)pyrene (3-OHBaP) (Wang et al. 2014a, b). 1-PYR has been linked to dietary exposures, whilst both 1-PYR and 2-naphthol are well correlated with smoking in a non-occupational population (Nethery et al. 2012; Srogi 2007). Urinary 3-OHBaP may be a suitable biomarker to assess BaP genotoxic exposure in humans (Marie-Desvergne et al. 2010; Oliveira et al. 2017). One decisive factor to take into account when determining urinary metabolites is sampling time, due to the high rate of biotransformation of these compounds (Cathey et al. 2018; Grova et al. 2017a, b). Taken together, those results suggest that it is better to use a combination of metabolites, since each metabolite gives an information about a single or few parental PAHs (Castano-Vinyals et al. 2004; Grova et al. 2017b; Hilton et al. 2017; Singh et al. 2008).

Other excretion routes, such as nails, hair, sweat or feathers, amongst others, have been also investigated regarding their content in m-PAHs, as a means of determining long-term exposure to these substances. In fact, some authors have pointed out that these matrices are more appropriate for the determination of HMW-PAHs (Grova et al. 2017b; Marie-Desvergne et al. 2010).

On the other hand, not only for assessing exposure but also the toxicological effect of PAHs, some other authors prefer to determine the amount of PAHs-DNA adducts in peripheral white blood cells, or their binding to plasmatic proteins, especially in occupational studies (Oliveira et al. 2017; Pleil et al. 2010). Other authors correlate the level of oxidative stress induced by PAHs as an indirect indicator of the carcinogenicity of these compounds (Singh et al. 2008). However, these studies of biomarkers have the disadvantage in that the analytical techniques are complex, have low sensitivity and do not allow deriving the global exposure to these compounds.

Finally, some authors consider that the direct measurement of PAHs in blood is the best way to estimate the total body burden and also the most realistic way to estimate exposure (Boada et al. 2015; Pleil et al. 2010). It has the disadvantage in that sampling is invasive, especially taking into account that WHO recommends that biomonitoring studies include mainly children, because it has been estimated that children aged 6–11 are the sector of the population most exposed to these compounds (Singh et al. 2013). In addition, and as we said before, it is possible to evaluate human exposure to PAHs indirectly, using bioindicator species. In these cases also blood is often the easiest sample to take, so comparison with human levels is simpler (Boada et al. 2015; Bucchia et al. 2015; Camacho et al. 2012b, 2014; Camacho et al. 2013b; García-Álvarez et al. 2014a, b; Luzardo et al. 2014).

In this sense, studies of the effects of environmental exposures on vegetables or animals can corroborate or support epidemiological studies in humans or in the environment. In these cases, the levels determined in these easy-to-sample species may reflect the exposure of a group of environmentally related species, rather than the individual exposure. Thus, the use of microbial bioindicators in order to evaluate contamination of some PAHs in agricultural soils (Niepceron et al. 2013) and in the gas and aqueous phases (Cho et al. 2014) has been reported. In the same way, moss,

lichens and plants have been used as passive phytomonitors instead of the active samplers and several studies have found promising results. In wildlife, some authors have proposed different species as possible sentinels of exposure. Some invertebrates have been studied. Amphipod (*Talitrus saltator*) appears to be a good bioindicator of this class of organic compounds in supralittoral zone (Ugolini et al. 2012). The possibility that molluscs are good bioindicators of the contamination of PAHs from the waters or sediments in mudflats of Malaysia (Tavakoly Sany et al. 2014) and mangrove oysters (*Crassostrea rhizophorae*) (Ramdine et al. 2012) has also been reported. Studies on oil spills such as those occurring on the northern Cantabrian sea and in Guanabara Bay, Brazil, respectively, concluded that barnacles are good indicators for oil spill evolution (Soares-Gomes et al. 2010; Vinas et al. 2009). Other species in the highest levels of the food chain have also been described as efficient indicators of recent pollution. Fuentes-Rios et al. (2005) determined that the cat shark is a good bioindicator for exposure to PAHs on the Chilean Pacific coast, showing good correlation with the concentration of pyrene in water and urinary 1-PYR. On Atlantic eastern coast and Mediterranean sea, several authors (Bucchia et al. 2015; Camacho et al. 2012a, 2013a, 2014, García-Álvarez et al. 2014a) investigated serum levels of PAHs in different populations of sea turtles (*Caretta caretta*) and bottlenose dolphins (*Tursiops truncata*) indicating that both species could be good indicators of local and recent pollution in the marine environment.

Since the iconic 'canary in the cage' began to be used to detect the presence of toxic gases in the coal mines, pets and other animals in the human immediate environment have been used as sentinels of human exposure to many other chemical classes. In this case, they were used as an early warning system, since the canary is more sensitive to carbon monoxide poisoning than humans and other domestic animals like cats, dogs, pigeons or rabbits. Livestock, including bees, cattle, horses, sheep and goats, can be good bioindicators for outdoor air, whilst pet cats and dogs can share the indoor air, water, food or even household dust. However, daily routine and diet, especially in people who are occupationally exposed, smokers or on some kind of diets, are confounding factors. The different metabolism and elimination capacity amongst the species should be also taken into account as confounding factors.

4.4 Pet Dogs as Sentinels forHuman Exposure to PAHs

Pet dogs are particularly interesting as sentinels for human exposure to PAH, given that they share the habitat with humans and they respond to toxic assaults similarly than their owners (Backer et al. 2001). As far as we know, there is only one research article that has assessed exposure to PAHs in dogs and humans to date (Ruiz-Suárez et al. 2016). In this study, the authors included blood samples from 87 pet dogs (46 males and 41 females, 0.5–13 years old) visiting the veterinary hospital of the Faculty of Veterinary Medicine of the University of Las Palmas de Gran Canaria (Canary Islands, Spain) for routine care. Only clinically normal animals (negative stool sample, negative result on a heartworm test and no overt disease) were included

in the study, after owners' consent. In parallel, human blood samples from 60 males and 40 females (19–34 years old) were collected from a blood bank during the same period that dogs' samples were drawn. For logistical reasons the researchers could not get blood from the owners of the same dogs included in the study. Even so it has been estimated that there are about six million domestic dogs in Spain and that more than 40% of Spanish homes have at least one dog, so the authors assumed that a high percentage of these blood donors share habitat with some dog.

In this research work the authors determined 21 PAHs, including the 13 c-PAHs and also 6 common m-PAHs (Table 4.1), by means of solid phase extraction and gas chromatography coupled to tandem triple-quadrupole mass spectrometry. In this research the authors detected the totality of the PAHs and m-PAHs in any of the samples, both in humans and dogs, with the only exception of benzo(a)pyrene, which was not detected in none of the dog plasma samples.

The compounds most frequently detected in both species were phenanthrene, fluorene and fluoranthene and 2-naphthol, which were present in nearly 100% of the samples. The frequencies of detection of the rest of the compounds of this chemical group were highly variable and different between the two species (Table 4.1). The mean values of ∑PAH21 were much lower in dogs than in humans (782.2 vs. 1623.3 ng/g lw, respectively). Regarding the c-PAHs, the authors considered only seven compounds (PAH7, benzo(a)anthracene, chrysene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene and indeno(1,2,3-c,d)pyrene), and the mean values were also much lower in dogs than in humans (6.8 vs. 21.9 ng/g lw, respectively). On the opposite, according to the authors' results, it seems that dogs may have a higher capacity of biotransformation of these compounds, because in parallel to the lower levels of untransformed PAHs, dogs also had higher levels of PAHs metabolites than humans, in whom the relationship was inverse (∑m-PAH = 198.1 and 131.6 ng/g lw in dogs and humans, respectively; $p < 0.0001$).

The importance of the employment of sentinel species for the assessment of human exposure to chemicals has been widely demonstrated for many chemical classes, since the sentinel species may reflect the actual human exposure of a given population, much more accurately than the comparison to other remote populations. However, it does not seem to be the case of pet dogs as sentinels of human exposure to PAHs, because the authors of the only study available in this regard found that there were many significant differences between these two species (Fig. 4.2), both in the levels of many parental compounds and in their metabolites. These results suggest that exposure of both species to this contaminant group could be different, but also may be indicating that dogs have a higher capacity to metabolise these compounds than humans. Obviously, to confirm this point, additional research is needed, but these results allowed the authors to hypothesise that the lower levels of PAHs detected in the plasma of dogs could be due to a higher rate of biotransformation and elimination thereof. Furthermore, as shown in Fig. 4.2c, neither the profiles of PAHs contamination were similar between dogs and humans, with a clear predominance of the four-ring compounds in humans and three-ring compounds in dogs. In fact, it is noteworthy that some compounds such as pyrene, which was

Table 4.1 Concentrations of individual PAHs and PAHs metabolites concentrations (ng/g lw) in dog (n = 87) and human (n = 100) serum samples from the Canary Islands, Spain

	Dog serum		Human serum		
	Mean ± SD	Freq. (%)	Mean ± SD	Freq. (%)	P^{*}
Benzo(a)anthracene	4.2 ± 5.1	12.6	6.0 ± 17.9	12.0	
Benzo(a)phenanthrene (chrysene)	5.4 ± 7.3	10.3	4.4 ± 17.1	13.0	
Benzo(a)pyrene	n.d.	0.0	4.4 ± 8.5	8.0	
Benzo(b)fluoranthene	4.6 ± 5.4	6.9	4.7 ± 9.2	10.0	
Benzo(k)fluoranthene	4.3 ± 6.9	11.9	17.9 ± 34.1	36.0	0.0015**
Dibenzo(a,h)anthracene	4.9 ± 6.1	2.3	4.43 ± 7.8	6.0	
Indeno(1,2,3-cd)pyrene	5.2 ± 7.4	2.3	5.2 ± 4.4	3.0	
Benzo(j)fluoranthene	4.5 ± 5.6	7.9	11.7 ± 3.7	14.0	0.0056**
Benzo(j,k)fluorene (fluoranthene)	6.6 ± 4.3	97.7	77.5 ± 26.4	99.0	<0.0001***
Dibenzo(a,e)pyrene	5.2 ± 5.6	1.2	4.3 ± 5.3	5.0	
Dibenzo(a,h)pyrene	6.3 ± 7.4	2.3	4.5 ± 5.8	6.0	
Dibenzo(a,l)pyrene	4.4 ± 6.1	1.2	6.2 ± 5.9	5.0	
5-Methylchrysene	6.3 ± 6.1	12.6	7.6 ± 17.9	14.0	
Acenaphthene	7.6 ± 25.5	13.8	8.7 ± 19. 2	16.0	
Acenaphtylene	51.2 ± 34.4	75.8	12.5 ± 6.3	6.0	<0.0001***
Anthracene	4.7 ± 26.2	4.6	6.8 ± 34.1	10.0	
Benzo(ghi)perylene	n.d.	0.0	4.5 ± 5.2	3.0	
Fluorene	76.9 ± 42.5	98.8	42.5 ± 17.2	98.0	<0.0001***
Phenanthrene	382.5 ± 0.21	100.0	313.3 ± 137.5	100.0	<0.0001***
Pyrene	7.6 ± 17.2	17.2	43.7 ± 25.9	94.0	<0.0001***
Naphtalene	34.1 ± 51.6	28.7	34.1 ± 59.6	41.0	
1-Naphthol	76.9 ± 69.8	79.3	5.2 ± 17.2	8.0	<0.0001***
2-Naphthol	95.1 ± 52.6	96.6	67.2 ± 23.5	98.0	<0.0001***
2-OH-Fluorene	5.8 ± 8.5	11.5	7.9 ± 12.3	21.0	0.1623
1-OH-Phenanthrene	17.4 ± 35.6	23.5	4.2 ± 6.6	10.0	0.0459*
7-OH-Benzo(c)fluorene	51.4 ± 8.5	36.8	4.5 ± 8.5	12.0	0.0089**
1-OH-Pyrene	4.5 ± 17.2	14.9	17.0 ± 43.5	6.0	0.4185
∑PAH7[a]	6.8 ± 17.2	53.2	21.9 ± 43.5	78.0	0.01**
∑PAH21[b]	782.2 ± 323.8	100.0	1623.5 ± 799.2	100.0	<0.0001***
∑m-PAH[c]	198.1 ± 110.5	100.0	131.6 ± 148.5	100.0	<0.0001***

P^{*}: Mann-Whitney U test
[a]∑PAH7, sum of carcinogenic PAHs
[b]∑PAH21, sum of 21 priority PAHs
[c]∑m-PAH, sum of 6 PAH metabolites

detected in almost 100% of the human samples, were barely detectable in 17% of samples from dogs, and yet, others such as acenaphthylene or 1-naphthol were much more frequently detected in dog plasma than in human plasma. Thus, in the light of the above, the authors concluded that the pet dogs do not seem to be good sentinels for human exposure to PAHs.

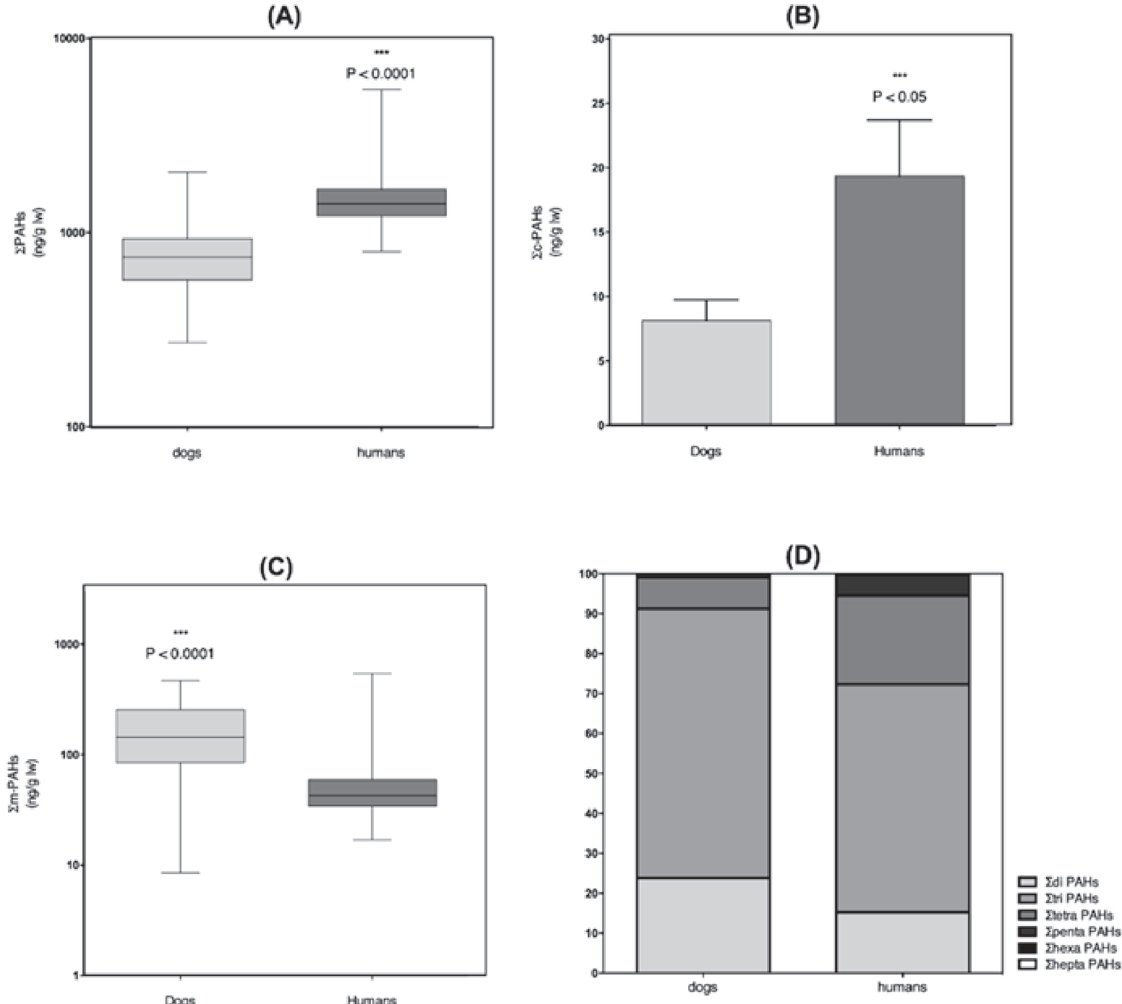

Fig. 4.2 Levels of PAHs in plasma samples. (**a**) (main body). Box plots of ∑PAH21 in dogs and humans. (**a**) (inset). Bar graph of ∑PAH7 (carcinogenic PAHs, median and interquartile range) in dogs and humans. (**b**) Box plots of ∑PAH metabolites in dog and humans. (**c**) Profile of distribution of PAHs in dogs and humans. 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. (**d**) distribution profile of PAH in dogs and humans

4.5 Pet Cats as Sentinels for Human Exposure to PAHs

As far as we know, there is no published study that explored the role of domestic cats as sentinels of human exposure to PAHs. However, the cat that lives inside the house is usually considered a good bioindicator, even better than the dog, to assess the exposure of man to the contaminants present in the domestic environment. This is mainly due to their grooming habits, which cause cats to ingest high amounts of household dust, with all the load of contaminants associated with it. Thus, in different publications, it has been indicated that this pet is ideal for evaluating human exposure to different kinds of contaminants. (Bost et al. 2016; Chow et al. 2015; Dirtu et al. 2013; Henriquez-Hernandez et al. 2017a). In addition, other studies have

shown that dietary exposure to different contaminants (including PAHs) is different between dogs and cats (Ruiz-Suarez et al. 2015), so although, as we said earlier, dogs do not seem to be good sentinels of human exposure to PAHs, cats present differential facts that could make them suitable for this purpose, so this possibility is worth investigating.

With the purpose of completing the information in this chapter, we decided to shed light on the question whether or not cats would be good sentinels of human exposure to PAHs. For this, we collected venous blood from a total of 25 cats that were recently admitted for routine health check-ups and vaccination in the clinical hospital of the Faculty of Veterinary Medicine of the University of Las Palmas de Gran Canaria. In parallel, blood was collected from 25 volunteers from the same faculty, from amongst the staff and the students of the same, all of them owners of cats (although not from the same cats participating in the study). The serum was obtained, and the PAHs were extracted by solid phase extraction following the procedure described elsewhere (Camacho et al. 2012a). In this work, we included only the 16 priority PAHs for the USEPA, whose analysis was performed by gas chromatography coupled to tandem triple-quadrupole mass spectrometry (Luzardo et al. 2013b). All human volunteers and cat owners provided their written informed consent to participate in this study.

We found only 8 out of the 16 compounds analysed both in humans and cats. In addition, acenaphthylene was also detected in cats, but not in humans. The summary of the results of this study is shown in Table 4.2. As it can be seen, the most frequently detected compounds were acenaphthene, phenanthrene and fluorene, with frequencies of 90% or more in both species. For the rest of the substances, the detection percentage between both species was highly variable. We want to highlight the differences found between cats and humans for chrysene and fluoranthene (percentages of detection of 18.2 vs. 90% and 31.3 vs. 100%, respectively). The median of the $\sum$16PAHs was similar in both species (1.93 vs. 2.08 ng/mL or 232 vs. 257 ng/g lw, respectively). However, although the total concentrations do not show significant differences between both species, when we focus on carcinogenic compounds for EFSA, the outlook changes radically, since these compounds were practically undetectable in the group of cats, whilst they were present in the group of cat owners (Fig. 4.3). Obviously, this is only a preliminary study, and the conclusions that derive from it should be taken with caution because of the low sample size. However, based on the results obtained, it could not be considered that the cat is the ideal sentinel to assess human exposure to PAHs, although it does seem to be better than dogs in this sense.

4.6 Conclusions

Based on the scarce existing bibliography and limitations of the study, it can be concluded that pet dogs and cats are not good sentinels of human exposure to PAHs. The analyses of parental compounds and metabolites in serum and their concentra-

Table 4.2 Concentrations of polycyclic aromatic hydrocarbons (ng/mL) in the whole series of cats (n = 22) and humans (n = 20)

Congener	Pet cats			Humans			P^a
	% detection	Median	p25th–p75th	% detection	Median	p25th–p75th	
Acenaphthylene	63.6	0.02	0.00–0.04	0	–	–	0.003
Acenaphthene	100.0	0.56	0.42–0.99	100.0	0.52	0.29–0.79	ns
Anthracene	31.0	0.00	0.00–0.07	80.0	0.13	0.05–0.20	0.008
Benzo(a)anthracene	0	–	–	0	–	–	na
Benzo(a)pyrene	0	–	–	0	–	–	na
Benzo(b) fluoranthene	0	–	–	0	–	–	na
Benzo(ghi)perylene	0	–	–	0	–	–	na
Benzo(k) fluoranthene	0	–	–	0	–	–	na
Chrysene	18.2	0.00	0.00–0.00	90.0	0.03	0.01–0.04	0.003
Dibenzo(ah) anthracene	0	–	–	0	–	–	na
Fluoranthene	31.8	0.00	0.00–0.03	100.0	0.05	0.03–0.07	0.001
Fluorene	100.0	0.16	0.09–0.41	90.0	0.12	0.03–0.21	ns
Indeno(123,cd) pyrene	0	–	–	0	–	–	na
Naphthalene	18.2	0.00	0.00–0.00	30.0	0.00	0.00–0.07	ns
Phenanthrene	100.0	1.16	0.57–2.30	100.0	1.22	0.68–1.44	ns
Pyrene	13.2	0.00	0.00–0.00	60.0	0.01	0.00–0.04	0.047

Abbreviations: *p25th–p75th* percentiles 25 and 75 of the distribution, *ns* non-significant, *na* not applicable
[a]Mann-Whitney U test

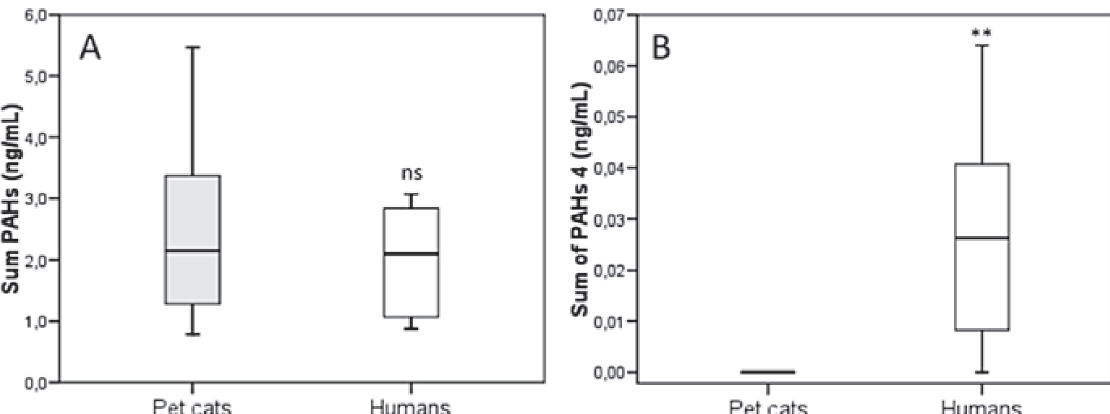

Fig. 4.3 Box plot showing the serum levels of sum of all PAHs (panel A) and sum of PAHs 4 (panel B), amongst cats (n = 22) and humans (n = 20). Sum PAHs included all the 16 congeners analysed. Sum of PAHs 4 included only benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene. P values were calculated with Mann-Whitney U test. The lines connect the medians, the boxes cover the 25th to 75th percentiles, and the minimal and maximal values are shown by the ends of the bars. Abbreviations: ns, non-significant. $**$, p = 0.003

tions and contamination profiles are not comparable between species. These results could indicate that different sources of exposure, such as smoking, occupational setting or food intake, in humans exist. In the analysis of PAH metabolites, higher levels in dogs suggest that they metabolise them more effectively than humans. Despite sharing a home and in some cases diet with humans, pets differ greatly from humans to consider them good sentinels for PAHs exposure.

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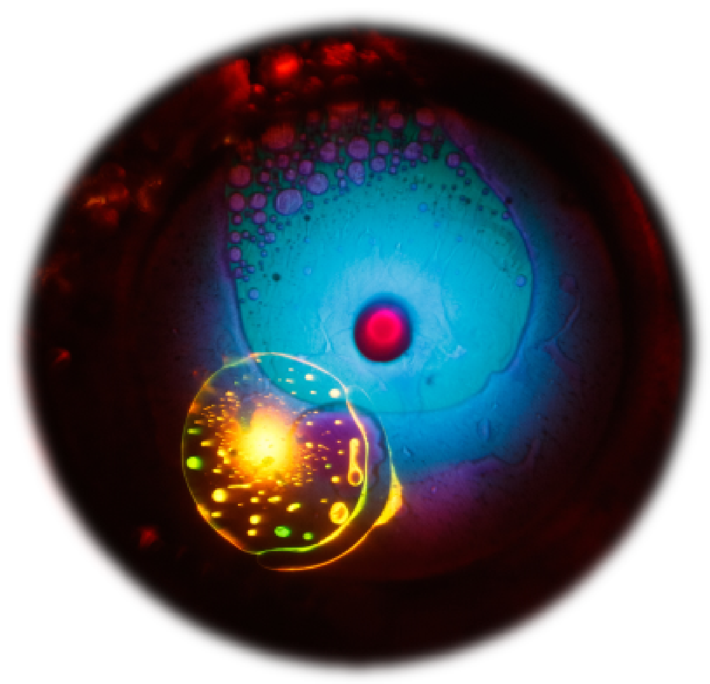
Publicación 5. Intensive livestock farming as a major determinant of the exposure to anticoagulant rodenticides in raptors of the Canary Islands (Spain)

La ganadería intensiva como principal determinante de la exposición a raticidas anticoagulantes en rapaces de Canarias (España)

Science of the Total Environment, 2021, 768: 144386

DOI: https://doi.org/10.1016/j.scitotenv.2020.14438



Science of the Total Environment 768 (2021) 144386

Contents lists available at ScienceDirect

Science of the Total Environment

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

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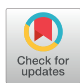

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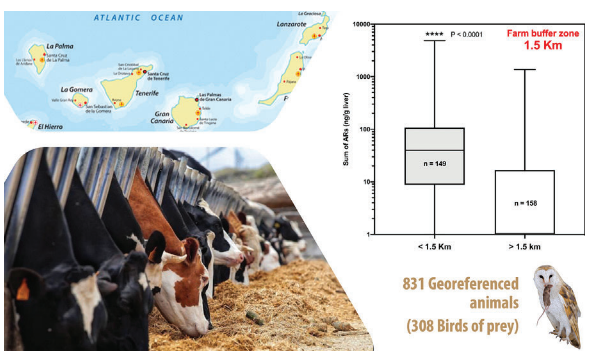
HIGHLIGHTS

- Exposure to AR in reptiles, mammals and non-raptor birds is mainly related to intentional poisoning.
- AR exposure appears as a threat for the conservation of the critically endangered Canary raven.
- Incidence of AR in raptors almost reach 60%, with the highest values detected in common kestrels
- First report of AR residues in Barbary Falcon and Eleonora's Falcon
- Raptors that live near livestock intensive farms are significantly more exposed to AR.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 September 2020
Received in revised form 18 November 2020
Accepted 4 December 2020
Available online 25 December 2020

Editor: Dimitra A Lambropoulou

Keywords:
Brodifacoum
Bromadiolone
Falco tinnunculus
Common buzzard

ABSTRACT

The Canary Islands (Spain) is a biodiversity hotspot, with more than 4500 registered endemic species. However, it is subject to high anthropogenic pressure that threatens its wildlife in various ways. In the context of forensic toxicological surveys, the presence of anticoagulant rodenticides (AR) has been investigated in the liver of 831 animal carcasses with georeferenced data from 2011 to May 2020. The high concentrations of toxic pesticides in carcasses and in baits found close to the corpses indicated that all the reptiles and most of the mammals tested positive for AR were intentionally poisoned, although mainly by other substances. The frequency of detection of AR in non-raptor birds ($n = 343$) was only 4.1%, being the Canary raven the most frequently affected species (7/97, 7.2%). On the contrary, in raptors ($n = 308$) the detection frequency was almost 60%, with an average of more than 2 ARs per animal. The highest concentrations were found in the common kestrel. We present for the first-time results of AR contamination in two species of raptors that are very rare in Europe, Eleonora's falcon ($n = 4$) and Barbary falcon ($n = 13$). The temporal trend of positive cases remains stable, but since the entry into force of the restriction to the concentration of the active ingredient in baits (<30 ppm), a decrease in the

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https://doi.org/10.1016/j.scitotenv.2020.144386
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Egyptian vulture
Owl

concentrations of these compounds in the raptors' liver has been detected. Conversely, we registered an increase in the number of ARs per animal. From the study of the geographic information system (GIS) it can be deduced that intensive livestock farms are an important determinant in the exposure of raptors to ARs. Those birds that have their territory near intensive production farms have higher levels of exposure than those of birds that live far from such facilities.

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1. Introduction

The annual use of anticoagulant rodenticides (AR) for rodents' control is estimated in thousands of tons (Hong et al., 2019). Most rodenticides belong to the group of anticoagulants, of which eight are EU-approved active ingredients (brodifacoum, bromadiolone, chlorophacinone, coumatetralyl, difenacoum, difethialone, flocoumafen and warfarin) (EC, 2017). These highly effective products interfere with the correct synthesis of the vitamin K-dependent clotting factors, thereby causing the rodents' death due to fatal bleeding (Watt et al., 2005). The second-generation products (SGARs; bromadiolone, difenacoum, brodifacoum, flocoumafen and difethialone) are highly resistant to degradation, reaching plasma half-lives of up to 91.7 days, and liver half-lives of up to 307.4 days, after a single exposure (Nakayama et al., 2019). After the exposure to SGARs, the exploratory behavior pattern of rodents changes, which results in a widening of its radius of action thus increasing the chances of being preyed upon (Frankova et al., 2019; Simon et al., 1994). All this leads to unintentional exposure to ARs in non-target wildlife, which has been documented in numerous reports around the world, many of them relating population declines to this exposure, especially in raptors (Nakayama et al., 2019). This threat was dramatically reflected in the article "*Killing rats is killing birds*", which had a substantial impact on public opinion (Lovett, 2012). However, almost ten years later, the threat continues, and the reported incidences in non-target species have barely diminished (Lohr and Davis, 2018; Lopez-Perea et al., 2015; Lopez-Perea et al., 2019; Nakayama et al., 2019; Seljetun et al., 2019). In 2014, our research group already presented results of a preliminary study in raptors of the Canary Islands (Ruiz-Suarez et al., 2014). In those findings we reported a frequency of ARs of around 61%, although with a high variability among species, which is a similar percentage to those reported in raptors from other regions of Spain (Lopez-Perea et al., 2019), and from the rest of the world (Nakayama et al., 2019).

It would seem logical to think that for wildlife exposed to these ARs, these animals must live in an area relatively close to the sites where these compounds are applied (Geduhn et al., 2016; Lopez-Perea et al., 2019). This has recently been demonstrated in areas of high human population and livestock densities (Alabau et al., 2020; Lopez-Perea et al., 2019). In the case of livestock farming (intensive confinement, in particular) it is especially striking, since it is estimated that in some areas >90% of them suffer from rat and mouse infestations (Endepols et al., 2003) because of its easy availability of food and shelter. The proliferation of rodents in these facilities provides excellent feeding opportunities for numerous species of opportunistic predators, which take advantage of anthropogenic environments, settling around farms, agricultural facilities and urban environments (Lopez-Perea et al., 2019). Nevertheless, on-farm application of rodenticides is often carried out irregularly, by personnel with little or no training at all in the application of pesticide products (in particular, on medium and small farms). The baiting points are often placed quite arbitrarily, and distributed based on subjective criteria, such as random detection of rodent activity (Endepols et al., 2003). In fact, this self-application of rodenticides by farmers is even facilitated by government agencies, given freely or subsidized, as part of the strategies to stimulate and support the livestock sector (Hong et al., 2019). Generally, a professional rodenticide service is only available in larger facilities. Partly due to the reasons outlined above, ARs have been pointed out by Lopez-Perea et al. (2019) as facilitators of what have been called "ecological traps" (Battin, 2004). Therefore, a habitat chosen by animals based on their chances of greater survival and better reproductive success may become a wrong option if an adverse environmental factor, such as the continued presence of ARs, is introduced (Battin, 2004; Lopez-Perea et al., 2019).

In the Canary Islands, the livestock sector is structured in two types of production: intensive livestock farming and traditional livestock farming. Intensive livestock farming, mainly dedicated to cattle, pigs and poultry, is normally found in the coastal strip of the islands, with a clear entrepreneurial character and higher technological level. Traditional livestock farms, mainly devoted to goats and sheep, are mostly located in the midlands, and characterized by a limited number of animals, whose diet is directly linked to agricultural activity and local fodder resources. Since 2010, our research group has received wildlife specimens from all the islands of the archipelago for the diagnosis of possible poisonings (more than 1300 animals recorded to date). Following the progressive implementation of the Poisoning Control and Prevention Strategy in the Canaries (BOC, 2014), the number of georeferenced incidents received has increased significantly. This fact allows us to locate with greater precision the habitat of these specimens and study the possible relationships with potential sources of exposure to chemicals and other stressors. Thus, the main objective of this research has been to investigate whether the presence of livestock activity, both intensive and traditional, is related to exposure to ARs in non-target wildlife.

2. Material and methods

2.1. Geographic context, sampling and ethical statement

This study was carried out in the Canary Islands, one of the so-called Outermost Regions of the European Union. This Spanish archipelago, located in the Atlantic Ocean off the northwest coast of Africa (between the coordinates 27° 37′ and 29° 25′ north latitude and 13° 20′ and 18° 10′ west longitude) consists of 8 inhabited islands and several uninhabited islets, all of volcanic origin. The archipelago occupies a land area of 7447 km^2 (20.4% of a total area of 36,567 km^2). The islands are home to marine and terrestrial ecosystems of great value, both ecologically and in terms of landscape.

Due to the evolutionary isolation and climate characteristics, the biota of the archipelago has evolved differently from that of the nearby continents (Africa and Europe), giving rise to high levels of endemism. According to the updated data, in the Canary Islands there are almost 4500 endemic species, which represents more than 27% of the total biodiversity registered (https://www.biodiversidadcanarias.es/biota/). At present, the archipelago has a stable population of 2.1 million inhabitants and receives more than of 12 million tourists per year, putting enormous pressure on its ecosystems. However, the distribution of this anthropogenic pressure on the territory is irregular, since large environmentally protected areas have been recognized by UNESCO and governmental authorities in this archipelago (4 national parks, 11 natural parks, 7 biosphere reserves, 43 terrestrial Special Protection Areas (SPAs), 11 marine SPAs, and 177 Special Areas of Conservation (SACs)). In total, around of 40% of the islands' surface is protected. Thus, there are densely populated areas with population centers of up to 3700 inhabitants/km^2 (usually located on the coastal strip), and

other municipalities with densities as low as 7 inhabitants/km^2 (Godenau et al., 2012).

In the context of the Poisoning Control and Prevention Strategy in the Canaries (BOC, 2014), from 2011 to May 2020, the ULPGC Toxicology Laboratory received more than 1300 animal bodies as well as baits and other sample types (containers, soil, plants, etc.…) for forensic toxicological evaluations. Of these, 831 animals had georeferenced information about the place where they were found and furthermore, the good state of conservation of the animals allowed the sampling of the liver. Additionally, 78 georeferenced baits were also received. Among the georeferenced animals received, 308 birds of prey stand out, as these were the object of a detailed geographic information system (GIS) analysis in the second part of this study. The series of raptors included individuals from 13 different species/subspecies: *Accipiter nisus granti* (n = 9); *Asio otus canariensis* (n = 68); *Buteo buteo insularum* (n = 53); *Circus aeruginosus* (n = 1); *Falco eleonorae* (n = 4); *Falco peregrinus pelegrinoides* (n = 13); *Falco subbuteo* (n = 1); *Falco tinnunculus canariensis* (n = 69); *Falco tinnunculus dacotiae* (n = 14); *Neophron percnopterus majorensis* (n = 67); *Pandion haliaetus* (n = 1); *Tyto alba alba* (n = 5); and *Tyto alba gracilirostris* (n = 3). The animals (and baits) were sent by environmental officers or patrols if found dead, or by wildlife recovery centers if they had been admitted alive but euthanized or death within a week of admission. All carcasses were kept frozen at −20 °C, until they were necropsied. No animals were sacrificed for the purpose of this study. Whenever possible, the cause of death was determined by clinical examination of the animals, supported by hematological, clinical-pathological, radiological or toxicological analyses, when required and performed by veterinarians at wildlife recovery centers, or by veterinarians assigned to our research center. The main cause of death of the animals, included in this study, was trauma (62,3% including mainly run over, collision with power lines or wind turbines, shooting, and falling from the nest), as well as intentional or unintentional poisoning (26.4%). The livers, as the main organ for accumulation and storage of rodenticides, were used for this study, (Dowding et al., 2010). Obtained during the necropsy, they were kept frozen at −20 °C until the preparation of the extraction and chemical analysis.

2.2. Analysis of anticoagulant rodenticides in the liver

Solvents (acetonitrile and methanol) of the highest purity available were used (>99. 9%, Honeywell, Morristown, NJ, USA). Ultrapure (UP) water was produced in the laboratory using a Gradient A10 Milli-Q System (Millipore, Molsheim, France). Standards for ARs and a procedural-internal standard (P-IS, (±)-Warfarin-d5) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). All standards were pure compounds (purity from 98% to 99.5%). Stock solutions of each compound at 1 mg/ml were prepared in acetonitrile and stored at −20 °C. From a mixture containing all the rodenticides at 10 μg ml^{-1} each in acetonitrile (ACN), diluted solutions from 0.1 ng/ml to 500 ng/ml were prepared and used for calibration curves.

During the period covered by this study, two different methods were used for the analysis of rodenticides, because at the end of 2015, there was a total renewal of the equipment of our research unit. For this reason, both methods are presented:

2.2.1. Sample preparation and chemical analysis (January 2011 to November 2015)

Briefly, 2 g of liver were homogenized with 5 ml of ultrapure water, and 10 μl of the P-IS (50 μg/ml in acetonitrile) were added. Then, 10 g of diatomaceous earth and 10 ml of dichloromethane/ethyl acetate/acetone mixture (50/30/20, v/v/v) were added and vigorously agitated for 5 min. Then, the sample was centrifuged at 4000*g*, the supernatant was subjected to evaporation under a nitrogen stream, redissolved in acetonitrile, filtered (0.2 μm) and subjected to a purification stage consisting of three consecutive freezing and centrifugation steps (mainly for fat removal). The resulting supernatant was used for the quantitative determination of AR, which was performed using a Thermo LC-MS/MS Accela Ultra instrument (Thermo Fisher Scientific Inc., USA). The detailed procedure can be found in Luzardo et al. (2014).

2.2.2. Sample preparation and chemical analysis (December 2015 to May 2020)

For the extraction, 1 g of liver was homogenized with 4 ml of ultrapure water, and from this homogenate 1 ml was taken, to which 10 μl of the P-IS were added. In this method a matrix-matched calibration curve was used, so the matrix (chicken or beef liver tested negative for the analytes of interest) was prepared in the same way, but the 1-ml aliquots were fortified to 12 increasing concentrations of the ARs mixture (0.1 to 50 ng/ml). After this, 2 ml of acidified acetonitrile (0,5% formic acid) were added, shaken vigorously (30 sg), and sonicated for 20 min. After this time, 480 mg of anhydrous magnesium sulphate and 120 mg of sodium acetate were added to each tube and shaken vigorously again (90 sg). The samples were centrifuged at 4200*g* for 5 min at 2 °C and the supernatant was collected and filtered through 0.2 μm (Chromafil PET-20/15, Macherey-Nagel, Düren, Germany) to be used directly for chromatographic analysis, without any additional purification steps. An Agilent 1290 UHPLC (Agilent Technologies, Palo Alto, USA) coupled to an Agilent 6460 triple-quadrupole mass spectrometer was used to separate and detect the analytes. The method was fully validated for liver tissue following the strict recommendations of the guide of Standard Practices for Method Validation in Forensic Toxicology (SWGTOX, 2013). Recoveries were between 80 and 120% for all the analytes. Chromatographic and acquisition conditions and basic procedural details can be found in Rial-Berriel et al. (2020).

2.3. Geospatial analysis of the data (GIS analysis)

The free and open source geographical information system software QGIS Desktop (version 3.12) was used for the spatial analysis of the data. The images were orthorectified and projected to the UTM 28 N zone based on the WGS84 Geographic Coordinates System. The base map for the location of the farms and the positive/negative raptor cases for AR was the default topographic map of the software (OpenStreetMap), on which the rest of the layers created from the geocoded data were superimposed. The data about the place where the carcass was found were collected by Canary Islands environmental patrols and obtained by GPS tracking. From these data, two vector layers were created, one for animals that tested positive for some AR residue, and another for those that tested negative. The geocoded data of the livestock farms were obtained from the metadata catalogue of the Department of Spatial Data Infrastructure of the Canary Islands Government (https://catalogo.idecanarias.es/geonetwork), and from the register of Canary Islands livestock farms, in order to find out the species farmed, the number of heads and type of farm (intensive or extensive). Due to the lack of data, poultry farms were not included. Regarding the other farms, a selection was made considering only the medium to large size holdings (more than 15 head of cattle or pigs, or more than 40 head of goats and/or sheep). From these data, 3 vectorial layers were created, differentiating each type of livestock: cattle, pigs and goats/sheep. For each of these layers, additional layers were created using the Buffer geoprocessing tool, in order to show the potential areas of influence. Four potential radii (0.5, 1, 1.5 and 2 km) were explored based on the described home range areas for the main species of raptors that inhabit the archipelago (Casagrande et al., 2008; Garcia-Heras et al., 2013; Kenward et al., 2018; Martínez and Zuberogoitia, 2004; Thomsen et al., 2014). Using the vector analysis tools of QGIS, a distance matrix between farms and cases was created. From this matrix, the shortest distance from the place where each carcass was found to the nearest farm was identified. Furthermore, the number of inhabitants (as of 1 January 2017) in the radius of 1.5 km^2 around each specimen was also recorded using the plug-in for QGIS available on the Canary Islands

Institute of Statistics website (https://plugins.qgis.org/plugins/istacqgis/) as well as the density of inhabitants per km^2 of the municipality in which each incident was recorded.

2.4. Statistical analyses

All the statistical analyses were done using GraphPad Prism v8.0 (GraphPad Software, CA, USA). The distribution of the variables included in this study was evaluated through Kolmogorov-Smirnov test. The concentration of the ARs and distances included in this study did not follow a normal distribution; therefore, the results, apart from the mean ± SD, are expressed in terms of the median and range. We employed the Levene's test to check the equality of variance among groups. This was important to know whether to employ nonparametric or parametric tests. The Levene's test was non-significant in all cases ($P > 0.05$), meaning that the requirement of homogeneity was met. Therefore, we preferred to test the statistical differences among groups using nonparametric tests, since these assess the median rather than the mean, which is appropriate given the relatively high number of non-detected values in some groups. Thus, Kruskal-Wallis and Mann-Whitney *U* tests were employed for general and pair-wise comparisons, respectively. However, as an additional check, the pair-wise comparisons were also done using the Student's *t*-test after log transformation of the data. For clarity we show the graphs and the results of nonparametric analyses in the main body of the article, and only one example of the analysis with transformed data in the supplementary material. P value of less than 0.05 (two-tailed) was considered to be statistically significant. Spearman's rank correlation coefficient was employed to study the statistical dependence between the ranking of minor-distance-to-farm and $\sum$AR of each animal, either for the whole series or for each of the species with more than 50 individuals separately.

The prevalence of AR exposure for each species was calculated as the percentage of animals with, at least, one residue detected in the liver. The variable response considered for the comparisons was the presence of $\sum$ARs in the liver (0/1). In the analyses of the positive cases, the use of a cut-off value for $\sum$ARs of 200 ng/g was also considered, since this value has been proposed as the threshold for serious toxic effects of ARs in some species (Thomas et al., 2011).

3. Results and discussion

3.1. Incidence of anticoagulant rodenticides in non-target animals of the Canary Islands: reptiles, mammals and non-raptor birds

3.1.1. Reptiles

It is striking that the highest levels of AR were found in reptiles, and that the only residue detected in these animals was brodifacoum. The possible reason for these high levels was that, according the forensic evidences collected from the cases, all the reptiles that tested positive ($n = 23$, Table 1) were the target of intentional poisoning. High concentrations of brodifacoum were detected in baits found close to the carcasses in 4 incidents (involving 18 out of the 23 lizards). However, none of these reptiles seemed to die as consequence of the exposure to brodifacoum, whose concentrations were much lower than those of other toxic chemicals also found in the carcasses and baits (the banned carbamates methomyl, carbofuran and aldicarb). All the positive reptiles were species of giant lizards, endemic to the Canary Islands (*Gallotia* spp.). Despite being protected species, these lizards are unwanted by a great number of farmers as they, in their search for water, eat the cultivated vegetables. Although the placement of drinking troughs would be the most effective way to minimize the damage they cause, these farmers consider their removal as more effective, which has put all these species in critical danger of extinction (BOE, 2010).

3.1.2. Mammals

After lizards, the next class in which we find the highest levels of ARs are mammals, with high or very high levels of brodifacoum, bromadiolone and difenacoum detected in many of the animals. It should be noted that, there are few species of wild mammals in the Canary Islands, they are small in size (shrews (2 species) and bats (7 species)), and on very rare occasions they are referred to our laboratory service for diagnosis of poisoning. Thus, the big majority of mammals that we have analyzed are dogs (mainly hunting or surveillance dogs), Moorish hedgehogs and feral cats. Most of them had also been target of intentional poisoning, in which rodenticides were part of a cocktail of substances that usually includes agricultural insecticides (carbofuran, aldicarb, chlorpyrifos…), as previously reported (Ruiz-Suarez et al., 2015). Just as we did with the lizards, we also analyzed numerous baits collected in connection with these incidents, and ARs were detected in 8 of them. In fact, in 19.2% of the baits analyzed in this research, ARs were detected.

The pattern exposure to ARs in the Canary Islands is mainly linked to the intentionality in poisonings, which coincides with what has been also described in this archipelago for other pesticides (Ruiz-Suarez et al., 2015), and for other non-anticoagulant rodenticides elsewhere (Bishop et al., 2016). However, this pattern differs considerably from that described by other authors for reptiles (Elliott et al., 2014; Johnston et al., 2005; Nakayama et al., 2019) and mammals (Alabau et al., 2020; Nakayama et al., 2019), and moreover, from the main objective of this work that focuses on secondary exposure, we decided to discard the specimens of these two classes, as well as the baits, for further analyses in this research.

3.1.3. Non-raptor birds

First, we compared the frequency, the concentrations and the number of ARs found in nonpredatory birds ($n = 343$) versus those of raptors ($n = 308$), and we found big differences between them (Fig. 1). ARs were detected in 4.7% of nonpredatory birds ($n = 16$) compared to almost 60% of raptors ($n = 176$), which also almost quadrupled the median concentration value of $\sum$ARs (Fig. 1). Moreover, the mean number ARs per individual differs significantly between both types of birds (2.15 vs. 1.1 in raptors and non-raptors, respectively, $P < 0.001$). These results contrast with the data reported by other authors with much higher frequencies of AR detection in non-raptor birds, even exceeding 50% of the animals analyzed (Eason et al., 2002; Lopez-Perea et al., 2019; Nakayama et al., 2019; Sanchez-Barbudo et al., 2012) but resemble those reported by other authors, mainly in waterbird studies (Lambert et al., 2007).

Table 1
Incidence and concentrations in liver of the anticoagulant rodenticides detected during investigation of the crimes against wildlife flora and fauna in the Canary Islands (2011–2020).

	Mammals (n = 118)			Reptiles (n = 62)			Birds (non-raptors) (n = 343)			Birds (raptors) (n = 308)			Baits and other evidences (n = 78)		
	Freq (%)	Med (ng/g)	Range	Freq (%)	Med (ng/g)	Range	Freq (%)	Med (ng/g)	Range	Freq (%)	Med (ng/g)	Range	Freq (%)	Med (ng/g)	Range
Brodifacoum	7.1	108.7	8.7–1181	62.7	526.3	118.3–934.2	3.9	4.4	1.1–65.7	48.7	23.8	0.2–1174	6.4	15,409	880–149,877
Bromadiolone	10.1	182.1	2.1–1571	–	–	–	2.5	13.6	0.9–32.1	41.3	30.6	0.2–4840	8.9	9900	317–15,540
Difenacoum	2.0	158.0	4.5–311.5	–	–	–	1.1	23.0	1.7–56.0	17.8	3.9	0.1–73.4	5.1	1465	730–21,567
Difetialone	1.0	1.3	–	–	–	–	–	–	–	1.4	1.8	0.5–29.2	–	–	–
Flocoumafen	–	–	–	–	–	–	–	–	–	3.9	0.9	0.2–127.7	–	–	–

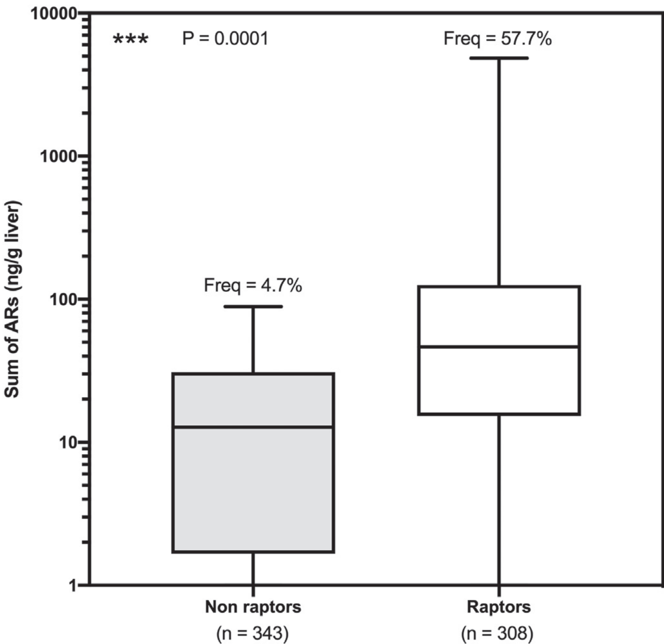


Fig. 1. Box and whiskers graph showing the comparison of anticoagulant rodenticides in the liver between wild birds (343 non-raptors vs. 308 raptors) (2011—2020). The lines show the medians, the boxes cover the 25th to 75th percentiles, and the minimal and maximal values are shown by the ends of the bars. The frequencies of positive cases are indicated for each group.

Among the non-predatory birds, the most affected was the Canary raven (*Corvus corax canariensis*, n = 7). Even so, in relation to the total number of individuals of this species that we have analyzed, the frequency of detection was low (7/97, 7.2%), and it does not coincide with what has been published by other authors who indicate that the common raven is one of the most vulnerable species to both primary and secondary AR exposure (Howald et al., 1999). This could be due to the fact that, unlike the continental species, in the diet of the canary raven the plant component predominates (Nogales and Nieves, 2007). Of the 7 cases of Canary ravens with ARs, 6 were animals that were poisoned after ingesting baits prepared with several toxic substances simultaneously, including ARs. The Canary raven is one of the most threatened species of the archipelago, catalogued as critically endangered (BOE, 2010), and a recent count has confirmed a previous estimate indicating that no more than 400 nesting pairs remain (Nogales and Nieves, 2007). Thus, our results are very relevant, as AR exposure appears as a significant threat to the conservation of this species.

The rest of the non-predatory birds that presented ARs were grey herons (*Ardea cinerea*, n = 2), stone-curlews (*Burhinus oedicnemus distinctus*, n = 3), European turtle doves (*Streptopelia turtur*, n = 2), and blackbirds (*Turdus merula cabrerae*, n = 2). In these last 4 birds, the consumption of poisoned bait was also found to be the primary cause of death, according the forensic evidences.

Taken together, our results indicate that in addition to the low incidence of ARs in nonpredatory birds, in most cases their appearance was clearly related to the intentional placement of toxic baits.

3.2. Incidence of anticoagulant rodenticides in non-target animals of the Canary Islands: birds of prey

We studied the frequencies and concentrations of ARs among the different species of raptors in the archipelago (Table 2). Only in 3 species we did not detect ARs, although it should also be noted that only one individual was received from each of them (*Pandion haliaetus*, *Falco subbuteo*, and *Circus aeruginosus*). Only 5 compounds were detected, and all of them from the SGAR group, which were, in order of frequency: brodifacoum, bromadiolone, difenacoum, flocoumafen and difethialone (Fig. 2). This is the same order that has been published in the most recent review of the ARs incidence in non-target animals (Nakayama et al., 2019).

The most frequently affected species were nocturnal raptors (represented in our series by three subspecies, *Asio otus canariensis*, *Tyto alba alba* and *Tyto alba gracilirostris*) (Supplementary Fig. 1). This high frequency of detection in nocturnal raptors coincides with that reported by other authors (Albert et al., 2010; Christensen et al., 2012; Lambert et al., 2007; Lopez-Perea et al., 2015; Sanchez-Barbudo et al., 2012; Stone et al., 2003) and also in the Canary Islands (Ruiz-Suarez et al., 2014), and is particularly worrisome since owls seem to have a deficiency in the metabolic activity of AR detoxification, which makes the half-life of these compounds longer in them than in other species of birds of prey (Rattner et al., 2014). Therefore, anthropogenic environments such as livestock farms could provide excellent feeding opportunities (Geduhn et al., 2016) and might also become ecological traps for them (Lopez-Perea et al., 2019). In the long-eared owl (*Asio otus*

Table 2
Concentrations of the anticoagulant rodenticides detected in liver by raptor species.

Species[a]	Positives/no. animals	Brodifacoum			Bromadiolone			Difenacoum			Difethialone			Flocoumafen		
		Freq (%)	Med (ng/g)	Range	Freq (%)	Med (ng/g)	Range	Freq (%)	Med (ng/g)	Range	Freq (%)	Med (ng/g)	Range	Freq (%)	Med (ng/g)	Range
Accipiter nisus granti	5/9 (55.6%)	55.5%	20.9	5.8–400.0	25%	1.5	0.2–2.8	25%	0.6	0.4–0.8	–	–	–	11.5%	0.22	–
Asio otus canariensis	51/68 (75.0%)	68.2%	23.8	2.1–240.0	44.4%	10.3	0.8–512.8	28.6%	3.7	0.7–73.4	3.2%	14.8	0.5–29.2	13.1%	0.9	0.3–4.8
Buteo buteo insularum	33/53 (62.3%)	62.3%	14.6	2.5–796.6	55.8%	33.7	1.3–187.6	20.9%	12.1	1.7–47.9	–	–	–	2.3%	12.7	–
Falco eleonorae	2/4 (50.0%)	50.0%	1.5	0.6–2.4	33.3%	0.4	–	–	–	–	–	–	–	–	–	–
Falco peregrinus pelegrinoides	6/13 (46.2%)	46.2%	6.0	1.2–26.2	25.0%	7.4	5.0–36.2	–	–	–	–	–	–	–	–	–
Falco tinnunculus (*canariensis* and *dacotiae*)	53/83 (63.9%)	63.9%	44.2	1.4–1174.0	58.3%	45.3	2.6–4840.3	22.2%	4.5	1.2–33.4	1.4%	2.4	–	2.8%	5.6	0.5–10.8
Neophron percnopterus majorensis	20/67 (29.9%)	29.9%	33.4	9.3–176.5	20.0%	33.2	2.8–112.5	4.6%	12.4	2.7–21.1	–	–	–	–	–	–
Tyto alba (*alba* and *gracilirostris*)	7/8 (87.5%)	87.5%	7.9	0.2–119.7	42.8%	1.6	0.2–33.4	28.6%	7.8	0.1–15.6	14.3%	1.6	–	–	–	–

[a] Additionally, the series of raptors included 1 individual of each one of the three species, *Pandion haliaetus*, *Falco subbuteo*, and *Circus aeruginosus*, but anticoagulant rodenticides were not detected in the liver of any of them.

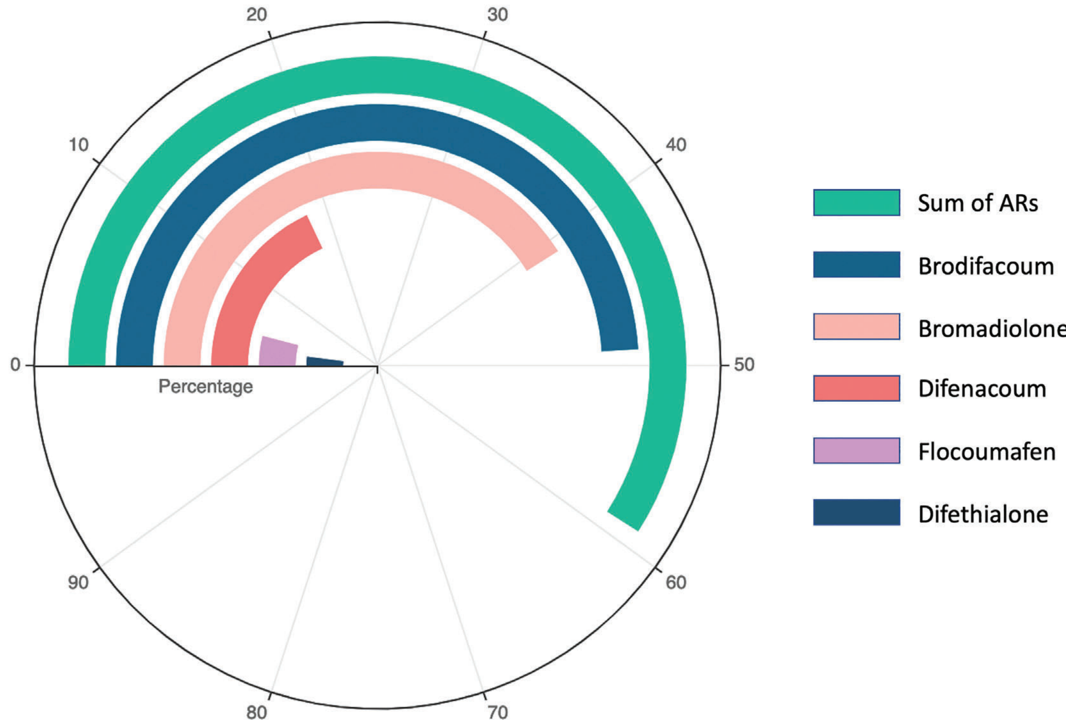


Fig. 2. Frequencies of anticoagulant rodenticides in raptor livers.

canariensis), the high frequency of detection of brodifacoum, which almost reached 70% of individuals, is also noteworthy, as well as the higher frequency of detection of flocoumafen of all the birds of prey included in this study (13%) (Fig. 3).

However, the highest concentrations were not found in these nocturnal species, but in common kestrels (*Falco tinnunculus*, median value $\sum AR = 102.4$ ng/g (median 768.4 ng/g)). In fact, of the 60 animals with levels $\sum AR > 100$ ng/g of the entire raptor series, 43.3%

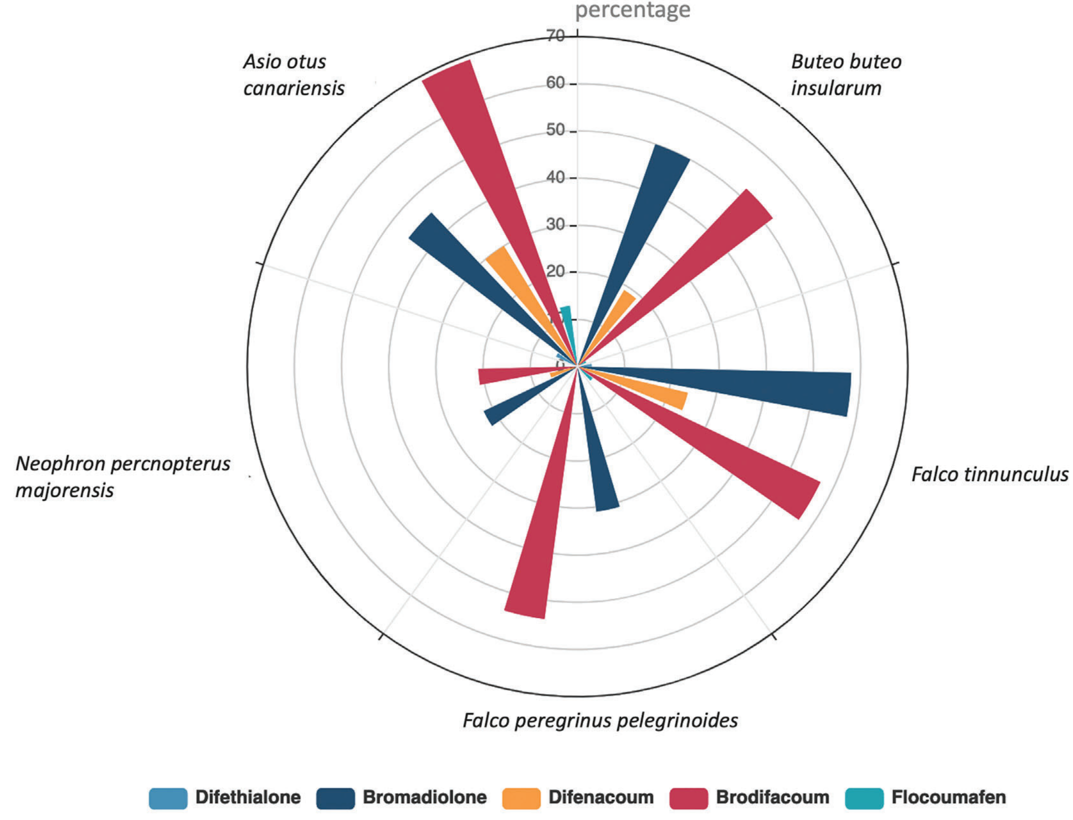


Fig. 3. Distribution of the frequency of detection of each of the anticoagulant rodenticides by species (only those with more than 10 individuals tested are shown).

were kestrels. This percentage rises to 68% in the case of raptors with more than 200 ng/g of $\sum$AR in the liver. In common kestrels, the most frequently detected compounds were brodifacoum and bromadiolone, both present simultaneously in most of the specimens (Fig. 3). Our results are consistent with those preliminarily reported in the Canary Islands (Ruiz-Suarez et al., 2014), and remain among the highest values reported for this species, similar to those described in the UK (Walker et al., 2013), but much lower than those reported in most of the available studies for this species (Christensen et al., 2012; Hughes et al., 2013; Lambert et al., 2007; Nakayama et al., 2019). Two out of the eleven subspecies of *Falco tinnunculus* known in the world are endemic to the Canary Islands, *Falco tinnunculus canariensis* and *Falco tinnunculus dacotiae*. No differences in the level of exposure to ARs were found between them. It has been established that the diet of both subspecies depends on the nesting area (Carrillo et al., 1994). In the Canary Islands, kestrels feed mainly on medium to large sized insects and small reptiles, but for individuals living in anthropogenic environments, it has been proven that mice can be an important part of their diet (*Mus* spp. would contribute up to 38% of the food biomass) (Carrillo et al., 2017; Carrillo et al., 1994). Therefore, as described in other areas, rodenticides (and other pesticides) would represent one of the main threats to the conservation of these subspecies (Buck et al., 2020; Mateo et al., 2000).

In common buzzards, ARs were frequently detected (mainly brodifacoum and bromadiolone, >50% of individuals, Table 2 and Fig. 3) and at moderately high concentrations (median $\sum$AR = 50.7 ng/g). Buzzards are represented in the Canary Islands by an endemic subspecies (*Buteo buteo insularum*). These concentrations are higher than those previously reported in the Canary Islands for this species (Ruiz-Suarez et al., 2014), although only 9 individuals were included in that study. The values we report here are very similar to those described in most of the studies available in the literature (Christensen et al., 2012; Hughes et al., 2013; Lambert et al., 2007; Lopez-Perea et al., 2015; Sanchez-Barbudo et al., 2012). The current report of exposure in 53 specimens is significant since this species has recently been identified as one of the key sentinel species for biomonitoring studies of contaminants at a pan-European level (Badry et al., 2020).

At the other extreme, as a species with a lower frequency of AR detection, we find the Egyptian Vulture (represented in the Canary Islands by a subspecies, *Neophron percnopterus majorensis*), with the high frequency of detection of brodifacoum being striking (Fig. 3). This is the only vulture in the archipelago, and similarly to what is described for other species of vultures on the continent; it is occasionally exposed to ARs and other agricultural pesticides, mainly to secondary exposure by ingesting the livers of the carrion on which they feed. The frequency of detection of ARs in these Egyptian Vultures was 29.2% (19 positives out of 65 specimens examined), which is similar to that described in the few studies available on exposure of vultures to ARs (Berny et al., 2015; Plaza et al., 2019; Sanchez-Barbudo et al., 2012).

Finally, we highlight the case of the Barbary Falcon (*Falco peregrinus pelegrinoides*) and Eleonora's Falcon (*Falco eleonorae*), as these were the species in which, although frequently detected, the concentrations of ARs were the lowest. In both species, brodifacoum was the main compound detected (Fig. 3). The Barbary Falcon is one of the least recorded birds of prey of Europe. It is known to feed mainly on pigeons and other medium-sized bird species (Rodríguez and Silverio, 2007). Given the almost null incidence of ARs that we have detected in the prey birds of the Barbary Falcon that we have analyzed throughout this decade, these results seem logical, with an average value for the $\sum$AR = 3.8 ng/g. Similarly, the Eleonora's Falcon (*Falco eleonorae*) also shown very low levels of ARs. Eleonora's Falcon is an uncommon bird species in the Canary Islands, with a population of only about 300 nesting pairs. They prey mainly on migrating small passeriform birds, but also on medium-size insects. Basing on their habitat and diet, the low levels of ARs detected seems very logical. However, in this decade we have only received 4 specimens, therefore no very firm conclusions can be drawn from the level of exposure of this species. Of these last two species (*Falco peregrinus pelegrinoides* and *Falco eleonorae*) the results we present are, as far as we know, the first reported in the world.

The results of the species with more than 10 specimens analyzed have been represented graphically in Supplementary Fig. 2, where the great differences in concentration found among them can be seen.

3.3. Temporal trend of the presence of anticoagulants in predatory birds

The temporal evolution of the cases of birds of prey received in our service since 2011, as well as the percentage of birds that tested positive for ARs were studied (Fig. 4). After irregular operation of the Poisoning Control and Prevention Strategy in the Canaries (BOC, 2014) in the early years, from 2016 onwards we have been receiving more and more cases, although the percentage of positive cases to ARs each year is around 60% with no clear trend, neither increasing nor decreasing. The increase in cases, not only of birds of prey, but of wildlife in general is very evident since 2017, mainly due to the higher degree of specialization of environmental officers, and above all due to the use of the first specialized anti-poison dog unit in the archipelago. It should be noted that in 2020, until May when we decided to stop the inclusion of cases for this study, we have already received more cases than the previous year. Our estimate is that by the end of this year, we could exceed 250 birds of prey tested, even considering the difficulties due to the COVID-19 pandemic. One reason for this could be that a second unit of anti-poison dogs began operating in the archipelago at the end of 2019.

With respect to the concentrations found, we divided the series into two groups, with the cases received before and after May 2018 (Fig. 5), since it was on that date that the reclassification of products formulated with anticoagulant active substances with concentrations equal to or greater than 30 ppm as reprotoxic compounds was made mandatory (Frankova et al., 2019). This reclassification led to significant restrictions with respect to its application and sale, with the result that the vast majority of baits applied since then are 30 ppm or below. Consequently, we wanted to see if the average concentrations in non-target fauna decreased. This seems to be the case, with significantly lower values ($P < 0.001$) in the cases after May 2018 (Fig. 5). However, the opposite occurred with the average number of ARs per animal (Fig. 5, inset). Although there is evidence that baits with < 30 ppm are just as effective as the more concentrated ones (50 ppm or more) (Frankova et al., 2019), this result could indicate that the simultaneous use of baits with different active ingredients to "ensure effectiveness" has increased.

3.4. GIS analysis

There are some works of spatial analysis of the exposure of raptors to ARs, which explore the influence of different stressors (Hindmarch et al., 2017). In this research we focused the GIS analysis only on the 308 raptors, to try to elucidate whether the application of rodenticides on livestock farms, mostly by farmers themselves in our archipelago, could play a role in the exposure of birds of prey to these compounds.

From the mere visual observation of the geolocated positive/negative cases on the map of the archipelago, everything seemed to indicate that the majority of positive cases were grouped near the areas of greatest density of farms (an example is shown Supplementary Fig. 3 showing Gran Canaria island). In the supporting article in Data in Brief "*Dataset on the concentrations of anticoagulant rodenticides in raptors from the Canary Islands with geographic information*", we provide the exact location of each case, the distance to the nearest farm (and the type of livestock), the island by island and species by species analyses, and the concentrations of the ARs found in each individual.

It was necessary to obtain the exact distances, for which a matrix of distances between all points (cases and farms) was obtained, selecting the shortest case-farm distance. Spearman's correlation analysis of these data pairs indicated a significant value of −0.5657 ($P < 0.0001$),

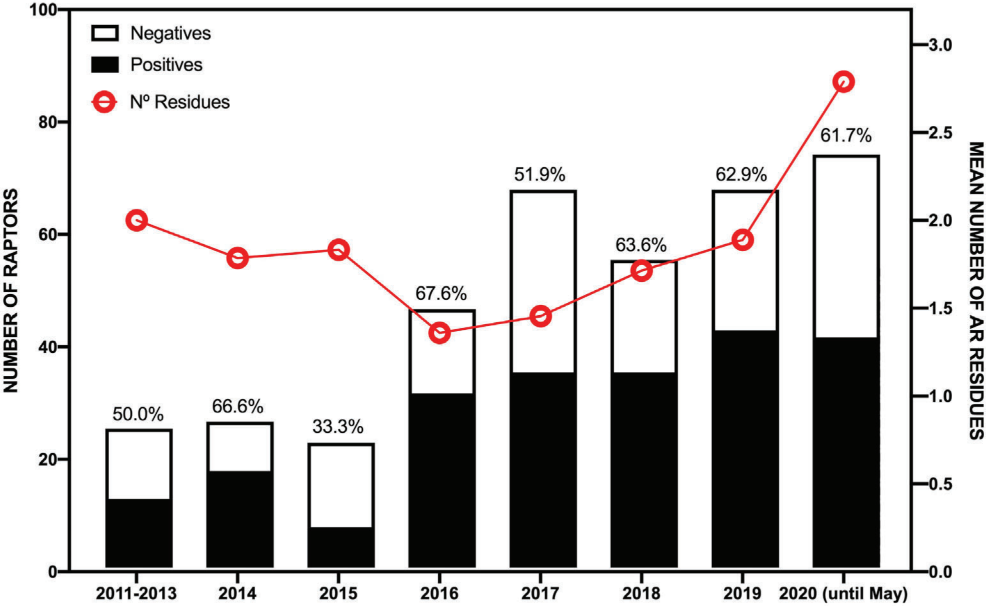


Fig. 4. Temporal trend of positive/negative for anticoagulant rodenticides cases (left Y-axis), and of the mean number of ARs per animal (right Y-axis).

indicating that, the greater the distance from the animal to the nearest farm, the lower the concentration of $\sum$AR (Fig. 6). The significant Spearman's inverse correlation was maintained when we analyzed the species with the highest number of individuals separately (*Falco tinnunculus*, Spearman's r −0.5451, P < 0.0001; *Buteo buteo*, Spearman's r −0.5094, P < 0.0001; *Asio otus canariensis*, Spearman's r −0.4855, P < 0.0001; *Neophron percnopterus majorensis*, Spearman's r −0.5682, P < 0.0001).

Although information is scarce on many of the Canarian raptor subspecies, we took into consideration the home-range areas described in other regions for the main species of raptors included in this study (Casagrande et al., 2008; Garcia-Heras et al., 2013; Kenward et al., 2018; Martínez and Zuberogoitia, 2004; Thomsen et al., 2014), and according to this we divided the data series in two groups, individuals that were found at greater or lesser distance from the nearest farm to the chosen cut-off point (0.5, 1, 1.5, and 2 km). In all cases we found

Fig. 5. Mean concentrations of the sum of anticoagulant rodenticides in the liver of raptors found before and after May 2018, which is the date of entry into force of EC (EU) 2016/1179 setting a maximum content of 30 ppm of active substance in rodenticide baits; (Insert) Mean ± SD of number of ARs per animal before and after May 2018.

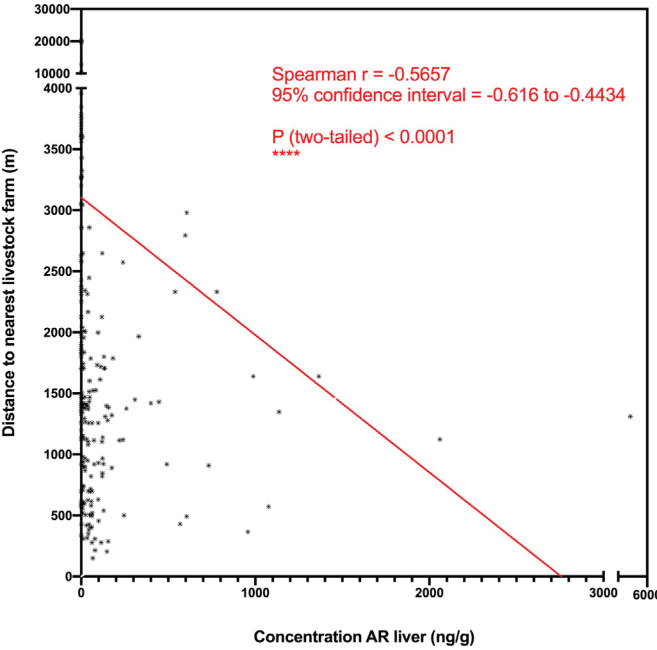


Fig. 6. Scatterplot and Spearman's correlation coefficient of the shortest distance the cases of raptors analyzed to the nearest farm and the concentration in liver of the sum of anticoagulant rodenticides of the cases (ng/g).

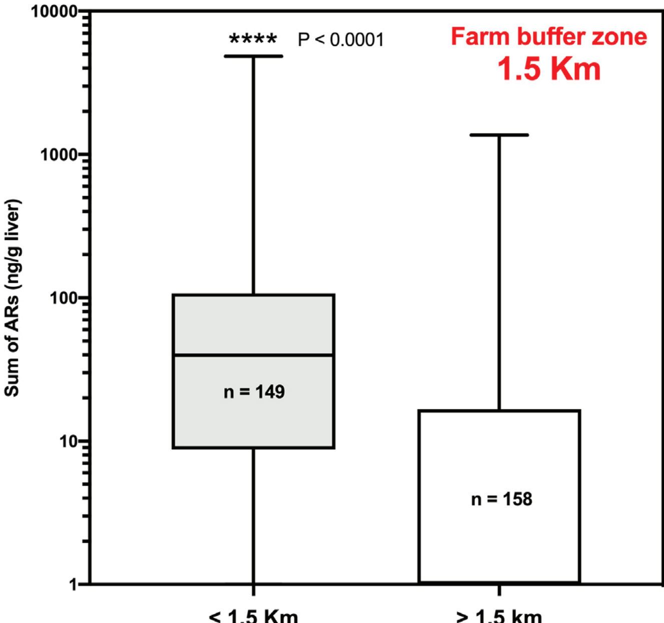


Fig. 7. Box and whiskers graph showing the comparison of anticoagulant rodenticides in the liver between those animals found less or more than 1.5 km from the nearest livestock farm. The lines show the medians, the boxes cover the 25th to 75th percentiles, and the minimal and maximal values are shown by the ends of the bars.

that the concentrations of the group of animals found closer to the farms were significantly higher than those found further away (Fig. 7 for a buffer of 1.5 km, Supplementary Fig. 3 for the analysis with the log transformed data for a buffer of 1.5 km, and Supplementary Fig. 4 for the rest of buffer zones). When we analyzed the farms separated by type of livestock, we found that the association with distance was maintained for farms that are dedicated to intensive production (pigs and cattle), whereas it was lost in those dedicated to extensive or more traditional production in the Canary Islands (sheep/goats) (Supplementary Fig. 5).

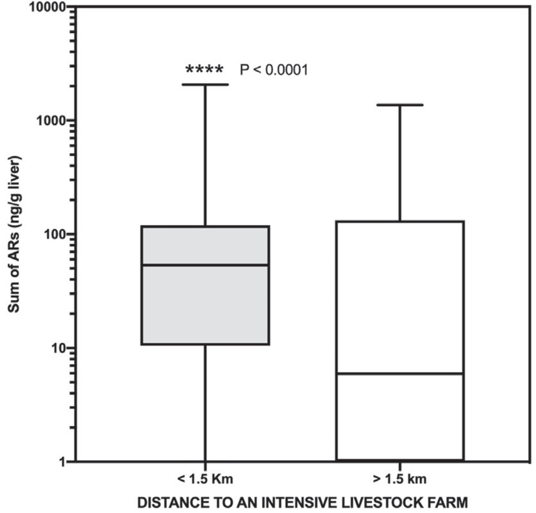
Finally, since human population density is considered to be another determining factor for AR, we subdivided again the series of birds into two groups, those sampled in municipalities with a low population density from those with a high density. The cut-off point was chosen based on the median of the population density distribution of the municipalities of the archipelago (120 inhabitants/km^2). The relationship with the distance near the farms remains significant in both cases (Fig. 8). In addition, using the Canary Islands population density GIS layer, the number of inhabitants within 1.5 km^2 around the sampling location of each bird was calculated, and the results (n° inhabitants/case) of the series of positive cases (1 or more AR detected) were compared with those of the series of negative cases. The difference was not statistically significant (Mann-Whitney *U* test). Although the secondary effects of anticoagulant exposure of non-target wildlife in human-dominated environments has been widely studied (Hong et al., 2019; Lopez-Perea et al., 2019; Quinn et al., 2012; Serieys et al., 2018), the distorting effect of population density on the results observed in this study could be ruled out.

4. Conclusions

This study reports incidence data of anticoagulant rodenticides in non-target species in the Canary Islands during the last decade. The high incidence of these compounds in birds of prey, particularly brodifacoum and bromadiolone, with about 60% of the birds of prey containing one or more ARs, is noteworthy. Since the entry into force of the restriction on the concentration of active ingredient in baits (<30 ppm), a decrease in the concentrations of these compounds in the liver has been detected, but also, conversely, an increase in the average number of ARs per animal.

From the GIS study, it can be deduced that intensive farms are a major determinant in the exposure of birds of prey to SGARs, and that those birds that opportunistically nest around intensive production farms are susceptible to be significantly more exposed than those of birds that live far from facilities of this type.

The results of this study should be taken into consideration by the authorities, with a view to restricting the free distribution and non-professional use of ARs, particularly the SGARs. In addition, they should implement education and awareness campaigns on the consequences of the uncontrolled application of rodenticides on the unique biodiversity of the Canary Islands, as well as promote the replacement of



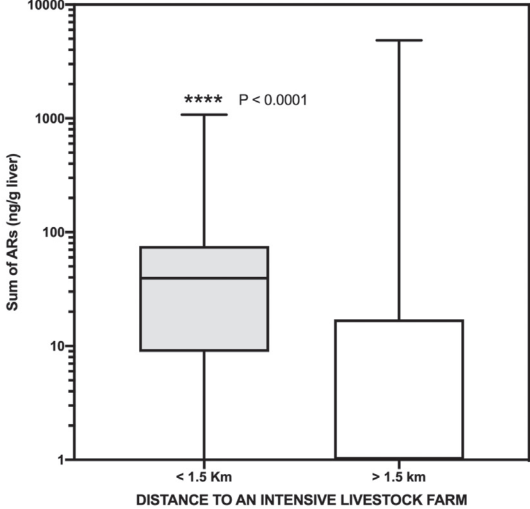


Fig. 8. Box and whiskers graph showing the comparison of anticoagulant rodenticides in the liver between those animals found less or more than 1.5 km from the nearest livestock farm, separated by municipalities with low population density (left) and high population density (right). Population density cut-off = 120 inhabitants/km^2. The lines show the medians, the boxes cover the 25th to 75th percentiles, and the minimal and maximal values are shown by the ends of the bars.

chemical control of rodent pests by alternative methods of physical and biological control where feasible.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.144386.

CRediT authorship contribution statement

Guarantor of integrity of the entire study: OPL
Study concepts and design: OPL
Literature research: CRB, AAD, MACP, ASP, AMM, OPL
Laboratory work: CRB, AAD, ASP, NRS, ARH, AMM, MZ, LAHH, LDB, OPL
Data analysis: CRB, AAD, NRS, MACP, AMM, OPL
Statistical analysis: LAHH, OPL
Manuscript preparation: CRB, AAD, MACP, ASP, AMM, MZ, OPL
Manuscript editing: CRB, AAD, MACP, ASP, AMM, MZ, OPL

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was supported by the University of Las Palmas de Gran Canaria via a doctoral grant to the first author Cristian Rial-Berriel (ULPGC-012-2016) and also by the Spanish Ministry of Education, Culture and Sports via a doctoral grant to author Andrea Acosta-Dacal (FPU16-01888).

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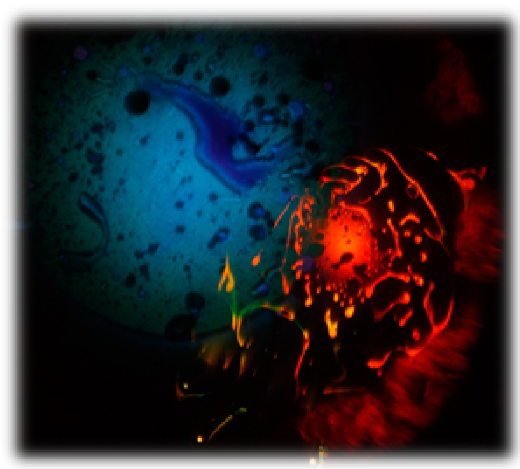
Publicación 6. Dataset on the concentrations of anticoagulant rodenticides in raptors from the Canary Islands with geographic information

Conjunto de datos de concentraciones de raticidas anticoagulantes en rapaces de Canarias con información geográfica

Data in Brief, 2021, 34: 106744

DOI: https://doi.org/10.1016/j.dib.2021.106744



Data in Brief 34 (2021) 106744

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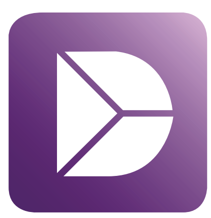

Data Article

Dataset on the concentrations of anticoagulant rodenticides in raptors from the Canary Islands with geographic information

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A R T I C L E I N F O

Article history:
Received 23 December 2020
Revised 7 January 2021
Accepted 8 January 2021
Available online 13 January 2021

A B S T R A C T

The dataset presented in this article supports "Intensive livestock farming as a major determinant of the exposure to anticoagulant rodenticides in raptors of the Canary Islands (Spain)" (Rial-Berriel et al., 2020). A Geographic Information

DOI of original article: 10.1016/j.scitotenv.2020.144386

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https://doi.org/10.1016/j.dib.2021.106744
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Keywords:
Geographic information system
Long-eared owl
Common kestrel
Common buzzard
Egyptian vulture
SGAR

System (GIS) analysis on the influence of the influence of livestock activity on exposure to anticoagulant rodenticides in raptors in the Canary Islands was performed. This dataset provides geographic information on the localization of each raptor (either positive or negative for anticoagulant rodenticides, $n = 308$), as well as the concentrations of each compound found in their livers. In addition, we present complementary analyses to those included in the main article, such as the detailed analysis of the farming activity influence on anticoagulant rodenticide exposure of raptors, by island and by raptor species.

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Specifications Table

Subject	Environmental Chemistry
Specific subject area	Census of raptor specimens for contaminant biomonitoring
Type of data	Figures (processed data), and the corresponding raw data (table)
How data were acquired	Ultra-high performance liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS) GPS devices
Data format	Raw and analysed
Parameters for data collection	The data were systematically collected in the course of forensic investigations of incidents affecting wildlife. The main conditions for starting the data collection were that: • The animal was a raptor bird nesting in the Canary Islands, • Found dead or died/euthanized within a week of its admission to a wildlife recovery center. • With fresh liver tissue available, • With georeferenced data of the location where the carcass or severely affected animal was found.
Description of data collection	All the available information about the animal (species and subspecies, age, sex, the possible cause of death, etc. . .), as well as the coordinates of the place where the animal/cadaver was found, collected with a centimetre precision GPS device by the environmental officers, were obtained from the reports of sample collection and the medical histories opened in the wildlife recovery centres of the Canary Islands. The quantification data of anticoagulant rodenticides in raptor were obtained analysing a series of 308 livers from January 2011 to May 2020.
Data source location	Institution: Toxicology Unit, Clinical Sciences Department, Universidad de Las Palmas de Gran Canaria City/Town/Region: Las Palmas de Gran Canaria (Gran Canaria, Canary Islands) Country: Spain
Data accessibility	With the article
Related research article	Intensive livestock farming as a major determinant of the exposure to anticoagulant rodenticides in raptors of the Canary Islands (Spain) https://doi.org/10.1016/j.scitotenv.2020.144386

Value of the Data

- The data that we present in this article are very useful to researchers who carry out biomonitoring of pesticides. They will also contribute in a very relevant way to the elaboration of the map of the exposure to rodenticides of raptors in Europe (LIFE APEX projects, European

Raptor Biomonitoring Facility), as well as to implement the Information Platform for Chemical Monitoring (IPCheM) database, which is the European Commission's reference access point for searching, accessing and retrieving chemical occurrence data collected and managed in Europe.
- By providing not only pollution data, but also the GPS coordinates of 308 raptor specimens, some of them belonging to species very rarely observed in Europe (the case of Eleanora's falcon and Barbary falcon), allows feeding databases, such as the Census of raptor specimens for pan-European contaminant biomonitoring.
- In addition, the censuses that are currently being carried out in Europe focus in a special way on those species that have a pan-European distribution, since the contamination data in raptors is crossed with those of the human population and in environmental samples of each country. In this work, we provide data from numerous specimens of the species that have been identified as being of greatest interest for this purpose: *Buteo buteo* ($n = 53$); *Falco tinnunculus* ($n = 83$); *Tyto alba* ($n = 8$); *Asio otus* ($n = 68$); as well as data from many other species of interest.

1. Data Description

The data presented here are part of a larger series of 831 animals that have been investigated for anticoagulant rodenticides in the Canary Islands over a decade (2011–2020), and which are presented in the article by Rial-Berriel et al. [1]. The data detailed at the individual level here focus on the 308 raptors included in that series. We have decided to include all the raptor specimens investigated, both those positive for anticoagulants and those negative, due to the importance of having georeferenced data of the specimens suitable for future analysis of contaminants at a pan-European level. Table 1 contains the details of all the individuals investigated, including species and subspecies, geographical data of their location (island, municipality, and GPS coordinates), and the individual concentrations of the 5 anticoagulant rodenticides detected in the series (brodifacoum, bromadiolone, difenacoum, difethialone, and flocoumafen). The shortest distance from the location of the bird of prey to the nearest cattle farm is also presented and including the type of livestock farmed.

Figs. 1 to 5 present a double panel each. On the one hand, we present the geographical location of all cases (positive and negative for anticoagulant rodenticides) on the map of each of the Canary Islands (with the exception of El Hierro, in which only 2 cases were recorded), and the relationship with the location of medium / large-sized livestock farms, surrounded by a 1.5 km buffer zone (which we consider to coincide with the average home range of the raptor species studied). Additionally, in each of these graphs, the comparative statistics of the distribution of anticoagulant rodenticide values between the groups of animals that were found inside or outside the aforementioned 1.5 km buffer zone of the livestock farms are also presented.

Additionally, detailed analysis is presented for those raptor species in which more than 50 individuals were analysed. In these analyses we present the comparative statistics of the distribution of anticoagulant rodenticide values between the groups of birds that were found inside or outside the 1.5 km-buffer zone of farms: common kestrel (*Falco tinnunculus*, Fig. 6); common buzzard (*Buteo buteo*, Fig. 7); long-eared owl (*Asio otus*, Fig. 8); and Egyptian vulture (*Neophron percnopterus*, Fig. 9).

2. Experimental Design, Materials and Methods

2.1. Sampling

This study was carried out in the Canary Islands, and the samples were taken in the context of the Poisoning Control and Prevention Strategy in the Canaries [2] from 2011 to May 2020.

Table 1
Identification of each bird included in the study, with detailed information about the location where the corpse was found, including GPS coordinates, and about the concentration of anticoagulant rodenticides detected (replicate analysis are provided).

RAPTOR SPECIES	YEAR	ISLAND	MUNICIPALITY	UTMX	UTMY	BRODIFACOUM measurement 1	BRODIFACOUM measurement 2	BROMADIOLONE measurement 1	BROMADIOLONE measurement 2	DIFENACOUM measurement 1	DIFENACOUM measurement 2	DIFETHIALONE measurement 1	DIFETHIALONE measurement 2	FLOCOUMAFEN measurement 1	FLOCOUMAFEN measurement 2	SUM OF RODENTICIDES (using averaged measurements)	№ RODENTICIDES	Distance to the nearest farm (m)	Type of livestock
Accipiter nisus granti	2020	Gran Canaria	Moya	454582	3104241	34.32	26.96	2.71	2.93	0.72	0.90			0.20	0.24	34.49	4	315	Goat/Sheep
Accipiter nisus granti	2020	Gran Canaria	Telde	458011	3096342	12.54	9.86	0.19	0.21	0.36	0.44					11.8	3	940	Cattle
Accipiter nisus granti	2019	Tenerife	Güimar	359845	3135597	448.00	352.00									400	1	1420	Cattle
Accipiter nisus granti	2019	Tenerife	Güimar	359845	3135597											0	0	1420	Cattle
Accipiter nisus granti	2019	Tenerife	Güimar	359845	3135597											0	0	1420	Cattle
Accipiter nisus granti	2020	Gran Canaria	Las Palmas de Gran Canaria	454183	3107904	2.45	2.87	0.22	0.24							2.89	2	1432	Cattle
Accipiter nisus granti	2019	Gran Canaria	Telde	459098	3096981	5.34	6.26									5.8	1	2040	Goat/Sheep
Accipiter nisus granti	2014	Gran Canaria	Moya	441131	3107838											0	0	2580	Goat/Sheep
Accipiter nisus granti	2016	Gran Canaria	Artenara	432827	3098128											0	0	3722	Goat/Sheep
Actitis hypoleucos	2015	Fuerteventura	Antigua	610682	3140741											0	0	7585	Pork
Asio otus canariensis	2020	Gran Canaria	Agüimes	452323	3084882	31.07	36.47			2.90	3.62	28.03	30.37	0.80	1.00	67.13	4	150	Goat/Sheep
Asio otus canariensis	2020	Gran Canaria	La Aldea de San Nicolás	447360	3104609	64.18	75.34	5.70	6.18	3.38	4.22					79.5	3	215	Pork
Asio otus canariensis	2018	La Gomera	San Sebastian de la Gomera	293178	3110572	20.56	25.64	37.15	40.25							61.8	2	277	Pork
Asio otus canariensis	2019	Gran Canaria	San Bartolomé de Tirajana	441414	3073783	34.44	42.96	2.02	2.18							40.8	2	348	Goat/Sheep
Asio otus canariensis	2019	Gran Canaria	Santa María de Guía	437346	3107889	21.18	26.42	3.65	3.95	3.56	4.44			0.45	0.56	32.1	4	420	Cattle
Asio otus canariensis	2020	Gran Canaria	San Mateo	445964	3099016	1.87	2.33	41.98	45.48							45.83	2	425	Pork
Asio otus canariensis	2020	Gran Canaria	Moya	444454	3110673	207.46	258.74	11.90	12.90					0.30	0.38	245.84	3	500	Goat/Sheep
Asio otus canariensis	2019	La Gomera	Vallehermoso	277429	3117336	8.10	10.10	15.17	16.43							24.9	2	504	Pork

(*continued on next page*)

Table 1 (*continued*)

RAPTOR SPECIES	YEAR	ISLAND	MUNICIPALITY	UTMX	UTMY	BRODIFACOUM measurement 1	BRODIFACOUM measurement 2	BROMADIOLONE measurement 1	BROMADIOLONE measurement 2	DIFENACOUM measurement 1	DIFENACOUM measurement 2	DIFETHIALONE measurement 1	DIFETHIALONE measurement 2	FLOCOUMAFEN measurement 1	FLOCOUMAFEN measurement 2	SUM OF RODENTICIDES (using averaged measurements)	№ RODENTICIDES	Distance to the nearest farm (m)	Type of livestock
Asio otus canariensis	2019	La Gomera	Alajeró	279237	3108868	26.21	20.59	34.37	37.23							59.2	2	504	Pork
Asio otus canariensis	2020	Gran Canaria	Agüimes	457380	3085646	8.40	6.60			1.25	1.55					8.9	2	520	Pork
Asio otus canariensis	2019	La Gomera	San Sebastian de la Gomera	291600	3110450	71.34	56.06									63.7	1	520	Pork
Asio otus canariensis	2020	Gran Canaria	Arucas	448896	3111130	44.12	34.66									39.39	1	523	Pork
Asio otus canariensis	2020	Gran Canaria	Las Palmas de Gran Canaria	457274	3101765	142.96	112.32	0.82	0.70					0.59	0.73	129.06	3	540	Pork
Asio otus canariensis	2018	Tenerife	Icod de los Vinos	332484	3137697	1.91	2.39	4.75	4.05	1.07	1.33					7.75	3	558	Cattle
Asio otus canariensis	2018	Tenerife	Garachico	328159	3138215	13.84	17.26			2.72	3.40					18.61	2	595	Cattle
Asio otus canariensis	2016	Gran Canaria	Santa Brígida	455403	3100630			9.85	8.39							9.12	1	599	Goat/Sheep
Asio otus canariensis	2019	Gran Canaria	Arucas	449698	3111641	11.04	13.76									12.4	1	611	Goat/Sheep
Asio otus canariensis	2019	Tenerife	Garachico	327088	3138866	39.34	49.06			13.08	16.32					58.9	2	615	Cattle
Asio otus canariensis	2019	Tenerife	Garachico	327088	3138866											0	0	615	Cattle
Asio otus canariensis	2019	Tenerife	Garachico	327088	3138866											0	0	615	Cattle
Asio otus canariensis	2019	Tenerife	Icod de los Vinos	333648	3138294	12.55	15.65	86.94	74.06	2.58	3.22					97.5	3	630	Cattle
Asio otus canariensis	2018	Tenerife	La Orotava	349264	3139111	21.46	26.76	15.66	13.34					2.35	2.93	41.25	3	700	Pork
Asio otus canariensis	2017	Gran Canaria	Santa Brígida	445895	3099567											0	0	700	Pork
Asio otus canariensis	2017	Gran Canaria	Santa Brígida	445379	3099148	20.92	26.09	42.01	35.79							62.4	2	700	Pork
Asio otus canariensis	2020	Gran Canaria	Las Palmas de Gran Canaria	453517	3107995	8.23	9.67									8.95	1	708	Goat/Sheep

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Table 1 (*continued*)

RAPTOR SPECIES	YEAR	ISLAND	MUNICIPALITY	UTMX	UTMY	BRODIFACOUM measurement 1	BRODIFACOUM measurement 2	BROMADIOLONE measurement 1	BROMADIOLONE measurement 2	DIFENACOUM measurement 1	DIFENACOUM measurement 2	DIFETHIALONE measurement 1	DIFETHIALONE measurement 2	FLOCOUMAFEN measurement 1	FLOCOUMAFEN measurement 2	SUM OF RODENTICIDES (using averaged measurements)	№ RODENTICIDES	Distance to the nearest farm (m)	Type of livestock
Asio otus canariensis	2019	Gran Canaria	Arucas	448362	3112107	47.05	55.23	1.62	1.38					1.25	1.55	54.04	3	717	Goat/Sheep
Asio otus canariensis	2019	Tenerife	Garachico	327929	3139211	9.14	10.74			3.45	3.67					13.5	2	740	Cattle
Asio otus canariensis	2017	Gran Canaria	Las Palmas de Gran Canaria	456453	3101685	111.81	131.25									121.53	1	846	Pork
Asio otus canariensis	2020	Gran Canaria	Galdar	437782	3109413	156.22	183.38	1.36	1.44			0.48	0.52	4.66	4.94	176.5	4	890	Cattle
Asio otus canariensis	2019	Gran Canaria	Telde	457693	3096377	16.04	18.84			4.95	5.25					22.54	2	898	Cattle
Asio otus canariensis	2020	Gran Canaria	Moya	444415	3109696	32.57	38.23	2.94	3.12	1.09	1.15	0.27	0.29			39.83	4	932	Pork
Asio otus canariensis	2020	Gran Canaria	Moya	443379	3110260	9.53	11.19									10.36	1	980	Goat/Sheep
Asio otus canariensis	2020	Gran Canaria	Santa María de Guía	438138	3110107	27.49	32.27	9.17	9.73	0.65	0.69					40	3	1104	Goat/Sheep
Asio otus canariensis	2016	Gran Canaria	Las Palmas de Gran Canaria	454870	3103678	136.75	160.53	65.42	69.46	2.70	2.86					218.86	3	1115	Goat/Sheep
Asio otus canariensis	2016	Gran Canaria	Las Palmas de Gran Canaria	454870	3103678	67.34	79.06									73.2	1	1115	Goat/Sheep
Asio otus canariensis	2020	Gran Canaria	Valsequillo	4513691	3095938											0	0	1140	Goat/Sheep
Asio otus canariensis	2019	Gran Canaria	Arucas	449842	3112531	5.04	3.96	4.46	4.74							9.1	2	1148	Goat/Sheep
Asio otus canariensis	2016	La Gomera	Vallehermoso	277601	3115588	149.18	117.22	22.41	23.79							156.3	2	1384	Pork
Asio otus canariensis	2019	La Gomera	Vallehermoso	276852	3117893	37.74	29.66			3.69	3.91					37.5	2	1395	Pork
Asio otus canariensis	2016	Gran Canaria	Tejeda	441406	3097942	264.19	207.57			71.23	75.63					309.31	2	1449	Cattle
Asio otus canariensis	2017	Gran Canaria	San Mateo	444207	3096578	11.91	9.35	32.64	34.66	3.72	3.96					48.12	3	1603	Cattle
Asio otus canariensis	2016	La Gomera	Alajeró	279309	3110804			10.89	11.57							11.23	1	1742	Pork
Asio otus canariensis	2018	Tenerife	Icod de los Vinos	331062	3137692	61.60	48.40									55	1	1786	Cattle
Asio otus canariensis	2018	Tenerife	Icod de los Vinos	334580	3140044	12.56	9.86	3.98	4.22							15.31	2	1955	Cattle
Asio otus canariensis	2019	Gran Canaria	Telde	458537	3096938											0	0	1970	Goat/Sheep
Asio otus canariensis	2017	Tenerife	La Orotava	349544	3140835	16.86	13.24									15.05	1	2008	Cattle
Asio otus canariensis	2017	Tenerife	La Orotava	349601	3140971			20.56	25.64							23.1	1	2008	Cattle
Asio otus canariensis	2017	Gran Canaria	San Mateo	445037	3093464											0	0	2250	Cattle
Asio otus canariensis	2018	Tenerife	Icod de los Vinos	332418	3140319	20.05	23.53			14.16	15.04					36.39	2	2314	Cattle

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Table 1 (*continued*)

RAPTOR SPECIES	YEAR	ISLAND	MUNICIPALITY	UTMX	UTMY	BRODIFACOUM measurement 1	BRODIFACOUM measurement 2	BROMADIOLONE measurement 1	BROMADIOLONE measurement 2	DIFENACOUM measurement 1	DIFENACOUM measurement 2	DIFETHIALONE measurement 1	DIFETHIALONE measurement 2	FLOCOUMAFEN measurement 1	FLOCOUMAFEN measurement 2	SUM OF RODENTICIDES (using averaged measurements)	№ RODENTICIDES	Distance to the nearest farm (m)	Type of livestock
Asio otus canariensis	2020	Gran Canaria	Arucas	448585	3107247											0	0	2430	Goat/Sheep
Asio otus canariensis	2017	Tenerife	Icod de los Vinos	333662	3140549	220.80	259.20									240	1	2573	Cattle
Asio otus canariensis	2011	Gran Canaria	Santa María de Guía	437802	3114283	68.89	80.87	456.37	569.17	10.28	10.92					598.25	3	2794	Goat/Sheep
Asio otus canariensis	2017	Tenerife	El Tanque	325534	3135980			5.87	7.33							6.6	1	3046	Cattle
Asio otus canariensis	2019	Gran Canaria	Telde	461042	3099842											0	0	3050	Goat/Sheep
Asio otus canariensis	2018	La Gomera	Vallehermoso	274597	3117378											0	0	3270	Pork
Asio otus canariensis	2020	Tenerife	Icod de los Vinos	331220	3135855											0	0	3286	Goat/sheep
Asio otus canariensis	2018	Tenerife	Icod de los Vinos	335500	3140260			2.79	3.47							3.13	1	3430	Cattle
Asio otus canariensis	2017	Tenerife	El Tanque	325537	3135796											0	0	3567	Cattle
Asio otus canariensis	2017	Tenerife	Santiago del Teide	322325	3126701											0	0	3620	Goat/Sheep
Asio otus canariensis	2016	Gran Canaria	San Mateo	441262	3098794											0	0	4095	Pork
Asio otus canariensis	2016	Gran Canaria	San Mateo	443791	3095962											0	0	4181	Pork
Asio otus canariensis	2020	Tenerife	Santa Cruz de Tenerife	380799	3156456											0	0	4556	Goat/sheep
Asio otus canariensis	2017	Tenerife	El Tanque	352118	3134946											0	0	4566	Pork
Asio otus canariensis	2020	Lanzarote	Teguise	642186	3212326											0	0	4788	Goat/sheep
Asio otus canariensis	2017	Tenerife	El Tanque	324844	3134546	6.32	7.88									7.1	1	5055	Cattle
Asio otus canariensis	2018	La Gomera	San Sebastian de la Gomera	284046	3116134	23.16	28.88									26.02	1	6260	Pork
Asio otus canariensis	2018	La Gomera	San Sebastian de la Gomera	284356	3116998					1.34	1.14					1.24	1	6260	Pork
Asio otus canariensis	2019	Fuerteventura	Pájara	587386	3136159	2.55	3.19	0.74	0.92							3.7	2	7889	Goat/Sheep
Buteo buteo insularum	2020	Gran Canaria	Ingenio	456965	3089566	7.92	9.88			1.84	1.56					10.6	2	310	Goat/Sheep
Buteo buteo insularum	2015	Gran Canaria	Valsequillo	449544	3094810	709.01	884.27	109.83	116.63	51.72	44.06					957.76	3	365	Pork
Buteo buteo insularum	2016	Gran Canaria	Valleseco	441656	3103060	19.14	23.87	31.72	33.68							54.2	2	400	Pork
Buteo buteo insularum	2014	Gran Canaria	Teror	445691	3102357											0	0	580	Goat/Sheep
Buteo buteo insularum	2014	Gran Canaria	Teror	445691	3102357	19.09	23.81	34.60	36.74							57.12	2	580	Goat/Sheep
Buteo buteo insularum	2018	La Gomera	Vallehermoso	278333	3116479	34.89	43.51									39.2	1	625	Pork

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Table 1 (*continued*)

RAPTOR SPECIES	YEAR	ISLAND	MUNICIPALITY	UTMX	UTMY	BRODIFACOUM measurement 1	BRODIFACOUM measurement 2	BROMADIOLONE measurement 1	BROMADIOLONE measurement 2	DIFENACOUM measurement 1	DIFENACOUM measurement 2	DIFETHIALONE measurement 1	DIFETHIALONE measurement 2	FLOCOUMAFEN measurement 1	FLOCOUMAFEN measurement 2	SUM OF RODENTICIDES (using averaged measurements)	№ RODENTICIDES	Distance to the nearest farm (m)	Type of livestock
Buteo buteo insularum	2019	Gran Canaria	San Mateo	446859	3098882	2.34	2.92	5.78	6.14							8.59	2	735	Pork
Buteo buteo insularum	2020	Gran Canaria	Arucas	447622	3113963	68.13	84.97	149.59	158.85	25.27	21.53					254.17	3	786	Pork
Buteo buteo insularum	2017	Fuerteventura	Antigua	602696	3139347	4.68	5.84	1.47	1.57							6.78	2	910	Pork
Buteo buteo insularum	2016	Gran Canaria	Valleseco	446323	3100270	340.67	267.67	181.97	193.23							491.77	2	919	Pork
Buteo buteo insularum	2016	Gran Canaria	Valleseco	446323	3100270											0	0	919	Pork
Buteo buteo insularum	2018	Gran Canaria	Arucas	450882	3109182									123.87	131.53	127.7	1	922	Goat/Sheep
Buteo buteo insularum	2018	La Palma	Mazo	228158	3162869			22.70	24.10							23.4	1	967	Cattle
Buteo buteo insularum	2018	La Palma	Mazo	228256	3162049			119.12	126.48							122.8	1	967	Cattle
Buteo buteo insularum	2020	Gran Canaria	Galdar	437673	3108975	134.89	105.99			3.46	2.94					123.64	2	1140	Pork
Buteo buteo insularum	2019	Gran Canaria	Las Palmas de Gran Canaria	456551	3106437	2.91	2.29	1.62	1.38							4.1	2	1150	Pork
Buteo buteo insularum	2014	Gran Canaria	Artenara	438018	3100502											0	0	1196	Goat/Sheep
Buteo buteo insularum	2020	Gran Canaria	San Mateo	444505	3099749	29.06	22.84	19.68	16.76	7.94	6.76					51.52	3	1234	Pork
Buteo buteo insularum	2017	La Palma	El Sauzal	362492	3146442	112.00	88.00	36.99	31.51	18.66	15.90					151.53	3	1279	Cattle
Buteo buteo insularum	2018	Tenerife	La Matanza de Acentejo	358634	3146709	16.89	13.27	132.95	113.25							138.18	2	1310	Pork
Buteo buteo insularum	2019	Gran Canaria	Telde	457380	3096726											0	0	1330	Goat/Sheep
Buteo buteo insularum	2015	Gran Canaria	Telde	454049	3097857			25.54	21.76							23.65	1	1375	Cattle
Buteo buteo insularum	2018	Tenerife	El Tanque	326925	3137290	6.23	4.89	16.96	14.44	8.50	7.24					29.13	3	1413	Cattle
Buteo buteo insularum	2013	Gran Canaria	Galdar	439904	3102452											0	0	1468	Pork
Buteo buteo insularum	2013	Gran Canaria	Galdar	439904	3102452	17.55	13.79	35.77	30.47							48.79	2	1468	Pork
Buteo buteo insularum	2011	Gran Canaria	San Mateo	445573	3099452			23.38	19.92							21.65	1	1469	Pork
Buteo buteo insularum	2017	Tenerife	La Victoria de Acentejo	357709	3144010	26.70	33.30	59.40	50.60							85	2	1493	Pork
Buteo buteo insularum	2019	Gran Canaria	San Bartolomé de Tirajana	446430	3086617	3.34	4.16									3.75	1	1503	Pork
Buteo buteo insularum	2017	Gran Canaria	San Mateo	444425	3093490	12.02	14.99	131.33	111.87							135.1	2	1705	Cattle
Buteo buteo insularum	2017	Gran Canaria	San Mateo	444425	3093490	9.97	12.43	96.55	82.25	34.67	29.53					132.7	3	1705	Cattle
Buteo buteo insularum	2014	Gran Canaria	Arucas	447168	3111519	56.03	69.89	47.69	40.63	25.11	21.39					130.37	3	1800	Goat/Sheep
Buteo buteo insularum	2014	Gran Canaria	Arucas	447168	3111519											0	0	1800	Goat/Sheep

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Table 1 (*continued*)

RAPTOR SPECIES	YEAR	ISLAND	MUNICIPALITY	UTMX	UTMY	BRODIFACOUM measurement 1	BRODIFACOUM measurement 2	BROMADIOLONE measurement 1	BROMADIOLONE measurement 2	DIFENACOUM measurement 1	DIFENACOUM measurement 2	DIFETHIALONE measurement 1	DIFETHIALONE measurement 2	FLOCOUMAFEN measurement 1	FLOCOUMAFEN measurement 2	SUM OF RODENTICIDES (using averaged measurements)	№ RODENTICIDES	Distance to the nearest farm (m)	Type of livestock
Buteo buteo insularum	2018	Tenerife	Buenavista del Norte	315335	3138528											0	0	1871	Pork
Buteo buteo insularum	2017	Gran Canaria	Valleseco	443073	3099523											0	0	2126	Pork
Buteo buteo insularum	2017	Gran Canaria	Valleseco	443073	3099523	29.01	36.19	80.24	68.36	11.62	12.58					119	3	2126	Pork
Buteo buteo insularum	2011	Gran Canaria	Tejeda	441863	3100156											0	0	2342	Pork
Buteo buteo insularum	2011	Gran Canaria	Tejeda	441863	3100156	8.65	10.79	11.84	12.82							22.05	2	2342	Pork
Buteo buteo insularum	2018	Tenerife	Buenavista del Norte	317195	3139973											0	0	2627	Pork
Buteo buteo insularum	2018	Tenerife	Buenavista del Norte	315602	3139247	9.46	11.80	105.79	114.61							120.83	2	2648	Pork
Buteo buteo insularum	2018	Tenerife	Buenavista del Norte	315913	3139006					9.41	10.19					9.8	1	2648	Pork
Buteo buteo insularum	2011	Gran Canaria	Tejeda	441807	3093073	12.57	15.67	30.84	33.40							46.24	2	2859	Pork
Buteo buteo insularum	2011	Gran Canaria	Tejeda	441807	3093073			1.27	1.37							1.32	1	2859	Pork
Buteo buteo insularum	2017	Gran Canaria	San Bartolomé de Tirajana	439141	3090223	2.23	2.78									2.5	1	3280	Pork
Buteo buteo insularum	2018	Tenerife	Buenavista del Norte	319935	3135622											0	0	3848	Pork
Buteo buteo insularum	2011	Gran Canaria	Artenara	436337	3097463											0	0	3960	Goat/Sheep
Buteo buteo insularum	2018	Fuerteventura	Puerto del Rosario	609021	3152412											0	0	4052	Pork
Buteo buteo insularum	2020	Tenerife	Guía de Isora	327433	3122925											0	0	4322	Goat/sheep
Buteo buteo insularum	2013	Gran Canaria	Artenara	426275	3101266											0	0	5249	Goat/Sheep
Buteo buteo insularum	2020	Lanzarote	Haría	617377	3211234											0	0	6322	Goat/sheep
Buteo buteo insularum	2020	Gran Canaria	Mogan	428829	3083419											0	0	6345	Goat/sheep
Buteo buteo insularum	2018	La Gomera	Hemigua	284932	3115997											0	0	6350	Pork
Buteo buteo insularum	2014	Gran Canaria	Tejeda	434124	3091498											0	0	8395	Pork
Buteo buteo insularum	2019	Gran Canaria	San Bartolomé de Tirajana	431763	3078873											0	0	12700	Pork
Circus aeruginosus	2019	Gran Canaria	Las Palmas de Gran Canaria	457499	3111772											0	0	4015	Pork
Falco eleonorae	2014	Gran Canaria	Tejeda	440918	3098515											0	0	4220	Goat/Sheep
Falco eleonorae	2019	Lanzarote	Arrecife	641223	3204522	3.18	3.74	0.41	0.45							3.89	2	4555	Pork
Falco eleonorae	2019	Lanzarote	Haria	645428	3238810	1.18	1.38									1.28	1	7345	Goat/sheep
Falco eleonorae	2016	Fuerteventura	Pájara	567311	3105914											0	0	19737	Pork

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Table 1 (*continued*)

RAPTOR SPECIES	YEAR	ISLAND	MUNICIPALITY	UTMX	UTMY	BRODIFACOUM measurement 1	BRODIFACOUM measurement 2	BROMADIOLONE measurement 1	BROMADIOLONE measurement 2	DIFENACOUM measurement 1	DIFENACOUM measurement 2	DIFETHIALONE measurement 1	DIFETHIALONE measurement 2	FLOCOUMAFEN measurement 1	FLOCOUMAFEN measurement 2	SUM OF RODENTICIDES (using averaged measurements)	№ RODENTICIDES	Distance to the nearest farm (m)	Type of livestock
Falco peregrinus pelegrinoides	2020	Gran Canaria	Artenara	449358	3101476	1.10	1.30									1.2	1	430	Goat/Sheep
Falco peregrinus pelegrinoides	2019	Lanzarote	Teguise	634517	3215084	5.80	6.80									6.3	1	1343	Pork
Falco peregrinus pelegrinoides	2019	Gran Canaria	Telde	462858	3097310	7.27	8.53	7.10	7.70							15.3	2	1420	Goat/Sheep
Falco peregrinus pelegrinoides	2019	Gran Canaria	Telde	462858	3097310											0	0	1420	Goat/Sheep
Falco peregrinus pelegrinoides	2018	Tenerife	Arico	352926	3115789	3.54	4.16	4.77	5.17							8.82	2	1710	Cattle
Falco peregrinus pelegrinoides	2020	Gran Canaria	Agüimes	453150	3086742											0	0	3213	Goat/sheep
Falco peregrinus pelegrinoides	2019	Gran Canaria	Agüimes	459145	3081405											0	0	3655	Goat/Sheep
Falco peregrinus pelegrinoides	2011	Lanzarote	Tinajo	626292	3212418	29.37	23.07	39.12	33.32							62.44	2	4002	Pork
Falco peregrinus pelegrinoides	2011	Lanzarote	Yaiza	620344	3207276											0	0	4684	Goat/Sheep
Falco peregrinus pelegrinoides	2018	Gran Canaria	Mogan	438231	3089822											0	0	6213	Cattle
Falco peregrinus pelegrinoides	2011	Lanzarote	Teguise	640224	3215519	6.33	4.97									5.65	1	6816	Pork
Falco peregrinus pelegrinoides	2018	Gran Canaria	Mogan	437655	3088876											0	0	7432	Cattle
Falco peregrinus pelegrinoides	2015	Fuerteventura	La Oliva	615073	3180976											0	0	10343	Goat/Sheep
Falco subbuteo	2019	Fuerteventura	La Oliva	568210	3105010											0	0	20240	Pork
Falco tinnunculus canariensis	2019	Gran Canaria	Santa Brígida	456500	3101889	101.55	79.79	25.51	21.73							114.29	2	278	Pork
Falco tinnunculus canariensis	2020	Gran Canaria	Arucas	448651	3109655	109.87	86.33	61.34	52.26							154.9	2	288	Goat/Sheep
Falco tinnunculus canariensis	2020	Gran Canaria	San Bartolomé de Tirajana	443196	3087108	6.61	5.19	33.37	28.43	30.14	32.66			11.66	9.94	79	4	308	Cattle
Falco tinnunculus canariensis	2020	Gran Canaria	Ingenio	460439	3086554	14.09	11.07	4.60	3.92	25.14	27.24					43.03	3	380	Cattle

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Table 1 (*continued*)

RAPTOR SPECIES	YEAR	ISLAND	MUNICIPALITY	UTMX	UTMY	BRODIFACOUM measurement 1	BRODIFACOUM measurement 2	BROMADIOLONE measurement 1	BROMADIOLONE measurement 2	DIFENACOUM measurement 1	DIFENACOUM measurement 2	DIFETHIALONE measurement 1	DIFETHIALONE measurement 2	FLOCOUMAFEN measurement 1	FLOCOUMAFEN measurement 2	SUM OF RODENTICIDES (using averaged measurements)	№ RODENTICIDES	Distance to the nearest farm (m)	Type of livestock
Falco tinnunculus canariensis	2020	Gran Canaria	Telde	461779	3097167	26.61	20.91	36.41	31.01	1.54	1.66			0.50	0.42	59.53	4	405	Goat/Sheep
Falco tinnunculus canariensis	2014	La Palma	Los Llanos de Aridane	216856	3173902	163.56	128.52	419.50	357.36	32.09	34.77					567.9	3	430	Pork
Falco tinnunculus canariensis	2014	Gran Canaria	Santa María de Guía	437007	3107606	97.52	76.62	560.20	477.20							605.77	2	492	Pork
Falco tinnunculus canariensis	2018	La Gomera	San Sebastian de la Gomera	293533	3109569	48.05	37.75			3.46	3.74					46.5	2	505	Pork
Falco tinnunculus canariensis	2014	La Palma	El Paso	224440	3161517											0	0	572	Goat/Sheep
Falco tinnunculus canariensis	2014	La Palma	El Paso	224440	3161517	28.13	35.09	1013.65	1076.35							1076.61	2	572	Goat/Sheep
Falco tinnunculus canariensis	2014	Gran Canaria	Valleseco	443613	3102482	47.56	59.32									53.44	1	695	Cattle
Falco tinnunculus canariensis	2020	Gran Canaria	Tejeda	444550	3092087	30.37	37.87	4.92	5.22	60.83	51.81	8.65	7.37			103.52	4	774	Cattle
Falco tinnunculus canariensis	2020	Gran Canaria	San Bartolomé de Tirajana	448717	3072935	22.70	28.31	26.00	27.60	1.94	1.66	2.59	2.21			56.5	4	820	Goat/Sheep
Falco tinnunculus canariensis	2020	Gran Canaria	Ingenio	456956	3088494	47.97	59.83	54.13	57.47	11.34	9.66					120.2	3	820	Goat/Sheep
Falco tinnunculus canariensis	2019	La Gomera	Hemigua	278774	3117473			14.74	15.66							15.2	1	894	Pork
Falco tinnunculus canariensis	2017	La Gomera	Alajeró	279727	3109537	605.20	754.80	50.44	53.56							732	2	909	Pork
Falco tinnunculus canariensis	2020	Gran Canaria	San Bartolomé de Tirajana	446416	3073529	5.74	7.16	1.12	1.18	1.19	1.01	2.91	2.47			11.39	4	911	Pork
Falco tinnunculus canariensis	2019	Gran Canaria	Las Palmas de Gran Canaria	457740	3100863	62.03	77.37	4.66	4.94							74.5	2	920	Cattle
Falco tinnunculus canariensis	2019	Gran Canaria	Arucas	447882	3112415	38.72	48.29	43.46	46.14	12.10	10.30					99.5	3	930	Goat/Sheep
Falco tinnunculus canariensis	2019	Gran Canaria	Santa Lucía de Tirajana	456584	3080365	15.31	19.09	18.33	19.47	1.51	1.29					37.5	3	950	Cattle

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Table 1 (*continued*)

RAPTOR SPECIES	YEAR	ISLAND	MUNICIPALITY	UTMX	UTMY	BRODIFACOUM measurement 1	BRODIFACOUM measurement 2	BROMADIOLONE measurement 1	BROMADIOLONE measurement 2	DIFENACOUM measurement 1	DIFENACOUM measurement 2	DIFETHIALONE measurement 1	DIFETHIALONE measurement 2	FLOCOUMAFEN measurement 1	FLOCOUMAFEN measurement 2	SUM OF RODENTICIDES (using averaged measurements)	№ RODENTICIDES	Distance to the nearest farm (m)	Type of livestock
Falco tinnunculus canariensis	2020	Gran Canaria	San Bartolomé de Tirajana	440413	3073126	1.25	1.55	7.84	9.78	0.52	0.44	0.54	0.58			11.25	4	1023	Cattle
Falco tinnunculus canariensis	2020	Gran Canaria	San Bartolomé de Tirajana	441025	3072520	179.10	223.38	26.99	33.67	9.54	8.12					240.4	3	1120	Goat/Sheep
Falco tinnunculus canariensis	2014	La Palma	Los Llanos de Aridane	215137	3165875			1833.40	2286.60							2060	1	1123	Goat/Sheep
Falco tinnunculus canariensis	2014	La Palma	El Paso	219297	3172571			20.60	25.70							23.15	1	1266	Goat/Sheep
Falco tinnunculus canariensis	2014	La Palma	El Paso	224341	3162500			4307.60	5372.40							4840	1	1311	Pork
Falco tinnunculus canariensis	2020	Gran Canaria	Arucas	447238	3106246	56.37	66.17	3.85	4.81	0.46	0.40					66.03	3	1321	Cattle
Falco tinnunculus canariensis	2014	Gran Canaria	Firgas	443790	3109207	818.42	960.76	219.49	273.75							1136.21	2	1348	Goat/Sheep
Falco tinnunculus canariensis	2017	Tenerife	Los Realejos	343970	3142281											0	0	1352	Pork
Falco tinnunculus canariensis	2017	Tenerife	Tacoronte	362702	3148625			12.64	15.76							14.2	1	1385	Cattle
Falco tinnunculus canariensis	2018	Tenerife	La Orotava	351940	3140026	1.29	1.51			1.57	1.67					3.02	2	1423	Pork
Falco tinnunculus canariensis	2015	Gran Canaria	Las Palmas de Gran Canaria	452395	3110907	71.41	83.83	328.14	409.26							446.32	2	1430	Goat/Sheep
Falco tinnunculus canariensis	2018	Tenerife	Icod de los Vinos	332042	3136867	3.52	4.14	4.57	5.69							8.96	2	1463	Cattle
Falco tinnunculus canariensis	2016	Gran Canaria	Santa Brígida	453909	3102429	1079.69	1267.47	170.49	212.63							1365.14	2	1640	Goat/Sheep
Falco tinnunculus canariensis	2016	Gran Canaria	Santa Brígida	453976	3102516	909.15	1067.27									988.21	1	1640	Goat/Sheep

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Table 1 (*continued*)

RAPTOR SPECIES	YEAR	ISLAND	MUNICIPALITY	UTMX	UTMY	BRODIFACOUM measurement 1	BRODIFACOUM measurement 2	BROMADIOLONE measurement 1	BROMADIOLONE measurement 2	DIFENACOUM measurement 1	DIFENACOUM measurement 2	DIFETHIALONE measurement 1	DIFETHIALONE measurement 2	FLOCOUMAFEN measurement 1	FLOCOUMAFEN measurement 2	SUM OF RODENTICIDES (using averaged measurements)	№ RODENTICIDES	Distance to the nearest farm (m)	Type of livestock
Falco tinnunculus canariensis	2017	La Gomera	Alajeró	279894	3110877	41.40	48.60	64.32	69.68							112	2	1718	Pork
Falco tinnunculus canariensis	2014	La Palma	Tazacorte	212224	3173709	11.93	14.01	75.64	81.94							91.76	2	1732	Goat/Sheep
Falco tinnunculus canariensis	2018	Tenerife	Garachico	329541	3139807	9.90	11.62	160.61	173.99	4.41	4.69					182.61	3	1788	Cattle
Falco tinnunculus canariensis	2018	Tenerife	El Tanque	325971	3138235	2.71	3.19	12.43	13.47							15.9	2	1836	Cattle
Falco tinnunculus canariensis	2018	Gran Canaria	Agaete	434493	3103475											0	0	1901	Goat/Sheep
Falco tinnunculus canariensis	2014	Gran Canaria	Telde	459249	3097315	11.92	14.00	281.54	305.00	23.90	25.38					330.87	3	1966	Goat/Sheep
Falco tinnunculus canariensis	2016	Gran Canaria	Galdar	438114	3103429			37.34	40.46							38.9	1	2167	Cattle
Falco tinnunculus canariensis	2017	Tenerife	Guía de Isora	324346	3123508	840.00	660.00	27.84	30.16							779	2	2332	Goat/Sheep
Falco tinnunculus canariensis	2017	Tenerife	Guía de Isora	324346	3123508	403.20	316.80	172.80	187.20							540	2	2332	Goat/Sheep
Falco tinnunculus canariensis	2018	La Gomera	San Sebastian de la Gomera	283452	3110317			581.76	630.24							606	1	2980	Pork
Falco tinnunculus canariensis	2017	Tenerife	Santiago del Teide	319264	3124971											0	0	3200	Goat/Sheep
Falco tinnunculus canariensis	2016	Gran Canaria	Las Palmas de Gran Canaria	458680	3104557	3.73	2.93	2.52	2.72							5.95	2	3601	Pork
Falco tinnunculus canariensis	2016	Gran Canaria	Las Palmas de Gran Canaria	458981	3106517											0	0	3601	Pork
Falco tinnunculus canariensis	2016	Gran Canaria	Las Palmas de Gran Canaria	458981	3106517											0	0	3601	Pork
Falco tinnunculus canariensis	2016	Gran Canaria	Las Palmas de Gran Canaria	458981	3106517											0	0	3601	Pork
Falco tinnunculus canariensis	2016	Gran Canaria	Las Palmas de Gran Canaria	458981	3106517											0	0	3601	Pork

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Table 1 (*continued*)

RAPTOR SPECIES	YEAR	ISLAND	MUNICIPALITY	UTMX	UTMY	BRODIFACOUM measurement 1	BRODIFACOUM measurement 2	BROMADIOLONE measurement 1	BROMADIOLONE measurement 2	DIFENACOUM measurement 1	DIFENACOUM measurement 2	DIFETHIALONE measurement 1	DIFETHIALONE measurement 2	FLOCOUMAFEN measurement 1	FLOCOUMAFEN measurement 2	SUM OF RODENTICIDES (using averaged measurements)	№ RODENTICIDES	Distance to the nearest farm (m)	Type of livestock
Falco tinnunculus canariensis	2018	Tenerife	El Tanque	325000	3136221											0	0	3685	Cattle
Falco tinnunculus canariensis	2020	La Palma	Mazo	225396	3180471											0	0	3876	Goat/sheep
Falco tinnunculus canariensis	2020	Tenerife	El Tanque	337685	3137794											0	0	3965	Cattle
Falco tinnunculus canariensis	2020	Gran Canaria	Tejeda	444533	3071230											0	0	4380	Goat/Sheep
Falco tinnunculus canariensis	2017	Tenerife	Santa Cruz de Tenerife	323246	3127519	728.00	572.00	121.18	103.22							762.2	2	4460	Goat/Sheep
Falco tinnunculus canariensis	2017	Tenerife	Santa Cruz de Tenerife	323246	3127519	350.45	275.35	191.05	162.75							489.8	2	4460	Goat/Sheep
Falco tinnunculus canariensis	2017	Tenerife	El Tanque	324534	3134856											0	0	5070	Cattle
Falco tinnunculus canariensis	2017	Tenerife	El Tanque	324448	3134743											0	0	5160	Cattle
Falco tinnunculus canariensis	2014	Gran Canaria	San Mateo	444338	3095625	534.00	666.00									600	1	5279	Goat/Sheep
Falco tinnunculus canariensis	2020	Gran Canaria	San Bartolomé de Tirajana	441804	3069539											0	0	5432	Goat/sheep
Falco tinnunculus canariensis	2018	Tenerife	Buenavista del Norte	320372	3129173											0	0	6152	Goat/Sheep
Falco tinnunculus canariensis	2017	La Gomera	Hermigua	284055	3117368											0	0	6200	Goat/Sheep
Falco tinnunculus canariensis	2017	Tenerife	El Tanque	323743	3134459			10.04	8.56							9.3	1	6321	Pork
Falco tinnunculus canariensis	2018	Tenerife	Vilaflor	338727	3115304											0	0	6427	Cattle
Falco tinnunculus canariensis	2018	La Gomera	Alajeró	282492	3102551	34.43	42.93									38.68	1	6508	Pork
Falco tinnunculus canariensis	2018	Tenerife	Santiago del Teide	325564	3130959											0	0	7848	Cattle

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Table 1 (*continued*)

RAPTOR SPECIES	YEAR	ISLAND	MUNICIPALITY	UTMX	UTMY	BRODIFACOUM measurement 1	BRODIFACOUM measurement 2	BROMADIOLONE measurement 1	BROMADIOLONE measurement 2	DIFENACOUM measurement 1	DIFENACOUM measurement 2	DIFETHIALONE measurement 1	DIFETHIALONE measurement 2	FLOCOUMAFEN measurement 1	FLOCOUMAFEN measurement 2	SUM OF RODENTICIDES (using averaged measurements)	№ RODENTICIDES	Distance to the nearest farm (m)	Type of livestock
Falco tinnunculus canariensis	2017	Tenerife	Santa Cruz de Tenerife	378838	3158973											0	0	10177	Pork
Falco tinnunculus canariensis	2017	Tenerife	Santa Cruz de Tenerife	378875	3158830											0	0	10177	Pork
Falco tinnunculus dacotiae	2019	Fuerteventura	Antigua	596424	3140592	24.83	30.97	31.64	26.96	2.04	2.16					59.3	3	497	Pork
Falco tinnunculus dacotiae	2019	Lanzarote	Haría	636900	3219658	190.10	237.10	49.46	42.14							259.4	2	1376	Pork
Falco tinnunculus dacotiae	2016	Lanzarote	Tinajo	629815	3214304			72.62	61.86							67.24	1	1493	Pork
Falco tinnunculus dacotiae	2019	Fuerteventura	La Oliva	608201	3172036											0	0	2312	Goat/Sheep
Falco tinnunculus dacotiae	2020	Fuerteventura	La Oliva	594156	3160227											0	0	2378	Cattle
Falco tinnunculus dacotiae	2020	Fuerteventura	Puerto del Rosario	604782	3153957											0	0	3156	Pork
Falco tinnunculus dacotiae	2019	Fuerteventura	Puerto del Rosario	609610	3152448	30.97	38.63	3.68	3.14	4.01	5.00					42.71	3	4104	Pork
Falco tinnunculus dacotiae	2011	Lanzarote	Tinajo	626294	3212311											0	0	4244	Pork
Falco tinnunculus dacotiae	2012	Lanzarote	Yaiza	613978	3208223											0	0	4480	Pork
Falco tinnunculus dacotiae	2019	Lanzarote	Teguise	640583	3216566											0	0	5617	Pork
Falco tinnunculus dacotiae	2020	La Gomera	Hemigua	287716	3114182											0	0	5789	Goat/sheep
Falco tinnunculus dacotiae	2017	Fuerteventura	Pájara	585926	3137560	11.13	13.88	3.15	3.35	1.07	1.33					16.95	3	8000	Goat/Sheep
Falco tinnunculus dacotiae	2020	Fuerteventura	Pájara	567931	3108609											0	0	8731	Goat/sheep
Falco tinnunculus dacotiae	2014	Fuerteventura	Pájara	581964	3137545	9.93	12.39									11.16	1	9275	Goat/Sheep

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Table 1 (*continued*)

RAPTOR SPECIES	YEAR	ISLAND	MUNICIPALITY	UTMX	UTMY	BRODIFACOUM measurement 1	BRODIFACOUM measurement 2	BROMADIOLONE measurement 1	BROMADIOLONE measurement 2	DIFENACOUM measurement 1	DIFENACOUM measurement 2	DIFETHIALONE measurement 1	DIFETHIALONE measurement 2	FLOCOUMAFEN measurement 1	FLOCOUMAFEN measurement 2	SUM OF RODENTICIDES (using averaged measurements)	№ RODENTICIDES	Distance to the nearest farm (m)	Type of livestock
Falco tinnunculus dacotiae	2018	Lanzarote	Yaiza	613971	3208465											0	0	9596	Pork
Neophron percnopterus majorensis	2016	Fuerteventura	Tuineje	596547	3123216	124.57	146.23			11.06	13.80					147.83	2	204	Pork
Neophron percnopterus majorensis	2013	Gran Canaria	Las Palmas de Gran Canaria	455338	3104877											0	0	338	Goat/Sheep
Neophron percnopterus majorensis	2016	Fuerteventura	Tuineje	596547	3123216	21.30	25.00	74.91	79.55							100.38	2	455	Pork
Neophron percnopterus majorensis	2020	Fuerteventura	Pájara	560849	3108460	12.31	14.45	5.92	6.28							19.48	2	942	Pork
Neophron percnopterus majorensis	2017	Fuerteventura	Betancuria	590940	3143771	36.52	42.88									39.7	1	1080	Goat/Sheep
Neophron percnopterus majorensis	2016	Fuerteventura	Puerto del Rosario	607315	3150965			15.20	16.14							15.67	1	1125	Pork
Neophron percnopterus majorensis	2016	Fuerteventura	Puerto del Rosario	606445	3156687	26.59	31.21			2.38	2.96					31.57	2	1125	Pork
Neophron percnopterus majorensis	2016	Fuerteventura	Puerto del Rosario	607315	3150965											0	0	1125	Pork
Neophron percnopterus majorensis	2015	Fuerteventura	La Oliva	604823	3166247											0	0	1257	Goat/Sheep
Neophron percnopterus majorensis	2015	Fuerteventura	La Oliva	604823	3166247			41.97	44.57							43.27	1	1257	Goat/Sheep
Neophron percnopterus majorensis	2015	Fuerteventura	La Oliva	604823	3166247											0	0	1257	Goat/Sheep
Neophron percnopterus majorensis	2015	Fuerteventura	La Oliva	604823	3166247	30.77	36.13	28.03	29.77							62.35	2	1257	Goat/Sheep
Neophron percnopterus majorensis	2015	Fuerteventura	La Oliva	604823	3166247											0	0	1257	Goat/Sheep
Neophron percnopterus majorensis	2015	Fuerteventura	La Oliva	604823	3166247	70.42	82.66			18.79	23.43					97.65	2	1257	Goat/Sheep
Neophron percnopterus majorensis	2019	Fuerteventura	Pájara	607688	3159978	13.34	15.66	2.72	2.88							17.3	2	1294	Pork

(*continued on next page*)

Table 1 (*continued*)

RAPTOR SPECIES	YEAR	ISLAND	MUNICIPALITY	UTMX	UTMY	BRODIFACOUM measurement 1	BRODIFACOUM measurement 2	BROMADIOLONE measurement 1	BROMADIOLONE measurement 2	DIFENACOUM measurement 1	DIFENACOUM measurement 2	DIFETHIALONE measurement 1	DIFETHIALONE measurement 2	FLOCOUMAFEN measurement 1	FLOCOUMAFEN measurement 2	SUM OF RODENTICIDES (using averaged measurements)	№ RODENTICIDES	Distance to the nearest farm (m)	Type of livestock
Neophron percnopterus majorensis	2017	Fuerteventura	Antigua	598898	3143771	162.38	190.62									176.5	1	1320	Pork
Neophron percnopterus majorensis	2016	Fuerteventura	Puerto del Rosario	590936	3142556											0	0	1371	Goat/Sheep
Neophron percnopterus majorensis	2016	Fuerteventura	Puerto del Rosario	590936	3142556			36.69	45.77							41.23	1	1371	Goat/Sheep
Neophron percnopterus majorensis	2016	Fuerteventura	Antigua	605541	3130700											0	0	1397	Cattle
Neophron percnopterus majorensis	2016	Fuerteventura	Antigua	605541	3130700	29.77	37.13	100.08	124.82							145.9	2	1397	Cattle
Neophron percnopterus majorensis	2016	Fuerteventura	Puerto del Rosario	604312	3150277			28.59	35.65							32.12	1	1402	Pork
Neophron percnopterus majorensis	2016	Fuerteventura	Puerto del Rosario	611720	3155801											0	0	1434	Pork
Neophron percnopterus majorensis	2016	Fuerteventura	Puerto del Rosario	611720	3155801	11.77	14.69	29.56	36.86							46.44	2	1434	Pork
Neophron percnopterus majorensis	2017	Fuerteventura	Puerto del Rosario	607305	3150431			40.65	50.69							45.67	1	1514	Pork
Neophron percnopterus majorensis	2018	Fuerteventura	Puerto del Rosario	608263	3160015	8.28	10.32	88.91	110.89							109.2	2	1614	Pork
Neophron percnopterus majorensis	2017	Fuerteventura	Puerto del Rosario	596714	3153054											0	0	1703	Pork
Neophron percnopterus majorensis	2017	Fuerteventura	Puerto del Rosario	596714	3153054			11.76	14.66							13.21	1	1703	Pork
Neophron percnopterus majorensis	2018	Fuerteventura	Tuineje	596478	3134487											0	0	1822	Goat/Sheep
Neophron percnopterus majorensis	2017	Fuerteventura	Tuineje	587076	3127897											0	0	1850	Goat/Sheep
Neophron percnopterus majorensis	2018	Fuerteventura	Puerto del Rosario	606387	3150575	86.06	107.34									96.7	1	1997	Pork
Neophron percnopterus majorensis	2019	Fuerteventura	Puerto del Rosario	609021	3150578											0	0	2374	Pork
Neophron percnopterus majorensis	2016	Fuerteventura	La Oliva	612467	3167795											0	0	3266	Goat/Sheep
Neophron percnopterus majorensis	2012	Fuerteventura	La Oliva	604784	3171358											0	0	3758	Goat/Sheep

(*continued on next page*)

Table 1 (*continued*)

RAPTOR SPECIES	YEAR	ISLAND	MUNICIPALITY	UTMX	UTMY	BRODIFACOUM measurement 1	BRODIFACOUM measurement 2	BROMADIOLONE measurement 1	BROMADIOLONE measurement 2	DIFENACOUM measurement 1	DIFENACOUM measurement 2	DIFETHIALONE measurement 1	DIFETHIALONE measurement 2	FLOCOUMAFEN measurement 1	FLOCOUMAFEN measurement 2	SUM OF RODENTICIDES (using averaged measurements)	№ RODENTICIDES	Distance to the nearest farm (m)	Type of livestock
Neophron percnopterus majorensis	2015	Fuerteventura	La Oliva	604823	3166247											0	0	3787	Goat/Sheep
Neophron percnopterus majorensis	2015	Fuerteventura	La Oliva	604823	3166247											0	0	3787	Goat/Sheep
Neophron percnopterus majorensis	2015	Fuerteventura	La Oliva	604823	3166247											0	0	3787	Goat/Sheep
Neophron percnopterus majorensis	2015	Fuerteventura	La Oliva	604823	3166247											0	0	3787	Goat/Sheep
Neophron percnopterus majorensis	2019	Fuerteventura	Puerto del Rosario	607885	3144275											0	0	4320	Goat/Sheep
Neophron percnopterus majorensis	2017	Fuerteventura	Puerto del Rosario	611889	3160520											0	0	4320	Pork
Neophron percnopterus majorensis	2016	Fuerteventura	La Oliva	611994	3169859											0	0	4322	Goat/Sheep
Neophron percnopterus majorensis	2016	Fuerteventura	La Oliva	599608	3168201											0	0	5137	Goat/Sheep
Neophron percnopterus majorensis	2017	Fuerteventura	Puerto del Rosario	609614	3144261											0	0	5311	Pork
Neophron percnopterus majorensis	2017	Fuerteventura	Tuineje	593053	3140264											0	0	5438	Goat/Sheep
Neophron percnopterus majorensis	2017	Fuerteventura	Antigua	608244	3142737											0	0	5783	Goat/Sheep
Neophron percnopterus majorensis	2017	Fuerteventura	Pájara	587946	3134649											0	0	5890	Goat/Sheep
Neophron percnopterus majorensis	2019	Lanzarote	Teguise	645605	3218612											0	0	6023	Goat/Sheep
Neophron percnopterus majorensis	2017	Fuerteventura	La Oliva	598129	3168975											0	0	6214	Pork
Neophron percnopterus majorensis	2019	Fuerteventura	La Oliva	597873	3166603											0	0	6251	Goat/Sheep
Neophron percnopterus majorensis	2017	Fuerteventura	Puerto del Rosario	605347	3134467											0	0	6450	Goat/Sheep
Neophron percnopterus majorensis	2017	Fuerteventura	Pájara	585159	3141910											0	0	6580	Goat/Sheep
Neophron percnopterus majorensis	2019	Fuerteventura	Puerto del Rosario	604194	3130953											0	0	6790	Goat/Sheep
Neophron percnopterus majorensis	2017	Fuerteventura	Tuineje	608844	3137625											0	0	7125	Goat/Sheep
Neophron percnopterus majorensis	2017	Fuerteventura	Pájara	583630	3134807											0	0	7230	Goat/Sheep

(*continued on next page*)

Table 1 (*continued*)

RAPTOR SPECIES	YEAR	ISLAND	MUNICIPALITY	UTMX	UTMY	BRODIFACOUM measurement 1	BRODIFACOUM measurement 2	BROMADIOLONE measurement 1	BROMADIOLONE measurement 2	DIFENACOUM measurement 1	DIFENACOUM measurement 2	DIFETHIALONE measurement 1	DIFETHIALONE measurement 2	FLOCOUMAFEN measurement 1	FLOCOUMAFEN measurement 2	SUM OF RODENTICIDES (using averaged measurements)	№ RODENTICIDES	Distance to the nearest farm (m)	Type of livestock
Neophron percnopterus majorensis	2018	Fuerteventura	Pájara	573786	3115739											0	0	7719	Pork
Neophron percnopterus majorensis	2015	Fuerteventura	La Oliva	604823	3166247											0	0	7750	Goat/Sheep
Neophron percnopterus majorensis	2015	Fuerteventura	La Oliva	604823	3166247											0	0	7750	Goat/Sheep
Neophron percnopterus majorensis	2020	Fuerteventura	Pájara	560849	3109790											0	0	8201	Goat/Sheep
Neophron percnopterus majorensis	2020	Fuerteventura	Pájara	561500	3108762											0	0	8201	Goat/Sheep
Neophron percnopterus majorensis	2020	Fuerteventura	Pájara	560847	3108070											0	0	8201	Goat/Sheep
Neophron percnopterus majorensis	2020	Fuerteventura	Pájara	562108	3108323											0	0	8201	Goat/Sheep
Neophron percnopterus majorensis	2020	Fuerteventura	Pájara	561541	3108920											0	0	8201	Goat/Sheep
Neophron percnopterus majorensis	2020	Fuerteventura	Pájara	562037	3108820											0	0	8201	Goat/Sheep
Neophron percnopterus majorensis	2011	Fuerteventura	Tuineje	590891	3124411	12.21	9.59	20.29	21.99							32.04	2	8240	Pork
Neophron percnopterus majorensis	2020	Fuerteventura	Pájara	582566	311811											0	0	8544	Goat/Sheep
Neophron percnopterus majorensis	2020	Fuerteventura	Puerto del Rosario	603683	3153678											0	0	9716	Goat/sheep
Neophron percnopterus majorensis	2015	Fuerteventura	Pájara	581745	3137832											0	0	9870	Goat/Sheep
Neophron percnopterus majorensis	2017	Fuerteventura	Pájara	562439	3109174											0	0	20147	Pork
Tyto alba alba	2020	Gran Canaria	Galdar	438947	3108983	14.29	11.23	3.08	3.34	1.11	1.39					17.22	3	811	Pork
Tyto alba alba	2019	Gran Canaria	Galdar	437861	3109172	5.60	4.40	1.54	1.66	13.88	17.32					22.2	3	867	Cattle
Tyto alba alba	2020	Gran Canaria	Galdar	432671	3116177	134.06	105.34									119.7	1	1104	Pork
Tyto alba alba	2020	Gran Canaria	Santa Brígida	451999	3100544											0	0	1480	Goat/Sheep
Tyto alba alba	2019	Gran Canaria	Las Palmas de Gran Canaria	450683	3107583	4.82	3.78									4.3	1	3325	Goat/Sheep
Tyto alba gracilirostris	2011	Lanzarote	Yaiza	623148	3198152	0.20	0.16	0.22	0.24	0.04	0.04					0.45	3	602	Pork
Tyto alba gracilirostris	2019	Fuerteventura	Puerto del Rosario	597146	3152290	12.21	9.59									10.9	1	1400	Pork
Tyto alba gracilirostris	2011	Lanzarote	Yaiza	619010	3203526	12.77	10.03	32.06	34.74			1.13	1.19			45.96	3	1423	Pork

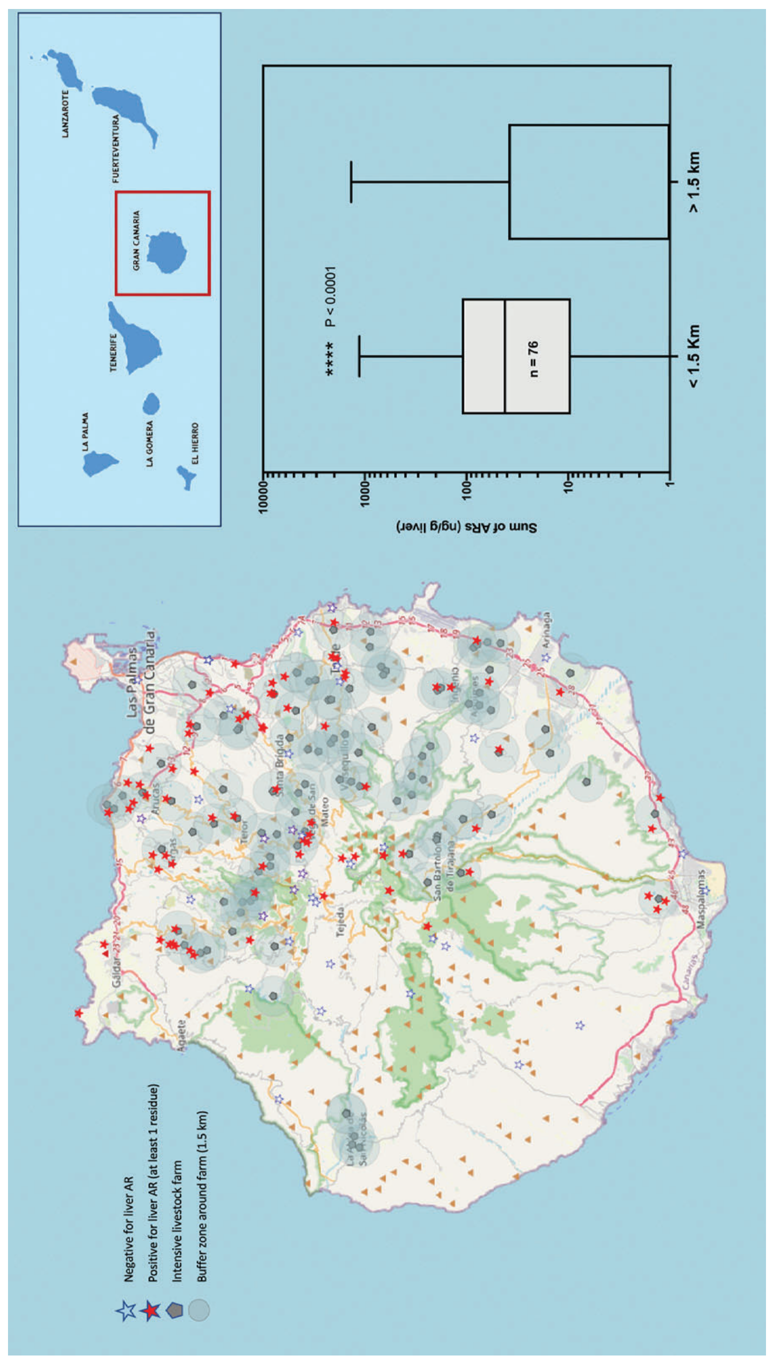

Fig. 1. Map of the Island of Gran Canaria. The location of the cases of raptors positive to oral anticoagulants (red stars), the negative ones (white stars) and the cattle farms surrounded by a buffer zone of 1.5 km radius are shown. On the right, a box and whiskers graph shows the statistical comparison between the two groups of animals, found inside or outside the buffer zone of the farms. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

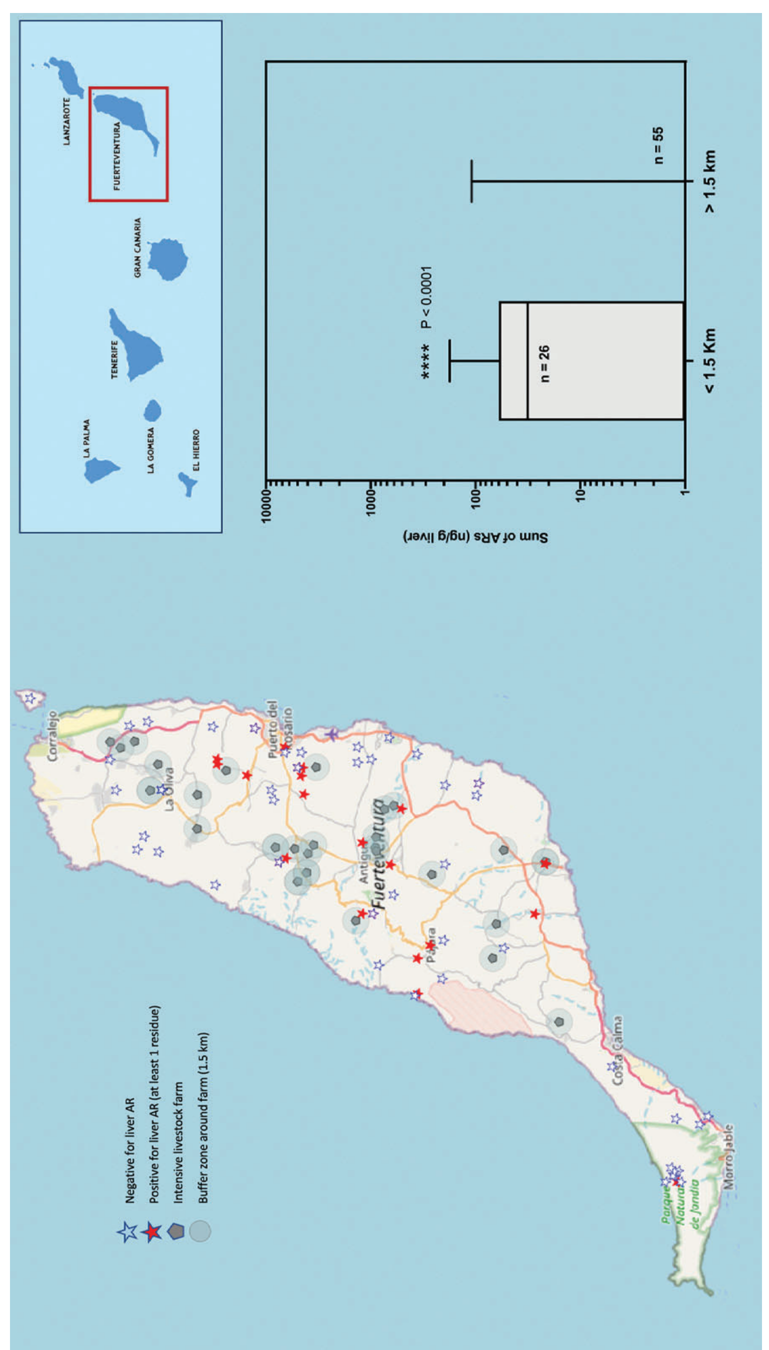

Fig. 2. Map of the Island of Fuerteventura. The location of the cases of raptors positive to oral anticoagulants (red stars), the negative ones (white stars) and the cattle farms surrounded by a buffer zone of 1.5 km radius are shown. On the right, a box and whiskers graph shows the statistical comparison between the two groups of animals, found inside or outside the buffer zone of the farms. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

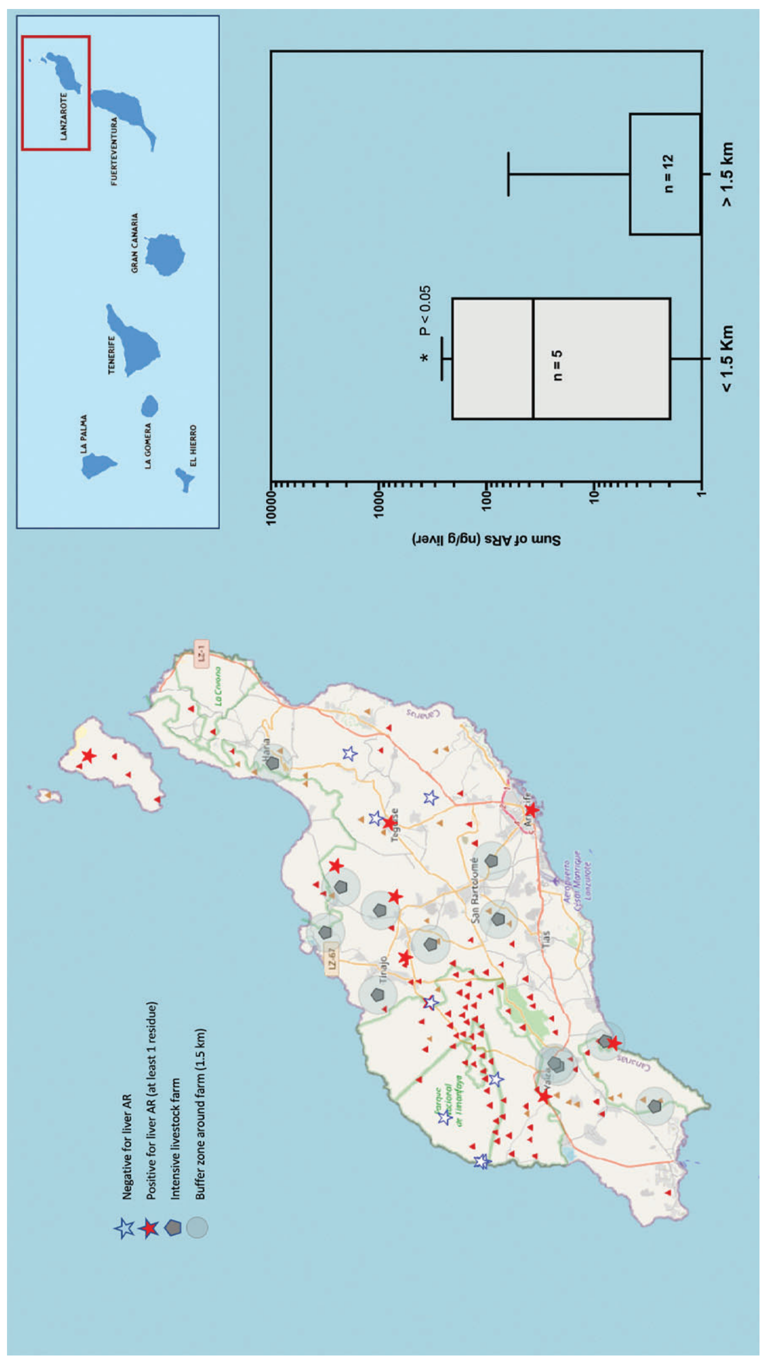

Fig. 3. Map of the Islands of Lanzarote and La Graciosa. The location of the cases of raptors positive to oral anticoagulants (red stars), the negative ones (white stars) and the cattle farms surrounded by a buffer zone of 1.5 km radius are shown. On the right, a box and whiskers graph shows the statistical comparison between the two groups of animals, found inside or outside the buffer zone of the farms. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

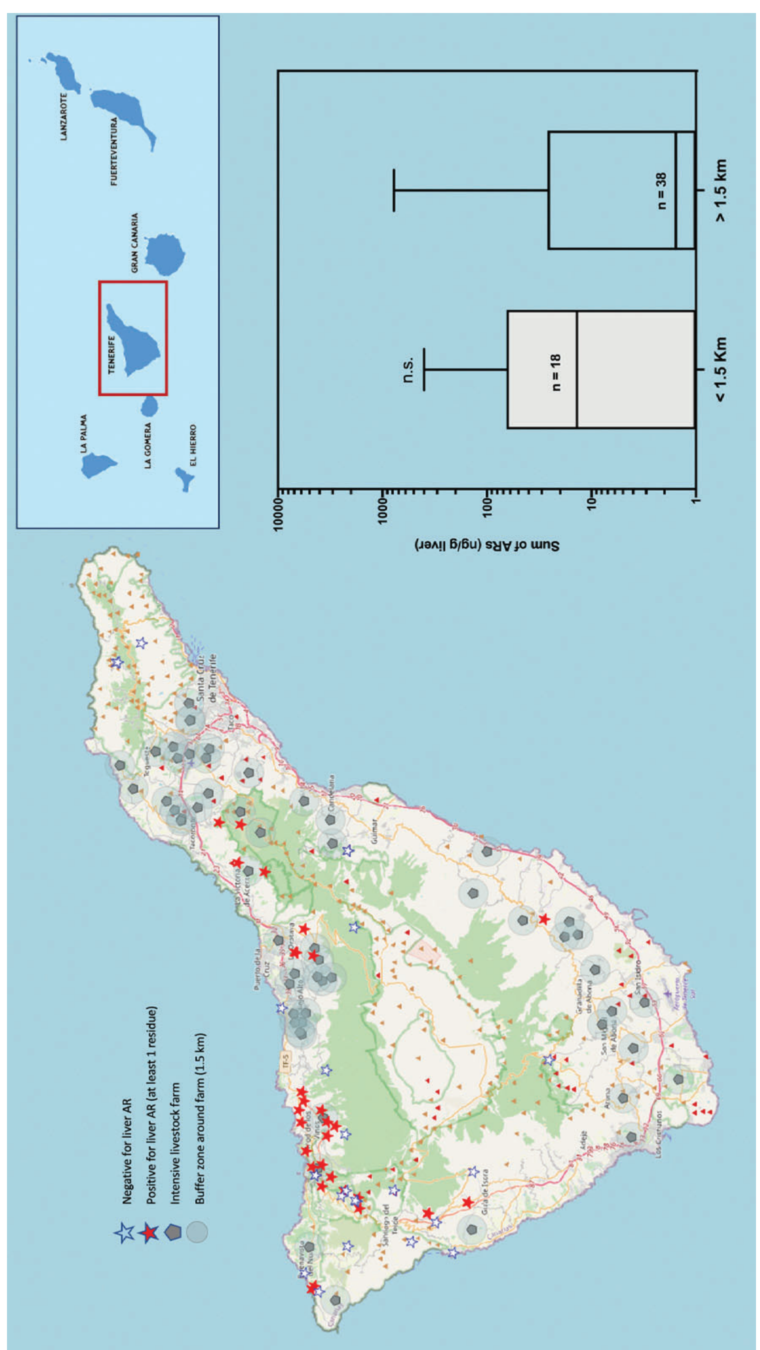

Fig. 4. Map of the Island of Tenerife. The location of the cases of raptors positive to oral anticoagulants (red stars), the negative ones (white stars) and the cattle farms surrounded by a buffer zone of 1.5 km radius are shown. On the right, a box and whiskers graph shows the statistical comparison between the two groups of animals, found inside or outside the buffer zone of the farms. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

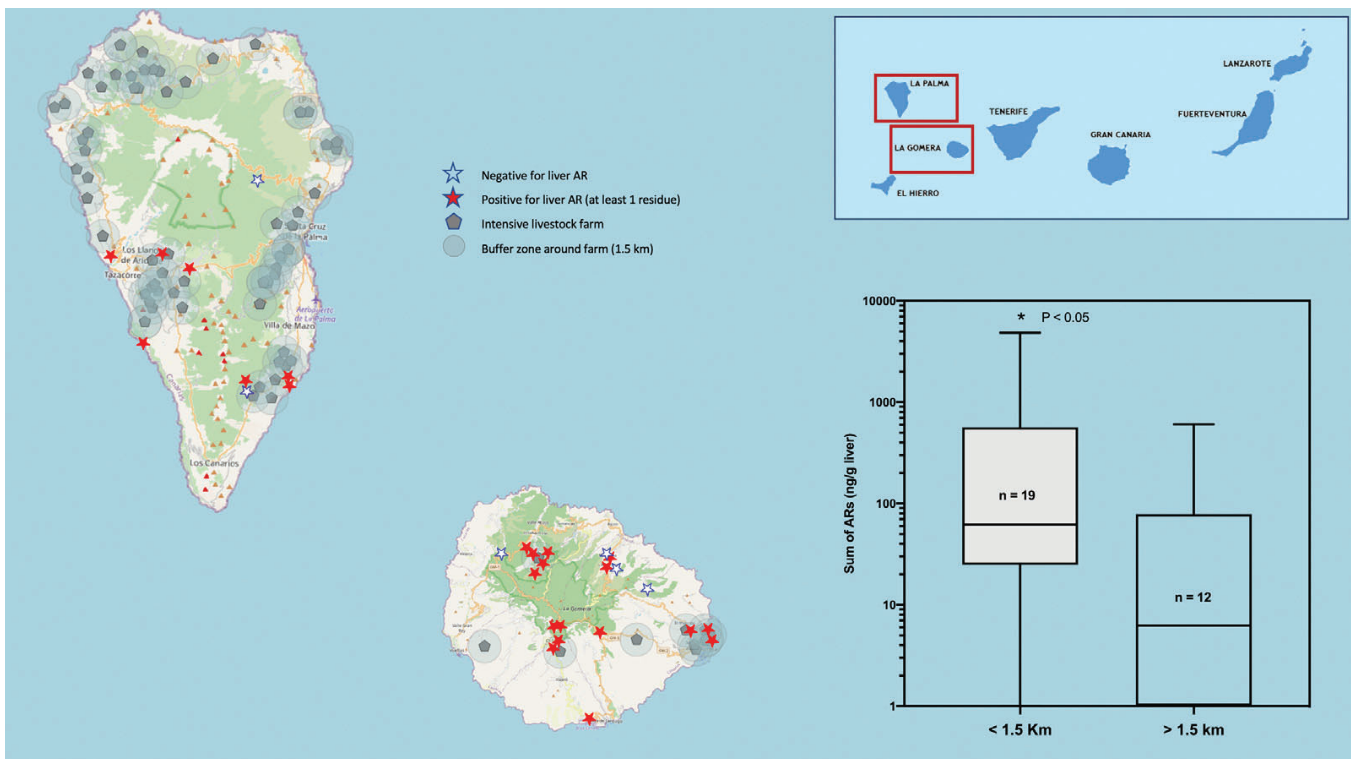

Fig. 5. Map of the Islands of La Palma and La Gomera. The location of the cases of raptors positive to oral anticoagulants (red stars), the negative ones (white stars) and the cattle farms surrounded by a buffer zone of 1.5 km radius are shown. On the right, a box and whiskers graph shows the statistical comparison between the two groups of animals, found inside or outside the buffer zone of the farms. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

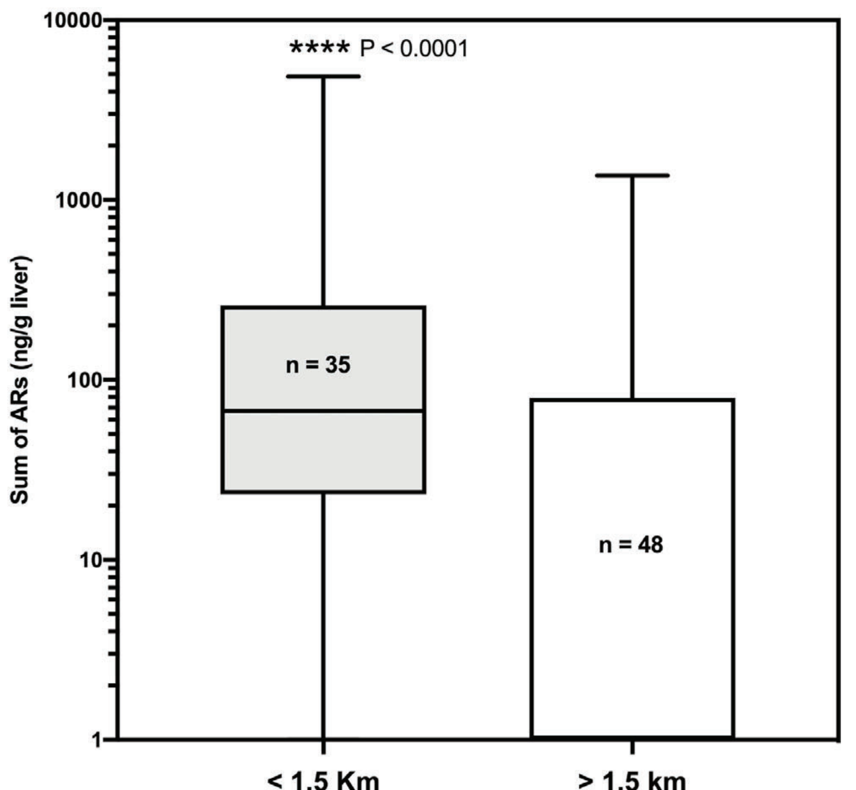

Fig. 6. Box and whiskers graph showing the statistical comparison between the two groups of common buzzards (*Buteo buteo insularum*), found inside or outside the buffer zone of the farms.

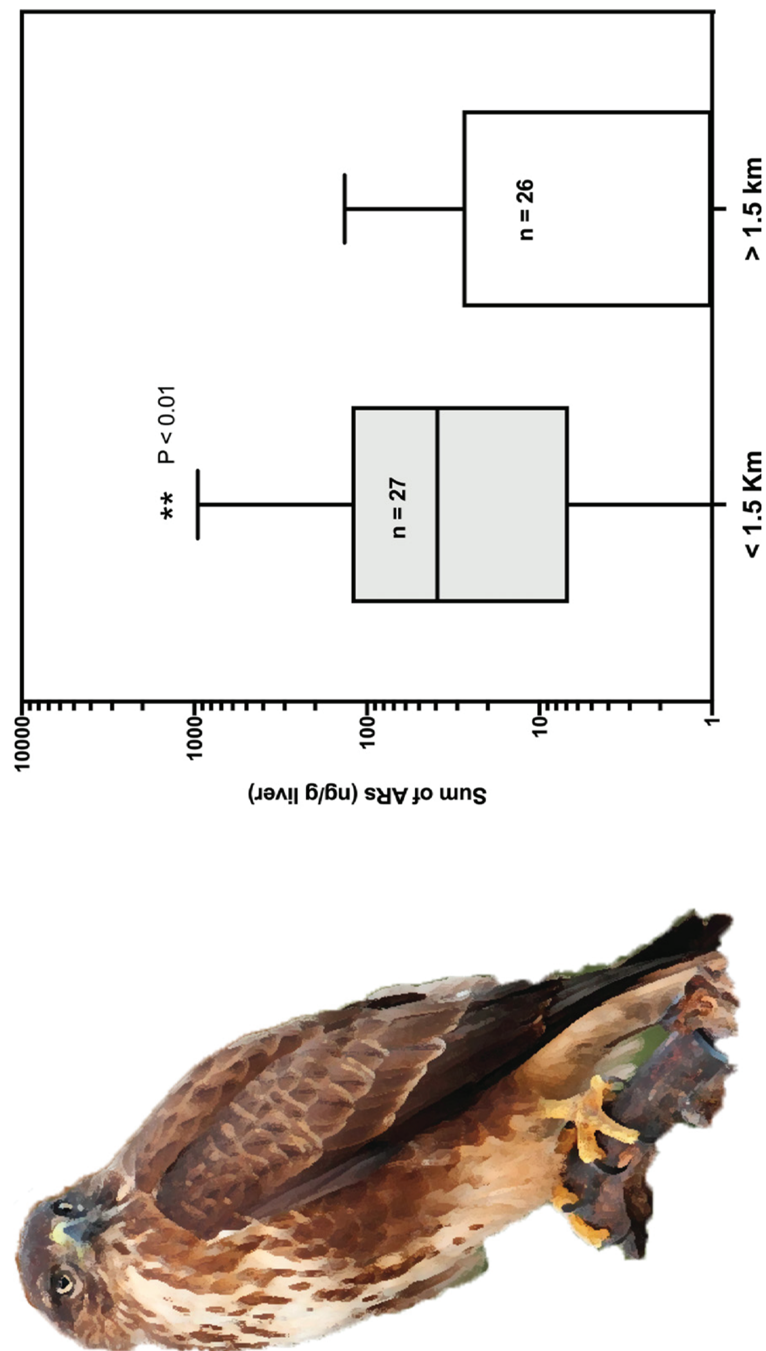

Fig. 7. Box and whiskers graph showing the statistical comparison between the two groups of common kestrels (*Falco tinnunculus canariensis* and *dacotiae*), found inside or outside the buffer zone of the farms.

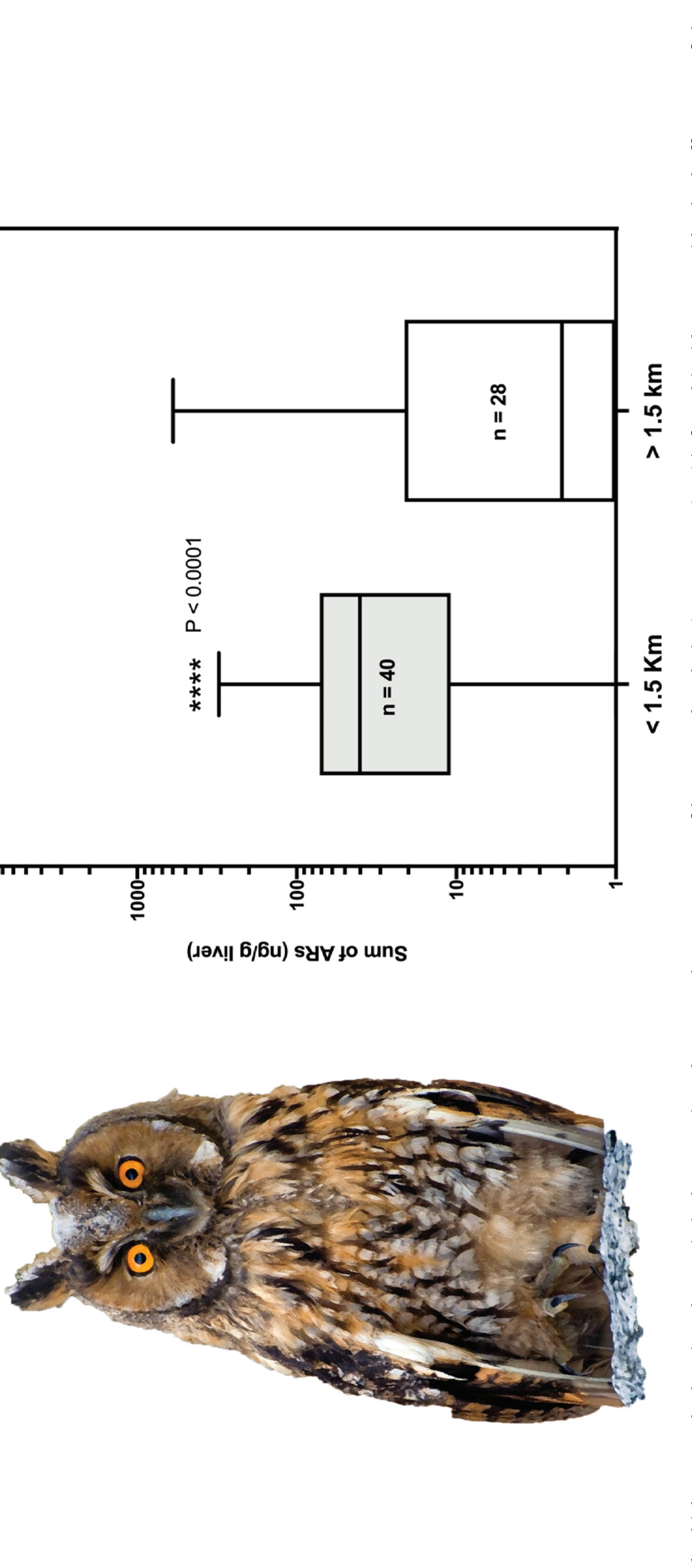

Fig. 8. Box and whiskers graph showing the statistical comparison between the two groups of long-eared owls (*Asio otus canariensis*), found inside or outside the buffer zone of the farms.

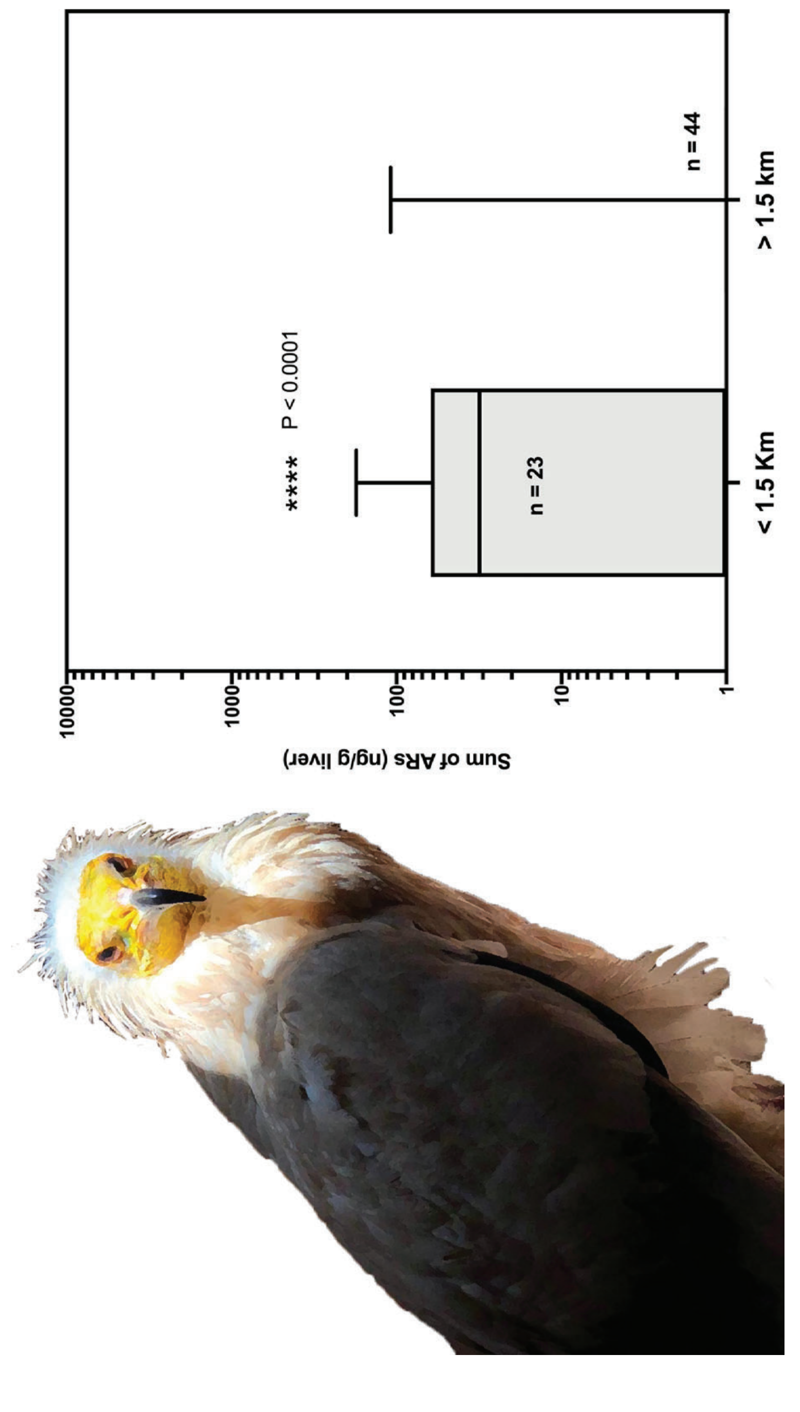

Fig. 9. Box and whiskers graph showing the statistical comparison between the two groups of Egyptian vultures (*Neophron percnopterus majorensis*), found inside or outside the buffer zone of the farms.

The corpses or liver samples were received in the ULPGC Toxicology Laboratory for forensic toxicological evaluations. Only those birds that had georeferenced information about the place where they were found and furthermore, the good state of conservation of the animals allowed the sampling of the liver were included. The series of raptors included 308 individuals from 13 different species/subspecies: *Accipiter nisus granti* ($n=9$); *Actitis hypoleucos* ($n=1$); *Asio otus canariensis* ($n=68$); *Buteo buteo insularum* ($n=53$); *Circus aeruginosus* (n = 1); *Falco eleonorae* (n = 4); *Falco peregrinus pelegrinoides* (n = 13); *Falco subbuteo* (n = 1); *Falco tinnunculus canariensis* ($n=69$); *Falco tinnunculus dacotiae* (n = 14); *Neophron percnopterus majorensis* (n = 67); *Tyto alba alba* (n = 5); and *Tyto alba gracilirostris* ($n=3$). The animals were sent by environmental officers or patrols if found dead, or by wildlife recovery centers if they had been admitted alive but euthanized or death within a week of admission. All carcasses were kept frozen at −20 °C, until they were necropsied. No animals were sacrificed for the purpose of this study. The livers, as the main organ for accumulation and storage of rodenticides, were used for this study [3]. Obtained during the necropsy, they were kept frozen at −20 °C until the preparation of the extraction and chemical analysis.

2.2. Chemical analyses

During these 10 years, we employed two extraction-detection methods for the quantitative analysis of all the anticoagulant rodenticides permitted in the EU (brodifacoum, bromadiolone, chlorophacinone, coumatetralyl, difenacoum, difethialone, flocoumafen and warfarin) [4]. All the solvents employed were of the highest purity available (>99. 9%, Honeywell, Morristown, NJ, USA). Ultrapure (UP) water was produced in the laboratory using a Gradient A10 Milli-Q System (Millipore, Molsheim, France). Standards for ARs and a procedural-internal standard (P-IS, (±)-Warfarin-d5) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). All standards were pure compounds (purity from 98% to 99.5%). The method employed from January 2011 to November 2015 was a solid-liquid extraction followed of a LC-MS/MS analysis using a Thermo LC-MS/MS Accela Ultra instrument (Thermo Fisher Scientific Inc., USA) as previously described [5]. The method employed from December 2015 to May 2020 consisted on an extraction based on the QuEChERS method (Anastassiades et al., 2003), which has been fully validated in our laboratory followed by a LC-MS/MS analysis using an Agilent 1290 UHPLC (Agilent Technologies, Palo Alto, USA) coupled to an Agilent 6460 triple-quadrupole mass spectrometer, according to the previously described procedure [6]. All the quantitative data were obtained from at least two independent measurements.

2.3. Geospatial analysis of the data (GIS analysis)

The data about the place where the carcass was found were collected by Canary Islands environmental patrols and obtained by GPS tracking. We employed the QGIS Desktop software (version 3.12) for the analyses of geospatial data. The images were projected to the UTM 28N zone based on the WGS84 Geographic Coordinates System. Several vectorial map layers were created for animals positive for anticoagulant rodenticides; animals negative for anticoagulant rodenticides, farms of pig/cattle/sheep/goat production, and 1.5 km-buffer zones for farms, and all these were superimposed on the base map (OpenStreetMap).

2.4. Statistical analysis

The statistical analyses were done using the software package using GraphPad Prism v8.0 (GraphPad Software, CA, USA). First, the adjustment to the series of data to normality was examined using the Kolmogorov–Smirnov test. The distributions of the anticoagulant rodenticides

did not adjust to normality and therefore, non-parametric tests were employed. Thus, we used the Mann–Whitney tests for the analysis. Probability levels of less than 0.05 (two tailed) were considered statistically significant.

Ethics Statement

All samples were collected after obtaining the corresponding permits and following the animal welfare protocols during the sampling.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

Acknowledgments

This research was supported by the University of Las Palmas de Gran Canaria via a doctoral grant to the first author Cristian Rial-Berriel (ULPGC-012-2016) and also by the Spanish Ministry of Education, Culture and Sports via a doctoral grant to the first co-author Andrea Acosta-Dacal (FPU16-01888).

CRediT Author Statement

Guarantor of integrity of the entire study: OPL
Study concepts and design: OPL
Literature research: CRB, AAD, MACP, ASP, AMM, OPL
Laboratory work: CRB, AAD, ASP, NRS, ARH, AMM, MZ, LAHH, LDB, OPL
Data analysis: CRB, AAD, NRS, MACP, AMM, OPL
Statistical analysis: LAHH, OPL
Manuscript preparation: CRB, AAD, MACP, ASP, AMM, MZ, OPL
Manuscript editing: CRB, AAD, MACP, ASP, AMM, MZ, OPL

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[3] http://www.scottishmink.org.uk/.
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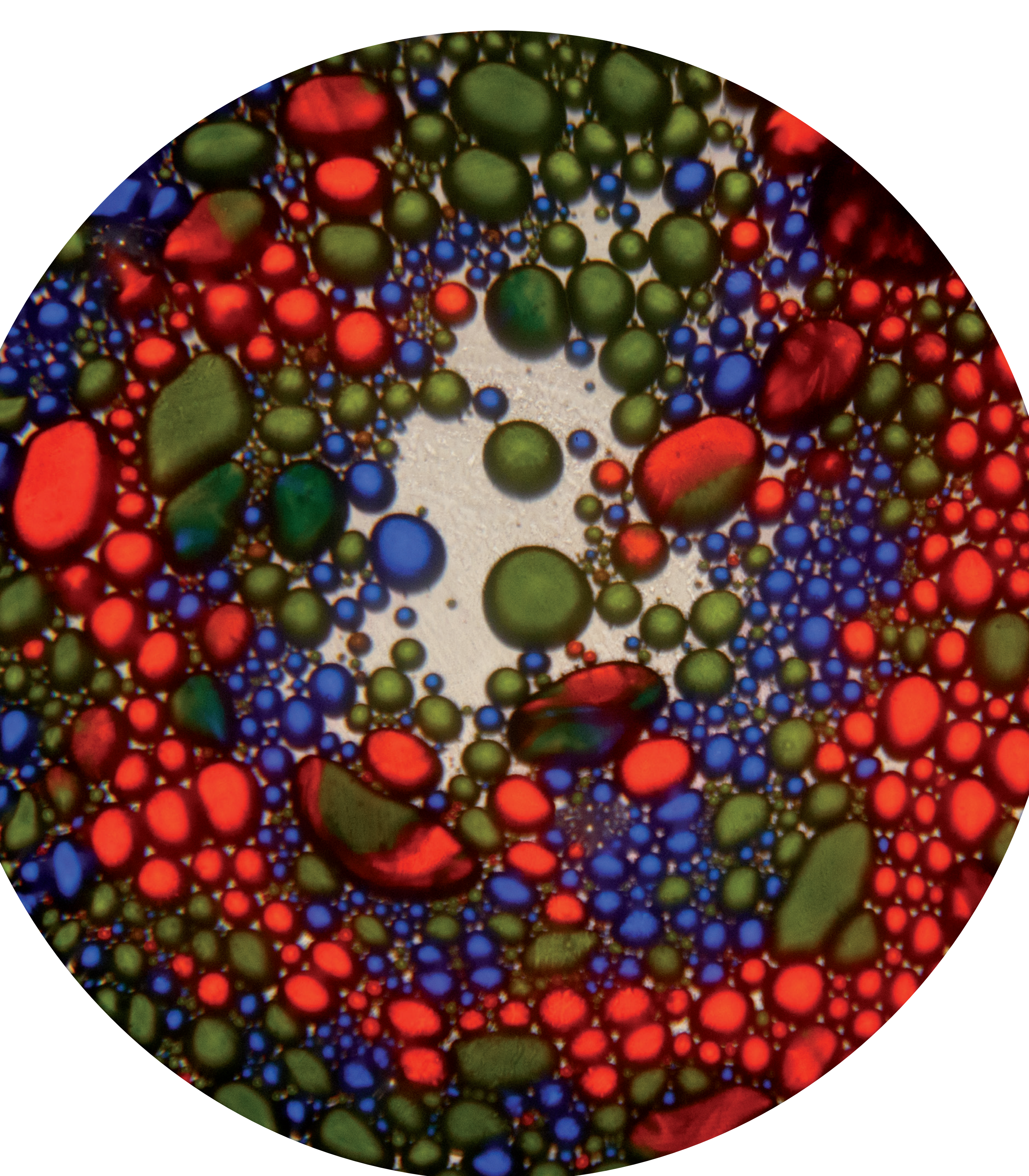
[6] C. Rial-Berriel, et al., Micro QuEChERS-based methodology for the simultaneous biomonitoring in whole blood of 360 toxicologically relevant pollutants for wildlife, Sci. Total Environ. 736 (2020) 139444.

Bloque C. Diagnóstico de envenenamientos de fauna silvestre de Canarias

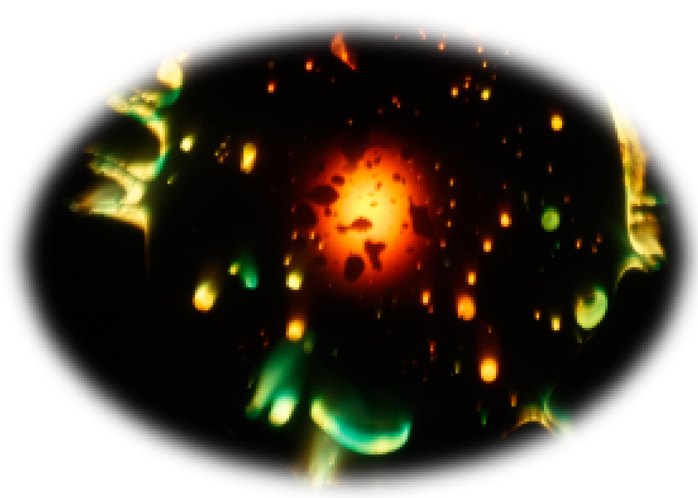


Publicación 7. Epidemiology of animal poisonings in the Canary Islands (Spain) during the period 2014-2021

Epidemiología de las intoxicaciones animales en Canarias (España) durante el período 2014-2021

Toxics, 2021, 9: 267

DOI: https://doi.org/10.3390/toxics9100267



Article

Epidemiology of Animal Poisonings in the Canary Islands (Spain) during the Period 2014–2021

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Citation: Rial-Berriel, C.; Acosta-Dacal, A.; Zumbado, M.; Henríquez-Hernández, L.A.; Rodríguez-Hernández, Á.; Macías-Montes, A.; Boada, L.D.; Travieso-Aja, M.d.M.; Martin-Cruz, B.; Suárez-Pérez, A.; et al. Epidemiology of Animal Poisonings in the Canary Islands (Spain) during the Period 2014–2021. *Toxics* **2021**, *9*, 267. https://doi.org/10.3390/toxics9100267

Academic Editor: Christopher J. Martyniuk

Received: 8 September 2021
Accepted: 9 October 2021
Published: 14 October 2021

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Abstract: Animal poisoning is one of the greatest conservation threats facing wildlife. In a preliminary study in the oceanic archipelago of the Canary Islands, we showed that the degree of threat from this circumstance was very high-even higher than that reported in other regions of continental Europe. Consequently, a legal framework for the effective prosecution of the crime of wildlife poisoning came into force in 2014 in this region. We present the results of the investigation of 961 animals and 84 baits sent to our laboratory for the diagnosis of animal poisonings during the period 2014–2021. We were able to identify poison as the cause of death in 251 animals and 61 baits. Carbofuran stands out as the main agent used in this archipelago. We have also detected an increasing tendency to use mixtures of several pesticides in the preparation of baits. The entry into operation of two canine patrols has led to the detection of more dead animals in the wild and a greater number of poisoned animals. The percentage of poison positives is significantly higher in areas with lower population density, corresponding to rural environments, as well as in areas with greater agricultural and livestock activity.

Keywords: banned pesticides; intentional poisoning; carbofuran; aldicarb; anticoagulant rodenticides; QuEChERS; LC-MS/MS; GC-MS/MS

1. Introduction

Poison remains one of the most important conservation threats faced by many wildlife species, and it also affects domestic animals frequently [1–12]. There are numerous chemicals that can affect animals, but perhaps the group of agricultural pesticides is the most important [13]. These compounds, commonly used and widespread in global food production, cause many accidental poisonings in non-target species [5,7,13,14], and accidental poisonings by these substances in humans are also frequently described [15,16]. Among the pesticides, rodenticides stand out, since they are directed against higher vertebrates-rodents- and can easily reach other non-target species that share their habitat [17]. Numerous studies

have indicated that rodenticides can harm non-target species of mammals, reptiles, and birds, particularly birds of prey, but also other species, which do not necessarily feed on rodents or small mammals that have ingested the poison, but granivorous birds that directly ingest the baits [18]. Affected animals suffer anticoagulant and hemodynamic effects that predispose the animal to death [17,19–22]. Apart from pesticides, there are other substances that can poison wildlife, such as industrial pollutants or veterinary drugs. Like pesticides, veterinary drugs have the potential for bioaccumulation and transfer through food webs. Each year, several thousand tons of active ingredients are used in animal husbandry [18], and a portion of these may eventually result in environmental and ecological impacts [23]. Wildlife exposure to pharmaceuticals can occur through contaminated water [24], agricultural soils, plants, and arthropods [25–27], and through excreta and carcasses of medicated livestock (i.e., supplemental feeding of threatened scavenger birds) [28,29].

If accidental exposure to chemicals can cause harm to animal health, the situation becomes dramatic when chemicals are intentionally used in the preparation of baits to kill animals. Among the chemicals that baits may contain, pesticides, including rodenticides, are the most used [8,12,30]. It has been estimated that pesticides are used illegally in up to 68% of all suspected animal poisoning cases [1,5,7,14]. Due to their high toxicity, several restrictions have been applied to many compounds that are currently banned or severely restricted in the EU. However, these are not the only compounds intentionally used for the purpose of killing animals, and there is growing evidence that veterinary drugs and chemicals other than agricultural pesticides are being used for this purpose [31–33].

The Canary Islands, where this study has focused, constitute a Spanish archipelago located in the Atlantic Ocean off the northwest coast of Africa (between coordinates 27° 37′ and 29°25′ north latitude and 13°20′ and 18°10′ west longitude) consisting of eight inhabited islands and several uninhabited islets, all of them of volcanic origin. This archipelago is home to marine and terrestrial ecosystems of great value, both ecological and scenic, and represents a hotspot of biodiversity, with a huge number of endemic species, due to the evolutionary isolation from nearby continents (Africa and Europe). According to updated data, there are almost 4500 endemic species in the Canary Islands, representing more than 27% of the total biodiversity recorded (https://www.biodiversidadcanarias.es/biota/, accessed on 7 September 2021).

The archipelago has extensive areas of its territory with different levels of environmental protection (around 40% of its surface area), but it also has other areas subject to intense anthropogenic pressure, since it has a stable population of 2.1 million inhabitants and receives more than 12 million tourists a year. In such a small territory, poison baiting is particularly dramatic, as a single poisoning event can severely damage the populations of endemic species with only a few tens or hundreds of individuals, to the point of bringing them to the brink of extinction. Unfortunately, poisoning is a common practice in this archipelago, as could be documented in a first study that covered 4 years (2010–2013) and that aimed to highlight this dramatic problem [4]. As a consequence of this preliminary study, in 2014, the Strategy for the Prevention and Control of Poisoning in the Canary Islands was approved [34]. This law articulates a series of measures for research, public awareness, and prosecution of the crime of wildlife poisoning in this European region. This law names the Toxicology laboratory of the University of Las Palmas de Gran Canaria as the reference laboratory for the official investigation of poisoning incidents in this region, as it had been in charge of the official investigation of such events since January 2014 [4,34]. Consequently, our laboratory began to receive the totality of the samples generated in the incidents investigated by the environmental police and has been developing all the necessary methodology to perform the most complete search possible of all toxic substances that usually affect or could affect the health of wildlife and domestic fauna [35–37].

In this article, we present the description and epidemiological study of the results of the diagnostic service of wildlife poisonings that we provide to the Government of the Canary Islands in the period 2014–2021 (until June), including the results of 961 animals and 84 baits that have been investigated during this period.

2. Materials and Methods

2.1. Sampling

Blood and liver samples from 961 animals received in our laboratory for diagnosis of possible poisoning were analyzed between January 2014 and June 2021. During this period, we also received 84 meat baits. Occasionally, we also received other samples, such as gastric contents, degraded carcasses, cadaveric fauna, and insects collected at the scene of the incident. When necessary, these samples were used to clarify results found in blood or liver. However, none of the results we present were obtained solely from these ancillary samples, so cases where blood or liver were not available have not been included. It is noteworthy that 424 animals belong to species or subspecies endemic to the Canary Islands, highlighting 307 birds of prey and 74 lizards (*Gallotia* spp.), the latter being critically endangered. The animals correspond to a total of 753 incidents investigated, giving an average of 1.3 animals affected per incident (although they ranged from one to 20 animals per incident). The animals investigated belonged to 63 different species, including 44 dogs and 49 cats. Table 1 (Results and Discussion section) lists the species in which at least one chemical as the cause of death was detected. All animals were collected in the context of investigations of possible environmental crimes by environmental agents and were transferred to the wildlife rehabilitation centers of Tafira (in Gran Canaria) or La Tahonilla (in Tenerife) where liver samples for toxicological analysis were collected during necropsy. Once collected, the samples were kept frozen until they were transferred to the Clinical and Analytical Toxicology Service (SERTOX) of the University of Las Palmas de Gran Canaria (ULPGC, Canary Islands, Spain), where they remained frozen at −24 °C until their analysis. Most of the animals were found dead in the field or in urban areas or died while in veterinary facilities. No animals were sacrificed for the purposes of this study, and no experiments were performed on or with live animal samples.

Table 1. Identification of pesticides in animals and baits from poisoning episodes occurred in the Canary Islands during the period 2014–2020.

	Principal Toxicant (s)				
	Carbofuran	**Aldicarb**	**Other AChE Inhibitors**	**Anticoag.**	**Others**
Wild animals					
Accipiter nisus				1	
Alectoris rufa			1	1	
Anas platyrrhynchos	5				
Ardea cinerea					2
Asio otus	3		3	14	2
Atelerix algirus		1		1	
Buteo buteo	11	2	2	12	
Chalcides simonyi				1	1
Columba livia	7		18		
Corvus corax	14		7		1
Pyrrhocorax pyrrhocorax barbatus	7				
Falco tinnunculus	6		5	21	
Gallotia galloti	8	1	3	9	
Larus michaellis	1				
Neophron percnopterus	12		1	2	2
Oryctolagus cuniculus			2	2	
Turdus merula	3	2	5		
Tyto alba				2	1
Domestic animals					
Cats	11	2	4	4	
Dogs	9	6	6	2	3
Baits and suspicious materials					
Meat or feed	24	9	16	8	4

2.2. Analytical Method

The methodology used for blood and liver samples allowed the search respectively for 360 and 351 compounds highly toxic to animals and has been fully validated according to international guidelines [38,39] and previously published by our group [3,35,36,40]. The complete list of analytes, as well as the technique used for their quantification, can be found in Appendix A. The extraction of blood and liver are based on the QuEChERS technique, although in our methods, a miniaturization of this technique has been performed, allowing the use of only 250 µL of blood [35,36] or 1 g of liver [3,40], without requiring any additional purification step, nor any change of solvent. For the rest of the samples (baits, gastric and intestinal contents, decomposing carcasses), a solid–liquid extraction was usually used, according to a procedure also previously published by our group [4,37], although in the case of these auxiliary samples, we consider the analyses to be semi-quantitative only.

The quantitative analysis was carried out in all cases by a combination of two complementary analyses, one by gas chromatography coupled to triple quadrupole mass spectrometry (GC-MS/MS) for the analysis of the more volatile compounds (mainly persistent organic pollutants and some less polar pesticides) and another analysis by liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS) for pharmaceuticals, rodenticides, and more polar pesticides. For GC-MS/MS, an Agilent 7890B gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) was used, which was equipped with two Agilent J&W HP-5MS (5% cross-linked phenyl-methyl-polysiloxane, Agilent Technologies) fused silica capillary columns, with a total length of 30 m (15 + 15 m), a film thickness of 0.25 µm, and 0.25 mm diameter each. The reason for using two columns joined by a purged joint was to allow the application of the backflushing technique that reduces background noise and prolongs the lifetime of the column. He 5.0 (99.999%) was used as the carrier gas, and N_2 6.0 (99.9999%) was used as the collision gas. Gases were from Linde (Dublin, Ireland). For LC-MS/MS, an Agilent 1290 Infinity II UHPLC instrument (Agilent Technologies, Palo Alto, CA, USA) was used. It was equipped with an InfinityLab Poroshell 120 column (2.1 mm × 100 mm, 2.7 µm), coupled to an in-line filter and a UHPLC guard column with the same characteristics as the analytical column. The detection and quantification of GC-separated compounds was performed with an Agilent 7010c mass spectrometer, and that of LC-separated compounds with an Agilent 6460 electrospray Jet Stream (AJS-ESI) mass spectrometer (Agilent Technologies, Palo Alto, CA, USA), which were both operated in dynamic multiple reaction monitoring (dMRM) mode, in positive (GC-MS/MS), and positive and negative (LC-MS/MS) polarities. All the technical details of the extractions, the programming of the equipment, and the results of the validation of the techniques can be found in the cited references [3,4,35–37].

All quantifications were performed against calibration curves prepared in matrix, blood, or liver as appropriate with a mixture of the 360 individual chemicals and the deuterated compounds used as procedural internal standards. All standards were of the highest purity available (93.1% to 99.8%) and purchased from various suppliers (A2S-Analytical Standard Solutions (Staint Jean D'Illac, France), Sigma-Aldrich (Augsburg, Germany), CPA Chem (Stara Zagora, Bulgaria), European Pharmacopoeia Reference Standards (Strasbourg, France), Accustandard (New Haven, CT, USA), and Dr. Ehrenstorfer (Augsburg, Germany)). The calibration curves were prepared with a minimum of 6 points, covering the range up to 2 µg/mL.

2.3. Statistical Analyses

All statistical analyses were performed with GraphPad Prism v9.2 software (GraphPad Software, San Diego, CA, USA). The distribution of the variables included in this study was evaluated using the Kolmogorov–Smirnov test. Given the nature of the cases investigated (animals due to poisoning), the concentrations of most of the chemicals detected did not follow a normal distribution, so the results presented in Table 2 are expressed in terms of median and range. For the same reason, we employed nonparametric tests to check for statistical differences between the concentrations found in the groups of animals, using the

median rather than the mean. For the study of determinants of poisoning, the dichotomous categorization (0/1) was used for the absence or presence of substances in a concentration that was compatible with the poisoning of the animals, *regardless* of whether one or more substances were involved. The association study between the different determinants and the outcome (poisoning vs. non-poisoning) was evaluated with the Chi-square test (χ^2). A *p*-value of less than 0.05 (two-tailed) was considered statistically significant in all the statistical analyses.

Table 2. Comparison of concentrations of chemicals identified in confirmed poisoning cases between wild and domestic animals.

	Wild Animals		Domestic Animals		
Chemical	**Median**	**Range (p25–p75)**	**Median**	**Range (p25–p75)**	***p***
Aldicarb	20,298.1 *	82.4–343,267.4	402.5	101.3–1913.2	0.0387
Alpha chloralose			3498.3		
Amobarbital	35,677				
Brodifacoum	1128.8 ****	585.6–3098.3	338.5	291.5–456.7	<0.0001
Bromadiolone	331.0	222.2–646.5	960.7 *	389.5–1902.1	0.0432
Carbofuran	1499.2	353.3–4412.7	3702.3	420.1–23,007.9	n.s.
Chlorpyrifos			8832.4		
Dimethoate	5589.2	1987.2–12,377.3			
Fenamiphos	7469.5				
Flocoumafen	1223.2	287.1–2298.6			
Imidacloprid			1034.5	877.6–3347.8	
Methiocarb			2336.5		
Methomyl	2155.6	788.3–21,887.3	2332.2	987.4–7886.5	n.s.
Oxamyl	556.8	344.3–12,443.8	766.3	677.8–8876.5	n.s.
Permethrin	381.2	227.4–1886.3	1500.2 *	998.4–5667.9	0.0234
Pirimiphos methyl	1223.4	556.7–3446.7			
Tetramethrin			921.6		

* $p < 0.05$; **** $p < 0.0001$

3. Results and Discussion

We studied the incidence of wildlife poisonings in the period from January 2014 to June 2021. We finally included liver or blood samples from 961 deceased animals and 84 baits. A total of 312 animals were referred with strong suspicion of poisoning, and of these, the presence of poisoning was confirmed in 223 animals (71.5%). The remaining 649 animals were sent to rule out the presence of poison, among other possible causes of death, and the poisoning was confirmed in 29 of them (4.5%). All baits were initially classified as intentionally supplied in the environment, and the presence of some type of poison was confirmed in 61 of them (72.6%). The total number of positive identifications represented 29.9% of the samples submitted during this period. The number of deceased animals in incidents where a chemical was detected in toxic concentrations was significantly higher than in negative cases (mean 2.53 ± 0.28 vs. 1.31 ± 0.15, $p < 0.0001$), as has been described in other works at the international level [6,7,40–43], and in the previous studies conducted in the Canary Islands [4,44].

In Table 1, we show the number of positive cases distributed by species and type of toxicants involved. The wildlife species with the highest number of positive cases was *Columba livia*, with 92.6% of the specimens submitted positive for poison (25/27). This can be explained by the fact that only pigeons for which a strong suspicion of poisoning had been received by our laboratory, in contrast with what occurred with other species. We also identified poisoning in a high number of *Falco tinnunculus canariensis* (32/108), *Gallotia* spp. (21/27), *Buteo buteo insularum* (27/52), *Neophron percnopterus majorensis* (17/49), *Asio otus canariensis* (22/84), *Corvus corax canariensis* (22/97), and *Pyrrhocorax pyrrhocorax barbatus* (7/10). These seven species or subspecies are endemic to the Canary Islands, and all of them are at a high degree of conservation threat, mainly due to human activities. Regarding domestic animals, we only received cases of dogs (26 positives out of 44 referrals) and

cats (21 positives out of 49 referrals). In the previous study period (2010–2013) [4], these two species represented the highest percentage in the total diagnoses. In the period we now present, the percentage of positives among domestic animals has fallen appreciably, while the percentage of positives among wild species has remained fairly similar over the years [4].

Seventeen different toxicants were detected, and most of them were pesticides, except for amobarbital, which was identified as the probable cause of death in one case (*Corvus corax canariensis*). According to our results, in the positive cases, a mean of 1.54 ± 0.38 toxicants per incident were detected. The most frequently detected chemical was carbofuran, which was present in 97 animals and 24 baits (38.7% of positive cases). This percentage is very similar to that previously reported in the Canary Islands [4], which would indicate that the habit of using this compound to get rid of nuisance animals has not diminished in the Canary Islands. It should be noted that carbofuran has been banned in the EU since 2007. Almost 15 years after its withdrawal from the market, there still seems to be an important stock of this compound in private farms in the archipelago, which has not been handed over to the authorities and which continues to be used illegally. The use of aldicarb, also banned in the EU for almost two decades [45], does not seem to have decreased either, as it was detected in almost 10% of the positive cases (in the previous period, it was present in 12.2% of the positive cases [4]). In addition, the percentage of cases in which the cause of death was an anticoagulant rodenticide has remained practically unchanged between both periods (29.9% in 2014–2020 vs. 29.1% in 2010–2013). However, regarding the rest of the detected compounds, we did find differences between both periods, although this was not too important. We detected a greater variety of substances involved (17 vs. 14) as well as a greater involvement of other acetylcholinesterase inhibitor insecticides, such as chlorpyrifos, pirimiphos methyl, dimethoate, oxamyl, or methomyl. We were struck by the fact that unlike what happened in the previous study period, in these years, we have detected an increase in the cases in which several poisons are detected simultaneously, which became more evident in the study of baits. In 35.8% of the cases, the baits were prepared with between three and five different compounds, all of them commonly used in Canary Island agriculture in the past but mostly also substances currently banned in the EU. This could be due to the fact that stocks of the more potent poisons that have traditionally been used may be running low in some places, and poisoners seek to maximize the effectiveness of the baits they prepare by mixing different substances of lower toxicity than the compounds previously used.

In Figure 1, we present in graphical form a comparison of the distribution of poisons that have affected wild and domestic fauna in the Canary Islands in both study periods; the present study corresponds to the investigations after the regional law against the poison in natural areas came into force [34], as compared to our previous report, in which the cases were received quite informally [4]. A slight change in the pattern of use of poisons is evident, particularly regarding baits. Possibly, this indicates that in certain parts of the archipelago, carbofuran and aldicarb have been depleted, and mixtures of other compounds that are assumed to be less potent individually are beginning to be used.

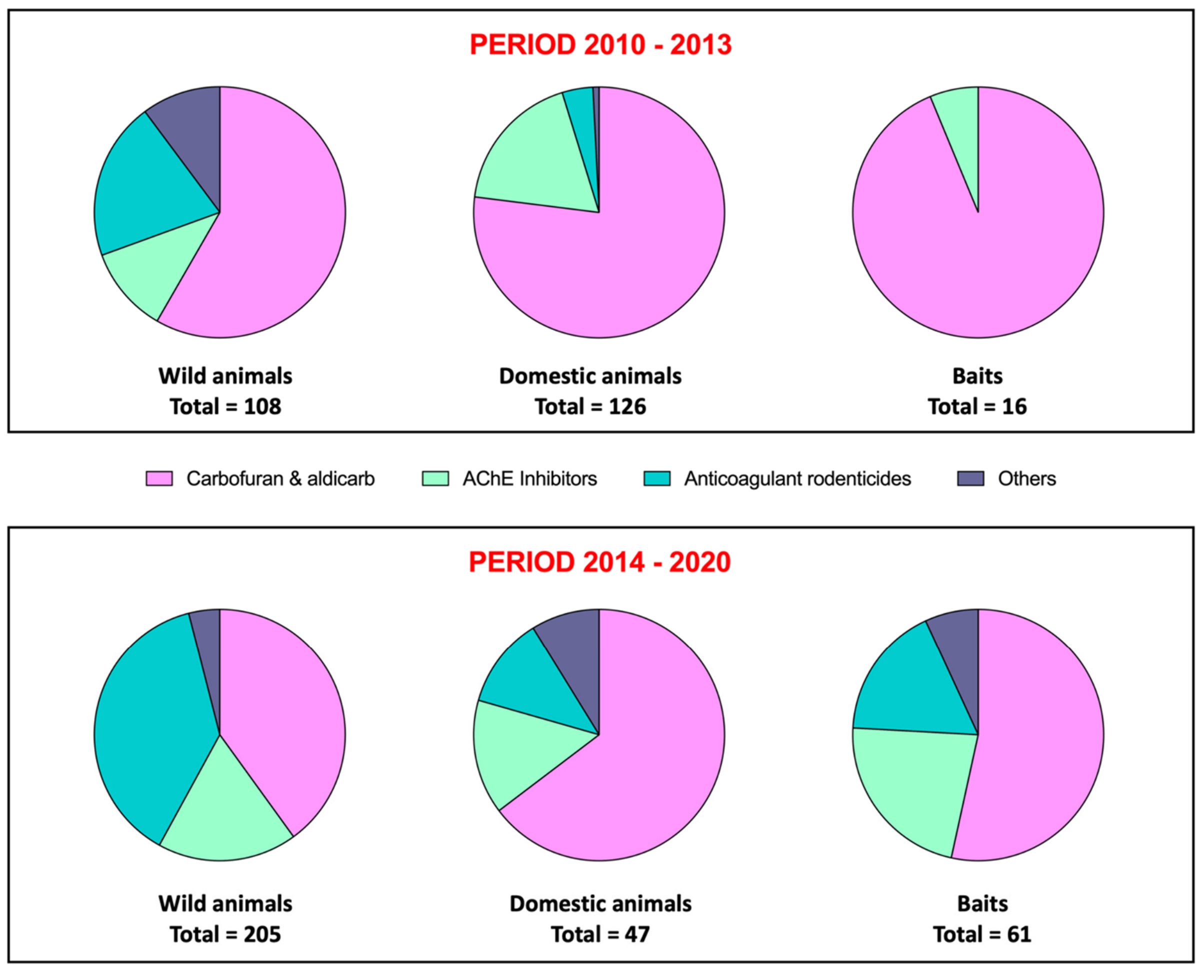

Figure 1. Comparison of the distribution of poisons involved in wildlife mortality cases grouped by type between the period 2010–2013 (upper panel) and the period 2014–2020 (lower panel).

With respect to the proportion of poison-positive cases among the total number of cases referred during this period, we found significant differences depending on whether they were wild or domestic animals. As shown in Figure 2, the percentage of positives among wildlife barely reached 20%, while in the case of dogs and cats, this percentage was more than double. This finding is not surprising, since it has been described that cats, and mainly dogs, when sick become much more visible to humans than wild animals, which, in these same circumstances, tend to seek refuge in their nests and burrows, and many of them are never found [46]. We highlight this fact because the cases of poison in wildlife could be much higher than what we have officially recorded from the samples submitted to us, because dying animals that hide are probably never found. As can be seen in the graph, in the baits analyzed, the percentage of positive identifications was very high (75%). This finding is also very logical, since when baits are sent to us, their appearance and location attract attention in most cases, and there are strong suspicions that they have been laced with poison, and virtually all these samples are submitted to the laboratory for investigation. Additionally, the poisons of interest are expected to be more concentrated and perhaps also more stable in the bait source compared to biological samples, so even if there is degradation, it is more likely that high enough concentrations will remain to allow detection.

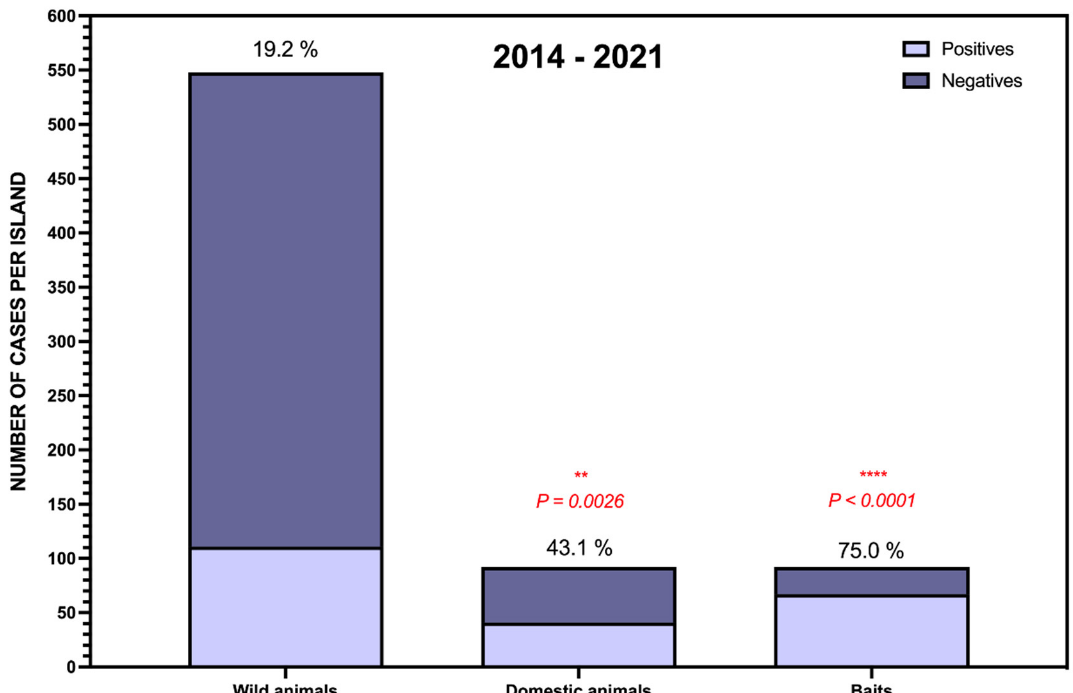

Figure 2. Proportion of positive cases among wild animals, domestic animals, and baits tested. ** $p < 0.01$; **** $p < 0.0001$.

During these years, the diagnosis of death by poisoning has been made based either on the comparison of the liver concentrations found with the data available in the literature, on the calculation of the toxic dose from the blood concentration and the apparent volume of distribution of the poison found (when these data were available), and on the clinical or presumptive findings of poisoning collected in the files by the environmental police agents or by the veterinarians who attended the animals. It was not always possible to attribute the death of the animal to the toxic substances found, which was either because the concentrations found did not seem sufficiently high or because the advanced state of degradation of the samples did not allow knowing if there was degradation of the chemical substances decreasing their concentration or because there were no reference data. Therefore, the doubtful cases have not been included as positive in this study. However, although the outcome does not change once a certain threshold concentration of a chemical is exceeded that is considered potentially lethal, we do consider that the finding of extremely high concentrations in liver would reflect exposure to massive doses of the poison, probably after ingestion of a bait, thus indirectly suggesting a pattern of intentionality in the poisoning. Obviously, intentionality cannot be inferred from concentrations alone, so we only state this as a working hypothesis based on our experience. According to this hypothesis, we wanted to compare whether there were also differences in the type of poisons affecting one or the other type of animal (wildlife vs. domestic) and the concentrations found for each of them in both types of animals (Table 2).

We found significant differences in the concentrations of some toxicants. Thus, the most striking case is that of aldicarb, which presented a median value about 40 times higher in the series of wildlife than in that of domestic animals ($p < 0.05$), with several animals of different incidents presenting massive concentrations of this substance in their liver. Something similar was observed with brodifacoum, which also presented significantly higher concentrations in the livers of wild animals than in domestic animals ($p < 0.0001$). In the first case, the use and even possession of aldicarb has been prohibited for almost 20 years [45], so that accidentality could be ruled out in all cases. In the case of brodifacoum, there is a limitation for its outdoor use in the agricultural environment, so this result was to some extent surprising. The high concentrations found in wild animals compared to dogs and cats probably suggests a pattern of intentionality, at least in part of the cases. Even more

so considering that with another frequently detected rodenticide, bromadiolone, just the opposite is true. Bromadiolone levels were significantly higher in the livers of intoxicated pets (Table 2). This second-generation anticoagulant is a legal and very commonly used rodenticide, both in urban and rural areas, including agriculture and livestock. It is more than likely that most of the cases involving this toxicant, both in domestic and wild animals, are due to accidental poisoning, probably secondary to the ingestion of poisoned rodents, rather than to the ingestion of baits. With respect to the other chemical substance for which there was a significant difference between wild and domestic animals, permethrin, it should be noted that this is a compound of particularly high toxicity to cats [47], which is the species with the highest liver concentrations. It is true that the literature describes that some cat poisonings are due to the accidental application of flea products labeled for dogs. It might be reasonable for well-meaning people to apply permethrin-containing dog products to cats with the goal of helping rather than harming them. However, it should also be noted that permethrin was also identified in two of the baits analyzed, so, at least in some cases, the high concentrations found could also point to a pattern of intentionality aimed at eliminating stray cats. Finally, some compounds were only detected in wildlife specimens, such as amobarbital, dimethoate, fenamiphos, flocoumafen, and pirimiphos methyl, while others were only detected in domestic animals, such as alpha chloralose, chlorpyrifos, imidacloprid, methiocarb, and tetramethrin, although some of the chemicals were detected only sporadically. Among them, it is worth highlighting the detection of amobarbital, since it represents the first case of poisoning by barbiturates recorded in the Canary Islands, unlike what has been reported recently for mainland Spain, where barbiturates were involved in up to 3.4% of the poisonings detected [31].

During this period, the number of positive cases per year has not shown a downward trend (Figure 3), remaining stable. What has increased is the number of samples received in our laboratory to rule out cases of poisoning, as this increase has been very noticeable from 2017. We previously pointed out that one of the biggest problems in the investigation of wildlife poisoning is that a large part of the cases may never be detected due to the elusive behavior of most species when they are seriously ill [46]. Precisely because of this, and in the context of the Canarian strategy against poison [34], two canine patrols trained in the detection of poisons and carcasses have come into operation: the first one was on the island of Gran Canaria, which began operating in 2017, and the second one came into operation in 2020 and covers the islands of Fuerteventura and Lanzarote. As can be seen in Figure 3, these milestones coincide with respective increases in the receipt of samples and the corresponding increase in the detection of poisoning cases. Even so, although there was a quantitative increase in the number of positive cases identified, this did not alter the proportion of cases in relation to the total number of samples received in the laboratory, remaining approximately the same. Only in the last period, from January 2020 onwards, has there been a slight increase in the number of positive cases detected, but it is too early to conclude whether this trend will continue over time. Future studies will test whether canine patrols contribute effectively to the visibility of wildlife poisoning cases, as has been described in other regions [46].

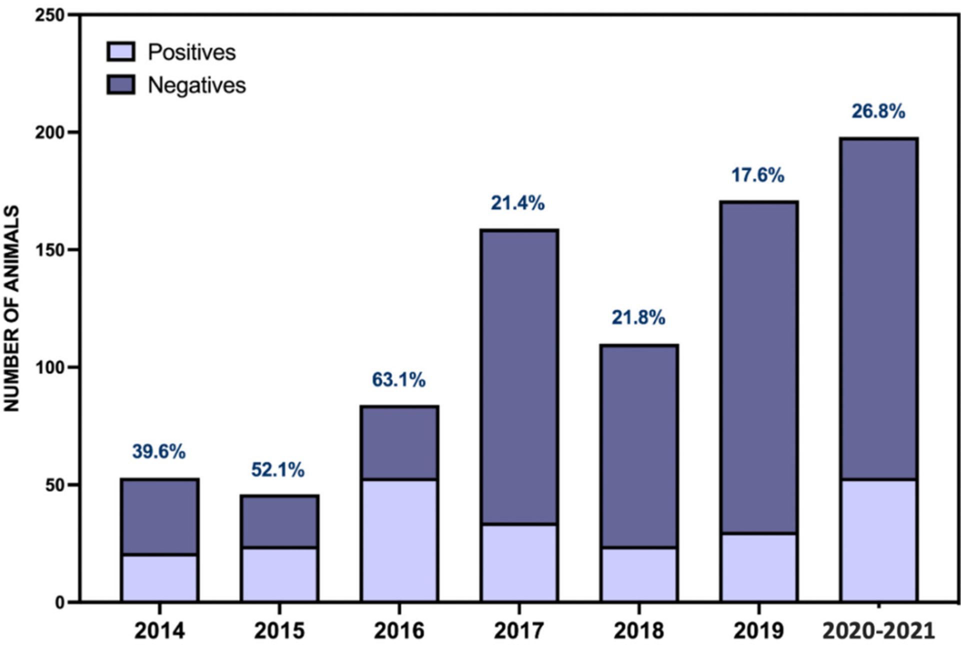

Figure 3. Temporal evolution of positive/negative cases for poisoning as cause of death.

The Canary Islands include eight inhabited islands, five islets, eight rocks, and the sea. The animals received in our service correspond only with the eight inhabited islands, although the smallest of all, La Graciosa, is usually considered together with Lanzarote, on which it has depended administratively until very recently (2018). Figure 4 represents how the cases received have been distributed in relation to the island on which the incident occurred. As can be seen, most of the cases were recorded on the island of Gran Canaria, which is where our laboratory is physically located. Probably, since it is an archipelago, this is due to logistical reasons, since it was easier to send the samples, especially during the first few years of operation. It is also noteworthy that one of the canine patrols, the first to become operational, is also based on this island. Although this patrol operates throughout the archipelago, it is true that the highest rate of interventions occurs on the island of Gran Canaria, so this undoubtedly influences this difference with respect to the rest of the archipelago. From the islands of Fuerteventura and, to a much lesser extent, Lanzarote, a good number of cases have also been received, especially in the last two years, also coinciding with the entry into operation of the second canine patrol, which is physically located on the island of Fuerteventura. Our results indicate that dogs trained in the detection of poisons are a valuable aid in detecting this serious environmental problem. With respect to the cases coming from the islands farthest from our laboratory (La Gomera, La Palma, and El Hierro), the high percentage of positives is striking, reaching 100% of those sent from the island of El Hierro. From our point of view, this reflects the logistical problems that have existed during these first years of operation of the Canary Islands anti-poison strategy, which has meant that the cases sent for investigation from these islands have been meticulously selected. The case of the island of Tenerife is noteworthy, since it is the most populated island of the archipelago, the one with the greatest agricultural and livestock activity, and one of the islands with the greatest biodiversity. However, the low rate of samples investigated on this island is surprising. This situation will probably change significantly in the next few years, since a third canine patrol is expected to start operating, which will be located on this island, and which will undoubtedly help to make visible the cases of poisoning that we believe are going unnoticed there.

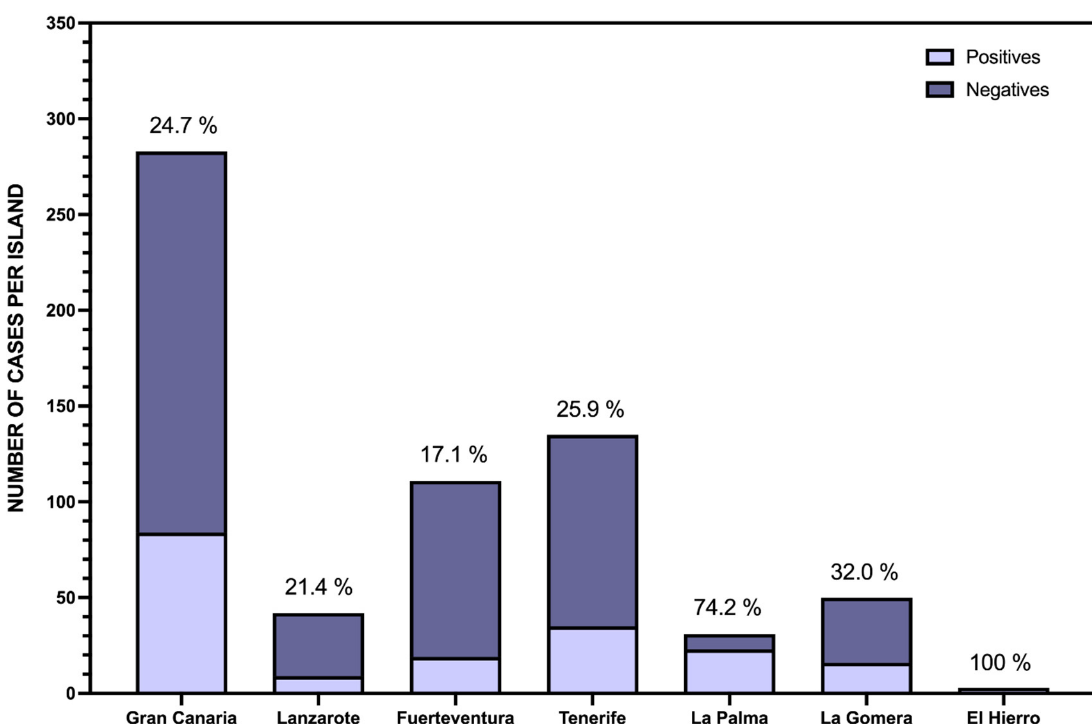

Figure 4. Distribution of poisoning cases diagnosed in relation to the total number of cases referred from each of the islands of the Canary archipelago. In the bar corresponding to Lanzarote, those from the island of La Graciosa have been included due to its administrative dependence on that island until 2018.

Finally, we wanted to study the main determinants of the pattern of poison use in the Canary Islands, using the available variables, mainly in relation to parameters related to the species, land use, and population density, as has been established in other research studies [21].

First, we studied the influence of habitat (urban vs. rural) and population density (Figure 5). We found that the number of animals referred from cities is significantly higher than those referred from smaller towns (based on the number of inhabitants of the municipality in which the carcass was found, cut-off point = 19,657 inhabitants (median value)), but the percentage of positive cases is the inverse, being significantly higher in animals referred from rural localities (Figure 5, left). Something very similar occurred with the population density (cut-off point = 161 inhabitants/km^2). The number of cases referred from less densely populated areas was lower, which was probably because it is more difficult to find the carcasses, but the percentage of positive cases among the animals referred from these areas (43.1%) was significantly higher than those referred from more populated areas (34.1%) (Figure 5, right). This finding seems quite logical to us, given that most of the chemical compounds we have detected are agricultural pesticides, so their availability in rural areas should be greater.

We also explored the relationship with agricultural and livestock activity in the municipalities where the carcasses that were sent to our laboratory were found (Figure 6). First, with respect to agricultural activity, we found that there was a relationship, with the percentage of positive cases being significantly higher in those municipalities with greater agricultural activity. Several cut-off points were used to calculate this (number of cultivated hectares, cultivated area surface (%), and cultivated area per inhabitant), and with all of them, statistical significance was maintained (Figure 6, left). We also find this result logical for the same reason discussed above: the availability of agricultural products, whether permitted or not, is closely related to agriculture. According to our results, the same is true for livestock activity, since the situation is repeated: the percentage of positive cases in municipalities with more livestock is significantly higher than in those with less

livestock activity. This significance was maintained for all the cut-off points tested (total number of livestock in the municipality; livestock density (number of heads/hectare); and livestock density/population density) (Figure 6, right).

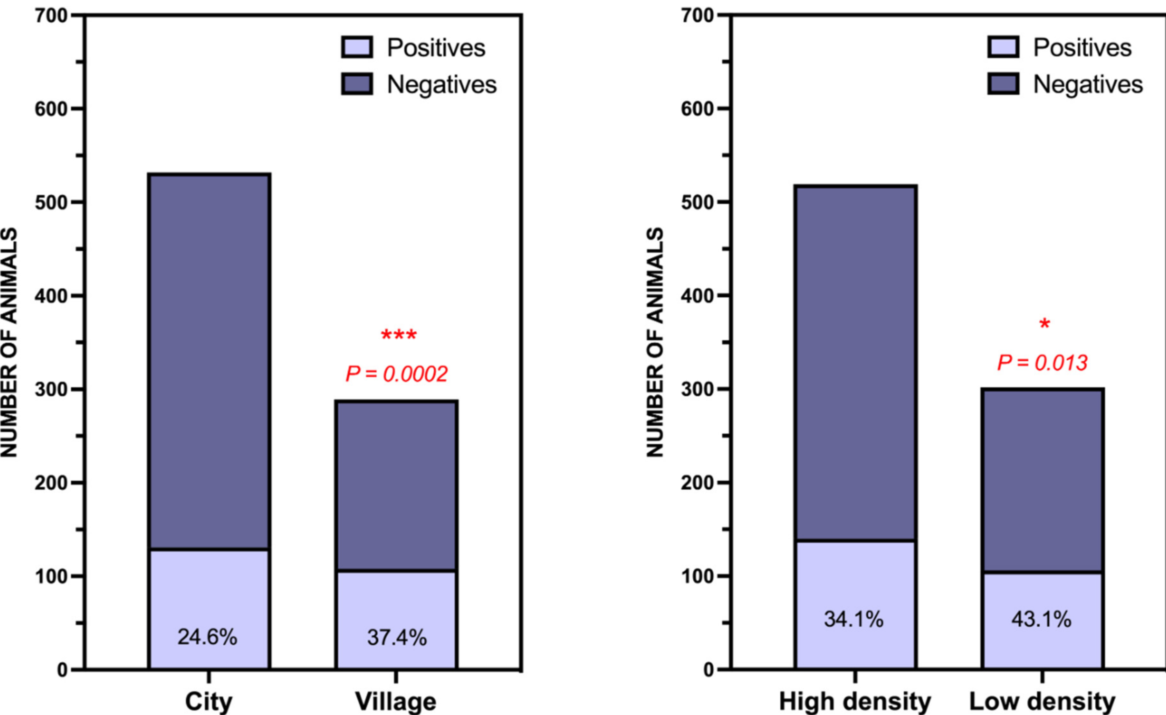

Figure 5. Study of the determinants of the incidence of wildlife poisoning in the Canary Islands. (Left) Type of locality according to the number of inhabitants (cut-off point = 19,657 inhabitants (median value)), (Right) Population density (cut-off point = 161 inhabitants/km^2 (median value). * $p < 0.05$; *** $p < 0.001$.

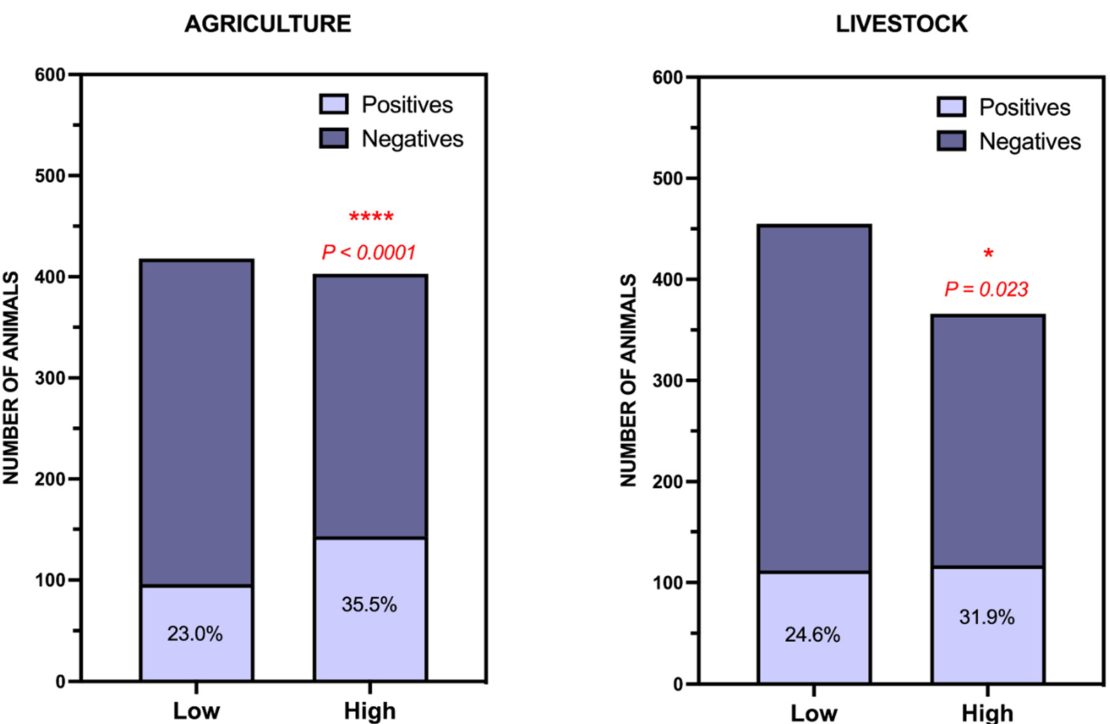

Figure 6. Study of the determinants of the incidence of wildlife poisoning in the Canary Islands. (Left) Influence of agricultural activity (cultivated area surface (%)) and (Right) Livestock influence (number of livestock per hectare). * $p < 0.05$; **** $p < 0.0001$.

In conjunction with our previous report of poisonings in the Canary Islands [4], our results indicate that the incidence of poisonings in this archipelago is very high and probably higher than in other European regions [7,9,14,41,48]. Moreover, the profile of toxicants that we have found suggests that many of these poisonings occur intentionally, given the high prevalence of substances whose use in agriculture would be illegal throughout the EU. Since this practice is highly detrimental to biodiversity, as well as a major public health problem, it is necessary that the authorities enact effective measures on the marketing of toxic chemicals, the control of stocks of banned chemicals, the implementation of educational programs and the effective criminal prosecution of poisoners to prevent, or at least minimize, the incidence of this harmful practice. From the literature, it can be assumed that the numbers reported in those studies only represent an approximation of the actual incidence of wildlife mortality, because it has been estimated that less than 10% of poisoning cases are detected and sent to a forensic laboratory [49]. This is especially relevant for wildlife because sick animals are often less visible and many die in nests, burrows, or inaccessible locations. The presence of canine patrols probably increases detection rates, as we have found in this study, but it is still quite likely that a good portion of cases will go undetected, particularly regarding wildlife. Whatever the case, our findings indicate that the actual incidence of poisoning mortality in the Canary Islands is very high and certainly higher than in other European regions.

We have observed a very slight decreasing trend in the use of prohibited substances, which is much lower than the progressively decreasing annual trend reported in other regions [50,51]. Carbofuran, aldicarb, and other banned AChE inhibitors were used extensively in agriculture in the Canary Islands, which are mainly associated with the cultivation of banana and other export vegetables. It is likely that there are still significant stocks of these banned substances on many farms, although there is also the possibility that they are still being acquired on the black market [50].

Although numerous measures have been taken to correct this problem in this region, it is probably too early to verify their efficacy. The authorities should take different measures to correct the circumstances that motivate the intentional poisoning of animals to curb, or at least minimize, this serious problem that seriously threatens biodiversity, animal welfare, and public health.

Author Contributions: Conceptualization, C.R.-B., A.S.-P., M.Á.C.-P. and O.P.L.; methodology, C.R.-B. and A.A.-D.; software, C.R.-B. and A.A.-D.; validation, C.R.-B., A.A.-D., A.M.-M., and Á.R.-H.; formal analysis, L.A.H.-H., C.R.-B., and O.P.L.; investigation, C.R.-B., A.A.-D., A.M.-M., Á.R.-H., B.M.-C.; resources, L.D.B. and O.P.L.; data curation, C.R.-B., M.Z. and O.P.L.; writing—original draft preparation, O.P.L., M.d.M.T.-A., and C.R.-B.; writing—review and editing, O.P.L., M.d.M.T.-A., and C.R.-B.; visualization, O.P.L.; supervision, O.P.L. and L.D.B.; project administration, O.P.L.; funding acquisition, O.P.L., M.Z., L.A.H.-H., and L.D.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the University of Las Palmas de Gran Canaria, grant number ULPGC-012-2016, to C. Rial-Berriel., and by the Spanish Ministry of Education, Culture and Sports, grant number FPU16-01888, to A. Acosta-Dacal. Part of this study (2018–2019) has also been supported by the CajaCanarias Foundation (VENECAN project, 2017REC08).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. List of compounds analyzed together with their group and technique of analysis.

Nº	Compound	Group [a]	Technique [b]	Nº	Compound	Group [a]	Technique [b]
1	4,4′-Dichlorobenzophenone	Pesticides, OCPs	GC	156	Pencycuron	Pesticides	LC
2	4,4′-Dicofol	Pesticides, OCPs	GC	157	Pendimethalin	Pesticides	LC
3	Abamectine	Pesticides	LC	158	Permethrin	Pesticides	GC
4	Acephate	Pesticides	LC	159	Phosalone	Pesticides	LC
5	Acetamiprid	Pesticides	LC	160	Phosmet	Pesticides	LC
6	Acrinathrin	Pesticides	LC	161	Phosmet oxon	Pesticides	LC
7	Aldicarb	Pesticides	LC	162	Phthalimide (Folpet deg)	Pesticides	GC
8	Aldicarb sulfone	Pesticides	LC	163	Pirimicarb	Pesticides	LC
9	Atrazine	Pesticides	LC	164	Pirimiphos ethyl	Pesticides	LC
10	Azinphos methyl	Pesticides	LC	165	Pirimiphos methyl	Pesticides	LC
11	Azoxystrobin	Pesticides	LC	166	Prochloraz	Pesticides	LC
12	Benalaxyl	Pesticides	LC	167	Procymidone	Pesticides	GC
13	Bendiocarb	Pesticides	LC	168	Profenofos	Pesticides	LC
14	Bifenthrin	Pesticides	GC	169	Propargite	Pesticides	LC
15	Bitertanol	Pesticides	LC	170	Propiconazole		
16	Boscalid	Pesticides	GC	171	Propoxur	Pesticides	LC
17	Bromopropylate	Pesticides	GC	172	Propyzamide (pronamide)	Pesticides	LC
18	Bromuconazole	Pesticides	LC	173	Proquinazid	Pesticides	LC
19	Bupirimate	Pesticides	LC	174	Prothioconazole-desthio	Pesticides	LC
20	Buprofezin	Pesticides	LC	175	Prothiophos	Pesticides	GC
21	Cadusafos	Pesticides	LC	176	Pyraclostrobin	Pesticides	LC
22	Carbaryl	Pesticides	LC	177	Pyrazophos	Pesticides	LC
23	Carbofuran	Pesticides	LC	178	Pyridaben	Pesticides	LC
24	Carbofuran-3-hydroxy	Pesticides	LC	179	Pyridaphenthion	Pesticides	LC
25	Chlorantraniliprole	Pesticides	LC	180	Pyrimethanil	Pesticides	GC
26	Chlorfenapyr	Pesticides	GC	181	Pyriproxifen	Pesticides	LC
27	Chlorobenzilate	Pesticides	GC	182	Quinalphos	Pesticides	LC
28	Chlorfenvinphos	Pesticides	LC	183	Quinoxyfen	Pesticides	LC
29	Chlorpropham	Pesticides	GC	184	Rotenone	Pesticides	LC
30	Chlorpyrifos	Pesticides	GC	185	Simazine	Pesticides	LC
31	Chlorpyrifos methyl	Pesticides	GC	186	Spirodiclofen	Pesticides	LC
32	Chlorthal dimethyl	Pesticides	GC	187	Spiromesifen	Pesticides	LC
33	Clofentezine	Pesticides	LC	188	Spirotetramat	Pesticides	LC
34	Clothianidin	Pesticides	LC	189	Spirotetramat-enol	Pesticides	LC
35	Coumachlor	Pesticides	LC	190	Spiroxamine	Pesticides	GC
36	Coumaphos	Pesticides	LC	191	Tebuconazole	Pesticides	LC
37	Cyazofamid	Pesticides	LC	192	Tebufenocide	Pesticides	LC
38	Cyflufenamid	Pesticides	LC	193	Tebufenpyrad	Pesticides	LC
39	Cyfluthrin	Pesticides	GC	194	Teflubenzuron (artifact 3)	Pesticides	GC
40	Cyhalothrin (lambda isomer)	Pesticides	LC	195	Tefluthrin	Pesticides	GC
41	Cymoxanil	Pesticides	LC	196	Telodrin	Pesticides	GC
42	Cypermethrin	Pesticides	GC	197	Terbufos	Pesticides	GC
43	Cyproconazole	Pesticides	LC	198	Terbuthylazine	Pesticides	LC
44	Cyprodinil	Pesticides	GC	199	Tetrachlorvinphos	Pesticides	LC
45	Deltamethrin	Pesticides	GC	200	Tetraconazole	Pesticides	LC

Table A1. *Cont.*

Nº	Compound	Group [a]	Technique [b]	Nº	Compound	Group [a]	Technique [b]
46	Demeton-S-methyl	Pesticides	LC	201	Tetradifon	Pesticides	GC
47	Demeton-S-methyl-sulfone (Dioxydemeton)	Pesticides	LC	202	Tetramethrin	Pesticides	GC
48	Diazinon	Pesticides	GC	203	Thiacloprid	Pesticides	LC
49	Dichlofluanid	Pesticides	GC	204	Thiamethoxam	Pesticides	LC
50	Dichloran	Pesticides	GC	205	Thiodicarb	Pesticides	LC
51	Diethathyl ethyl	Pesticides	LC	206	Tolclofos methyl	Pesticides	GC
52	Diethofencarb	Pesticides	LC	207	Tolylfluanid	Pesticides	GC
53	Difenoconazole	Pesticides	LC	208	Triadimefon	Pesticides	LC
54	Diflubenzuron	Pesticides	LC	209	Triadimenol	Pesticides	LC
55	Diflufenican	Pesticides	LC	210	Triazophos (hostathion)	Pesticides	LC
56	Dimethenamide	Pesticides	LC	211	Trichlorfon	Pesticides	LC
57	Dimethoate	Pesticides	LC	212	Trifloxystrobin	Pesticides	LC
58	Dimethomorph	Pesticides	LC	213	Triflumizole	Pesticides	LC
59	Diniconazole-M	Pesticides	LC	214	Triflumuron	Pesticides	LC
60	Dinocap	Pesticides	LC	215	Trifluralin	Pesticides	GC
61	Diphenylamine	Pesticides	LC	216	Triticonazole	Pesticides	LC
62	Endosulfan alfa	Pesticides, OCPs	GC	217	Vinclozolin	Pesticides	GC
63	Endosulfan beta	Pesticides, OCPs	GC	218	Zoxamide	Pesticides	LC
64	EPN	Pesticides	LC	219	Aldrin	OCPs	GC
65	Epoxiconazole	Pesticides	LC	220	Dichlorodiphenyldichlo roethane (p,p′ DDD)	OCPs	GC
66	Esfenvalerate	Pesticides	GC	221	Dichlorodiphenyldichloro ethylene (p,p′ DDE)	OCPs	GC
67	Ethion	Pesticides	LC	222	Dieldrin	OCPs	GC
68	Ethofumesate	Pesticides	GC	223	Endrin	OCPs	GC
69	Ethoprophos	Pesticides	LC	224	Heptachlor	OCPs	GC
70	Etofenprox	Pesticides	LC	225	Hexachlorobenzene	OCPs	GC
71	Etoxazole	Pesticides	LC	226	Hexachlorocyclohexane (alpha)	OCPs	GC
72	Famoxadone	Pesticides	LC	227	Hexachlorocyclohexane (gamma, lindane)	OCPs	GC
73	Fenamidone	Pesticides	LC	228	Hexachlorocyclohexano (beta)	OCPs	GC
74	Fenamiphos	Pesticides	LC	229	Hexaclorociclohexano (delta)	OCPs	GC
75	Fenamiphos sulfone	Pesticides	LC	230	Mirex	OCPs	GC
76	Fenamiphos sulfoxide	Pesticides	LC	231	PCB 28	PCBs	GC
77	Fenarimol	Pesticides	GC	232	PCB 52	PCBs	GC
78	Fenazaquin	Pesticides	LC	233	PCB 77	PCBs	GC
79	Fenbuconazole	Pesticides	LC	234	PCB 81	PCBs	GC
80	Fenbutatin oxide	Pesticides	LC	235	PCB 101	PCBs	GC
81	Fenitrothion	Pesticides	GC	236	PCB 105	PCBs	GC
82	Fenoxycarb	Pesticides	LC	237	PCB 114	PCBs	GC
83	Fenpropathrin	Pesticides	LC	238	PCB 118	PCBs	GC
84	Fenpropimorph	Pesticides	LC	239	PCB 123	PCBs	GC
85	Fenpyroximate	Pesticides	LC	240	PCB 126	PCBs	GC
86	Fenthion	Pesticides	LC	241	PCB 138	PCBs	GC
87	Fenthion oxon	Pesticides	LC	242	PCB 153	PCBs	GC
88	Fenthion oxon sulfone	Pesticides	LC	243	PCB 156	PCBs	GC

Table A1. *Cont.*

Nº	Compound	Group [a]	Technique [b]	Nº	Compound	Group [a]	Technique [b]
89	Fenthion oxon sulfoxide	Pesticides	LC	244	PCB 157	PCBs	GC
90	Fenthion sulfone	Pesticides	LC	245	PCB 167	PCBs	GC
91	Fenthion sulfoxide	Pesticides	LC	246	PCB 169	PCBs	GC
92	Fenvalerate	Pesticides	GC	247	PCB 180	PCBs	GC
93	Fipronil	Pesticides	LC	248	PCB 189	PCBs	GC
94	Fipronil sulfide	Pesticides	GC	249	PBDE 28	PBDEs	GC
95	Fluazinam	Pesticides	LC	250	PBDE 47	PBDEs	GC
96	Flubendiamide	Pesticides	LC	251	PBDE 85	PBDEs	GC
97	Flucythrinate	Pesticides	GC	252	PBDE 99	PBDEs	GC
98	Fludioxonil	Pesticides	LC	253	PBDE 100	PBDEs	GC
99	Flufenoxuron	Pesticides	LC	254	PBDE 153	PBDEs	GC
100	Fluopyram	Pesticides	LC	255	PBDE 154	PBDEs	GC
101	Fluquinconazole	Pesticides	LC	256	PBDE 183	PBDEs	GC
102	Flusilazole	Pesticides	LC	257	Acenaphthene	PAHs	GC
103	Flutolanil	Pesticides	LC	258	Acenaphthylene	PAHs	GC
104	Flutriafol	Pesticides	LC	259	Anthracene	PAHs	GC
105	Fluvalinate tau	Pesticides	LC	260	Benzo[a]anthracene	PAHs	GC
106	Fonofos	Pesticides	GC	261	Benzo[b]fluoranthene	PAHs	GC
107	Fosthiazate	Pesticides	LC	262	Chrysene	PAHs	GC
108	Hexaconazole	Pesticides	LC	263	Fluoranthene	PAHs	GC
109	Hexaflumuron	Pesticides	LC	264	Fluorene	PAHs	GC
110	Hexythiazox	Pesticides	LC	265	Naphthalene	PAHs	GC
111	Imidacloprid	Pesticides	LC	266	Phenanthrene	PAHs	GC
112	Indoxacarb	Pesticides	LC	267	Pyrene	PAHs	GC
113	Iprodione	Pesticides	GC	268	Brodifacoum	ARs	LC
114	Iprovalicarb	Pesticides	LC	269	Bromadiolone	ARs	LC
115	Isocarbophos	Pesticides	GC	270	Coumatetralyl	ARs	LC
116	Isofenphos methyl	Pesticides	LC	271	Difenacoum	ARs	LC
117	Isoprothiolane	Pesticides	LC	272	Difetihalone	ARs	LC
118	Kresoxim methyl	Pesticides	LC	273	Flocoumafen	ARs	LC
119	Linuron	Pesticides	LC	274	Warfarin	ARs	LC
120	Lufenuron	Pesticides	LC	275	Albendazole	PhACs	LC
121	Malaoxon	Pesticides	LC	276	Cefuroxima axetil	PhACs	LC
122	Malathion	Pesticides	LC	277	Chloramphenicol	PhACs	LC
123	Mandipropamid	Pesticides	LC	278	Cloxacillin	PhACs	LC
124	Mefenoxam (metalaxyl-M)	Pesticides	LC	279	Cortiscosterone 21 acetate	PhACs	LC
125	Mepanipyrim	Pesticides	LC	280	Dexamethasone	PhACs	LC
126	Metaflumizone	Pesticides	LC	281	Diclofenac	PhACs	LC
127	Metalaxyl	Pesticides	GC	282	Eprinomectin	PhACs	LC
128	Metaldehyde	Pesticides	LC	283	Fenbendazole	PhACs	LC
129	Metconazole	Pesticides	LC	284	Flunixin	PhACs	LC
130	Methamidophos	Pesticides	LC	285	Imipenem	PhACs	LC
131	Methidathion	Pesticides	LC	286	Josamycin	PhACs	LC
132	Methiocarb	Pesticides	LC	287	Ketoprofen	PhACs	LC
133	Methiocarb sulfone	Pesticides	LC	288	Mebendazole	PhACs	LC
134	Methiocarb sulfoxide	Pesticides	LC	289	Mefenamic acid	PhACs	LC
135	Methomyl	Pesticides	LC	290	Metronidazole	PhACs	LC
136	Methomyl oxime	Pesticides	LC	291	Moxidectin	PhACs	LC
137	Methoxyfenozide	Pesticides	LC	292	Naproxen	PhACs	LC

Table A1. *Cont.*

Nº	Compound	Group [a]	Technique [b]	Nº	Compound	Group [a]	Technique [b]
138	Metrafenone	Pesticides	LC	293	Oxfendazole	PhACs	LC
139	Mevinphos (phosdrin)	Pesticides	LC	294	Penicilina V	PhACs	LC
140	Monocrotophos	Pesticides	LC	295	Sulfacetamide	PhACs	LC
141	Myclobutanil	Pesticides	LC	296	Sulfacloropiridacine	PhACs	LC
142	N,N-Dimethyl-N′-p-tolylsulphamide (DMST)	Pesticides	LC	297	Sulfadiacine	PhACs	LC
143	N,N-dimethylformamidine (DMF)	Pesticides	LC	298	Sulfadimetoxine	PhACs	LC
144	Nuarimol	Pesticides	LC	299	Sulfadoxine	PhACs	LC
145	Ofurace	Pesticides	LC	300	Sulfameracine	PhACs	LC
146	Omethoate	Pesticides	LC	301	Sulfametacine	PhACs	LC
147	Oxadixyl	Pesticides	LC	302	Sulfametizole	PhACs	LC
148	Oxamyl	Pesticides	LC	303	Sulfametoxazole	PhACs	LC
149	Oxamyl oxime	Pesticides	LC	304	Sulfametoxipiridacine	PhACs	LC
150	Oxyfluorfen	Pesticides	GC	305	Sulfamonomethoxine	PhACs	LC
151	Paclobutrazol	Pesticides	LC	306	Sulfanilamide	PhACs	LC
152	Paraoxon methyl	Pesticides	GC	307	Sulfapiridine	PhACs	LC
153	Parathion ethyl	Pesticides	GC	308	Sulfaquinoxaline	PhACs	LC
154	Parathion methyl	Pesticides	GC	309	Sulfisoxazole	PhACs	LC
155	Penconazole	Pesticides	LC	310	Tolfenamic acid	PhACs	LC

[a] PBDE—Polybrominated diphenyl ethers, OCP—Organochlorine pesticides, PAH—Polycyclic aromatic hydrocarbon, PCB—Polychlorinated biphenyl, PhACs—Pharmaceuticals Active Compounds, Ars—Anticoagulant Rodenticides, P-IS—Procedural Internal Standard. [b] Gas chromatography (GC) or liquid chromatography (LC), both coupled with tandem triple quadrupole mass spectrometry.

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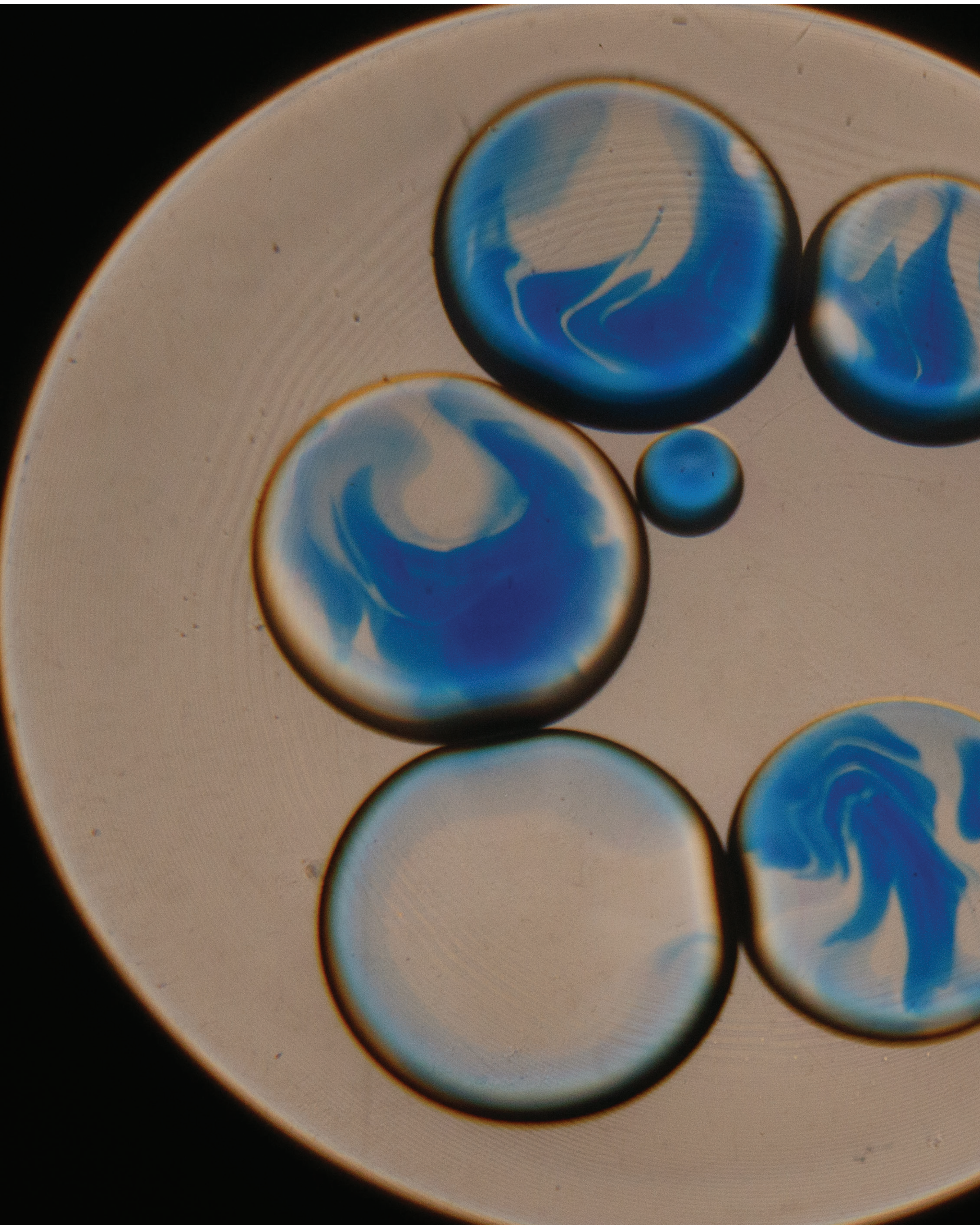
VII. CONCLUSIONES/CONCLUSIONS



CONCLUSIONES

1. Nuestro trabajo ha servido para validar los métodos de extracción, basados en una extracción simple (QuEChERS), y detección y cuantificación en cromatografía líquida y de gases acoplada a espectrometría de masas de triple cuadrupolo (LC-MS/MS y GC-MS/MS para el análisis simultaneo de 360 y 351 analitos, para las matrices de sangre e hígado, respectivamente, en pequeños volúmenes.

2. Las técnicas desarrolladas permiten analizar simultáneamente compuestos de diferentes clases y familias químicas, tales como contaminantes orgánicos persistentes, medicamentos, y biocidas de diferentes tipos, los cuales habitualmente han requerido análisis independientes.

3. Como parte del proceso de verificación de la aplicabilidad de los métodos, hemos analizado series de muestras reales, que han permitido verificar la idoneidad de los métodos desarrollados para estudios de biomonitorización, de seguridad alimentaria, así como para el diagnóstico laboratorial de envenenamientos de fauna.

4. Nuestros estudios de biomonitorización han demostrado la elevada incidencia de la exposición a rodenticidas anticoagulantes en las especies no objetivo, detectándose varios de estos compuestos simultáneamente en alrededor del 60% muestras de hígado de especies de fauna silvestre de las islas canarias. Nuestros datos demuestran una clara influencia de la ganadería intensiva en la exposición de la fauna silvestre a los rodenticidas anticoagulantes.

5. Algunos COPs, como el DDE, o algunos PAHs, siguen estando presentes en muestras de animales vivos, tanto en sangre, lo que podría indicar una exposición reciente, como en hígado, donde se encuentran mayor cantidad de contaminantes, como son BDEs, PCBs, organoclorados o PAHs.

6. Los medicamentos como AINEs y antibióticos, parecen no ser un problema para la salud de la fauna silvestre estudiada, ya que no se han detectado cantidades relevantes de estos compuestos en hígado y sangre.

7. Finalmente, nuestro trabajo ha permitido actualizar la base de envenenamientos de fauna silvestre de Canarias, desde el año 2014 al 2021. Durante este periodo hemos analizado un total de 961 animales afectados y 84 cebos, y la incidencia de envenenamientos en Canarias sigue siendo muy alta. El carbofurano y el aldicarb siguen siendo las sustancias más usadas, seguidos por los rodenticidas anticoagulantes, a la vez que han aumentado los casos en los que se detectan varias sustancias activas simultáneamente.

CONCLUSIONS

1. Our work has supported the data to validate the extraction methods, based on a simple extraction (QuEChERS), and detection and quantification by liquid and gas chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS and GC-MS/MS) for the simultaneous analysis of 360 and 351 analytes, for blood and liver, respectively, in small sample volumes.

2. The methods developed allow the simultaneous analysis of compounds of different classes and chemical families, such as persistent organic pollutants, pharmaceuticals, and biocides of different types, which have usually required independent analysis.

3. As part of the process of verifying the applicability of the methods, we have analysed series of real samples, which have made it possible to verify the suitability of the methods developed for biomonitoring and food safety studies, as well as for the laboratory diagnosis of poisonings.

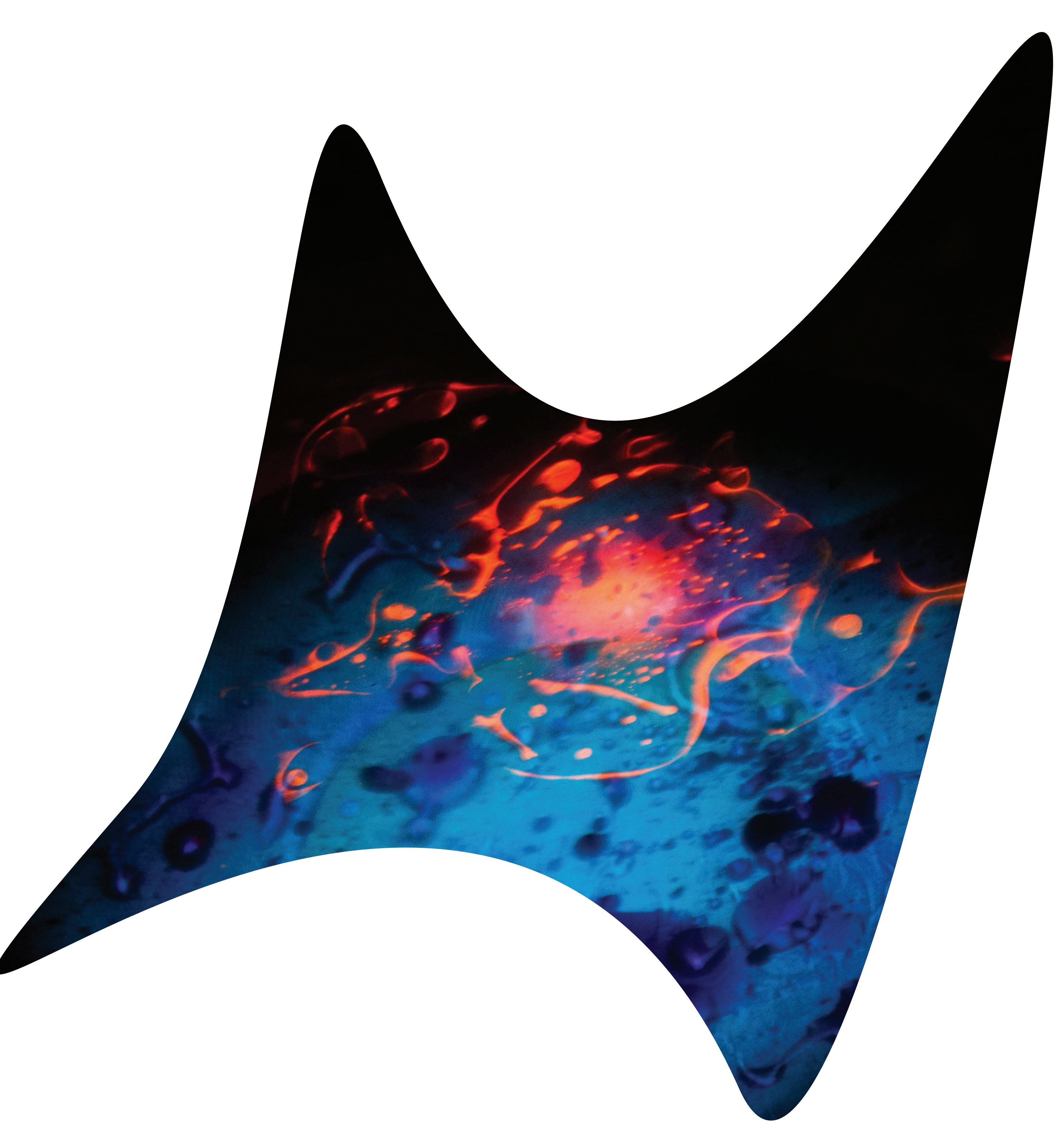
4. Our biomonitoring studies have demonstrated the high incidence of exposure to anticoagulant rodenticides in non-target species, with several of these compounds being detected simultaneously in around 60% liver samples from wildlife species from the Canary Islands. Our data demonstrate a clear influence of intensive livestock farming on the exposure of wildlife to anticoagulant rodenticides.

5. Some POPs, such as DDE, or some PAHs, are still present in samples of live animals, both in blood, which could indicate a recent exposure, and in the liver, where there are more contaminants, such as BDEs, PCBs, organochlorines, or PAHs.

6. Medicaments such as NSAIDs and antibiotics seem not to be a health problem for the wildlife studied, since relevant amounts of these compounds have not been detected in liver or in blood.

7. Finally, our work has made it possible to update the Canary Islands wildlife poisoning database, from 2014 to 2021. During this period, we have analysed a total of 961 affected animals and 84 baits, and the incidence of poisonings in the Canary Islands continues to be very high. Carbofuran and aldicarb continue to be the most used substances, followed by anticoagulant rodenticides, while the cases in which several active substances are detected simultaneously have increased.

VIII. ANEXOS

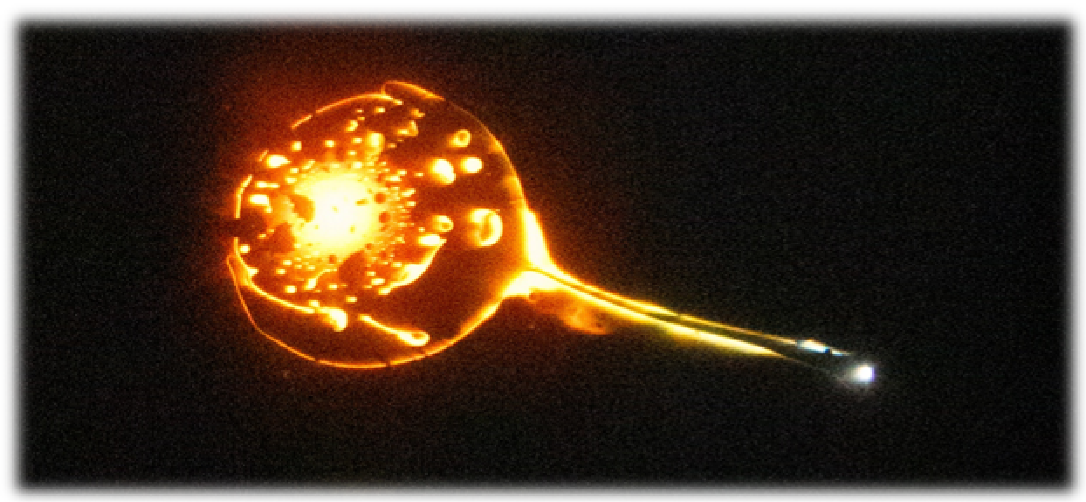


Publicación 8. Incidence of 49 elements in the blood and scute tissues of nesting hawksbill turtles (*Eretmochelys imbricata*) in Holbox Island

Incidencia de 49 elementos en la sangre y los tejidos del escudo de las tortugas carey (Eretmochelys imbricata) en la isla Holbox

Regional Studies in Marine Science, 2021, 41: 101566

DOI: https://doi.org/10.1016/j.rsma.2020.101566

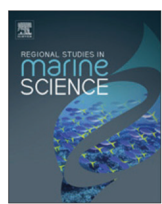


Regional Studies in Marine Science 41 (2021) 101566

Contents lists available at ScienceDirect

Regional Studies in Marine Science

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



Incidence of 49 elements in the blood and scute tissues of nesting hawksbill turtles (*Eretmochelys imbricata*) in Holbox Island

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ARTICLE INFO

Article history:
Received 17 February 2020
Received in revised form 22 November 2020
Accepted 23 November 2020
Available online 4 December 2020

Keywords:
Hawksbill
Sea turtle
Rare earth elements
Caribbean Region
Inorganic emergent pollutants
Elements

ABSTRACT

Due to progressive urban development along the Mexican Caribbean coastline, it is crucial to gauge the impact of anthropogenic contamination of marine ecosystems through biomonitoring procedures. In the current study, we quantified the concentration of 49 inorganic elements in the blood and scute tissues of clinically healthy nesting hawksbill sea turtles (*Eretmochelys imbricata*). The elements were classified into four groups: Group A: essential mineral elements with toxic potential; Group B: non-essential elements with high toxicity; Group C: toxic non-essential minority elements and Trace Elements (TE); and Group D: rare-earth elements (REE) and other TE. Almost all the samples in both tissues showed perceptible levels of all the quantified elements. The only element identified with a correlation between blood and scute was arsenic (As), which could indicate a fast excretion through this type of keratinized tissue. The bio-accumulation of inorganic elements is a complex process, requiring the simultaneous examination of different tissues to evaluate the exposure. Our study reinforces the usefulness of scute tissue as a non-invasive sampling technique for the evaluation of persistent pollutants in marine turtles.

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1. Introduction

Biomonitoring inorganic elements as persistent pollutants has become an important tool to provide baseline measurements for further studies on the health status of marine life. Being aware of the contamination levels in developed areas enables timely decision-making for wildlife management and conservation (Patino-Martinez et al., 2014).

Hawksbill turtles are selective feeders. Sponges comprise 95.3% of their diet throughout the Caribbean Region, while the rest consists of jellyfish, mollusks, fish, marine algae, crustaceans, and other sea plants and benthic invertebrates. They forage in benthic habitats, over coral reefs, rock outcroppings, seagrass pastures, and mangrove-fringed bays (Bjorndal, 1996). These coastal habitats are often in close proximity to sources of persistent inorganic pollutants, which make their way into the marine environment from industrial, domestic and agricultural sources (Ehsanpour et al., 2014).

No studies on the biomonitoring of sea turtles to date have included rare-earth elements (REE). There is growing concern regarding environmental pollution produced by REE and other trace elements (TE), which are extensively and increasingly employed in the manufacture of consumer electronics and new technologies, so some authors consider these REE as emerging pollutants to be considered in biomonitoring studies (Goodenough et al., 2017; Deetman et al., 2018). Although they have not been classified as toxic or priority pollutants for the marine ecosystem, some studies have reported REE with regard to health effects, toxicity and concentration in different species and tissues

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https://doi.org/10.1016/j.rsma.2020.101566
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(Pagano et al., 2015a,b), providing evidence of adverse effects, including inflammation, oxidative stress and tissue damage in liver, lungs and kidneys in medium-term exposure (Pagano et al., 2012).

In the current study we evaluate the concentrations of 49 environmentally relevant inorganic elements of anthropogenic origin, including REE and TE in nesting hawksbill turtles (*Eretmochelys imbricata*) using blood and scute tissues to provide the baseline data for future studies of monitoring and contamination assessment risk programs in the Caribbean Region.

2. Materials and methods

Between May through June 2018, we collected 19 blood and scute samples of clinically healthy nesting hawksbill sea turtles (*Eretmochelys imbricata*) from the beaches of Holbox Island, Quintana Roo State, an important hawksbill nesting area. The study site is located along the coast between the coordinates 21°33'42.3"N; 87°20'13.8"W and 21°35'35.8"N; 87°07'11.8"W, covering a total length of 24 km. The sampling permits (Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) were issued by the Secretary for Natural Resources Management in Mexico. The research methods and animal welfare measures were evaluated and approved by our local institutional Ethical Committee, at the Veterinary Medicine and Zootechnic Faculty (FMVZ), National Autonomous University of Mexico (UNAM). To avoid double sampling by recapture, individual turtles were marked with flipper tags and series numbers were registered.

2.1. Blood sampling

Whole blood samples (5 ml) were collected from the cervical sinus using single-use needles (21 gauge), plastic syringes, and blood collection tubes containing lithium heparin to avoid clotting (Campbell, 2012). The dorsal neck region was previously wiped clean and disinfected (gauze with ethanol 70° and neck with povidone-iodine 3%) to reduce sample contamination and health risk in the specimens. The samples were collected when the turtles returned to the sea after laying eggs to minimize any disturbance to nesting. In addition, a complete visual physical examination was performed, and the size of the turtles was evaluated based on the curve carapace length and width. During fieldwork and transportation to the processing site, the samples were kept with a coolant gel (−4 °C) in ice chests in the Toxicology Laboratory at the FMVZ-UNAM.

2.2. Scute sampling

According to modified protocols (Bjorndal et al., 2010), supracaudal scute samples (<1 g) were collected after successfully blood sampling the specimens and cleaning the carapace. The region was previously wiped clean and disinfected with a sequence of gauze with ethanol 70° and gauze with povidone-iodine 3% to reduce sample contamination or health risk to the specimens. This is a proven non-invasive procedure, which does not affect the health or physical condition of the specimen (Bjorndal et al., 2010; Komoroske et al., 2011, 2012).

2.3. Analysis of elements

The whole blood and scute samples were frozen at −20 °C until analysis. In the Toxicology Laboratory at the FMVZ-UNAM, the blood samples were homogenized by manual shaking oscillation and the scute samples were washed with deionized water and a brush with plastic bristles to eliminate any superficial material from the environment. A 1 g fraction of whole blood and 0.5 g of scute were used for the quantification of the elements. These samples were digested by the humid digestion process in 2 ml of nitric acid at 60% and 0.5 ml of hydrogen peroxide at 10% following the NOM-117-SSA1-1994 methodological standards. Once completely digested, the processed samples were filtered with Whatman No. 2 filter paper and diluted. The digested samples were sent to the Clinical and Analytical Toxicology Laboratory of the Las Palmas de Gran Canaria University, where they were stored at −4 °C till their analysis. All the samples were received in perfect condition and correctly identified.

We determined the concentration levels of 49 elements, which were classified according to their biological and toxicological importance (Goyer and Clarkson, 2001) into four groups: Group A: essential mineral elements with toxic potential: Co (cobalt), Cr (chromium), Cu (copper), Fe (iron), Mn (manganese), Mo (molybdenum), Ni (nickel), Se (selenium) and Zn (zinc). Group B: non-essential elements with high toxicity: Al (aluminum), As (arsenic), Be (beryllium), Cd (cadmium), Pb (lead) and Hg (mercury). Group C, toxic non-essential minority elements: Ag (silver), Au (gold), Ba (barium), Bi (bismuth), Ga (gallium), Pd (palladium), Pt (platinum), Sb (antimony), Sn (tin), Sr (strontium), Th (thorium), Ti (titanium), Tl (thallium), U (uranium) and V (vanadium). Group D: rare-earth elements (REE) and other trace elements (TE): Ce (cerium), Dy (dysprosium), Eu (europium), Er (erbium), Gd (gadolinium), Ho (holmium), In (indium), La (lanthanum), Lu (lutetium), Nb (niobium), Nd (neodymium), Os (osmium), Pr (praseodymium), Ru (ruthenium), Sm (samarium), Ta (tantalum), Tb (terbium), Tm (thulium), Y (yttrium) and Yb (ytterbium).

For the element analyses, we employed an Agilent 7900 ICP-MS (Agilent Technologies, Tokyo, Japan) equipped with standard nickel cones, Ultra High Matrix Introduction (UHMI) system, and a Cross-Flow Nebulizer with a make-up gas port (X400 Nebulizer, Savillex Corporation, Eden Prairie, MN, USA). We followed the previously validated procedure in our laboratory, using certified reference materials (González-Antuña et al., 2017). Two standard curves (twelve points, 100–0.005 ng/mL) were made to avoid interferences between elements: a) one using a commercial multi-element mixture (CPA Chem Catalog number E5B8.K1.5N.L1, 21 elements, 100 mg/L, 5% HNO_3) containing for all the inorganic elements (metals, metalloids, and non-metals) b) multi-element mixture tailor-made in our laboratory, which contained the REE and TE most frequently employed in the high-tech industry. The concentrations of elements below the limit of detection (LOD) were assigned a zero (0) value and all the metal concentrations were expressed as micrograms per gram of wet weight ($\mu g/g^{-1}$ of w.w.).

2.4. Statistical analysis

Database management and statistical analysis were performed using R software (R-3.5.2 version). The mean, standard deviation, median, and range were determined for each parameter. Because the data were not normally distributed, the statistical analyses involved the use of non-parametric tests. The differences among the tissues were tested through Wilcoxon signed-rank test in the paired analysis. In addition, continuous variables were analyzed by the Spearman correlation test. The P values of less than 0.05 (two-tail) were considered statistically significant.

3. Results and discussion

The complete profile and *P values* of all inorganic elements in blood and scute is shown in Fig. 1, the distribution of blood and scute concentrations is listed in the order of highest to lowest concentration of the element between both tissues.

Group A, corresponding to essential elements, showed the highest concentrations. For the great majority of elements, the scute concentrations were higher than those found in the blood, except for Fe, Se, Be, Hg, Ag, Ce, and Os. These findings support previous reports, suggesting that scute in sea turtles acts as a repository where metals are stored after they have been metabolized and excreted into a keratinized tissue (Komoroske et al., 2011, 2012; Bezerra et al., 2013). Moreover, it is also indicative of longer-term exposure and useful for monitoring concentrations of inorganic elements (Jakimska et al., 2011a,b). These findings are consistent with those reported in other studies (Komoroske et al., 2011, 2012; Bezerra et al., 2013).

Essential minerals are required for physiological processes in the body and are commonly found in high concentrations in different tissues (Cortés-Gómez et al., 2017; Jakimska et al., 2011a,b). To date, there have been no known studies in nesting hawksbill turtles that have used scute as a biomonitoring tissue, and to our knowledge, ours is the first study to analyze inorganic mineral concentrations of scute in hawksbill turtles. Table 1 summarizes the concentrations of the elements found in blood and scute tissues.

Concentrations within Group A (essential mineral elements with toxic potential) are summarized in Table 1, where no elements were found below LOD. Median elemental values in the blood were ranked as: Fe > Zn > Se > Cu > Ni > Mn > Mo > Cr > Co, while those in scute were ranked as: Fe > Zn > Ni > Cu > Cr > Mn > Se > Mo > Co. Elements found to be in the greatest concentrations in blood were metals preferentially associated with red blood cells (Fig. 1) relative to whole blood (Goyer and Clarkson, 2001). In other studies of different species of sea turtles (Camacho et al., 2014; Jerez et al., 2010; Ley-Quiñonez et al., 2011), Zn has been reported to be the most abundant element present in different tissues.

Hawksbill sea turtles are one of the least-studied species regarding their contamination status, as only the levels of inorganic elements in the Pacific (Anan et al., 2001; Suzuki et al., 2017), Persian Gulf (Ehsanpour et al., 2014), East Wider Caribbean Region (Dyc et al., 2015) and Atlantic (Camacho et al., 2014) populations have been reported. A study on nesting hawksbill turtles (Ehsanpour et al., 2014) had reported Cu, Zn and TE elements in whole blood. However, the results were originally published in dry weight and no moisture content was reported by the authors. Compared to other studies, the concentrations of Cr, Cu, Mn, Ni, Se and Zn in whole blood of juvenile hawksbills were found at a rate of 4.4 to 4.7 times lower (Camacho et al., 2014) than those reported in the present study, while Ni and Se presented a greater difference, with 37 and 12.3 times lower respectively.

These differences could be related to geographic variations in the diet, age, and physiological status. Juvenile hawksbills typically consume what is available at the pelagic zone, whereas adults, who feed in coastal waters, subsist on a diet comprised primarily of sponges. Some studies have indicated that bio-accumulation of Se by sponges could be due to the high dependence of the species on the element for growth (Müller et al., 2005). As these sponges represent a significant portion of adult hawksbills' diet, this relationship probably accounts for the high levels of Se found in their tissues.

Some authors like Ley-Quiñonez et al. (2011) mention the difficulty in establishing whether the high concentrations of Se in sea turtles can be considered a toxic factor; however, selenomethionine is considered to be the primary form of organic selenium relevant for bio-accumulation and toxicity in wildlife (Dyc et al., 2015, 2016). It has been suggested that the presence of Se in sea turtle eggs could be toxic for the embryo (Lam et al., 2006), and a mother-embryo metal transfer has been demonstrated in sea turtles (Paez-Osuna et al., 2010a,b, 2011; Dyc et al., 2015). The registration of high concentration should not be underestimated, as much remains to be learned regarding Se toxicity for reptiles and marine wildlife. The essential elements (Cu, Cr, Mn, Ni, Se, and Zn) play vital roles in tissue metabolism and growth. However, the negative effects of some of these elements on sea turtles are not well known.

Concentrations within Group B (non-essential elements with high toxicity) are summarized in Table 1, where only beryllium (Be) was present above LOD in 56% of blood samples and below LOD in all scute samples. All other elements were present in both tissues. Most of the values in this group were similar to others published for different species (Cortés-Gómez et al., 2017). The median elemental values in the blood and scute were ranked as: Al > As > Pb > Cd > Hg > Be.

It is noteworthy that the levels of As in scute and blood were not significantly different ($P = 0.7019$), which could indicate a fast excretion through this type of keratinized tissues such as scute (Goyer and Clarkson, 2001). The arsenic concentrations in blood (median 0.82 $\mu g/g^{-1}$ in w.w.) were found at double the levels reported in juvenile hawksbills (Camacho et al., 2014). The arsenic compounds in seawater are mainly comprised of arsenates including arsenite, methylarsonic acid, and dimethylarsinic acid (DMA) (Saeki et al., 2000), where DMA tended to be the most common arsenic compound found in sea turtles and in higher concentrations in hawksbill turtles (Saeki et al., 2000). These arsenic compounds bio-accumulate more intensively in sea turtles than in other marine animals, and the explanation for it could be that their preys contain high levels of arsenic or that the metabolic processes are different for sea turtles (Jakimska et al., 2011a). Although DMA is less toxic, it may be necessary to investigate the influences of DMA on different tissues of sea turtles due to the DNA damage induced by free radicals of DMA metabolites, dimethylarsine and molecular oxygen in other species (Okada and Yamanaka, 1994).

The concentrations within Group C (toxic non-essential minority elements TE) are summarized in Table 1, where no elements were found below LOD. The median elemental values in the blood were ranked as: Sr > Ba > Sn > V > Ti > Sb > U > Ag > Bi > Pd > Tl > Th > Pt > Ga > Au; while those in scute were ranked as: Sr > Ba > Sn > Ti > V > Sb > Pd > U > Bi > Pt > Ag > Ga > Tl > Th > Au. The scute concentrations of these elements were higher than those found in the blood except for Ag.

The most studied TE in sea turtles to date are Ag, Ba, Sb, Sn, Sr, Ti, Tl, and V (Komoroske et al., 2011; Labrada-Martagón et al., 2011; McFadden et al., 2014; Carneiro da Silva et al., 2016; Villa et al., 2017). These concentrations were lower than those in our study. The mechanism of action underlying the adverse effects of TE is largely unknown in many cases.

The concentrations within Group D (REE and other TE) are summarized in Table 1, where only osmium (Os) was found below LOD in half of the samples of blood, and 44% of scute samples. The median elemental values in the blood were ranked as: Ce > La > Nd > Y > In > Gd > Pr > Nb > Sm > Dy > Yb > Eu > Er > Ho > Tb > Lu > Tm > Os > Ta; while those in scute ranked as: Ce > La > Nd > Y > In > Nb > Pr > Sm > Gd > Dy > Yb > Er > Eu > Ho > Tb > Ta > Lu > Tm > Os. The scute concentrations of these elements were higher than those found in the blood, except for Ce and Os. Most of the REE studied to date in other species are mainly Ce, La, Pr, Nd, Ho, and Tb; these elements have been shown to produce cytogenetic abnormalities, induce a differential expression of the genes involved in immune response to inflammation, apoptosis, oxidative stress, and tissue damage on the kidneys, liver, and lungs (Cheng et al., 2014; Pagano et al., 2015a,b).

In both tissues, cerium (Ce) was the element of this group found at the highest concentrations. It is relevant to mention that this element has been described to be toxic in Sprague-Dawley

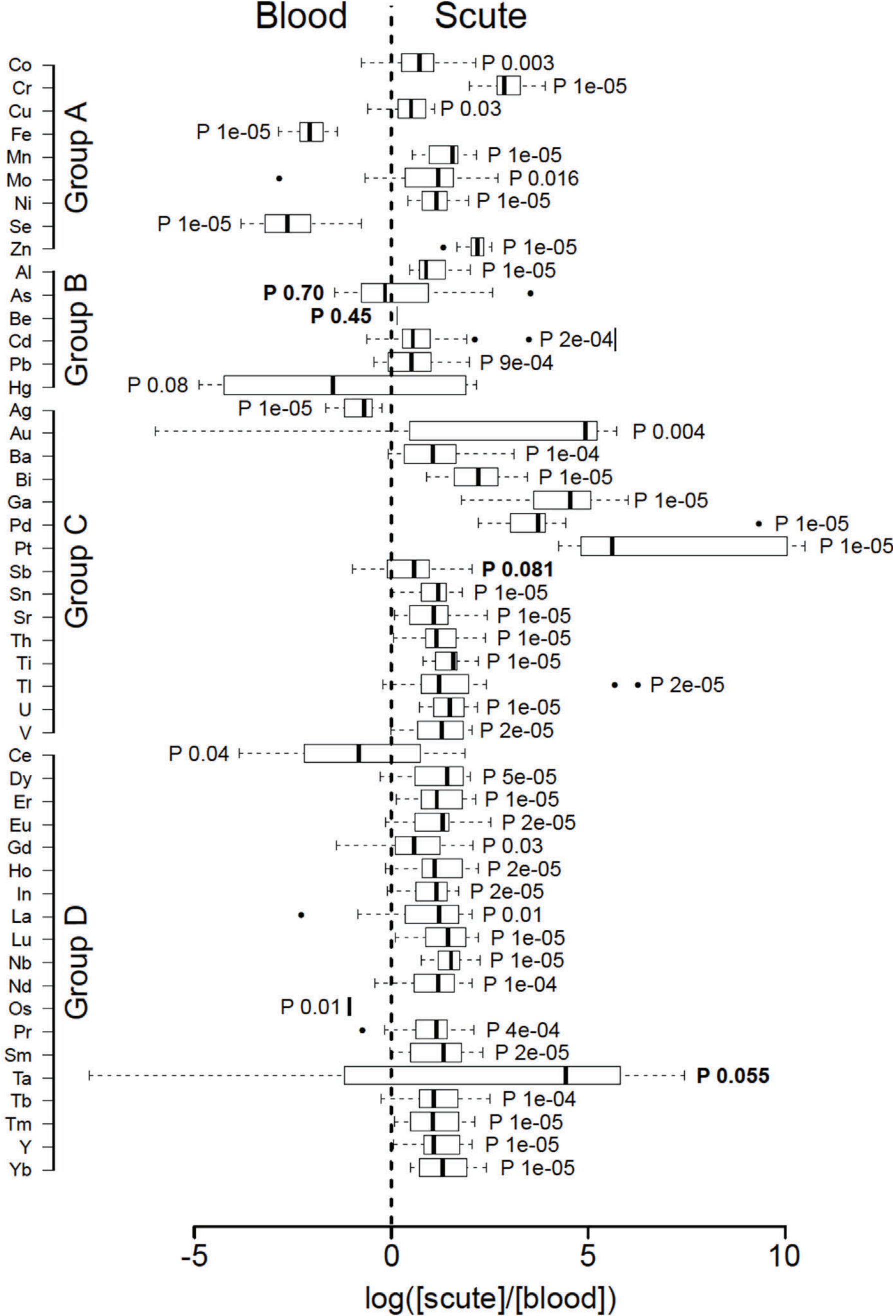

Fig. 1. Distribution of blood and scute concentrations in groups A, B, C, and D. Statistical significance (*P* values) is indicated between blood and scute values in the paired analysis. The Wilcoxon signed-rank test was performed for each element. The central line in each box plot represents the median value; the box denotes the data spread from 25% to 75%, and the whiskers reflect 10%–90%, upper and lower whiskers (largest and smaller data numbers) are noted with dotted line in each box plot. The outliers for each distribution are plotted. Box plots are side orientated (left or right) in the order of highest to lowest concentration of the element between blood (left) and scute (right). In this figure we can observe that for the great majority of the elements analyzed scute concentration were higher than those found in the blood.

rats, causing liver tissue damage (hydropic degeneration of the hepatocytes, dilation of the sinusoids, portal inflammation, and fibrosis of the liver) (Nalabotu et al., 2011). The second most abundant element was lanthanum (La), for which toxicity has also been demonstrated. Thus, some studies have demonstrated that La causes nephrotoxicity (histopathological changes in the kidneys, changes in lipid peroxidation levels, increased activity of oxidative stress, decreased superoxide dismutase activity) in mice (Zhao et al., 2013), and neurotoxicological consequence of long-term exposure, disturbance of the homeostasis of trace elements, enzymes, and neuro-transmitter systems in the brain of rats (Feng et al., 2006).

Some authors like Wang and Yamada (2006) have reported that REE are present at low concentrations in marine systems in a normal way. Considerable field observations and laboratory experiments have been conducted to study the marine cycling of REE (Wang and Yamada, 2006; Zheng et al., 2016). Despite these efforts, substantial uncertainties remain about the processes that control the distribution in the ocean, the ability to interpret patterns, the influence of physical transport, the bio-geochemical processes, and the different sources responsible for additional plumes, like those reported in 2016 by Zheng et al. in the tropical South Atlantic current about the abnormal increase of Ce from

Table 1
Inorganic elements concentrations (μg/g-1 en w.w.) in blood and scute tissues of nesting hawksbill turtles of the Yum-Balam Biosphere Reserve, Holbox, Mexico.

	Blood		Scute		Bio-magnification[a]
	Median ± SD	Mean (Range)	Median ± SD	Mean (Range)	
Group A: essential mineral elements with toxic potential concentrations					
Co	0.01 ± 0.01	0.01 (n.d.–0.03)	0.02 ± 0.01	0.02 (0.01–0.4)	2
Cr	0.04 ± 0.04	0.05 (0.03–0.18)	0.94 ± 0.32	0.96 (0.5–1.54)	17.5
Cu	0.79 ± 0.88	1.10 (0.49–3.69)	1.45 ± 0.47	1.48 (0.77–2.40)	1.7
Fe	180.07 ± 36.39	181.99 (108.21–250.41)	22.75 ± 10.25	24.74 (11.33–49.55)	0.1
Mn	0.14 ± 0.05	0.14 (0.08–0.26)	0.57 ± 0.20	0.59 (0.24–0.91)	4.5
Mo	0.08 ± 0.47	0.20 (0.02–2.07)	0.18 ± 0.09	0.21 (0.11–0.44)	3.3
Ni	0.74 ± 0.47	0.88 (0.35–2.39)	2.40 ± 0.69	2.57 (1.68–4.08)	3.2
Se	7.25 ± 6.31	8.55 (1.16–23.54)	0.50 ± 0.23	0.50 (0.15–1.11)	0.1
Zn	9.64 ± 1.68	9.87 (7.67–13.28)	92.9 ± 23.46	85.52 (39.80–124.14)	8.8
Group B: non-essential elements with high toxicity concentrations					
Al	1.81 ± 0.85	1.90 (0.90–3.58)	4.46 ± 2.22	5.20 (2.42–11.30)	2.4
As	0.82 ± 0.69	0.81 (0.05–2.82)	0.68 ± 0.68	0.80 (0.34–3.40)	0.9
Be	0.02 ± 0.01	0.02 (n.d. – 0.05)	0.02 ± 0.01	0.02 (n.d.–0.04)	0
Cd	0.01 ± 0.01	0.01 (n.d.–0.02)	0.01 ± 0.01	0.02(n.d.-0.04)	1.7
Pb	0.12 ± 0.10	0.16 (0.06–0.46)	0.22 ± 0.09	0.24 (0.11–0.47)	1.7
Hg	0.01 ± 0.005	0.005 (n.d.–0.02)	0.01 ± 0.02	0.01 (n.d.–0.07)	0.9
Group C: toxic non-essential minority elements, TE concentrations					
Ag	0.01 ± 0.01	0.02 (0.01–0.04)	0.01 ± 0.005	0.01 (n.d.–0.02)	0.5
Au	0.005 ± 0.005	0.005 (n.d.–0.01)	0.01 ± 0.01	0.01 (n.d.–0.02)	138.8
Ba	0.63 ± 0.41	0.78 (0.27–1.93)	1.71 ± 1.71	2.49 (0.90–6.12)	2.9
Bi	0.005 ± 0.003	0.005 (n.d.–0.01)	0.02 ± 0.01	0.02 (0.01-0.1)	9.2
Ga	0.005 ± 0.003	0.005 (n.d–0.02)	0.005 ± 0.01	0.01 (n.d.–0.02)	95.3
Pd	0.005 ± 0.004	0.005 (n.d.–0.008)	0.03 ± 0.02	0.03 (0.01–0.07)	41.7
Pt	0.005 ± 0.005	0.0 (n.d.–0.005)	0.02 ± 0.01	0.02 (0.01–0.03)	270.7
Sb	0.02 ± 0.02	0.3 (0.01–0.08)	0.04 ± 0.02	0.04 (0.01–0.09)	1.8
Sn	0.23 ± 0.06	0.23 (0.13–0.35)	0.8 ± 0.36	0.74 (0.29–1.37)	3.3
Sr	1.44 ± 0.44	1.48 (0.77–2.53)	3.67 ± 3.28	4.83 (1.62–15.49)	3
Th	0.005 ± 0.005	0.0 (n.d.–0.005)	0.01 ± 0.005	0.0 (n.d.–0.01)	3.2
Ti	0.04 ± 0.01	0.05 (0.03–0.07)	0.19 ± 0.08	0.21 (0.08–0.37)	4.8
Tl	0.005 ± 0.005	0.0 (n.d.–0.005)	0.01 ± 0.005	0.01 (n.d.–0.01)	3.4
U	0.01 ± 0.002	0.01 (0.005–0.01)	0.03 ± 0.01	0.03 (0.01–0.05)	4.4
V	0.04 ± 0.02	0.05 (0.02–0.09)	0.12 ± 0.08	0.15 (0.07–0.35)	3.6
Group D: REE and other TE concentrations					
Ce	0.04 ± 0.08	0.06 (0.003–0.31)	0.01 ± 0.01	0.02 (0.01–0.07)	0.4
Dy	0.005 ± 0.005	0.0 (n.d.–0.01)	0.005 ± 0.03	0.01 (n.d.–0.02)	4.1
Er	0.005 ± 0.003	0.0 (n.d.–0.01)	0.08 ± 0.005	0.01 (n.d.–0.03)	3.2
Eu	0.005 ± 0.005	0.0 (n.d.–0.02)	0.005 ± 0.03	0.01 (n.d.–0.03)	3.7
Gd	0.01 ± 0.01	0.01 (n.d.–0.02)	0.02 ± 0.01	0.01 (n.d.–0.03)	1.8
Ho	0.005 ± 0.003	0.0 (n.d.–0.01)	0.08 ± 0.005	0.01 (n.d.–0.03)	3
In	0.001 ± 0.0003	0.001(0.001-0.002)	0.003 ± 0.002	0.003(0.001-0.01)	3.1
La	0.005 ± 0.003	0.0 (n.d.–0.01)	0.08 ± 0.005	0.01 (n.d.–0.03)	3.4
Lu	0.01 ± 0.002	0.01 (0.005–0.01)	0.03 ± 0.01	0.03 (0.01–0.05)	4.3
Nb	0.005 ± 0.005	0.0 (n.d.–0.005)	0.01 ± 0.005	0.0 (n.d.–0.01)	4.6
Nd	0.005 ± 0.003	0.0 (n.d.–0.01)	0.08 ± 0.005	0.01 (n.d.–0.03)	3.3
Os	0.02 ± 0.01	0.02 (n.d. – 0.05)	0.02 ± 0.01	0.02 (n.d.–0.04)	0.4
Pr	0.005 ± 0.005	0.0 (n.d.–0.005)	0.01 ± 0.005	0.0 (n.d.–0.01)	3.2
Sm	0.005 ± 0.005	0.0 (n.d.–0.005)	0.01 ± 0.005	0.0 (n.d.–0.01)	3.8
Ta	0.005 ± 0.003	0.005 (n.d–0.02)	0.005 ± 0.01	0.01 (n.d.–0.02)	83.4
Tb	0.005 ± 0.003	0.0 (n.d.–0.01)	0.08 ± 0.005	0.01 (n.d.–0.03)	3
Tm	0.006 ± 0.004	0.0 (n.d.–0.02)	0.07 ± 0.006	0.01 (n.d.–0.04)	2.9
Y	0.02 ± 0.005	0.005 (n.d.–0.005)	0.06 ± 0.006	0.009 (n.d.–0.1)	2.9
Yb	0.005 ± 0.003	0.0 (n.d.–0.01)	0.08 ± 0.005	0.01 (n.d.–0.03)	3.7

[a]Proportion of the biomagnified element in scute in relation to the amount recorded in blood, values ≤ 1 represent elements that were found in greater proportion in blood.
n.d. Non detected.

unknown sources, although all processes are recognized to be important for REE in marine systems.

These elements can bio-accumulate in marine invertebrates such as zooplankton and initiate a trophic chain transfer factor (TTF) (Palmer et al., 2006). Other studies in aquaculture on marine systems have reported REE concentrations in fish and bivalves (Squadrone et al., 2016), some of them with very similar values (*Crassostrea gigas* Ce median, 0.02 μg/g^{-1} in w.w.) to the scute concentrations of this study (see Table 1). Due to the lack of understanding regarding the toxicology of these emerging inorganic elements, the potential effect of these concentrations should not be underestimated.

4. Conclusion

Owing to their unique suite of ecological and life-history attributes, sea turtles are excellent model organisms for contamination risk assessment programs and studies. With the advent of precise equipment capable of detecting pollutants at very low levels, inorganic elements in blood and scute can be accurately determined using non-invasive and non-destructive sampling techniques in wild populations.

Scute is a useful tissue for bio-monitoring concentrations of inorganic elements with an indication of longer-term exposure. However, the toxicokinetics are probably metal-specific and need future studies to facilitate the use of carapace tissues as non-lethal biomarkers of the bioaccumulation of inorganic elements. In order to properly measure the concentrations of Fe, Se, Be, Hg, Ag, Ce, and Os, it is recommended to use blood tissue instead of scute, while the rest of the elements are better identified through scute for the determination and bio-monitoring of inorganic pollutants.

The rapid development and widespread application of REE and TE technologies in industrialized countries necessitate additional information on the potential health effects derived from possible exposure to these compounds.

CRediT authorship contribution statement

Maribel Escobedo Mondragón: Conceptualization, Investigation, Data Curation, Formal analysis, Writing - original draft. **Octavio P. Luzardo:** Methodology, Software, Validation, Funding acquisition. **Manuel Zumbado:** Methodology, Software, Validation. **Ángel Rodríguez-Hernández:** Methodology, Software, Validation. **Cristian Rial Berriel:** Methodology, Software, Validation. **Héctor Vicente Ramírez-Gomez:** Sofware, Formal analysis. **Carlos González-Rebeles Islas:** Writing - review & editing, Visualization, Supervision. **Roberto F. Aguilar Fisher:** Writing - review & editing, Visualization, Supervision. **J. Rene Rosiles Martínez:** Methodology, Visualization, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to acknowledge the non-governmental organization "Pronatura Península de Yucatan A.C." which provided valuable logistics support, with special recognition to the biologists Jalil Mezquita Ruíz and René Salinas Salazar, the local wildlife authorities CONANP, and the national environmental authorities SEMARNAT and DGVS. We are grateful to the people of Toxicology Unit of University of Las Palmas de Gran Canaria and National Autonomous University of Mexico (UNAM) for the funding and analyze of the samples, and the Georgia Sea Turtle Center for donate sampling materials for the research. Also thank the National Science and Technology Council for its funding support within the National Quality Postgraduate Program in UNAM.

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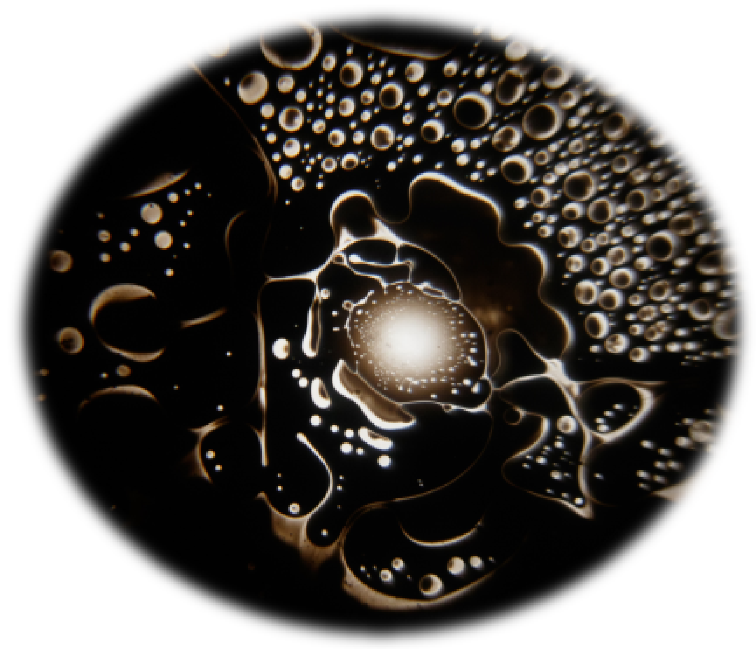
Publicación 9. Postmortem investigations on leatherback sea turtles (*Dermochelys coriacea*) stranded in the Canary Islands (Spain) (1998–2017): Evidence of anthropogenic impacts

Investigaciones post mortem sobre tortugas laúd (Dermochelys coriacea) varadas en las Islas Canarias (España) (1998-2017): evidencia de impactos antropogénicos

Marine Pollution Bulletin, 2021, 167: 112340

DOI: https://doi.org/10.1016/j.marpolbul.2021.112340



Marine Pollution Bulletin 167 (2021) 112340

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

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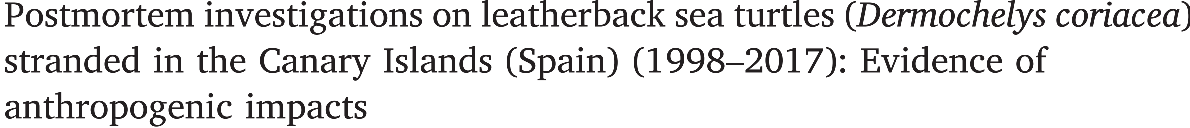

Postmortem investigations on leatherback sea turtles (*Dermochelys coriacea*) stranded in the Canary Islands (Spain) (1998–2017): Evidence of anthropogenic impacts

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A R T I C L E I N F O

Keywords:
Dermochelys coriacea
Leatherback sea turtle
Pathology
Inorganic elements
Pollutants
Canary Islands

A B S T R A C T

Opportunities for postmortem studies on leatherback sea turtles (*Dermochelys coriacea*) are infrequent due to their predominantly pelagic life history. In this study, the pathological findings and causes of mortality of 13 leatherback turtles stranded in the Canary Islands, Spain, from 1998 to 2017, are described. In addition, concentrations of Se, As, Cd, Pb, Hg, 15 rare earth elements (REE) and other 4 minor elements (ME), 41 persistent organic pollutants, and 16 polycyclic aromatic hydrocarbons in hepatic samples from 5 leatherbacks were determined. 84.62% of the turtles died possibly due to anthropogenic causes (entanglement/fishing interaction - 46.15%; boat strike - 23.07%; plastic ingestion - 15.38%). Although Se, As, and Cd were found at higher hepatic concentrations than those reported for leatherbacks from other locations, no acute lesions were detected. This is the first report of exposure to REE-ME in sea turtles. Organic contaminant hepatic concentrations were generally low or undetectable.

Two families and seven species of sea turtles are currently recognized (Pritchard, 1997). The globally distributed leatherback sea turtle (*Dermochelys coriacea*) is the most ancient extant species, the sole living member of the family Dermochelyidae, and the largest living chelonian (Pritchard, 1997). It is listed as Vulnerable according to the IUCN Red List, but there are important differences among the seven subpopulations of leatherbacks recognized in different ocean basins (Wallace et al., 2013). Leatherbacks belonging to the East Pacific Ocean, West Pacific Ocean, Southwest Atlantic Ocean, and Southwest Indian Ocean subpopulations are considered Critically Endangered, whereas leatherbacks from the Northeast Indian Ocean and Southeast Atlantic Ocean subpopulations are included in the category Data Deficient (IUCN, 2020). Leatherbacks observed around the coasts of the Canary Islands belong to the Northwest Atlantic Ocean subpopulation, considered Endangered (IUCN, 2020).

Major recognized global threats to leatherbacks have an anthropogenic origin: incidental capture in fishing gear targeting other species (fisheries bycatch) (Gilman and Huang, 2017; Hamelin et al., 2017), direct utilization of turtles or eggs for human use (Spotila et al., 2000), and coastal development affecting critical turtle habitat (Wallace and Saba, 2009). When a new assessment framework was developed to define the global conservation priorities using categories of paired risk and threats scores for all subpopulations, marine pollution and debris was only scored as affecting three populations of leatherbacks (Wallace et al., 2011); in fact, there are fewer studies investigating inorganic elements and organic contaminants in leatherback turtles than in other sea turtle species such as loggerhead turtles (*Caretta caretta*) and green turtles (*Chelonia mydas*) (Orós et al., 2009; Perrault, 2014; Cortés-Gómez et al., 2017).

When compared with other sea turtle species such as loggerheads and green turtles, there are few reports describing necropsy findings in leatherback turtles (Stacy et al., 2015; Ferguson et al., 2016; Santos-Costa et al., 2020). Opportunities for necropsy and other postmortem studies are infrequent due to their predominantly pelagic life history, and the subsequent difficulty in accessing fresh carcasses (Stacy et al., 2015).

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https://doi.org/10.1016/j.marpolbul.2021.112340
Received 25 December 2020; Received in revised form 1 April 2021; Accepted 5 April 2021
Available online 15 April 2021
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Since 1994, the Veterinary Faculty at the University of Las Palmas de Gran Canaria (ULPGC), in collaboration with the Tafira Wildlife Rehabilitation Center (TWRC) (Cabildo de Gran Canaria), has been conducting a survey on the pathology and causes of mortality among sea turtles stranded on the coasts of the Canary Islands. Because the most common species around the Canary Islands is the loggerhead turtle, the majority of postmortem studies were focused on this species (Orós et al., 2005; Camacho et al., 2013a; Inurria et al., 2019).

The aim of this study was to describe the pathological findings and causes of mortality of leatherback sea turtles stranded on the coasts of the Canary Islands, Spain, from 1998 to 2017. In addition, chemical analyses were carried out to determine the concentrations of Se, As, Cd, Pb, Hg, 15 rare earth elements (REE) and other 4 minor elements (ME), 41 persistent organic pollutants (POPs), and 16 polycyclic aromatic hydrocarbons (PAHs) in hepatic samples from 5 leatherback turtles.

A total of 17 leatherback turtles were submitted to the TWRC from 1998 to 2017. Four turtles were excluded from the study due to their advanced autolytic status. Except for 3 turtles that died during the rehabilitation period (< 36 h), the rest of the animals were already admitted dead.

Necropsies and pathological studies were performed using the procedures previously described (Orós and Torrent, 2001; Orós et al., 2005). Samples from gross lesions and from spleen were cultured on a variety of selective and non-selective media (Oxoid Ltd., Basingstoke, UK), including blood agar, Mac-Conkey agar, Baird Parker agar for staphylococci, and Sabouraud Dextrose agar for fungi and yeasts. Bacteria were identified based on the biochemical profile (API 20 E, API 20 NE, and API 20 Staph, BioMérieux, Marcy-l'Étoile, France).

Hepatic concentrations of 24 inorganic elements, including Se as essential trace element, the four major toxic elements (As, Cd, Pb, Hg) from the ATSDR Substance Priority List (ATSDR, 2019), 15 rare earth elements (Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sm, Tb, Tm, Yb, Y) and other 4 minor elements (Ga, In, Nb, Ta) were determined. A total of 57 analytes belonging to three relevant groups of organic contaminants were also selected for this study. The 23 organochlorine pesticides (OCPs) and metabolites included were the diphenyl-aliphatics (methoxychlor, *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, *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 polychlorinated biphenyls (PCBs), 12 dioxin-like congeners (IUPAC numbers# 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189), and six non-dioxin-like congeners (IUPAC numbers# 28, 52, 101, 138, 153 and 180) were included. Finally, we also included the 16 EPA (Environmental Protection Agency) priority PAHs, often targeted for measurement in environmental samples (naphthalene, acenaphtylene, acenaphtene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]anthracene and benzo[*ghi*]perylene).

For the analysis of inorganic elements, pure standards of elements in acid solution (5% HNO3, 100 mg/L) were purchased from CPA Chem (Stara Zagora, Bulgary). Two standard curves (ten points, 0.005–20 ng/mL) were made to avoid interferences between elements: a) one using a commercial multi-element mixture (CPA Chem Catalog number E5B8•K1.5N.L1, 100 mg/L, 5% HNO3) containing all the essential elements and main heavy metals; and b) other multi-element mixture tailor-made in our laboratory from individual elements (CPA Chem), which contained the REE and ME, as previously reported (Henríquez-Hernández et al., 2017). The liver samples were acid digested using a Milestone Ethos Up microwave (Milestone, Bologna, Italy). All the samples were processed in duplicate, and for this two 150 mg-portions of each turtle's liver were carefully weighted into the digestion vessels, and 3.5 mL of Milli-Q water and 1.mL of concentrated sub-boiling HNO_3 (65%) were added to each sample. Digestion conditions were according to the following program: Step 1: 1800–100–5 [power (W) – temperature (°C) – time (min)]; Step 2: 1800–150–5; Step 3: 1800–200–8, and Step 4: 1800–200–7. After cooling, the digested samples were transferred into 50 mL plastic bottles and diluted up to 7.5 mL with Milli-Q water. Finally, an aliquot of each sample was taken and the internal standards [ISTD solution was composed by Sc (scandium), Ge (germanium), Rh (rhodium), and Ir (iridium) at a stock concentration of 20 mg/mL each] were added for the analysis. Blanks were prepared in the same way as the sample.

An Agilent 7900 ICP-MS (Agilent Technologies, Tokyo, Japan) with standard nickel cones, MicroMist glass concentric nebulizer, and Ultra High Matrix Introduction (UHMI) system was used for these measurements. The Integrated Sample Introduction System (ISIS) was configured for discrete sampling. The UHMI system was operated in robust mode. The 4th generation Octopole Reaction System (ORS4) was operated in helium (He) mode to reduce polyatomic interferences. A tuning solution consisting in a mix of cesium, cobalt, lithium, magnesium, thallium, and yttrium was used before the analysis for optimization of instrumentation. Quantification of the elements was made in the MassHunter v.4.2. ICP-MS Data Analysis software (Agilent Technologies).

The entire procedure was validated prior to its use in the analyses of samples. Recoveries obtained ranged from 89 to 128% for REE and other elements used in high tech devices, and from 87% to 118% for ATSDR's toxic heavy elements and trace elements. Linear calibration curves were found for all elements (regression coefficients ≥0.998). Instrumental LODs and LOQs were calculated as the concentration of the element that produced a signal that was three and ten times higher than that of the averaged blanks, respectively (Supplementary Table 1). The accuracy and precision of this method was assessed using fortified alkaline solution (0.05, 0.5, and 5 ng/mL) in substitution of sample. In general, the calculated relative standard deviations (RSD) were lower than 13% for all the elements at the lowest level of fortification. The precision improved at the highest level of concentration, as it was lower than 5% for all elements.

A fully validated method was employed for the analysis of organochlorine pesticides and PCBs (Luzardo et al., 2014b). The scope of the method was subsequently extended to PAHs and validated in-house (in terms of accuracy, precision and recovery) (Luzardo et al., 2014a). For the extraction, 1 g of liver was homogenized with 4 mL of ultrapure water, and from this homogenate 1 mL was taken, to which 10 μL of the P-IS were added. In this method, a matrix-matched calibration curve was used, so the matrix (chicken or beef liver tested negative for the analytes of interest) was prepared in the same way, but the 1-mL aliquots were fortified to 12 increasing concentrations of the POPs mixture (0.05 to 50 ng/mL). After this, 2 mL of acidified acetonitrile (0.5% formic acid) were added, shaken vigorously (30 sg), and subjected to an ultrasound bath for 20 min (Selecta, Barcelona, Spain). After this time, 480 mg of anhydrous magnesium sulphate and 120 mg of sodium acetate were added to each tube and shaken vigorously again (90 sg). The samples were centrifuged at 4200g for 5 min at 2 °C and the supernatant was collected and filtered through 0.2 μm (Chromafil PET-20/15, Macherey-Nagel, Düren, Germany) to be used directly for chromatographic analysis, without any additional purification steps. An Agilent 1290 UHPLC (Agilent Technologies, Palo Alto, USA) coupled to an Agilent 6460 triple-quadrupole mass spectrometer was used to separate and detect the analytes. Validation parameters are given in Supplementary Table 2. Chromatographic and acquisition conditions and basic procedural details can be found in Rial-Berriel et al. (2020).

The mean ± standard deviation of the straight carapace length (SCL), curved carapace length (CCL) and curved carapace width (CCW) were 147.70 ± 23.09 cm (range, 123–210 cm), 149.10 ± 22.50 cm (range, 127–213 cm) and 86.50 ± 13.60 cm (range, 74.40–120 cm), respectively. On the basis of SCL and sexual maturity estimated by gonadal visualization, all specimens were adult or subadult, and all the turtles

Table 1
Pathological and microbiological findings and suspected causes of death in 13 leatherback sea turtles (*Dermochelys coriacea*) stranded in the Canary Islands.

Turtle	Gross lesions	Histological lesions	Microbiology	Suspected cause of death
1	Ulcerative/purulent dermatitis (neck/flipper)	Purulent dermatitis Interstitial pneumonia	*Vibrio alginolyticus* (lung)	Entanglement
2	Ulcerative/purulent dermatitis (flipper) Ileocecal diverticulitis	Purulent dermatitis Edema (lung) Ileocecal diverticulitis	*Proteus* spp. (ileocecal diverticulum)	Entanglement
3	Multifocal granulomatous hepatitis / splenitis / pneumonia Ileocecal diverticulitis	Multifocal granulomatous hepatitis / splenitis / pneumonia Renal thrombosis Ileocecal diverticulitis	*Serratia marcescens* (liver, spleen, lung) *Morganella morganii* (ileocecal diverticulum)	Septicemia
4	Ulcerative/purulent dermatitis (neck/flipper)	Purulent dermatitis Edema (lung)		Entanglement
5	Ulcerative/purulent dermatitis (flipper) Ileocecal diverticulitis	Purulent dermatitis Edema (lung) Hydropericardium Ileocecal diverticulitis	*Proteus* spp. (ileocecal diverticulum)	Entanglement
6	Skull fractures/Brain hemorrhage Ileocecal diverticulitis	Brain hemorrhage/acute inflammation Ileocecal diverticulitis	*Morganella morganii* (ileocecal diverticulum)	Boat strike
7	Ulcerative/purulent dermatitis (flipper)	Purulent dermatitis Edema (lung) Hydropericardium		Entanglement
8	Traumatic injury (carapace)	Necrosis (lung)		Boat strike
9	Intestinal obstruction (plastic bag)	Edema (gastrointestinal serosa)		Plastic ingestion
10	Intestinal perforation	Fibrinous intestinal serositis/celomitis		Plastic ingestion
11	Skull fractures/Brain hemorrhage	Brain hemorrhage/acute inflammation		Boat strike
12	Fibrinopurulent perihepatitis Ileocecal diverticulitis	Fibrinopurulent perihepatitis Acute interstitial nephritis Ileocecal diverticulitis	*Morganella morganii* (liver, ileocecal diverticulum)	Septicemia
13	Ulcerative/purulent dermatitis (flippers) Hook injury (flipper) Small plastic piece (stomach)	Purulent dermatitis Edema (lung) Hydropericardium		Entanglement/fish hook

were female. Pathological and microbiological findings are given in Table 1. Suspected causes of death (Table 1) were entanglement ($n = 6$) (Fig. 1), boat strike ($n = 3$) (Fig. 2), plastic ingestion ($n = 2$) (Fig. 3), and septicemia (n = 2) (Fig. 4). Ileocecal diverticulitis (without intestinal obstruction) was observed in 5 turtles (Fig. 5).

Hepatic concentrations of Se, As, Cd, Pb, and Hg are given in Table 2. Among the ATSDR list of toxic elements, As and Cd were the ones with the highest concentrations.

Hepatic concentrations of rare earth elements and other minor elements (Ga, In, Nb, and Ta) are given in Table 3. The mean concentration of cerium was the highest, doubling the mean concentration of the next element, lanthanum.

Hepatic concentrations of POPs and PAHs detectable in at least one turtle are given in Table 4. The rest of pollutants analyzed and not expressed in the Table had values below detection limit. Among the POPs, the highest values were those detected for *p,p'*-DDE, followed by PCB 138. In the case of PAHs, detectable concentrations of naphthalene and fluoranthene were only found, respectively, in two turtles (leatherbacks #9 and #12).

Our study confirms two relevant aspects derived from the pelagic habits of this sea turtle species and its physiological characteristics. Firstly, the extreme difficulties to carry out a successful rehabilitation in these animals have been in evidence; it was not possible to rehabilitate any of the three leatherbacks that were admitted alive, and they died in a short time. However, during a similar period of time (1998–2014), 86.29% of loggerhead turtles admitted alive to the TWRC were successfully released (Orós et al., 2016). Due to their pelagic habits, when they strand on the coasts, leatherbacks often do so either dead or in terminal condition; in addition, the size and weight of these animals makes it extremely difficult to handle and house them in adequate facilities in rehabilitation centers. Secondly, the difficulties to carry out postmortem studies on a high number of animals are also highlighted, as stated by other authors (Stacy et al., 2015; Ferguson et al., 2016).

In our survey, 46.15% of leatherbacks ($n = 6$) died possibly due to entanglement in fishing gear. Although interactions with the Canarian artisanal fishery are infrequent, potential interactions between leatherbacks and fisheries can take place in waters off due to use of 'trasmallos' (as in loggerheads, with 50.81% of strandings caused by entanglement) (Orós et al., 2016), a fishing net used to catch several fish species. Entanglement was a significant cause of injury in several surveys (Stacy et al., 2015; Hamelin et al., 2017; Archibald and James, 2018). More recently, in an unusual mortality event in Brazil involving 23 leatherbacks, cardiorespiratory collapse by asphyxia due to entanglement in nets was the most likely cause of death (Santos-Costa et al., 2020); the most prevalent lesions were cutaneous lesions around the neck and flippers, generalized congestion, and pulmonary edema (Santos-Costa et al., 2020).

In our survey, only the turtle #13 had hook-compatible lesions in a front flipper. Bycatch in pelagic longline fisheries is one of the most important anthropogenic threats to leatherback turtle populations (Gilman and Huang, 2017; Hamelin et al., 2017). An international survey showed that 50,000 leatherbacks were likely taken as pelagic longline bycatch in 2000 (Lewison et al., 2004). Leatherbacks are more likely to be foul-hooked in the head, shoulders, flippers, or carapace than to swallow hooks, while ingestion of hooks is more frequent in other sea turtle species (Blades et al., 2019). Indeed, during a similar period of time (1998–2014), 221 loggerheads were admitted to the TWRC due to ingestion of hooks and monofilament lines (Orós et al., 2016).

Several strategies have been implemented in various countries to mitigate this bycatch. Large circle hooks have been shown to reduce bycatch primarily by reducing the chances of a turtle swallowing the hook (Gilman and Huang, 2017; Blades et al., 2019). Use of fish bait (vs. squid) has been found to significantly reduce the bycatch probability (Gilman and Huang, 2017; Swimmer et al., 2017). Bycatch reduction may also be gained for leatherbacks by mitigation of color of lightsticks in longline gear (Swimmer et al., 2017) or using light sources flickered at >16 Hz (Crognale et al., 2008).

In our survey, 23.07% of leatherbacks ($n = 3$) died due to boat strikes. During a similar period of time (1998–2014), 97 loggerheads

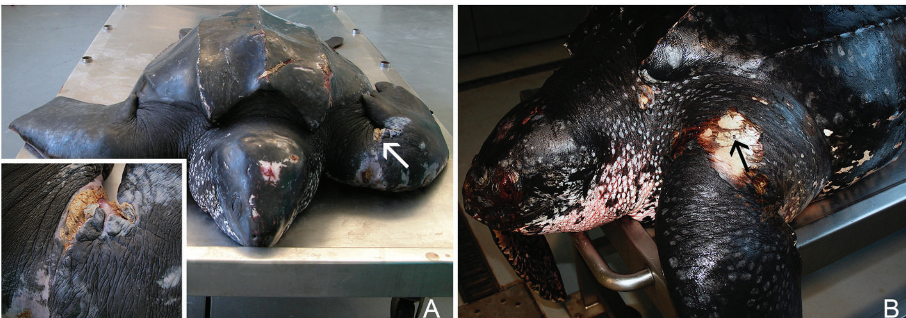

Fig. 1. (A) Leatherback turtle (#5) showing ulcerative and purulent skin lesions (arrow) around the left front flipper caused by entanglement in fishing gear. Inset: detail of the lesion from a dorsal view. (B) Leatherback turtle (#13) showing a penetrating injury (arrow) in the left front flipper possibly caused by a hook.

Fig. 2. Severe skull fractures in a leatherback turtle (#11) possibly caused by boat strike.

were admitted to the TWRC due to boat strikes (Orós et al., 2016). Involvement of vital organs such as lungs and kidneys (because the anatomical location, dorsally attached to the carapace), and brain, explains the generally poor prognosis for turtles with severe traumatic injuries in the carapace and skull (Orós et al., 2005). The prevalence of boat strike injuries varies according to the subpopulations analyzed: whereas boat strikes were the main causes of leatherback strandings in Algeria (Belmahi et al., 2020), in a recent study on characterization of watercraft-related mortality of sea turtles in Florida, authors estimated that 4–6 leatherbacks died annually due to boat strike injuries; however, taking into account that only about 10–20% of turtles that died likely washed ashore, authors suggested that the overall annual mortality may be 5–10 times greater than that represented by strandings (Foley et al., 2019).

In our survey, 15.38% of leatherbacks ($n = 2$) died due to digestive lesions caused by plastic ingestion. According to the Ocean Conservancy, 150 million tonnes of plastic are currently in the oceans, and, as stated by the World Economic Forum, eight million tonnes infiltrate the oceans per year (Aretoulaki et al., 2020). Especially in the case of leatherbacks, plastic may be mistaken for jellyfish (Mrosovsky et al., 2009). Ingestion of plastics can cause intestinal obstruction and other intestinal lesions, dietary dilution, malnutrition, and increased buoyancy resulting in poor health, reduced growth rates and reproductive output, or death; in addition, plastics can accumulate contaminants from the marine environment, such as heavy metals and PCBs (Nelms et al., 2016).

In a review of 408 necropsy records of leatherback turtles (1885–2007), plastic was found in the gastrointestinal tract in 34% of cases, although lethal effects were relatively infrequent (8.7%) (Mrosovsky et al., 2009). Other recent studies have not reported the ingestion of plastics, or only in very few animals, possibly due to the low number of leatherbacks analyzed (Clukey et al., 2017; Rizzi et al., 2019). The ingestion of significant quantities of plastic debris may not be lethal for leatherbacks, especially if it can be expelled (Barreiros and Barcelos, 2001); however, perforation of the gastric mucosa by a plastic fragment,

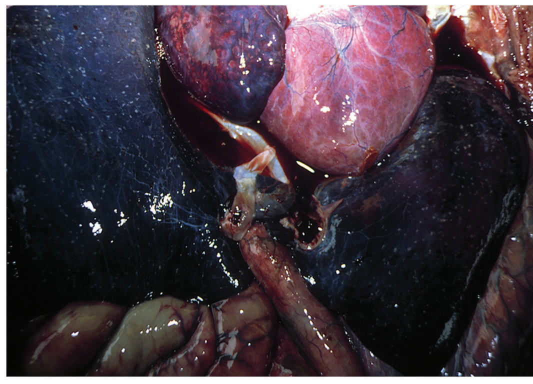

Fig. 4. Severe multifocal granulomatous hepatitis in a leatherback turtle (#3).

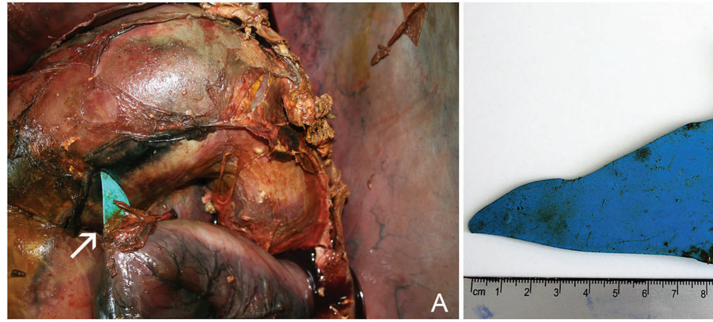

Fig. 3. (A) Intestinal perforation (arrow) in a leatherback turtle (#10) caused by a hard, sharp piece of plastic; note also the severe fibrinous intestinal serositis. (B) Note the size of the plastic piece causing the intestinal perforation.

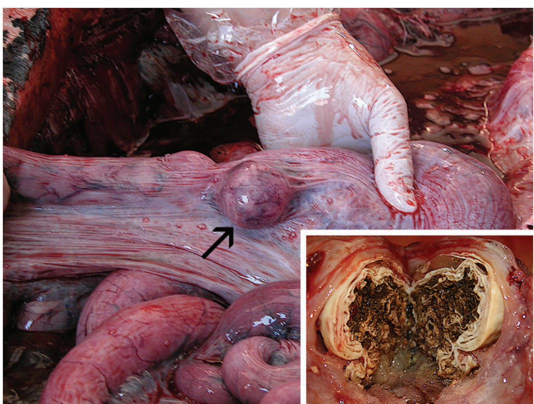

Fig. 5. Ileocecal diverticulum (arrow) in a leatherback turtle (#5). Inset: the diverticulum was filled with firm yellow caseous exudate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Concentrations of inorganic elements (μg/g wet wt.) detected in liver samples from 5 adult female leatherback sea turtles (*Dermochelys coriacea*) stranded in the Canary Islands.

	Turtle 9	Turtle 10	Turtle 11	Turtle 12	Turtle 13	Mean
Essential trace element						
Selenium (Se)	29.5	11.55	13.28	22.61	11.18	17.62
ATSDR list of toxic elements						
Arsenic (As)	3.61	39.20	11.55	22.89	1.39	15.73
Cadmium (Cd)	25.56	15.05	7.04	7.24	3.78	11.73
Lead (Pb)	1.21	0.03	0.78	0.31	0.32	0.53
Mercury (Hg)	1.08	0.15	0.31	0.18	0.12	0.37

ATSDR: Agency of Toxic Substances and Disease Registry.

hemorrhagic gastroenteritis, and obstruction of the intestinal lumen by plastic fragments were reported in a leatherback stranded in Italy (Poppi et al., 2012).

In our survey, 15.38% of leatherbacks (n = 2) possibly died due to septicemia. During a similar period of time (1998–2014), 103 loggerheads were admitted to the TWRC due to infectious diseases, and they had a high unassisted mortality rate (25.49%) during the hospitalization period (Orós et al., 2016). *Serratia marcescens* has been associated with hepatic lesions in loggerheads (Orós et al., 2005); more recently, it has also been isolated from captive green turtles with ulcerative stomatitis (Vega-Manriquez et al., 2018). *Morganella morganii* has been isolated from cloacal samples of rehabilitated green turtles in Australia (Ahasan et al., 2017) and loggerheads in Italy (Pace et al., 2019). It was also isolated from three leatherbacks with large intestinal diverticulitis (Stacy et al., 2015).

Solitary large intestinal diverticulitis was a very frequent necropsy finding in leatherbacks in North America (Stacy et al., 2015); it was unrelated to the cause of death or clinical disease in any of the cases, although authors suggested the possibility of perforation or obstruction (Stacy et al., 2015). Two of the turtles with ileocecal diverticulitis in our survey (leatherbacks #3 and #12) died due to septicemia, but only in the leatherback #12 the microorganism isolated from the diverticulum and the hepatic and renal lesions was the same, *Morganella morganii*.

Therefore, when causes of mortality were established in our survey, 84.62% of the turtles died possibly due to anthropogenic causes (entanglement/fishing interaction - 46.15%; boat strike - 23.07%; plastic ingestion - 15.38%); obviously, the absence of cases of predation as a natural cause of death skews our results. When 1860 loggerhead turtles admitted to the TWRC during a similar period of time (1998–2014) were analyzed, anthropogenic causes of mortality were detected in 71.70% of the turtles, although in 20.4% of the total admitted turtles the cause of death could not be determined (Orós et al., 2016).

There are very few reports of pollutants in liver samples from leatherbacks due to limited opportunities for necropsy (Davenport and Wrench, 1990; Godley et al., 1998; Caurant et al., 1999; Mckenzie et al., 1999; Orós et al., 2009; Perrault, 2012; Poppi et al., 2012). The number of studies for the detection of pollutants in blood from leatherbacks is greater, although if compared to other sea turtle species, they are still scarce (Perrault et al., 2011; Keller et al., 2012; Perrault et al., 2013).

In addition, several reasons make it difficult to investigate the effects of environmental pollutants on sea turtles: (i) sea turtles often harbor various pollutants from the sea and their synergistic effect is unknown in most cases, (ii) the biological effects of pollutants are a function of the concentration or contaminant burden and it is difficult to detect the

Table 3
Concentrations of rare earth elements (RRE) and other minor elements (ME) (ng/g wet wt.) detected in liver samples from 5 adult female leatherback sea turtles (*Dermochelys coriacea*) stranded in the Canary Islands.

	Turtle 9	Turtle10	Turtle 11	Turtle 12	Turtle 13	Mean
Rare earth element						
Cerium (Ce)	27.64	1.36	12.66	6.82	5.32	10.76
Dysprosium (Dy)	0.71	0.04	0.15	0.12	0.09	0.22
Erbium (Er)	0.41	0.02	0.07	0.06	0.04	0.12
Europium (Eu)	0.19	0.01	0.06	0.04	0.03	0.07
Gadolinium (Gd)	1.03	0.06	0.28	0.18	0.14	0.34
Holmium (Ho)	0.16	0.01	0.03	0.03	0.02	0.05
Lanthanum (La)	15.68	0.70	6.44	3.37	2.96	5.83
Lutetium (Lu)	0.04	0.001	0.02	0.005	0.003	0.01
Neodymium (Nd)	5.55	0.31	2.12	1.32	0.10	1.88
Praseodymium (Pr)	1.47	0.09	0.58	0.35	0.29	0.55
Samarium (Sm)	0.83	0.05	0.28	0.18	0.13	0.29
Terbium (Tb)	0.13	0.01	0.03	0.02	0.02	0.04
Thulium (Tm)	0.05	0.003	0.01	0.007	0.005	0.01
Ytterbium (Yb)	0.24	0.01	0.05	0.03	0.03	0.07
Yttrium (Y)	6.36	0.32	1.20	0.10	0.66	1.73
Minor element						
Gallium (Ga)	2.86	2.90	1.41	1.17	0.89	1.85
Indium (In)	0.05	0.08	0.05	0.02	0.01	0.04
Niobium (Nb)	0.37	0.44	0.22	0.06	0.12	0.24
Tantalum (Ta)	0.02	0.02	0.01	0.007	0.01	0.01
∑REE-ME	63.79	6.43	25.67	13.89	10.87	24.13

Table 4
Concentrations of persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs) (ng/g wet wt.) detected in liver samples from 5 adult female leatherback sea turtles (*Dermochelys coriacea*) stranded in the Canary Islands.

	Turtle 9	Turtle 10	Turtle 11	Turtle 12	Turtle 13	Mean
POPs[a]						
p,p'-DDD	BDL	0.21	BDL	BDL	BDL	0.04
p,p'-DDE	4.67	11.23	0.32	9.63	4.11	5.99
Hexachlorobenzene	1.17	0.97	BDL	3.21	2.11	1.49
β-hexachlorocyclohexane	BDL	1.07	BDL	BDL	BDL	0.21
PCB 52	BDL	3.13	BDL	BDL	1.15	0.86
PCB 138	1.11	BDL	BDL	BDL	2.13	0.65
PCB 153	2.09	BDL	0.87	BDL	2.11	1.01
PCB 180	4.88	1.17	2.18	0.45	2.91	2.32
BDE 99	BDL	1.23	BDL	BDL	BDL	0.25
PAHs[b]						
Naphthalene	2.63	BDL	BDL	BDL	BDL	0.526
Fluoranthene	BDL	BDL	BDL	13.44	BDL	2.69

BDL: below detection limit.
[a] 23 organochlorine pesticides and 18 polychlorinated biphenyls were analyzed (see Material and methods).
[b] 16 polycyclic aromatic hydrocarbons were analyzed (see Material and methods).

effects if the animals die from another cause, and (iii) experimental studies are especially difficult to carry out in these protected reptiles (Orós et al., 2021).

Our results indicate that Se levels in leatherback turtles stranded in the Canary Islands are higher than those found in leatherbacks from other geographical areas (Table 5) (Davenport and Wrench, 1990; Godley et al., 1998; Perrault, 2012; Poppi et al., 2012). Se is a trace element naturally found in high-protein marine food sources, and it is considered an essential element. However, at high concentrations it can be toxic, and even fatal (Perrault et al., 2011). The studies of Se levels in leatherbacks have been aimed at trying to explain their low hatching and emergence success compared to other sea turtle species (Perrault et al., 2011, 2013; Perrault, 2014). Se serves to detoxify the Hg body burden, and a positive correlation of Se and Se/Hg with leatherback turtle hatching and emergence success has been found (Perrault et al., 2011). Accordingly, we found low Hg levels in our leatherbacks, although these levels were higher than those found in loggerhead sea turtles stranded in the Canary Islands (Torrent et al., 2004). Also, Pb levels found in our leatherback turtles were lower than those found in other studies (Table 5) (Godley et al., 1998; Poppi et al., 2012), including those carried out in the Canary Islands with samples from loggerheads (Torrent et al., 2004).

In our study, As concentrations were higher to those detected in leatherbacks from other geographical areas (Table 5) (Davenport and Wrench, 1990; Godley et al., 1998; Poppi et al., 2012) and similar to the mean As concentration found in loggerheads stranded in the Canary Islands (Torrent et al., 2004). However, whereas hepatic lesions such as severe diffuse vacuolar hepatic degeneration, and multifocal necrotizing hepatitis were reported in three loggerhead turtles stranded in the Canary Islands with high As hepatic concentrations (Torrent et al., 2004), no hepatic lesions attributable to As were found in our leatherbacks.

It is remarkable the high mean Cd concentration (11.73 μg/g) detected in our leatherbacks, especially if compared to other studies (Table 5) (Davenport and Wrench, 1990; Caurant et al., 1999; Poppi et al., 2012). Godley et al. (1998) also reported a high Cd hepatic concentration in a leatherback stranded in United Kingdom. A mean Cd hepatic concentration of 2.53 μg/g was reported in loggerheads stranded in the Canary Islands, without evidence of hepatic lesions (Torrent et al., 2004). Histological lesions attributable to Cd contamination were also not detected in the leatherback turtles in this study.

To the best of our knowledge, there are no reports of exposure to rare earth elements and other minor elements in sea turtles. REEs are a group of metals comprised of 15 lanthanides, yttrium, and scandium, which have been named "the vitamins of modern industry" due to their use in a wide range of industrial processes (Rim, 2016). The consequent generation of huge amounts of electronic waste represents an environmental problem that has been rarely analyzed in wildlife (Censi et al., 2013; Sánchez-Virosta et al., 2020). It is remarkable the high $\sum$REE-ME concentration (63.79 ng/g) found in the leatherback #9, especially Ce and La concentrations (27.64 and 15.68 ng/g, respectively). In addition, leatherbacks #9, #11, #12, and #13 had higher hepatic Ce concentrations than the maximum levels found in eagle owls (*Bubo bubo*) inhabiting polluted environments in southeastern Spain (Sánchez-Virosta et al., 2020). Several toxicity tests on REEs, mostly Ce, La, and Gd, have been conducted in laboratory animals, although long-term REE exposure studies have not been reported (Rim, 2016). Unfortunately, studies on REE-associated toxicity, especially for marine organisms, are very

Table 5
Concentrations of metals (mean ± SD, range, μg/g wet wt.) detected in liver samples from leatherback sea turtles (*Dermochelys coriacea*) from different geographical areas.

Source	Number of turtles	Location	Se	As	Cd	Pb	Hg
Davenport and Wrench, 1990	1	UK	1.41[a]	0.58[a]	0.22[a]	0.12[a]	0.39[a]
Edmonds and Francesconi, 1994	1	Australia		1.2			
Godley et al., 1998	1	UK	6.5	2.6	28	4.3	0.37
Caurant et al., 1999	18	France (Atlantic)	–	–	6.84 ± 3.66 0.60–14.7	–	–
Perrault, 2012	14	USA (Atlantic)	8.34 ± 1.91 5.77–12.9	–	–	–	0.48 ± 0.38 0.07–1.44
Poppi et al., 2012	1	Italy (Adriatic)	12.57	2.13	5.68	16.37	20.4
This study	5	Canary Islands	17.62 ± 8.11 11.18–29.5	15.73 ± 15.59 1.39–39.20	11.73 ± 8.77 3.78–25.56	0.53 ± 0.46 0.03–1.21	0.37 ± 0.4 0.12–1.08

[a] Concentration in dry weight.

scarce, preventing an adequate comparison. Oral et al. (2010) demonstrated that Ce and La affect sea urchin embryogenesis at concentrations in the micromolar range. We do not know the possible effects on sea turtles, especially in these animals that died from other known causes, but their detection at relevant concentrations for the first time is worrying, possibly adding to the effects caused by other pollutants.

In our survey, organic contaminant hepatic concentrations were low or undetectable. As reported by Mckenzie et al. (1999) when analyzing two leatherbacks stranded in UK, *p,p'*-DDE was present at the greatest concentrations; individual leatherbacks from our study showed higher *p,p'*-DDE concentrations than those (6.5 and 1.7 ng/g wet weight) reported by Mckenzie et al. (1999). According to other surveys in sea turtles, *p,p'*-DDE is the pesticide found in the greatest concentrations due to its highly persistent nature (Monagas et al., 2008; Camacho et al., 2013b).

In our study, the predominant PCB congeners were PCB 180 and PCB 153. Mckenzie et al. (1999) reported PCB 153 as the one detected at the highest values. The only leatherback turtle previously studied in the Canary Islands showed much higher levels of PCBs (251 and 114 ng/g wet weight for PCB 153 and PCB 180, respectively) (Orós et al., 2009). Mean PCB concentrations in liver samples from 30 loggerheads stranded in the Canary Islands were also higher, reaching up to 915 ng/g wet weight for PCB 153 (Orós et al., 2009); although almost all loggerheads with severe septicemia had high levels of PCBs, it was difficult to establish a clear association between PCB concentrations and causes of death because no acute lesions exclusively attributed to PCBs were detected (Orós et al., 2009). In our study, with much lower levels of PCBs, associated acute lesions were not observed either, and chronic effects of PCBs are much difficult to demonstrate.

Camacho et al. (2012) reported highest blood concentrations of fluoranthene in loggerhead turtles stranded due to crude oil ingestion when compared to loggerheads stranded due to other causes. No signs of crude oil ingestion were observed in the leatherback #12, in which fluoranthene was detected (13.44 ng/g wet weight). Godley et al. (1998) reported very low or undetectable PAH concentrations in the liver of a leatherback turtle stranded in UK, with $\sum$PAH concentrations of 5.5 ng/g wet weight. Attending to the very low degree of biomagnification of PAHs, authors suggest that PAH concentrations in sea turtles are directly related to recent exposure to waters or food contaminated by PAHs (Camacho et al., 2013b). PAHs come from burning fossil fuels, not only from oil spills, and no hepatic lesions attributed to PAHs were detected in the leatherback #12.

As mentioned above, the biological effects of contaminants are a function of the concentration and, although contaminant concentrations measured in our leatherbacks did not produce acute toxic effects, sublethal or chronic effects may be occurring in this species. In addition, (i) there are evidences of maternal transfer of contaminants in this species (Perrault et al., 2011; Stewart et al., 2011), and (ii) physiological parameters in nesting leatherbacks (susceptible to be altered by low concentrations of some contaminants) correlate with hatching and emergence success (Perrault et al., 2012), already the lowest of all those reported for sea turtles.

In conclusion, this survey is the first focused on postmortem studies on leatherbacks stranded in the Canary Islands. Despite the low number of studied turtles in comparison with other species of turtles also analyzed in the Canary Islands, it is remarkable the negative anthropogenic impact, possibly causing the death of 84.625% of the stranded leatherback turtles. Although we found some inorganic elements (Se, As, Cd) at higher hepatic concentrations than those reported for leatherbacks from other geographical areas, no acute lesions were detected. This is also the first report of exposure to rare earth elements and other minor elements in sea turtles, and their detection at relevant concentrations in some of our turtles deserves to be studied in depth. Finally, organic contaminant hepatic concentrations were generally low or undetectable.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.112340.

CRediT authorship contribution statement

Jorge Orós: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing - original draft. **María Camacho:** Formal analysis, Methodology. **Pascual Calabuig:** Methodology, Resources. **Cristian Rial-Berriel:** Formal analysis, Methodology. **Natalia Montesdeoca:** Formal analysis, Investigation. **Soraya Déniz:** Formal analysis, Methodology. **Octavio P. Luzardo:** Investigation, Resources, Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank all the staff of the Tafira Wildlife Rehabilitation Center (Cabildo Insular de Gran Canaria).

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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RESUMEN

La biodiversidad mundial está gravemente amenazada, y uno de esos peligros son las sustancias químicas nocivas que provienen del ambiente. Muchas de estas sustancias han sido creadas con numerosos fines, ya sea como como aislantes térmicos y eléctricos, o como biocidas, medicamentos y productos de higiene o con fines industriales, entre otros. Otras, como las dioxinas, los hidrocarburos aromáticos policíclicos o los metales tóxicos son inherentes a la industrialización humana y a la combustión. Los contaminantes orgánicos persistentes comprenden un grupo amplio de tóxicos que son capaces de permanecer durante grandes periodos de tiempo, incluso décadas, en el medioambiente y los seres vivos, produciendo efectos nocivos a concentraciones muy bajas, como cáncer, malformaciones y alteraciones endocrinas. Los pesticidas y fitosanitarios se usan sobre todo en ganadería y agricultura, aunque también en entornos domésticos. Muchos tienen efectos agudos sobre los artrópodos, y a dosis mayores, pueden producir intoxicaciones agudas en también en vertebrados. La mayoría no suelen persistir más de unas semanas en el ambiente, pero existen otros biocidas con efectos cancerígenos y genotóxicos. Los rodenticidas anticoagulantes se han usado ampliamente en todo el mundo con el fin de eliminar poblaciones de roedores y otros pequeños mamíferos en el campo. Se ha constatado la entrada a la cadena trófica de estos compuestos en aves rapaces, no diana, ya que existen rodenticidas que persisten en el ambiente y se biomagnifican. Por otra parte, los medicamentos como los AINEs pueden ser muy tóxicos para aves de carroña como buitres, que se pueden alimentar de canales de ganado contaminadas, y los antibióticos pueden generar mayores resistencias bacterianas, con lo que se complicaría su uso para infecciones posteriores. Muchas de estas sustancias se usan ilícitamente para producir la muerte por envenenamiento de animales no deseados en entornos rurales y urbanos. La mayor parte de las sustancias usadas son altamente tóxicas, que se llegan a mezclar para producir mayores lesiones.

Los estudios de biomonitorización de sustancias químicas en seres vivos necesitan del desarrollo de una metodología que nos pueda brindar información sobre la mayor cantidad de sustancias posible y de su integración en los ecosistemas. A su vez, la seguridad alimentaria y la monitorización de muestras ambientales son cada vez más restrictivas en cuanto a residuos de plaguicidas, medicamentos y contaminantes persistentes. Por lo tanto, el desarrollo de métodos de extracción y cuantificación para un gran número de sustancias, que sean sensibles, de rutina, robustos y económicos, es un requisito indispensable. En este estudio de tesis doctoral, hemos desarrollado, validado y aplicado metodología basada en QuEChERS como técnica de extracción, y en cromatografías líquida y de gases acopladas a espectrometría de masas como técnicas de detección y cuantificación. Así, hemos desarrollado técnicas para la detección cuantitativa simultánea de 360 compuestos tóxicos en sangre y de 351 en hígado, la mayoría de ellos a concentraciones ultra traza (< 1 ng/g). Hemos aplicado Los métodos desarrollados los a estudios de biomonitorización y de diagnóstico de venenos de fauna silvestre y doméstica de Canarias.