UNIVERSITAT DE BARCELONA

Biomonitoreo de contaminantes de preocupación emergente en especies invasoras y nativas para la evaluación del riesgo ecológico en ecosistemas acuáticos

Diana Patricia Manjarrés Lopez

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BIOMONITOREO DE CONTAMINANTES DE PREOCUPACIÓN EMERGENTE EN ESPECIES INVASORAS Y NATIVAS PARA LA EVALUACIÓN DEL RIESGO ECOLÓGICO EN ECOSISTEMAS ACUÁTICOS

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Biomonitoratge de contaminants de preocupació emergent en espècies invasores i natives per a l'avaluació del risc ecològic en ecosistemes aquàtics

Diana Patricia Manjarrés López

Memoria presentada por compendio de artículos para optar al título de Doctora por la Universidad de Barcelona

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HACEN CONSTAR,

Que la presente memoria presentada para optar al título de Doctora, titulada: **"Biomonitoreo de contaminantes de preocupación emergente en especies invasoras y nativas para la evaluación del riesgo ecológico en ecosistemas acuáticos",** que se traduce al catalán como "Biomonitoratge de contaminants de preocupació emergent en espècies invasores i natives per a l'avaluació del risc ecològic en ecosistemes aquàtics", ha sido realizada por la **Sra. Diana Patricia Manjarrés López** bajo su dirección en el grupo Environmental and Water Chemistry for Human Health (ONHEALTH) del IDAEA-CSIC, y que los resultados presentados son fruto de la investigación realizada por la mencionada doctoranda.

Y para que así conste, expedimos y firmamos el presente certificado

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¡La suerte del gavilán no la tiene el garrapatero!

Mi mamá

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RESUMEN

La contaminación de ecosistemas acuáticos derivada de actividades antropogénicas puede generar graves consecuencias ambientales, como la degradación de la calidad del agua, la pérdida de biodiversidad, la acumulación de contaminantes en la cadena trófica, efectos directos sobre la biota acuática y, en general, el deterioro de los hábitats. Estos impactos varían según el tipo de contaminante, las tasas de emisión, su potencial de bioacumulación en los distintos compartimentos ambientales y la vulnerabilidad de las especies presentes. En particular, los contaminantes de preocupación emergente (CECs), como los compuestos activos de productos farmacéuticos, productos de cuidado personal, plaguicidas y sustancias industriales, representan una creciente amenaza para los ecosistemas acuáticos. Estos compuestos son recientemente identificados, no están completamente regulados y carecen de información precisa en las evaluaciones previas sobre contaminación ambiental.

En este contexto, la presente tesis propuso el uso de estrategias de biomonitoreo basadas en especies acuáticas invasoras y nativas como herramienta clave para evaluar el riesgo ecológico en ecosistemas acuáticos. Para ello, se analizó la presencia, bioacumulación y riesgo ecológico de 173 CECs en matrices biológicas de distintas especies acuáticas, incluidos peces, cangrejos de río y almejas. Se emplearon metodologías analíticas avanzadas, basadas en cromatografía de líquidos acoplada a la espectrometría de masas de alta resolución (LC-HRMS), para desarrollar dos métodos robustos que permitieron el análisis de CECs a niveles traza, destacando la importancia de la técnica de limpieza por extracción en fase sólida dispersiva (d-SPE) para eliminar interferencias en los extractos y garantizar la precisión de los resultados.

La investigación se llevó a cabo en ecosistemas acuáticos con diferentes niveles de intervención humana, como el río Llobregat (Barcelona) y el Parque Natural de L'Albufera (Valencia). En el río Llobregat, se evaluó la distribución de CECs en los órganos de cuatro especies de peces, identificando a la carpa común como una especie clave para el biomonitoreo debido a su carácter invasor. Esta especie fue incluida en un segundo estudio que permitió evaluar el riesgo ecológico en este ecosistema. En el Parque Natural de L'Albufera, se investigó el potencial de especies invasoras, como el cangrejo de río rojo americano, la almeja asiática y el pez percasol, para el biomonitoreo de CECs, centrándose en su capacidad para resistir y bioacumular compuestos químicos. Un experimento de exposición en condiciones

ambientales reales permitió establecer patrones de acumulación diferenciados por especie, lo que facilitó el análisis del riesgo ecológico de este humedal costero de alta importancia ambiental.

Se evidenció que las especies invasoras, como la carpa común y la almeja asiática, tienen una capacidad de acumulación mayor de CECs en comparación con las especies nativas, lo que sugiere que pueden ser utilizadas eficazmente como biomonitores para evaluar la calidad ambiental de distintos tipos de ecosistemas. Además, se identificaron CECs que no habían sido reportados previamente en las especies invasoras, como el 5-metil-1H-benzotriazol, la amantadina, el benzotriazol, la cotinina, la hidroclorotiazida, el bezafibrato, el bisfenol A, el sulfonato de perfluorononanoico, el ácido perfluorononanoico y el sulfonato de perfluorooctanoico.

Los resultados presentados en esta tesis, destacaron que las especies invasoras no solo permiten evaluar la acumulación de CECs en ambientes afectados por la contaminación, sino que su inclusión en estudios de biomonitoreo también contribuiría a reducir la presión sobre las especies nativas. En conclusión, esta tesis representó un avance en el biomonitoreo ambiental, proporcionando herramientas analíticas avanzadas y un enfoque integrado para evaluar el riesgo ecológico asociado a los CECs en ecosistemas acuáticos, lo que amplía el conocimiento para mejorar las estrategias de mitigación y gestión de la contaminación.

ABSTRACT

The contamination of aquatic ecosystems due to human activities causes severe consequences, including water quality degradation, biodiversity loss, pollutant accumulation in food chains, and habitat deterioration. These impacts depend on pollutant type, bioaccumulation potential, and species vulnerability. Among these, contaminants of emerging concern (CECs) such as pharmaceuticals active compounds, pesticides, and industrial compounds are particularly worrisome due to limited regulation and insufficient data in previous environmental pollution assessments.

This thesis explored biomonitoring strategies using invasive and native aquatic species to assess ecological risks in aquatic ecosystems. The study analyzed 173 CECs in biological matrices from fish, crayfish, and clams. Advanced techniques, including liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) and d-SPE cleanup, ensured accurate trace-level analysis.

The research was conducted in aquatic ecosystems with different levels of human intervention, such as the Llobregat River (Barcelona) and L'Albufera Natural Park (Valencia). In the Llobregat river, the distribution of CECs was evaluated in the organs of four fish species, identifying the common carp as a key species for biomonitoring due to its invasive nature. This species was included in a second study to assess the ecological risk in this ecosystem. In L'Albufera Natural Park, the potential of invasive species, such as the American red crayfish, the Asian clam and the pumpkinseed sunfish, for biomonitoring of CECs was investigated, focusing on their ability to resist and bioaccumulate chemical compounds. An exposure experiment under real environmental conditions allowed the establishment of species-differentiated accumulation patterns, which facilitated the ecological risk analysis of this coastal wetland of high environmental importance.

Findings showed invasive species, particularly common carp and Asian clam, accumulate CECs more effectively than native species, making them valuable for biomonitoring. Previously unreported CECs in invasive species, such as benzotriazole, cotinine, bisphenol A, and certain PFAS, were identified. The results highlight the dual benefits of using invasive species: providing insights into pollution levels and reducing pressure on native species.

This research advances biomonitoring methodologies and ecological risk

assessment of CECs in aquatic ecosystems. It offers innovative tools and knowledge to improve pollution mitigation strategies and sustainable ecosystem management.

RESUM

La contaminació dels ecosistemes aquàtics derivada d'activitats antropogèniques pot generar greus conseqüències ambientals, com la degradació de la qualitat de l'aigua, la pèrdua de biodiversitat, l'acumulació de contaminants a la cadena tròfica, efectes directes sobre la biota aquàtica i, en general, el deteriorament dels hàbitats. Aquests impactes varien segons el tipus de contaminant, les taxes d'emissió, el seu potencial de bioacumulació en els diferents compartiments ambientals i la vulnerabilitat de les espècies presents. En particular, els contaminants emergents (CECs), com els compostos actius de productes farmacèutics, productes de cura personal, plaguicides i substàncies industrials, representen una amenaça creixent per als ecosistemes aquàtics. Aquests compostos són recentment identificats, no estan completament regulats i manquen d'informació precisa en les avaluacions prèvies sobre contaminació ambiental.

En aquest context, aquesta tesi proposa l'ús d'estratègies de biomonitoratge basades en espècies aquàtiques invasores i natives com a eina clau per avaluar el risc ecològic en ecosistemes aquàtics. Per això, es va analitzar la presència, bioacumulació i risc ecològic de 173 CECs en matrius biològiques de diferents espècies aquàtiques, incloent-hi peixos, crancs de riu i petxines. Es van emprar metodologies analítiques avançades, basades en cromatografia de líquids acoblada a la espectrometria de masses d'alta resolució (LC-HRMS), per desenvolupar dos mètodes robustos que van permetre l'anàlisi de CECs a nivells de traça, destacant la importància de la tècnica de neteja d-SPE per eliminar interferències als extracts i garantir l'exactitud dels resultats.

La recerca es va dur a terme en ecosistemes aquàtics amb diferents nivells d'intervenció humana, com el riu Llobregat (Barcelona) i el Parc Natural de l'Albufera (València). Al riu Llobregat, es va avaluar la distribució de contaminants emergents als òrgans de quatre espècies de peixos, identificant la carpa comuna com a espècie clau per al biomonitoratge degut al seu caràcter invasor. Aquesta espècie es va incloure en un segon estudi que va permetre avaluar el risc ecològic en aquest ecosistema. Al Parc Natural de l'Albufera, es va investigar el potencial d'espècies invasores, com el cranc de riu roig americà, la petxina asiàtica i el peix percasol, per al biomonitoratge de CECs, centrant-se en la seva capacitat per resistir i bioacumular compostos químics. Un experiment d'exposició en condicions ambientals reals va permetre establir patrons d'acumulació diferenciats per espècie, facilitant l'anàlisi

del risc ecològic d'aquest aiguamoll costaner d'alta importància ambiental.

Es va evidenciar que les espècies invasores, com la carpa comuna i la petxina asiàtica, tenen una capacitat d'acumulació més gran de CECs en comparació amb les espècies natives, la qual cosa suggereix que poden ser utilitzades eficaçment com a biomonitors per avaluar la qualitat ambiental de diferents tipus d'ecosistemes. A més, es van identificar CECs que no havien estat reportats prèviament a les espècies invasores, com el 5-metil-1H-benzotriazol, l'amantadina, el benzotriazol, la cotinina, la hidroclorotiazida, el bezafibrat, el bisfenol A, l'àcid perfluorononanoic, el sulfonat de perfluorononanoic i el sulfonat de perfluorooctanoic.

Els resultats presentats en aquesta tesi van destacar que les espècies invasores no només permeten avaluar l'acumulació de CECs en ambients afectats per la contaminació, sinó que la seva inclusió en estudis de biomonitoratge també contribuiria a reduir la pressió sobre les espècies natives. En conclusió, aquesta tesi ha representat un avenç en el biomonitoratge ambiental, proporcionant eines analítiques avançades i un enfocament integrat per avaluar el risc ecològic associat als CECs en ecosistemes aquàtics, ampliant així el coneixement per millorar les estratègies de mitigació i gestió de la contaminació.

ABREVIATURAS Y ACRÓNIMOS

A

ADME	Absorción, Distribución, Metabolismo y Excreción
AIF	*All Ion Fragmentation* Fragmentación de todos los iones
APCI	*Atmospheric Pressure Ionization* Ionización química a presión atmosférica
APPI	*Atmospheric Pressure Photoionization Ionization* Fotoionización a presión atmosférica
ASE	*Accelerated Solvent Extraction* Extracción acelerada con disolventes
ATC	*Anatomical Therapeutic Classification* Clasificación anatómica, terapéutica

B

BAF	*Bioaccumulation factor* Factor de bioacumulación
BCF	*Bioconcentration factor* Factor de bioconcentración

C

CCL	*Drinking water Contaminant Candidate List* Lista de Candidatos a Contaminantes del Agua Potable
CECs	*Contaminants of Emerging Concern* Contaminantes de preocupación emergente

D

DDA	*Data Dependent Acquisition* Adquisición dependiente de datos
DHD	Dosis diarias definidas por 1000 habitantes
DIA	*Data Independent Acquisition* Análisis independiente de datos
DMA	Directiva Marco del Agua
DR	Determinaciones reglamentarias
d-SPE	*Dispersive Solid Phase Extraction* Extracción en fase sólida dispersiva

E

ECHA	*European Chemicals Agency* Agencia Europea de sustancias y mezclas químicas
ECOSAR	*Ecological Structure Activity Relationships* Relaciones ecológicas estructura-actividad
EDAR	Estación Depuradora de Aguas Residuales
EDCs	*Endocrine Disrupting Chemicals* Compuestos disruptores endocrinos

EM	Efecto Matriz
EPA	*Environmental Protection Agency* Agencia de Protección Ambiental de Estados Unidos
ESI	*Electrospray Ionization* Ionización por electrospray

F

FDA	*Food and Drug Administration* Administración de alimentos y medicamentos de los Estados Unidos
FODA	Análisis de Fortalezas, Oportunidades, Debilidades y Amenazas
FWHM	*Full Width at Half Maximum* Medida del ancho completo del pico a media altura

G

GC	*Gas Chromatography* Cromatografía de gases
GCB	*Graphitized Carbon Black* Adsorbente carbón negro grafitizado
GPC	*Gel Permeation Chromatography* Cromatografía de permeación en gel

H

HRMS	*High Resolution Mass Spectrometry* Espectrometría de masas de alta resolución

I

IDA	*Information-Dependent Acquisition* Adquisición dependiente de la información
INERIS	*Institut National de L'environnement Industriel et des Risques* Instituto Nacional Francés de medio ambiente y riesgos industriales

K

K_{ow}	*Octanol-Water partition coefficient* Coeficiente de partición octanol-agua

L

LC	*Liquid chromatography* Cromatografía de líquidos
LOD	*Limit of Detection* Límite de detección
LOQ	*Limit of Quantification* Límite de cuantificación

M

MAE	*Microwave- Assisted Extraction* Extracción asistida con microondas
MCL	*Maximum Contaminant Levels* Niveles máximos individuales de contaminantes

MRM	*Multiple Reaction Monitoring* Monitoreo de reacciones múltiples
m/z	Relación masa/carga

N

NCA	Normas de Calidad Ambiental
NPDWR	*PFAS National Primary Drinking Water Regulation* Reglamento Nacional sobre Agua Potable Primaria – PFAS

O

OPFRs	*Organophosphate Flame Retardants* Retardantes de llama organofosforados

P

PAHs	*Polycyclic Aromatic Hydrocarbons* Hidrocarburos aromáticos policíclicos
PBPK	*Physiologically Based Pharmacokinetic model* Modelo farmacocinético de base fisiológica
PCBs	*Polychlorinated Biphenyls* Bifenilos policlorados
PCP	*Personal Care Products* Productos de cuidado personal
PFAA	*Perfluoroalkyl Acids* Ácidos perfluoroalquilo
PFAS	*Per and polyfluorinated alkyl substances* Sustancias per y polifluoroalquiladas
PFCA	*Perfluoroalkyl carboxylic acids* Ácidos perfluoroalquil carboxílicos
PFSA	*Perfluoroalkanesulfonic acids* Ácidos perfluoroalcanosulfónicos
PhACs	*Pharmaceutical Active Compounds* Compuestos activos de productos farmacéuticos
PLE	*Pressurized Liquid Extraction* Extracción con líquidos a presión
PNECs	*Predicted No-effect Concentrations* Concentraciones previstas sin efecto
PRM	*Parallel Reaction Monitoring* Monitoreo de Reacciones Paralelas
PSA	*Primary Secondary Amine* Adsorbente amina secundaria principal

Q

Q	*Quadrupole* Cuadrupolo
QqQ	*Triple Quadrupole* Triple cuadrupolo
QSAR	*Quantitative Structure-Activity Relationship* Relación Cuantitativa Estructura-Actividad

QuEChERS	*Quick, Easy, Cheap, Effective, Rugged, and Safe* Rápido, fácil, económico, efectivo, robusto y seguro

R

REACH	*Registration, evaluation, authorization, and restriction of chemicals* Reglamento sobre registro, evaluación, autorización y restricción de sustancias químicas
RQ	*Risk Quotient* Cociente de riesgo

S

SAX	*Strong Anion Exchange* Adsorbente de intercambio iónico fuerte
SDWA	*Safe Drinking Water Act* Ley de Agua Potable Segura de Estados Unidos
SIM	*Selected ion Monitoring* Monitoreo de iones seleccionados
SLE	*Solid-liquid extraction* Extracción sólido-líquido
SPE	*Solid Phase Extraction* Extracción en fase sólida
SWATH	*Sequential Window Acquisition of All Theorical Fragment-ion Spectra* Adquisición secuencial de ventanas de todos los espectros teóricos de fragmentos iónicos

T

TOF	*Time of Flight* Analizador de masas de tiempo de vuelo
TPs	*Transformation Products* Productos de transformación

U

UAE	*Ultrasound-Assisted Extraction* Extracción asistida con ultrasonidos

Z

Z-Sep	Adsorbente de gel de sílice recubierta con óxido de circonio

ESTRUCTURA DE LA TESIS

Esta tesis doctoral es presentada mediante el compendio de cinco artículos científicos, que se despliegan en seis capítulos principales. En el Capítulo 1, se presenta una revisión general de los contaminantes de preocupación emergente (CECs del inglés *Contaminants of Emerging Concern*) objeto de estudio (compuestos activos de productos farmacéuticos, plaguicidas, sustancias per y polifluoroalquiladas, retardantes de llama organofosforados y otros compuestos de uso industrial), abordando en primera medida su definición, uso, origen, producción, principales fuentes de emisión hacia los ecosistemas acuáticos y sus efectos sobre el medio ambiente. Posteriormente se definen los principales procesos de absorción, distribución, metabolismo y excreción de los CECs por parte de la fauna acuática que se encuentra expuesta en las masas de agua: peces de río, crustáceos y almejas de agua dulce. Se incluye una revisión sobre la legislación aplicable para algunos CECs incluidos en esta tesis respecto a niveles de concentración máxima en agua para consumo humano y niveles de referencia para la protección de organismos acuáticos. Se presenta una revisión sobre el uso de especies acuáticas para el biomonitoreo de CECs, haciendo énfasis en la importancia de incluir las especies invasoras en los estudios de evaluación de la contaminación ambiental. Finalmente se presenta el estado del arte de los métodos analíticos comúnmente utilizados para el análisis de CECs en matrices biológicas, y la metodología de análisis de riesgo adaptada para definir el estado ambiental de los ecosistemas acuáticos evaluados.

En el Capítulo 2 se incluyen el objetivo general y los objetivos específicos de esta tesis doctoral. Seguidamente, en el Capítulo 3, se presentan las metodologías analíticas desarrolladas para el análisis de los 173 CECs en especies nativas e invasoras. En este capítulo se informa detalladamente de las etapas a través de las cuales se logró la obtención de dos métodos robustos que permitieron el análisis a niveles traza de los analitos en diferentes matrices biológicas, incluidas órganos y fluidos corporales de peces y tejido blando de cangrejos de río y almejas, centrándose en el pretratamiento de las muestras, el método de extracción y la técnica de limpieza. Como resultado de este trabajo, se presentan dos publicaciones:

1. Publicación No. 1: **Manjarrés, D. P.,** Montemurro, N., & Pérez, S. (2022). Development of a USE/d-SPE and targeted DIA-Orbitrap-MS acquisition methodology for the analysis of wastewater-derived organic pollutants in fish tissues and body fluids. MethodsX, 9, 101705. https://doi.org/10.1016/J.

MEX.2022.101705

2. Publicación No. 2: **Manjarrés-López, D. P.,** Vitale, D., Callejas-Martos, S., Usuriaga, M., Picó, Y., Pérez, S., & Montemurro, N. (2023). An effective method for the simultaneous extraction of 173 contaminants of emerging concern in freshwater invasive species and its application. Analytical and Bioanalytical Chemistry. https://doi.org/10.1007/s00216-023-04974-3

En el Capítulo 4, se aplica uno de los métodos desarrollados para evaluar la distribución de CECs, principalmente compuestos activos de productos farmacéuticos en órganos y tejidos corporales de cuatro especies de peces de río salvajes: el barbo del Ebro, la lisa, la anguila europea y la carpa común. Este estudio, presentado en la Publicación No. 3 permitió la priorización de la carpa común para el biomonitoreo de CECs en el Río Llobregat, debido a que se trata de una especie invasora en la que se detectaron las concentraciones más altas de los analitos de interés. A partir de estos hallazgos, el capítulo incluye una segunda publicación en la que se amplió el número de individuos analizados de la carpa común para una evaluación más amplia, precisa y representativa de la zona de estudio. Además, se realizó una comparación de los factores de bioacumulación medidos con los factores de bioconcentración predichos a partir de un modelo farmacocinético de base fisiológica y se efectuó un análisis de riesgo ecológico, que permitió una definición clara de la calidad ambiental del ecosistema evaluado. Este segundo estudio, se llevó a cabo en colaboración con el Centro Helmholtz de investigación Medioambiental (Helmholtz Centre for Environmental Research – UFZ, Alemania), como resultado de una estancia de investigación predoctoral. Los resultados se presentan en la Publicación No. 4.

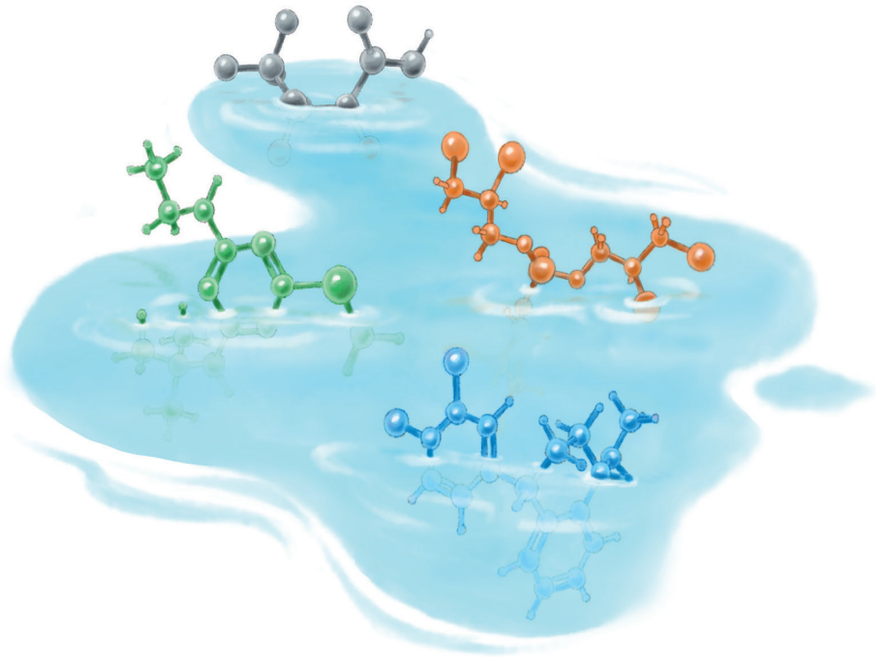
3. Publicación No. 3: **Manjarrés-López, D.P.,** Peña-Herrera, J. M., Benejam, L., Montemurro, N., & Pérez, S. (2023). Assessment of wastewater-borne pharmaceuticals in tissues and body fluids from riverine fish. Environmental Pollution, 324, 121374. https://doi.org/10.1016/J.ENVPOL.2023.121374

4. Publicación No. 4: **Manjarrés-López, Diana P.,** Montemurro, N., Ulrich, N., Ebert, R. U., Jahnke, A., & Pérez, S. (2024). Assessment, distribution, and ecological risk of contaminants of emerging concern in a surface water-sediment-fish system impacted by wastewater. Science of The Total Environment, 935, 173358. https://doi.org/10.1016/J.SCITOTENV.2024.173358

En el Capítulo 5, se aplica el segundo método analítico publicado y descrito en el capítulo 3, para la evaluación del potencial de las especies invasoras seleccionadas (almeja asiática, cangrejo de río rojo americano y pez percasol) en el biomonitoreo de CECs en ecosistemas acuáticos con un alto grado de intervencion antrópica. Para esto incialmente se detalla el origen, propagación e impactos de las especies invasoras y se realiza una revisión de algunos estudios de biomonitoreo de CECs en los que se han usado. La Publicación No. 5 incluida en este capítulo muestra los resultados obtenidos tras la realización de un experimento de exposición en condiciones ambientales reales, en el que se determinaron los patrones de acumulación de contaminantes en función de las caracteristicas de cada especie y las diferencias en comparación con una especie nativa (almeja petxinot). Asi mismo se calcularon factores de bioacumulación por especie y se realizó una análisis de riesgo ecológico que no sólo permitió establecer el grado de vulnerabilidad del ecosistema, sino que también facilitó la priorización de especies para el biomonioreo.

5. Publicación No. 5: **Manjarrés-López, D.P.,** Martínez-Megías, C., Vitale, D., Picó, Y., Rico, A., & Pérez, S. (2024). Occurrence, bioaccumulation, and ecological risk assessment of contaminants of emerging concern using native and invasive species as biomonitors. Environmental Research (Enviado).

Finalmente, en el Capítulo 6 se presentan las conclusiones de esta tesis doctoral, con base a las discusiones desarrolladas en los capítulos 3, 4 y 5.



CAPÍTULO 1

INTRODUCCIÓN GENERAL

INTRODUCCIÓN GENERAL

1.1. Los contaminantes de preocupación emergente y el medio ambiente

De acuerdo con la Agencia de Protección Ambiental de los Estados Unidos (EPA del inglés *Environmental Protection Agency)*, los CECs son sustancias químicas que anteriormente no se detectaban en el agua, pero que actualmente se encuentran a niveles significativamente diferentes a los esperados (EPA, 2024a). Los CECs no solo abarcan contaminantes recientes debido a la fabricación de nuevos productos o la incorporación de nuevas sustancias en los procesos de producción, sino también aquellos cuya presencia en el medio ambiente ya se conocía pero que ahora generan mayor interés (Xabadia et al., 2021). Dentro de esta definición, se pueden establecer dos categorías: i) contaminantes nuevos, es decir compuestos o moléculas recientemente descubiertos o que acaban de aparecer en la literatura científica, y ii) contaminantes de interés emergente cuya existencia ya se conocía, pero que no se podían analizar por limitaciones analíticas y para los que la nueva información muestra riesgos asociados al medio ambiente y la salud humana que anteriormente se desconocían (Sauvé & Desrosiers, 2014).

Dentro de esta clase de contaminantes se incluyen una amplia gama de compuestos como productos de cuidado personal (PCP del inglés *personal care product)*, compuestos activos de productos farmacéuticos (PhACs del inglés *pharmaceutical active compounds*), sustancias per y polifluoroalquiladas (PFAS del inglés *per and polyfluorinated alkyl substances*), tensoactivos, plastificantes, plaguicidas, retardantes de llama organofosforados (OPFRs del inglés *organophosphate flame retardants*), y nanomateriales que podrían impactar negativamente las formas de vida terrestres, acuáticas y a la salud humana (Feng et al., 2023; Kumar et al., 2022; Pastorino & Ginebreda, 2021).

La liberación de CECs en el medio ambiente se puede originar a partir de fuentes difusas como la contaminación atmosférica y la escorrentía de aguas pluviales (Feng et al., 2023; Kumar et al., 2022), o a partir de fuentes directas como las originadas a través de actividades industriales y/o por su presencia en aguas residuales (agrícolas, domésticas o industriales). Se considera que las aguas residuales municipales tratadas en estaciones depuradoras de aguas residuales (EDAR) y posteriormente su vertido en masas de agua superficial, son la principal fuente puntual de este tipo

de contaminantes en ecosistemas acuáticos (Gogoi et al., 2018). Esta condición está asociada a las limitaciones que aún existen en las tecnologías implementadas para su eliminación por parte de las EDAR (Xabadia et al., 2021).

Los CECs abarcan un amplio abanico de propiedades fisicoquímicas. Dependiendo de su naturaleza pueden transportarse, transformarse, aglomerarse, degradarse y generar impactos ambientales en función de su naturaleza química. Algunos CECs pueden persistir en los sedimentos durante períodos prolongados, lo que resulta en su bioacumulación en organismos bentónicos (Feng et al., 2023). Gracias a los avances en técnicas analíticas como la cromatografía de líquidos y la espectrometría de masas de alta resolución (LC-HRMS del inglés *Liquid chromatography - High resolution mass spectrometry*) (Richardson, 2007) se han reportado CECs a lo largo del ciclo hidrológico y se ha confirmado su presencia en aguas subterráneas, agua superficiales y de bebida (Kumar et al., 2022), sedimentos y fauna acuática.

El objeto de estudio de esta tesis, se centra en el análisis de cinco grupos de CECs: PhACs y algunos de sus metabolitos (86 compuestos), plaguicidas y sus metabolitos o TPs (52 compuestos), PFAS (20 compuestos), OPFRs (11 compuestos) y otros compuestos de uso industrial (5 compuestos) (Fig. 1.1). A continuación, se aborda brevemente a que hace referencia cada grupo de CECs incluyendo sus propiedades físico-químicas, usos, producción, las fuentes principales mediante las cuales se incorporan en el medio ambiente y efectos.

1.1.1. Compuestos activos de productos farmacéuticos

Los PhACs, están diseñados para curar y prevenir enfermedades, además de mejorar la calidad de vida humana y animal. Estos compuestos presentan una amplia diversidad en cuanto a estructuras químicas, aplicaciones y metabolismo tanto en humanos como animales, y consecuentemente, interactúan de diversa manera en el medio ambiente (Tijani et al., 2015). Los PhACs se clasifican según el sistema europeo de clasificación anatómica, terapéutica (ATC del inglés *Anatomical therapeutic classification*), que los distingue en función del sistema u órgano afectado, efecto farmacológico, indicaciones terapéuticas y estructura química (AEMPS, 2024).

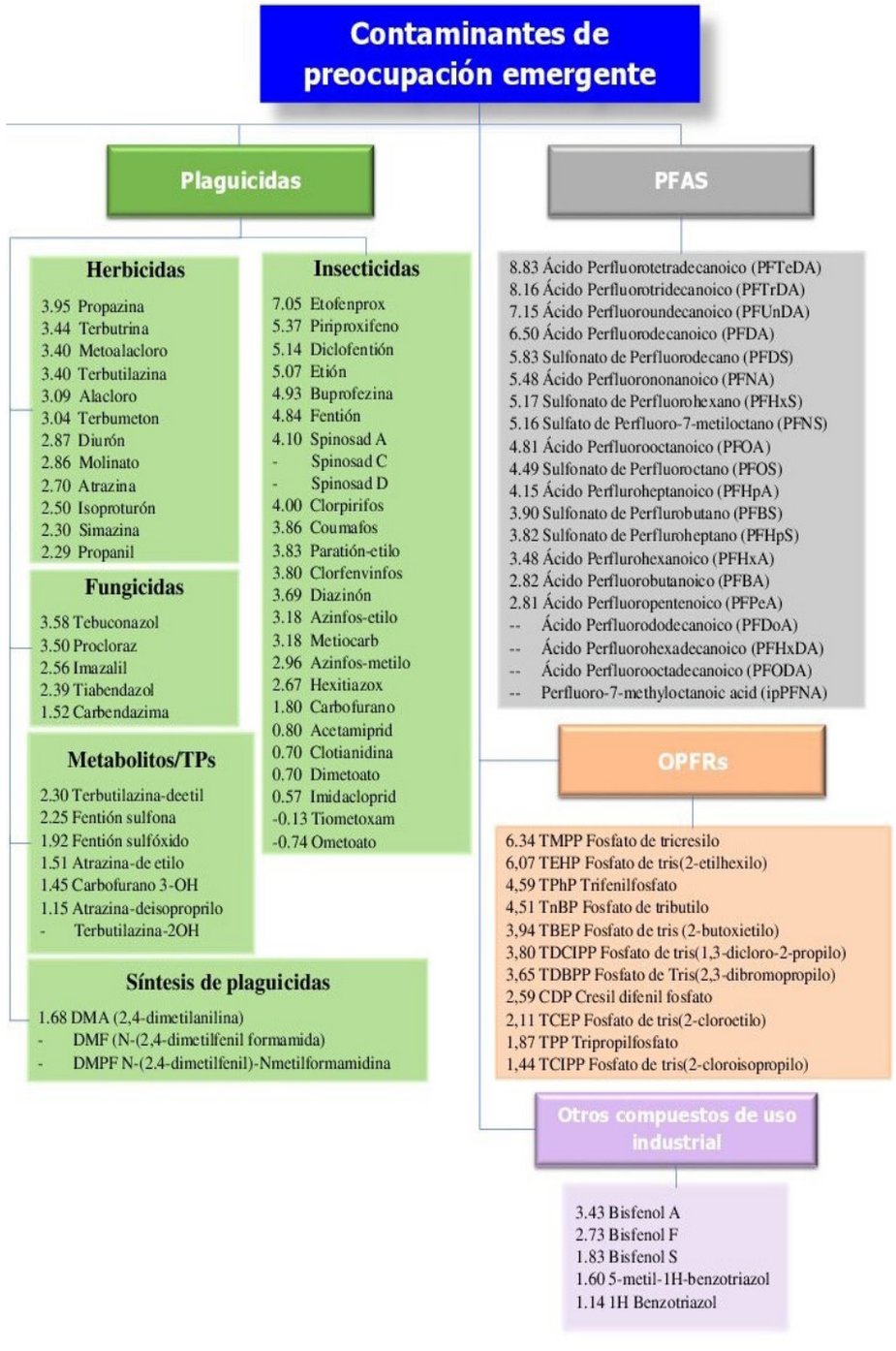
En el mercado existen más de veinte mil medicamentos aprobados por la Administración de alimentos y medicamentos de Estados Unidos (FDA del inglés *Food and Drug Administration*) (Matthee et al., 2023). De acuerdo con la Agencia

española de medicamentos y productos farmacéuticos, en España se consumen más de 500 dosis diarias definidas por 1000 habitantes (DHD) de medicamentos, teniendo en cuenta el número de envases dispensados en oficinas de farmacias con cargo al Sistema nacional de salud (AEMPS, 2024). Desde el año 2010 el consumo de antidepresivos, antidiabéticos, analgésicos y opioides se ha visto incrementado alcanzando valores de 96,5, 87,6, 52,8 y 22,3 DHD respectivamente (datos del 2022) (Fig. 1.2).

La mayoría de estos medicamentos pueden administrarse por vía oral o intravenosa. Una vez en el cuerpo humano, los PhACs pueden metabolizarse parcialmente y una parte puede ser excretada a través de la orina o las heces. El uso creciente de productos farmacéuticos, combinada con su metabolismo incompleto en el cuerpo, ha llevado a la detección de PhACs o sus metabolitos en el medio ambiente (Martínez-Megías et al., 2024; Miller et al., 2018; Nozaki et al., 2023; Ramirez et al., 2009; Świacka et al., 2022; Tanoue et al., 2015; Valdés et al., 2014; Xie et al., 2019; Zheng et al., 2022). Los PhACs entran a las EDAR a través de diversas rutas, como las aguas residuales domésticas, fosas sépticas, vertederos, efluentes industriales, aguas residuales urbanas y prácticas agrícolas. Algunos de estos contaminantes son estables y difíciles de degradar mediante los sistemas de tratamiento convencionales, y su presencia en el medio ambiente varían de un país a otro, dependiendo de factores como el patrón de consumo y el tamaño de la población (Tijani et al., 2015).

Se han realizado diversos estudios eco-toxicológicos que demuestran los efectos de los PhACs sobre la fauna acuática. Por ejemplo, compuestos como los antidepresivos pueden afectar el comportamiento, la reproducción, el desarrollo y la supervivencia de invertebrados y vertebrados (Richardson & Kimura, 2020). Tras un estudio de la exposición del pez rutilo (*Rutilus rutilus)* a aguas residuales tratadas durante 15 días, se encontró que los PhACs neuro-activos se concentraron en diferentes regiones del cerebro (David et al., 2018). También se ha demostrado que la venlafaxina tiene el potencial de reducir la supervivencia de los peces (Richardson & Kimura, 2020) y puede generar malformaciones en las larvas del pez cebra (Ribeiro et al., 2022), mientras que la sertralina y el oxazepam alteran su comportamiento, además de retrasar la locomoción y el aprendizaje (Brodin et al., 2017). Otros fármacos como el citalopram, propranolol, codeína y trimetazidina afectaron significativamente la forma del cuerpo y las escamas de peces analizados en ríos cercanos a un área suburbana de Budapest (Staszny et al., 2021). Asimismo, se ha indicado que los

Fig. 1.1. *CECs analizados en esta tesis, organizados en categorías y de acuerdo a su polaridad, del menos polar al más polar, según su coeficiente de partición octanol-agua (K_{ow}) indicado a la izquierda de cada compuesto*



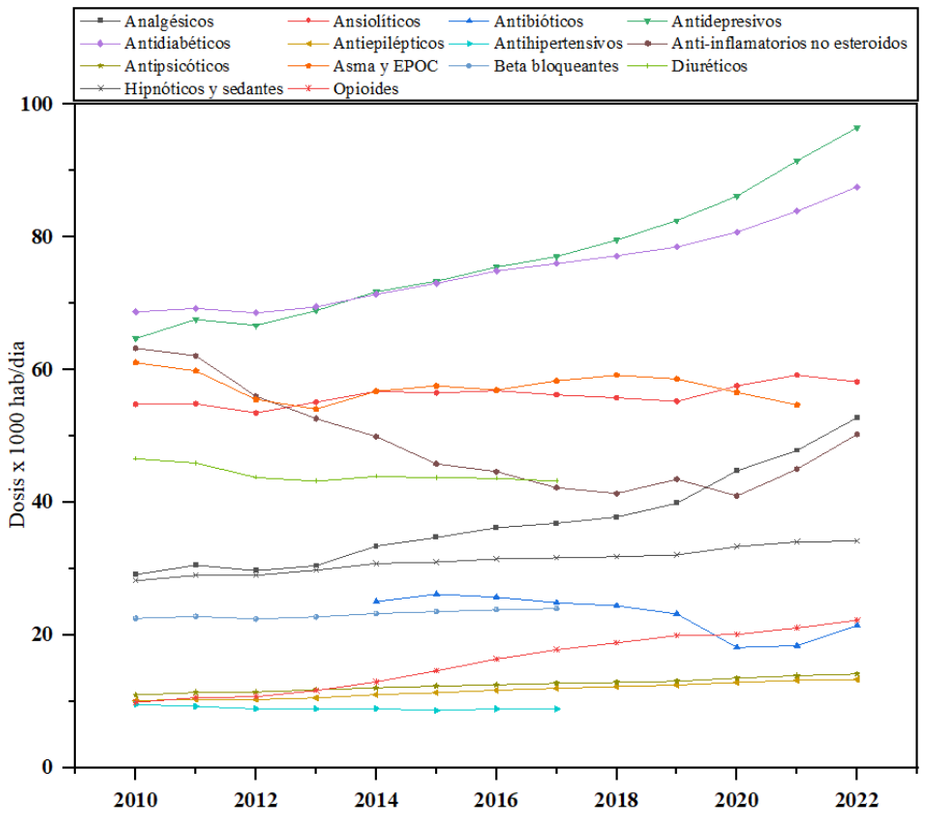


Fig. 1.2. *Consumo anual de diferentes clases de medicamentos en España 2010-2022 (AEMPS, 2024)*

bloqueadores β presentan un peligro potencial para los ecosistemas acuáticos (Wick et al., 2009) ya que pueden reducir el crecimiento y/o fecundidad en peces (Huggett et al., 2002; Owen et al., 2007).

1.1.2. Plaguicidas

Según la definición propuesta por la Comisión Europea, un plaguicida es una sustancia que previene, destruye o controla organismos nocivos (denominados plagas), o enfermedades. Además, protege las plantas y productos vegetales durante su producción, almacenamiento y transporte. De acuerdo a su acción específica, se clasifican en herbicidas, fungicidas, insecticidas, acaricidas, nematicidas, molusquicidas, reguladores del crecimiento, repelentes, rodenticidas y biocidas, entre otros (CE, 2024).

En 2021, a nivel mundial, el uso total de plaguicidas en la agricultura fue de 3,5

millones de toneladas (Mt) de ingredientes activos, lo que representó un aumento del 4% en un año, del 11% en una década y una duplicación desde 1990. Las exportaciones de plaguicidas alcanzaron 7,1 Mt, valoradas en 43.200 millones de dólares. Comparando la década más reciente con la de 1990, la aplicación mundial de herbicidas aumentó un 53%, el de fungicidas y bactericidas un 111%, y el de insecticidas un 44%. El uso de plaguicidas por hectárea de cultivo fue de 2,26 kg, un aumento del 4% respecto al 2020 (FAO, 2023).

América ha sido el continente con el mayor consumo de plaguicidas desde la década de 1990, alcanzando una tasa de crecimiento del 191%, seguido por Asia, Europa, África y Oceanía. Brasil fue el mayor usuario mundial de plaguicidas en 2021 con 720 kilo-toneladas (kt), seguido de Estados Unidos (457 kt), Indonesia (283 kt), China (245 kt) y Argentina (242 kt). Otros grandes consumidores fueron la Federación Rusa (97 kt), Canadá (92 kt), España (76 kt), Francia (70 kt) y Australia (63 kt). En Europa, el uso de plaguicidas aumentó en un 4%, de 486 kt en 2020 a 505 kt en 2021 y, el uso de plaguicidas por hectárea fue de 1,75 kg en 2021, el cual se encuentra por debajo de la media mundial (FAO, 2023).

En la Unión Europea los fungicidas y bactericidas fueron los plaguicidas más vendidos en el año 2022. Durante el período comprendido entre 2011 y 2022, el volumen total de ventas de plaguicidas superó las 300 kt anuales. Alemania, España, Francia e Italia representaron conjuntamente más de dos tercios del volumen total de ventas (EUROSTAT, 2024). Desde el 2011 la comercialización de plaguicidas en España ha estado por encima de las 60 kt anuales, siendo los fungicidas y bactericidas los más utilizados (Fig. 1.3) (MAPAMA, 2024).

El transporte de los plaguicidas hacia las masas de agua puede producirse a través del drenaje subsuperficial, la lixiviación, la escorrentía superficial y la deriva de pulverización (Cosgrove et al., 2019). Este movimiento depende de los patrones de drenaje, las propiedades del plaguicida, las precipitaciones, la actividad microbiana, la superficie de tratamiento y la tasa de aplicación (Mensah et al., 2014).

Una vez en el medio acuático, el efecto de los plaguicidas sobre los organismos depende de sus propiedades fisico-químicas como por ejemplo la solubilidad. Los plaguicidas solubles en agua son más móviles, mientras que los plaguicidas liposolubles pueden biomagnificarse en la red trófica (Mojiri et al., 2020). La presencia de plaguicidas en el medio acuático puede causar efectos directos e indirectos en la fauna. Los efectos directos están relacionados con la acción fisiológica del

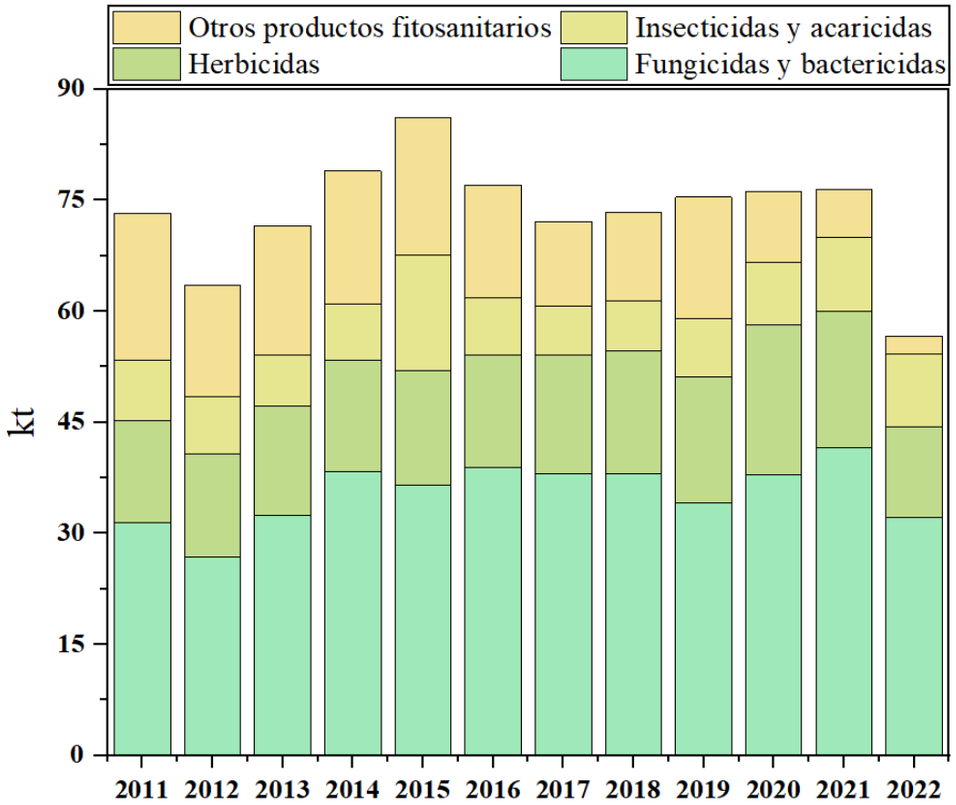


Fig. 1.3. Cantidades de plaguicidas comercializadas por los titulares autorizados en España 2011-2022 con base en los informes anuales de las encuestas de comercialización de productos fitosanitarios (MAPAMA, 2024)[1]

plaguicida dentro del organismo, como por ejemplo el aumento de la mortalidad de organismos acuáticos, cambios en el comportamiento natatorio de los peces, alteraciones en la morfología de algunas especies, pérdida de peso, alteraciones en los ciclos de reproducción (cambios hormonales), pérdida de la capacidad de evitar depredadores, reducción del desarrollo y la eclosión de los huevos de peces, entre otros (Bhat et al., 2022).

Los efectos indirectos de los plaguicidas en el ecosistema incluyen la alteración de los hábitats y del suministro de alimentos para los animales. Algunos plaguicidas, al ingresar a las masas de agua, promueven la proliferación de algas, lo cual puede desequilibrar el ecosistema y reducir la disponibilidad de plantas e insectos que

[1] Entre los años 2016 y 2019 los acaricidas se incluyeron en el grupo de otros productos fitosanitarios. A partir de 2022 se excluyen de los informes las sustancias activas coadyuvantes que están fuera del ámbito de la encuesta.

servirían como alimento y refugio para otras especies (Friberg-Jensen et al., 2003). Esto es especialmente crítico para los peces más jóvenes, quienes dependen de las plantas acuáticas para protegerse de los depredadores. Cuando se aplican herbicidas cerca de estos entornos, la vegetación disminuye, reduciendo así la cobertura y el refugio que estas especies necesitan para su supervivencia (Bhat et al., 2022).

1.1.3. Sustancias per y polifluoroalquiladas

Los PFAS se definen como sustancias fluoradas que contienen al menos un metilo o metileno fluorado, sin ningún átomo de hidrógeno, cloro, bromo o yodo unido. Estos compuestos han recibido especial atención desde la década del 2000 debido a la información sobre sus peligros y a la confirmación de la presencia en el medio ambiente del PFOA y el PFOS (Wang et al., 2021). Se han documentado más de 6300 PFAS, siendo los ácidos perfluoroalquilcarboxílicos (PFCA) y los ácidos perfluoroalcanosulfónicos (PFSA) los más estudiados (Richardson & Kimura, 2020).

Los ácidos perfluoroalquilo (PFAA), un subconjunto de los PFAS, se denominan a menudo PFAS terminales, ya que los precursores de PFAS tienden a descomponerse para formarlos en el medio ambiente. Dentro de los PFAA, existen dos clases principales sobre las que se informa frecuentemente en la literatura: los ácidos PFSA y PFCA. Las principales diferencias dentro de estas clases de compuestos están relacionadas con la longitud de la cadena fluorada y el grupo funcional principal (Lewis et al., 2022).

Los PFAS tienen una amplia variedad de aplicaciones debido a su alta estabilidad térmica y química, resultado de la fuerza de sus enlaces carbono-flúor, así como su capacidad para reducir la tensión superficial, su hidrofobicidad, lipofobicidad, resistencia al calor y bajo coeficiente de fricción. Estos compuestos se encuentran en revestimientos de fluoro-telómeros para productos resistentes al agua, la suciedad, el aceite y las manchas, en espumas contra incendios, y en la producción industrial. También se emplean en impermeabilizantes, tensioactivos, antiadherentes para utensilios de cocina, y en muchos productos domésticos comunes. El sector textil es uno de los mayores consumidores de PFAS y sus precursores, seguido por la industria de envases de papel y los bienes de consumo postventa (Lewis et al., 2022).

A pesar de sus beneficios para los humanos, los PFAS se consideran contaminantes ambientales debido a que pueden incorporarse en el medio ambiente a lo largo de

todo su ciclo de vida, desde su producción y cadenas de suministro hasta el uso y la eliminación de productos industriales y de consumo. Estos compuestos pueden ingresar al medio ambiente a través de la lixiviación en vertederos, la pulverización de espuma formadora de película acuosa, el tratamiento de aguas residuales y las emisiones al aire en las instalaciones de fabricación. El agua es el principal medio de transferencia de PFAS entre compartimentos ambientales y la biota, debido a su solubilidad parcial, lo que contribuye a su difusión (Nayak et al., 2023).

La longitud de la cadena alquílica y los grupos funcionales de los PFAS influyen en su toxicidad y su capacidad de bioacumulación (Ma et al., 2022). Estos CECs pueden acumularse en organismos acuáticos y causar de efectos adversos que incluyen toxicidad reproductiva, estrés oxidativo, alteraciones metabólicas, toxicidad inmunológica, toxicidad del desarrollo, daño celular y necrosis (Nayak et al., 2023). Se ha demostrado que la exposición al PFOS ha provocado alteraciones en los tejidos de bivalvos, afectación de las glándulas digestivas e inhibición del comportamiento de sifonamiento[1] (Yan Liu et al., 2020; Ma et al., 2022).

1.1.4. Retardantes de llama organofosforados

Los retardantes de llama son compuestos químicos incorporados a materiales combustibles para prevenir incendios o retrasar su inicio y propagación, interrumpiendo o dificultando el proceso de combustión. Los retardantes de llama se dividen en tres categorías principales según su composición química en inorgánicos, halogenados (bromados y clorados) y OPFRs, caracterizados por la presencia de fósforo unido a grupos orgánicos (Van der Veen & de Boer, 2012). Los retardantes de llama también se dividen en dos categorías según su uso: reactivos y aditivos. Los reactivos introducen grupos ignífugos en el material mediante reacciones químicas, mejorando así su resistencia a la llama. En contraste, los aditivos actúan principalmente añadiendo sustancias ignífugas a los materiales combustibles (Yao et al., 2021). Los OPFRs, al ser aditivos, solo se mezclan con los materiales que los contienen y no se unen químicamente a ellos, lo que facilita su liberación en el medio ambiente circundante (Rodríguez et al., 2006).

La introducción de los OPFRs en el mercado surgió debido a la prohibición de los retardantes de llama bromados, como los éteres de difenilo poli-bromados, que fueron catalogados como contaminantes orgánicos persistentes por el Convenio de

[1] Acción mediante la cual los bivalvos toman agua de su entorno y filtran nutrientes, oxígeno, y partículas.

Estocolmo en el 2008 (Pantelaki & Voutsa, 2019). También se consideran sustitutos de los bifenilos policlorados (PCBs del inglés *polychlorinated biphenyls*), que son persistentes en el medio ambiente y tóxicos para humanos y animales (Yao et al., 2021).

Los OPFRs se utilizan ampliamente en diversos productos de consumo, como textiles, electrónica, productos químicos, pegamentos, placas de circuito impreso, materiales industriales y muebles, para prevenir el riesgo de incendio (Pantelaki & Voutsa, 2019; Yao et al., 2021). El consumo de OPFRs en el mercado global aumentó de 108.000 a 186.000 toneladas entre 1995 y 2001 (Van der Veen & de Boer, 2012) y para el 2016, representaron el segundo grupo de retardantes de llama más producido, alcanzando el 18% del mercado total en todo el mundo (Pantelaki & Voutsa, 2019).

Los OPFRs no están unidos químicamente a los materiales y pueden liberarse fácilmente al medio ambiente por volatilización, abrasión y disolución (Pantelaki & Voutsa, 2019). La vida media de los OPFRs en fase de partículas (entre 3,5 y 14 días), es más larga que en fase gaseosa (inferior a 2 días), lo cual sugiere un posible transporte atmosférico de largo alcance, permitiéndoles viajar distancias significativas en la atmósfera y alcanzar regiones remotas (Liu et al., 2014). Si a esto se le suma la naturaleza variable de sus sustituyentes que les permite abarcar una amplia gama de propiedades físicas y químicas, que van desde muy polares hasta altamente hidrófobas, su presencia en diversos medios ambientales como el agua, aire y sedimento se ve facilitada (Yao et al., 2021).

Como es común en los CECs se ha demostrado que la eliminación de OPFRs en las EDAR no es del todo efectiva, por lo cual los efluentes tratados también se consideran una fuente importante de su libración hacia el medio ambiente acuático (Pantelaki & Voutsa, 2019). Dentro de los efectos de los OPFRs en los organismos acuáticos se encuentran el daño al tejido nervioso, obstaculización de la transmisión de neurotransmisores y neurotoxicidad (Yao et al., 2021).

Como se ha señalado, cada grupo de CECs incluido en esta tesis presenta distintos usos y mecanismos de transporte hacia el medio acuático. No obstante, todos comparten una fuente común de entrada en masas de agua superficial: los efluentes de EDAR, que facilitan su incorporación directa. La Fig. 1.4 resume la información más relevante para cada grupo de CECs de acuerdo a la revisión realizada.

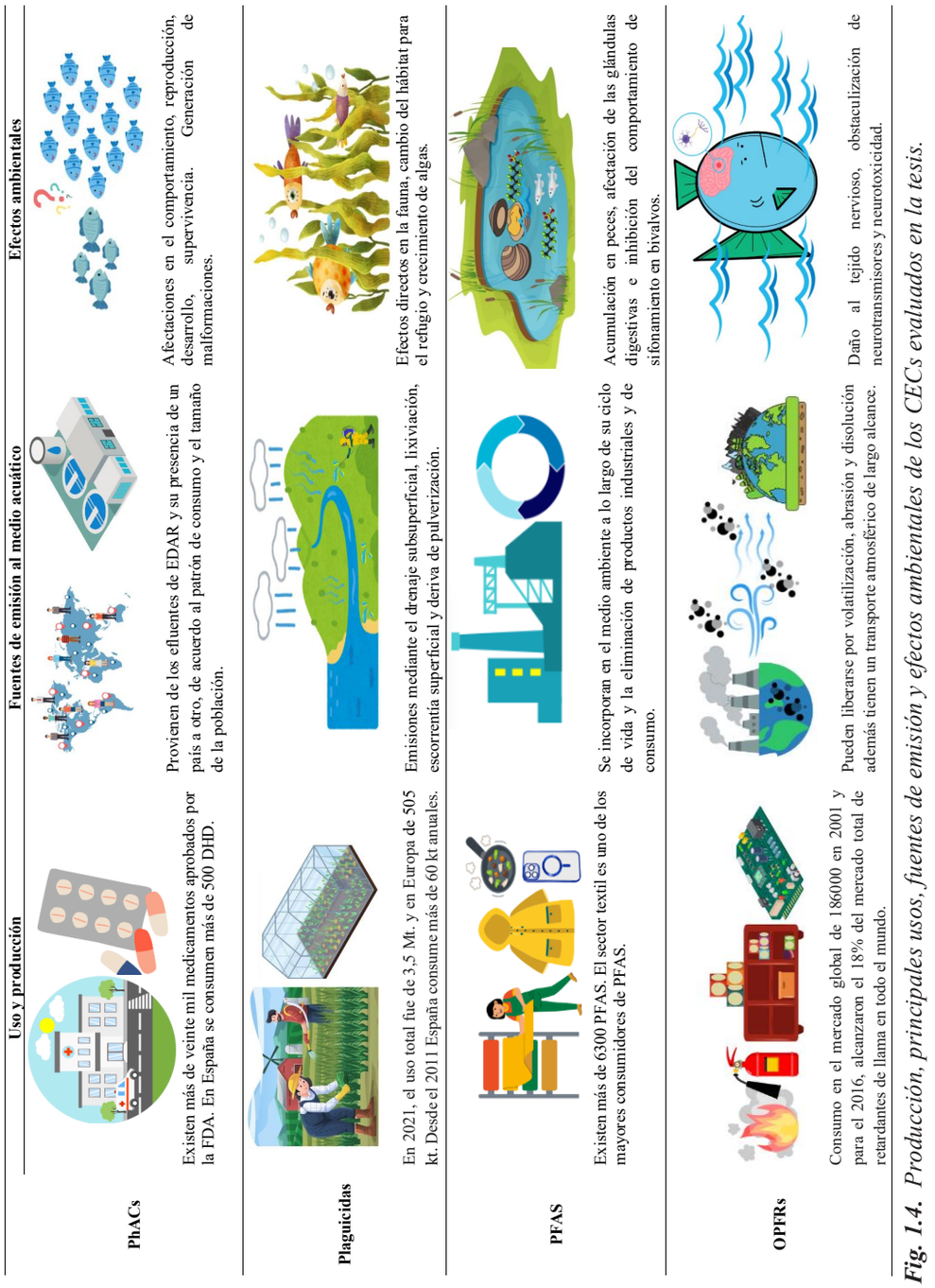


Fig. 1.4. *Producción, principales usos, fuentes de emisión y efectos ambientales de los CECs evaluados en la tesis.*

Debido a que los CECs logran incorporarse con éxito en los ecosistemas acuáticos, resulta fundamental comprender los procesos a través de los cuales los organismos los absorben y se ven afectados. En el siguiente apartado, se amplía la información sobre los diferentes mecanismos de entrada de los CECs de acuerdo a las características propias de cada especie y en función de su naturaleza química.

1.2. Absorción, distribución, metabolismo y excreción de contaminantes de preocupación emergente en fauna acuática de agua dulce

Una vez que los CECs se integran en los ecosistemas de agua dulce, pueden interactuar con los organismos acuáticos, siendo absorbidos de diversas formas. La absorción de estos compuestos por parte de la fauna que habita en las masas de agua depende de las propiedades fisicoquímicas de cada compuesto y de las características específicas de cada especie. La diversidad en términos de biología y ecología, como el taxón, el tamaño, el ciclo de vida, tipo de respiración, dieta, estrategia reproductiva y la preferencia de hábitat pueden influir en la absorción y determinar la sensibilidad de las especies a los contaminantes (Meredith-Williams et al., 2012; Rubach et al., 2011).

La absorción y acumulación de sustancias químicas también depende de factores fisiológicos (Rubach et al., 2011). Los efectos tóxicos resultantes de la exposición y la posibilidad de acumularlos o no, están relacionados con las concentraciones del compuesto en el agua y se determinan por una interacción compleja entre la absorción, la distribución en el cuerpo de los organismos, el metabolismo y la excreción (ADME) (Tierney et al., 2013). A continuación, se describen brevemente estos cuatro procesos fundamentales para los vertebrados (peces: *Cyprinus carpio*, L*iza ramada, Luciobarbus graellsii, Anguilla anguilla* y *Lepomis gibbossus*) e invertebrados acuáticos como el cangrejo de río rojo americano (*Procambarus clarkii*) y almejas de río (*Corbicula fluminea* y *Anodonta cygnea*) que son las especies que se han utilizado en esta tesis para el biomonitoreo de los CECs.

1.2.1. Peces de río

Absorción: el potencial de absorción de los contaminantes existe en todas las células epiteliales que están en contacto con el agua y los alimentos, pero es mayor en los epitelios respiratorio y gastrointestinal. Varias características de los tejidos epiteliales aumentan sus tasas de absorción: una gran superficie, una corta distancia de difusión y una elevada perfusión sanguínea. Los contaminantes atraviesan los

epitelios a través de la difusión pasiva simple, difusión facilitada, transporte activo y endocitosis (Tierney et al., 2013).

La tasa de absorción dérmica puede variar según el grosor de la piel y la presencia de escamas, siendo generalmente mayor en peces juveniles o larvas, donde la piel es la principal vía de captación de oxígeno hasta que las branquias se desarrollan completamente (Rombough, 2002). La mayoría de compuestos se absorben a través de las vías branquial o gastrointestinal. La absorción por ambas vías se considera como una transferencia a través de una serie de compartimentos acuosos y lipídicos. Primero, el compuesto debe llegar al epitelio y atravesar varias barreras de difusión, incluida la membrana celular, hasta llegar a la sangre (Gobas et al., 1986).

Las sustancias químicas hidrosolubles con un log K_{ow} <1 están fácilmente biodisponibles para el epitelio branquial; sin embargo, su absorción es muy baja porque tienen una baja permeabilidad celular. En contraste, la eficiencia de captación por las branquias es alta y relativamente constante para los compuestos con valores de log K_{ow} entre 4 y 6 (McKim et al., 1985). Por otro lado, el epitelio gastrointestinal es una vía importante de absorción, especialmente para compuestos muy hidrófobos disueltos en lípidos alimentarios. Las sustancias químicas hidrófobas, que se absorben por difusión pasiva, pueden sortear las barreras difusionales acuosas en el intestino al asociarse con micelas de ácidos grasos durante la pinocitosis (Ekelund, 1989).

Distribución: la distribución de sustancias químicas en los peces ocurre a través de sus sistemas circulatorios tras la absorción. El plasma, que es el componente acuoso de la sangre, permite que muchos de los compuestos hidrofílicos se disuelvan y sean transportados en esta matriz. En cambio, los compuestos hidrófobos que no se disuelven fácilmente en el plasma, tienden a asociarse con otros componentes de la sangre, como el colesterol y los triglicéridos, así como con constituyentes macrocelulares como las membranas celulares, pero especialmente con las proteínas plasmáticas (Tierney et al., 2013).

Los compuestos con elevado peso molecular unidos a proteínas encuentran dificultades para salir de los capilares, lo que limita su disponibilidad para distribuirse en el espacio extravascular y en los tejidos. Por otro lado, las interacciones hidrofóbicas son reversibles, permitiendo la liberación del compuesto cuando otra biomolécula o componente tisular muestra mayor afinidad por la proteína plasmática. Una vez liberada, la sustancia química puede difundirse fácilmente entre las células, hasta

alcanzar un equilibrio entre los tejidos y las proteínas plasmáticas. En resumen, la distribución tisular depende principalmente de las propiedades fisicoquímicas del compuesto, del flujo sanguíneo hacia un tejido específico y de la afinidad del compuesto por los componentes tisulares (Clark et al., 1990).

Metabolismo: la capacidad de biotransformación influye significativamente en las tasas de acumulación y eliminación de sustancias químicas, lo cual afecta a los tiempos de vida media biológicos, la persistencia y la carga corporal. En peces, la eliminación eficaz de sustancias químicas depende de su fracción libre en el plasma y ser excretados a través de branquias, el tracto gastrointestinal y los riñones. Las sustancias muy lipofílicas pueden no disolverse adecuadamente en el medio acuoso o pueden ser reabsorbidas por las células a través de procesos de retrodifusión desde los medios excretores como la orina, la bilis o las heces. El órgano con las mayores concentraciones de enzimas de biotransformación es el hígado (Kapoor et al., 1973).

Excreción: los peces eliminan sustancias principalmente a través de los riñones, el sistema hepatobiliar (excreción fecal) y las branquias. Los lugares de captación como las branquias pueden actuar como lugares de excreción, aprovechando la bidireccionalidad del flujo químico siempre que los gradientes de actividad (concentración o fugacidad) favorezcan la salida de sustancias al exterior (Tierney et al., 2013).

En la excreción renal, la filtración glomerular y la secreción tubular activa son procesos fundamentales. La filtración glomerular es el principal mecanismo para eliminar pequeños compuestos disueltos en el plasma, que son ultrafiltrados hacia los túbulos renales a través de los poros glomerulares. Los ácidos débiles pueden atravesar las células tubulares y ser excretados al atrapar iones en la orina. Se han detectado diversos compuestos y sus metabolitos en la orina de peces, como plaguicidas y PhACs (Christiansen et al., 1996).

La excreción hepatobiliar implica que los metabolitos producidos en el hígado, así como el compuesto original, pueden ser transportados por la sangre hacia los riñones o a la bilis para su expulsión en las heces. Algunos compuestos se almacenan en la vesícula biliar como un reservorio hasta que son liberados durante la digestión de alimentos, siendo finalmente expulsados del organismo en forma de materia fecal. En los peces, la excreción a través de la bilis probablemente representa la ruta más significativa de eliminación (Tierney et al., 2013).

La excreción branquial es regulada principalmente por la hidrofobicidad de las moléculas. Por ejemplo, los contaminantes hidrofílicos son excretados más eficientemente a través de las branquias. Sin embargo, los compuestos muy hidrofóbicos no se eliminan significativamente por este medio (Thomas & Rice, 1981). Las propiedades del medio acuático también influyen. Los compuestos orgánicos ionizables pueden ser excretados más eficazmente si el pH del agua facilita la difusión a través de las membranas branquiales (Erickson et al., 2006). Cuando la excreción branquial no es suficiente, los peces pueden recurrir a biotransformaciones para convertir las sustancias en metabolitos más solubles en agua, que son luego eliminados por el hígado y los riñones (Tierney et al., 2013).

1.2.2. Crustáceos de río

Absorción: la ingesta de alimentos puede considerarse la principal ruta de absorción de contaminantes por parte de los crustáceos, que se alimentan de detritos vegetales y animales, macrófitos y organismos vivos como moluscos, insectos, anélidos, nemátodos, gusanos, renacuajos y alevines (Anandkumar et al., 2020; Loureiro et al., 2015). El hábitat bentónico en el que se distribuyen algunas especies, junto con su dieta omnívora pueden llevar a la acumulación de sustancias químicas si los organismos en los niveles tróficos inferiores de la cadena alimentaria están contaminados con metales pesados, biotoxinas, u otro tipo de contaminantes (Bian, H. et al., 2023; Bian, J. et al., 2024). No obstante, también pueden absorber sustancias químicas a través de las branquias y transportarlas a otros órganos a través de la hemolinfa (Bian, H. et al., 2023).

El tipo y composición de las superficies corporales de las especies puede afectar las tasas de difusión de contaminantes por contacto directo con el agua (Boudou et al., 1991). Algunos estudios han demostrado que mecanismos genéticos, como la muda del exoesqueleto, permiten regular la absorción y acumulación de sustancias tóxicas en estas especies (Peng et al., 2024). Sin embargo, los cangrejos de río consumen como primer alimento la quitina, el principal constituyente de la cutícula desprendida durante la muda, debido a su alto contenido de calcio (Gu¨ & Vogt, 2018). De esta manera, los compuestos excretados o regulados durante la muda pueden ser reabsorbidos mediante la ingesta del exoesqueleto.

Distribución: en diversos estudios sobre la presencia de metales pesados en cangrejos de río, se ha observado una tendencia de acumulación predominante en el hepatopáncreas, seguida por la glándula antenal, las branquias, los tejidos del

exoesqueleto y, en menor medida, en el músculo abdominal. Esta distribución se debe a que el hepatopáncreas es un órgano metabólicamente muy activo, responsable de secuestrar contaminantes y contribuir al proceso de desintoxicación (Bian, J. et al., 2024; Goretti et al., 2016). Además, tiene la capacidad de concentrar metales pesados provenientes de la hemolinfa y el tracto digestivo, almacenándolos en vacuolas intracelulares. En consecuencia, los niveles más altos de bioacumulación se han observado en este órgano (Belanger et al., 2017).

Metabolismo: el hepatopáncreas tiene la función de desintoxicar compuestos, además de producir enzimas digestivas (Belanger et al., 2017). Este órgano, caracterizado por su alto contenido en grasas y proteínas, desempeña funciones fundamentales de metabolismo, inmunidad, respuesta al estrés y desintoxicación. La observación de la acumulación de compuestos en el hepatopáncreas sugiere un proceso de biotransformación similar al observado en peces. Por ejemplo, los PFAS de cadena larga sufren una degradación parcial en homólogos de cadena corta dentro del hepatopáncreas, mientras que los de cadena corta se metabolizan más rápidamente (Bian, J. et al., 2024).

Excreción: los crustáceos de río poseen un sistema excretor bien desarrollado, localizado en la parte anterior al corazón y equipado con una vejiga. Esta vejiga está conectada a un nefro-poro, situado justo debajo de los ojos, a través del cual se libera su contenido (Belanger et al., 2017). En cuanto a la eliminación de sustancias químicas tóxicas, los cangrejos de río pueden deshacerse de estos compuestos a través del proceso de muda. Se estima que las concentraciones de OPFRs en el músculo y el hepatopáncreas pueden reducirse a largo plazo durante su crecimiento, ya que estos compuestos son transferidos al exoesqueleto (Peng et al., 2024).

Se ha demostrado por ejemplo que el cangrejo de río tiene la capacidad de reducir la formación de tumores inducidos por el medio ambiente, gracias a su eficiente metabolismo y capacidad excretora. En el caso del carcinógeno benzo[a]pireno, el cangrejo de río puede acumular grandes cantidades en su hígado y hepatopáncreas, pero los metabolitos de fase I de este compuesto se convierten rápidamente en metabolitos de fase II, que son finalmente excretados por la orina. Esto reduce la acumulación de aductos de ADN que promueven la formación de tumores (Gu¨ & Vogt, 2018; Jewell et al., 1997). Esta capacidad podría estar relacionada con su alta tolerancia a estresores ambientales, lo que facilita su adaptación y supervivencia en zonas contaminadas.

1.2.3. Bivalvos de agua dulce

Absorción: los organismos bentónicos, y en particular los bivalvos filtradores, se distribuyen en la interfaz agua-sedimento de los ambientes acuáticos. Los bivalvos pueden filtrar grandes volúmenes de agua, procesando microalgas, bacterias y restos orgánicos, y eliminando partículas tanto de la columna de agua como de los sedimentos depositados. A través de los procesos de alimentación por filtración y alimentación por depósito, estos bivalvos pueden acumular contaminantes disueltos en el agua o desorbidos de la materia particulada. La ingesta de sedimentos y/o partículas es una de las principales vías por las que los contaminantes se acumulan en sus tejidos (Bjork, 1995; Fan et al., 2017; Ismail et al., 2014; Rong et al., 2021).

Las tasas de filtración varían en función de factores como la disponibilidad de alimento, el sexo, el ciclo reproductivo, el pH, la temperatura, la turbidez y la velocidad del agua (Hills et al., 2020). La absorción de contaminantes depende de si estos están disueltos libremente en el agua o asociados a partículas. Cuando los contaminantes están disueltos libremente y la concentración de partículas es baja, la principal vía de exposición es a través de las branquias. En cambio, cuando la concentración de partículas es alta, la tasa de bombeo disminuye, y la fracción de contaminantes asociada a partículas aumenta, estando mayoritariamente disponible para la absorción intestinal. Esto ocurre mediante difusión pasiva, impulsada por una mayor fugacidad en el tracto gastrointestinal (Bjork, 1995).

Para los bivalvos que se alimentan de sedimentos, se estima que la absorción a través del intestino a partir de sólidos ingeridos representa entre el 54 y el 63% de la absorción total (Bjork, 1995). El tamaño de las partículas desempeña un papel vital en la disponibilidad de alimento. En comparación con otros bivalvos, *C. fluminea*, por ejemplo, prefiere materiales más pequeños, lo cual está asociado con su tamaño corporal y las características estructurales de su aparato filtrador. El límite superior del tamaño de las partículas para la alimentación filtrante efectiva de *C. fluminea* es de aproximadamente 20-25 µm. Las partículas más grandes o las altas concentraciones de partículas facilitan la producción de pseudoheces, que reducen la tasa de filtración de la almeja asiática (Li et al., 2023).

Distribución: la acumulación de compuestos orgánicos e inorgánicos es diferenciada. Por ejemplo, se ha demostrado que la masa visceral, las branquias y el manto de *C. fluminea* son los principales tejidos de almacenamiento de metales, cuya distribución depende de la presencia de sitios de unión específicos en los tejidos. En

cambio, para algunos PhACs, la distribución está determinada por la partición entre el citosol y los lisosomas, que pueden secuestrar fácilmente compuestos con grupos amino y de basicidad débil (Li et al., 2023). Se ha demostrado que compuestos básicos como el propanolol, pueden experimentar una mayor bioacumulación debido a la atracción de la molécula cargada positivamente hacia los fosfolípidos de la membrana celular cargados negativamente. Esta interacción electroestática facilita la acumulación de propanolol en el tejido del bivalvo mediante procesos de sorción de membrana, independientes de la partición lipídica (Ismail et al., 2014).

Para compuestos lipofílicos como los hidrocarburos aromáticos policíclicos (PAHs del inglés *polycyclic aromatic hydrocarbons*), la cinética de acumulación en *C. fluminea* está relacionada con la desorción de partículas del sedimento. La absorción de PAHs asociados al sedimento es muy rápida durante las primeras 24 horas, alcanzando su máximo para los compuestos de bajo peso molecular; sin embargo, esta disminuye con el tiempo. La absorción logarítmica de PAHs está inversamente relacionada con el log K_{ow}. Los PAHs con valores de K_{ow} bajos o pesos moleculares bajos se vuelven biológicamente inasequibles más rápidamente y se excretan con mayor eficacia. En términos generales, la distribución de PAHs en tejidos de *C. fluminea* sigue la tendencia: branquias > masa visceral > músculo, mientras que los contaminantes orgánicos persistentes se acumulan mayoritariamente en el músculo, seguido de la masa visceral y, finalmente, las branquias (Guo & Feng, 2018).

Metabolismo: los bivalvos tienen sistemas enzimáticos menos activos para la biotransformación de contaminantes químicos que los peces, y a pesar de que los bivalvos acumulan contaminantes con facilidad, los metabolizan y eliminan más lentamente que los vertebrados (Johnson et al., 2013). Sin embargo, existen pocos datos que examinen la capacidad metabólica de los bivalvos para biotransformar contaminantes orgánicos. Los resultados disponibles indican que las enzimas presentes en las glándulas digestivas pueden ser las principales responsables de las posibles transformaciones (Ismail et al., 2014).

Excreción: el órgano más comúnmente utilizado por los bivalvos para la excreción es la glándula digestiva. Este órgano tiene un papel importante en la regulación y eliminación de xenobióticos y es el órgano principal del metabolismo y acumulación. Las células epiteliales que recubren los túbulos de la glándula digestiva son sensibles al efecto dañino de muchos contaminantes como los metales pesados (Guo & Feng, 2018).

Respecto a la eliminación de contaminantes de su organismo, los bivalvos realizan un proceso denominado depuración, que ocurre cuando se introducen en agua limpia. Este proceso consiste en liberar los contaminantes que absorbieron nuevamente a la columna de agua (Ismail et al., 2014). Los principales factores responsables de la tasa de depuración son el tipo de alimento, la temperatura, la biomasa tisular individual, la concentración de alimento disponible y las longitudes de la concha. Por ejemplo para individuos pequeños de *C. fluminea*, la tasa de aclaramiento es menor a 2 mL min^{-1} ind^{-1}, mientras que para los más grandes es de 6 mL min^{-1} ind^{-1} y varía entre 40 y 120 mL min^{-1} g^{-1} (Li et al., 2023).

La tasa de depuración también depende del contaminante. Por ejemplo, los mejillones *Mytilus* expuestos a PCBs y PAHs necesitan hasta dos meses para eliminar estos compuestos y alcanzar los niveles de fondo que presentan los mejillones no expuestos, mientras que el diazepam es eliminado en tres días (Pruell et al., 1986). Los bivalvos tienden a retener ciertos compuestos, como TCC y propranolol, en sus tejidos, liberándolos parcialmente al agua durante la depuración. Otros compuestos, como TDCPP y diurón, no se retienen; en su lugar, son excretados en pseudoheces durante la exposición inicial y también liberados durante la depuración (Ismail et al., 2014).

1.2.4. Distribución en biota por familia de contaminantes analizados

Respecto a las propiedades fisicoquímicas de los compuestos, el K_{ow} es una de las principales propiedades utilizadas para estimar la absorción y acumulación de compuestos orgánicos neutros (Meredith-Williams et al., 2012). Adicionalmente, el peso molecular y la lipofília de los compuestos, son propiedades que están inversamente relacionados con su solubilidad en agua, lo que afecta la cantidad de compuesto disponible para la absorción. Los compuestos con valores de log $K_{ow} \geq 3$ son potencialmente bioacumulables, ya que, a medida que aumenta su coeficiente de partición, se incrementa la acumulación en membranas y tejidos biológicos lipofílicos (Matthee et al., 2023). Adicionalmente, cada clase o familia de compuestos tiene propiedades intrínsecas que pueden favorecer su absorción y acumulación. A continuación, se discuten brevemente para las familias de CECs incluidas en la tesis.

Los **PhACs** son compuestos biológicamente activos que generalmente están diseñados para interactuar con órganos diana (humanos o animales). Si los organismos en el medioambiente poseen receptores similares al órgano diana

asociado a la acción terapéutica, el medicamento también puede afectarlos (Meredith-Williams et al., 2012). La absorción pasiva desde la fase acuosa a los organismos es impulsada por un gradiente de concentración, donde los compuestos neutros se difunden más rápido a través de las membranas lipídicas. Por eso el pH ambiental es crucial para determinar el estado de ionización del compuesto en el medioambiente, determinando su paso por la membrana. Del mismo modo, el pH de los fluidos corporales determina el equilibrio entre la forma neutra y cargada del compuesto dentro del organismo, lo cual afecta a su interacción con los órganos diana, particularmente para aquellos con valores de pKa entre 5 y 9 (Matthee et al., 2023).

Los **plaguicidas**, al igual que los PhACs, se absorben en los organismos acuáticos mediante la respiración y por vía oral (Bhat et al., 2022), pero principalmente por vía dérmica a través del tegumento (membrana que cubre el cuerpo del animal) (Pawan Kumar et al., 2023). Cada plaguicida tiene sus propiedades distintas, que determinan su riesgo potencial de manera específica (Bhat et al., 2022). Los insecticidas son considerados la clase de plaguicida más tóxica (Aktar et al., 2009), debido a que se han demostrado efectos inmediatos en organismos como los peces (Ray & Shaju, 2023). Los órganos de peces que experimentan los mayores efectos tóxicos de los plaguicidas son el hígado, los riñones y las branquias (Pawan Kumar et al., 2023).

Los **PFAS** comparten la característica de tener múltiples enlaces fluoro-carbono muy estables, lo que les hace particularmente persistentes y móviles. Los pKa de los PFAA de cadena larga oscila entre menos de cero y 1, lo que indica que están casi completamente ionizados a un pH ambiental y biológicamente relevante (Goss, 2008). A pesar de estar ionizados, los PFAS son biodisponibles y los PFAA de cadena larga pueden acumularse en medios biológicos específicos hasta niveles equivalentes a la acumulación de lípidos de los compuestos persistentes neutros.

Los PFAS de cadena larga son más propensos a adherirse a partículas de sedimento o materia orgánica y, tienen un gran potencial de bioacumulación, mientras que los de cadena corta suelen encontrarse en la fase acuosa del medio ambiente (Nayak et al., 2023). La ramificación o linealidad de su cadena y sus grupos funcionales pueden influir en su eliminación; por ejemplo, se ha demostrado que los isómeros lineales se eliminan más rápidamente que los isómeros ramificados (Benskin et al., 2009). Los fosfolípidos y las proteínas son cruciales para la distribución y acumulación de PFAS en los tejidos (De Silva et al., 2021) y tienden a tener una gran afinidad por la

albúmina sérica y las proteínas fijadoras de ácidos grasos (Shi et al., 2012).

En el caso de los **OPFRs,** también su estructura química es la que determina su modo de acumulación específico en peces (Bekele et al., 2018), pero, en general, su absorción es a través de las branquias y está relacionado con el contacto directo con partículas en suspensión en el agua. Una vez que están dentro del organismo, se distribuyen en tejidos metabólicamente activos en el caso de los peces, mientras que en las aves, por ejemplo, son más propensos a acumularse en tejidos inactivos y con mayor contenido de grasa (Yao et al., 2021).

Como se ha podido observar a lo largo de esta sección, existen diferentes mecanismos que favorecen la acumulación de CECs en especies acuáticas. Frente a esta situación, las autoridades ambientales han tomado algunas medidas con el objetivo de reducir la presión de este tipo de sustancias en ecosistemas acuáticos. En el siguiente apartado se abordan las de mayor relevancia.

1.3. Vigilancia de contaminantes de preocupación emergente en el medio ambiente

La mayoría de sustancias químicas consideradas como CECs no están reguladas ni sujetas a un seguimiento rutinario o a un régimen de control de emisiones (Dulio et al., 2018). Sin embargo, en la actualidad, principalmente en los países desarrollados, existen regulaciones ambientales que, con el ánimo de proteger los recursos hídricos han incluido algunos CECs. Por ejemplo, en Estados Unidos, se ha designado una lista de contaminantes candidatos que se centra específicamente en los contaminantes no regulados en el agua potable, conocida como la Lista de Candidatos a Contaminantes del Agua Potable (CCL del inglés *drinking water contaminant candidate list*). Por otro lado, la Unión Europea ha establecido la Directiva Marco del Agua (DMA) para todos los países miembros y se ha implementado el Reglamento sobre registro, evaluación, autorización y restricción de sustancias químicas (REACH del inglés *Registration, evaluation, authorization, and restriction of chemicals*) de la Agencia Europea de sustancias y mezclas químicas (ECHA del inglés *European chemicals agency*) (Feng et al., 2023). A continuación, se describen brevemente.

Lista de Candidatos a Contaminantes del Agua Potable: la CCL de agua potable en Estados Unidos incluye sustancias químicas que no están sujetas a normativas nacionales vigentes, pero que se sabe o se prevé que pueden estar presentes en los sistemas públicos de abastecimiento de agua. Estos contaminantes pueden necesitar

regulación futura según la Ley de Agua Potable Segura (SDWA del inglés *Safe Drinking Water Act*) (EPA, 2024b) por lo cual se requiere que la EPA publique una CCL cada cinco años. Para esto, se recopilan datos de las empresas de suministro de agua potable y se determina si un contaminante aparece con frecuencia y en concentraciones que justifiquen su análisis. A partir de la CCL, se deben seleccionar al menos cinco candidatos para su regulación. La primera CCL se publicó en marzo de 1998 (Richardson, 2007).

Hasta la fecha, se han elaborado cinco CCL y se han realizado cuatro determinaciones reglamentarias (DR). Tras la DR4, publicada en febrero de 2021, la EPA tomó decisiones finales para regular el PFOS y PFOA, en el agua potable. La CCL5 fue publicada en noviembre de 2022 y aún no tiene determinaciones reglamentarias. Incluye 66 sustancias químicas, entre las cuales se encuentran algunos de los CECs evaluados en esta tesis (EPA, 2024b) (Tabla 1.1).

Como resultado de la implementación continua de las CCL, el 26 de abril de 2024 se publicó el Reglamento Nacional sobre Agua Potable Primaria – PFAS (NPDWR del inglés *PFAS National primary drinking water regulation*). Esta normativa de la EPA establece los niveles máximos individuales de contaminantes (MCL del inglés *maximum contaminant levels*) para el PFOA, PFOS, PFHxS, PFNA y HFPO-DA. El reglamento entró en vigencia el 25 de junio de 2024 (EPA, 2024a).

Directiva Marco del Agua (Directiva 2000/60/CE): es una normativa esencial de la Unión Europea, adoptada el 23 de octubre de 2000, cuyo objetivo es proteger y mejorar la calidad del agua en todos los Estados miembros. Esta directiva establece un marco común para la gestión de las aguas superficiales y subterráneas con la finalidad de alcanzar un buen estado ecológico y químico en todas las masas de agua. Entre sus principales objetivos se encuentran la prevención y reducción de la contaminación, la promoción del uso sostenible del agua, la protección del medio acuático y la mejora del estado de los ecosistemas acuáticos. Para ello, la directiva propuso la implementación de un sistema de monitoreo regular del estado de las masas de agua y la eficacia de las medidas implementadas, además de introducir límites para ciertos contaminantes (CE, 2000).

Esta normativa también aborda la identificación y regulación de sustancias prioritarias que representan un riesgo significativo para el medio acuático. Para mantenerse actualizada, ha sido revisada varias veces, incorporando nuevos compuestos, entre los cuales se incluyen algunos CECs (Tabla 1.1). Hasta la fecha, se

han realizado cinco actualizaciones principales a la lista de sustancias prioritarias:

- Decisión n.º 2455/2001/CE: se adoptó la primera lista de sustancias prioritarias.
- Directiva 2008/105/CE: introdujo Normas de Calidad Ambiental (NCA) para sustancias prioritarias y otros contaminantes en aguas superficiales continentales y otras aguas superficiales. Establece valores límites para la concentración media anual de las sustancias, es decir, la máxima concentración permitida de la sustancia a lo largo de un año, así como una concentración máxima admisible, que representa la máxima concentración permitida en cualquier momento con el objetivo de evitar efectos tóxicos agudos.
- Directiva 2013/39/UE: actualizó la lista de sustancias prioritarias y sus NCA e incluyó limites en biota para algunos de los compuestos.
- Directiva (UE) 2018/840: incluyó nuevos compuestos a la lista de observación de contaminantes emergentes a efectos de seguimiento, estableció los métodos analíticos indicativos y los limites mínimos aceptables de detección del método.
- Decisión De Ejecución (UE) 2022/1307: actualizó la lista de vigilancia de sustancias en las aguas superficiales de la Unión Europea, y propone la realización de un monitoreo adecuado y actualizado.

Adicionalmente, como parte de la ejecución de la DMA, en el artículo 8 de la Directiva sobre Normas de Calidad Ambiental (Directiva 2008/105/CE) (CE, 2008), se estableció la denominada "Watchlist" de sustancias para las que deben recopilarse datos de seguimiento a escala de la UE a fin de proporcionar datos adecuados para su posible inclusión en la lista de sustancias prioritarias (Backhaus, 2023). La Comisión ha actualizado la lista de vigilancia en una serie de decisiones de ejecución, primero en 2015 (CE, 2015), luego en 2018 (CE, 2018), 2020 (EC, 2020) y nuevamente en 2022 (CE, 2022).

En virtud de la DMA, los países de la Unión han desarrollado sus propios programas y estrategias para cumplir con sus objetivos. En España, por ejemplo, se han implementado los Planes Hidrológicos de Cuenca y se han elaborado diversas normativas y directivas para restringir el uso de ciertos compuestos y establecer límites de concentración en el agua, enfocadas principalmente en proteger el agua destinada al consumo humano. El Real Decreto 140/2003 fija un valor paramétrico

de 0,1 µg L^{-1} como concentración máxima permisible para los plaguicidas que estén presentes en los suministros de agua potable y de 0,5 µg L^{-1} como concentración total de plaguicidas medidos por muestra (BOE, 2003).

Dentro de los compuestos evaluados en esta tesis, el grupo de los plaguicidas es el que presenta un marco normativo más desarrollado y criterios de calidad más específicos sobre su presencia en el agua (Tabla 1.1). Esto se debe principalmente a que sus efectos sobre el medio ambiente y la vida acuática se estudian desde hace más de seis décadas, intensificándose tras la publicación del libro "Silent Spring" de Rachel Carson en 1962.

En cuanto a la normativa relacionada con los límites establecidos para los OPFRs, en Estados Unidos varias organizaciones medio ambientales han presentado una petición a la EPA para realizar pruebas adicionales que permitan determinar si el TCEP, TClPP y TDClPP representan un riesgo significativo para la salud o el medio ambiente, debido a su posible toxicidad y persistencia (Pantelaki & Voutsa, 2019). Sin embargo actualmente la regulación existente solo se ha enfocado en el establecimiento de valores límite específicos para su presencia en juguetes destinados a niños menores de tres años (CE, 2014).

Criterios de calidad específicos para la vida acuática

Como se señaló anteriormente, el objetivo principal de las normativas y directivas que proponen límites específicos para algunos compuestos considerados CECs, es garantizar la calidad y seguridad del agua destinada al consumo humano. No obstante, dentro de este contexto, se han propuesto límites de concentración basados en estudios toxicológicos que establecen las concentraciones a partir de las cuales podrían presentarse efectos en organismos acuáticos.

En Estados Unidos el grupo de trabajo OW/ORD Emerging Contaminants elaboró un libro blanco titulado: "Aquatic life criteria for contaminants of emerging concern, Part I: general challenges and recommendations", para identificar y abordar los desafíos técnicos relacionados con la derivación de criterios de calidad del agua para proteger la vida acuática (EPA, 2008). Esto, junto con los avances logrados con la SDWA, ha permitido que la EPA elabore una tabla que presenta las concentraciones recomendadas para la protección de la vida acuática en agua dulce y salada (Xabadia et al., 2021). El objetivo es que los gobiernos estatales utilicen esta tabla como guía para desarrollar sus propios estándares (EPA, 2024c).

Tabla 1.1. *Directivas y listas de seguimiento en las que están incluidos algunos de los CECs objeto de estudio en esta tesis.*

Directiva o Lista de seguimiento	Compuestos	Niveles establecidos	
Decisión de ejecución (UE) 2455/2001/CE del Parlamento Europeo y del Consejo por la que se aprueba la lista de sustancias prioritarias en el ámbito de la política de aguas (CE, 2000)	Alacloro, atrazina, diurón, isoproturon, simazina clorfenvinfos, clorpirifos	No aplica	
		NCA-MA ($\mu g\ L^{-1}$)	**NCA-CMA ($\mu g\ L^{-1}$)**
Directiva 2008/105/CE del Parlamento Europeo y del Consejo, relativa a las normas de calidad ambiental en el ámbito de la política de aguas (CE, 2008)	Alacloro	0.3	0.7
	Atrazina	0.6	2.0
	Diurón	0.2	1.8
	Isoproturon	0.3	1.0
	Simazina	1.0	4.0
	Clorfenvinfos	0.1	0.3
	Clorpirifos	0.03	0.1
Directiva 2013/39/UE del Parlamento Europeo y del Consejo por la que se modifican las Directivas 2000/60/CE y 2008/105/CE en cuanto a las sustancias prioritarias en el ámbito de la política de aguas (CE, 2013)	Terbutrina	0.065	0.34
	PFOS Biota 9.1 µg Kg^{-1}	$6.5x10^{-4}$	36
Directiva 2014/79/UE de la Comisión por la que se modifica, en lo que respecta al TCEP, el TCPP y el TDCP relativa a la seguridad de los juguetes (CE, 2014)	TCEP	5 µg Kg^{-1}	
Incluidos en la Watchlist mediante la Decisión de ejecución (UE) 2015/495 (CE, 2015)	Diclofenaco, claritromicina, Eritromicina, acetamiprida, clotianidina, imidacloprid, metiocarb, tiometoxam	No aplica	
Incluidos en la Watchlist mediante la Decisión de ejecución (UE) 2018/840 (CE, 2018)	Claritromicina, ciprofloxacina acetamiprida, imidacloprid, metiocarb,	No aplica	
Incluidos en la Watchlist mediante la Decisión de ejecución (UE) 2020/1161 (CE, 2020)	Venlafaxina, trimetoprima, sulfametoxazol, O-desmetilvenlafaxina, fluconazol, imazalil, procloraz, tebuconazol	No aplica	
Incluidos en la Watchlist mediante la Decisión de ejecución (UE) 2022/1307 (CE, 2022)	Venlafaxina, trimetoprima, sulfametoxazol, metformina, o-desmetilvenlafaxina, fipronil, fluconazol	No aplica	
Incluidos en la CCL 4 de la EPA (EPA, 2024b)	PFOA, PFOS	No aplica	
Incluidos en la CCL5 de la EPA (EPA, 2024b)	O-desmetilvenlafaxina, fipronil, fluconazol carbendazima, tebuconazol, atrazina-deetilo, atrazina-deisoproprilo, diurón, propanil, propazina clorpirifos, diazinón, dimetoato, TnBP, TCEP, bisfenol A	No aplica	
		CMC ($\mu g\ L^{-1}$)	**CCC ($\mu g\ L^{-1}$)**
Criterio de calidad para la vida acuática de agua dulce recomendado por la EPA (EPA, 2024b)	Clorpirifos	0.083	0.041
	Diazinón	0.17	0.17
	Paratión	0.065	0.013
		MCL (ng L^{-1})	
PFAS National Primary Drinking Water Regulation de la EPA (EPA, 2024a)	PFNA	10	
	PFHxS	10	
	PFOS	4.0	
Convenio de Estocolmo	PFOS, PFOA	No aplica	

NCA-MA (Norma de calidad ambiental – Media anual); NCA-CMA (Norma de calidad ambiental – Concentración máxima admisible) para aguas continentales; CMC (Concentración máxima criterio (aguda)); CCC (Concentración Continua Criterial (crónica)) para agua dulce.; MCL (Niveles máximos de contaminantes).

En Europa, durante el 2005, la Comisión Europea financió el proyecto NORMAN con el objetivo de facilitar un intercambio de datos más rápido y de mayor alcance sobre la aparición y los efectos de los CECs en el medio ambiente y mejorar la validación y armonización de métodos comunes de muestreo y medición. NORMAN se puede considerar como en una red permanente de grupos de trabajo y de laboratorios de referencia y centros de investigación, en el que colaboran sinérgicamente el mundo académico, la industria, los organismos de normalización y las ONG (Dulio et al., 2018).

Dentro de las actividades que realiza la red, se encuentra la recopilación y evaluación de estudios de ecotoxicidad para la derivación y armonización de los niveles umbral de concentración prevista sin efecto (PNECs del inglés *Predicted No-effect concentration*) (Dulio et al., 2018). Actualmente la base de datos de eco-toxicología NORMAN recopila los PNECs para más de 90.000 sustancias en agua dulce, agua marina, sedimentos, peces y moluscos tanto de agua dulce como marinos (NORMAN, 2024). En la Tabla 1.2. se muestran los PNECs de los compuestos incluidos en esta tesis.

Tabla 1.2. *Valores PNEC más bajos para los compuestos analizados en esta tesis en agua superficial, sedimentos, peces y moluscos de agua dulce, de acuerdo con la base de datos de ecotoxicología de la NORMAN.*

Categoría	Compuestos	Número CAS	Agua superficial (µg L^{-1})	Sedimento (µg Kg^{-1}) d.w.	Peces (µg Kg^{-1}) w.w.	Molusco (µg Kg^{-1}) w.w.
Antidepresivos/ ansiolíticos/ sedantes/ hipnóticos	Alprazolam	2898-97-7	0.077	2.39	21.4	5.34
	Bromazepam	1812-30-2	0.59	12.5	8.26	2.07
	Citalopram	59729-33-8	16	1923	1645	411
	Clozapina	5786-21-0	0.18	5.02	389	97.2
	Diazepam	439-14-5	0.29	11.6	14.2	3.54
	Fluoxetina	54910-89-3	0.1	21.2	37.8	9.45
	Lorazepam	846-49-1	0.096	7.41	8.95	2.24
	Midazolam	59467-70-8	0.12	231	17.5	4.38
	Oxazepam	604-75-1	0.37	7.19	19.9	4.97
	Paroxetina	61869-08-7	20	1882	3019	755
	Pentobarbital	76-74-4	49.5	392	117	42.1
	Quetiapina	111974-69-7	10	573	102	25.5
	Sertralina	79617-96-2	0.0094	2.08	1.32	0.33
	Temazepam	846-50-4	0.071	4.07	4.12	1.03
	Venlafaxina	93413-69-5	0.88	29.8	87.8	22
	Zolpidem	82626-48-0	0.18	18	3.48	0.87
Analgésicos/anti-inflamatorios	Acetaminofén	103-90-2	46	182	306	76.4
	Codeína	76-57-3	7.19	69580	453	113
	Diclofenaco	5307-86-5	0.05	14.3	27.8	6.94
	Ácido flufenámico	30-78-9	0.4	35.6	4.65	1.16
	Ibuprofeno	31121-93-4	1.14	9.63	239	59.8
	Ketoprofeno	22071-15-4	2.1	75.4	15.1	3.78
	Ácido mefenámico	61-68-7	1	36.4	15.1	3.78
	Propifenazona	479-92-5	0.8	28.3	20.2	5.04
	Ácido salicílico	69-72-7	18	62	22500	5625
Antibacteriales	Claritromicina	81103-11-9	0.12	269	0.47	0.12
	Irgasan - Triclosán	3380-34-5	0.11	45.9	1170	293
	Oxitetraciclina	79-57-2	10	16.6	4.03	1.01
	Triclocarbán	101-20-2	0.0011	0.23	0.37	0.093
	Trimetoprima	738-70-5	120	872	3665	916
Antibióticos	Cloranfenicol	56-75-7	2.73	19.9	3.6	0.9
	Ciprofloxacina	85721-33-1	0.089	1.49	0.62	0.15
	Eritromicina	114-07-8	0.3	657	1.01	0.25
	Flumequina	42835-25-6	1.5	74.9	9.12	2.28
	Furazolidona	67-45-8	2.5	15	5.88	1.47
	Ácido nalidíxico	389-08-2	4.66	55.4	11.9	2.98
	Sulfadimetoxina	122-11-2	1.21	5.07	2.44	0.61
	Sulfametoxazina	57-68-1	30	260	62.7	15.7
	Sulfametoxazol	723-46-6	0.6	3.67	5.95	1.49
	Sulfapiridina	144-83-2	0.46	4.13	1.15	0.29
Anticonvulsivo	Acridona	578-95-0	-	-	-	-
	Carbamazepina	298-46-4	2	56.9	19.8	4.96
	Lamotrigina	84057-84-1	8	83.5	212	53
Antidiabéticos	Metformina	657-24-9	160	331	311	77.7
	Sitagliptina	486460-32-6	84	1391	1232	308
Anti-hipertensivos	Diltiazem	34933-06-7	0.23	17.3	1.91	0.48
	Losartán	114798-26-4	78	52103	1552	388
	Telmisartán	144701-48-4	49	311142	2068	517
	Valsartán	137862-53-4	560	0	1064	266
Antivirales	Amantadina	768-94-5	25	2781	1506	376
	Oseltamivir	196618-13-0	13	50.4	27.1	6.78
Diuréticos	Furosemida	54-31-9	31.3	98.6	132	33
	Hidroclorotiazida	58-93-5	100	420	135	33.8
Reguladores lipídicos	Atorvastatina	134523-00-5	8.5	101500	649	162
	Bezafibrato	41859-67-0	2.3	1586	13.6	3.4
	Ácido clofíbrico	882-09-7	16.5	97	69.1	17.3
PhAC Metabolitos/ Productos de transformación	Benzoilecgonina	519-09-5	-	-	-	-
	CBZ-10,11-epóxido	36507-30-9	2.57	116	9.68	2.42
	Cocaetileno	529-38-4	1.55	148	17.6	4.4
	Cotinina	486-56-6	10	55.3	27.6	6.9
	Fipronil desulfinilo	205650-65-3	0.031	2.92	30.4	7.59
	Fipronil sulfuro	120067-83-6	0.012	0.33	17	4.25
	Fipronil sulfona	120068-36-2	0.013	0.13	0.36	0.091
	N-acetilsulfametoxazol	18607-98-2	4.9	65.7	15.9	3.96
	N-desmetilcitalopram	62498-67-3	0.5	24	9.2	2.3
	Norfluoxetina	83891-03-6	1.7	565	227	56.7
	O-desmetilvenlafaxina	93413-62-8	0.88	13.5	39.5	9.86
	Oseltamivir carboxilato	No disponible	-	-	-	-
	Ácido valsartán	164265-78-5	320	1180	1032	258

Tabla 1.2. *Continuación.*

Categoría	Compuestos	Número CAS	Agua superficial (µg L^{-1})	Sedimento (µg Kg^{-1}) d.w.	Peces (µg Kg^{-1}) w.w.	Molusco (µg Kg^{-1}) w.w.
Opioides	Metadona	76-99-3	0.59	87.7	125	31.2
	Morfina	57-27-2	5.38	54991	333	83.3
Estimulantes	Cafeína	58-08-2	1.2	5.61	1.92	0.48
	Cocaína	50-36-2	2.46	143	17.2	4.29
	Nicotina	54-11-5	5.45	36.3	46.9	11.7
Agentes bloqueadores β	Atenolol	29122-68-7	150	2480	15.8	3.94
	Bisoprolol	66722-44-9	92	17850	2900	725
	Metoprolol	37350-58-6	8.6	557	215	53.9
	Sotalol	3930-20-9	6.52	41.7	15.5	3.89
	Tramadol	27203-92-5	8.65	256	515	129
Otros PhACs	Carazolol	57775-29-8	0.27	19.7	2.81	0.7
	Fipronil	120068-37-3	0.00077	0.017	0.019	0.0047
	Fluconazol	86386-73-4	1.04	4.32	14.5	3.62
	Loratadina	79794-75-5	5.3	1127	404	101
	Omeprazol	73590-58-6	18.1	861	268	67
	Ondansetrón	99614-02-5	0.99	28.4	24.4	6.09
	Salbutamol	18559-94-9	1000	6863	4822	1205
	Verapamilo	52-53-9	2.53	19670	504	126
	Warfarina	81-81-2	1.2	56	26.3	6.59
Fungicidas	Carbendazima	10605-21-7	0.44	5.57	26.9	6.72
	Imazalil	35554-44-0	0.87	232	189	47.1
	Procloraz	67747-09-5	1.56	0.3	0.4	0.1
	Tebuconazol	107534-96-3	0.24	5.48	9.31	2.33
	Tiabendazol	148-79-8	1.2	105	20.3	5.07
Herbicidas	Alacloro	15972-60-8	0.3	3.3	10.5	2.62
	Atrazina	1912-24-9	0.6	0.2	1527	382
	Diurón	330-54-1	0.07	0.98	7.89	1.97
	Isoproturón	34123-59-6	0.3	1.97	31.4	7.85
	Metoalacloro	51218-45-2	0.2	3.17	8.12	2.03
	Molinato	2212-67-1	3.8	21.7	82.7	20.7
	Propanil	709-98-8	0.2	1.78	16.6	4.15
	Propazina	139-40-2	0.18	2.52	3.33	0.83
	Simazina	122-34-9	1	7.82	0.05	0.013
	Terbumeton	33693-04-8	1.7	34.9	11.8	2.95
	Terbutilazina	5915-41-3	0.22	2.62	1.28	0.32
	Terbutrina	886-50-0	0.065	2.38	2.41	0.6
Insecticidas	Acetamiprida	135410-20-7	0.024	0.18	0.46	0.11
	Azinfos-etil	2642-71-9	0.0011	0.022	0.016	0.0039
	Azinfos-metil	86-50-0	0.0034	0.037	0.25	0.063
	Buprofezina	69327-76-0	0.99	72.4	116	29.1
	Carbofurano	1563-66-2	0.016	0.07	0.75	0.19
	Clorfenvinfos	470-90-6	0.1	1.62	25.5	6.38
	Clorpirifos	2921-88-2	0.00046	0.11	9.51	2.38
	Clotianidina	210880-92-5	0.007	0.013	0.043	0.011
	Cumafós	56-72-4	0.0007	0.04	0.3	0.075
	Diazinón	333-41-5	0.01	0.29	1.61	0.4
	Diclofentión	97-17-6	0.0041	0.39	5.37	1.34
	Dimetoato	60-51-5	0.07	0.17	0.024	0.0059
	Etión	563-12-2	0.00048	0.27	0.31	0.077
	Etofenprox	80844-07-1	0.00054	0.85	2.11	0.53
	Fentión	55-38-9	0.004	0.31	1.01	0.25
	Hexitiazox	78587-05-0	0.025	7.65	15.5	3.87
	Imidacloprid	138261-41-3	0.013	0.042	0.024	0.006
	Metiocarb	2032-65-7	0.002	0.024	0.043	0.011
	Ometoato	1113-02-6	0.004	0.0084	0.0031	0.00077
	Paratión-etilo	56-38-2	0.005	0.4	0.99	0.25
	Piriproxifeno	95737-68-1	0.0015	0.65	1.34	0.34
	Spinosad A	131929-60-7	0.0027	11.9	0.029	0.0072
	Spinosad D	131929-63-0	0.062	267	1.7	0.42
	Tiametoxam	153719-23-4	0.042	0.12	0.29	0.071
Plaguicidas Metabolitos/ Productos de transformación	Atrazina-deetil	6190-65-4	0.26	1.66	1.76	0.44
	Atrazina-Deisopropril	1007-28-9	0.39	1.62	2.42	0.61
	Carbofurano 3-OH	16655-82-6	4.3	56.5	50	12.5
	Fentión sulfona	3761-42-0	0.0095	0.1	0.025	0.0064
	Fentión sulfóxido	3761-41-9	0.24	7.21	0.35	0.088
	Terbumetón desetílico	30125-64-5	0.065	0.64	0.16	0.039
	Terbutilazina-2OH	66753-07-9	0.0073	0.03	0.018	0.0044
	Terbutilazina-desetil	30125-63-4	0.25	2.14	2.18	0.55
Síntesis de plaguicidas	DMA	95-68-1	8.44	41.9	47.9	12
	DMF	68-12-2	22800	37856	18111	4528

Tabla 1.2. Continuación.

Categoría	Compuestos	Número CAS	Agua superficial (µg L^{-1})	Sedimento (µg Kg^{-1}) d.w.	Peces (µg Kg^{-1}) w.w.	Molusco (µg Kg^{-1}) w.w.
PFAS	DMPF	33089-74-6	10.3	78.4	69.6	17.4
	PFUnDA	2058-94-8	0.13	103	223	55.9
	PFDA	335-76-2	0.17	3.51	8.15	2.04
	PFNA	375-95-1	1	142	165	41.3
	PFHxS	82382-12-5	0.15	17.2	0.88	0.22
	PFOA	335-67-1	0.18	6.04	0.41	0.1
	PFOS	4021-47-0	0.07	1.34	51.7	12.9
	PFHpA	375-85-9	0.5	53.5	46.6	11.6
	PFBS	29420-49-3	0.45	2.63	19.6	4.89
	PFHxA	307-24-4	140	7602	6901	1725
	PFBA	375-22-4	27.8	166	185	46.3
	ipPFNS	No disponible	-	-	-	-
	ipPFNA	15899-31-7	0.19	35.1	21.7	5.41
	PFDS	335-77-3	0.16	154	212	52.9
	PFDoDA	307-55-1	0.11	485	1494	373
	PFHxDA	67905-19-5	0.078	583	369	92.3
	PFODA	16517-11-6	0.069	453	25.8	6.44
	PFPeA	2706-90-3	3.91	24.8	110	27.5
	PFTeDA	376-06-7	0.083	954	1366	341
	PFTrDA	72629-94-8	0.1	940	41	10.2
	PFHpS	375-92-8	0.48	30	218	54.4
OPFRs	TMPP	1330-78-5	0.16	0	26.9	6.72
	TEHP	78-42-2	0.039	24.3	1.93	0.48
	TPhP	115-86-6	0.17	14.8	465	116
	TnBP	126-73-8	37	542	1224	306
	TBEP	78-51-3	24	842	138	34.5
	TDCIPP	13674-87-8	1.1	20.5	13.2	3.31
	TDBPP	126-72-7	0.14	17.1	67.7	16.9
	CDP	26444-49-5	0.064	0	0	0
	TCEP	115-96-8	65	151	83.7	20.9
	TPP	513-08-6	2.32	22.8	13.1	3.27
	TCIPP	13674-84-5	260	11.1	2065	516
Otros compuestos de uso industrial	5-metil-1H-Benzotriazol	136-85-6	150	705	915	229
	1H-Benzotriazol	95-14-7	19	76.4	109	27.2
	Bisfenol A	80-05-7	0.24	15.1	13.1	3.28
	Bisfenol F	620-92-8	5.44	179	232	58
	Bisfenol S	46765-03-1	11.4	185	36.2	9.06

1.4. Seguimiento de la contaminación ambiental a partir de especies acuáticas

Con el objetivo de evaluar la presión ambiental como consecuencia de la presencia de CECs en los ecosistemas de agua dulce, el biomonitoreo de la fauna acuática se ha utilizado ampliamente. Esto se debe a su capacidad para absorber y acumular sustancias químicas como se presentó en la sección 1.2. El análisis de los CECs en el medio acuático tiene varios propósitos, entre los cuales se incluyen el estudio de la presencia de contaminantes en el medio ambiente y la identificación del riesgo que estos representan para la biota (Álvarez-Ruiz & Picó, 2020).

En la Fig. 1.5 (A) se muestran las especies más ampliamente utilizadas para detectar la presencia de CECs en ecosistemas acuáticos. Los peces y los bivalvos son los grupos de especies más analizados. El uso de bivalvos es generalizado debido a su naturaleza sedentaria (Álvarez-Ruiz & Picó, 2020; Berthet, 2015), mientras que los peces son preferidos por su relevancia como producto alimenticio y por su facilidad de captura.

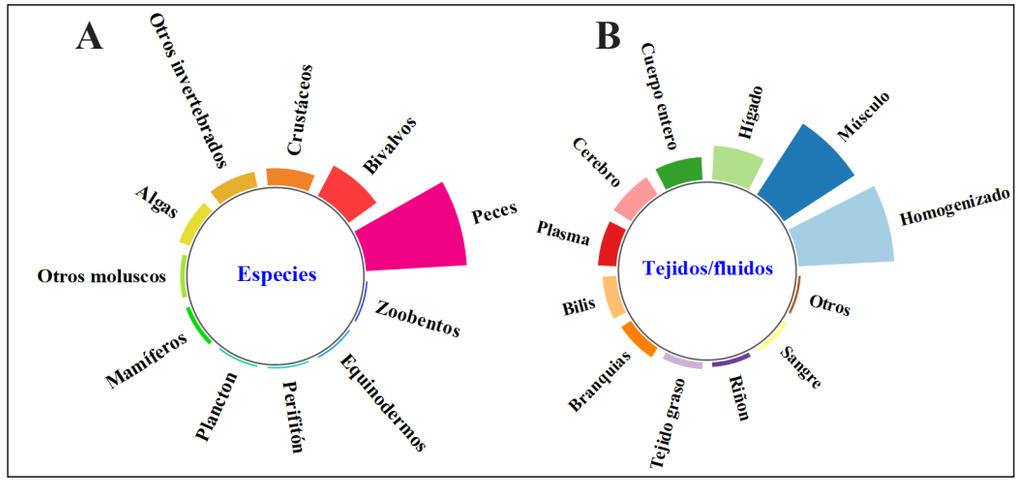


Fig. 1.5. A. *Especies acuáticas más usadas en estudios de biomonitoreo de CECs.* ***B.*** *Tejidos y fluidos corporales de especies acuáticas que se analizan con mayor frecuencia. Las gráficas muestran los resultados basados en 93 estudios revisados por Álvarez-Ruiz & Picó., (2020)*

En la mayoría de los estudios de biomonitoreo, se analiza el cuerpo completo de la especie seleccionada, por lo que los datos reportados provienen mayoritariamente de muestras catalogadas como homogenizadas (Fig. 1.5 (B)) (Álvarez-Ruiz & Picó, 2020). Debido a que los contaminantes pueden provocar efectos directos al interactuar con compartimentos biológicos específicos del organismo (Rubach et al., 2011), el análisis multi-órgano proporciona mayor información para determinar el factor de bioacumulación (BAF del inglés *bioaccumulation factor*) específico de cada compuesto y los riesgos asociados con su presencia en diferentes partes del cuerpo. Este enfoque ha sido aplicado en peces debido a la facilidad de disección, siendo el músculo el órgano más analizado por su relación directa con el consumo de filetes en la dieta humana (Matthee et al., 2023; Miller et al., 2018). Esta línea de investigación se desarrolla en mayor detalle en el capítulo 4 a partir del análisis de peces salvajes.

Aunque el biomonitoreo de CECs se ha realizado principalmente con organismos acuáticos locales de los ecosistemas a evaluar, el análisis de especies invasoras también podría revelar la presencia de contaminantes que no se detectan comúnmente. Esto se debe a que estas especies tienden a tener una mayor tolerancia a la contaminación y, por ende, podrían acumular niveles más altos de contaminantes en sus organismos sin provocar mortalidad aguda. Sin embargo, los estudios de biomonitoreo de CECs que emplean este enfoque aún son limitados. A continuación, se amplía la información sobre las características de las especies invasoras, resaltando su importancia para llevar a cabo estudios de biomonitoreo de

CECs.

Definición: en primera medida, es importante comprender la problemática ambiental de las especies acuáticas invasoras, y para esto, es fundamental tener claras tres definiciones: i) Especie autóctona: especies nativas que han evolucionado en el entorno local o que han llegado allí por medios naturales, sin intervención humana (Corrales et al., 2020; Havel et al., 2015). ii) Especie exótica: se refiere a una especie que ha cruzado barreras geográficas, desde su área de distribución natural e introducida en un nuevo entorno y iii) Especie invasora: se trata de una especie exótica que no solo logra persistir en su nuevo entorno, sino que también se reproduce y expande su distribución, adaptándose con éxito al nuevo ecosistema (Corrales et al., 2020; Havel et al., 2015). Las especies exóticas se convierten en invasoras si presentan madurez sexual temprana, alta fecundidad, falta de depredadores y amplia tolerancia ambiental, representando una amenaza, tanto a nivel ecológico como económico y social (Corrales et al., 2020; Darrigran et al., 2002; Gallardo et al., 2016).

Causas: las actividades humanas son los principales impulsores de la propagación y establecimiento de especies invasoras, rompiendo las barreras biogeográficas a través del comercio de larga distancia e introduciendo intencionalmente algunas especies o transportando otras de manera accidental (Havel et al., 2015). La rápida globalización y el incremento del comercio y transporte en las últimas décadas han acelerado las invasiones biológicas (Corrales et al., 2020). Entre las actividades que facilitan la propagación de especies exóticas se encuentran la repoblación con especies acuáticas, el comercio y almacenamiento de peces para pesca recreativa (Rahel, 2002), el uso de especies exóticas como cebos de pesca, el comercio de acuarios en el que se incluyen variedad de peces, invertebrados y plantas acuáticas, y la liberación no controlada de especies en ecosistemas inapropiados, generalmente por personas sin conocimiento (Padilla & Williams, 2004). Asimismo, los grandes buques que transportan agua de lastre juegan un papel significativo, ya que se ha documentado que pueden transportar cientos de especies acuáticas. Se estima que alrededor del 75% de las especies introducidas en los Grandes Lagos Laurentinos (Estados Unidos) desde 1970 han llegado a través de esta vía (Havel et al., 2015).

Si al transporte directo de especies se le suman además las interacciones con otro tipo de estresores ambientales como el cambio climático, la degradación y pérdida del hábitat, la sobreexplotación y la contaminación, el éxito de invasión por parte de las especies exóticas pude verse facilitado y comprometer la integridad de los

ecosistemas (Dudgeon et al., 2006). Esto se debe a que al verse alterados los hábitats acuáticos, se reduce la riqueza de especies y, por lo tanto, la resistencia biótica a la invasión (Havel et al., 2015). Esta situación, ha provocado que en España hayan declaradas 182 especies exóticas invasoras, dentro de las cuales 21 corresponden a especies de peces y 12 a crustáceos (MITECO, 2024).

Impacto: las especies invasoras pueden causar impactos en el medio ambiente por la interacción biótica directa o por interacción abiótica indirecta. Las interacciones con las comunidades bióticas autóctonas pueden generar competencia o depredación, disminuyendo la diversidad y abundancia de las especies residentes. La interacción indirecta produce cambios en la estructura del hábitat. Los hábitats invadidos pueden presentar un aumento de la turbidez del agua, la concentración de nitrógeno y materia orgánica, que están relacionados con la capacidad de los invasores para aumentar la eutrofización. Especies invasoras como algunos tipos de bivalvos pueden agotar las comunidades planctónicas y disminuir la abundancia de macrófitos e invertebrados bentónicos debido a su capacidad filtradora (Gallardo et al., 2016).

La disminución de la diversidad de especies afecta a todos los niveles tróficos, y se ha demostrado que una menor diversidad reduce la estabilidad de la red trófica. Incluso pequeñas disminuciones en la abundancia de especies nativas, podrían conducir a extinciones funcionales y dar como resultado la reorganización de la red alimentaria (Gallardo et al., 2016; Rooney & McCann, 2012).

Los ecosistemas de agua dulce tienen una mayor biodiversidad por superficie que los ecosistemas marinos y terrestres, y desempeñan un papel activo en el ciclo de nutrientes y del agua (Havel et al., 2015). Dada su complejidad, estudiar el impacto de las especies invasoras en estos ecosistemas es un reto debido a: i) la naturaleza dinámica y la alta conectividad de los ecosistemas acuáticos, en los que las barreras hidrológicas funcionan a escalas espaciales mucho mayores que en los ecosistemas terrestres, y ii) las limitaciones de las herramientas metodológicas, que incluyen incertidumbres en los estudios de campo derivadas de la baja detectabilidad de especies y la limitada disponibilidad de datos sobre la distribución de hábitats y especies (Corrales et al., 2020).

Además de los impactos ecológicos, las invasiones biológicas pueden causar impactos económicos. Un estudio realizado en ecosistemas acuáticos de Estados Unidos estimó un costo anual aproximado de 7,7 mil millones de dólares asociados

con los daños causados por especies invasoras y su control. Estos costos están asociados principalmente con peces introducidos (5,4 mil millones), moluscos (2,2 mil millones) y malezas acuáticas (0,1 mil millones) (Pimentel et al., 2005).

Tolerancia a la contaminación ambiental: además de los impactos ecológicos que generan las especies invasoras en los ecosistemas de agua dulce, estos hábitats también son vulnerables a la contaminación orgánica e inorgánica, alteraciones en el ciclo hidrológico y los efectos del cambio climático. Se ha observado que las especies invasoras tienen una notable capacidad para aclimatarse rápidamente a condiciones fluctuantes y poseen una amplia tolerancia al estrés ambiental. Por ello, es probable que las especies invasoras sean más resistentes en entornos contaminados en comparación con las especies nativas de los ecosistemas invadidos (Bielen et al., 2016; Havel et al., 2015).

Las especies invasoras están reemplazando cada vez más a las especies nativas, especialmente en hábitats transformados o afectados por actividades antrópicas, lo que abre la posibilidad de utilizar estas especies para el biomonitoreo de la contaminación. Por ejemplo, peces invasores como el *guppy* o pez misionero (*Poecilia reticulata*) han reemplazado a las especies autóctonas en áreas altamente contaminadas, demostrando una mayor tolerancia a la contaminación (Gomes-Silva et al., 2020).

Varios estudios han demostrado que las especies autóctonas son más sensibles a ambientes contaminados que las invasoras. Karatayev et al., (2009) observaron que más del 35% de las especies nativas de invertebrados acuáticos en América del Norte solo pueden vivir en áreas con excelente calidad de agua y son intolerantes a la contaminación orgánica. En contraste, las especies invasoras parecen tolerar concentraciones moderadas de contaminantes orgánicos.

En un experimento de exposición que se llevó a cabo en un dique de la Bahía de San Francisco (Estados Unidos) se demostró que tras tres días de exposición a cobre a través de placas incrustantes, la estructura de la comunidad y la riqueza de especies autóctonas descendió en un 40% al aumentar las concentraciones, mientras que el conjunto de especies invasoras no cambió significativamente (Crooks et al., 2011).

Tras la exposición de especies de bivalvos nativos (*Anodontites trapesialis*) e invasores (*Limnoperna fortunei*) a dos tipos diferentes de estresantes ambientales

como el cobre y el herbicida glifosato, El Haj et al., (2019) observaron que en las especies exóticas el cobre y el herbicida empezaron a generar efectos citotóxicos a concentraciones superiores a 9000 µg L^{-1} y 5000 µg L^{-1} respectivamente, mientras que en las especies nativas fue a partir de 900 µg L^{-1} y 50 µg L^{-1}. Esto supone que las especies invasoras podrían bioacumular concentraciones más altas de contaminantes en comparación con las especies nativas, debido a que el umbral a partir del cual empiezan a presentarse efectos es hasta 100 veces mayor.

Características como un crecimiento rápido y la alta fecundidad permiten a los bivalvos invasores, como *C. fluminea,* recuperarse rápidamente tras disturbios, facilitando su éxito en hábitats inestables y contaminados. Esto los convierte en colonizadores efectivos en ecosistemas sujetos a interferencias humanas como el dragado, la canalización y la contaminación (Havel et al., 2015; McMahon, 2002).

En un estudio sobre las concentraciones relativas de metales pesados en macro-decápodos de agua dulce en Italia, se detectó que *P. clarkii* mostró una acumulación significativamente más alta para la mayoría de los metales analizados, que el cangrejo de río de pinzas blancas autóctono (*Austropotamobius pallipes)*, y el cangrejo de río *Potamon fluviatile* (Souty-Grosset et al., 2016). Para realizar estudios de biomonitoreo utilizando organismos biológicos, es esencial desarrollar métodos analíticos que permitan obtener datos confiables sobre las concentraciones de estos contaminantes. En el siguiente apartado se proporciona información detallada sobre este tema.

1.5. Métodos analíticos para la detección de contaminantes de preocupación emergente en matrices biológicas

El análisis de CECs en biota acuática ha representado un desafío en química analítica debido a que son matrices ambientales complejas, al amplio rango de propiedades fisicoquímicas en el que se encuentran los compuestos de interés y, a las bajas concentraciones a las que se pueden detectar los analitos (Sandoval et al., 2024). Las matrices biológicas tienen un alto contenido de proteínas y lípidos que pueden ser extraídos con solventes orgánicos y aumentar o disminuir la señal analítica de los contaminantes de interés (Álvarez-Ruiz & Picó, 2020).

La co-extracción de compuestos biológicos naturales no objetivo en matrices biológicas puede afectar negativamente la separación y resolución de los analitos de interés mediante cromatografía de líquidos. Algunos efectos no deseados incluyen la

co-elución con otros contaminantes y la degradación de la columna cromatográfica debido a la presencia de lípidos en los extractos. Además, los componentes de la matriz presentes en los extractos inyectados podrían provocar cambios en el tiempo de retención y el ensanchamiento de los picos (Sørensen et al., 2015).

Para superar estas barreras, los métodos analíticos abordan principalmente cuatro pasos que permiten la obtención de datos fiables: i) pretratamiento de la muestra, ii) método de extracción más apropiado de acuerdo a las propiedades fisicoquímicas de los compuestos de interés, iii) limpieza o purificación de los extractos para reducir las interferencias y, iv) separación cromatográfica y análisis instrumental (Sandoval et al., 2024). A continuación, se describen brevemente cada uno de los pasos.

1.5.1. Pretratamiento de muestras

El principal objetivo del pretratamiento de las muestras es evitar su deterioro o las pérdidas de analitos debido a la degradación (Pareja et al., 2022). Para esto se deben diseñar estrategias de muestreo y condiciones de conservación y almacenamiento adecuadas. Es necesario conservar las muestras sólo en recipientes controlados y limpios, y su manipulación debe ser mínima en cualquier paso del protocolo de pretratamiento. El almacenamiento a largo plazo de muestras de tejidos debe realizarse en completa oscuridad y a temperaturas inferiores a -20 °C (Sørensen et al., 2015).

En el caso de biota como los peces, cuando se realiza análisis multi-órgano, la disección de los órganos debe llevarse a cabo lo más pronto posible y su almacenamiento debe realizarse al menos a -40 °C. Por otro lado, las matrices líquidas, como la sangre o bilis, deberían ser tomadas preferiblemente en campo y almacenarse a -80 °C. Se ha informado que matrices sólidas como cerebro, hígado o músculo no requieren pretratamientos adicionales para el análisis de PhACs (Liu, J. et al., 2014; Zhao, J.L. et al., 2015).

Un pretratamiento ampliamente utilizado, especialmente cuando la muestra consiste en el cuerpo completo de una especie, es la homogenización. Durante los análisis multi-órgano también es necesario homogenizar individualmente los órganos disectados. La homogenización garantiza la distribución uniforme de los CECs en la muestra. Generalmente, se eliminan las espinas y escamas de los peces y se conserva solo el tejido blando en el caso de cangrejos y almejas. Del mismo modo, cuando la muestra proviene de varios individuos, la homogenización asegura

su representatividad (Álvarez-Ruiz & Picó, 2020).

La homogenización puede llevarse a cabo con enfoque húmedo o seco en función del procedimiento de extracción posterior. La homogenización en húmedo se realiza normalmente en un medio acuoso o en un disolvente orgánico, mientras que la seca se consigue mezclando la muestra con una sal desecante (Sørensen et al., 2015). Durante la homogenización, algunos lípidos y tejidos podrían deteriorarse. Para evitar la degradación de la muestra y la producción de peróxidos, que puedan afectar los analitos objetivo, es recomendable moler la muestra congelada o añadir hielo seco o nitrógeno líquido durante esta etapa (Pareja et al., 2022). Otro tipo de pretratamiento es la liofilización, utilizado para eliminar el agua de la muestra y determinar el peso seco para el análisis de los CECs. Tras la liofilización, las muestras suelen triturarse para homogenizar la matriz, obteniendo así un polvo fino para el análisis (Álvarez-Ruiz & Picó, 2020).

Finalmente, para matrices biológicas líquidas, la centrifugación es el pretratamiento más importante, debido a que elimina sólidos en suspensión y otras posibles interferencias. En muestras de sangre, por ejemplo, se utiliza para eliminar el hematocrito, utilizando el plasma o el suero para el análisis (Álvarez-Ruiz & Picó, 2020).

1.5.2. Métodos de extracción

El objetivo principal de un proceso de extracción es maximizar el rendimiento de los compuestos de interés sin o con un impacto mínimo en sus propiedades, mientras se minimiza la extracción de compuestos no deseados (Tiwari, 2015). La mayoría de las técnicas utilizadas para extraer CECs de biota acuática se basan en la partición de los analitos entre la matriz de la muestra (sólida) y su transferencia a una fase líquida, generalmente un disolvente orgánico (Núñez et al., 2017).

La transferencia de los analitos hacia el disolvente se facilita mediante la aplicación de energía al sistema, ya sea a través de calor, ultrasonidos, sonicación por microondas o presión. Un paso inherente a las técnicas de extracción con disolventes es la necesidad de concentrar el disolvente antes del análisis. Esto se logra comúnmente mediante métodos de evaporación, que incluyen el uso de calor y/o la exposición de los extractos a una corriente de gas inerte, a menudo nitrógeno (Sørensen et al., 2015). A continuación, se describen brevemente las principales técnicas de extracción basadas en disolventes utilizadas para el análisis de CECs en

biota acuática y se presenta un análisis comparativo en la Tabla 1.3.

Extracción sólido-líquido (SLE del inglés *solid-liquid extraction)*: la SLE se basa en la partición de los analitos de una muestra de biota hacia un disolvente orgánico, sin aplicar energía adicional, utilizando únicamente agitación manual. Aunque este método es apreciado por su simplicidad, la extracción sólo con disolventes puede no ser suficiente para alcanzar niveles adecuados de recuperación de analitos presentes en bajas concentraciones (Sørensen et al., 2015). Además, la ausencia de energía puede limitar la capacidad del método para separar los analitos objetivo de los componentes afines de la matriz, por lo que su uso se recomienda principalmente en análisis donde se espera que las concentraciones estén por encima de los límites de detección instrumentales (Núñez et al., 2017).

Extracción Soxhlet: la extracción Soxhlet es una técnica que emplea disolventes a temperatura de ebullición y a presión atmosférica (Saini & Keum, 2018). Se trata de un proceso que consume mucho tiempo y requiere grandes cantidades de disolventes, lo que aumenta el costo de la extracción (Saini & Keum, 2018; Sørensen et al., 2015). Con el objetivo de mejorar la eficiencia del método Soxhlet convencional, se han desarrollado diversas modificaciones, como la aplicación de altas presiones, la asistencia por ultrasonidos y la asistencia por microondas (Sørensen et al., 2015).

Extracción con líquidos a presión (PLE de inglés *presurized liquid extraction*): la PLE es una técnica habitual para muestras de biota de gran tamaño, y se conoce comercialmente como extracción acelerada con disolventes (ASE del inglés *aceletared solvent extraction*) (Sørensen et al., 2015). La PLE emplea disolventes a altas temperaturas y presiones, siempre por debajo de sus puntos críticos, manteniendo el disolvente en estado líquido durante todo el proceso (Alvarez-Rivera et al., 2020).

Extracción asistida con ultrasonidos (UAE del inglés *ultrasound-assisted extraction*): la UAE se basa en el proceso de cavitación generado por la radiación ultrasónica. Esta radiación se transmite a través de un medio, generando una perturbación que, al repetirse periódicamente, produce ciclos de expansión y compresión en las moléculas, dando lugar a la formación y colapso de burbujas. La implosión de estas burbujas provoca cambios en la temperatura y la presión, lo que facilita la penetración del disolvente en la matriz y aumenta la transferencia de masa de los analitos al disolvente (Seidi & Yamini, 2012; Tiwari, 2015). Entre las variables que afectan la eficiencia de la extracción se encuentran el tipo de disolvente, el

tiempo de sonicación, el tamaño de la muestra y la relación sólido/líquido. La UAE se considera una técnica respetuosa con el medio ambiente debido a que reduce el consumo de disolventes y la generación de residuos (Albero et al., 2019).

Extracción asistida con microondas (MAE del inglés *microwave-assisted extraction*): la extracción MAE se basa en el efecto directo de las microondas sobre las moléculas del sistema de extracción, mediante la conducción iónica y la rotación dipolar (Sanchez-Prado et al., 2015). A diferencia de la extracción convencional, los principios físicos de la MAE permiten que la irradiación por microondas sea más eficiente. La vaporización del agua intracelular deshidrata la pared celular, reduciendo su resistencia y, junto con el incremento de la presión intracelular, conduce a la ruptura de las paredes y membranas celulares, facilitando el paso de los analitos al solvente extractivo (Cavalloro et al., 2021).

Método de extracción por QuEChERS Rápido, fácil, económico, efectivo, robusto y seguro (del inglés *Quick, Easy, Cheap, Effective, Rugged, and Safe*): este método multi-residuo, fue desarrollado inicialmente para la determinación de residuos de plaguicidas en productos alimentarios. Lo novedoso del método radica en la combinación de procedimientos de extracción y limpieza utilizando acetonitrilo, sales y adsorbentes que demostraron ser altamente efectivos (González-Curbelo et al., 2015). El método QuEChERS se ha adaptado para la extracción de CECs de diversas matrices, como la biota acuática, debido a su simplicidad y a la ausencia de equipos costosos (Pareja et al., 2022).

1.5.3. Limpieza o purificación de extractos

El desarrollo de técnicas de limpieza en muestras de biota acuática es especialmente desafiante debido a la similitud química entre los componentes de la matriz y los analitos objetivo. Estos pueden compartir peso molecular, grupos funcionales o polaridad, lo que dificulta su separación mediante métodos tradicionales (Sørensen et al., 2015). Las técnicas de extracción en organismos acuáticos tienden a ser poco selectivas, extrayendo no solo los analitos, sino también compuestos endógenos como especies iónicas, compuestos altamente polares, y metabolitos similares. Por ello, es crucial eliminar estos componentes co-extraídos empleando procedimientos de limpieza específicos durante o después de la extracción para minimizar los efectos negativos (Núñez et al., 2017).

La biota, especialmente los invertebrados y los peces, contiene entre un 10% y

Tabla 1.3. Comparación de los principales métodos de extracción basados en disolventes usados para el análisis de CECs en fauna acuática.

	UAE	SLE	Soxhlet	PLE	MAE	QuEChERS
Tiempo de extracción	2 – 60 min	3 – 2880 min	3 – 24 h	5 – 30 min	3- 30 min	<30 min
Tamaño de muestra	0.1 – 10 g	0.1 – 30 g	1 – 30 g	1 – 100 g	1 – 10 g	1 – 10 g
Cantidad de solvente	1 – 20 mL	10 – 500 mL	100 – 500 mL	10 – 100 mL	10 – 40 mL	2 – 10 mL
Costo de inversión	Bajo	Bajo	Moderado	Alto	Moderado	Bajo
Ventajas	Extracción simultánea de muestras. Fácil de usar y económico. Se puede adaptar a diferentes tipos de muestras y compuestos. No se requieren grandes cantidades de muestra.	Simplicidad y flexibilidad. Adecuado para métodos de rutina en el que se esperen altas concentraciones.	El sistema de recirculación continua permite una extracción exhaustiva de analitos.	Rápido. Automatizado. Diferentes tamaños de celda, de 1-100 mL que permiten variar la cantidad de muestra.	Rápido. Extracción simultánea de muestras. Automatizado.	Rápido y sencillo. Versátil. No requiere el uso de equipos. Requiere un paso extra para reducir el contenido de agua en la muestra.
Desventajas	Se pueden requerir varios ciclos de sonicación incrementando el tiempo total de extracción.	Lento. Altos volúmenes de disolvente. Al no usar energía adicional durante la extracción puede tener bajas eficiencias de recuperación.	Lento. Altos volúmenes de disolvente. Grandes cantidades de muestra para obtener recuperaciones eficientes. Requiere la limpieza del equipo tras cada extracción. Puede presentar degradación térmica de los compuestos.	Requiere del control de varias variables que pueden afectar la extracción como la presión y la temperatura. Operatividad complicada. Puede requerir 2 o 3 ciclos de extracción, lo que implica mayor cantidad de disolvente.	El solvente de extracción debe ser capaz de absorber microondas. Es necesario esperar el enfriamiento de las muestras para continuar. Puede presentar degradación térmica de los compuestos.	Sólo funciona cuando la muestra tiene un contenido de agua del 80% o más elevado. Es más efectivo para muestras con menos del 2% de contenido de grasa. Los kits comerciales vienen en plástico que restringe el uso de algunos disolventes. El uso de PSA como paso de limpieza promueve la hidrólisis de ácidos débiles ya que las aminas tienden a elevar el pH final del extracto.

Fuente: Adaptado y actualizado de (Sparr Eskilsson & Björklund, 2000)

15% de proteínas, y su contenido de grasas puede llegar al 30% o 35%. La presencia de estas proteínas y grasas puede generar interferencias en la señal durante el análisis de las muestras (Álvarez-Ruiz & Picó, 2020). Para reducir estas interferencias, se han implementado diversas técnicas de limpieza. A continuación, se describen brevemente las más comunes:

Extracción en fase sólida (SPE del inglés *solid phase extraction*): el principio de la limpieza por SPE implica una partición de los analitos entre dos fases: una fase líquida (matriz de la muestra o extracto) y una fase sólida (adsorbente) (Rejczak & Tuzimski, 2015). El proceso de limpieza consta de cuatro etapas: i) Evaporación del extracto y posterior dilución antes de cargarlo al cartucho (Núñez et al., 2017); ii) Acondicionamiento del cartucho, se activan los grupos funcionales del adsorbente mediante un disolvente; iii) Adición de la muestra, el extracto o matriz atraviesa el adsorbente, reteniendo tanto los analitos como algunas interferencias ; y iv) Elución, se separan los analitos de las interferencias utilizando un disolvente que desorbe los analitos de interés, dejando atrás las interferencias (Lucci et al., 2012).

Extracción en fase sólida dispersiva (dSPE del inglés *dispersive solid phase extraction*): para la limpieza con d-SPE se añade una alícuota del extracto obtenido en un tubo de centrífuga que contiene una pequeña cantidad de adsorbente SPE. La mezcla se agita para homogenizar el material adsorbente con el extracto y luego se centrifuga para separar el adsorbente y extraer una alícuota del sobrenadante resultante para su análisis. El adsorbente utilizado en la etapa de limpieza d-SPE se selecciona para retener los compuestos no deseados y co-extraídos de la matriz, permitiendo que los analitos de interés permanezcan en la fase líquida (Rejczak & Tuzimski, 2015).

Cromatografía de permeación en gel (GPC del inglés *gel permeation chromatography*): la GPC es un método consolidado para la limpieza de matrices de origen vegetal y animal, especialmente para purificar extractos complejos. Las columnas de GPC están compuestas por microesferas poliméricas porosas que permiten la separación por exclusión de tamaño (Núñez et al., 2017; Y. Picó, 2012; Sørensen et al., 2015).

1.5.4. Separación cromatográfica y análisis instrumental

Una vez obtenidos los extractos limpios, el último paso es su análisis. En este apartado se profundiza sobre la separación cromatográfica y la adquisición de

datos. Las técnicas más comunes para la separación y detección de CECs son la LC y la cromatografía de gases (GC del inglés *gas cromatography*), acoplada a la espectrometría de masas (MS del inglés *mass spectrometry*). Estas técnicas permiten una identificación tanto cualitativa como cuantitativa de los compuestos de interés. El uso de la HRMS ha permitido además aplicar enfoques de selección amplia y no dirigida para la identificación de compuestos desconocidos o sospechosos, haciendo uso de bases de datos sin necesidad de estándares de referencia (Álvarez-Ruiz & Picó, 2020).

En esta tesis se aplicaron dos enfoques de evaluación de datos a través de HRMS: el análisis dirigido y el análisis de sospechosos. En el análisis dirigido lo más común es comenzar con un escaneo MS1 de amplio rango de *m/z* para obtener la masa exacta de los iones precursores presentes en la muestra, lo que se conoce como barrido completo MS1 (*Full scan MS*^{1}*)*. El full scan es seguido por un segundo experimento en el que se pueden aislar un grupo de iones precursores conocidos dentro de un rango de masas reducido, con el fin de generar espectros específicos de iones producto para esos iones. Los experimentos MS^2 no dirigidos permiten que todos los iones precursores que eluyen en un momento dado pasen a la celda de colisión, generando espectros de iones producto mixtos, lo que se denomina fragmentación de todos los iones (AIF, por sus siglas en inglés *all ion fragmentation*). Otro enfoque para generar iones producto a partir de muestras complejas es la adquisición dependiente de datos (DDA, del inglés *data dependent acquisition*), en la que los espectros MS^2 solo se generan para iones precursores que superan un umbral determinado (Turnipseed, 2024).

Alternativamente, se pueden muestrear y fragmentar secuencialmente conjuntos limitados de iones precursores, a través del análisis independiente de datos (DIA, del inglés *data independent acquisition*) sin necesidad de superar un valor umbral. En esta tesis, el análisis dirigido de datos se basó en este tipo de adquisición, pero generando un DIA-dirigido a través de la incorporación de una lista de inclusión.

En el análisis de sospechosos los iones precursores encontrados en las muestras por HRMS, se comparan con las masas exactas teóricas de compuestos en bibliotecas o bases de datos específicas (Turnipseed, 2024). Las masas exactas de los iones producto, los tiempos de retención y los perfiles isotópicos de los analitos, se suelen utilizar como criterios de confirmación.

Dependiendo de la cantidad de información disponible para la comparación, el

análisis de sospechosos puede detectar un elevado número de compuestos, lo que dificulta la diferenciación entre hallazgos reales de falsos positivos. Esto puede ser un proceso complicado y consumir mucho tiempo, a menudo requiriendo un análisis de seguimiento con patrones de referencia (Turnipseed & Casey, 2024). Para asegurar la identificación de compuestos detectados a partir de una análisis de sospechosos, Schymanski et al., (2014) propuso unos niveles de confianza que van desde el nivel 5 (en donde existe coincidencia de la *m/z* exacta del compuesto, pero que carece de información para asignar su fórmula química), hasta el nivel 1 (en donde la estructura del compuesto es confirmada con un patrón de referencia y hay coincidencia entre su *m/z* exacta, la *m/z* de sus precursores y fragmentos y el tiempo de retención). Estos niveles de confianza, fueron aplicados tras la realización de un análisis de sospechosos en el capítulo 4. En la Fig. 1.6 se presenta un esquema de los dos enfoques de evaluación de datos utilizados en la tesis, incluyendo los niveles de confianza tras el análisis de sospechosos.

Respecto a los instrumentos para la detección, los analizadores más utilizados en el análisis de matrices biológicas acuáticas son el cuadrupolo tiempo de vuelo (QTOF del inglés *Quadrupole Time of Flight*), el orbitrap (Q-Orbitrap) (Álvarez-Ruiz & Picó, 2020; Richardson & Kimura, 2020) y los de triple cuadrupolo (QqQ) (Jakimska et al., 2014). Durante el desarrollo de esta tesis se hizo uso de dos equipos: el sistema hibrido Q-TOF-MS X500R (SCIEX) y el Orbitrap Q-Exactive-MS™ (Thermo Fischer Scientific). En la Tabla 1.4 se describen sus características principales.

Finalmente, en la Tabla 1.5 se presenta el estado del arte de los diferentes métodos analíticos para el análisis de CECs en fauna acuática reportados en los últimos cinco años, detallando el pretratamiento de la muestra y las técnicas de extracción, limpieza y detección.

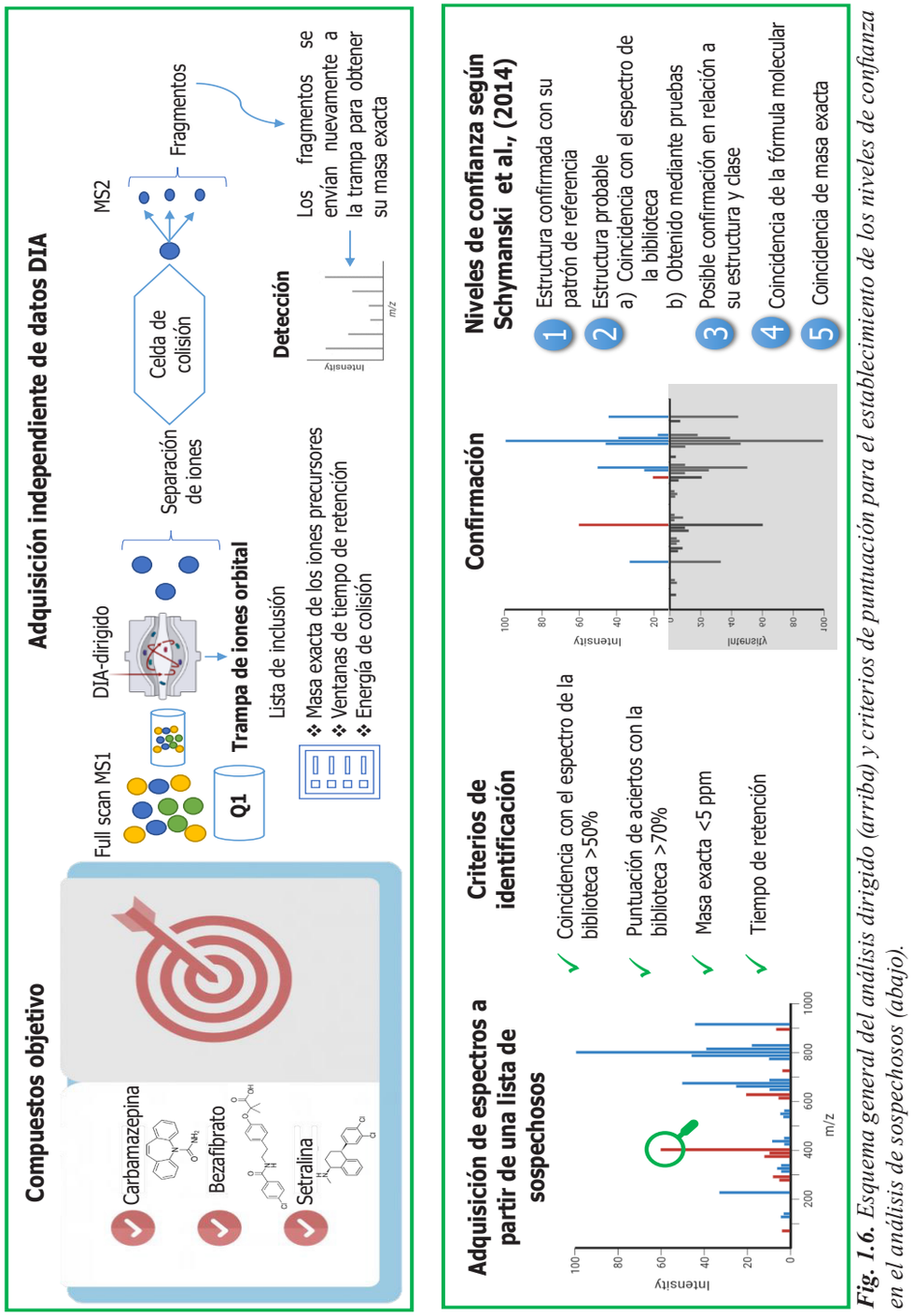
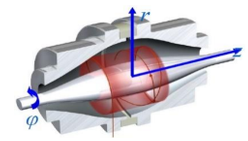


Fig. 1.6. *Esquema general del análisis dirigido (arriba) y criterios de puntuación para el establecimiento de los niveles de confianza en el análisis de sospechosos (abajo).*

Tabla 1.4. *Cuadro comparativo: Q-TOF-MS X500R (SCIEX) y Orbitrap Q-Exactive™ (Thermo Fischer Scientific)*

Características	Q-TOF-MS X500R (SCIEX)	Orbitrap Q-Exactive-MS™ (Thermo Fisher Scientific)
Resolución	30.000 FWHM	60.000-140.000 FWHM
Velocidad de adquisición MS/MS	100 espectros/s	12 espectros/s
Precisión de masa	<2 ppm	<1 ppm
Rango de masa	Hasta *m/z* 40.000 con selección de iones precursores entre *m/z* 20 y 2.250	Rango de masa entre *m/z* 50 y 6.000
Fuentes de ionización	Fuente de iones Turbo V con una sonda Twin Spayer de ionización por ESI y una sonda Twin Sprayer de ionización APCI	Fuentes ESI; Fuente de ionización combinada APCI/APPI
Analizador de masas	El analizador mide el tiempo que un ion tarda en desplazarse por un tubo de vuelo desde un pulsador hasta el detector. La separación de los iones depende de su velocidad, la cual está determinada por su *m/z*.	 Es un dispositivo de trampa de iones que emplea la transformada de Fourier para generar el espectro *m/z*. Los iones oscilan alrededor de un electrodo central debido a la aplicación de un campo eléctrico y su separación se realiza según su frecuencia de oscilación.
Métodos de adquisición	*Full scan TOF-MS:* Detecta todos los iones en un rango de *m/z* especificado. *MS/MS*: Selecciona un ion precursor en el cuadrupolo y lo fragmenta en la celda de colisión. *MRM:* Adquisición dirigida que monitorea pares específicos de iones precursor/producto. Proporciona alta especificidad. *IDA:* Combina escaneos de MS y MS/MS en un solo experimento. Selecciona automáticamente los iones más intensos detectados en un espectro MS para su fragmentación. *SWATH:* Fragmenta todos los iones en intervalos definidos de *m/z* a lo largo de un rango específico.	*Full Scan:* Detecta todos los iones en un rango de *m/z* específico. *AIF* Fragmenta todos los iones en el rango de *m/z* seleccionado y analiza tanto los precursores como los fragmentos. *MS/MS:* El cuadrupolo selecciona un ion precursor específico para fragmentarlo en la celda de colisión. *dd-MS²*: Combina un barrido completo con escaneos MS/MS de iones seleccionados automáticamente en función de su intensidad. *SIM:* Monitorea iones específicos seleccionados por el cuadrupolo. *PRM:* Variante dirigida del modo MS/MS, donde los fragmentos de un ion precursor seleccionado se analizan en paralelo.

Fuente: (Álvarez-Ruiz & Picó, 2020; Kind et al., 2018; Peña-Herrera, 2020; SCIEX, 2024; ThermoFisher, 2024) .

FWHM: Medida del ancho completo del pico a media altura del inglés Full Width at Half Maximum; Full scan: barrido completo; ESI: Ionización por electrospray del inglés electrospray ionization; APCI: Ionización química a presión atmosférica del inglés Atmospheric Pressure Ionization; APPI: Fotoionización a presión atmosférica del inglés Atmospheric Pressure Photoionization Ionization; MRM: Monitoreo de reacciones múltiples del inglés Multiple reaction monitoring; IDA: Adquisición dependiente de la información del inglés Information-dependent acquisition; SWATH: Adquisición secuencial de ventanas de todos los espectros teóricos de fragmentos iónicos del inglés Sequential window acquisition of all theorical fragment-ion spectra; dd-MS2: Adquisición dependiente de datos del inglés Data-Dependent Acquisition; SIM: Monitoreo de iones seleccionados del inglés Selected ion monitoring; PRM: Monitoreo de Reacciones Paralelas del inglés Parallel reaction monitoring.

Tabla 1.5. *Métodos analíticos basados en la extracción con disolventes y técnicas de limpieza usados para la detección de CECs en biota acuática entre el 2019 y el 2024*

Tipo de CECs	Especie	Pretratamiento	Método de extracción	Técnica de limpieza	Detección	Referencia
PhACs	Peces	Liofilización	SLE (Homogenizador de perlas)	SPE (HLB)	UPLC-MS QTRAP	(Castaño-Ortiz et al., 2024)
PhACs	Peces	Homogenización tejido fresco con perlas de zirconio	SLE	-	LC-MS/MS TSQ Quantiva	(Duarte et al., 2023)
PhACs	Peces	Homogenización tejido fresco con acetato/acetileno	UAE	SPE (HLB)	UPLC-MS/MS Xevo	(Zheng et al., 2022)
PhACs	Peces	Homogenización tejido fresco	UAE	CAFS[1]/d-SPE	HPLC-MS/MS QTRAP	(Mu et al., 2022)
PhACs	Peces	Homogenización tejido fresco	SLE + agitación	Centrifugación	UHPLC-MS/MS QqQ	(Cerveny et al., 2021)
PhACs	Peces	Homogenización tejido fresco	QuEChERS	d-SEP	UHPLC-MS/MS Orbitrap	(Kalogeropoulou et al., 2021)
PhACs	Peces	Liofilización	PLE	GPC	UPLC-MS	(Martínez-Morcillo et al., 2020)
PhACs	Peces	Liofilización	Disrupción celular	SPE (Gel de sílice)	LC-MS/MS QTRAP	(Boulard et al., 2020)
PhACs	Peces	Homogenización tejido fresco con TissueLyser	SLE	Filtración con filtro de celulosa regenerada (Labicom)	LC-HRMS Orbitrap	(Grabicová et al., 2020)
PhACs	Peces	Homogenización tejido fresco	UAE	d-SPE	LC-MS/MS QToF	(Peña-Herrera et al., 2020)
PhACs	Peces	Homogenización tejido fresco	UAE	SPE (Cartuchos de sílice recubierto de circonia)	LC-MS/MS QTRAP	(Tanoue et al., 2020)
PhACs	Peces	Homogenización tejido fresco	QuEChERS	-	UHPLC-MS/MS QqQ	(Pemberthy M et al., 2020)
PhACs	Peces	Homogenización tejido fresco	SLE + agitación	Centrifugación	UHPLC-MS/MS QqQ	(Cerveny et al., 2020)
PhACs	Peces	Liofilización	UAE	SPE (HLB)	UHPLC-MS/MS QqQ	(Xie et al., 2019)
PhACs	Peces	Liofilización	MAE	SPE (PPR[2])	UHPLC-MS/MS QqQ	(Santana-Viera et al., 2019)
PhACs	Peces	Liofilización	QuEChERS	d-SPE	UPLC-MS/MS QToF	(Peña-Herrera et al., 2019)
PhACs	Peces	Homogenización tejido fresco	SLE + vórtex + centrifugación	SPE (columna de gel de alúmina)	HPLC-Curva de calibración externa	(Lan et al., 2019)
PhACs	Peces	Homogenización + liofilización	PLE	GPC	UPLC-MS/MS QTRAP	(Rojo et al., 2019)

[1] CAFS del inglés Cheap analytical filtration syringe
[2] PPR del inglés Phree Phospholipid Removal

Tabla 1.5. *Continuación.*

Tipo de CECs	Especie	Pretratamiento	Método de extracción	Técnica de limpieza	Detección	Referencia
PhACs	Peces	Homogenización tejido fresco	SLE	Centrifugación	UHPLC-MS/MS QqQ	(McCallum et al., 2019)
PhACs	Peces	Homogenización tejido fresco (pool de órganos)	UAE	Centrifugación + filtración con filtro de membrana de nailon	HPLC	(Gasca-Pérez et al., 2019)
PhACs	Peces, ostras y camarones	Homogeneización tejido fresco	QuEChERS	d-SPE	LC-HRMS Orbitrap	(Kim et al., 2023)
PhACs	Peces y bivalvos	Liofilización	QuEChERS	d-SPE	HPLC-MS/MS QqQ	(Mello et al., 2022)
PhACs	Peces y moluscos	Homogenización tejido fresco	QuEChERS	SPE (ITSP[3])	UHLC-MS/MS QqQ	(Godfrey et al., 2022)
PhACs	Peces, crustáceos	Liofilización + pulverización	SLE	Filtración con filtros de jeringa de politetrafluoroetileno	UPLC-MS Xevo	(Miossec et al., 2020)
PhACs	Peces, crustáceos	Liofilización	UAE	SPE (Oasis MCX + Oasis MAX)	HPLC-MS QTRAP	(Ruan et al., 2020)
PhACs	Almeja asiática	Homogeneización tejido fresco con batidora de mano	SLE (Mesa giratoria)	Filtración con filtros de jeringa de membrana hidrofóbica de teflón	LC-MS/MS Tándem	(Burket et al., 2019)
PhACs	Almejas	Homogenización tejido fresco con agua y acetonitrilo	QuEChERS	d-SPE	LC-MS/MS QqQ	(Rodrigues et al., 2019)
PhACs	Mejillones, caracoles	Congelación	Soxhlet	SPE (HLB)	UPLC-MS Xevo	(Ojemaye & Petrik, 2022)
PhACs	Mejillones y ostras	Homogenización tejido fresco	SLE	SPE (HLB Prime)	UHPLC-MS/MS Xevo	(Maskrey et al., 2021)
PhACs	Mejillones, cangrejos	Liofilización	PLE	SPE (HLB)	UPLC-MS	(Martínez-Morcillo et al., 2020)
PhACs	Mejillones		SLE (sistema de dispersión, agitación, homogeneización y molienda IKA ULTRA TURRAX)	SPE (Discovery DSC-18 + cartuchos C18)	HPLC-detección fluorimétrica y de matriz de diodos	(Mezzelani et al., 2020)
Drogas ilícitas	Mejillones	Liofilización	UAE	-	HPLC-MS/MS QqQ	(Fontes et al., 2021)
Drogas ilícitas	Mejillones	Homogenización tejido fresco	QuEChERS	d-SPE	HPLC-MS/MS QTRAP	(López-García et al., 2019)
Plaguicidas	Peces	Homogenización tejido fresco	UAE	Centrifugación	IC-MS/MS Orbitrap	(Chiesa et al., 2019)
Plaguicidas	Peces	Homogenización tejido fresco	UAE	Filtración en filtro de celulosa	IC-MS/MS Orbitrap	(Chiesa et al., 2019)

[3] ITSP del inglés in-tip solid-phase

Tabla 1.5. *Continuación.*

Tipo de CECs	Especie	Pretratamiento	Método de extracción	Técnica de limpieza	Detección	Referencia
Plaguicidas	Peces	-	QuEChERS	d-SPE	LC-MS/MS QqQ	(Barbieri et al., 2019)
PFAS	Peces	Homogenización en batidora manual	UAE	Hidrolización de grasas y proteína con proteasa y lipasa + 5 Ml de hexano y centrifugación antes de la extracción	LC-MS/MS	(Y. M. Lee et al., 2020)
PFAS	Peces	Trituración y liofilización	Digestión alcalina	Cromatografía de turboflujo	GC-MS/MS	(Pico et al., 2019)
PFAS	Peces	Trituración	UAE (disruptor celular ultrasónico)	Centrifugación	UPLC-MS/MS Xevo-TQD	(Jie Liu et al., 2019)
PFAS	Peces	Liofilización y trituración	SLE	d-SPE	UHPLC-MS/MS QqQ	(Vidal et al., 2019)
PFAS	Cangrejo de rio	Homogenización tejido fresco	SLE	SPE (PSA)	LC-MS/MS QTRAP	(J. Bian et al., 2024)
PFAS	Almeja asiática	Homogeneización tejido fresco haciendo uso de un procesador de ultrasonido	Micro- QuEChERS	Congelamiento de la capa acuosa + SPE (Captiva-EMR) + d-SPE + filtración con filtro de jeringa de nailon	UHPLC-MS/MS QqQ	(Koban et al., 2024)
PFAS	Almeja asiática	Homogenización tejido blando	UAE	-	UHPLC-MS/MS QqQ	(Yan Liu et al., 2020)
PFAS	Almeja asiática, mejillones, cangrejos de río, peces	Homogeneización tejido fresco	UAE	SPE (Oasis WAX)	UPLC-MS/MS	(Penland et al., 2020)
PFAS	Peces, crustáceos, cefalópodos	Liofilización	Digestión alcalina	SPE (Oasis WAX)	UPLC-MS/MS QqQ	(Pan et al., 2021)
OPEs	Peces	Liofilización	PLE	SPE (columna multicapa con alúmina neutra, gel de sílice neutra, Florisil, PSA y sulfato de sodio)	GC-MS	(Bekele et al., 2021)
OPFRs y sus metabolitos	Peces	Liofilización	UAE	d-SPE	GC-MS/MS	(Tang et al., 2019)
OPFRs	Cangrejo de rio	Homogenización tejido fresco	UAE	SPE (CNWBOND Florisil)	UPLC-MS/MS QTRAP	(Peng et al., 2024)
PhACs, plaguicidas, compuestos de uso industrial, PFAS	Peces	Homogenización y liofilización	UAE	SPE (Captiva ND-Lipid filtres)	LC-MS/MS Orbitrap	(Musatadi et al., 2020)
PhACs, plaguicidas, PFAS, drogas ilícitas	Peces	Homogenización tejido fresco	QuEChERS	d-SPE	UHPLC-MS/MS QqQ	(Vitale et al., 2021)

Tabla 1.5. *Continuación.*

Tipo de CECs	Especie	Pretratamiento	Método de extracción	Técnica de limpieza	Detección	Referencia
Plaguicidas, fármacos veterinarios y PCB	Peces	-	QuEChERS	SPE (ITSP)	UHPLC-MS/MS QTRAP	(Ninga et al., 2021)
PhACs, drogas ilícitas	Peces	Homogenización tejido fresco	SLE + mesa giratoria	Filtración con filtros de jeringa Acrodisc® de super-membrana hidrofóbica de teflón	LC-MS/MS QqQ	(Ondarza et al., 2019)
Plaguicidas, compuestos de uso industrial	Peces	Trituración y liofilización	QuEChERS	-	LC-MS/MS	(Pico et al., 2019)
PhACs y retardantes de flama halogenados	Peces	Trituración y liofilización	PLE	GPC	LC-MS/MS GC-MS/MS	(Pico et al., 2019)
PhACs, PFAS	Peces	Liofilización	Soxhlet	SPE (HLB)	UHPLC-MS/MS QqQ	(Ojemaye & Petrik, 2019)
Plaguicidas	Peces	Liofilización y trituración	PLE	Columnas de gel de sílice acidificada + multicapa + Florisil	HRGC (del inglés higth resolution gas chromatography)-HRMS	(X. Zhao et al., 2019)
Plaguicidas, hidrocarburos aromáticos policíclicos	Peces	Liofilización	PLE	SPE (Florisil)	GC-MS/MS QqQ	(Molbert et al., 2019)
PhACs, plaguicidas, compuestos de uso industrial, EDCs	Peces	Homogenización tejido fresco	UAE	SPE (LiChrolut)	GC-MS Q	(Azzouz et al., 2019)
PFAS, OPFRs	Peces	Trituración	UAE	SPE (Strata-X)	LC-MS/MS QqQ	(Lorenzo et al., 2019)
PhACs, plaguicidas, BPA	Peces, caracoles	Liofilización	QuEChERS	SPE (Strata-X)	HPLC-MS QTRAP	(Omar et al., 2019)
Compuestos de uso industrial, Antimicrobianos	Mejillones	Liofilización	UAE	d-SPE	UHPLC-MS/MS Xevo	(García-Fernández et al., 2022)
PhACs, plaguicidas, PFAS, drogas ilícitas	Mejillones	Homogenización tejido fresco	QuEChERS	d-SPE	HPLC-MS/MS QTRAP	(Álvarez-Ruiz et al., 2021)
Plaguicidas, PCB, PAHs	Mejillones	Homogenización tejido fresco	UAE	SPE (Florisil) + sulfato sódico anhídrido	GC-MS/MS QqQ	(J. Lee et al., 2020)
Plaguicidas, compuestos de uso industrial, PFAS, PhACs	Peces y mejillones	Liofilización y trituración	UAE	SPE (HLB)	LC-MS/MS QqQ	(Mijangos et al., 2019)
PhACs, plaguicidas, drogas ilícitas	Crustáceos	Liofilización + homogeneización en TissueLyser	UAE	SPE (Strata-X, alúmina-N y HLB)	LC-MS QqQ	(Miller et al., 2019)

1.6. Análisis de riesgo ecológico

Una vez obtenidas las concentraciones de los CECs en las diferentes matrices de interés, es fundamental interpretar los resultados para determinar si los valores obtenidos podrían tener relevancia ambiental en los ecosistemas acuáticos. Un enfoque común para este análisis es la evaluación de riesgo ecológico, que permite identificar posibles efectos adversos de contaminantes específicos en función de su concentración.

La evaluación de riesgo ecológico se define como el procedimiento por el cual se estiman los efectos adversos probables o reales de contaminantes y/o actividades antropogénicas sobre los ecosistemas y sus componentes (Depledge & Fossi, 1994). Su principal objetivo es la protección de los ecosistemas, considerando su estructura, funcionamiento y servicios (Amiard & Amiard-Triquet, 2015). Este tipo de evaluaciones han ganado importancia al reconocerse que sustancias que no representan riesgo para la salud humana, podrían generar afectaciones serias sobre los recursos naturales (Van der Oost et al., 2003).

El proceso de evaluación de riesgos puede resumirse en seis pasos clave: i) identificación de peligros; ii) evaluación de los efectos; iii) evaluación de la exposición; iv) caracterización del riesgo; v) clasificación de los riesgos y vi) reducción de los riesgos y monitoreo (Van Leeuwen & Hermens, 1995). En la Fig. 1.7 se explica brevemente en que consiste cada paso y se relacionan con la información presentada en los capítulos 4 y 5.

El paso de caracterización del riesgo, se ha llevado a cabo tradicionalmente comparando las concentraciones ambientales previstas o medidas con su valores PNECs, los cuales se basan en datos de ecotoxicidad experimentales (Nozaki et al., 2023). Diferentes estudios han realizado el análisis de riesgo ecológico calculando los PNECs de los compuestos que analizaron a partir de los valores LC50 y EC50 reportados para diferentes PhACs en peces (Ginebreda et al., 2010; Liu, J. et al., 2014, 2015, 2018; Ojemaye & Petrik, 2022; Staszny et al., 2021; Zheng et al., 2022), OPFRs en Daphnia magna (Cristale et al., 2013), PhACs y PFAS en peces (Ojemaye & Petrik, 2019) o usando directamente los proporcionados por las bases de datos para pesticidas, PFAS, PhACs y OPFRs (Yolanda Picó et al., 2021). Otros análisis de riesgo se han realizado a partir de la medición o predicción de las concentraciones plasmáticas de PhACs en peces y su comparación con concentraciones biológicamente efectivas en humanos, es decir, las concentraciones plasmáticas terapéuticas más

bajas (Nozaki et al., 2023; Sumpter & Margiotta-Casaluci, 2022).

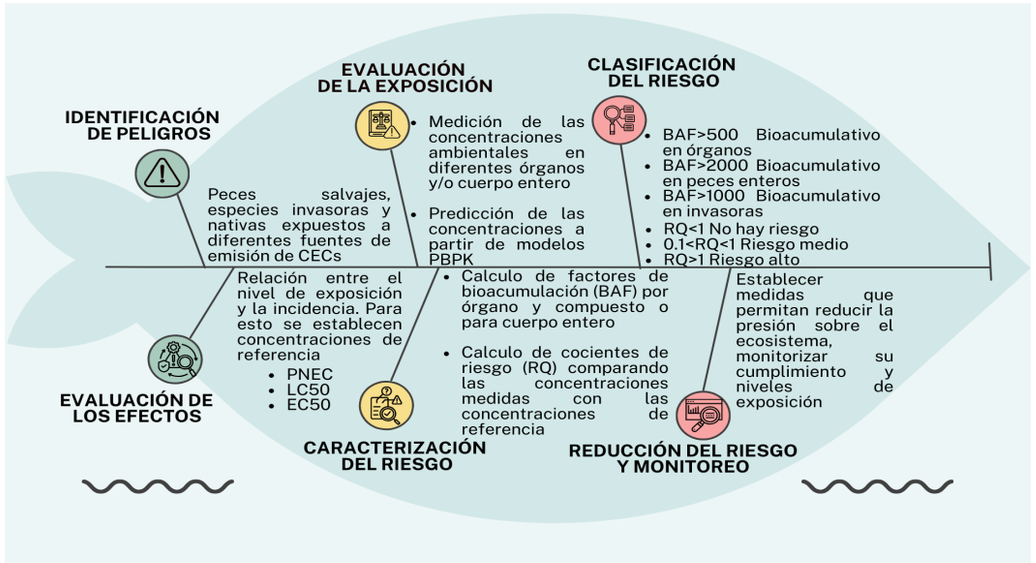


Fig. 1.7. *Pasos para la evaluación de riesgo ecológico. LC50: Concentración letal 50, es decir concentración a la que un compuesto causa la muerte del 50% de la población en pruebas de toxicidad aguda. EC50: Concentración efectiva, es decir concentración a la que un compuesto causa efectos adversos en el 50% de la población analizada. PBPK: Modelos farmacocinéticos de base fisiológica, por sus siglas en inglés Physiologically based pharmacokinetic model*

Sin embargo, actualmente existen diferentes bases de datos para consultar valores de referencia. Una de ellas es la base de datos de Relaciones ecológicas estructura-actividad (ECOSAR por sus siglas en inglés *Ecological Structure Activity Relationships*), que es una herramienta que utiliza modelos QSAR para predecir la toxicidad aguda y crónica de una sustancia química (EPA, 2024d).

La ECHA tiene una base de datos que incluye las concentraciones máximas admisibles para sustancias y contaminantes prioritarios en el agua (ECHA, 2024). En Francia, el Instituto nacional del medio ambiente industrial y de riesgos (INERIS por sus siglas en francés *Institut national de l'environnement industriel et des risques*) ha diseñado un portal de sustancias químicas en el que se informa sobre sus propiedades toxicológicas, eco-toxicológicas y fisicoquímicas (INERIS, 2024).

Adicionalmente, a nivel europeo se cuenta con la base de datos de eco-toxicología de la red NORMAN, que fue elegida en esta tesis para realizar el análisis de riesgo ecológico debido a que cuenta con los PNEC de todos los compuestos objetivo en esta tesis, incluyendo los diferentes compartimentos ambientales evaluados, como agua superficial, sedimentos, peces y moluscos (Tabla 1.2).

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CAPÍTULO 2

OBJETIVOS

OBJETIVOS

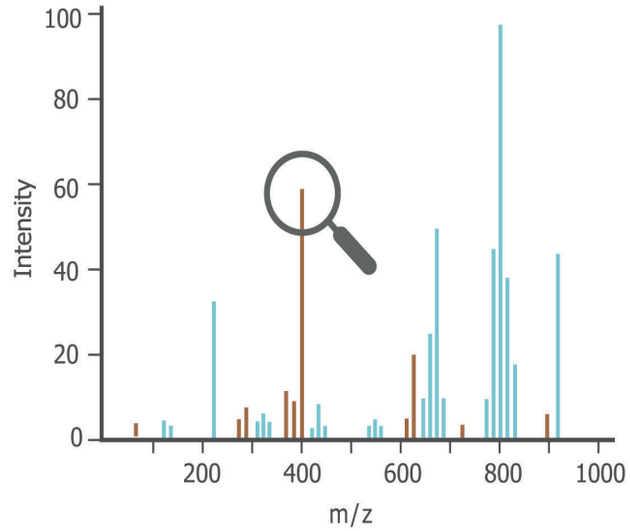
El objetivo general de esta tesis doctoral es ***evaluar la distribución y bioacumulación de contaminantes de preocupación emergente en especies acuáticas de agua dulce, incluyendo especies nativas e invasoras como biomonitores de la contaminación ambiental, para determinar el riesgo ecológico de ecosistemas acuáticos sometidos a presiones antrópicas.*** Para la obtención de este objetivo general, se plantean, además, los siguientes objetivos específicos:

- Desarrollar métodos analíticos que permitan el análisis simultáneo de contaminantes de preocupación emergente de diferentes clases químicas en matrices biológicas de diversa naturaleza, garantizando parámetros analíticos como precisión, sensibilidad y exactitud.

- Priorizar órganos y fluidos corporales centinela para evaluar la acumulación de contaminantes de preocupación emergente en peces salvajes a través de un análisis de distribución en diferentes especies.

- Evaluar la distribución de contaminantes de preocupación emergente en órganos de peces y especies invasoras, a través de factores de bioacumulación experimentales y/o predichos.

- Evaluar el potencial de especies invasoras como biomonitores y su uso para la evaluación del riesgo ecológico mediante un análisis comparativo de los patrones de acumulación respecto a especies nativas.



CAPÍTULO 3

METODOLOGÍAS ANALÍTICAS PARA LA DETERMINACIÓN DE CONTAMINANTES DE PREOCUPACIÓN EMERGENTE EN PECES Y MACROINVERTEBRADOS

METODOLOGÍAS ANALÍTICAS PARA LA DETERMINACIÓN DE CONTAMINANTES DE PREOCUPACIÓN EMERGENTE EN PECES Y MACROINVERTEBRADOS

3.1. Introducción

Para llevar a cabo estudios de biomonitoreo que reflejen con precisión las clases de compuestos químicos y sus concentraciones en ecosistemas acuáticos, es esencial desarrollar métodos analíticos robustos. En este capítulo se presentan dos métodos analíticos para el análisis de CECs en órganos de peces (hígado, riñón, cerebro, músculo, corazón y piel) y el tejido blando de macroinvertebrados (almejas y cangrejos de río). Los métodos desarrollados tienen como base común el pretratamiento de la muestra, el método de extracción y la técnica de limpieza (Fig. 3.1). A continuación, se detallan las características transversales a los dos métodos.

Fig. 3.1. *Esquema general de los métodos analíticos desarrollados para el análisis de CECs en órganos de peces y macroinvertebrados*

3.1.1. Pretratamiento de la muestra

Debido a la naturaleza de las diferentes matrices a analizar, se decidió pretratar la muestra mediante homogenización en tejido fresco. Esta técnica de pretratamiento ha sido ampliamente utilizada para el análisis de biota acuática debido a que permite obtener muestras uniformes y consistentes, simplifica y reduce los costos del método analítico al eliminar pasos adicionales como la liofilización y reduce las pérdidas de analitos sensibles a los cambios de temperatura (Bian et al., 2024; Mu et al., 2022; Peng et al., 2024).

El uso de instrumentos que optimizan la homogenización y permiten una ruptura más eficiente de los tejidos, es preferible a la homogenización manual. Por ello, dispositivos como el TissueLyser LT (QIAGEN GmbH, Germany), diseñados para la disrupción de muestras de tamaño pequeño, se utilizan cada vez más en los

métodos analíticos (Duarte et al., 2023; Gil-Solsona et al., 2021; Grabicová et al., 2020; Miller et al., 2019).

El TissueLyser LT empleado en el desarrollo de los métodos analíticos propuestos en esta tesis, es un molino de microesferas compacto que permite la disrupción y homogenización simultánea de muestras de tejido fresco. Funciona mediante la agitación a alta velocidad de las muestras en tubos de microcentrífuga de 2 mL junto con microesferas de acero inoxidable. Este instrumento facilita a través de una acción mecánica, la ruptura eficiente de las membranas celulares, incluso en tejidos difíciles de lisar, como corazón y cerebro, liberando así los compuestos presentes en su interior para su posterior análisis (Qiagen, 2024).

3.1.2. Extracción con disolvente asistida con ultrasonidos

Una vez establecido el protocolo para el pretratamiento de la muestra, se seleccionó la UAE como el método de extracción a implementar debido a sus ventajas respecto a los demás. En el proceso de extracción se puede hacer uso de baño ultrasónico o sondas ultrasónicas. Las sondas son sistemas más potentes que los baños, ya que tienen una mayor intensidad y están sumergidas en la muestra, concentrando la energía en una pequeña área. Esto potencia el efecto de cavitación y permite tiempos de extracción más cortos. Sin embargo, el baño ultrasónico es más comúnmente utilizado debido a su bajo costo, su facilidad de operación y su capacidad para la extracción simultánea de muestras (Pérez & Albero, 2023), por lo cual, esta segunda opción fue la elegida en lo métodos aquí desarrollados.

Una desventaja de la UAE es que, después del proceso de extracción, es necesario realizar una centrifugación para separar el extracto de la parte sólida de la muestra, lo que incrementa el tiempo total del proceso. Generalmente la UAE, incluyendo la centrifugación, toma entre 10 y 30 min, y suele requerir dos o tres ciclos para obtener resultados óptimos (Núñez et al., 2017). Sin embargo, una de sus principales ventajas es que puede considerarse una técnica de extracción en frío, que ésta más asociada a los principios de la química verde, ya que la temperatura durante el proceso es comparativamente baja, y no afecta la estabilidad de los compuestos extraídos. Además, el ultrasonido es fácil de usar, versátil y flexible, y requiere una inversión relativamente baja en comparación con otras técnicas como la ASE o la extracción con fluidos supercríticos (Núñez et al., 2017; Tiwari, 2015).

En la Tabla 3.1. se presenta información detallada de estudios que han utilizado

la UAE para la detección de CECs en peces, bivalvos y cangrejos. La mayoría de estos estudios se centran en el análisis de PhACs en el músculo y órganos de peces. En estos casos, las disoluciones de acetonitrilo con agua o metanol son las más utilizadas para la extracción de PhACs. Por otro lado, el metanol es el disolvente más usado para la extracción de plaguicidas, mientras que para los PFAS y OPFRs se han empleado distintas combinaciones de disolventes. Es importante destacar que una de las ventajas de la UAE es su capacidad para lograr extracciones eficientes utilizando pequeñas cantidades de muestra. En los estudios referenciados, las cantidades oscilaron entre 0.5 y 1.0 g.

3.1.3. Técnica de limpieza basada en la extracción en fase sólida dispersiva

Se han desarrollado diversas técnicas de limpieza de extractos para reducir el efecto matriz y eliminar interferencias. Aunque la SPE es la técnica de limpieza más utilizada en estudios de evaluación de CECs en biota acuática, en esta tesis se decidió usar la d-SPE. La d-SPE es una técnica de limpieza basada en la adición de un extracto disolvente en un tubo que contiene un material adsorbente disperso, con el propósito de eliminar compuestos de la matriz o contaminantes interferentes del extracto. Después de agitar y mezclar, el adsorbente que contiene las interferencias retenidas se separa de la fase líquida mediante centrifugación. El adsorbente se elige para retener compuestos no deseados, co-extraídos con la matriz, y permitir que los analitos de interés permanezcan en la fase líquida (Rejczak & Tuzimski, 2015; Sørensen et al., 2015).

Para muestras con alto contenido de grasa, se recomiendan adsorbentes como C_{18}, NH_2 (radical amino), amina secundaria principal (PSA del inglés *primary secondary amine)*, carbón negro grafitizado (GCB del inglés *graphitized carbon black*) o de intercambio iónico fuerte (SAX del inglés *strong anion exchange*) en diversas combinaciones. PSA se utiliza comúnmente para eliminar azúcares y ácidos grasos y C_{18} para eliminar lípidos residuales (Álvarez-Ruiz & Picó, 2020; Núñez et al., 2017; Sørensen et al., 2015).

Esta técnica de limpieza ofrece varias ventajas sobre la SPE clásica, como la eliminación del paso de acondicionamiento, la ausencia de colectores o dispositivos de vacío o presión simplificando el proceso al evitar problemas de control de flujo. La d-SPE no requiere un paso de elución ni dilución, utiliza menos adsorbente y por ende requiere de un volumen menor de extracto, es más rápida, económica y fácil de implementar (Rejczak & Tuzimski, 2015; Sørensen et al., 2015).

Tabla 3.1. *Estudios en los que se ha usado UAE cómo método de extracción para el análisis de CECs en biota acuática entre 2019 – 2024.*

Compuestos analizados	Matriz	Cantidad de muestra (g)	Disolvente	Cantidad de disolvente	Tiempo en ultrasonidos	Referencia
15 PhACs	Peces (cerebro, hígado, riñón, branquias, músculo, intestino, bilis, plasma)	0.5 w.w.	Acetato/acetileno	3.5 mL	5 min (previamente vórtex por 20 min)	(Zheng et al., 2022)
18 PhACs	Pez (músculo)	2.0 w.w.	Agua/acetonitrilo acidificado	10 mL	10 min	(Mu et al., 2022)
47 PhACs	Pez (músculo)	0.5 w.w.	Acetonitrilo/isopropanol acidificado	1 mL	10 min	(Peña-Herrera et al., 2020)
72 PhACs	Pez entero	0.1 w.w.	Metanol/acetonitrilo + buffer ácido acético	1 mL	15 min	(Tanoue et al., 2020)
4 PhACs	Peces, crustáceos (músculo)	2.0 d.w.	Metanol/agua mili Q	8 mL	20 min	(Ruan et al., 2020)
Carbamazepina	Peces (hígado, branquias, cerebro)	-	Agua desionizada + cloroformo	5 mL + 10 mL	10 min	(Gasca-Pérez et al., 2019)
34 PhACs	Peces y mariscos (músculo, parte blanda)	0.2 d.w.	Acetonitrilo/agua	8 mL	2 ciclos de 15 min	(Xie et al., 2019)
Cocaína y su metabolito	Mejillones	10 d.w.	Acetonitrilo + Acetonitrilo + buffer de fosfato	10 mL 10 mL	2 ciclos de 30 min	(Fontes et al., 2021)
Glifosato y sus metabolitos	Pez entero	1.0 w.w.	Metanol acidificado/agua desionizada acidificada	3 mL/7 mL	15 min	(Chiesa et al., 2019)
21 PFAS	Peces (músculo)	1.0 w.w.	sulfato de hidrógeno de tetra butil amonio + 0.25 M de carbonato de sodio/bicarbonato de sodio	1 mL + 2mL	10 min	(Y. M. Lee et al., 2020)
10 PFAS	Peces (branquias, músculo, estómago, hígado)	1.0 w.w.	Acetonitrilo	2 mL	10 min	(J. Liu et al., 2019)
14 PFAS	Almeja asiática, cangrejos de río, peces, moluscos (tejido blando)	1.0 w.w.	Hidróxido de sodio/metanol	8 mL	30 min	(Penland et al., 2020)
PFOS	Almeja asiática (branquias, manto, músculos aductores, masa visceral)	0.2 w.w.	Sulfato de hidrógeno de tetra butil amonio 0.5 M + carbonato de sodio 0.25 M + metil-terc-butil éter	1 mL + 2 mL + 5 mL	2 ciclos de 20 min	(Y. Liu et al., 2020)
29 PFAS	Peces, cangrejos de rio (hígado, músculo)	2.0 d.w.	Acetonitrilo	5 mL + 4 mL	2 ciclos de 30 min	(Langberg et al., 2020)
7 OPFRs y sus metabolitos	Peces (músculo, hígado, riñón, gónadas, branquias, cerebro, intestino, suero)	0.3 d.w.	Acetonitrilo	5 mL	2 ciclos de 5 min	(Tang et al., 2019)
7 OPFRs	Cangrejos de río (exoesqueleto-cabeza, exoesqueleto-espalda, músculo abdominal, hepatopáncreas)	0.5 w.w.	Diclorometano	8 mL	15 min	(Peng et al., 2024)
112 PhACs, 60 plaguicidas, 4 compuestos de uso industrial, 4 PFAS	Pez (músculo)	0.5 d.w.	Acetonitrilo frío	10 mL	2 min	(Musatadi et al., 2020)

Tabla 3.1. *Continuación.*

Compuestos analizados	Matriz	Cantidad de muestra (g)	Disolvente	Cantidad de disolvente	Tiempo en ultrasonidos	Referencia
4 plaguicidas, 1 compuesto de uso industrial, 4 PFAS, 30 PhACs	Peces (músculo, hígado, branquias, cerebro, plasma, bilis) y mejillones (músculo)	0.5 d.w.	Metanol/agua milliQ	7 mL	0.5 min en baño de agua a 0 °C	(Mijangos et al., 2019)
21 PFAS, 9 OPFRs	Peces (pez entero)	1.0 w.w.	Metanol	10 mL	3 ciclos de 15 min	(Lorenzo et al., 2019)
1 PhACs, 11 plaguicidas, 1 compuestos de uso industrial, 11 EDCs[a]	Peces, ostras, cangrejos	2.0 w.w.	Acetonitrilo	8 mL	10 min	(Azzouz et al., 2019)
107 compuestos (plaguicidas, PhACs y drogas ilícitas)	Crustáceos	0.2 d.w.	Acetonitrilo/agua acidificado	2 mL	15 min	(Miller et al., 2019)
7 compuestos de uso industrial 6 PhACs	Mejillones	0.5 d.w.	Acetonitrilo + metanol	2 mL + 2 mL	2 ciclos de 5 min	(García-Fernández et al., 2022)
23 OCPs (incluidos plaguicidas), 8 PCBs, 35 PAHs	Mejillones	0.5 w.w.	Agua + Diclorometano/metanol Hexano/metanol	15 mL + 3 mL 3 mL	2 ciclos de 30 min	(J. Lee et al., 2020)

[a] Disruptores endocrinos (del inglés Endocrine disruptors)

w.w: peso húmedo; d.w: peso seco

En los métodos desarrollados, los d-SEP seleccionados fueron los de Supelco, quienes lanzaron al mercado los adsorbentes Z-Sep (gel sílice recubierta con óxido de circonio ZrO) y Z-Sep+ (una combinación de ZrO y C_{18}) como alternativas al uso de PSA (Fig. 3.2). Estas fases dispersivas son más efectivas que los adsorbentes tradicionales en la extracción de grasa y pigmentos y ofrecen una mayor recuperación de analitos y reproducibilidad (Rejczak & Tuzimski, 2015).

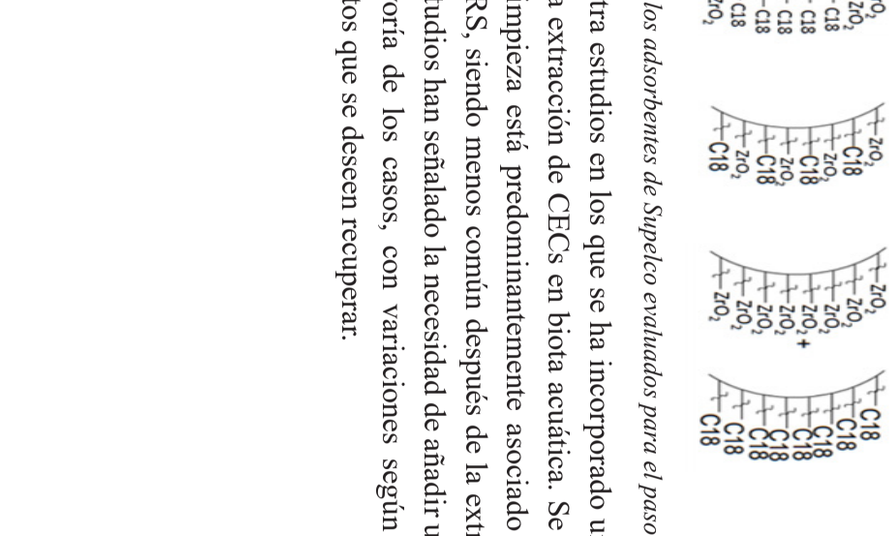


Fig. 3.2. *Estructuras de los adsorbentes de Supelco evaluados para el paso de limpieza*

La Tabla 3.2 muestra estudios en los que se ha incorporado un paso de limpieza con d-SPE durante la extracción de CECs en biota acuática. Se destaca que el uso de esta técnica de limpieza está predominantemente asociado con el método de extracción QuEChERS, siendo menos común después de la extracción por UAE o SLE. Además, los estudios han señalado la necesidad de añadir un paso de limpieza adicional en la mayoría de los casos, con variaciones según el tipo de matriz analizada y los analitos que se deseen recuperar.

Tabla 3.2. *Estudios en los que se ha usado d-SPE cómo técnica de limpieza para el análisis de CECs en biota acuática entre 2019 – 2024.*

Compuestos	Matriz	Cantidad de muestra (g)	Método de extracción	Tipo de d-SEP	Limpieza adicional	Referencia
18 PhACs	Peces (músculo)	2.0 w.w.	UAE	C18 asistida por CAFS	0.5 mL agua acidificada + filtración con membrana 0.22µm	(Mu et al., 2022)
23 PhACs	Pez (músculo)	1.0 w.w.	QuEChERS	Z-Sep^{+}	No	(Kalogeropoulou et al., 2021)
47 PhACs	Pez (músculo)	0.5 w.w.	UAE	Z-Sep/C18	No	(Peña-Herrera et al., 2020)
21 PhACs	Pez (músculo)	1.0 d.w.	QuEChERS	Material lipídico EMR[a]	Eliminación de agua con cloruro de sodio y sulfato de magnesio	(Peña-Herrera et al., 2019)
52 PhACs	Peces, ostras y camarones (músculo, tejido blando)	1.0 w.w.	QuEChERS	PSA, C18 y sulfato de magnesio	Filtración con filtro de acetato de celulosa de vidrio	(Kim et al., 2023)
11 PhACs	Pez (músculo), bivalvos	0.5 d.w.	QuEChERS	Sulfato de magnesio + Z-Sep	No	(Mello et al., 2022)
24 PhACs	Almejas	1.0 w.w.	QuEChERS	d-SPE P/N: JO4020	5 mL n-hexano + vórtex	(Rodrigues et al., 2019)
35 drogas ilícitas	Mejillones (tejido blando)	10 w.w.	QuEChERS	PSA, C18 y sulfato de magnesio	Almacenamiento y centrifugación a -20 °C por 4 horas para precipitar las sales restantes y separar lípidos	(López-García et al., 2019)
52 plaguicidas	Peces (músculo)	10 -	QuEChERS	PSA, C18 y sulfato de magnesio	No	(Barbieri et al., 2019)
2 PFAS	Peces (músculo, cerebro, hígado, riñón)	0.25 d.w.	SLE	PSA y C18	No	(Vidal et al., 2019)
12 PFAS	Almeja asiática (tejido blando)	0.2 w.w.	Micro-QuEChERS	PSA, C18, GCB	Congelamiento de la capa acuosa + SPE (Captiva-EMR) + d-SPE + filtración con filtro de jeringa de nailon	(Koban et al., 2024)
7 OPFRs y sus metabolitos	Peces (músculo, hígado, riñón, gónadas, branquias, cerebro, intestino, suero)	0.3 d.w.	UAE	PSA, C18	Cartuchos de Florisil	(Tang et al., 2019)
8 PhACs, 5 plaguicidas, 5 PFAS, 2 drogas ilícitas	Peces (músculo, hígado)	1.0 w.w.	QuEChERS	EMR-lipid	Filtración con filtros de nailon	(Vitale et al., 2021)
11 PhACs, 5 plaguicidas, 5 PFAS, 2 drogas ilícitas	Mejillones	1.0 w.w.	QuEChERS	Z-Sep^{+} Negro de carbón grafitado	No	(Álvarez-Ruiz et al., 2021)
7 compuestos de uso industrial 6 PhACs	Mejillones	0.5 d.w.	UAE	C18	Sonicación 5 min	(García-Fernández et al., 2022)

[a] EMR del inglés Enhanced Matrix Removal

3.2. Metodología y resultados

Con el objetivo de ampliar el trabajo previamente realizado en nuestro grupo de investigación, en el cual se validó un método de extracción para el análisis de 47 PhACs (principalmente antidepresivos) en músculo de peces (Peña-Herrera et al., 2020), se desarrolló un método nuevo basado en la combinación de la extracción mediante UAE y limpieza con d-SPE para la determinación de 81 PhACs.

El método se diseñó para el análisis de diferentes órganos y fluidos corporales de peces de agua dulce incluyendo piel, hígado, riñón, músculo, corazón, cerebro, plasma y bilis. Los resultados se presentan en la Publicación No 1: ***Manjarrés, D. P.,*** *Montemurro, N., & Pérez, S. (2022). Development of a USE/d-SPE and targeted DIA-Orbitrap-MS acquisition methodology for the analysis of wastewater-derived organic pollutants in fish tissues and body fluids. MethodsX, 9, 101705. https://doi.org/10.1016/J.MEX.2022.101705*

Debido a que los resultados obtenidos tras la validación del método fueron satisfactorios para la mayoría de los compuestos y matrices, se decidió extender su aplicación a diferentes especies acuáticas, incluyendo almejas, cangrejos de río y peces enteros pequeños homogenizados. El objetivo fue incluir en el análisis especies invasoras que podrían mostrar la presencia de una mayor variedad de compuestos además de PhACs, como OPFRs, PFAS y plaguicidas, debido a que estas especies se caracterizan por su gran capacidad de adaptación y tolerancia a la contaminación.

En este contexto, el método inicialmente desarrollado fue optimizado para garantizar la recuperación de analitos de diferentes propiedades físicoquímicas en matrices con diversos contenidos lipídicos. Para ello, se evaluaron tres solventes de extracción y tres d-SPE para la eliminación de co-extractos de la matriz y limpieza de los extractos. Los resultados obtenidos se presentan en la Publicación No. 2: ***Manjarrés-López, D. P.,*** *Vitale, D., Callejas-Martos, S., Usuriaga, M., Picó, Y., Pérez, S., & Montemurro, N. (2023). An effective method for the simultaneous extraction of 173 contaminants of emerging concern in freshwater invasive species and its application. Analytical and Bioanalytical Chemistry. https://doi.org/10.1007/s00216-023-04974-3*

3.2.1. PUBLICACIÓN No. 1

Development of a USE/d-SPE and targeted DIA-Orbitrap-MS acquisition methodology for the analysis of wastewater-derived organic pollutants in fish tissues and body fluids

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Method Article

Development of a USE/d-SPE and targeted DIA-Orbitrap-MS acquisition methodology for the analysis of wastewater-derived organic pollutants in fish tissues and body fluids

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A B S T R A C T

Pharmaceuticals (PhACs) are partially removed during wastewater treatment and end up in the receiving waters. As a result, aquatic biota is continuously exposed to a wide range of potentially hazardous contaminants such as PhACs. Therefore, fish could be a good bio indicator to give a direct measure of the occurrence of PhACs in the aquatic environment. In this study, a robust analytical method has been optimized and validated for the determination of 81 organic compounds, mainly PhACs, in seven wild fish tissue types (liver, muscle, pancreas, kidney, skin, heart, and brain) and two body fluids (plasma and bile). Solid samples extraction was performed combining a procedure based on bead beating tissue homogenization plus ultrasound extraction followed by dispersive solid-phase extraction (dSPE) clean-up using zirconia and C18 sorbents for solid matrices, whereas bile and plasma were diluted.

The key aspects of this method are:

• The method facilitated the simultaneous extraction of 81 PhACs of a wide range of polarity (logP from -4.9 to 5.6) in tissues with variable lipid content.

• The validation was performed using *Cyprinus carpio* at 20 ng g^{-1} and 200 ng g^{-1} for solid tissues, 50 ng mL^{-1} and 500 ng mL^{-1} for plasma, and 100 ng mL^{-1} and 1000 ng mL^{-1} for bile. Analyte detection was performed in LC-HRMS Q-Exactive Orbitrap system with full scan and targeted data-independent acquisition (DIA) mode for high-confidence and sensitive quantitation in either (+) or (-) ESI mode.

• The majority of compounds presented recoveries between 40% and 70% and relative standard deviations (RSD) below 30%.

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ARTICLE INFO
Method name: * ***METFISH*: Analysis** method of **PHA**Cs from **FIsH** t**ISS**ues/organs by USE/d-SPE coupled with targeted D**IA-MS** acquisition
Keywords: Pharmaceuticals: Fish organs, LC-HRMS, Targeted data independent acquisition
Article history: Received 10 December 2021; Accepted 11 April 2022; Available online 16 April 2022

Specifications table

Subject Area	Chemistry
More specific subject area	Environmental Analytical Chemistry – Organic contaminants
Method name	* ***METFISH*: Analysis** method of **PHA**Cs from **FIsH** t**ISS**ues/organs by USE/d-SPE coupled with targeted D**IA-MS** acquisition
Name and reference of original method	Peña-Herrera J.M., Montemurro N., Barceló D., Pérez S. Combining quantitative and qualitative approaches using Sequential Window Acquisition of All Theoretical Fragment-Ion methodology for the detection of pharmaceuticals and related compounds in river fish extracted using a sample miniaturized method. Journal of Chromatography A, 461009 (2020) [1]. Peña-Herrera J.M., Montemurro N., Barceló D., Pérez S. Analysis of pharmaceuticals in fish using ultrasound extraction and dispersive SPE clean-up on QuE Z-Sep/C18 followed by LC-QToF-MS detection [2]. Aceña, J., Pérez, S., Eichhorn, P. Solé, M., Barceló, D.1. Metabolite profiling of carbamazepine and ibuprofen in *Solea senegalensis* bile using high-resolution mass spectrometry. Anal Bioanal Chem 409, 5441–5450 (2017) [3]. Zhang X., Danaceau J., Chambers E. Quantitative analysis of THC and its metabolites in plasma using Oasis PRiME HLB for toxicology abd forensic laboratories. Waters Corporation (2016) [4].
Resource availability	Thermo Scientific Xcalibur Software v. 4.1.31.9.

*Method details

Currently, there are several analytical methods for the extraction of organic pollutants that mainly focus on their extraction in fish muscle [5–7] or in whole fish homogenate [8–10] because these can be obtained in sufficient amounts even from small species and are easy to produce, respectively. Analyzing a single tissue or homogenate fish does not allow to obtain information on the distribution of the contaminants within the organism. This, however, is important as the analytes may distribute unevenly with lipophilic compounds being more prone to be retained in tissues rich in fat (e.g brain or liver) and hydrophilic ones being more dominant in blood. With this in mind, we present a methodology for the extraction of 81 wastewater-derived organic pollutants, mainly pharmaceuticals and their metabolites, from various fish tissues, plasma, and bile. The extraction was carried out combining bead beating tissue homogenization with ultrasound-solvent assisted extraction solvent followed by a zirconia-based cleanup step (Z-Sep/C18). Separation and quantitation of the analytes were achieved on a Waters Acquity UPLC system coupled with an Orbitrap Q-Exactive™ mass spectrometer. Acquisition was performed in full-scan mode followed by a targeted-DIA-MS where the accurate mass of each compound was fixed in a narrow retention time window. Data analysis and processing were performed with Thermo Scientific Xcalibur v. 4.1.31.9. The proposed extraction method includes easy to follow steps and is efficient in terms of time (preparation of 12 samples in less than two hours) when compared to other techniques for the extraction of PhACs in solid samples such as Solvent Assisted Extraction (ASE) (between 10 and 30 min by sample), Microwave Assisted Extraction (MAE) (between 5 and 20 min) or Soxhlet (360 min) [11].

Chemicals, reagents, and other materials

- *Material for sample preparation:*

BenchMixer XLQ QuEChERS Vortexer (Benchmark Scientific, Sayreville NJ, U.S.)
Knife mill with stainless steel grinding chamber (Grindomix GM 200, Retsch GmbH, Haan, Germany)

Precision balance (Mettler Toledo)
5810 R centrifuge (Eppendorf AG, Hamburg, Germany)
2-mL Eppendorf tubes (Eppendorf AG, Hamburg, Germany)
TissueLyzer LT Sample Disruptor (QIAGEN GmbH, Germany)
Stainless Steel Beads, 5 mm (QIAGEN GmbH, Germany)
Ultrasound bath (Fisherbrand® FB15064, Waltham, MA, USA)
TurboVap® LV (Biotage. Uppsala, Sweden)

- *Reagents for standards and samples preparation:*

LC-MS grade acetonitrile (MeCN) (≥99.9 %) (Merck Darmstadt, Germany)
LC-MS grade methanol (MeOH) (≥99.9 %) (Merck Darmstadt, Germany)
LC-MS grade dimethyl sulfoxide (DMSO) (≥99.9 %) (Merck Darmstadt, Germany)
LC-MS grade HPLC water (H_2O) (Merck Darmstadt, Germany)

- *Reagents for the extraction procedure and clean up step:*

LC-MS grade isopropanol (iPrOH) (≥ 99.9 %) (Merck Darmstadt, Germany)
Hydrochloric acid 0.1 N (Merck, Darmstadt, Germany)
QuE Z-Sep/C18 Supel™ QuE (Supelco/Sigma-Aldrich, St. Louis, MO, USA)

- *Reagents for mobile phases preparation:*

Optima™ LCMS Grade acetonitrile (Fisher Chemical, Fisher Scientific SL, Madrid, Spain)
Optima™ LCMS Grade water (Fisher Chemical, Fisher Scientific SL, Madrid, Spain)
Acetic and formic acids (98 %) (Sigma-Aldrich, St. Louis, MO, USA)
Ammonium acetate (Sigma-Aldrich, St. Louis, MO, USA)
Ammonium fluoride (Fisher Chemical, Fisher Scientific SL, Madrid, Spain)

- *Reference standards:*

Highly pure (> 90 %) reference standards of PhACs and deuterated standards were purchased from Sigma-Aldrich (St. Louis, MO, USA), Toronto Research Chemicals (Toronto, Ontario, Canada), or Santa Cruz Biotechnology (Dallas, TX). Depending on the compound solubility, individual stock solutions (5 mg L^{-1} or 10 mg L^{-1}) were prepared in HPLC water, MeOH, or DMSO. A complete list of all the chemical standards used, their physicochemical properties, and the solvent in which they were prepared are reported in Table S1 of the Supplementary Material. Working solution mixtures used for validation were prepared by serial dilution in MeOH at concentrations of 200 ng mL^{-1} and 2000 ng mL^{-1}, whereas the deuterated standards mixture was prepared by serial dilution in MeOH at a concentration of 1000 ng mL^{-1} and added to the samples as surrogate standards at 12.5 ng mL^{-1} for solid tissues and 25 ng mL^{-1} for body fluids. All solutions were stored at −20 °C.

Sampling and sample pre-treatment

The solid tissues and body fluids used for the method validation and matrix-matched calibration curves were obtained from a pool of carp (*Cyprinus carpio*) acquired from a fish farm. In addition, to determine the method applicability a sampling campaign was carried out in Sant Joan Despí, in the lower part of the Llobregat River (Catalonia, Spain) in accordance with the technical requirements established by the Biodiversity and Animal Protection Service of the Department of Agriculture, Livestock, Fisheries, Food and Environment of the Generalitat of Catalonia. Four fish species (*Anguilla Anguilla, Cyprinus carpio, Barbus graellsii*, and *Liza ramada*) were captured downstream of the WWTP outlet. The wild fishes were caught by electro-fishing (200–350 V, 2–3 A, fully rectified triphasic DC). At the sampling location, blood from each individual (2 mL) was extracted with a syringe containing 10 µL of lithium heparin to avoid clotting. Once in the laboratory, blood plasma was prepared by centrifugation of 1 mL aliquots for 10 min at 2500 rpm and 4 °C. The supernatant was transferred to a clean Eppendorf tube and centrifuged again for 10 min at 2500 rpm and 4 °C. Finally, 500 µL of the resulting plasma was stored at -80 °C until analysis. The fish were transported at 4 °C to the laboratory. Bile was immediately treated and stored at -40 °C, to prevent any resorption or rupture

of the gallbladder. Then the fish were dissected and the organs/tissues of interest were carefully separated. They were pre-homogenized using a knife mill with a stainless-steel grinding chamber [M&M] and stored at -40 °C until use.

Analyte extraction protocol

A pool of the samples from the individuals of *Cyprinus carpio* was made for each matrix, they were analyzed to verify the absence of the target analytes and were used for method validation and for the preparation of matrix-matched calibration curves.

Solid matrices (muscle, brain, kidney, pancreas, liver, skin, heart)

1 Put 0.5 g of fresh pre-homogenized sample in a 2 mL Eppendorf tube.
2 Spike the sample with 12.5 µL of deuterated standard mixture (1000 ng mL^{-1}) used as surrogate standards (12.5 ng mL^{-1} in the final extract). The concentration levels at which the reference standards were spiked for validation are specified in Note #1 (see at the end of this section).
3 Vortex the sample for 90 s using the BenchMixer XLQ QuEChERS Vortexer.
4 Let the tube rest for 30 min.
5 Add two stainless steel beads to the tube and homogenize for 90 s (skin: 180 s) in a TissueLyzer Sample Disruptor set at 50 Hz.
6 Vortex the Eppendorf tube again for 60 s using the BenchMixer XLQ QuEChERS Vortexer.
7 Let the tube equilibrate for 15 min.
8 Add 1 mL of the extraction solvent: MeCN/iPrOH (3:1) + 0.1% formic acid.
9 Vortex for 60 s.
10 Sonicate for 10 min.
11 Centrifuge for 12 min at 14000 rpm and -2 °C.
12 Transfer 750 µL of the supernatant to a vial containing 70 mg QuE Z-Sep/C18 sorbent for clean-up.
13 Vortex for 60 s. It is recommended not to exceed the indicated time due to possible hydrolysis of polar compounds.
14 Centrifuge again for 6 min at 14000 rpm and -2 °C.
15 Transfer 500 µL of the supernatant to a 2 mL glass HPLC vial.
16 Evaporate to dryness under a gentle stream of nitrogen.
17 Reconstitute with 500 µL of 5 mM ammonium acetate/MeCN (9:1) and vortex for 60 s prior to injection into the HPLC-ESI-HRMS.

Plasma

1 Put 100 µL of plasma in an Eppendorf tube.
2 Add 387.5 µL of cold MeCN and spike the sample with 12.5 µL of deuterated standard mixture (1000 ng mL^{-1}) for its use as surrogate (corresponding to 25 ng mL^{-1} in the final extract). The concentration levels at which the reference standards were spiked for validation are specified in Note #2.
3 Vortex for 60 s.
4 Centrifuge at for 10 min at 8000 rpm and 4 °C
5 Transfer 400 µL of the supernatant to a glass HPLC vial.
6 Evaporate to dryness under a gentle stream of nitrogen.
7 Reconstitute with 500 µL of 5 mM ammonium acetate /MeCN (9:1) and vortex for 60 s prior to injection into the HPLC-ESI-HRMS.

Bile

1 Put 50 µL of bile in an Eppendorf tube.

2 Add 300 µL of HCl 0.1 N, 137.5 µL of cold MeCN and spike the sample with 12.5 µL of deuterated standard mixture (1000 ng mL^{-1}) for its use as surrogate (corresponding to 25 ng mL^{-1} in the final extract). The concentration levels at which the reference standards were spiked for validation are specified in Note #3.
3 Vortex for 60 s.
4 Centrifuge for 10 min at 14000 rpm and 4 °C.
5 Transfer 300 µL of the supernatant to a glass HPLC vial and add 200 µL of acetone to facility the evaporation.
6 Evaporate to dryness under a gentle stream of nitrogen.
7 Reconstitute with 500 µL of 5 mM ammonium acetate /MeCN (9:1).

***Note* #1:** For the validation method, solid tissues samples were spiked with 50 µL of 200 ng mL^{-1} or 2000 ng mL^{-1} reference standard mixture (corresponding 20 ng g^{-1} (level 1) or 200 ng g^{-1} (level2) fresh weight, respectively).

Note #2: For plasma validation 100 µL of the sample was added at 375 µL of cold MeCN and 25 µL of 200 ng mL^{-1} or 2000 ng mL^{-1} reference standard mixture for two levels of validation (corresponding to 50 ng mL^{-1} (level 1) and 500 ng mL^{-1} (level 2) respectively).

Note #3: For bile validation 50 µL of the sample was added at 300 µL of HCl 0.1 N, 125 µL of cold MeCN, and 25 µL of 200 ng mL^{-1} or 2000 ng mL^{-1} reference standard mixture (corresponding to 100 ng mL^{-1} (level 1) and 1000 ng mL^{-1} (level 2), respectively).

Instrumental analysis

Chromatographic separation was carried out with a Waters Acquity UPLC system (Water, Milford, MA) under the conditions shown in Table 1. Detection was performed using an Orbitrap Q-Exactive™ mass spectrometer (Thermo Fischer Scientific, San Jose, CA, USA) and the acquisition was in full-scan mode followed by a targeted DIA that combines the monoisotopic mass of each compound with short retention time (RT) windows to obtain high-quality confirmation fragment ions (MS2). For this, an inclusion list was prepared containing the *m/z* of all the precursor ions of interest, and the MS2 scanning events were carried out sequentially through selected RT windows associated with each precursor ion. The MS parameters for the selected method are listed in Table 1. Furthermore, an inclusion list with accurate mass and RT window for each target compound was used to generate as many micro-scanning events as shown in Table S2.

Data analysis and processing were performed with Thermo Scientific Xcalibur v. 4.1.31.9. For the quantification, matrix-matched calibration curves were prepared at 8 concentration levels in the range of 0.5 ng mL^{-1} to 500 ng mL^{-1}, including deuterated standard concentration at each point at 12.5 ng mL^{-1} for solids tissues and 25 ng mL^{-1} for body fluids. The calibration curve was constructed by linear weighted least-squares regression (1/x as weighting factor). The deuterated standards associated for each compound are shown in Table S1.

Method validation

According to [12] the performance of the extraction method (n=3) was evaluated in terms of accuracy (recovery study), precision (% RSD), matrix effect (ME), limits of detection (LOD), and limits of quantification (LOQ) for all matrices. Recovery studies were performed in triplicate at 50 ng mL^{-1} and 500 ng mL^{-1} for plasma, 100 ng mL^{-1} and 1000 ng mL^{-1} for bile, and 20 ng g^{-1} and 200 ng g^{-1} for solid matrices.

Extraction efficiency

In order to evaluate the extraction efficiency, relative recoveries were determined by comparing the peak area of the samples spiked before the extraction method, to the peak area of the ones spiked after the extraction method. The blank extract area (sample without spiking) was subtracted, as shown

Table 1
Analytical platform and respective conditions.

Waters Acquity I-Class UPLC®	
Columns	Phenomenex Reversed-phase EVO C18 KINETEX column (100 × 2.1 mm, 2.6 μm) supplied with precolumn Phenomenex p/n: AJO-9000
Mobile phase A	ESI (+): 5 mM of ammonium acetate and 0.1% acetic acid ESI (-): 2 mM ammonium fluoride[1]
Mobile phase B	MeCN
Flow rate	0.2 mL min^{-1}
Run time	19 min
Injection volume	10 μL
Gradient profile	(1) 0.0 min 5% A (2) 10.0 min 30% A (3) 13.3 min 65% A (4) 15.5 min 100% A (5) 17.7 min 5% A (6) 19.0 min 5% A
Auto sample Temp.	10°C
Flow diverted to waste	Flow diverted to waste 18.0 - 19.0 min
Weak Needle wash	H_2O:/MeCN (95:5, v/v)
Strong Needle wash	MeOH 100%
Seal wash	H_2O/MeOH (70:30, v/v)
Thermo Orbitrap Q-Exactive™ mass spectrometer	
Spray voltage	3.5 kV (ESI +), -2.5 kV (ESI -)
Sheath gas (N_2 >95%)	35 (ESI +), 25 (ESI -)
Resolving power	70,000 FWHM
Capillary temperature	350°C
Aux Gas (N_2 >95%)	10
Auxiliary gas heater temp	250°C
S-Lens RF Level	60
Overall method settings	
Chrom. Peak width (FWHM)	12 s
Full MS - SIM	
Microscans	1
Resolution	35,000
AGC target	3e6
Maximum IT	150 ms
Number of scan ranges	1
Scan range	90 to 1000 m/z
Spectrum data type	Profile
DIA	
Microscans	1
Resolution	17.500
AGC target,	2e5
Maximum IT	auto
Loop count	1
MSX count	1
MSX isochronus ITs	On
Isolation window	1.5 m/z
Isolation offset	0.0 m/z
Spectrum data type	Profile
(N) CE / stepped (N) CE	nce: 30

[1] Ammonium fluoride was used because this additive improves the negative ionization. This is due to the high electronegativity of the fluoride ion [27]. Sensitivity is enhanced because in the gas phase fluoride ions capture protons from neutral analytes giving to HF and the formation of $[M+F]^-$ and $[M+FHF]^-$ ions clusters [28]. The use of ammonium fluoride has been previously reported by [29] for PhACs analysis.

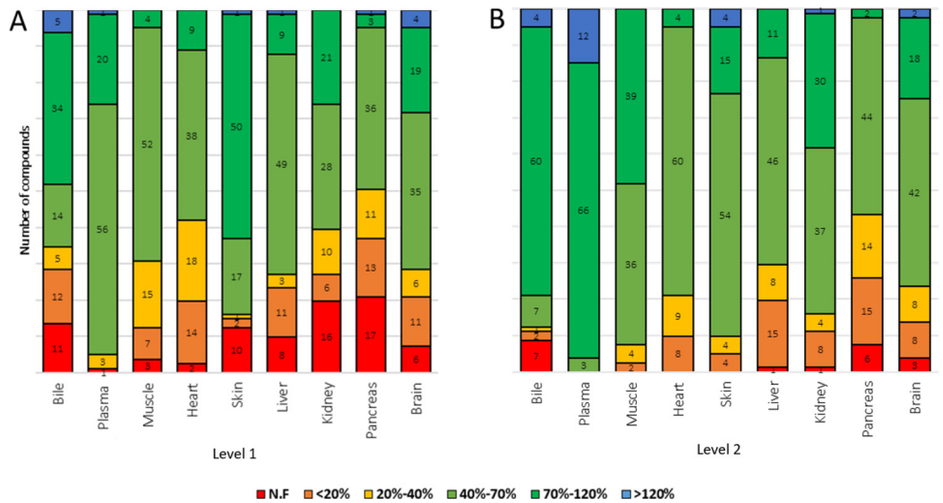

Fig. 1. Recoveries obtained for all matrices at the two validation levels. **A**: Level 1 (100 ng mL^{-1} for bile, 50 ng mL^{-1} for plasma and 20 ng g^{-1} for solid tissues). **B**: Level 2 (1000 ng mL^{-1} for bile, 500 ng mL^{-1} for plasma and 200 ng g^{-1} for solid tissues).

in Eq. (1).

$$RR\ (\%) = \left(\frac{Area_{\ pre-spiked} - Blank\ extract}{Area_{\ post-spiked} - Blank\ extract} \right) x100 \quad (1)$$

Fig. 1 shows the recovery ranges obtained for the 81 compounds. Most compounds displayed recoveries between 40% and 70% for all matrices, except for skin, bile, and plasma where the majority of compounds recovered in the range of 70% to 120%. The high recoveries obtained in bile and plasma can be attributed to the fact that are fewer complex matrices (see Fig. 2). Additionally, the validation levels for these two matrices are greatest (100 ng mL^{-1} and 50 ng mL^{-1} for the level 1 respectively).

At level 2, the number of compounds with recoveries between 70 to 120% increases substantially in bile, plasma, and muscle (see Fig. 1), while the number of compounds not found is smaller. On the other hand, the liver, kidney, and pancreas are more complex matrices where for approximately 30% of the compounds recoveries below 40% were obtained, especially for negative ionization compounds.

Overall, the compounds that obtained the highest average recoveries and were recovered in a percentage higher than 70% in most matrices were sotalol, caffeine, fluconazole, hydrochlorothiazide, and pentobarbital for the first validation level and trimethoprim, acridone, bromazepam, sitagliptin, N-acethyl-smx, CBZ-10,11-epoxide, hydrochlorothiazide, chloramphenicol, pentobarbital, and fipronil for the second validation level.

Flufenamic acid was the only compound not recovered in any matrix for the first level of validation, however, for the second level it was possible to recover it in bile, plasma, muscle, skin, and kidney in a percentage higher than 40%. Atorvastatin was only recovered in bile and plasma for the first level of validation, while in the second level it was recovered in all matrices except the pancreas. Ciprofloxacin and omeprazole only presented acceptable recovery values in plasma for the first level of validation, but at the second level, ciprofloxacin was recovered in all matrices except the brain, and omeprazole was recovered in muscle, heart, skin, and brain with values higher than 45%. In general, oxytetracycline and norfluoxetine were the compounds that presented the lowest recoveries in the two levels of validation for all matrices except in the plasma and skin.

In relation to the compounds with the best recoveries (>90%) obtained in each of the matrices evidenced that in the first validation level in bile are sertraline (99%), ketoprofen (93%), sulfamethazine (99%), nalidixic acid (93%), flumequine (93%), oxazepam (103%), propyphenazone (91%),

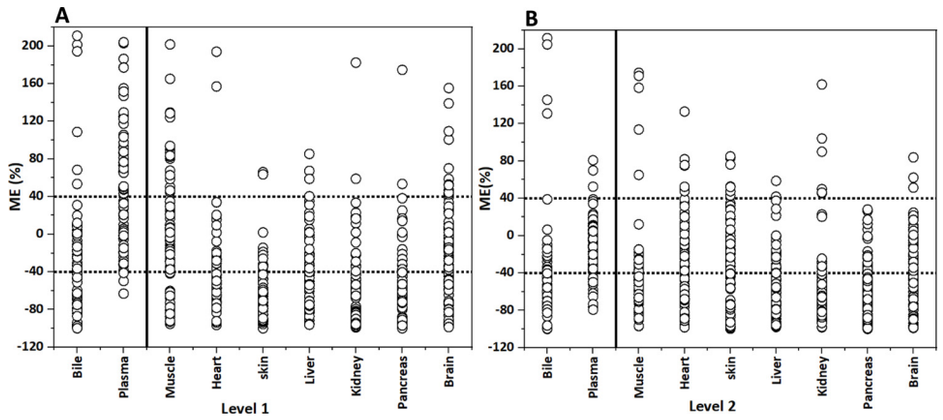

Fig. 2. Matrix effect obtained by all tissues and body fluids for the two validation levels. **A**: Level 1 (100 ng mL^{-1} for bile, 50 ng mL^{-1} for plasma and 20 ng g^{-1} for solid tissues). **B**: Level 2 (1000 ng mL^{-1} for bile, 500 ng mL^{-1} for plasma and 200 ng g^{-1} for solid tissues).

temazepam (94%), pentobarbital (110%), bisphenol A (100%), and atorvastatin (92%). While in plasma, compounds such as sertraline (105%), paroxetine (91%), and norfluoxetine (93%) stand out. The number of compounds with recoveries greater than 90% increased to 49% for the second validation level in bile and to 77% for plasma.

In solid tissues for the first validation level, sertraline and fipronil sulfide stand out in muscle (99%) (95%) and skin (102%) (95%) respectively. In the kidney, the highest recoveries were obtained for temazepam (96%) and diazepam (109%); in the pancreas, acetaminophen (110%) and, in the brain, sulfamethoxazole (101%) and bromazepam (106%). For the second validation level, furazolidone (97%) and erythromycin (105%) stand out in the muscle; fluoxetine (118%), sertraline (106%), loratadine (91%), bromazepam (100%), paroxetine (96%) and norfluoxetine (99%) in the skin and; clarithromycin (110%), diazepam (93%), verapamil (110%), desmethylcitalopram (102%), alprazolam (91%) and atorvastatin (95%) for the kidney. In the heart and in the liver, no compound was recovered a percentage greater than 90% for any of the validation levels. In the case of the liver, the low recoveries may be associated with the fact that this organ, which is the main metabolic organ of fish [13,14], has a higher lipid content than in the whole body homogenates [15,16] and retains a greater amount of blood [17]. The heart also has a high blood content which interferes with the suppression of the analyte signal, as can be seen in Fig. 2.

Matrix effects

To evaluate the matrix effect (suppression or enhancement of the analyte signal), the ratio of the peak area of the analyte spiked into the final extracts (*Area post-spiked*) is compared to the peak area of the analyte in a solvent (*Area in solvent*) at the same concentration. The blank extract area was subtracted according to Eq. (2):

$$ME\ (\%) = 100\ x\left(\frac{Area_{\ post-spiked} - Blank\ extract}{Area\ in\ solvent} - 1\right) \quad (2)$$

For most of the compounds, a strong signal suppression was observed, especially in the heart, kidney, liver, and pancreas, where more than 40% of the compounds presented a suppression of less than -40%. However, for the other matrices, the ME was calculated within an acceptable range, -40% to +40%, with a larger number of compounds within this range for the higher level. Plasma was the only matrix that displayed a strong signal enhancement (>40%) for most of the compounds, but only for the first validation level, since at the second level, more than 70% of the compounds had an acceptable

Table 2
LOD and LOQ estimated for each matrix.

Matrix	LOD (ng g^{-1})	LOQ (ng g^{-1})
Bile[1]	0.02–28.71	0.08-95.69
Plasma[1]	0.01–1.56	0.03–5.20
Muscle	0.01–9.27	0.02–30.90
Heart	0.0004–3.85	0.001–12.82
Skin	0.004–3.46	0.01–11.54
Liver	0.01–2.66	0.03–8.87
Kidney	0.01–14.91	0.02–49.70
Pancreas	0.001–8.55	0.004–28.49
Brain	0.004–14.10	0.01–47.00

[1] Units in ng mL^{-1}.

ME. Most compounds with negative ionization had strong signal suppression (close to -100 %) in the most complex matrices (liver, kidney and, pancreas).

In general, the recoveries obtained for most compounds were between 40% and 70% and the ME was signal suppression of the analytes. Similar recoveries and ME values were obtained by [9] and [15] for liver; by [18] and [19] for muscle and plasma. Matrices such as heart, skin, and pancreas were validated here for the first time.

Detection and quantification limits

The detection and quantification limits were estimated according to [20] based on the average of the signal-to-noise ratio (*S/N*) that the analytes showed in the pre-spiked samples and the spiked samples concentration (*C*), according to Eq. (3) and Eq. (4). Table 2 shows the LOD and LOQ ranges of the extraction method.

$$LOD = \frac{3\ x\ C}{S/N} \quad (3)$$

$$LOQ = \frac{10\ x\ C}{S/N} \quad (4)$$

The highest LOD and LOQ were calculated for oxytetracycline compound. Muscle 9.27 ng g^{-1} and 30.90 ng g^{-1}, heart 3.85 ng g^{-1} and 12.82 ng g^{-1}, kidney 14.91 ng g^{-1} and 49.70 ng g^{-1}, brain 14.10 ng g^{-1} and 47.00 ng g^{-1}, and in bile 10.90 ng mL^{-1} and 36.35 ng mL^{-1}, coinciding with its low percentage recovery in most matrices.

The recoveries, RSDs, ME, LOD and LOQ for the two validation levels for each compound and matrix, are reported in Tables S3, S4, and S5.

Method robustness

According to [12] the method robustness can be expressed as the relative standard deviation (RSD%) of the data obtained. In this study, the robustness was expressed as a function of the calculated RSD of the recoveries (samples analyzed in triplicate) for each validation level. In addition, RSD <30% and recovery >40% for the two validation levels were established as acceptability criteria to define the compounds that can be reliably determined in each matrix. Table 3 shows the compounds that met the range of acceptability for each of the matrices.

For the plasma 78 of the compounds satisfy the established criteria, while in the pancreas the number of compounds within the acceptable range was 33. However, it is important to remark that since our multi-matrix method, it is valid to expect that these values fluctuate from one matrix to another, as a consequence of the intrinsic characteristics of each one, without compromising the robustness of the method.

Table 3
PhACs within acceptable range.[1]

PhACs	Muscle	Heart	Skin	Liver	Kidney	Pancreas	Brain	Plasma	Bile
Benzotriazole	√	√	√	x	x	x	√	√	√
5-methyl-Benzotriazole	√	√	√	√	√	x	√	√	√
Acetaminophen	x	x	√	x	x	x	x	√	x
Acridone	√	√	√	√	√	x	√	√	√
Alprazolam	√	√	√	x	√	x	x	√	√
Amantadine	√	√	√	√	√	√	√	√	√
Atenolol	√	√	√	√	√	√	√	√	√
Atorvastatin	x	x	x	x	x	x	x	√	√
Benzoylecgonine	√	√	√	√	√	√	√	√	√
Bezafibrate	√	√	√	√	√	x	√	√	√
Bisphenol-A	x	x	x	√	x	x	x	√	√
Bromazepam	x	√	√	√	√	√	√	√	√
Caffeine	√	√	√	√	√	√	√	√	√
Carazolol	x	x	√	√	√	x	√	√	x
Carbamazepine	√	√	√	√	√	√	x	√	√
CBZ-10,11-epoxide	√	√	√	√	√	√	√	√	x
Clarithromycin	√	√	√	x	√	√	√	√	x
Chloramphenicol	√	√	√	√	√	x	√	√	√
Ciprofloxacin	x	x	x	x	x	x	x	√	√
Clofibric_Acid	√	√	√	√	√	√	√	√	√
Coca-ethylene	√	√	√	x	√	√	x	√	x
Cocaine	√	√	√	x	√	x	√	√	x
Codeine	√	√	√	√	√	x	√	√	√
Cotinine	√	√	√	√	x	x	√	√	√
Desmethylcitalopram	√	x	x	x	√	x	x	√	x
Diazepam	√	√	√	x	√	x	x	√	√
Diclofenac	x	x	x	√	x	x	√	√	x
Diltiazem	√	√	√	√	√	√	x	√	x
Erythromycin	√	√	√	√	√	x	√	√	x
Fipronil	√	√	√	x	√	x	x	√	√
Fipronil_Desulfinyl	x	x	x	x	√	x	x	√	x
Fipronil_Sulfide	x	x	√	x	x	x	x	√	x
Fipronil_sulfone	√	x	x	x	x	x	x	√	x
Fluconazole	√	√	√	√	√	√	√	√	√
Flufenamic_Acid	x	x	x	x	x	x	x	x	x
Flumequine	x	√	x	x	x	√	x	√	√
Fluoxetine	√	x	√	√	√	x	√	√	x
Norfluoxetine	x	x	x	x	x	x	x	√	x
Furazolidone	√	√	√	x	√	√	√	√	√
Furosemide	x	x	√	√	x	x	√	√	√
Hydrochlorothiazide	√	√	√	√	√	√	√	√	√
Ibuprofen	√	√	√	√	x	√	x	√	√
Irgasan	x	x	√	x	x	x	x	√	x
Ketoprofen	√	√	√	√	√	√	√	√	√
Lamotrigine	√	√	√	√	x	x	x	√	√
Loratadine	x	x	√	x	x	x	x	√	√
Lorazepam	√	√	√	√	√	x	√	√	x
Losartan	x	x	x	x	x	x	x	√	√
Mefenamic acid	x	x	x	x	x	x	√	√	x
Metformin	x	√	√	x	x	x	√	x	x
Metoprolol	√	√	√	√	√	√	√	√	√
Midazolam	x	x	√	x	√	x	x	√	x
Morphine	x	x	x	x	x	x	x	√	√
Nadilixic Acid	x	x	x	x	x	√	x	√	√
Omeprazole	x	x	x	x	x	x	x	√	x
Oseltamivir_phospate	√	x	√	√	√	x	√	√	x
Oseltamivir-CBX	√	√	x	√	√	√	x	√	√
Oxazepam	x	√	√	√	√	√	√	√	√
Oxytetracycline	x	x	x	x	x	x	x	x	x

(*continued on next page*)

Table 3 (*continued*)

PhACs	Muscle	Heart	Skin	Liver	Kidney	Pancreas	Brain	Plasma	Bile
Paroxetine	x	x	√	x	x	x	x	√	x
Pentobarbital	√	√	√	√	√	√	√	√	√
Propyphenazone	√	√	√	√	√	√	√	√	√
Salbutamol	x	√	√	√	√	√	√	√	√
Sertraline	√	√	√	√	√	√	√	√	√
Sitagliptin	√	√	√	√	√	x	√	√	x
Sotalol	√	√	√	√	√	√	√	√	√
Sulfadimethoxine	√	x	√	√	x	x	√	√	√
Sulfamethazine	√	x	√	√	x	x	√	√	√
Sulfamethoxazole	√	x	√	√	x	x	√	√	√
N-acethyl_SMX	√	√	√	√	√	√	√	√	√
Sulfapyridine	√	x	√	√	x	x	√	√	√
Temazepam	√	√	√	√	√	√	x	√	√
Tramadol	√	√	√	√	√	√	√	√	x
Triclocarban	x	x	√	x	x	x	x	√	x
Trimethoprim	√	√	√	√	√	√	√	√	√
Valsartan	√	x	√	√	x	x	x	√	√
Valsartan Acid	x	x	x	x	x	x	x	√	√
Venlafaxine	√	√	√	√	√	√	√	√	x
O-Desmethylvenlafaxine	√	√	√	√	√	√	√	√	√
Verapamil	x	x	√	x	√	√	x	√	√
Warfarin	√	√	√	√	√	x	√	√	√
Total of compounds with acceptable range (√)	**52**	**48**	**63**	**49**	**49**	**33**	**47**	**78**	**52**

[1] The parameters for which an acceptable range was determined were that the compound obtained a recovery greater than 40% and an RSD less than 30% for the two validation levels. (√) Meets acceptable range. (*x*) Does not meet acceptable range.

Occurrence of the target analytes in fish samples

In order to evaluate the applicability of the validated method, tissues and body fluids from 4 wild fish (*Cyprinus carpio, Liza ramada, Barbus graellsii, and Anguilla Anguilla*) were analyzed. Quantification was performed by the internal standard method with matrix-matched calibration standards. The present method compensates for recovery losses and ionization suppression/enhancement effects [21,22]. Therefore, the deuterated standards were spiked as surrogates at the beginning of the extraction at a concentration of 12.5 ng mL^{-1} for solid tissues and 25 ng mL^{-1} for body fluids as previously indicated.

As a result, 11 PhACs were detected. The kidney and pancreas presented the higher detection frequency with 12 and 10 positive detections, respectively. Sertraline was detected in all individuals and in all matrices except bile, with a concentration range between 5.7 ng g^{-1} (heart) and 1010.2 ng g^{-1} (pancreas). Similar concentrations of sertraline in fish tissues have been reported previously. A maximum concentration (cmax) of 110 ng g^{-1} in the brown trout liver collected downstream of a WWTP in the USA has been reported by [23]. A cmax of 44 ng g^{-1} in the kidney of brown trout caught in a river in the southern Czech Republic was reported by [24]. Diltiazem and venlafaxine were detected in most matrices although only in *Liza ramada*. Diltiazem concentrations were less than 10 n g^{-1} in all matrices (brain, kidney, liver, pancreas and plasma), whereas venlafaxine was quantified in a range from 27.4 ng g^{-1} (skin) to 153.8 ng g^{-1} (brain). The presence of diltiazem has been reported by other authors [25] in the brain of fish with a Cmax of 1.5 ng g^{-1} and a Cmax of 34 ng g^{-1} in the muscle of *Leuciscus cephalus* captured from the Sava river in Slovenia has been reported by [5]. Othe authors [26] reported a Cmax of 20.8 ng g^{-1} for venlafaxine in the liver of rainbow trout exposed to undiluted effluent from a Swedish municipal WWTP for 13 days.

Acridone (643.5 ng mL^{-1}), caffeine (50.2 ng g^{-1}), ketoprofen (1.1 ng g^{-1}), and loratadine (3.0 ng mL^{-1}) were only detected in one matrix (bile, brain, kidney, heart, and plasma) respectively. Fluoxetine was detected in the brain (5.2 ng g^{-1}) which was also detected in the kidney (22.9 ng g^{-1}).

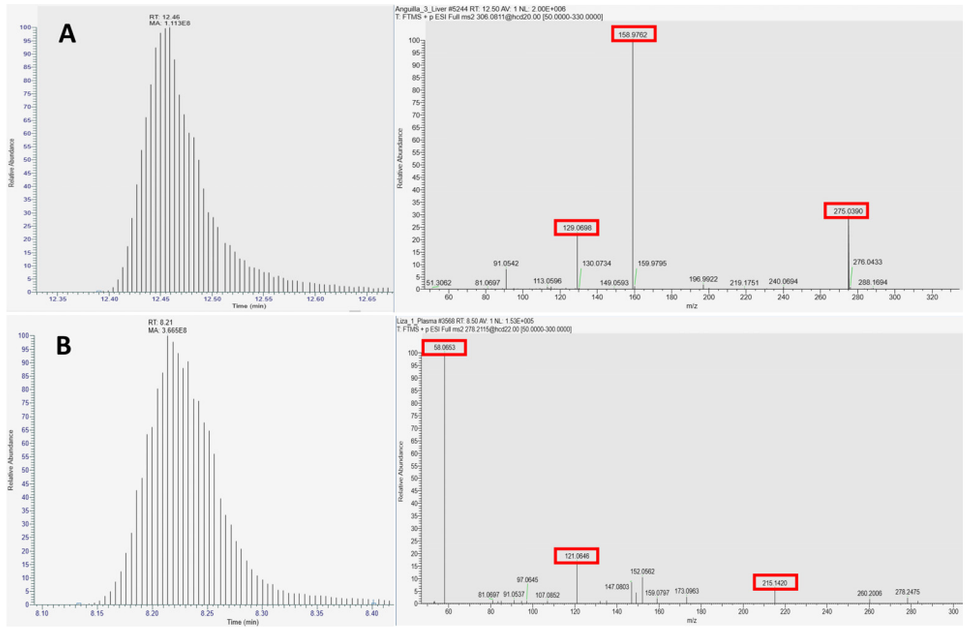

Fig. 3. Chromatograms and their corresponding MS2 spectra for two of the compounds detected in fish tissues and body fluids. Characteristics product ions are highlighted in a red box. **A**: Chromatogram and MS2 spectra for venlafaxine detected in *Anguilla anguilla* liver. **B**: Chromatogram and MS2 spectra for sertraline detected in *Liza ramada* plasma.

Clarithromycin was detected in the kidney (2.5 ng g^{-1}) and pancreas (6.8 ng g^{-1}). Metoprolol was detected in kidney (105.5 ng g^{-1}), skin (13.6 ng g^{-1}) and heart (1.3 ng g^{-1}) and sotalol was detected in skin (4.3 ng g^{-1}), liver (5.4 ng g^{-1} to 7.9 ng g^{-1}), pancreas (3.1 ng g^{-1}) and plasma (<LOQ). Fig. 3 shows chromatograms and their corresponding MS2 spectra for two of the compounds detected in fish tissues and body fluids.

Conclusions

Overall, the extraction method developed showed recoveries between 40% and 70 % and satisfactory analytical parameters for most of the validated fish tissues/organs and for a wide range of compounds with different physicochemical properties. This method will allow the evaluation of the distribution of PhACs in fish exposed to wastewater and select a subset of matrices that provide sensitive detection of these compounds in unprotected fish.

Tissues and body fluids from four fish species were analyzed. Due to the complexity of the matrices, especially the pancreas, liver, and heart (which presented the lowest recovery values and highest analyte suppression), it was decided to perform the quantification with the internal standard method with deuterated standards as surrogates at the beginning of the extraction and to use matrix-matched calibration to ensure the reliability of the calculated data. The application of this approach together with the extraction method developed allowed the detection and quantification of 11 PhACs in similar concentrations to previous studies.

Declaration of Competing Interests

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2022.101705.

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INFORMACIÓN COMPLEMENTARIA
PUBLICACIÓN No. 1

Development of a USE/d-SPE and targeted DIA-Orbitrap-MS acquisition methodology for the analysis of wastewater-derived organic pollutants in fish tissues and body fluids

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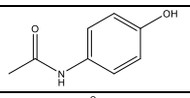
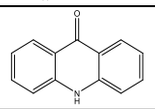
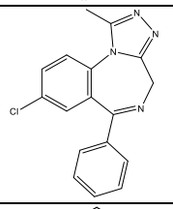
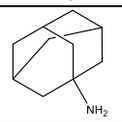
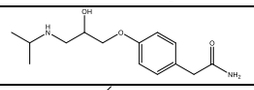
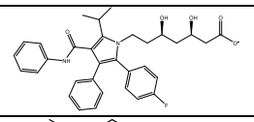
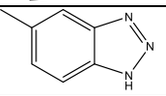
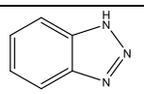
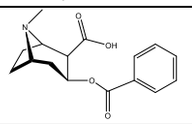
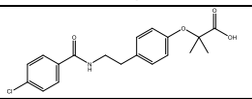
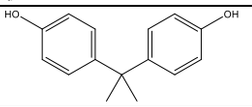
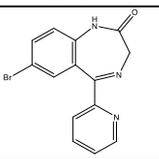
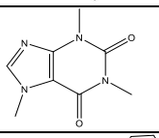
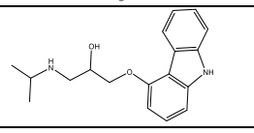
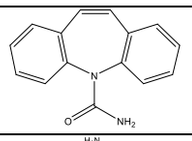
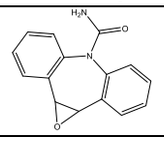
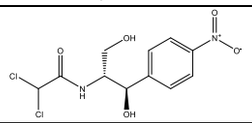
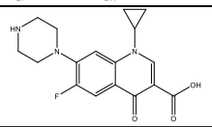
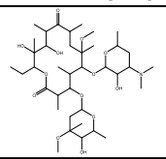
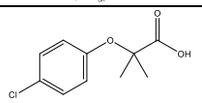
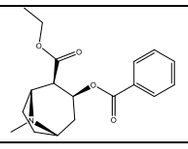
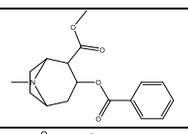
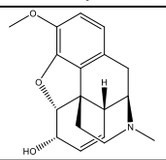
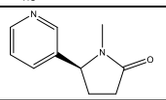
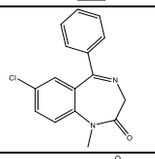
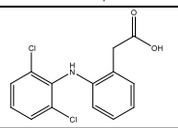
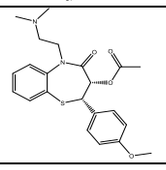
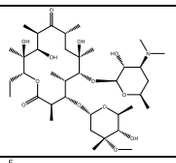
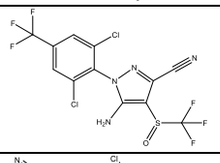
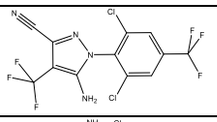
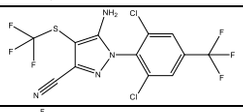
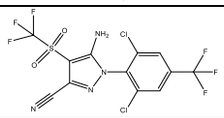
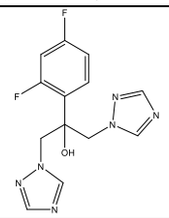
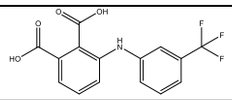
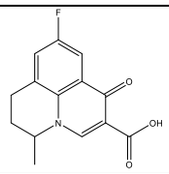
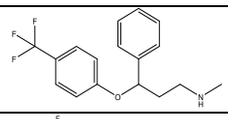
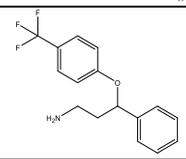
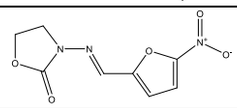
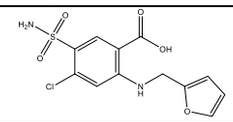
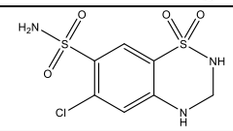
Table S1 Target compounds for the method validation

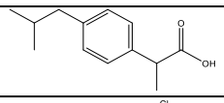
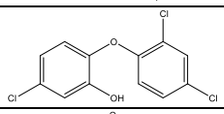
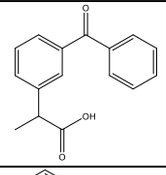
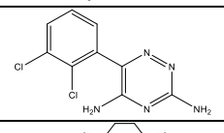
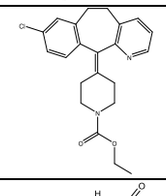
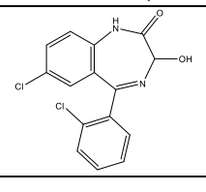
PhACs	Classification ATC[1]	CAS number[1]	Formula	Molecular weigth (g mol[1])[1]	Log P[2]	Standard preparation solvent[3]	Estructure	Associated deuterated standar[4]
Acetaminophen	Analgesic	103-90-2	$C_8H_9NO_2$	151.16	0.91	MeOH		Acetaminophen-d4
Acridone	Anticancer activity	578-95-0	$C_{13}H_9NO$	195.22	4.20	DMSO		Carbamazepine-d10
Alprazolam	Anxiolytic	28981-97-7	$C_{17}H_{13}ClN_4$	308.77	3.02	MeOH		Alprazolam-d5
Amantadine	Parkinson's treatment	768-94-5	$C_{10}H_{17}N$	151.14	1.47	MeOH		Erythromycin-13CD3
Atenolol	Cardiovascular system	29122-68-7	$C_{14}H_{22}N_2O_3$	266.34	0.43	MeOH		Codeine-d3
Atorvastatin	Lipid regulator	134523-00-5	$C_{33}H_{35}FN_2O_5$	558.64	5.39	MeOH		Lorazepam-d4
5-methyl-benzotriazole	Metabolit of benzotratzole	136-85-6	$C_7H_7N_3$	133.15	1.81	MeOH		Benzotriazole-d4
Benzotriazole	Antibacterial antifungal	95-14-7	$C_6H_5N_3$	119.12	1.30	MeOH		Benzotriazole-d4
Benzoylecgonine	Metabolit of cocaine	519-09-5	$C_{16}H_{19}NO_4$	289.33	-0.59	EtOH		Tramadol-13CD3
Bezafibrate	Lipid regulator	41859-67-0	$C_{19}H_{20}ClNO_4$	361.82	3.99	MeOH		Bezafibrate-d4
Bisphenol A	Plastic production	80-05-7	$C_{15}H_{16}O_2$	228.29	4.04	MeOH		Bisphenol A-d8
Bromazepam	Anxiolytic	1812-30-2	$C_{14}H_{10}BrN_3O$	316.15	2.54	MeOH		Carbamazepine-d10
Caffeine	Psychoanaleptic	58-08-2	$C_8H_{10}N_4O_2$	194.19	-0.55	MeOH		Trimethoprim-d3
Carazolol	Veterinary medicine	57775-29-8	$C_{18}H_{22}N_2O_2$	298.38	2.71	DMSO		Venlafaxine-d6

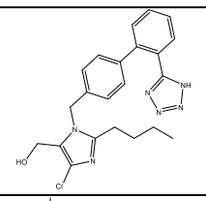
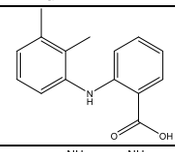
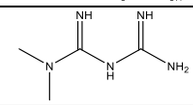
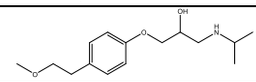
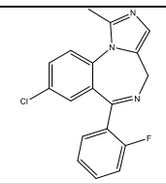
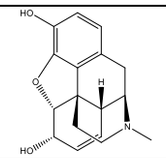
PhACs	Classification ATC[1]	CAS number[1]	Formula	Molecular weigth (g mol[1])[1]	Log P[2]	Standard preparation solvent[3]	Estructure	Associated deuterated standar[4]
Carbamazepine	Antiepileptic	298-46-4	$C_{15}H_{12}N_2O$	236.27	2.77	MeOH		Carbamazepine-d10
Carbamazepine-10,11-epoxide	Metabolite of carbamazepine	36507-30-9	$C_{15}H_{12}N_2O_2$	252.27	1.97	MeOH		Carbamazepine-d10
Chloramphenicol	Antibacterial	56-75-7	$C_{11}H_{12}Cl_2N_2O_5$	323.13	0.88	MeOH		Ibersartan-d6
Ciprofloxacin	Antibacterial	85721-33-1	$C_{17}H_{18}FN_3O_3$	331.35	-0.81	MeOH		Ciprofloxacin-d8
Clarithromycin	Antibacterial	81103-11-9	$C_{38}H_{69}NO_{13}$	747.95	3.24	MeOH		Diazepam-d5
Clofibric acid	Metabolite of clofibrate	882-09-7	$C_{10}H_{11}ClO_3$	214.64	2.90	MeOH		Diclofenac13C6
Cocaethylene	Metabolite of Cocaine	529-38-4	$C_{18}H_{23}NO_4$	317.38	2.64	MeOH		Cocaine-d5

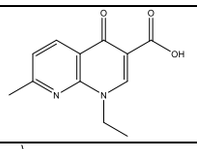
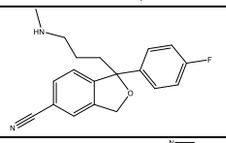
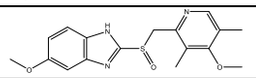
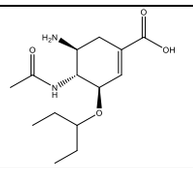
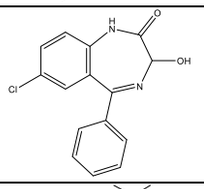
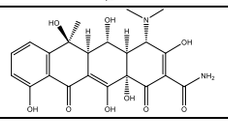
PhACs	Classification ATC[1]	CAS number[1]	Formula	Molecular weigth (g mol[1])[1]	Log P[2]	Standard preparation solvent[3]	Estructure	Associated deuterated standar[4]
Cocaine	CNS stimulant	50-36-2	$C_{17}H_{21}NO_4$	303.35	2.28	MeOH		Cocaine-d5
Codeine	Opiod -Psycholeptic	76-57-3	$C_{18}H_{21}NO_3$	299.36	1.34	MeOH		Codeine-d3
Cotinine	Nervous system drug	486-56-6	$C_{10}H_{12}N_2O$	176.22	0.21	MeOH		Cotinine-d3
Diazepam	Psycholeptic	439-14-5	$C_{16}H_{13}ClN_2O$	284.74	3.08	MeOH		Diazepam-d5
Diclofenac	Antiinflammatory	15307-86-5	$C_{14}H_{11}Cl_2NO_2$	296.15	4.26	MeOH		Diclofenac-13C6
Diltiazem	Calcium Chanel blocker	42399-41-7	$C_{22}H_{26}N_2O_4S$	414.52	2.73	MeOH		Carbamazepine-d10

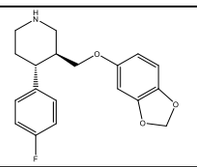
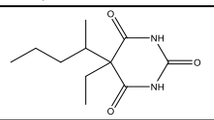
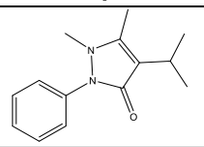
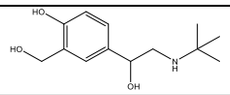
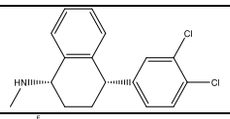
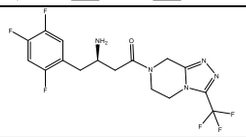
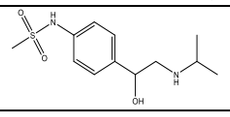
PhACs	Classification ATC[1]	CAS number[1]	Formula	Molecular weigth (g mol[1])[1]	Log P[2]	Standard preparation solvent[3]	Estructure	Associated deuterated standar[4]
Erythromycin	Antibiotic	114-07-8	$C_{37}H_{67}NO_{13}$	733.93	2.60	MeOH		Lorazepam-d4
Fipronil	Insecticide-antiparasitic	120068-37-3	$C_{12}H_4Cl_2F_6N_4OS$	437.15	4.49	MeCN		Fipronil-13C3
Fipronil Desulfinyl	Metabolite of Fipronil	205650-65-3	$C_{12}H_4Cl_2F_6N_4$	389.08	4.53	MeCN		Fipronil-13C3
Fipronil sulfide	Metabolite of Fipronil	120067-83-6	$C_{12}H_4Cl_2F_6N_4S$	421.15	5.62	MeCN		Fipronil-13C3
Fipronil sulfone	Metabolite of Fipronil	120068-36-2	$C_{12}H_4Cl_2F_6N_4O_2S$	453.15	4.60	MeCN		Fipronil-13C3
Fluconazole	Antifungal	86386-73-4	$C_{13}H_{12}F_2N_6O$	306.27	0.56	DMSO		Fluconazole-13C3

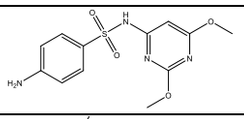
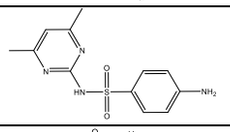
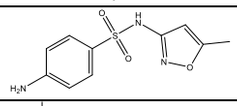
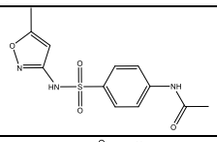
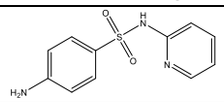
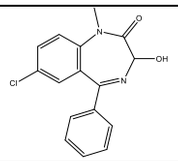
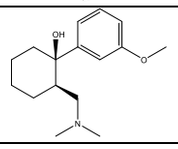
PhACs	Classification ATC[1]	CAS number[1]	Formula	Molecular weigth (g mol[1])[1]	Log P[2]	Standard preparation solvent[3]	Estructure	Associated deuterated standar[4]
Flufenamic acid	Anti-inflammatory	530-78-9	$C_{14}H_{10}F_3NO_2$	281.23	5.25	DMSO		Bromazepam-d4
Flumequine	Antibiotic	42835-25-6	$C_{14}H_{12}FNO_3$	261.25	2.42	MeOH		Carbamazepine-d10
Fluoxetine	Psychoanaleptics	54910-89-3	$C_{17}H_{18}F_3NO$	309.33	4.17	MeOH		Diazepam-d5
Norfluoxetine	Metabolite of Fluoxetine	57226-68-3	$C_{16}H_{16}F_3NO$	295.30	3.70	MeOH		Bromazepam-d4
Furazolidone	Antibiotics/Antibacterials	67-45-8	$C_8H_7N_3O_5$	225.16	0.87	MeOH		Sulfamethazine-d4
Furosemide	Diuretic	54-31-9	$C_{12}H_{11}ClN_2O_5S$	330.74	1.75	MeOH		Furosemide-d5
Hydrochlorothiazide	Diuretic	58-93-5	$C_7H_8ClN_3O_4S_2$	297.74	-0.58	MeOH		Hydrochlorothiazide -13CD2

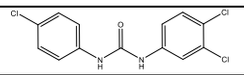
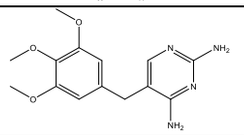
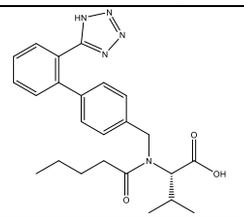
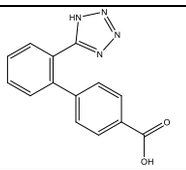
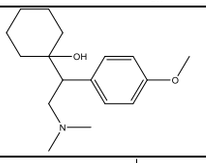
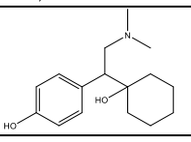
PhACs	Classification ATC[1]	CAS number[1]	Formula	Molecular weigth (g mol[1])[1]	Log P[2]	Standard preparation solvent[3]	Estructure	Associated deuterated standar[4]
Ibuprofen	Antiinflammatory	15687-27-1	$C_{13}H_{18}O_2$	206.29	3.84	MeOH		Ibuprofen-d3
Irgasan	Antibacterial Antifungal	3380-34-5	$C_{12}H_7Cl_3O_2$	289.50	4.98	MeOH		Triclosan-13C6
Ketoprofen	Antiinflammatory and antirheumatic	22071-15-4	$C_{16}H_{14}O_3$	254.28	3.61	MeOH		Diazepam-d5
Lamotrigine	Antiepileptic	84057-84-1	$C_9H_7Cl_2N_5$	256.09	1.93	MeOH		Lamotrigine-13C3
Loratadine	Antihistamine	79794-75-5	$C_{22}H_{23}ClN_2O_2$	382.88	5.2	DMSO		Bezafibrate-d4
Lorazepam	Anxiolytic	846-49-1	$C_{15}H_{10}Cl_2N_2O_2$	321.20	4.06	MeOH		Lorazepam-d4

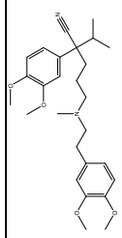
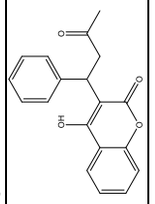
PhACs	Classification ATC[1]	CAS number[1]	Formula	Molecular weigth (g mol[1])[1]	Log P[2]	Standard preparation solvent[3]	Estructure	Associated deuterated standar[4]
Losartan	Antihypertensive activity	114798-26-4	$C_{22}H_{23}ClN_6O$	422.90	4.06	MeOH		Valsartan-d3
Mefenamic acid	Non-steroidal anti-inflammatory drug (NSAID)	61-68-7	$C_{15}H_{15}NO_2$	241.29	5.40	MeOH		Bezafibrate-d4
Metformin	Drugs used in diabetes	1115-70-4	$C_4H_{11}N_5$	129.16	-0.92	MeOH		Metformin-d6
Metoprolol	Cardiovascular system	37350-58-6	$C_{15}H_{25}NO_3$	267.36	1.76	MeOH		Metropolol-d7
Midazolam	Hypnotics and sedatives	59467-70-8	$C_{18}H_{13}ClFN_3$	325.78	3.97	MeOH		Carbamazepine-d10
Morphine	Analgesic and opioids	57-27-2	$C_{17}H_{19}NO_3$	285.34	0.9	MeOH		Morphine-d3

PhACs	Classification ATC[1]	CAS number[1]	Formula	Molecular weigth (g mol[1])[1]	Log P[2]	Standard preparation solvent[3]	Estructure	Associated deuterated standar[4]
Nalidixic acid	Antibiotics Antibacterials	389-08-2	$C_{12}H_{12}N_2O_3$	232.235	1.01	Chloroform		Venlafaxine-d6
N-Desmethylcitalopram	Metabolite of Citalopram	97743-99-2	$C_{19}H_{19}FN_2O$	310.15	3.38	MeOH		Midazolam-13C6
Omeprazole	Proton-pump inhibitor	73590-58-6	$C_{17}H_{19}N_3O_3S$	345.40	2.43	MeOH		Oseltamivir-d3
Oseltamivir carboxylate	Metabolite of oseltamivir	187227-45-8	$C_{14}H_{24}N_2O_4$	284.35	-1.84	MeOH		Oseltamivir-d3
Oxazepam	Psycholeptics and anxiolytics	604-75-1	$C_{15}H_{11}ClN_2O_2$	286.71	2.92	EtOH		Carbamazepine-d10
Oxytetracycline	Antimicrobial	79-57-2	$C_{22}H_{24}N_2O_9$	460.43	-4.87	DMSO		Bromazepam-d4

PhACs	Classification ATC[1]	CAS number[1]	Formula	Molecular weigth (g mol[1])[1]	Log P[2]	Standard preparation solvent[3]	Estructure	Associated deuterated standar[4]
Paroxetine	Pychoanaleptics and antidepressants	61869-08-7	$C_{19}H_{20}FNO_3$	329.37	3.15	DMSO		Bromazepam-d4
Pentobarbital	Hypnotics and sedatives	76-74-4	$C_{11}H_{18}N_2O_3$	226.28	1.89	EtOH		Pentobarbital-d5
Propyphenazone	Analgesic and antipyretic	479-92-5	$C_{14}H_{18}N_2O$	230.31	2.35	MeOH		Carbamazepine-d10
Salbutamol	Short-acting β2 adrenergic receptor agonist	18559-94-9	$C_{13}H_{21}NO_3$	239.31	1.4	EtOH		Codeine-d3
Sertraline	Psychoanaleptics and antidepressants	79617-96-2	$C_{17}H_{17}Cl_2N$	306.23	5.15	MeOH		Diazepam-d5
Sitagliptin	Drugs used in diabetes	486460-32-6	$C_{16}H_{15}F_6N_5O$	407.31	1.26	MeOH		Sitagliptin-d4
Sotalol	B-blocking agent	3930-20-9	$C_{12}H_{20}N_2O_3S$	272.36	-0.40	DMSO		Codeine-d3

PhACs	Classification ATC[1]	CAS number[1]	Formula	Molecular weigth (g mol[1])[1]	Log P[2]	Standard preparation solvent[3]	Estructure	Associated deuterated standar[4]
Sulfadimethoxine	Antibiotic Antibacterial	122-11-2	$C_{12}H_{14}N_4O_4S$	310.33	0.23	DMSO		Venlafaxinde-d6
Sulfamethazine	Antibiotic Antibacterial	57-68-1	$C_{12}H_{14}N_4O_2S$	278.33	0.65	MeOH		Sulfamethazine-d4
Sulfamethoxazole	Bacteriostatic and antibacterial	723-46-6	$C_{10}H_{11}N_3O_3S$	253.28	0.79	MeOH		Sulfamethoxazole-13C6
N-acetylsulfamethoxazole	Metabolite of Sulfamethoxazole	21312-10-7	$C_{12}H_{13}N_3O_4S$	295.32	0.86	MeOH		Sulfamethoxazole-13C6
Sulfapyridine	Veterinary Pharmaceutical	144-83-2	$C_{11}H_{11}N_3O_2S$	249.29	1.01	DMSO		Trimethoprim-d3
Temazepam	Antidepressant, sedative, hypnotic and anticonvulsant	846-50-4	$C_{16}H_{13}ClN_2O_2$	300.74	2.19	DMSO		Diazepam-d5
Tramadol	Analgesics/Anesthetics	123154-38-1	$C_{16}H_{25}NO_2$	263.37	2.45	MeOH		Tramadol-13CD3

PhACs	Classification ATC[1]	CAS number[1]	Formula	Molecular weigth (g mol[1])[1]	Log P[2]	Standard preparation solvent[3]	Estructure	Associated deuterated standar[4]
Triclocarban	Antibacterial	101-20-2	$C_{13}H_9Cl_3N_2O$	315.60	4.93	MeOH		Ibuprofen-d3
Trimethoprim	Antibacterial	738-70-5	$C_{14}H_{18}N_4O_3$	290.32	1.28	MeOH		Trimethoprim-d3
Valsartan	Cardiovascular system	137862-53-4	$C_{24}H_{29}N_5O_3$	435.52	4.59	MeOH		Valsartan-d3
Valsartan acid	Metabolite of valsartan	164265-78-5	$C_{14}H_{10}N_4O_2$	266.25	2.56	MeOH		Venlafaxine-d6
Venlafaxine	Psychoanaleptic	93413-69-5	$C_{17}H_{27}NO_2$	277.40	2.74	MeOH		Venlafaxine-d6
O-Desmethylvenlafaxine	Metabolite of venlafaxine	93413-62-8	$C_{16}H_{25}NO_2$	263.19	2.29	DMSO		Venlafaxine-d6

PhACs	Classification ATC[1]	CAS number[1]	Formula	Molecular weigth (g mol^{-1})[1]	Log P[2]	Standard preparation solvent[3]	Estructure	Associated deuterated standar[4]
Verapamil	Calcium channel blocking agent	52-53-9	$C_{27}H_{38}N_2O_4$	454.60	5.04	MeOH		Diazepam-d5
Warfarin	Anticoagulant	81-81-2	$C_{19}H_{16}O_4$	308.30	2.74	MeOH		Bezafibrato-d4

[1] Classification, CAS number, molecular weight: pubchem.
[2] Log P: drugbank.com and ChemDraw.
[3] Solvent in which the reference standard was prepared.
[4] Associated standard deuterate for sample quantification.

Table S2. Inclusion list

Target Compound	Precursor mass (*m/z*)	Polarity	Micros-scanning (min)		Collision energy
			Start	End	
1H_Benzotriazole	120.05562	Positive	4.7	5.7	15 (CE)
5-methyl-1-H-Benzotriazole	134.07127	Positive	7.0	8.0	25 (CE)
Acetaminophen	152.07061	Positive	2.6	3.6	20 (CE)
Acridone	196.07569	Positive	10.0	11.0	30 (NCE)
Alprazolam	309.09015	Positive	12.2	13.2	37 (CE)
Amantadine	152.14338	Positive	4.5	5.5	15 (CE)
Atenolol	267.17032	Positive	2.4	3.4	25 (CE)
Atorvastatin	557.24572	Negative	12.9	14.1	50 (CE)
Benzeilecgonina	290.13868	Positive	5.4	6.4	26 (CE)
Bezafibrate	362.11536	Positive	13.0	14.0	14 (CE)
Bisphenol-A	227.10775	Negative	12.5	13.7	31 (CE)
Bromazepam	316.00800	Positive	10.0	11.0	31 (CE)
Caffeine	195.08765	Positive	4.1	5.1	27 (CE)
Carazolol	299.17540	Positive	8.4	9.4	40 (NCE)
Carbamazepine	237.10224	Positive	11.4	12.4	23 (CE)
CBZ-10,11-epoxide	253.09715	Positive	9.2	10.2	20 (CE)
Chlarithromycin	748.48417	Positive	12.0	13.0	34 (CE)
Chloramphenicol	321.00510	Negative	8.8	10.0	15 (CE)
Ciprofloxacin	332.14050	Positive	5.4	6.4	25 (CE)
Clofibric_Acid	213.03240	Negative	7.4	8.6	10 (CE)
Coca-ethylene	318.16998	Positive	8.5	9.5	25 (CE)
Cocaine	304.15433	Positive	7.1	8.1	30 (CE)
Codeine	300.15942	Positive	3.2	4.2	42 (CE)
Cotinine	177.10224	Positive	1.4	2.4	28 (CE)
Desmethylcitalopram	311.15597	Positive	10.2	11.2	( )
Diazepam	285.07892	Positive	13.3	14.3	35 (CE)
Diclofenac	294.00890	Negative	11.8	13.3	( )
Diltiazem	415.16860	Positive	10.9	11.9	24 (CE)
Erythromycin	734.46852	Positive	10.9	11.9	29 (CE)
Fipronil	434.93143	Negative	14.5	15.7	20 (CE)
Fipronil_Desulfinyl	386.96440	Negative	14.7	15.9	10 (CE)
Fipronil_Sulfide	418.93650	Negative	14.9	16.1	25 (CE)
Fipronil_sulfone	450.92630	Negative	14.9	16.1	25 (CE)
Fluconazole	307.11134	Positive	6.6	7.6	20 (CE)
Flufenamic acid	282.07419	Positive	14.5	15.5	( )
Flumequine	262.08740	Positive	11.6	12.6	32 (CE)
Fluoxetine	310.14133	Positive	12.0	13.0	30 (NCE)
Furazolidone	226.04585	Positive	6.0	7.0	18 (CE)
Furosemide	329.00040	Negative	7.0	8.5	( )
Hydrochlorothiazide	295.95720	Negative	4.0	5.2	29 (CE)
Ibuprofen	205.12340	Negative	12.6	14.1	10 (CE)
Irgasan	286.94389	Negative	14.9	16.1	20 (CE)
Ketoprofen	255.10157	Positive	12.8	13.8	25 (CE)
Lamotrigine	256.01513	Positive	5.8	6.8	40 (CE)
Loratadine	383.15211	Positive	13.9	14.9	( )
Lorazepam	321.01921	Positive	12.1	13.1	19 (CE)
Losartan	423.16946	Positive	12.4	13.4	20 (CE)
Mefenamic_acid	240.10300	Negative	12.6	14.1	19 (CE)
Metformin	130.10872	Positive	0.8	1.8	15 (CE)
Metoprolol	268.19072	Positive	6.3	7.3	21 (CE)
Midazolam	326.08548	Positive	10.0	11.0	38 (CE)
Morphine	286.14377	Positive	1.6	2.6	40 (CE)
N-acethyl_SMX	296.06995	Positive	8.6	9.6	24 (CE)
NadilixicAcid	233.09207	Positive	11.2	12.2	32 (CE)

Table S3. Analytical parameters for muscle, heart and skin

Matrix		Muscle						Heart						Skin					
		20 (ng g^{-1})		200 (ng g^{-1})		LOD (ng g-1)	LOQ (ng g-1)	20 (ng g^{-1})		200 (ng g^{-1})		LOD (ng g-1)	LOQ (ng g-1)	20 (ng g^{-1})		200 (ng g^{-1})		LOD (ng g-1)	LOQ (ng g-1)
PhACs	RT	R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)		
Metformin	1.26	36 (21)	-95	61 (1)	-97	0.13	0.44	63 (4)	-96	58 (1)	-96	0.19	0.63	64 (12)	-95	45 (13)	-95	0.29	0.97
Cotinine	1.86	52 (5)	-7	66 (3)	-51	0.01	0.04	63 (6)	-67	56 (11)	-39	0.01	0.04	72 (1)	-52	63 (5)	-14	0.01	0.03
Morphine	2.00	<20 (16)	-35	<20 (19)	-70	0.41	1.37	<20 (1)	-72	<20 (21)	-56	1.88	6.26	37 (2)	-76	34 (4)	-38	0.15	0.49
Salbutamol	2.59	36 (15)	34	63 (3)	-47	0.02	0.06	61 (8)	-60	46 (6)	22	0.04	0.14	72 (4)	-47	59 (6)	35	0.05	0.16
Sotalol	2.71	51 (3)	10	72 (4)	-51	0.01	0.04	72 (4)	-58	61 (8)	5	0.02	0.06	75 (4)	-47	62 (5)	6	0.03	0.11
Atenolol	2.82	51 (1)	50	68 (4)	-43	0.01	0.05	71 (9)	-52	60 (10)	10	0.02	0.06	74 (2)	-39	60 (6)	29	0.02	0.07
Acetaminophen	3.10	27 (5)	-12	25 (7)	-67	0.18	0.62	31 (8)	-71	<20 (13)	-17	1.13	3.76	51 (7)	-66	40 (7)	-15	0.14	0.46
Codeine	3.63	48 (9)	45	68 (7)	-45	0.02	0.07	61 (14)	-57	55 (9)	-13	0.03	0.10	71 (6)	-52	57 (1)	18	0.03	0.10
Hydrochlorothiazide	4.36	49 (6)	-5	73 (4)	-29	0.06	0.20	67 (12)	-38	58 (12)	-24	0.10	0.32	84 (5)	-22	57 (17)	-29	0.10	0.32
Caffeine	4.58	65 (4)	129	73 (5)	-40	0.03	0.12	70 (5)	-41	57 (16)	82	0.05	0.17	77 (4)	298	67 (3)	83	0.06	0.20
Sulfapyridine	4.77	45 (4)	67	75 (14)	-57	0.03	0.09	34 (12)	-81	36 (7)	-4	0.07	0.23	75 (17)	-73	63 (4)	32	0.03	0.11
Amantadine	4.89	62 (2)	202	71 (1)	-32	0.02	0.08	75 (1)	34	56 (4)	34	0.03	0.10	56 (0.3)	-14	56 (1)	-3	0.04	0.14
Trimethoprim	4.92	48 (9)	4	72 (12)	-59	0.03	0.09	64 (7)	-62	56 (9)	-34	0.04	0.13	73 (5)	-75	49 (6)	-42	0.05	0.15
1H_Benzotriazole	5.11	46 (20)	5	82 (2)	175	0.03	0.11	68 (8)	-72	43 (12)	-32	0.30	1.00	66 (3)	-62	60 (1)	-38	0.04	0.13
Oseltamivir-CBX	5.31	41 (2)	59	51 (1)	-31	0.19	0.64	58 (12)	-29	43 (11)	51	0.15	0.49	41 (7)	-25	37 (3)	43	0.10	0.34
Oxytetracycline	5.50	<20 (1)	-14	<20 (1)	-44	9.27	30.90	<20 (1)	-74	<20 (1)	-72	3.85	12.82	- (-)	-	<20 (1)	-56	-	-
Ciprofloxacin	5.76	<20 (0.5)	17	28 (37)	-18	0.10	0.32	<20 (4)	-46	50 (6)	-55	0.02	0.06	<20 (1)	-89	<20 (4)	-32	0.80	2.67
Benzoylecgonine	5.77	48 (2)	124	67 (6)	-40	0.01	0.03	65 (7)	-46	59 (8)	25	0.02	0.05	67 (5)	-33	58 (3)	47	0.01	0.04
O-Desmethylvenlafaxine	5.79	47 (14)	21	73 (11)	-48	0.01	0.02	60 (13)	-65	54 (8)	-18	0.01	0.02	73 (6)	-64	57 (2)	-4	0.01	0.03
Sulfamethazine	6.17	48 (4)	129	78 (12)	-46	0.05	0.17	39 (12)	-74	40 (7)	10	0.11	0.37	74 (17)	-66	66 (1)	53	0.01	0.03
Lamotrigine	6.19	44 (23)	-42	82 (18)	-83	0.02	0.08	41 (19)	-93	42 (11)	-76	0.06	0.19	83 (12)	-95	42 (16)	-76	0.02	0.08
Furazolidone	6.36	50 (2)	10	97 (6)	-63	0.01	0.04	61 (6)	-51	50 (17)	-19	0.02	0.06	77 (9)	-48	67 (0.4)	-11	0.01	0.04
Tramadol	6.63	47 (22)	25	70 (5)	-53	0.01	0.03	72 (1)	-49	59 (7)	-15	0.01	0.03	73 (3)	-62	55 (3)	-19	0.02	0.06
Metoprolol	6.66	49 (20)	63	75 (8)	-47	0.01	0.04	72 (9)	-42	59 (11)	20	0.01	0.03	75 (3)	-64	56 (7)	5	0.02	0.06

Matrix		Muscle						Heart						Skin					
		20 (ng g^{-1})		200 (ng g^{-1})		LOD (ng g-1)	LOQ (ng g-1)	20 (ng g^{-1})		200 (ng g^{-1})		LOD (ng g-1)	LOQ (ng g-1)	20 (ng g^{-1})		200 (ng g^{-1})		LOD (ng g-1)	LOQ (ng g-1)
PhACs	RT	R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)		
Fluconazole	7.00	54 (5)	21	70 (4)	-33	0.04	0.15	70 (6)	-42	60 (14)	-12	0.07	0.23	76 (3)	-35	66 (2)	-7	0.13	0.43
5-methyl-1H_Benzotriazole	7.41	57 (1)	6	84 (5)	545	0.02	0.06	49 (5)	-74	43 (11)	-33	0.03	0.11	66 (5)	-64	54 (4)	-25	0.03	0.09
Cocaine	7.44	56 (4)	-2	72 (12)	-58	0.02	0.05	73 (6)	-53	63 (4)	-29	0.02	0.05	83 (13)	-72	46 (1)	-30	0.02	0.07
Clofibric_Acid	7.46	40 (7)	7	54 (6)	-25	0.13	0.42	46 (16)	-8	42 (19)	7	0.51	1.69	67 (15)	-19	50 (12)	-0.5	0.15	0.50
Sitagliptin	7.79	53 (10)	-18	71 (14)	-66	0.04	0.12	45 (14)	-57	49 (10)	-4	0.06	0.19	65 (7)	-86	48 (10)	-44	0.16	0.52
Furosemide	7.94	32 (1)	-28	55 (5)	-44	0.30	0.50	31 (11)	-36	30 (16)	-40	0.45	1.52	67 (20)	-42	74 (18)	-73	0.62	2.07
Sulfamethoxazole	8.03	46 (1)	80	77 (13)	-47	0.05	0.17	35 (10)	-65	38 (10)	-9	0.12	0.40	76 (13)	-52	65 (2)	28	0.07	0.24
Venlafaxine	8.52	54 (16)	-18	71 (16)	-66	0.01	0.04	65 (10)	-53	59 (8)	-32	0.01	0.03	74 (9)	-79	48 (10)	-52	0.02	0.05
Carazolol	8.78	34 (33)	-41	71 (18)	-80	0.04	0.15	<20 (17)	-63	<20 (4)	-47	0.18	0.60	76 (12)	-93	55 (19)	-84	0.20	0.67
Coca-ethylene	8.83	69 (18)	-34	73 (20)	-66	0.03	0.09	66 (11)	-50	58 (6)	-31	0.03	0.10	81 (13)	-82	43 (3)	-51	0.03	0.11
Oseltamivir_phospate	8.87	51 (10)	46	71 (18)	-47	0.05	0.16	32 (20)	-26	44 (6)	75	0.07	0.23	68 (3)	-76	46 (15)	-6	0.04	0.13
N-acethyl_SMX	8.93	56 (3)	90	73 (1)	-29	0.07	0.24	71 (5)	-35	58 (13)	39	0.03	0.10	78 (2)	-22	70 (2)	52	0.05	0.17
Chloramphenicol	9.19	47 (8)	-7	74 (5)	-33	0.02	0.06	60 (14)	-18	59 (11)	-12	0.04	0.14	84 (16)	-32	64 (22)	-33	0.03	0.11
Omeprazole	9.42	<20 (1)	-93	59 (10)	-97	7.89	26.32	- (-)	-	62 (28)	-98	0.28	0.94	- (-)	-	64 (12)	-100	-	-
CBZ-10,11-epoxide	9.61	53 (9)	165	71 (6)	-17	0.03	0.10	64 (9)	-26	60 (8)	76	0.01	0.05	76 (8)	-26	66 (2)	85	0.01	0.03
Valsartan Acid	9.90	22 (12)	86	43 (11)	-39	0.31	1.04	30 (22)	-83	31 (14)	-15	2.87	9.58	43 (7)	-79	35 (2)	21	3.46	11.54
Sulfadimethoxine	10.22	47 (2)	93	77 (17)	-53	0.06	0.19	31 (15)	-78	36 (5)	-11	0.10	0.35	77 (20)	-77	64 (1)	28	0.02	0.06
Midazolam	10.43	43 (36)	-83	71 (2)	-72	0.04	0.15	37 (11)	-70	55 (7)	-82	0.09	0.30	71 (19)	-95	58 (10)	-94	0.14	0.48
Acridone	10.44	48 (16)	-4	70 (7)	-47	0.02	0.07	57 (7)	-56	54 (12)	-20	0.02	0.05	75 (7)	-66	61 (1)	-30	0.01	0.03
Desmethylcitalopram	10.54	74 (30)	-80	81 (0.3)	-84	0.14	0.45	31 (22)	-71	49 (6)	-70	0.03	0.11	<20 (1)	-100	55 (15)	-91	0.07	0.25
Bromazepam	10.59	38 (34)	-3	67 (25)	158	0.12	0.41	52 (25)	16	55 (17)	47	0.27	0.89	99 (17)	-86	100 (3)	-38	0.17	0.55
Diltiazem	11.25	50 (35)	-63	69 (24)	-78	0.02	0.07	42 (16)	-44	45 (4)	-57	0.03	0.10	82 (23)	-92	51 (12)	-85	0.06	0.19
Erythromycin	11.32	48 (0.9)	-60	105 (3)	-68	0.11	0.37	59 (15)	-77	64 (6)	-67	0.08	0.26	51 (7)	-95	47 (11)	-86	1.27	4.23
Nalidixic Acid	11.57	<20 (25)	-26	36 (30)	-45	0.04	0.12	35 (9)	-49	53 (27)	-60	0.02	0.07	59 (19)	-75	<20 (4)	-43	0.12	0.39
Pentobarbital	11.74	55 (5)	-4	69 (4)	-38	0.05	0.16	64 (12)	-24	56 (15)	-10	0.05	0.17	84 (6)	-41	67 (3)	-42	0.04	0.14

Matrix		Muscle						Heart						Skin					
		20 (ng g^{-1})		200 (ng g^{-1})		LOD (ng g-1)	LOQ (ng g-1)	20 (ng g^{-1})		200 (ng g^{-1})		LOD (ng g-1)	LOQ (ng g-1)	20 (ng g^{-1})		200 (ng g^{-1})		LOD (ng g-1)	LOQ (ng g-1)
PhACs	RT	R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)		
Paroxetine	11.81	31 (44)	-87	59 (23)	-81	0.10	0.33	<20 (2)	-58	28 (19)	-85	0.75	2.50	78 (14)	-92	96 (17)	-97	0.18	0.62
Carbamazepine	11.85	46 (24)	7	71 (8)	-47	0.01	0.02	59 (8)	-51	56 (11)	-5	0.02	0.05	79 (5)	-64	64 (0.3)	-20	0.01	0.03
Flumequine	12.05	<20 (31)	-16	59 (2)	-21	0.06	0.19	42 (10)	-36	57 (23)	-44	0.03	0.09	83 (27)	-75	20 (4)	-36	0.10	0.34
Verapamil	12.26	46 (39)	-81	72 (23)	-82	0.24	0.80	32 (17)	-28	51 (4)	-72	-	-	107 (18)	-92	76 (19)	-94	0.15	0.51
Oxazepam	12.32	35 (6)	82	65 (8)	-36	0.07	0.23	56 (8)	-31	50 (8)	20	0.12	0.41	74 (12)	-57	52 (9)	-4	0.02	0.08
Norfluoxetine	12.32	- (-)	-	38 (33)	-90	2.20	7.32	<20 (1)	-83	24 (19)	-91	1.16	3.87	- (-)	-	99 (17)	-98	-	-
Propyphenazone	12.37	49 (28)	-18	70 (5)	-60	0.01	0.04	64 (9)	-59	56 (10)	-30	0.00	0.01	75 (5)	-69	67 (5)	-40	0.004	0.01
Fluoxetine	12.49	42 (24)	-92	62 (21)	-86	0.06	0.20	<20 (8)	-65	46 (19)	-89	0.17	0.58	64 (19)	-93	118 (20)	-98	0.56	1.88
Clarithromycin	12.50	53 (14)	-67	70 (24)	-79	0.69	2.29	50 (24)	-46	63 (9)	-68	0.02	0.07	86 (13)	-92	45 (18)	-88	0.09	0.29
Sertraline	12.54	99 (1)	-83	53 (25)	-69	0.38	1.28	41 (21)	20	59 (22)	-87	0.16	0.54	102 (17)	-61	106 (12)	-98	0.33	1.11
Lorazepam	12.55	46 (0.9)	30	65 (10)	-36	-	-	49 (14)	-26	55 (7)	14	0.0004	0.001	78 (8)	-63	64 (5)	-16	0.10	0.34
Diclofenac	12.61	23 (1)	-62	49 (16)	-58	0.35	1.15	<20 (1)	-57	<20 (0.4)	-53	-	-	- (-)	-	74 (2)	-75	0.51	1.70
Alprazolam	12.62	62 (20)	-29	64 (20)	-55	0.02	0.06	59 (15)	-56	60 (3)	-22	0.01	0.05	66 (23)	-76	60 (1)	-50	0.05	0.18
Losartan	12.98	36 (9)	-87	79 (16)	65	0.68	2.26	<20 (1)	-94	47 (11)	-91	1.32	4.40	- (-)	-	49 (5)	-84	0.01	0.03
Temazepam	13.08	51 (4)	46	68 (12)	-38	0.04	0.13	55 (11)	-20	55 (7)	31	0.04	0.14	74 (7)	-59	61 (2)	-9	0.01	0.04
Bisphenol-A	13.11	25 (1)	-21	46 (14)	-31	0.14	0.46	<20 (5)	-48	<20 (5)	-46	0.04	0.12	- (-)	-	70 (6)	-69	0.08	0.25
Atorvastatin	13.27	- (-)	-	61 (32)	-63	-	-	<20 (1)	-92	54 (23)	-86	-	-	- (-)	-	20 (23)	21	-	-
Ketoprofen	13.31	50 (4)	94	66 (4)	-45	0.01	0.04	59 (7)	-43	55 (16)	52	0.04	0.14	81 (18)	-62	57 (10)	76	0.04	0.12
Mefenamic acid	13.33	20 (30)	-88	43 (1)	-71	0.33	1.09	<20 (1)	-73	<20 (19)	-82	2.18	7.26	- (-)	-	88 (4)	-79	2.00	6.68
Ibuprofen	13.35	55 (3)	35	89 (13)	-50	0.18	0.61	59 (23)	157	45 (9)	-37	0.28	0.92	110 (11)	66	71 (6)	-57	0.39	1.29
Bezafibrate	13.45	40 (6)	20	62 (2)	-25	0.02	0.06	55 (8)	-28	51 (15)	2	0.08	0.27	72 (10)	-34	54 (0.1)	14	0.47	1.58
Valsartan	13.72	46 (6)	-73	49 (11)	425	0.04	0.12	31 (9)	-78	37 (18)	-65	0.71	2.36	61 (5)	-72	48 (0.2)	-41	0.16	0.55
Diazepam	13.76	53 (18)	-62	68 (12)	-69	0.02	0.06	42 (10)	-61	55 (7)	-54	0.07	0.25	74 (8)	-85	60 (5)	-73	0.01	0.02
Warfarin	13.87	51 (0.9)	29	69 (5)	-44	0.01	0.04	51 (10)	-50	47 (10)	11	0.01	0.05	80 (10)	-61	66 (2)	7	0.02	0.08
Loratadine	14.45	<20 (1)	-92	58 (19)	-88	0.03	0.09	24 (14)	194	59 (8)	-88	0.04	0.13	72 (13)	-79	91 (19)	-97	0.24	0.79

Matrix		Muscle						Heart						Skin					
		20 (ng g^{-1})		200 (ng g^{-1})		LOD (ng g-1)	LOQ (ng g-1)	20 (ng g^{-1})		200 (ng g^{-1})		LOD (ng g-1)	LOQ (ng g-1)	20 (ng g^{-1})		200 (ng g^{-1})		LOD (ng g-1)	LOQ (ng g-1)
PhACs	RT	R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)		
Flufenamic_Acid	14.93	- (-)	-	43 (14)	-66	3.48	11.60	- (-)	-	<20 (1)	-89	-	-	- (-)	-	61 (1)	-93	-	-
Fipronil	15.11	56 (23)	-65	75 (24)	-15	0.09	0.29	40 (15)	-53	71 (7)	-57	0.03	0.09	143 (11)	-92	135 (5)	-77	0.02	0.05
Fipronil_Desulfinyl	15.27	70 (32)	-80	73 (21)	12	0.03	0.11	34 (24)	-42	76 (6)	-69	0.02	0.07	<20 (1)	-91	126 (6)	-74	0.12	0.39
Triclocarban	15.44	60 (32)	84	49 (27)	114	0.08	0.28	<20 (21)	707	62 (11)	133	0.08	0.26	71 (24)	63	105 (6)	-	0.08	0.26
Fipronil sulfone	15.44	73 (29)	-77	59 (15)	246	0.04	0.14	34 (24)	34	81 (8)	-63	0.01	0.05	78 (31)	-43	137 (8)	-23	2.20	7.32
Fipronil Sulfide	15.47	95 (32)	-85	80 (20)	171	0.02	0.07	32 (19)	10	86 (10)	-68	0.02	0.06	95 (29)	-71	143 (7)	-56	0.02	0.06
Irgasan	15.51	68 (1)	-37	46 (35)	-26	0.20	0.67	<20 (1)	-50	28 (17)	20	0.29	0.95	83 (7)	-89	115 (4)	-	0.04	0.12

Table S4. Analytical parameters for liver, kidney and pancreas

Matrix		Liver						Kidney						Pancreas					
		20 (ng g-1)		200 (ng g-1)		LOD (ng g-1)	LOQ (ng g-1)	20 (ng g-1)		200 (ng g-1)		LOD (ng g-1)	LOQ (ng g-1)	20 (ng g-1)		200 (ng g-1)		LOD (ng g-1)	LOQ (ng g-1)
PhACs	RT	R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)		
Metformin	1.26	- (-)	-	46 (4)	-98	0.78	2.59	<20 (1)	-99	57 (1)	-98	0.99	3.31	- (-)	-	51 (17)	-98	-	-
Cotinine	1.86	61 (2)	-72	48 (10)	-72	0.03	0.11	25 (2)	-77	81 (2)	-80	0.03	0.10	33 (6)	-80	44 (11)	-77	0.05	0.15
Morphine	2.00	<20 (1)	-82	22 (16)	-83	0.78	2.59	<20 (1)	-85	<20 (15)	-84	0.53	1.76	<20 (1)	-90	<20 (1)	-90	0.52	1.73
Salbutamol	2.59	60 (3)	-25	53 (4)	-31	0.06	0.20	53 (20)	-47	53 (2)	-59	0.04	0.13	45 (12)	-45	50 (16)	-49	0.05	0.16
Sotalol	2.71	64 (7)	-22	56 (5)	-41	0.03	0.11	76 (1)	-55	57 (2)	-57	0.02	0.05	56 (9)	-52	66 (14)	-61	0.02	0.07
Atenolol	2.82	59 (2)	-31	58 (4)	-38	0.03	0.08	65 (2)	-47	64 (2)	-62	0.03	0.09	59 (11)	-51	74 (13)	-64	0.04	0.15
Acetaminophen	3.10	145 (20)	-80	<20 (28)	-77	0.79	2.64	32 (35)	-88	<20 (10)	-85	0.96	3.21	110 (16)	-88	<20 (9)	-84	0.30	1.00
Codeine	3.63	60 (1)	-27	53 (8)	-38	0.02	0.08	47 (8)	-42	52 (2)	-56	0.02	0.08	24 (23)	-64	51 (19)	-52	0.06	0.18
Hydrochlorothiazide	4.36	76 (8)	-50	71 (1)	-57	0.10	0.35	54 (18)	-66	58 (1)	-43	0.01	0.03	51 (4)	-54	61 (28)	-63	0.17	0.56
Caffeine	4.58	75 (3)	67	58 (4)	42	0.03	0.09	68 (10)	59	67 (3)	45	0.05	0.18	59 (9)	53	60 (13)	7	0.12	0.40
Sulfapyridine	4.77	47 (10)	-21	56 (12)	-18	0.09	0.29	27 (4)	-65	49 (7)	-82	0.11	0.36	24 (21)	-43	25 (3)	-37	0.12	0.39
Amantadine	4.89	46 (15)	415	43 (15)	-1	0.01	0.05	60 (1)	182	60 (4)	-34	0.04	0.12	41 (9)	175	42 (18)	-24	0.03	0.09
Trimethoprim	4.92	62 (1)	-52	60 (13)	-59	0.02	0.05	62 (2)	-52	77 (5)	-76	0.07	0.23	43 (16)	-75	50 (21)	-74	0.08	0.27
1H_Benzotriazole	5.11	<20 (1)	-60	55 (17)	-48	0.14	0.47	- (-)	-	66 (0.2)	23	0.06	0.20	- (-)	-	25 (38)	-51	0.37	1.24
Oseltamivir-CBX	5.31	56 (7)	35	55 (3)	37	0.02	0.07	53 (2)	19	54 (1)	-34	0.16	0.55	44 (11)	25	45 (23)	7	0.21	0.70
Oxytetracycline	5.50	<20 (1)	-66	<20 (1)	-46	-	-	- (-)	-	<20 (1)	-28	14.91	49.70	<20 (1)	-39	<20 (0.2)	-17	3.56	11.86
Ciprofloxacin	5.76	<20 (1)	-54	<20 (1)	-73	-	-	- (-)	-	31 (40)	-47	1.24	4.12	<20 (1)	-78	38 (39)	-80	0.02	0.06
Benzoylecgonine	5.77	65 (2)	6	60 (3)	-16	0.03	0.09	56 (5)	-21	61 (1)	-53	0.03	0.09	51 (10)	-16	59 (10)	-31	0.07	0.23
O-Desmethylvenlafaxine	5.79	67 (3)	-39	59 (11)	-45	0.02	0.06	68 (2)	-55	58 (2)	-60	0.01	0.04	51 (14)	-60	47 (1)	-57	0.02	0.07
Sulfamethazine	6.17	55 (4)	-3	64 (10)	-5	0.06	0.19	35 (5)	-48	52 (8)	-75	0.07	0.24	25 (23)	-21	25 (2)	-21	0.15	0.49
Lamotrigine	6.19	50 (8)	-84	72 (26)	-89	0.07	0.22	65 (9)	-93	<20 (15)	-81	0.20	0.66	<20 (6)	-95	<20 (8)	-92	0.10	0.34
Furazolidone	6.36	38 (9)	-33	38 (1)	-36	0.03	0.11	43 (4)	-49	54 (2)	-57	0.01	0.04	62 (9)	-64	67 (14)	-65	0.04	0.15
Tramadol	6.63	67 (3)	-23	62 (10)	-43	0.02	0.07	75 (1)	-43	61 (1)	-59	0.01	0.03	63 (16)	-53	47 (16)	-61	0.02	0.08
Metoprolol	6.66	69 (7)	15	65 (16)	-22	0.02	0.05	75 (1)	-9	63 (4)	-51	0.01	0.05	59 (16)	-27	57 (15)	-35	0.04	0.14

Matrix		Liver						Kidney						Pancreas					
		20 (ng g-1)		200 (ng g-1)		LOD (ng g-1)	LOQ (ng g-1)	20 (ng g-1)		200 (ng g-1)		LOD (ng g-1)	LOQ (ng g-1)	20 (ng g-1)		200 (ng g-1)		LOD (ng g-1)	LOQ (ng g-1)
PhACs	RT	R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)		
Fluconazole	7.00	75 (4)	23	67 (4)	-19	0.06	0.20	46 (12)	-22	62 (2)	-48	0.06	0.20	41 (11)	-26	58 (13)	-35	0.08	0.27
5-methyl-1H_Benzotriazole	7.41	65 (4)	-31	66 (10)	-54	0.14	0.45	50 (1)	-77	70 (1)	162	0.04	0.14	<20 (8)	-45	24 (75)	-53	0.05	0.17
Cocaine	7.44	59 (12)	-42	37 (10)	-53	0.04	0.12	57 (3)	-52	76 (4)	-69	0.01	0.03	61 (7)	-63	56 (32)	-66	0.05	0.15
Clofibric_Acid	7.46	69 (13)	19	67 (1)	-18	0.08	0.25	46 (6)	-48	60 (1)	-29	0.02	0.06	42 (10)	0.1	54 (22)	-33	0.15	0.49
Sitagliptin	7.79	62 (2)	-20	75 (21)	-35	0.08	0.27	70 (6)	-41	84 (9)	-75	0.05	0.18	27 (0.5)	-65	53 (27)	-58	0.18	0.60
Furosemide	7.94	58 (17)	-55	67 (3)	-64	0.80	2.65	35 (14)	-63	46 (4)	-52	0.21	0.69	22 (13)	-58	46 (41)	-62	0.31	1.02
Sulfamethoxazole	8.03	59 (4)	-1	65 (12)	-16	0.10	0.34	25 (39)	-57	52 (9)	-79	0.03	0.09	28 (20)	-33	40 (1)	-35	0.18	0.59
Venlafaxine	8.52	58 (14)	-43	40 (18)	-62	0.03	0.09	87 (1)	-55	81 (10)	-72	0.01	0.03	82 (15)	-72	71 (30)	-76	0.06	0.20
Carazolol	8.78	48 (6)	-71	66 (30)	-81	0.12	0.40	69 (11)	-90	43 (17)	-88	0.24	0.80	<20 (1)	-97	<20 (28)	-95	1.02	3.41
Coca-ethylene	8.83	42 (10)	-43	29 (31)	-61	0.07	0.24	64 (0.1)	-55	84 (10)	-73	0.03	0.10	56 (15)	-68	69 (20)	-73	0.04	0.14
Oseltamivir_phospate	8.87	54 (12)	31	54 (15)	0.3	0.03	0.11	57 (1)	23	82 (10)	-63	0.01	0.04	38 (15)	-32	35 (14)	-28	0.07	0.24
N-acethyl_SMX	8.93	74 (2)	40	70 (1)	22	0.08	0.27	67 (1)	12	60 (2)	-43	0.07	0.23	56 (12)	17	64 (17)	-3	0.10	0.35
Chloramphenicol	9.19	78 (15)	-26	84 (2)	-47	0.05	0.17	55 (13)	-65	64 (3)	-43	0.02	0.08	50 (1)	-38	63 (34)	-53	0.01	0.03
Omeprazole	9.42	- (-)	-	- (-)	-	-	-	- (-)	-	- (-)	-	-	-	- (-)	-	- (-)	-	-	-
CBZ-10,11-epoxide	9.61	71 (3)	59	68 (6)	29	0.02	0.08	74 (2)	17	73 (2)	-44	0.02	0.08	53 (13)	14	57 (19)	-0.5	0.03	0.09
Valsartan Acid	9.90	<20 (1)	-54	48 (15)	-33	0.62	2.06	- (-)	-	53 (9)	-82	1.23	4.12	<20 (1)	-3	<20 (1)	26	-	-
Sulfadimethoxine	10.22	60 (8)	-6	75 (13)	-25	0.07	0.25	25 (9)	-62	58 (8)	-87	0.33	1.11	<20 (30)	-52	<20 (8)	-47	0.06	0.19
Midazolam	10.43	40 (22)	-91	34 (79)	-94	0.21	0.70	64 (0.4)	-93	88 (26)	-83	0.13	0.42	<20 (4)	-98	44 (37)	-97	0.78	2.61
Acridone	10.44	69 (4)	-42	70 (11)	-46	0.02	0.06	76 (5)	-68	74 (6)	-67	0.05	0.18	41 (23)	-70	47 (34)	-66	0.16	0.54
Desmethylcitalopram	10.54	44 (8)	-76	35 (24)	-89	0.34	1.12	71 (9)	-87	101 (23)	-90	0.74	2.46	<20 (1)	-97	34 (14)	-96	-	-
Bromazepam	10.59	65 (7)	-33	76 (17)	-21	0.16	0.53	75 (1)	-20	83 (12)	104	0.15	0.49	55 (22)	-38	55 (12)	12	0.11	0.36
Diltiazem	11.25	44 (22)	-74	51 (28)	-83	0.07	0.22	78 (3)	-79	80 (25)	-81	0.04	0.12	44 (24)	-93	63 (23)	-90	0.30	0.99
Erythromycin	11.32	63 (21)	-82	94 (20)	-86	0.15	0.50	89 (10)	-90	65 (9)	-84	0.42	1.39	<20 (1)	-93	36 (21)	-79	0.83	2.78
Nalidixic Acid	11.57	<20 (20)	-58	<20 (17)	-70	0.38	1.28	25 (32)	-82	61 (16)	-59	0.05	0.18	50 (13)	-70	53 (16)	-74	0.17	0.57
Pentobarbital	11.74	83 (6)	-22	71 (8)	-52	0.05	0.18	67 (18)	-33	70 (3)	-42	0.08	0.25	73 (7)	-37	61 (20)	-59	0.25	0.82

Matrix		Liver						Kidney						Pancreas					
		20 (ng g-1)		200 (ng g-1)		LOD (ng g-1)	LOQ (ng g-1)	20 (ng g-1)		200 (ng g-1)		LOD (ng g-1)	LOQ (ng g-1)	20 (ng g-1)		200 (ng g-1)		LOD (ng g-1)	LOQ (ng g-1)
PhACs	RT	R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)		
Paroxetine	11.81	- (-)	-	<20 (1)	-97	-	-	- (-)	-	51 (31)	-92	0.79	2.62	- (-)	-	- (-)	-	-	-
Carbamazepine	11.85	72 (11)	-33	67 (19)	-40	0.01	0.05	86 (1)	-59	78 (7)	-64	0.02	0.06	51 (14)	-63	50 (28)	-55	0.02	0.07
Flumequine	12.05	<20 (9)	-46	<20 (16)	-72	0.51	1.69	29 (27)	-82	69 (23)	-48	0.04	0.14	56 (21)	-73	65 (23)	-72	0.20	0.66
Verapamil	12.26	43 (12)	-87	59 (57)	-92	0.55	1.83	65 (3)	-83	110 (30)	-87	0.13	0.43	40 (7)	-97	51 (29)	-96	0.53	1.77
Oxazepam	12.32	59 (4)	-0.1	54 (28)	-15	0.09	0.30	85 (2)	-35	74 (8)	-54	0.09	0.30	48 (20)	-49	51 (19)	-41	0.28	0.92
Norfluoxetine	12.32	<20 (1)	-96	<20 (1)	-98	-	-	- (-)	-	<20 (1)	-98	-	-	- (-)	-	- (-)	-	-	-
Propyphenazone	12.37	67 (0.6)	-40	62 (21)	-51	0.01	0.03	81 (2)	-60	74 (5)	-68	0.01	0.02	46 (24)	-64	56 (28)	-62	0.001	0.004
Fluoxetine	12.49	44 (27)	-95	48 (27)	-97	0.02	0.08	42 (0.5)	-97	70 (30)	-94	1.03	3.42	<20 (1)	-100	30 (11)	-99	-	-
Clarithromycin	12.50	66 (7)	-78	62 (54)	-85	0.07	0.24	109 (0.1)	-84	110 (12)	-82	0.14	0.47	51 (29)	-92	56 (30)	-90	0.12	0.42
Sertraline	12.54	64 (30)	-53	40 (30)	-94	1.58	5.26	40 (1)	-98	48 (27)	-86	0.69	2.30	52 (28)	-95	40 (30)	-99	0.24	0.80
Lorazepam	12.55	63 (0.8)	-3	65 (19)	-20	0.08	0.26	80 (9)	-43	78 (11)	-54	0.70	2.32	<20 (4)	-59	50 (20)	-54	0.69	2.32
Diclofenac	12.61	41 (10)	-80	46 (5)	-83	0.28	0.93	- (-)	-	43 (17)	-78	0.67	2.22	- (-)	-	<20 (1)	-94	-	-
Alprazolam	12.62	52 (12)	-26	55 (33)	-56	0.04	0.14	81 (2)	-65	91 (17)	-70	0.02	0.05	43 (26)	-70	57 (37)	-70	0.04	0.12
Losartan	12.98	- (-)	-	55 (29)	-94	1.30	4.34	- (-)	-	140 (18)	-85	1.01	3.36	- (-)	-	<20 (1)	-93	-	-
Temazepam	13.08	64 (5)	2	63 (20)	-15	0.05	0.16	96 (1)	-39	83 (9)	-56	0.09	0.29	54 (17)	-56	51 (26)	-45	0.20	0.66
Bisphenol-A	13.11	54 (6)	-54	43 (5)	-75	0.17	0.58	<20 (1)	-89	<20 (14)	-66	0.52	1.75	- (-)	-	<20 (1)	-92	-	-
Atorvastatin	13.27	- (-)	-	101 (23)	-55	0.65	2.15	- (-)	-	95 (25)	-87	0.94	3.14	- (-)	-	<20 (1)	17	-	-
Ketoprofen	13.31	64 (8)	85	69 (4)	59	0.08	0.27	68 (3)	33	76 (6)	-61	0.11	0.36	40 (14)	38	53 (23)	28	0.31	1.02
Mefenamic acid	13.33	- (-)	-	8 (5)	-84	1.76	5.86	- (-)	-	<20 (28)	-85	1.14	3.79	- (-)	-	<20 (1)	-100	-	-
Ibuprofen	13.35	52 (23)	45	65 (13)	-71	0.25	0.83	- (-)	-	57 (11)	-52	0.37	1.23	140 (10)	-72	42 (18)	-78	0.33	1.10
Bezafibrate	13.45	60 (14)	-7	68 (6)	-10	0.04	0.15	64 (8)	-29	70 (4)	-42	0.08	0.28	<20 (45)	-40	51 (27)	-23	0.27	0.89
Valsartan	13.72	46 (16)	-72	48 (27)	-72	0.51	1.72	<20 (1)	-90	75 (10)	50	0.12	0.41	25 (1)	-61	<20 (20)	-48	0.49	1.63
Diazepam	13.76	64 (5)	-76	59 (38)	-79	0.05	0.16	109 (0.5)	-86	93 (17)	-81	0.01	0.02	46 (35)	-92	37 (25)	-86	0.07	0.23
Warfarin	13.87	71 (6)	-16	66 (24)	-23	0.03	0.09	77 (0.1)	-54	79 (6)	-64	0.02	0.05	35 (19)	-53	36 (11)	-46	0.06	0.21
Loratadine	14.45	34 (2)	-96	<20 (40)	-96	0.87	2.90	<20 (2)	-96	61 (39)	-24	0.07	0.22	<20 (1)	-100	20 (19)	-99	2.92	9.72

Matrix		Liver						Kidney						Pancreas					
		20 (ng g-1)		200 (ng g-1)		LOD (ng g-1)	LOQ (ng g-1)	20 (ng g-1)		200 (ng g-1)		LOD (ng g-1)	LOQ (ng g-1)	20 (ng g-1)		200 (ng g-1)		LOD (ng g-1)	LOQ (ng g-1)
PhACs	RT	R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)		
Flufenamic_Acid	14.93	- (-)	-	<20 (1)	-96	-	-	- (-)	-	52 (26)	-82	3.11	10.36	- (-)	-	- (-)	-	-	-
Fipronil	15.11	50 (26)	-71	48 (33)	-75	0.41	1.36	70 (6)	-90	53 (16)	-38	0.01	0.03	34 (10)	-91	43 (18)	-85	0.30	1.00
Fipronil_Desulfinyl	15.27	32 (13)	-75	34 (38)	-79	0.16	0.52	68 (12)	-94	36 (21)	-33	0.07	0.22	59 (28)	-97	33 (5)	-90	0.83	2.75
Triclocarban	15.44	<20 (1)	35	<20 (1)	-	0.62	2.05	- (-)	-	40 (50)	20	0.56	1.88	- (-)	-	- (-)	-	-	-
Fipronil sulfone	15.44	8 (7)	-36	10 (45)	-40	0.19	0.63	- (-)	-	21 (41)	90	1.53	5.08	<20 (1)	-100	- (-)	-	0.24	0.81
Fipronil Sulfide	15.47	9 (8)	-54	13 (39)	-66	0.04	0.13	- (-)	-	21 (35)	46	0.61	2.02	- (-)	-	39 (39)	-88	0.65	2.17
Irgasan	15.51	- (-)	-	<20 (1)	-	2.66	8.87	49 (1)	-96	<20 (63)	-66	1.76	5.87	- (-)	-	- (-)	-	8.55	28.49

Table S5. Analytical parameters for brain, plasma and bile

Matrix		Brain						Plasma						Bile					
		20 (ng g^{-1})		200 (ng g^{-1})		LOD (ng g^{-1})	LOQ (ng g^{-1})	50 (ng mL^{-1})		500 (ng mL^{-1})		LOD (ng mL^{-1})	LOQ (ng mL^{-1})	100 (ng mL^{-1})		1000 (ng mL^{-1})		LOD (ng mL^{-1})	LOQ (ng mL^{-1})
PhACs	RT	R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)		
Metformin	1.26	53 (8)	-74	46 (0.1)	-79	0.01	0.05	37 (7)	-50	61 (2)	-76	0.05	0.16	36 (14)	-96	90 (1)	-97	1.09	3.62
Cotinine	1.86	69 (3)	5	64 (3)	-0.2	0.01	0.04	63 (3)	66	102 (4)	-5	0.01	0.04	62 (9)	-59	97 (3)	-76	0.17	0.56
Morphine	2.00	<20 (43)	-16	26 (7)	-21	0.34	1.15	52 (8)	86	104 (3)	-10	0.10	0.32	161 (17)	-84	102 (5)	-80	0.53	1.75
Salbutamol	2.59	64 (1)	53	71 (2)	22	0.03	0.11	53 (29)	-33	119 (4)	19	0.04	0.13	108 (7)	-78	107 (1)	-72	0.30	0.98
Sotalol	2.71	71 (2)	39	76 (3)	11	0.01	0.04	74 (4)	106	116 (4)	2	0.02	0.08	131 (13)	-74	124 (2)	-71	0.05	0.15
Atenolol	2.82	77 (6)	34	73 (5)	22	0.02	0.05	67 (5)	124	113 (5)	13	0.03	0.09	138 (11)	-69	114 (1)	-67	0.07	0.23
Acetaminophen	3.10	<20 (78)	-23	35 (35)	-19	0.16	0.55	69 (0.4)	22	113 (5)	-30	0.15	0.50	<20 (12)	355	98 (5)	-83	0.40	1.35
Codeine	3.63	62 (2)	28	63 (12)	7	0.03	0.09	66 (4)	93	111 (5)	4	0.04	0.13	81 (17)	-38	101 (3)	-52	0.16	0.52
Hydrochlorothiazide	4.36	79 (10)	-52	82 (16)	-60	0.10	0.35	70 (17)	-39	89 (12)	-58	0.34	1.15	84 (11)	-17	94 (1)	-34	0.08	0.25
Caffeine	4.58	72 (6)	101	74 (4)	62	0.03	0.10	65 (7)	186	108 (5)	52	0.06	0.19	88 (15)	-40	100 (0.3)	-58	0.19	0.62
Sulfapyridine	4.77	67 (20)	-45	50 (3)	-28	0.07	0.24	73 (1)	51	114 (6)	10	0.07	0.23	60 (16)	-26	77 (3)	-28	0.11	0.37
Amantadine	4.89	74 (18)	155	68 (1)	21	0.03	0.09	61 (9)	203	105 (5)	23	0.02	0.07	52 (29)	-65	90 (4)	-83	1.01	3.35
Trimethoprim	4.92	67 (3)	5	70 (3)	-11	0.02	0.06	64 (6)	67	106 (5)	-12	0.02	0.07	180 (5)	-81	134 (1)	-74	0.22	0.74
1H_Benzotriazole	5.11	79 (6)	-42	63 (17)	-42	0.05	0.17	75 (14)	-4	96 (9)	-32	0.09	0.29	59 (10)	-17	80 (3)	212	0.19	0.64
Oseltamivir-CBX	5.31	39 (12)	24	34 (37)	9	0.32	1.06	58 (1)	87	89 (8)	14	0.54	1.82	70 (8)	-1	96 (1)	-24	0.53	1.78
Oxytetracycline	5.50	- (-)	-	- (-)	-	14.10	47.00	39 (8)	42	88 (5)	20	1.19	3.95	<20 (1)	-96	69 (23)	-78	10.90	36.35
Ciprofloxacin	5.76	<20 (19)	-58	<20 (24)	-48	2.14	7.12	61 (10)	92	102 (4)	10	0.04	0.13	166 (6)	-94	100 (9)	-87	2.68	8.94
Benzoylecgonine	5.77	47 (14)	70	50 (28)	19	0.02	0.08	66 (5)	147	109 (4)	13	0.02	0.07	81 (14)	-21	99 (2)	-39	0.05	0.16
O-Desmethylvenlafaxine	5.79	62 (6)	7	72 (0.3)	-5	0.004	0.01	60 (8)	66	102 (4)	-3	0.01	0.03	72 (11)	-65	101 (2)	-73	0.13	0.45
Sulfamethazine	6.17	70 (17)	-26	51 (4)	-21	0.35	1.17	77 (2)	97	116 (6)	21	0.06	0.19	98 (14)	-41	93 (5)	-23	0.11	0.36
Lamotrigine	6.19	57 (34)	-48	57 (6)	-46	0.02	0.08	61 (3)	19	96 (6)	-37	0.04	0.15	58 (5)	-98	92 (11)	-98	6.91	23.03
Furazolidone	6.36	67 (23)	-4	78 (10)	-6	0.01	0.03	60 (8)	46	102 (7)	-17	0.02	0.08	82 (12)	-37	87 (2)	-44	0.03	0.09
Tramadol	6.63	66 (8)	11	67 (5)	-14	0.03	0.09	60 (9)	74	103 (4)	-2	0.01	0.03	60 (4)	-46	52 (1)	-75	0.91	3.04

Matrix		Brain						Plasma						Bile					
		20 (ng g^{-1})		200 (ng g^{-1})		LOD (ng g^{-1})	LOQ (ng g^{-1})	50 (ng mL^{-1})		500 (ng mL^{-1})		LOD (ng mL^{-1})	LOQ (ng mL^{-1})	100 (ng mL^{-1})		1000 (ng mL^{-1})		LOD (ng mL^{-1})	LOQ (ng mL^{-1})
PhACs	RT	R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)		
Metoprolol	6.66	67 (7)	59	72 (3)	24	0.01	0.02	67 (6)	125	111 (4)	17	0.03	0.08	81 (27)	-68	104 (8)	-78	1.05	3.51
Fluconazole	7.00	71 (3)	-10	65 (3)	-20	0.05	0.17	66 (6)	35	107 (5)	-25	0.08	0.26	73 (13)	5	83 (1)	-13	0.13	0.45
5-methyl-1H_Benzotriazole	7.41	70 (14)	-37	59 (16)	-43	0.03	0.10	65 (9)	-5	98 (10)	-31	0.03	0.10	55 (7)	2	75 (1)	-	0.12	0.40
Cocaine	7.44	60 (20)	-4	48 (16)	-20	0.03	0.10	56 (8)	76	102 (6)	-6	0.02	0.08	<20 (1)	-87	84 (11)	-96	2.70	8.98
Clofibric_Acid	7.46	70 (12)	-55	71 (15)	-64	0.60	2.00	66 (9)	-32	97 (15)	-62	0.78	2.61	87 (14)	12	90 (2)	-21	0.42	1.41
Sitagliptin	7.79	54 (27)	-8	55 (23)	-10	0.11	0.37	65 (4)	65	104 (4)	7	0.41	1.37	- (-)	-	82 (4)	-98	26.58	88.60
Furosemide	7.94	49 (6)	-51	51 (6)	-56	0.36	1.19	64 (14)	-20	106 (14)	-47	0.52	1.74	72 (17)	-32	91 (1)	-45	0.60	2.00
Sulfamethoxazole	8.03	101 (7)	-14	139 (0.03)	-19	0.06	0.19	66 (1)	72	108 (5)	7	0.09	0.32	86 (15)	-7	89 (9)	-41	0.14	0.48
Venlafaxine	8.52	57 (26)	-1	51 (24)	-23	0.01	0.04	60 (14)	-2	120 (8)	-22	0.02	0.06	- (-)	-	67 (18)	-100	11.16	37.19
Carazolol	8.78	45 (25)	6	41 (19)	-7	0.05	0.16	67 (7)	129	115 (6)	11	0.03	0.10	- (-)	-	- (-)	-	-	-
Coca-ethylene	8.83	66 (1)	-2	40 (37)	-29	0.02	0.06	61 (9)	47	106 (7)	-11	0.03	0.10	- (-)	-	- (-)	-	28.71	95.69
Oseltamivir_phospate	8.87	61 (18)	53	53 (29)	25	0.04	0.12	80 (8)	151	121 (4)	39	0.05	0.17	- (-)	-	- (-)	-	15.64	52.13
N-acethyl_SMX	8.93	73 (3)	42	76 (4)	21	0.12	0.39	64 (7)	103	108 (5)	11	0.52	1.72	45 (13)	31	146 (4)	-62	0.20	0.67
Chloramphenicol	9.19	81 (9)	-64	80 (14)	-65	0.04	0.14	64 (17)	-41	102 (16)	-62	0.14	0.47	78 (13)	-11	99 (3)	-48	0.12	0.40
Omeprazole	9.42	20 (16)	-86	48 (38)	-85	0.58	1.93	79 (4)	10	127 (8)	-37	0.17	0.55	<20 (1)	-100	<20 (1)	-100	23.36	77.86
CBZ-10,11-epoxide	9.61	69 (7)	109	75 (3)	51	0.02	0.06	65 (7)	177	106 (5)	37	0.03	0.09	- (-)	-100	123 (6)	-98	1.00	3.33
Valsartan Acid	9.90	46 (14)	-15	47 (35)	-1	1.38	4.62	55 (9)	204	87 (7)	81	0.70	2.33	58 (11)	-65	98 (7)	-45	1.25	4.17
Sulfadimethoxine	10.22	70 (9)	-28	47 (0.2)	-30	0.06	0.18	67 (1)	81	108 (5)	11	0.09	0.29	58 (10)	-17	57 (4)	-47	0.26	0.86
Midazolam	10.43	39 (56)	-75	<20 (1)	-87	0.19	0.63	66 (8)	-1	110 (7)	-39	0.02	0.08	- (-)	-	- (-)	-	1.55	5.16
Acridone	10.44	71 (7)	-18	64 (2)	-25	0.03	0.11	63 (10)	43	105 (6)	-13	0.02	0.07	73 (12)	-30	82 (1)	-47	0.04	0.13
Desmethylcitalopram	10.54	<20 (51)	-33	31 (14)	-41	0.03	0.11	68 (5)	47	111 (7)	-17	0.03	0.09	- (-)	-	- (-)	-	-	-
Bromazepam	10.59	106 (6)	-59	51 (6)	5	0.25	0.84	74 (7)	33	115 (7)	10	0.34	1.13	69 (2)	201	79 (11)	-	0.55	1.83
Diltiazem	11.25	58 (25)	-18	45 (26)	-46	0.07	0.24	64 (9)	49	109 (6)	-10	0.01	0.05	<20 (1)	-100	<20 (1)	-100	-	-
Erythromycin	11.32	47 (16)	-72	69 (16)	-73	0.09	0.30	66 (13)	28	105 (6)	-19	0.18	0.60	- (-)	-	- (-)	-	-	-

Matrix		Brain						Plasma						Bile					
		20 (ng g^{-1})		200 (ng g^{-1})		LOD (ng g^{-1})	LOQ (ng g^{-1})	50 (ng mL^{-1})		500 (ng mL^{-1})		LOD (ng mL^{-1})	LOQ (ng mL^{-1})	100 (ng mL^{-1})		1000 (ng mL^{-1})		LOD (ng mL^{-1})	LOQ (ng mL^{-1})
PhACs	RT	R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)		
Nalidixic Acid	11.57	<20 (21)	-12	<20 (51)	-61	0.80	2.66	64 (11)	48	108 (4)	-10	0.03	0.09	93 (12)	-42	96 (0.3)	-35	0.10	0.34
Pentobarbital	11.74	78 (10)	-34	78 (7)	-44	0.13	0.45	68 (12)	-1	106 (11)	-39	0.18	0.59	109 (16)	-23	98 (1)	-33	0.05	0.18
Paroxetine	11.81	<20 (1)	-96	- (-)	-65	0.33	1.09	90 (5)	32	138 (7)	-31	0.04	0.12	<20 (1)	-1	105 (2)	-99	5.92	19.73
Carbamazepine	11.85	38 (18)	47	52 (20)	17	0.02	0.08	67 (14)	51	112 (4)	-12	0.01	0.05	82 (13)	-33	93 (3)	-43	0.09	0.29
Flumequine	12.05	26 (29)	-7	<20 (1)	-56	0.46	1.53	64 (11)	70	110 (4)	-3	0.02	0.07	93 (11)	-24	102 (3)	-10	0.07	0.24
Verapamil	12.26	57 (36)	-35	20 (58)	-52	0.10	0.33	73 (6)	50	120 (5)	-17	0.01	0.04	80 (15)	-47	76 (2)	-60	0.10	0.33
Oxazepam	12.32	67 (0.1)	14	52 (20)	-27	0.24	0.80	59 (5)	87	110 (6)	2	0.10	0.35	102 (15)	20	63 (15)	7	0.12	0.41
Norfluoxetine	12.32	- (-)	-	- (-)	-	-	-	92 (14)	-19	150 (2)	-45	0.16	0.54	<20 (1)	10	<20 (1)	-64	-	-
Propyphenazone	12.37	66 (15)	-14	57 (24)	-22	0.03	0.09	63 (9)	29	105 (7)	-23	0.01	0.04	90 (11)	-73	52 (5)	-76	0.07	0.22
Fluoxetine	12.49	40 (28)	-87	42 (27)	-69	0.75	2.50	89 (9)	6	140 (10)	-37	0.03	0.09	<20 (1)	-42	79 (4)	-70	0.14	0.47
Clarithromycin	12.50	60 (13)	-28	43 (15)	-55	0.07	0.22	65 (12)	17	108 (6)	-35	0.05	0.17	- (-)	-	81 (16)	-96	-	-
Sertraline	12.54	60 (10)	-16	66 (25)	-38	1.52	5.07	105 (9)	147	143 (8)	-3	0.04	0.12	98 (17)	109	78 (6)	-37	0.07	0.23
Lorazepam	12.55	68 (2)	44	57 (18)	-19	0.18	0.59	62 (9)	117	106 (7)	10	0.19	0.64	20 (15)	-17	98 (1)	-75	1.92	6.38
Diclofenac	12.61	62 (11)	-71	71 (5)	-69	0.24	0.80	69 (17)	-24	109 (12)	-52	0.25	0.84	<20 (1)	-18	89 (19)	-79	1.39	4.62
Alprazolam	12.62	60 (7)	29	39 (63)	-13	0.02	0.06	60 (6)	123	106 (5)	6	0.02	0.07	42 (10)	-63	99 (2)	-83	0.32	1.08
Losartan	12.98	<20 (1)	-93	<20 (1)	-87	0.46	1.53	58 (2)	-63	107 (7)	-65	1.02	3.39	67 (15)	-51	92 (1)	-	0.36	1.21
Temazepam	13.08	72 (2)	52	60 (31)	-28	0.02	0.08	62 (7)	155	106 (6)	34	0.07	0.23	93 (13)	-18	98 (2)	-36	0.11	0.36
Bisphenol-A	13.11	31 (4)	-49	51 (9)	-51	0.06	0.21	72 (16)	33	120 (10)	-17	0.04	0.12	99 (3)	211	98 (5)	39	-	-
Atorvastatin	13.27	<20 (1)	-73	48 (16)	-75	0.91	3.05	54 (8)	-28	126 (23)	-49	0.10	0.33	91 (4)	-75	82 (5)	-18	1.91	6.35
Ketoprofen	13.31	62 (11)	139	67 (8)	84	0.11	0.35	62 (6)	233	109 (7)	70	0.03	0.09	92 (12)	-	99 (2)	-	0.11	0.36
Mefenamic acid	13.33	142 (1)	-91	60 (1)	-89	-	-	61 (18)	4	107 (17)	-33	0.07	0.24	23 (19)	2	82 (17)	-55	0.10	0.33
Ibuprofen	13.35	- (-)	-	86 (3)	-94	1.59	5.29	187 (15)	152	65 (20)	-73	1.13	3.76	73 (15)	8	81 (4)	-37	0.21	0.70
Bezafibrate	13.45	61 (14)	8	63 (6)	-4	0.01	0.02	60 (6)	51	105 (6)	-12	0.13	0.43	88 (14)	12	95 (2)	-5	0.12	0.41
Valsartan	13.72	65 (19)	-79	49 (40)	-68	0.20	0.66	54 (4)	21	98 (7)	-12	0.17	0.57	74 (15)	53	88 (4)	-	0.12	0.41

Matrix		Brain						Plasma						Bile					
		20 (ng g^{-1})		200 (ng g^{-1})		LOD (ng g^{-1})	LOQ (ng g^{-1})	50 (ng mL^{-1})		500 (ng mL^{-1})		LOD (ng mL^{-1})	LOQ (ng mL^{-1})	100 (ng mL^{-1})		1000 (ng mL^{-1})		LOD (ng mL^{-1})	LOQ (ng mL^{-1})
PhACs	RT	R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)			R (RSD) (%)	ME (%)	R (RSD) (%)	ME (%)		
Diazepam	13.76	86 (2)	-53	44 (61)	-76	0.01	0.02	60 (4)	-2	104 (7)	-27	0.03	0.09	93 (14)	-46	101 (1)	-48	0.02	0.08
Warfarin	13.87	62 (26)	22	74 (6)	1	0.01	0.04	60 (6)	77	103 (7)	5	0.03	0.11	80 (16)	-36	84 (2)	-40	0.05	0.17
Loratadine	14.45	<20 (1)	-80	<20 (1)	-99	0.28	0.95	70 (6)	-12	115 (9)	-36	0.03	0.09	78 (19)	-13	101 (1)	-14	0.02	0.08
Flufenamic_Acid	14.93	- (-)	-	- (-)	-	3.58	11.94	- (-)	-	66 (13)	-79	1.56	5.20	<20 (1)	-26	93 (14)	-55	8.70	29.00
Fipronil	15.11	<20 (1)	-97	62 (37)	-96	0.44	1.48	66 (21)	-19	114 (16)	-45	0.02	0.08	49 (18)	68	98 (5)	131	0.08	0.26
Fipronil_Desulfinyl	15.27	166 (49)	-95	48 (18)	-97	1.07	3.56	71 (18)	-31	113 (19)	-50	0.04	0.12	25 (19)	195	99 (4)	146	0.08	0.26
Triclocarban	15.44	<20 (1)	-83	<20 (1)	-34	0.16	0.55	88 (9)	-	119 (15)	-	0.08	0.27	<20 (16)	-	46 (19)	205	0.66	2.20
Fipronil sulfone	15.44	- (-)	-99	67 (9)	-98	0.98	3.28	80 (17)	-1	124 (14)	-20	0.03	0.11	<20 (8)	-	100 (8)	-	0.12	0.39
Fipronil Sulfide	15.47	- (-)	-99	47 (21)	-98	1.19	3.98	78 (16)	-15	117 (15)	-35	0.08	0.27	<20 (2)	-	97 (5)	-	0.14	0.46
Irgasan	15.51	- (-)	-	24 (13)	-90	3.74	12.46	83 (12)	177	121 (17)	233	0.09	0.29	33 (24)	-	37 (22)	-	0.27	0.89

3.2.2. PUBLICACIÓN No. 2

An effective method for the simultaneous extraction of 173 contaminants of emerging concern in freshwater invasive species and its aplication

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RESEARCH PAPER

An effective method for the simultaneous extraction of 173 contaminants of emerging concern in freshwater invasive species and its application

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Abstract
A robust and efficient extraction method was developed to detect a broad range of pollutants of emerging interest in three freshwater invasive species: American red crab (*Prokambarus clarkii*), Asian clam (*Corbicula fluminea*), and pumpkinseed fish (*Lepomis gibbosus*). One native species, "petxinot" clam (*Anodonta cygnea*), was also evaluated. Invasive species are often more resistant to contamination and could be used in biomonitoring studies to assess the effect of contaminants of emerging concern on aquatic ecosystems while preserving potentially threatened native species. So far, most extraction methods developed for this purpose have focused on analyzing fish and generally focus on a limited number of compounds, especially analyzing compounds from the same family. In this sense, we set out to optimize a method that would allow the simultaneous extraction of 87 PhACs, 11 flame retardants, 21 per- and polyfluoroalkyl substances, and 54 pesticides. The optimized method is based on ultrasound-assisted solvent extraction. Two tests were performed during method development, one to choose the extraction solvent with the best recovery efficiencies and one to select the best clean-up. The analysis was performed by high-performance liquid chromatography coupled to high-resolution mass spectrometry. The method obtained recoveries between 40 and 120% and relative standard deviations of less than 25% for 85% of the analytes in the four validated matrices. Limits of quantification between 0.01 ng g^{-1} and 22 ng g^{-1} were obtained. Application of the method on real samples from the Albufera Natural Park of Valencia (Spain) confirmed the presence of contaminants of emerging concern in all samples, such as acetaminophen, hydrochlorothiazide, tramadol, PFOS, carbendazim, and fenthion. PFAS were the group of compounds with the highest mean concentrations. *C. fluminea* was the species with the highest detection frequency, and *P. clarkii* had the highest average concentrations, so its use is prioritized for biomonitoring studies.

Keywords Aquatic contamination · Perfluoroalkyl substances · Pharmaceuticals · Aquatic fauna · LC-MS/MS · Ultrasound-assisted solvent extraction

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Introduction

The detection of contaminants of emerging concern (CECs) such as pharmaceutically active compounds (PhACs), pesticides, perfluoroalkyl substances (PFAS), and organophosphorus flame retardants (OPFR) in freshwater samples [1–13] has been gaining importance in recent years because their presence represents a risk to the environment [14]. These contaminants are diffusely released into the environment through runoff water or outlet water from wastewater treatment plants (WWTPs). Once in the water cycle, they can be taken up by aquatic organisms and may bioaccumulate in tissues. Their analysis in aquatic biota could provide

more insight into pollution relationships than measuring discrete levels in water or sediment samples [3, 15].

The process of globalization, industrialization, deliberate introduction of exotic species for food production, and climate change facilitate the spread of species beyond their natural habitats where they encounter favorable conditions, e.g., due to the absence of predators. When invasive species easily adapt to a new environment, they can become dominant and displace the ecological niche of native fauna. This is the case of the American red crab (*Procambarus clarkii*), the Asian clam (*Corbicula fluminea*), and the pumpkinseed fish (*Lepomis gibbosus*), which have become invasive species in Mediterranean wetlands, including the Albufera Natural Park (Valencia, Spain). *P. clarkii* is a crayfish native to North America, which was introduced for commercial purposes in the 1970s in the Guadalquivir marshes (Southwest Spain). The economic benefits of its introduction encouraged its exploitation in areas with similar habitats. The climatic conditions of the southern Iberian Peninsula and the absence of natural predators allowed this species' vast and rapid expansion [16, 17]. *C. fluminea* is a clam native to East Asia that has become a dominant invasive species in the Iberian Peninsula, mainly in tidally influenced lotic habitats [18]. *L. gibbosus* is native to North America and was introduced in Europe in 1880 along with other sport fishing species and as an ornamental species. The oldest documented presence in the Iberian Peninsula was between 1910 and 1913, identified in Lake Banyoles, northern Spain [19].

Invasive species could be used as integrative bioindicators to assess exposure to chemical in freshwater ecosystems. Due to their high adaptability, invasive species tend to have greater tolerance to environmental stresses. *P. clarkii*, for example, is more resilient and a better competitor than the native *Austropotamobius pallipes*, a highly endangered European freshwater crayfish. It can withstand lower oxygen levels, higher temperatures (30 °C), and high water pollution [16]. The use of invasive species in biomonitoring studies would reduce pressure on native endangered species. Invasive species populations are usually larger and their use for environmental analysis would not generate alterations in ecosystems. However, most of the active biomonitoring of CECs in freshwater ecosystems has been performed on fish and macroinvertebrates. Furthermore, most of the studies focus on a small number of compounds [20]. Due to the low expected levels of CECs and the complexity of the matrix, sensitive and robust analytical methods are essential. Ultrasound-assisted solvent extraction (UAE) is one of the most common extraction methods for CECs in aquatic biota [9, 12, 21–32]. Extraction of complex biological matrices typically features a clean-up step to ensure removal of co-extracted matrix components that otherwise may interfere in the quantification. The clean-up methods widely used in this type of analysis are solid-phase extraction (SPE) and dispersive solid-phase extraction (d-SPE) [20].

In this regard, we aimed to develop and validate a multiresidue method that allows for simultaneous extraction of four groups of CECs (87 PhACs, 54 pesticides, 21 PFAS, and 11 OPFRS) and their subsequent detection and quantification in three invasive species from the Albufera Natural Park (red swamp crayfish, Asian clam, and pumpkinseed fish) and the native species "petxinot" (*Anodonta cygnea*). The conditions of (a) sample extraction and (b) clean-up of the extracts were optimized in separate experiments. For the former, different extraction solvents were tested to maximize recovery efficiencies, while for the latter, different d-SPE materials were compared with respect to their effectiveness in reducing matrix effects (ME) in the LC-MS. The extraction solvents evaluated were **A** acetonitrile (MeCN) + 0.1% formic acid (F.A), **B** isopropanol/acetonitrile (iPrOH/MeCN) (1:3) + 0.1% F.A, **C** water/methanol (H_2O/MeOH) (7:3) + 1% F.A, and **D** 100% MeOH. The d-SPE sorbents evaluated were as follows: **A** Z-Sep, **B** Z-sep$^+$, and **C** Z-Sep/C_{18}. Once the extraction method was validated, it was used to analyze real samples collected in the Albufera Natural Park.

Materials and methods

Chemicals and standards

The chemical standards (Table S1) as well as the isotopically labeled standards (IS) (Table S2) were purchased from Sigma-Aldrich (St. Louis, MO, USA), Sigma-Aldrich (Steinheim, Germany), Toronto Chemicals Research (Toronto, Ontario, Canada), CDN Isotopes (Pointe-Claire, Quebec, Canada), Santa Cruz Biotechnology (Dallas, TX), Wellington (Ontario, Canada), and Cymit Quimica S.L. (Barcelona, Spain). LC-MS grade MeCN (≥ 99.9%), iPrOH (≥ 99.9%), HPLC grade water, and MeOH (≥ 99.9%) used as extraction solvents were obtained from J.T.Baker (Fisher Scientific SL, Madrid, Spain). F.A (≥ 96%, ACS reagent) was obtained from Sigma-Aldrich. The d-SPE sorbents (Z-Sep/C_{18}, Z-Sep$^+$, and Z-Sep) were obtained from Supel TM QuE, Supelco/Sigma-Aldrich, St. Louis, MO, USA. MeCN and water (Optima™ LCMS Grade) used for the preparation of the mobile phases were obtained from Fisher Chemical. Ammonium acetate and ammonium fluoride were obtained from Sigma-Aldrich and Fisher Chemical (Fisher Scientific SL, Madrid, Spain). The standard mixtures used for the calibration curve were prepared in serial dilutions from a stock solution of 2 µg mL^{-1}. A matrix-matched calibration curve was prepared by spiking the extracts to obtain ten points, from 0.1 to 500 ng g^{-1} (wet weight, w.w).

Sample collection and pretreatment

The species used to develop, optimize, validate, and evaluate the applicability of the extraction method were collected at the Albufera Natural Park. This ecosystem is a coastal lagoon with marine water influences, as well as wastewater impacts, but at present, it is mainly a freshwater system. It is located 10 km south of Valencia (Spain) and covers an area of 210 km^2. It constitutes a natural refuge for diverse biological species, and activities such as agriculture (mainly rice fields), fishing, hunting, urban, and industrial construction interact there [33].

To reduce costs and analytical time, a native species (*A. cygnea*) and an invasive species (*P. clarkii*) were selected for the development of the extraction method. The specimens were obtained from a fish farm located in the Natural Park. Of the analytes studied, PFAS have the longest half-life. This is why the samples were subjected to a 14-day cleanup process according to the depuration rates reported for PFAS in oysters [34] and aquatic invertebrates [35]. The depuration was carried out in 26-L tanks with a closed circuit of clean water and a filtration module to eliminate contaminants and provide the cleanest possible matrix. Once the analytical method was developed, it was validated for four species.

To demonstrate the method applicability, real samples of the three invasive species were collected: *C. fluminea* was collected in the irrigation channels of Rec de Tonyiner (351 specimens divided into groups of 50 to obtain seven pooles samples plus a sample of a single larger clam), *P. clarkii* was captured in the areas surrounding the rice paddies of "Sollana" and "El Palmar" (32 specimens divided according to their weight to obtain 17 pooled samples), and *L. gibbosus* from the "Sueca-El Palmar" canals and the fish farm settling ponds (13 specimens divided into seven samples according to their weight). In contrast to high population of invasive species, the native species was not sample to protect the existing population.

For the sample pretreatment, the soft tissue of each species was obtained, removing the shell in the case of clams and the exoskeleton in the case of crabs, while the whole organism was used in the case of fish. The tissues were homogenized using a knife mill with a stainless steel grinding chamber (Grindomix GM 200, Retsch GmbH, Haan, Germany) and stored at −40 °C until extraction.

Method development

The extraction protocol implemented was based on a method previously reported by [26] for fish tissues. In this case, the extraction efficiency of four solvents was evaluated: **A** MeCN + 0.1% F.A facilitates the recovery especially of more polar compounds; **B** iPrOH/MeCN (1/3) + 0.1% F.A tends to improve the recovery of less polar compounds; **C** H_2O/MeOH (7/3) +1% F.A has shown satisfactory recoveries under acidic conditions; and **D** 100% MeOH tends to obtain good recoveries, especially of more lipophilic compounds. We evaluated solvents under acidic conditions because most compounds ionize in positive mode. Only PFAS and 18 PhACs ionize in negative mode (Table S1). However, according to [36], PFAS had satisfactory recoveries with acidified solvents. Nevertheless, to favor the ionization of negative compounds, ammonium fluoride was used in the mobile phases during chromatography, as shown in Table 1.

In brief, for the extraction 500 mg of homogenized soft tissue was weighed into a 2-mL Eppendorf tube. Then, 50 µL of the working solution containing the analytes (200 ng mL^{-1}) was added to the sample to obtain a concentration of 10 ng mL^{-1} in the final extract, and allowed to stand under the fume hood for 30 min. Samples were homogenized again by placing two stainless steel beads (5 mm Ø) into the Eppendorf and operating a TissueLyzer LT sample disruptor (Quiagen, Germany) at 50 Hz for 90 s, and allowed to stand for 15 min. One milliliter of the extraction solvent to be evaluated was added (A, B, C, or D); the samples were vortexed for 90 s, sonicated for 10 min (Fisherbrand® FB15064, Waltham, MA, USA), and centrifuged for 12 min at −9 °C and 20854 G-force (Eppendorf AG 5810 R Hamburg, Germany). Finally, 500 µL of the supernatant was transferred to an HPLC vial, evaporated to dryness (TurboVap® LV, Biotage. Uppsala, Sweden), and reconstituted in 500 µL 5mM ammonium acetate in water/MeCN (9/1).

After selecting the extraction solvent with the most efficient recoveries and the lowest relative standard deviation (RSD), a second series of experiments were performed in which different d-SPE sorbents were evaluated to clean the extracts and remove interfering co-eluents from the matrix. The Z-Sep sorbent has previously demonstrated its utility for removing matrix components from samples containing a large number of both polar and non-polar analytes [12, 36, 40]. The common sorbent that all Z-Sep variants have is silica gel and zirconium dioxide, which is an amphoteric oxide that presents multiple interaction pathways with matrix components. The zirconium-coated silica particles selectively remove fat and pigments from sample extracts. Three different sorbents of this type were tested. **A** The standard Z-Sep sorbent is a mixture of two sorbents, C_{18} and zirconium dioxide-coated silica. The ratio of SiO_2+ZrO_2:C_{18} is 2:5. Z-Sep is used for hydrophobic analytes. Lipid retention is based on Lewis acid-base interactions between hydroxyl groups and zirconium. **B** Z-Sep$^+$ sorbent consists of zirconia and double-bonded C_{18} on the same silica particles and is suggested for cleaning samples with more than 15% fat content, and **C** the Z-Sep/C_{18} sorbent is a combination of Z-Sep and Discovery® DSC-18 particles. Its use is recommended for analyzing samples with less than 15% fat content. Lipid

Table 1 Chromatographic and analytical conditions by compounds group

Compounds	Conditions	LC-MS/MS	Elution gradient			Reference
			Time (min)	Aqueous phase (%)	Organic phase (%)	Flow (mL min^{-1})	
PhACs	*Column:* EVO C18 KINETEX (100 × 2.1 mm; 2.6 µm. Phenomenex). *Mobile phases:* H_2O 5mM ammonium acetate + 0.1% F.A and MeCN for ESI (+) H_2O 2mM NH_4F and MeCN for ESI (-)	Waters Acquity UPLC system (Waters. Milford. MA). Orbitrap Q-Exactive™ mass spectrometer (Thermo Fischer Scientific, San Jose, CA,USA)	0.0	95	5	0.2	[26]
			0.3	95	5	0.2	
			10	70	30	0.2	
			13.3	35	65	0.2	
			15.5	0	100	0.2	
			17.3	0	100	0.2	
			17.7	95	5	0.2	
			19.0	95	5	0.2	
OPFRs	*Column:* KINETEX XB-C18 (50.0 × 2.1 mm. 1.7 µm. Phenomenex. Torrance. USA) *Trap Column:* ZORBAX Eclipse Plus C18 (4.6 × 30 mm; 3.5 µm. Agilent. Santa Clara. USA). *Mobile phases:* H_2O + 0.1% F.A and MeOH +0.1% F.A	Thermo Scientific™ Vanquish™ UHPLC system. Thermo Scientific™ Orbitrap Exploris 120™ mass spectrometry	0.0	70	30	0.4	[37]
			0.5	79	30	0.4	
			12	2	95	0.4	
			18	2	98	0.4	
			18.1	70	30	0.4	
			28	70	30	0.4	
Pesticides	*Column:* Luna C18 (15.0 cm × 0.21 cm. 3 µm. Phenomenex. Torrance. USA). *Mobile phases:* H_2O 5mM ammonium formate + 0.1% F.A MeOH 5mM ammonium formate + 0.1% F.A		0.0	50	50	0.4	[38]
			10.0	17	83	0.4	
			12.0	17	83	0.4	
			12.5	2	98	0.4	
			15.5	2	98	0.4	
			15.6	50	50	0.4	
			25.6	50	50	0.4	
PFAS	*Column:* Kinetex® EVO C18 KINETEX (100 × 2.1 mm; 2.6 µm. Phenomenex. Torrance. USA). *Mobile phases:* H_2O + 4 mM NH_4F and MeOH + 4 mM NH_4F for ESI (-)	UHPLC Agilent 1260 HPLC (degasser, quaternary pump, and column oven) Eksigent ULC 100 HTC-xt autosampler. Sciex TRIPLE QUAD 6500+ mass (Sciex, Concord, Ontario, Canada)	0.0	95	5	0.3	[39]
			0.5	70	30	0.3	
			12	5	95	0.3	
			18	5	95	0.3	
			30	95	5	0.3	

retention is based on hydrophobic interactions between triglycerides and C_{18}.

For the development of the second experiment, the same protocol was followed, and a clean-up step was added to the procedure in which 750 µL of the supernatant was transferred to a vial containing the d-SPE to be evaluated (A, B, or C). It was vortexed for 1 min and centrifuged for 6 min at 20854 G-force and −2 °C. Five hundred microliters of the supernatant was transferred to an HPLC vial, evaporated, and reconstituted as described above. All samples were prepared in triplicate.

LC-HRMS/MS analysis

Information related to the chromatographic separation, the mobile phases used for the positive and negative ionization modes, the elution gradient, the source conditions, and any detailed information regarding the methodology LC-HRMS/MS are summarized in Table 1. Data acquisition for method validation was performed with Thermo Scientific Xcalibur 4.1.31.9. Software (Thermo Fisher Scientific). Quantification was performed with a matrix-matched approach and following the internal standard method. Samples were spiked with 12.5 µL of IS (2 µg mL^{-1}) used as surrogates before the extraction for quantification purposes. We used Trace Finder 5.1 software (Thermo Fisher Scientific) to quantify the analytes in the real samples. The presence of the precursor ion was confirmed by applying the following criteria: signal-to-noise ratio >10, mass accuracy of 5 ppm, equivalent retention time of 30 s compared to the reference standard, and presence of the product ion with the highest relative abundance in the MS/MS spectrum.

Validation protocols

According to the results obtained for solvent extraction and clean-up sorbent experiments, the method with the best recoveries for most of the compounds of the four groups of analytes was selected (MeCN + 0.1% F.A + the Z-Sep/C_{18} sorbent). The method was validated at two concentration levels (10 ng g^{-1} and 100 ng g^{-1}). The validation was performed for the four species: *C. fluminea*, *P. clarkii*, *L. gibbosus*, and *A. cygnea*. The performance of the extraction method was expressed in terms of recovery (%), precision (RSD), limits of detection and quantification (LOD and LOQ), and matrix effects (ME) for each specie. The accuracy and precision of the method were calculated based on the analysis of three replicates.

Recoveries (*R*, %) were determined by comparing the peak area of the analytes in the samples spiked with standards before the extraction with the peak area of analytes in the samples spiked after the extraction. The values were normalized by the area of corresponding IS, according to Eq. (1).

$$R(\%) = 100\left(\frac{(\text{ABE} - \text{Blanck extract})/\text{AIS}}{(\text{AAE} - \text{Blanck extract})/\text{AIS}}\right) \quad (1)$$

where **ABE** is the area of the analyte before extraction, **AAE** is the area of the analyte after extraction, and **AIS** is the area internal standard.

To evaluate the ME on the analyte signal in the MS, blank extracts were spiked at the same concentration as the recovery studied (10 ng g^{-1} and 100 ng g^{-1}). The relative ME was calculated by comparing the peak area of the analyte spiked after the extraction, with its peak area of a solution prepared in aliquots of 0.5 mL of injection solvent: 5mM ammonium acetate in water/MeCN (9/1) according to Eq. (2).

$$ME(\%) = 100x\left(\frac{\text{AAE} - \text{Blank extract}}{\text{Area in solvent solution}} - 1\right) \quad (2)$$

We used the matrix-matched calibration curve approach to determine the LOD and LOQ of the method by calculating the standard deviation of the calibration curve and dividing it by the slope, according to Eqs. (3) and (4).

$$LOD\left(ngg^{-1}\right) = \left(\frac{3.S_b}{\text{b}}\right) \quad (3)$$

$$LOQ\left(ngg^{-1}\right) = \left(\frac{10.S_b}{\text{b}}\right) \quad (4)$$

where $\boldsymbol{S_b}$ is the standard deviation of calibration curve and **b** is the slope.

The calibration series was prepared at ten concentration levels from 0.1 to 500 ng g^{-1}, and 12.5 µL of IS (1 µg mL^{-1}) was added to each calibration level. Linearity was evaluated by calculating the coefficient of determination (R^2). The calibration curve was constructed by weighted least squares linear regression (1/*x* as weighting factor) and was also used for quantification purposes.

Quality assurance and quality control (QA/QC)

Samples used for development, method validation, and preparing the matrix-matched calibration curves underwent a depuration process for 14 days in fish tanks with clean water to remove contamination. The stainless steel beads used in the soft tissues' homogenization step were washed with ethanol and acetone and placed in an ultrasonic bath for 15 min for each solvent to avoid the contamination of samples. The other materials used during the extraction process were single-use. Additional blank extracts spiked with reference standards (100 ng g^{-1}) were tested, as quality control after every eight samples to evaluate method performance.

Similarly, solvent blanks (MeOH) were analyzed in every four samples to verify the absence of carryover effect in the column. An interval of 30 s was an acceptable difference between the chromatographic retention times of the calibration curve and those of the samples. Moreover, a mass error of <5 ppm has been established to quantify the compounds detected in the real samples. The R^2, IS, and linearity range for each compound used for quantification purposes are shown in Table S2.

Results and discussion

Extraction solvent optimization

The method development was based on previous work to analyze PhACs in complex matrices such as fish tissues/organs [12, 30]. The expected result of the present method was to extend the simultaneous extraction of CECs (87 PhACs, 54 pesticides, 21 PFAS, and 11 OPFRS) in matrices with differences in the percentage of lipid content. Considering the variability of the physicochemical properties of the analytes, we initially tested four mixtures of extraction solvents: **A** MeCN + 0.1% F.A, **B** iPrOH:MeCN (1:3) + 0.1% F.A, **C** H_2O:MeOH (7:3) + 1% F.A, and **D** 100% MeOH. The results of the extraction efficiencies for the four groups of compounds spiked at a concentration level of 20 ng g^{-1} (w.w.) for one of the invasive species (*P. clarkii*) and the native species (*A. cygnea*) are shown in Fig. 1. The results of extraction efficiency for each compound according to each extraction solvent are reported in Table S3.

The results obtained show variability in the extraction efficiency by solvent and group of compounds. Considering an acceptable recovery between 40 and 120% as a reference, the PhACs group obtained the best extraction efficiencies, achieving the recovery of 84 analytes with solvent **B** and 80 with solvent **A** in the *A. cygnea* and 71 analytes with solvent **B** and 59 with solvent **A** in the *P. clarkii* respectively. In the case of PFAS, the same extraction efficiency was obtained with solvent **A** and solvent **B** for the *P. clarkii*, recovering 18 analytes. In contrast, for the *A. cygnea*, the highest number of PFAS was recovered with solvent **D** (19 analytes), followed by solvent **A** (18 analytes) and solvent **B** (14 analytes). For OPFRS, the best extraction efficiencies were obtained with solvents **C** and **D** (nine analytes each) in the *A. cygnea* and with solvents **B** (nine analytes) and **A** (eight analytes) for *P. clarkii*. Finally, pesticides obtained the lowest extraction efficiency. In the *P. clarkii*, recovery of 33 analytes was achieved with solvent B and 28 analytes with solvent **A**, whereas for the *A. cygnea*, solvents **A** and **D** recovered the highest number of analytes (27 each one). In general terms, solvent **A** and **B**´s extraction efficiencies are acceptable for most compounds assessed. In this sense, we decided to select solvent **A** (**MeCN + 0.1% F.A**) to continue with the clean-up test because the calculated RSDs for most of the analytes are below 20% for the two matrices. In contrast, solvent **B** showed higher extraction uncertainty among the triplicates evaluated.

Clean-up sorbent optimization

One of the main challenges during extraction method development for complex biota matrices is the co-extraction of

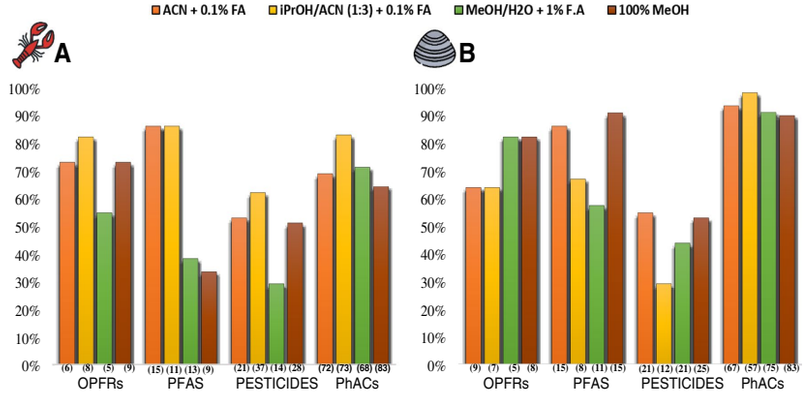

Fig. 1 Solvent extraction test results. **A** *P. clarkii*. **B** *A. cygnea*. Bars indicate the percentage of compounds with an extraction efficiency between 40 and 120%. The percentage was calculated according to the total number of analytes in each compound group: OPRFs (11), PFAS (21), pesticides (54), and PhACs (87). (Numbers in brackets): analytes with RSD ≤ 20%.

matrix compounds such as lipids, proteins, and other compounds that may interfere with the signal of the target analytes. A clean-up step was added after the extraction stage to avoid these interferences during analysis. Three different sorbents were tested: **A** Z-Sep, **B** Z-Sep$^+$, and **C** Z-Sep/C_{18}. The percentage of compounds for which an acceptable recovery efficiency (40 to 120%) was obtained is shown in Fig. 2. For PFAS, pesticides, and PhACs, most compounds were recovered after the cleaning step with sorbent **C**, in both *P. clarkii* and *A. cygnea*. For OPFRs, there were differences. In *P. clarkii*, the best results were obtained with sorbent **A**, whereas in *A. cygnea*, the best results were with sorbent **B**. However, satisfactory recoveries were obtained with sorbent **C**, with RSD below 20% (Table S3). The number of compounds recovered within an acceptable range was increased by including the extracts clean-up with the sorbent **C**, mainly for the pesticide group, where 12 more analytes were retrieved in the *P. clarkii* and 15 more in the *A. cygnea*.

During this experiment, we evaluated the percentage of matrix removed by each Z-sep tested. For this, we compared the amount of matrix remaining after extraction with MeCN + 0.1% F.A with the amount removed after the cleaning step. Thus, sorbent **A** reduced 14% of the matrix interferents in *A. cygnea* and 24% in *P. clarkii*. Sorbent **B** reduced 55% of the matrix in both species, and finally, sorbent **C** was able to eliminate 42% of interferents in *A. cygnea* and 17% in *P. clarkii*. The higher percentage obtained with sorbent **B** is probably due to the fact that this sorbent also favored the elimination of the pigments present in the extracts.

Overall, the use of cleanup generated a reduction in enhancement of several compounds during solvent testing by increasing the number of compounds within the ME range considered acceptable (>−40% and <40%) (Fig. S1). In the case of OPFRS, this effect was greater with sorbent **B** in *P. clarkii* and sorbent **C** in *A. cygnea*. Likewise, for the PhACs, the cleanup with sorbent **B** increased the number of compounds within the acceptable range of ME, going from 53 analytes in the solvent test to 63 for *P. clarkii* and 59 to 85 in *A. cygnea*. PFAS and pesticides obtained the best results with sorbent **C** for both species. Sorbent **C** included three more compounds in *P. clarkii* within the acceptable range and nine more in *A. cygnea.*

Another essential criterion for choosing the cleaning sorbent depends on the fat content of the matrix to be analyzed. We calculated the fat percentage of the four species according to [41]. Thus, we obtained that the fat rate (w.w) for *A. cygnea* was 2.2%, 2.3% for *L. gibossus*, 3% for *P. clarkii*, and 0.7% for *C. fluminea*. Relating to the results obtained, we decided to include Z-sep/C_{18} in the cleaning step because the sorbent allowed the recovery of the highest number of compounds in all matrices. Additionally, Z-sep/C_{18} obtained the lowest RSD values when calculating the recovery efficiencies and allowed the reduction of the matrix interferences without compromising the recoveries. The satisfactory results obtained for the Z-sep/C_{18} sorbent coincide with the expectations, considering that the percentage of fat calculated for the species is less than 15%.

Method validation

Validation of the optimized extraction method, i.e., using **MeCN + 0.1% F.A** as extraction solvent and **Z-Sep/C_{18}**

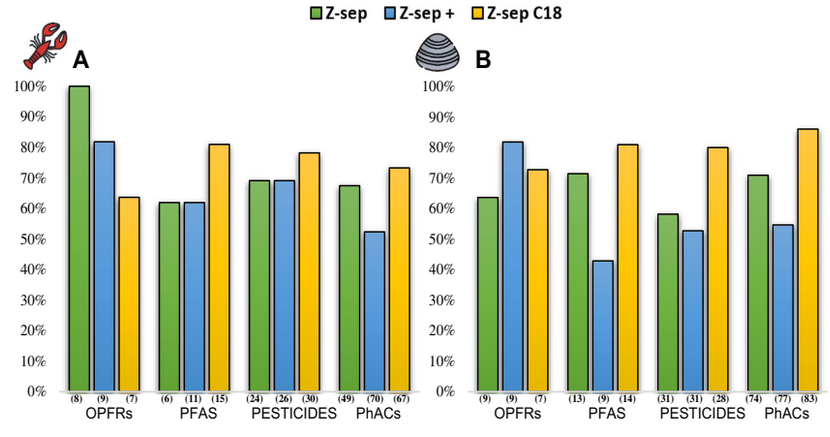

Fig. 2 Clean-up step test results. **A** *P. clarkii*. **B** *A. cygnea*. Bars indicate the percentage of compounds with an extraction efficiency between 40 and 120%. The percentage was calculated according to the total number of analytes in each compound group: OPRFs (11), PFAS (21), pesticides (54), and PhACs (87). (Numbers in brackets): analytes with RSD ≤ 20%.

sorbent during the clean-up step, was performed to confirm whether the procedure allows reliable analytical results. Validation was performed by evaluating the accuracy, linearity, precision, LOD, and LOQ defined in the "Validation protocols" section. The method was validated for the four species of interest: *P. clarkii, A. cygnea, C. fluminea*, and *L. gibbosus*. Accuracy and precision were calculated from three repeated injections for each kind of matrix spiked at 10 ng g^{-1} and 100 ng g^{-1} (w.w).

Recoveries and robustness

To define the compounds that can be reliably detected with the developed extraction method, acceptability criteria were established, based on the recovery and RSD values calculated during validation. Thus, detecting an analyte was considered reliable if the recovery was between 40 and 120% and its RSD was <25% in at least one of the validation levels. Table S4 shows the calculated values. Fig. 3 summarizes the recoveries by compound group and matrix.

Seven of the 11 validated OPFRS (green diamonds) were recovered with the established acceptability criteria in all four species. The other four OPFRS are outside the criteria in at least one species, mainly because their recovery is higher than 120% (TPP, the TnBP, the TDBPP, and the TBEP). Recoveries higher than the established maximum are probably due to an enhancement of the analyte response due to co-extractants (matrix effect). This effect is more marked in *P. clarkii* and *L. gibossus* (Fig. 4). Similar results have been obtained for the TPP in eel [25].

Of the 21 validated PFAS (blue boxes), only two are outside the acceptability criteria: PFPeA in *P. clarkii* with a recovery greater than 120% and PFHpS in *C. fluminea* with a recovery less than 40%. We consider that these two particular cases depend exclusively on the interaction of the matrix with the analyte at the time of ionization since their recoveries were satisfactory in the other species. In the case of PFPeA, the calculated ME in *P. clarkii* was an outlier in the data series (146%). The increase of its signal after ionization may have induced its high recovery (136%). The opposite effect occurred for PFHpS in *C. fluminea* (*R*, 34% and ME, −52%).

Regarding the pesticides (yellow triangles), 46 of the 54 compounds validated were within the range of acceptability in all species. Eight pesticides were outside the field, but each for a specific species. Ethion and isoproturon obtained recoveries greater than 120% and less than 40%, respectively, only in *P. clarkii.* The recovery of atrazine was above the acceptable range for *A. cygnea* and metolachlor below the range for *C. fluminea*. In *L. gibossus*, the buprofezine, azinphos-ethyl, propanil, and pyriproxyfen were not recovered at either validation level.

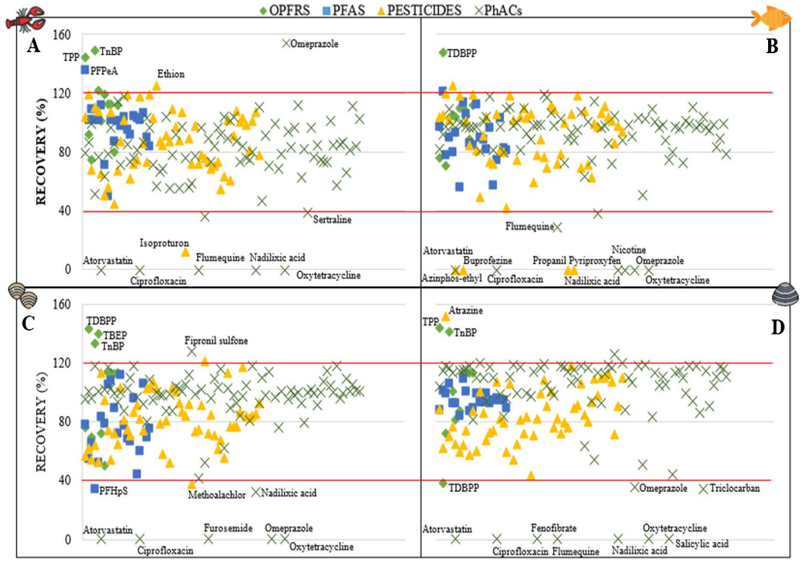

Fig. 3 Recoveries in all matrices for level 2: 100 ng g^{-1}. **A** *P. clarkii*, **B** *L. gibossus,* **C** *C. fluminea*, **D** *A. cygnea*. Compounds between the red lines obtained recoveries within the acceptable range (40–120%). The names of the compounds outside the acceptable range at the two validation levels are shown.

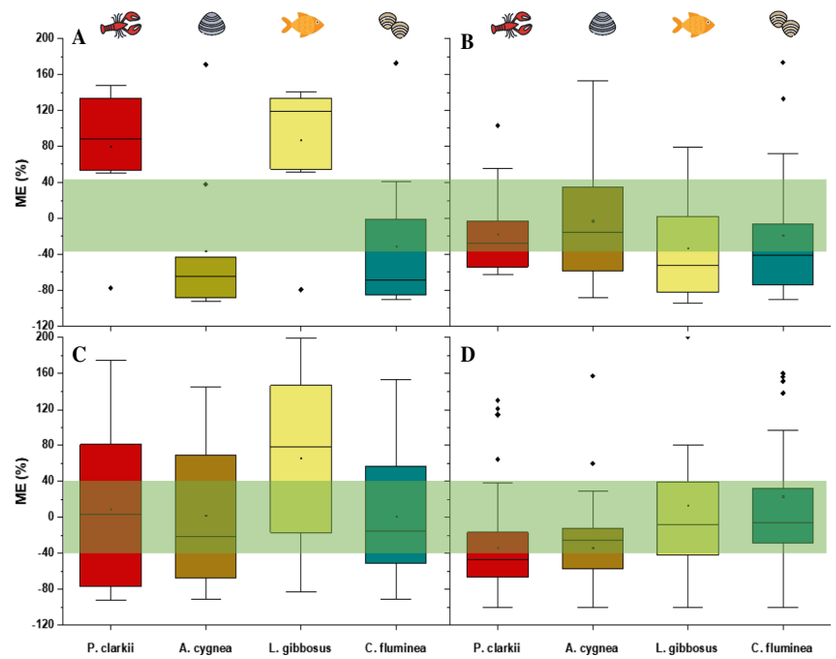

Fig. 4 Matrix effects for level 1 of validation (10 ng g^{-1}). **A**. OPFRs, **B**. PFAS, **C**. Pesticides, **D**. PhACs. The green band shows the acceptable ME range (-40% to 40%).

Finally, 75 of the 87 validated PhACs (green crosses) met the quality criteria. Five PhACs were not recovered in any species: atorvastatin, ciprofloxacin, nalidixic acid, oxytetracycline, and omeprazole. Flumequine was only recovered within the acceptable range in *C. fluminea*. Sertraline and nicotine recovered below 40% in *P. clarkii* and *L. gibossus*, respectively. Fipronil sulfone and furosemide were not validated in *C. fluminea*. Fenofibrate and salicylic acid were not recovered in *A. cygnea*. The zero-recovery rate during validation of ciprofloxacin, nalidixic acid, and oxytetracycline was related to their high polarity (log *P*, −0.87, 1.01, and −4.87, respectively) (Table S1). This condition allowed them to be recovered with greater than 50% efficiency with H_2O:MeOH (7:3) + 1% F.A in *P. clarkii* and *C. fluminea* during the solvent tests (Table S4). However, prioritizing MeCN + 0.1% F.A due to its better results for most of the analytes, the recovery of these latter compounds was compromised.

Matrix effects

During the analysis, matrix components present in the extracts can affect the quantification of the target analytes by increasing or suppressing their signal under ESI conditions in biological sample extracts [42]. The water content of *P. clarkii*, *A. cygnea*, *C. fluminea*, and *L. gibbosus* is close to 90% of its body weight with a fat content of 11.8%, 10.9%, 6.2%, 9.4% on a dry matter basis, respectively. In addition, their tissues, especially clams, are rich in proteins, carbohydrates including mucoproteins, glycoproteins, glycolipids or sphingolipids, and acid mucopolysaccharides, and pigments which carry out an intense chemical and biological activity. Unfortunately, all of these matrix components are inevitably co-extracted and present in the final extracts and are prone to altering the ionization efficiency of the analytes in the ion source. Although in most cases, this leads to suppression of the signal, compared to that obtained for a standard solution at the same concentration, in the case of OPFRS in *P. clarkii*, and in *L. gibbosus*, it is possible to observe an enhancement. According to [42], the higher ME could be related to the coelution of lipophilic matrix components, which apparently had not been completely removed during cleanup procedure. The pesticides showed a greater dispersion of the ME calculated in all matrices. In this case, no clear trend is evident in either ion polarity. Furthermore, this variation in ME values could be attributed to the relatively high log *P* variability of the

Table 2 Comparison with other UAE methods

Solvent and clean-up used in UAE method	Matrix	Validated compounds	LOQ (ng g^{-1}, w.w)	Recovery (%)	RSD (%)	Reference
Solvent: MeCN/MeOH + acetic acid-ammonium acetate (1 mL) Clean-up: HybridSPE®-phospholipid cartridge	Fish homogenate	72 PhACs and Personal care products	0.02–4.3	38–120	0.2–20	[29]
Solvent: Ice-cold MeCN/MeOH (1/1) (5 mL) Clean-up: Oasis HLB cartridge	Plasma, liver, brain fish	17 PhACs and Personal care products	0.02–8.7	90–110	1.2–10	[31]
Solvent: MeCN/H_2O (8:2) (8 mL) Clean-up: Oasis PRiME HLB cartridge	Fish mussel and whole-body shellfish	34 PhACs and Personal care products	0.03–5.6	43–127	-	[32]
Solvent: n-heptane + MeCN (9 mL) Clean-up: silica gel SPE cartridge	Fish muscle and liver	63 PhACs and their metabolites	0.1–48.2	69–133	-	[22]
Solvent: MeCN/H_2O (3/1) + 0.1% acetic acid (2 mL) Clean-up: Strata Alumina-N cartridge coupled to an Oasis HLB cartridge	Crustacean	67 PhACs and pesticides	0.1–25	43–100	7–22	[28]
Solvent: EtAct/MeCN (1/1) + 10 g anhydrous sodium sulfate (20 mL) Clean-up: GPC clean-up and d-SPE	Fish muscle	33 pesticides	0.001–0.04	57–110	2–20	[23]
Solvent: hexane/acetone (1/1) (15 mL) Clean-up: none	Fish	14 OPFRs	0.07–7	47–98	2.4–16	[24]
Solvent: MeOH (10 mL) Clean-up: STRATA-X cartridge	Fish homogenate	9 OPFRs	0.02–0.3	47–123	1–23	[25]
Solvent: hexane:acetone (1/1) (15 mL) Clean-up: SPE (tandem basic alumina with C18)	Dried fish	16 OPFRs	0.34–12	45–115	1–25	[43]
Solvent: H_2O/MeCN (1/9) (1.5 mL) Clean-up: HybridSPE® Phospholipid Ultra cartridge	Mussels, clams, fish	11 PFAS	0.1–0.9	40–133	4–19	[27]

pesticides (0.13–7.05). On the contrary, PFAS and PhACs tended to have more similar values despite the large log *P* variability especially of PhACs (−4.87 to 5.62). In these two groups, there was a higher percentage of analytes with an ME between −40 and 40% in most matrices, which implies a lower sensitivity to matrix interferences. Forty-three percent of all validated compounds obtained ME values between −40 and 40% in the *C. fluminea*, which means that in this species, the influence of the matrix tends to be lower than in the other species analyzed. ME calculated for each group of compounds and in each of the matrices are summarized in Fig. 4 whereas they are individually detailed in Table S4.

Linearity, LOD, and LOQ

The LOD and LOQ of the method were calculated from the calibration curve of each matrix using Eqs. (3) and (4). Pesticides were the compounds with the highest LOQ values for some of the compounds (>14 ng g^{-1}), while for PFAS, the calculated limits reached lower concentrations (<9 ng g^{-1}) for most of the analytes. The LODs of OPFRs ranged from 0.04 to 4 ng g^{-1} and LOQs ranged from 0.1 to 12 ng g^{-1}. The PhACs obtained LOD values between 0.01 and 7 ng g^{-1}, and LOQ between 0.02 and 22 ng g^{-1}. The LOD and LOQ for each group of compounds in each matrix are shown in Table S4.

Table 3 Compounds detected in the invasive species analyzed. Concentrations are expressed in ng g^{-1} (w.w). *n*, number of samples analyzed by species; *<LOQ*, less than limit of quantification; *Mean*, average concentration was calculated based on positive samples; for samples with data <LOQ, the value of the calculated limit of quantification divided by 2 was used; *Max*, maximum concentration calculated; *DF*, detection frequency

CECs		*P. clarkii* *n=17*				*C. fluminea* *n=8*				*L. gibbosus* *n=7*			
		Mean (ng g^{-1})	Median (ng g^{-1})	Max (ng g^{-1})	DF	Mean (ng g^{-1})	Median (ng g^{-1})	Max (ng g^{-1})	DF	Mean (ng g^{-1})	Median (ng g^{-1})	Max (ng g^{-1})	DF
PhACs	5-Methyl-1H-Benzotriazole	<LOQ	0.5	<LOQ	76%	<LOQ	<LOQ	<LOQ	100%	<LOQ	<LOQ	<LOQ	100%
	Acetaminophen	<LOQ	1.1	10	100%	<LOQ	<LOQ	<LOQ	100%	2	0.3	8	100%
	Benzotriazole	<LOQ	1.1	<LOQ	12%	<LOQ	<LOQ	<LOQ	100%	<LOQ	<LOQ	<LOQ	100%
	Benzoylecgonine	-	-	-	-	<LOQ	<LOQ	<LOQ	38%	-	-	-	-
	Bisphenol-A	-	-	-	-	4	4	11	100%	<LOQ	<LOQ	<LOQ	50%
	Caffeine	6	1	25	88%	<LOQ	<LOQ	<LOQ	100%	<LOQ	<LOQ	<LOQ	13%
	Carazolol	<LOQ	0.8	<LOQ	18%	-	-	-	-	-	-	-	-
	Carbamazepine	<LOQ	0.4	<LOQ	29%	<LOQ	<LOQ	<LOQ	38%	<LOQ	<LOQ	<LOQ	100%
	CBZ-10,11-epoxide	<LOQ	0.2	<LOQ	41%	<LOQ	<LOQ	0.3	13%	-	-	-	-
	Citalopram	-	-	-	-	-	-	0.1	88%	<LOQ	<LOQ	<LOQ	100%
	Cocaine	1	0.5	4	35%	<LOQ	<LOQ	<LOQ	100%	<LOQ	<LOQ	<LOQ	38%
	Cotinine	6	3	37	100%	<LOQ	<LOQ	8	100%	-	-	-	-
	Diltiazem	<LOQ	-	<LOQ	18%	-	-	-	-	-	-	-	-
	Fluconazole	<LOQ	0.1	0.3	29%	<LOQ	<LOQ	0.7	100%	-	-	-	-
	Fluoxetine	<LOQ	-<LOQ	<LOQ	6%	<LOQ	<LOQ	0.4	100%	-	-	-	-
	Hydrochlorothiazide	<LOQ	-	<LOQ	100%	<LOQ	<LOQ	-	-	<LOQ	<LOQ	<LOQ	100%
	Ketoprofen	-	-	-	-	-	-	-	-	7.4	7.4	7.4	14%
	Methadone	<LOQ	-	<LOQ	6%	<LOQ	<LOQ	-	-	-	-	-	-
	N-Desmethylcitalopram	<LOQ	0.6	<LOQ	6%	<LOQ	<LOQ	-	-	-	-	-	-
	Nicotine	-	-	-	-	<LOQ	<LOQ	-	-	-	-	-	-
	O-Desmethylvenlafaxine	0.4	0.3	1.5	94%	<LOQ	<LOQ	<LOQ	100%	-	-	-	-
	Paroxetine	-	-	-	-	<LOQ	<LOQ	0.1	100%	-	-	-	-
	Pentobarbital	9	9	17	47%	3	3	3.5	100%	3	0.3	12	71%

Table 3 (continued)

CECs		*P. clarkii* n=17				*L. gibbosus* n=7				*C. fluminea* n=8			
		Mean (ng g^{-1})	Median (ng g^{-1})	Max (ng g^{-1})	DF	Mean (ng g^{-1})	Median (ng g^{-1})	Max (ng g^{-1})	DF	Mean (ng g^{-1})	Median (ng g^{-1})	Max (ng g^{-1})	DF
	Propyphenazone	<LOQ	<LOQ	<LOQ	18%	-	-	-	-	-	-	-	-
	Quetiapine	-	-	-	-	-	-	-	-	<LOQ	<LOQ	<LOQ	75%
	Salbutamol	<LOQ	0.3	0.6	82%	-	-	-	-	<LOQ	<LOQ	<LOQ	63%
	Salicylic acid	-	-	-	-	5	3.2	18	100%	5	5	9.5	100%
	Sertraline	-	-	-	-	-	-	-	-	7	6.4	8	100%
	Sulfadimethoxine	<LOQ	0.2	<LOQ	6%	-	-	-	-	-	-	-	-
	Sulfapyridine	<LOQ	0.3	<LOQ	6%	-	-	-	-	-	-	-	-
	Tramadol	<LOQ	0.4	<LOQ	100%	<LOQ	0.2	<LOQ	100%	0.6	0.6	0.9	100%
	Trimethoprim	<LOQ	0.7	<LOQ	18%	-	-	-	-	3	3	3	13%
	Valsartan	1	1	1	24%	-	-	-	-	-	-	-	-
	Venlafaxine	<LOQ	0.3	<LOQ	94%	-	-	-	-	<LOQ	0.1	<LOQ	100%
	Zolpidem	<LOQ	<LOQ	<LOQ	6%	-	-	-	-	-	-	-	-
OPFR	TMPP	<LOQ	0.2	0.3	24%	-	-	-	-	-		-	-
	TPP	-	-	-	-	-	-	-	-	<LOQ	<LOQ	<LOQ	13%
PFAS	iPFNS	-	-	-	-	-	-	-	-	37	37	37	25%
	IpPFNA	<LOQ	<LOQ	<LOQ	12%	<LOQ	<LOQ	<LOQ	86%	<LOQ	2	5	83%
	PFBA	6	4.8	13	94%	6	6	9	100%	-	-	-	-
	PFBS	1	1	2.5	29%	0.8	0.9	1	86%	2	2	2.5	100%
	PFDA	2	1.4	3.5	82%	0.7	0.8	1	100%	0.8	1	1	75%
	PFDoDA	21	21	21	12%	<LOQ	<LOQ	<LOQ	29%	-	-	-	-
	PFDS	-	-	-	-	18	18	20	57%	20	15	41	50%
	PFHpA	0.4	0.3	0.9	65%	<LOQ	<LOQ	<LOQ	43%	<LOQ	0.8	<LOQ	63%
	PFHpS	10	4.5	27	41%	22	22	22	14%	-	-	-	-
	PFHxA	<LOQ	0.5	2	82%	<LOQ	2	4	86%	6	5	16	88%
	PFHxDA	<LOQ	<LOQ	<LOQ	41%	22	17	43	86%	<LOQ	0.9	<LOQ	50%
	PFHxS	2	1.8	5	94%	0.9	0.9	1.5	100%	0.06	0.02	0.2	88%
	PFNA	<LOQ	<LOQ	<LOQ	94%	-	-	-	-	<LOQ	<LOQ	<LOQ	100%
	PFOA	2	1.8	3	94%	12	9.6	28	100%	<LOQ	0.1	0.2	88%
	PFODA	73	73	74	35%	13	13	13	43%	-	-	-	-
	PFOS	17	9.7	60	88%	3.5	1	19	100%	<LOQ	<LOQ	<LOQ	100%
	PFPeA	-	-	-	-	<LOQ	<LOQ	<LOQ	100%	-	-	-	-
	PFTeDA	-	-	-	-	86	86	91	29%	-	-	-	-

Table 3 (continued)

CECs		*P. clarkii* n=17				*L. gibbosus* n=7				*C. fluminea* n=8			
		Mean (ng g^{-1})	Median (ng g^{-1})	Max (ng g^{-1})	DF	Mean (ng g^{-1})	Median (ng g^{-1})	Max (ng g^{-1})	DF	Mean (ng g^{-1})	Median (ng g^{-1})	Max (ng g^{-1})	DF
	PFTrDA	191	195	200	24%	13	12.6	13	29%	-	-	-	-
	PFUnDA	0.5	0.2	2	76%	<LOQ	0.1	0.2	86%	<LOQ	<LOQ	<LOQ	88%
Pesticides	Acetamiprid	-	-	-	-	<LOQ	5	7	100%	<LOQ	3.6	15	88%
	Alachlor	-	-	-	-	<LOQ	<LOQ	<LOQ	14%	4	4	4	13%
	Atrazine-desiso-propryl	<LOQ	<LOQ	<LOQ	29%	<LOQ	<LOQ	<LOQ	43%	<LOQ	<LOQ	<LOQ	13%
	Carbendazim	<LOQ	<LOQ	<LOQ	12%	1	0.5	4	100%	<LOQ	<LOQ	<LOQ	100%
	Carbofuran-3-hy-droxy	-	-	-	-	5	4.7	8	43%	12	12	19	100%
	Diazinon	-	-	-	-	<LOQ	<LOQ	<LOQ	14%	-	-	-	-
	Dichlofenthion	42	42	43	29%	-	-	-	-	-	-	-	-
	Diuron	-	-	-	-	<LOQ	<LOQ	<LOQ	29%	<LOQ	<LOQ	<LOQ	75%
	DMA	<LOQ	7	15	18%	17	17	19	43%	15	14	18	88%
	Etofenprox	-	-	-	-	<LOQ	<LOQ	<LOQ	29%	-	-	-	-
	Fenthion	51	51	51	6%	51	52	52	100%	52	52	52	100%
	Imidacloprid	<LOQ	2	2	65%	-	-	-	-	-	-	-	-
	Methiocarb	24	24	24	6%	24	24	24	86%	24	24	24	100%
	Molinate	<LOQ	0.8	0.8	24%	-	-	-	-	<LOQ	<LOQ	<LOQ	13%
	Propanil	<LOQ	<LOQ	<LOQ	12%	-	-	-	-	<LOQ	<LOQ	<LOQ	13%
	Propazine	<LOQ	<LOQ	<LOQ	18%	-	-	-	-	-	-	-	-
	Spinosyn A	6	6	6	35%	-	-	-	-	-	-	-	-
	Spinosyn D	-	-	-	-	-	-	-	-	3	3	3	13%
	Tebuconazole	<LOQ	<LOQ	<LOQ	6%	<LOQ	2	<LOQ	86%	<LOQ	<LOQ	<LOQ	25%
	Terbuthylazine-2-hydroxy	<LOQ	<LOQ	<LOQ	12%	-	-	-	-	<LOQ	<LOQ	<LOQ	50%
	Terbutryn	8	8	8	12%	8	8	8	43%	-	-	-	-

Comparison with other extraction methods

Table 2 shows the LOQs, recoveries, and RSDs of PhACs, pesticides, OPFRs, and PFASs obtained with different extraction methods based on UAE. The analytical values obtained with the method optimized by us are comparable with those previously reported for analysis in aquatic biota. However, the advantage offered by our method is the possibility of extracting a larger number of compounds from different chemical families with the same extraction solvent. The clean-up used does not compromise the recoveries of the analytes and it is a fast and simple step. Additionally, our method meets the requirements of green chemistry by using only 1 mL of solvent while most reported methods use more than 5 mL. We have successfully developed a single extraction method for the simultaneous analysis of CECs in four different matrices.

Quantification of CECs in real biota samples

The developed method was successfully applied to analyze the target analytes in three invasive species captured in the Albufera Natural Park (Valencia, Spain). We quantified using matrix-matched calibration curves for each matrix and the internal standard method described in the "LC-HRMS/MS analysis" section. Only compounds with reliable values during validation, i.e., those with recoveries between 40 and 120% and RSD < 25%, were included in the analysis (Table S4).

Table 3 shows the CECs detected in at least one of the samples analyzed. Thirty-five PhACs, 21 pesticides, 20 PFAS, and two OPFRs were detected. The compounds with the highest mean concentrations were the PFAS perfluorotridecanoic acid (PFTrDA) (191 ng g^{-1} in P. *clarkii*) and perfluoro-n-tetradecanoic acid (PFTeDA) (86 ng g^{-1} in *L. gibbosus*). The pesticide fenthion had the highest concentration in *C. fluminea* (52 ng g^{-1}). PhACs were detected at concentrations <10 ng g^{-1} (w.w).

The presence of PFTrDA and PFTeDA has been previously reported in *Perca fluviatillis* liver (22 ng g^{-1} and 12 ng g^{-1}, respectively) [5]. The results for PFTrDA were similar to those obtained by us in *L. gibbosus* homogenate (13 ng g^{-1}), but it is higher for PFTeDA. Propanil is a widely used herbicide in rice crops, is susceptible to biodegradation, is not persistent, and is easily transformed into various sub-products [44]. Salmo gairdneri has been shown to readily metabolize propanil forming at least ten sub-products [45]. Its presence in the analyzed samples does not suggest a harmful effect. On the other hand, fenthion is a broad-spectrum organophosphate pesticide used on numerous crops. The U.S. Environmental Protection Agency (EPA) has classified it as a restricted-use pesticide due to its toxic effects on birds, reptiles, and fish [46]. A significant decrease in protein was demonstrated in the middle mantle of *Lamellidens corrianus* when exposed to nominal (7 ng g^{-1}) and lethal (14 ng g^{-1}) concentrations of fenthion [47]. This compound was detected in 100% of the *L. gibbosus* and *C. fluminea* samples at concentrations above lethal concentrations. The concentrations detected suggest its general use in rice crops present in the Albufera, since it is a pesticide that allows the control of the rice borer (*Chilo suppressalis*), which is one of the most important pests in the rice fields of Valencia [48]. In this context, it would be interesting to extend the studies of fenthion bioaccumulation in these species.

In the case of PhACs, similar concentrations of acetaminophen, caffeine, carbamazepine, and fluoxetine have been previously reported in *P. clarkii* and clams *Ruditapes decussatus* caught in their habitat [17, 47]. PhACs such as acetaminophen (widely used over-the-counter analgesic); hydrochlorothiazide (used for the hypertension treatment); and tramadol (opioid painkiller) were the compounds with the highest detection frequency, detected in 100% of the samples analyzed.

As regards C. *fluminea,* 24 of the 78 compounds reported in Table 3 were detected in all samples of this species, but most of them were not quantifiable. The compounds with the highest mean concentrations in this species were fenthion (52 ng g^{-1}), sertraline (7 ng g^{-1}), and PFBS (2 ng g^{-1}). The high detection frequency in this species may be related to its ability to filter water and its anatomy, which could facilitate the absorption of compounds. *C. fluminea* has been shown to have a high accumulation rate of contaminants such as heavy metals [49]. Due to their biofiltration capacity, they have been able to eliminate up to 30% of carbamazepine and lorazepam concentrations during PhACs removal trials from wastewater [50]. Although *C. fluminea* was the species with the highest detection frequency, *P. clarkii* reported the highest maximum concentrations, especially for the PFAS group, so biomonitoring of this class of compounds with this species would be pretty interesting for future studies.

Conclusions

An ultrasound-assisted extraction with MeCN + 0.1% F.A as solvent, followed by a clean-up step with Z-sep/C_{18}, and subsequent quantification by HPLC-MS/MS provided the best compromise of high precision and matrix effects for the studied CECs in invasive species in a coastal wetland. The method allowed the simultaneous extraction of four groups of contaminants with different physicochemical properties (PhACs, pesticides, PFASs, and OPFRs) in different species. This method proved suitable for the correct recovery of most compounds with a low RSD. The application of the method allowed the detection of 78 compounds from different CECs families in freshwater species samples. The results obtained for *C. fluminea* and *P. clarkii* showed their capacity to accumulate contaminants and its potential to be used in biomonitoring studies.

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Declarations

Ethical approval Ethics approval was not required for this research.

Consent to participate All authors carefully read and approved the study.

Consent for publication All authors are agreed to publish the manuscript.

Source of biological material Samples were provided by the Fish Research Centre "El Palmar," Generalitat Valenciana (Valencia, Spain).

Statement on animal welfare No applications are required for testing with invertebrates. Fish experiments were performed according to the "Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes" and the Spanish legislation (Royal Decree 53/2013, from the 1st of February, which establishes the basic regulations that are applicable for the protection of animals used for experiments and other scientific uses, including teaching).

Competing interests The authors declare no competing interests.

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INFORMACIÓN COMPLEMENTARIA
PUBLICACIÓN No. 2

An effective method for the simultaneous extraction of 173 contaminants of emerging concern in freshwater invasive species and its aplication

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SUPPLEMENTARY INFORMATION

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Table S1.1. Pharmaceuticals (PhACs) list. CE: Collision energy; RT: Retention time.

PhACs	Mode	Precursor mass (*m/z*)	Confirming fragment (*m/z*)	CE (V)	RT (min)	Log P
5-methyl-1H-Benzotriazole	+	134.0713	79.0542	25	7.29	1.81
Acetaminophen	+	152.0706	110.0600	20	2.94	0.91
Acridone	+	196.0757	167.0721	30	10.41	4.20
Alprazolam	+	309.0901	281.0713	37	12.56	3.02
Amantadine	+	152.1434	135.1167	15	4.69	1.47
Atenolol	+	267.1703	190.0861	25	2.67	0.43
Atorvastatin	-	557.2457	278.1350	50	13.12	5.39
Benzotriazole	+	120.0556	65.0386	15	5.00	1.30
Benzoylecgonine	+	290.1387	168.1018	26	5.65	-0.59
Bezafibrate	+	362.1154	316.1096	14	13.41	3.99
Bisphenol-A	-	227.1078	211.0760	31	12.96	4.04
Bromazepam	+	316.0080	209.0945	31	10.42	2.54
Caffeine	+	195.0877	138.0661	27	4.48	-0.55
Carazolol	+	299.1754	116.1068	40	8.74	2.71
Carbamazepine	+	237.1022	194.0963	23	11.70	2.77
CBZ-10.11-epoxide	+	253.0972	180.0806	20	9.46	1.97
Chloramphenicol	-	321.0051	152.0353	15	8.93	0.88
Chlorpromazine	+	319.1030	86.0964	19	12.49	4.54
Ciprofloxacin	+	332.1405	288.1506	25	5.76	-0.81
Citalopram	+	325.1711	109.0446	26	10.78	3.76
Clarithromycin	+	748.4841	158.1174	34	12.43	3.24
Clofibric acid	-	213.0324	126.9955	30	6.91	2.90
Coca-ethylene	+	318.1700	196.1330	25	8.76	2.64
Cocaine	+	304.1543	182.1174	30	7.34	2.28
Codeine	+	300.1594	165.0697	42	3.50	1.34
Cotinine	+	177.1022	80.0494	28	1.70	0.21
Diazepam	+	285.0789	193.0885	35	13.69	3.08
Diclofenac	-	294.0094	221.1544	-	12.31	4.26
Diltiazem	+	415.1686	178.0319	24	11.24	2.73
Erythromycin	+	734.4685	158.1174	29	11.29	2.60
Fenofibrate	+	361.1201	233.0364	12	16.07	5.28
Fipronil	-	434.9314	249.9580	20	14.97	4.49
Fipronil Desulfynil	-	386.9644	350.9877	10	15.14	4.53
Fipronil sulfide	-	418.9365	261.9570	25	15.36	5.62
Fipronil sulfone	-	450.9263	183.0270	25	15.34	4.60
Fluconazole	+	307.1113	310.1219	20	6.87	0.56
Flumequine	+	262.0874	244.0766	32	11.93	2.42
Fluoxetine	+	310.1413	265.1577	30	12.42	4.17
Furazolidone	+	226.0459	95.0366	18	6.18	0.87
Furosemide	-	329.0004	204.9845	-	7.58	1.75
Hydrochlorothiazide	-	295.9572	268.9455	29	4.14	-0.58
Indomethacin	-	356.0695	297.0561	-	12.55	3.53
Ketoprofen	+	255.1016	105.0334	25	13.23	3.61
Lamotrigine	+	256.0151	172.9667	40	6.12	1.93
Loratadine	+	383.1521	337.1099	-	14.30	5.20
Lorazepam	+	321.0192	275.0138	19	12.48	4.06
Losartan	+	423.1695	207.0917	20	12.95	4.06
Mefenamic acid	-	240.1030	196.1134	19	13.12	5.40
Metformin	+	130.1087	60.0560	15	1.14	-0.92
Methadone	+	310.2165	265.1587	18	12.29	5.01
Metoprolol	+	268.1907	116.1068	21	6.54	1.76
Midazolam	+	326.0855	291.1166	38	10.30	3.97
Morphine	+	286.1438	201.0909	40	1.86	0.90
N-acethyl SMX	+	296.0700	134.0600	24	8.81	0.86
Nalidixic acid	+	233.0921	205.0606	32	11.37	1.01
N-Desmethylcitalopram	+	311.1554	109.0447	-	10.55	3.38
Nicotine	+	163.1230	117.0573	22	1.44	1.17
Norfluoxetine	+	296.1257	105.0700	18	12.28	3.70
O-Desmethylvenlafaxine	+	264.1958	246.1851	21	5.68	2.29
Omeprazole	+	346.1220	198.0583	-	9.28	2.43
Oseltamivir	+	313.2122	166.0864	12	8.78	1.16
Oseltamivir-CBX	+	285.1809	138.0551	38	5.19	-1.84
Oxazepam	+	287.0582	241.0524	25	12.21	2.92
Oxytetracycline	+	461.1555	426.1171	35	5.39	-4.87
Paroxetine	+	330.1500	192.1183	27	11.86	3.15
Pentobarbital	-	225.1245	125.9001	30	11.47	1.89
Propyphenazone	+	231.1492	189.1021	30	12.24	2.35
Quetiapine	+	384.1740	253.0794	30	10.36	2.81
Salbutamol	+	240.1594	148.0756	-	2.37	1.40
Salicylic acid	-	137.0244	93.0345	16	2.99	1.98
Sertraline	+	306.0811	158.9762	20	12.47	5.15
Sitagliptin	+	408.1254	174.0524	24	7.70	1.26
Sotalol	+	273.1268	213.0690	24	2.43	-0.40
Sulfadimethoxine	+	311.0809	156.0771	-	10.04	0.23
Sulfamethazine	+	279.0910	204.0436	22	5.98	0.65
Sulfamethoxazole	+	254.0594	156.0115	20	7.84	0.79
Sulfapyridine	+	250.0645	156.0112	22	4.60	1.01
Temazepam	+	301.0738	255.0684	-	12.99	2.19
Tramadol	+	264.1958	58.0654	15	6.50	2.45
Triclocarban	-	312.9708	159.9724	35	15.35	4.93
Trimethoprim	+	291.1452	230.1161	29	4.83	1.28
Valsartan	+	436.2343	207.0915	15	13.68	4.59
Valsartan acid	-	265.0731	165.0706	35	3.95	2.56
Venlafaxine	+	278.2115	58.0654	22	8.39	2.74
Verapamil	+	455.2904	165.0909	27	12.26	5.04
Warfarin	-	307.0976	161.0244	24	11.58	2.74
Zolpidem	+	308.1757	235.1230	30	8.16	3.02

Table S1.2. Organophosphorus flame retardants (OPFRs) list. CE: Collision energy; RT: Retention time.

OPFRs	Mode	Precursor mass (*m/z*)	Confirming fragment (*m/z*)	CE (V)	RT (min)	Log P
TCEP Tris(2-chloroethyl) phosphate	+	284.9612	100.15	20	10.7	2.11
TBEP Tris (2-butoxyethyl) phosphate	+	399.2506	-	20	-	3.94
TPP Tripropyl phosphate	+	225.125	84.33	20	15	1.87
TDBPP Tris(2.3-dibromopropyl) phosphate	+	692.5881	120.04	20	17.2	3.65
TEHP Tris(2-ethylhexyl) phosphate	+	435.3598	113.02	20	22.8	6.07
TMPP Tricresyl phosphate	+	369.125	192.08	20	18.4	6.34
TPhP Triphenyl phosphate	+	327.0781	117.05	20	16.8	4.59
TDCIPP Tris(1.3-dichloro-2-propyl) phosphate	+	428.8912	80.13	20	16.7	3.8
TCIPP Tris(2-chloroisopropyl) phosphate	+	327.0081	80.01	20	14.9	1.44
CDP Cresyl diphenyl phosphate	+	341.0937	167.02	20	17.4	2.59
TnBP tributyl phosphate	+	267.172	-	20	-	4.51

Table S1.3. Pesticides list. CE: Collision energy; RT: Retention time.

Pesticides	Mode	Precursor mass (*m/z*)	Confirming fragment (*m/z*)	CE (V)	RT (min)	Log P
Acetamiprid	+	223.0745	223.0739	20	2.57	0.80
Alachlor	+	270.1255	69.1177	15	9.26	3.09
Atrazine	+	216.1011	174.0539	20	6.33	2.70
Atrazine-deethyl	+	188.0698	146.0226	20	3.46	1.51
Atrazine-Deisopropryl	+	174.0541	146.0229	20	2.63	1.15
Azinphos-ethyl	+	346.0444	132.0443	20	9.06	3.18
Azinphos-Methyl	+	318.0131	132.0442	20	7.18	2.96
Buprofezine	+	306.1635	201.1055	20	12.62	4.93
Carbendazim	+	192.0768	60.0505	20	2.21	1.48
Carbofuran	+	222.1125	165.0908	20	4.71	1.80
Carbofuran 3-OH	+	238.1074	163.0751	20	2.72	1.45
Chlorfenvinphos	+	358.9768	159.0419	20	10.51	3.80
Chlorpyriphos	+	349.9336	197.9270	20	13.58	4.00
Chlothianidin	+	250.0160	168.0463	20	2.66	0.70
Coumaphos	+	363.0217	306.9590	20	10.54	3.86
Diazinon	+	305.1083	153.1019	35	10.54	3.69
Dichlofenthion	+	313.9695	279.2323	5	13.20	5.14
Dimethoate	+	230.0069	198.9645	20	2.98	0.70
Diuron	+	233.0243	72.0444	35	6.94	2.87
DMA (2,4-dimethylaniline)	+	122.0976	107.0745	20	1.39	1.68
DMF (N-(2,4-dimethylphenyl formamide)	+	60.0570	-	20	-	-
DMPF N-(2.4-dimethylphenyl)-Nmethylformamidine	+	150.0913	123.0799	20	3.99	-
Ethion	+	384.9949	230.9737	20	13.23	5.07

Pesticides	Mode	Precursor mass (*m/z*)	Confirming fragment (*m/z*)	CE (V)	RT (min)	Log P
Etofenprox	+	394.2377	359.2004	20	15.13	7.05
Fenthion	+	279.0273	108.0443	10	-	4.84
Fenthion sulfone	+	311.0171	297.0011	20	5.37	2.25
Fenthion sulfoxide	+	295.0222	279.9984	20	4.55	1.92
Fipronil	+	436.9460	-	30	-	4.00
Hexythiazox	+	353.1085	228.0242	20	13.66	2.67
Imazalil	+	297.0556	255.0089	20	4.88	2.56
Imidacloprid	+	256.0596	209.0586	20	2.47	0.57
Isoproturon	+	207.4149	165.1020	20	6.28	2.50
Methiocarb	+	226.0896	170.1034	20	7.79	3.18
Methoalachlor	+	284.1412	253.1179	20	9.38	3.40
Molinate	+	188.1104	126.0912	20	8.25	2.86
				20		-
Omethoate	+	214.0997	196.0188		1.40	0.74
Parathion-ethyl	+	292.0403	123.1172	10	7.98	3.83
Prochloraz	+	376.0381	308.0002	20	10.62	3.50
Propanil	+	218.0134	161.9867	10	8.23	2.29
Propazine	+	230.1167	188.0696	20	7.63	3.95
Pyriproxyfen	+	322.1488	227.1066	20	13.10	5.37
Simazine	+	202.0854	146.0226	20	4.54	2.30
Spinosad A	+	732.4681	142.1223	20	9.80	4.10
Spinosad C	+	-	-	20	-	-
Spinosad D	+	746.4838	142.1225	20	10.73	-
Tebuconazole	+	308.1524	70.0397	20	10.30	3.70
Terbumeton	+	226.1662	170.1034	20	5.40	3.04
Terbumeton deethyl	+	198.1349	142.0723	20	3.50	-
Terbuthylazine	+	230.1167	174.0538	30	7.90	3.40
Terbuthylazine-2OH	+	212.1506	-	20	2.40	-
Terbuthylazine-deethyl	+	202.0854	146.0226	30	4.99	2.30
Terbutryn	+	242.1439	187.0833	20	8.15	3.66
Thiabendazole	+	202.0433	175.0337	20	2.55	2.39
				20		-
Thiomethoxam	+	292.02656	211.0646		2.17	0.13

Table S1.4. Perfluoroalkyl substances (PFAS) list. CE: Collision energy; RT: Retention time.

PFASs	Mode	Precursor mass (*m/z*)	Product ions (*m/z*)	CE (V)	RT (min)	Log P
Perfluorobutanoic acid (PFBA)	-	213	169	-12	3.18	--
Perfluoropentenoic acid (PFPeA)	-	263	219	-5	8.9	--
Perflurohexanoic acid (PFHxA)	-	313	269/119	-12/-10	6.74	3.48
Perfluroheptanoic acid (PFHpA)	-	363	319/169	-12/-10	7.94	4.15
Perfluorooctanoic acid (PFOA)	-	413	369/169	-14/-14	8.87	4.81
Perfluorononanoic acid (PFNA)	-	463	419/169	-14/-14	9.65	5.48

PFASs	Mode	Precursor mass (*m/z*)	Product ions (*m/z*)	CE (V)	RT (min)	Log P
Perfluoro-7-methyloctanoic acid (i.p-PFNA)	-	463	419/269	-14/-14	9.94	--
Perfluorodecanoic acid (PFDA)	-	513	469/269	-16/-16	10.31	--
Perfluoroundecanoic acid (PFUnDA)	-	563	519/269	-18/-18	10.88	--
Perfluorododecanoic acid (PFDoA)	-	613	569/269	-5/-10	11.38	--
Perfluorotridecanoic acid (PFTrDA)	-	663	619/169	-12/-24	11.88	--
Perfluorotetradecanoic acid (PFTeDA)	-	714	669/169	-12/-25	12.18	--
Perfluorohexadecanoic acid (PFHxDA)	-	814	769/169	-8/-28	12.79	--
Perfluorooctadecanoic acid (PFODA)	-	914	869/169	-8/-29	13.38	--
Perflurobutane sulfonate (PFBS)	-	299	99/80	-58/-58	5.54	--
Perfluorohexane sulfonate (PFHxS)	-	399	99/80	-78/-66	8.04	--
Perfluroheptane sulfonate (PFHpS)	-	449	99/80	-88/-88	8.89	--
Perfluoroctane sulfonate (PFOS)	-	499	99/80	-108/-108	9.63	4.49
Perfluoro-7-methyloctane sulfonate (ipPFNS)	-	427	99/80	-45/-78	10.10	--
Perfluorodecane sulfonate (PFDS)	-	599	99/80	-118/-118	10.83	--

Table S2.1. Quality parameters for quantification purposes of PhACs

PhACs	IS	*Procambarus Clarkii*		*Corbicula fluminea*		*Lepomis gibbosus*	
		R^2	Linearity range (ng g^{-1})	R^2	Linearity range (ng g^{-1})	R^2	Linearity range (ng g^{-1})
5-methyl-1H_Benzotriazole	Benzotriazole-D4	0.999	0.5-500	0.999	0.5-500	0.999	0.5-500
Acetaminophen	Acetaminophen-D4	0.990	10-500	0.997	2-500	0.999	2-500
Acridone	Carbamazepine-D10	0.994	0.1-500	0.996	0.2-200	0.999	0.1-200
Alprazolam	Alprazomal-D5	0.999	1-500	0.995	0.5-500	0.999	1-500
Amantadine	Trimethoprim-D9	0.997	1-500	0.998	0.2-500	0.996	0.2-500
Atenolol	Atenolol-D7	1.000	2-500	1.000	0.2-500	0.999	0.5-500
Benzoylecgonine	Sulfamethazine-D4	0.994	2-500	0.990	1-200	0.993	0.5-200
Benzotriazole	Benzotriazole-D4	0.998	1-500	0.999	0.5-500	0.996	1-500
Bezafibrate	Bezafibrate-D4	1.000	1-500	0.999	1-500	0.998	1-500
Bisphenol-A	Bisphenol-A-D8	0.999	0.2-500	0.998	0.5-500	0.998	0.5-500
Bromazepam	Bromazepam-D4	0.995	1-500	0.992	1-500	0.991	2-500
Caffeine	Caffeine-13C3	0.995	10-500	0.997	0.5-500	0.997	0.5-500
Carazolol	Carbamazepine-D10	0.990	0.5-500	0.998	0.2-500	0.999	1-500
Carbamazepine	Carbamazepine-D10	0.997	0.2-500	0.995	0.2-200	0.999	0.2-200
CBZ-10,11-epoxide	Carbamazepine-D10	0.999	0.1-500	0.998	0.1-200	0.994	0.2-200
Chloramphenicol	Ibuprofen-D3	0.992	1-500	0.994	0.5-200	0.997	0.5-500
Chlorpromazine	Chlorpromazine-D3	0.998	1-500	0.999	0.5-500	0.999	0.5-500
Ciprofloxacin	Lamotrigine-13CD3	0.990	2-500	0.993	0.1-500	0.997	0.5-500
Citalopram	Carbamazepine-D10	0.996	1-500	0.997	0.5-200	0.994	2-500
Clarithromycin	Carbamazepine-D10	0.993	2-500	0.996	0.5-200	0.992	2-500
Clofibric Acid	Furosemide-D5	0.997	2-500	0.992	1-200	0.999	1-500
Coca-ethylene	Cocaine-D5	0.994	0.5-500	0.997	0.1-500	0.992	0.2-500
Cocaine	Cocaine-D5	0.998	1-500	0.997	0.1-500	0.999	1-500
Codeine	Codeine-D3	0.998	2-500	0.999	0.2-500	0.999	0.1-500
Cotinine	Cotinine-D3	0.998	0.1-500	0.999	0.5-500	0.998	1-500
Diazepam	Bezafibrate-D4	0.998	0.1-500	0.999	0.2-500	0.998	0.5-500
Diclofenac	Diclofenac-13C6	0.999	1-500	0.999	2-500	1.000	10-500
Diltiazem	Carbamazepine-D10	0.996	0.5-500	0.998	0.2-500	0.995	0.5-500
Erythromycin	Erythromycin-13CD3	0.998	1-500	0.998	0.5-500	1.000	1-500
Fenofibrate	Fenofibrate-D6	0.995	10-500	0.998	10-500	0.999	10-500
Fipronil	Fipronil-13C3	0.995	1-500	0.999	1-200	0.992	2-200
Fipronil desulfynil	Fipronil-13C3	0.996	1-500	0.996	1-200	0.995	1-500
Fipronil sulfide	Fipronil-13C3	0.994	10-500	0.996	1-500	0.998	10-500
Fipronil sulfone	Fipronil-13C3	0.999	2-500	0.996	1-200	0.995	10-500
Fluconazole	Fluconazole-13C3	0.999	0.1-500	0.998	0.5-500	0.999	0.5-500
Flumequine	Carbamazepine-D10	0.995	0.2-500	0.995	0.1-500	0.994	0.2-500
Fluoxetine	Fluoxetine-D5	0.999	1-500	0.998	0.1-500	0.999	0.5-500

PhACs	IS	*Procambarus Clarkii*		*Corbicula fluminea*		*Lepomis gibbosus*	
		R^2	Linearity range (ng g^{-1})	R^2	Linearity range (ng g^{-1})	R^2	Linearity range (ng g^{-1})
Furazolidone	Carbamazepine-D10	0.998	1-500	0.996	0.5-100	0.992	0.5-500
Furosemide	Furosemide-D5	0.998	1-500	0.999	10-500	0.999	2-500
Hydrochlorothiazide	Hydrochlorothiazide-13CD2	0.999	0.1-500	1.000	0.2-500	0.997	0.2-500
Indomethacin	Indomethacin-D4	0.998	10-500	0.999	10-500	0.999	10-500
Ketoprofen	Ketoprofen-D3	0.997	2-500	0.999	10-500	0.999	10-500
Lamotrigine	Lamotrigine-13CD3	0.999	1-500	0.999	0.5-500	0.994	0.5-500
Loratadine	Carbamazepine-D10	0.997	1-500	0.990	0.5-500	0.994	1-500
Lorazepam	Lorazepam-D4	0.996	1-500	0.998	0.5-500	0.999	1-500
Losartan	Ibesartan-D6	0.998	1-500	0.973	10-500	0.998	10-500
Mefenamic acid	Ibuprofen-D3	0.998	1-500	0.996	1-500	0.998	1-500
Metformin	Metformin-D6	0.988	20-500	0.998	0.5-500	0.999	2-500
Methadone	Carbamazepine-D10	0.991	1-500	0.997	0.2-200	0.994	1-500
Metoprolol	Metoprolol-D7	0.999	1-500	0.999	1-500	0.999	0.2-500
Midazolam	Midazolam-13C6	0.998	0.5-500	0.996	0.5-500	0.999	0.2-500
Morphine	Morphine-D3	0.996	2-500	0.999	1-500	0.998	2-500
N-acethyl_SMX	Venlafaxine-D6	0.998	0.5-500	0.994	0.5-500	0.991	1-500
Nalidixin_acid	Venlafaxine-D6	0.997	0.2-500	0.998	0.1-200	0.995	0.2-500
N-Desmethylcitalopram	Carbamazepine-D10	0.994	0.5-500	0.992	0.5-500	0.990	1-500
Nicotine	Nicotine-D4	0.998	20-500	0.999	2-500	0.996	10-500
Norfluoxetine	Norfluoxetine-D6	0.997	10-500	1.000	1-500	1.000	10-500
O-desmethylvenlafaxine	Venlafaxine-D6	0.996	0.5-200	0.993	0.1-200	0.994	0.2-200
Omeprazole	Carbamazepine-D10	0.995	10-200	0.998	20-500	0.504	10-500
Oseltamivir	Oseltamivir-D3	0.998	2-500	0.999	0.2-500	0.998	1-500
Oseltamivir-CBX	Oseltamivir-D3	0.998	2-500	0.998	0.2-200	0.996	2-500
Oxazepam	Oxazepam-D5	0.999	0.1-500	0.999	0.5-500	0.997	0.5-500
Oxytetracycline	Trimethoprim-D9	0.997	10-500	0.993	10-500	0.987	10-500
Paroxetine	Paroxetine-D4	0.997	2-500	0.998	0.1-500	0.999	0.2-500
Pentobarbital	Pentobarbital-D5	0.992	1-500	0.998	0.5-500	1.000	1-500
Propyphenazone	Carbamazepine-D10	0.999	0.1-200	0.999	0.1-200	0.997	0.2-200
Quetiapine	Bromazepam-D4	0.996	0.5-500	0.999	0.2-500	0.999	0.5-500
Salbutamol	Salbutamol D6	0.993	0.1-200	0.990	0.5-500	0.997	0.2-500
Salicylic_acid	Sotalol-D6	0.996	0.1-500	0.997	2-500	0.996	0.1-500
Sertraline	Sertraline-D3	0.996	1-500	1.000	0.1-500	0.997	1-100
Sitagliptin	Sitagliptin-D4	0.998	2-500	1.000	0.5-500	1.000	1-500
Sotalol	Sotalol-D6	0.996	2-200	0.999	0.2-500	0.998	0.2-500
Sulfadimethoxine	Bromazepam-D4	0.997	0.1-500	0.995	0.2-500	0.996	0.2-500
Sulfamethazine	Sulfamethazine-D4	0.997	1-500	0.999	2-500	0.999	0.5-500
Sulfamethoxazole	Venlafaxine-D6	0.999	1-500	0.999	0.5-500	0.999	0.5-500

PhACs	IS	*Procambarus Clarkii*		*Corbicula fluminea*		*Lepomis gibbosus*	
		R^2	Linearity range (ng g^{-1})	R^2	Linearity range (ng g^{-1})	R^2	Linearity range (ng g^{-1})
Sulfapyridine	Trimethoprim-D9	0.999	1-500	0.998	0.5-500	0.997	0.2-500
Temazepam	Oxazepam-D5	0.999	0.5-500	0.999	0.2-500	0.999	0.2-500
Tramadol	Tramadol-13CD3	0.997	0.5-500	0.999	0.1-500	0.999	0.1-500
Triclocarban	Fipronil-13C3	0.993	10-500	0.997	1-500	0.985	2-500
Trimethoprim	Trimethoprim-D9	0.996	1-500	0.998	0.1-500	0.998	0.2-500
Valsartan	Valsartan-D3	0.999	0.1-500	0.998	2-500	1.000	10-500
Valsartan acid	Valsartan acid D4	0.996	2-500	0.999	2-500	0.997	10-500
Venlafaxine	Venlafaxine-D6	0.998	0.1-500	0.999	0.1-500	0.999	0.1-500
Verapamil	Fluoxetine-D5	0.996	1-500	0.999	0.5-500	0.992	10-500
Warfarin	Pentobarbital-D5	0.998	1-500	0.991	0.5-200	0.992	0.2-200
Zolpidem	Venlafaxine-D6	0.996	0.5-500	0.994	0.5-500	0.996	1-500

Table S2.2. Quality parameters for quantification purposes of OPFRs

OPFRs	IS	*Procambarus Clarkii*		*Corbicula fluminea*		*Lepomis gibbosus*	
		R^2	Linearity range (ng g^{-1})	R^2	Linearity range (ng g^{-1})	R^2	Linearity range (ng g^{-1})
TPP	D12-TCEP	n.d.	0.5-500	0.998	0.5-500	0.996	0.5-500
TDBPP	D15-TPhP	1,000	1-500	0.999	0.5-500	0.999	0.5-500
CDP	D15-TPhP	n.d.	20-500	0.998	20-500	0.997	20-500
TnBP	D15-TPhP	n.d.	0.2-500	0.999	0.2-500	0.998	0.2-500
TBEP	D15-TPhP	0.990	0.2-500	0.991	0.2-500	0.990	0.2-500
TMPP	D15-TPhP	0.990	0.5-500	0.995	0.5-500	0.992	0.2-500
TEHP	D15-TPhP	0.995	1-500	0.996	2-500	0.996	1-500
TCEP	D12-TCEP	0.998	0.2-500	0.995	0.2-500	0.990	0.2-500
TClPP	D18-TClPP	1,000	2-500	0.998	2-500	0.999	2-500
TDClPP	D15-TDClPP	0.998	2-500	0.999	1-500	0.997	1-500
TPhP	D15-TPhP	0.996	0.2-500	0.997	0.2-500	0.995	0.2-500

Table S2.3. Quality parameters for quantification purposes of Pesticides. The IS used were Chlorfenvinphos-d10 and Chlorpyrifos-d10. For the other analytes, quantification was performed with an external standard.

Pesticides	*Procambarus Clarkii*		*Corbicula fluminea*		*Lepomis gibbosus*	
	R^2	Linearity range (ng g^{-1})	R^2	Linearity range (ng g^{-1})	R^2	Linearity range (ng g^{-1})
Acetamiprid	0.998	10-500	0.997	10-500	0.996	10-500
Alachlor	0.997	2-500	0.997	10-500	0.997	20-500

Pesticides	*Procambarus Clarkii*		*Corbicula fluminea*		*Lepomis gibbosus*	
	R^2	Linearity range (ng g^{-1})	R^2	Linearity range (ng g^{-1})	R^2	Linearity range (ng g^{-1})
Atrazine	0.998	10-500	0.998	10-500	0.999	10-500
Atrazine-desethyl	0.998	10-500	0.999	10-500	0.999	2-500
Atrazine-desisopropryl	0.995	10-500	0.997	10-500	0.987	10-500
Azinphos-ethyl	0.997	20-500	0.998	2-500	0.999	20-500
Azinphos-methyl	0.994	10-500	0.996	20-500	0.997	10-500
Buprofezin	0.999	20-500	0.999	20-500	0.999	20-500
Carbendazim	0.997	2-500	0.997	10-500	0.996	1-500
Carbofuran	0.998	10-500	0.999	20-500	0.999	10-500
Carbofuran-3-hydroxy	0.998	10-500	0.998	10-500	0.999	10-500
Chlorfenvinphos	0.996	20-500	0.997	10-500	0.999	10-500
Chlorpyrifos	0.994	10-500	0.998	10-500	0.999	20-500
Chlothianidin	0.992	10-500	0.999	10-500	0.998	10-500
Coumaphos	0.997	20-500	0.998	10-500	0.999	2-500
Diazinon	0.996	20-500	0.998	10-500	0.997	20-500
Dichlofenthion	0.993	20-500	0.995	20-500	0.991	20-500
Dimethoate	0.997	10-500	0.998	20-500	0.998	2-500
Diuron	0.997	20-500	0.997	20-500	0.997	20-500
DMA	0.996	20-500	0.998	10-500	0.995	20-500
DMF	0.992	10-500	0.996	10-500	0.996	10-500
DMPF	0.997	10-500	0.998	10-500	0.996	10-500
Ethion	0.999	1-500	0.999	1-500	0.999	2-500
Etofenprox	0.994	20-500	0.997	20-500	0.999	20-500
Fenthion	0.992	20-500	0.996	10-500	0.997	20-500
Fenthion-sulfone	0.998	10-500	0.998	2-500	0.998	1-500
Fenthion sulfoxide	0.995	10-500	0.996	10-500	0.991	2-500
Fipronil	0.994	0.5-500	0.997	0.2-500	0.999	0.2-500
Hexythiazox	0.996	10-500	0.998	2-500	0.998	10-500
Imazalil	0.997	20-500	0.999	2-500	0.999	20-500
Imidacloprid	0.998	10-500	0.999	10-500	0.999	10-500
Isoproturon	0.997	10-500	0.998	2-500	0.997	2-500
Methiocarb	0.998	10-500	0.998	10-500	0.993	10-500
Metolachlor	0.998	10-500	0.999	10-500	0.998	2-500
Molinate	0.996	2-500	0.998	10-500	0.997	2-500
Omethoate	0.997	20-500	0.998	10-500	0.998	20-500
Parathion-ethyl	0.995	20-500	0.997	20-500	0.992	20-500
Prochloraz	0.997	20-500	0.999	10-500	0.999	10-500
Propanil	0.997	20-500	0.998	20-500	0.991	20-500
Propazine	0.997	10-500	0.998	2-500	0.996	2-500
Pyriproxyfen	0.996	20-500	0.997	20-500	0.999	20-500
Simazine	0.997	10-500	0.998	10-500	0.997	2-500
Spinosyn A	0.995	10-500	0.996	10-500	0.994	10-500
Spinosyn C	0.994	10-500	0.994	10-500	0.994	2-500
Spinosyn D	0.995	10-500	0.997	2-500	0.995	10-500
Tebuconazole	0.997	10-500	0.998	10-500	0.997	10-500
Terbumeton	0.995	0.5-500	0.997	0.5-500	0.999	0.2-500
Terbumeton deethyl	0.996	0.5-500	0.994	0.5-500	0.999	0.2-500
Terbuthylazine	0.998	0.5-500	0.998	0.5-500	0.998	0.2-500
Terbuthylazine-2-hydroxy	0.997	2-500	0.998	2-500	0.998	2-500
Terbuthylazine-desethyl	0.998	2-500	0.999	10-500	0.999	2-500
Terbutryn	0.992	10-500	0.995	10-500	0.992	2-500
Thiabendazole	0.996	10-500	0.996	10-500	0.999	10-500
Thiamethoxam	0.997	10-500	0.998	10-500	0.999	10-500

Table S2.4. Quality parameters for quantification purposes of PFAS

PFAS	IS	*Procambarus Clarkii*		*Corbicula fluminea*		*Lepomis gibbosus*	
		R^2	Linearity range (ng g^{-1})	R^2	Linearity range (ng g^{-1})	R^2	Linearity range (ng g^{-1})
PFPeA	PFBA-D	0.990	10-500	0.991	10-500	0.992	10-500
PFBS	PFHxS-D	0.995	1-500	0.998	2-500	0.997	1-500
PFHpA	PFBA-D	0.996	0.5-500	0.993	2-500	0.998	2-500
PFHpS	PFHxS-D	0.991	0.2-500	0.995	1-500	0.998	0.5-500
IpPFNA	PFNA-D	0.992	10-200	0.990	10-500	0.998	10-500
FOUEA	PFDoDA-D	0.993	10-500	0.994	10-500	0.992	10-500
iPFNS	PFOS-D	0.995	0.5-500	0.998	20-500	0.990	0.5-500
PFDS	PFOS-D	0.990	10-500	0.990	10-500	0.994	10-500
PFTrDA	PFUnDA-D	0.992	0.2-500	0.997	0.2-500	1.000	0.2-500
PFTeDA	PFUnDA-D	0.993	20-500	0.990	20-500	0.990	20-500
PFHxDA	PFUnDA-D	0.990	0.5-500	0.990	20-500	0.994	20-500
PFODA	PFUnDA-D	0.998	10-500	0.999	10-500	0.992	10-500
PFBA	PFBA-D	0.991	10-500	0.996	10-500	0.995	10-500
PFHxA	PFHxA-D	0.995	1-500	0.995	10-500	0.996	10-500
PFHxS	PFHxS-D	0.991	0.1-500	0.990	0.1-500	0.997	0.1-500
PFOA	PFOA-D	0.990	0.2-500	0.992	0.2-500	0.997	0.2-500
PFOS	PFOS-D	0.993	1-500	0.997	10-500	0.992	2-500
PFNA	PFNA-D	0.997	10-500	0.998	10-500	0.995	10-500

PFAS	IS	*Procambarus Clarkii*		*Corbicula fluminea*		*Lepomis gibbosus*	
		R^2	Linearity range (ng g^{-1})	R^2	Linearity range (ng g^{-1})	R^2	Linearity range (ng g^{-1})
PFDA	PFDA-D	0.995	0.2-500	0.990	0.2-500	0.992	0.2-500
PFUnDA	PFUnDA-D	0.990	0.5-500	0.991	0.5-500	0.993	0.5-500
PFDoDA	PFDoDA-D	1.000	20-500	0.997	20-500	0.995	20-500

Table S2. Solvent and clean-up test results

		EXTRACTION SOLVENT TEST																					
		Anodonta cygnea												*Procambarus clarkii*									
		ACN + 0.1% FA			iPrOH/ACN (1:3) + 0.1% FA			H_2O:MeOH (7:3) + 1% F.A			MeOH			ACN + 0.1% FA			iPrOH/ACN (1:3) + 0.1% FA			H_2O:MeOH (7:3) + 1% F.A			MeOH		
CECs		Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)
OPFRs	TPP	131	19	-92	63	4	-89	61	17	110	45	11	-59	131	32	107	63	9	133	17	36	34	100	0.1	-97
	TDBPP	25	18	-67	130	7	-8	22	27	140	35	34	-71	79	80	139	135	2	119	135	23	-66	100	1	-97
	CDP	<LOQ	-	-88	<LOQ	-	-91	<LOQ	-	-99	87	9	-27	136	-	-99	<LOQ	-	-93	<LOQ	-	-82	100	1	-56
	TnBP	128	21	-89	120	25	-92	77	6	140	95	11	-80	109	10	43	90	4	118	68	31	-67	100	0.1	-97
	TBEP	87	1	-46	126	31	-80	84	21	141	99	0.3	-97	88	33	67	93	15	126	55	37	-94	100	0.01	-97
	TMPP	68	15	-97	59	8	-96	50	43	-96	69	23	-75	107	16	126	97	29	106	10	2	-74	100	0.01	-97
	TEHP	74	12	-70	37	29	-73	62	23	110	39	9	-85	100	1	141	97	4	129	80	14	-41	82	21	-68
	TCEP	100	0	166	100	0	166	99	2	132	80	14	-41	100	14	53	92	10	47	79	5	128	22	8	133
	TCIPP	100	1	-47	100	0	-47	74	13	136	79	5	-51	67	27	-85	76	33	-87	67	23	-51	37	17	-54
	TDCIPP	100	0	-97	100	0	-97	58	33	-31	67	23	-55	99	1	46	98	0.4	45	99	0.1	-98	136	46	-16
	TPhP	100	0	35	100	0.1	34	91	4	76	77	11		116	38	96	85	25	70	50	21	-30	73	12	-35
PFAS	PFPeA	68	9	145	64	36	167	101	8	141	66	17	71	82	24	-62	109	36	-68	53	9	-63	25	31	-69
	PFBS	91	16	-64	41	36	-50	56	31	-50	<LOQ	-	-86	89	12	-10	65	9	-7	43	12	-4	76	3	-9
	PFHpA	79	17	30	53	29	29	74	24	7	78	2	45	82	35	-27	78	16	-37	16	4	-44	146	32	-68
	PFHpS	66	23	-40	21	21	-61	15	32	-34	86	12	-8	82	18	-35	67	13	-62	6	13	-42	141	23	-79
	ipPFNA	72	10	-25	39	36	-46	30	23	-25	70	4	6	92	13	-15	80	33	-53	30	1	-18	132	6	-75
	FOUEA	82	14	-13	70	2	-48	92	1	-73	73	4	41	92	13	-15	80	-	-93	<LOQ	-	-93	<LOQ	-	-84
	ipPFNS	<LOQ	-	-91	<LOQ	-	-95	<LOQ	-	-91	<LOQ	-	-42	31	6	-71	43	-	-97	24	6	-89	133	12	-96
	PFDS	69	40	-95	92	1	-97	73	3	-96	77	28	-52	40	11	-91	101	11	-92	48	1	-94	151	21	-95
	PFTiDA	80	2	-90	95	0.5	-92	73	2	-89	74	6	-50	72	10	-88	93	1	-92	48	1	-94	<LOQ	-	-84
	PFTeDA	<LOQ	-	-96	<LOQ	-	-92	<LOQ	-	-97	58	22	-71	<LOQ	-	-96	<LOQ	-	-83	<LOQ	-	-91	<LOQ	-	-86
	PFHxDA	<LOQ	-	-97	<LOQ	-	-97	<LOQ	-	-80	<LOQ	-	-87	62	1	-95	<LOQ	-	-98	<LOQ	-	-89	<LOQ	-	-86
	PFODA	99	4	-82	99	1	-82	99	0.2	-82	41	15	-36	88	1	-80	100	0.1	-82	78	0.01	-77	101	1	-82
	PFBA	94	23	-73	64	34	126	82	15	159	65	13	158	94	13	44	78	19	41	63	8	77	100	9	58
	PFHxA	93	6	28	56	21	52	82	13	31	76	5	60	74	37	-17	75	7	16	130	29	-64	76	5	13
	PFHxS	89	15	-7	54	21	-13	62	29	-35	74	5	11	98	26	-42	89	12	-22	36	23	-16	98	5	36
	PFOA	92	1	79	83	33	42	93	9	47	74	5	118	92	5	36	92	13	42	63	5	73	92	<LOQ	-
	PFOS	74	9	-66	31	15	-81	30	9	-75	51	18	-15	113	17	-54	74	25	-66	31	15	-83	74	<LOQ	-
	PFNA	76	9	-23	47	22	-53	29	9	-35	53	31	-69	108	5	48	76	3	-4	62	20	-63	76	<LOQ	-
	PFDA	92	3	-4	93	5	-18	86	4	-14	89	2	52	82	10	-44	76	9	-23	47	22	-53	76	<LOQ	-
	PFUnDA	65	15	-88	56	8	-95	29	35	-88	55	39	-61	87	10	7	91	4	-96	56	8	-95	91	<LOQ	-
	PFDoDA	<LOQ	-	-96	<LOQ	-	-96	<LOQ	-	-95	59	19	-59	136	46	-16	100	0	35	100	0.1	34	136	46	-16
PESTICIDES	Acetamiprid	52	31	141	131	31	110	<LOQ	-	-95	24	24	-20	60	14	82	92	12	145	<LOQ	-	-84	3	-	-40
	Alachlor	60	14	82	92	12	145	<LOQ	-	-80	29	13	114	34	5	49	107	66	3	<LOQ	-	-96	<LOQ	-	-98
	Atrazine	70	5	38	129	6	25	<LOQ	-	-80	11	35	-51	74	31	25	112	35	-0.4	<LOQ	-	-64	<LOQ	-	-78
	Atrazine-Deethyl	74	31	25	112	35	-0.4	<LOQ	-	-76	21	12	-24	<LOQ	-	-99	<LOQ	-	-83	<LOQ	-	-92	<LOQ	-	-94
	Atrazine-Desisopropyl	<LOQ	-	114	105	7	141	<LOQ	-	-92	40	29	-29	26	34	114	105	7	141	<LOQ	-	-93	<LOQ	-	-94
	Azinphos-ethyl	<LOQ	-	-99	<LOQ	-	-83	<LOQ	-	-93	140	20	-80	<LOQ	-	-93	<LOQ	-	-81	<LOQ	-	-77	109	39	-72
	Azinphos-Methyl	<LOQ	-	-85	<LOQ	-	-90	<LOQ	-	-99	<LOQ	-	-82	<LOQ	-	-85	<LOQ	-	-90	<LOQ	-	-69	<LOQ	-	-96
	Buprofezine	<LOQ	-	-81	<LOQ	-	-89	<LOQ	-	-91	<LOQ	-	-96	<LOQ	-	-81	<LOQ	-	-89	<LOQ	-	-91	<LOQ	-	-83
	Carbendazim	61	25	-7	20	-	-23	50	13	7	41	15	1	61	12	-50	44	14	-60	21	17	-67	<LOQ	-	-95
	Carbofuran	61	-	-84	<LOQ	-	-60	21	9	-13	52	28	117	61	-	-96	60	14	-67	72	14	-69	<LOQ	-	-95
	Carbofuran 3-OH	33	40	-80	<LOQ	-	28	48	-	-28	140	13	-64	118	6	-80	171	6	140	106	6	-80	75	30	-90
	Chlorfenvinphos	55	5	140	64	26	64	64	-	-115	66	22	34	139	-	-80	47	-	-90	<LOQ	-	-92	69	-	141
	Chlorpyriphos	44	6	-99	112	-	-84	<LOQ	-	-91	62	7	63	99	29	-80	117	24	135	37	28	-58	79	14	96
	Clothianidin	72	-	-81	<LOQ	-	-99	112	6	-10	58	4	16	94	23	-73	72	9	-41	143	17	-41	99	14	96
	Coumaphos	<LOQ	-	-84	<LOQ	-	-99	<LOQ	-	-91	108	18	7	76	9	-23	47	24	135	45	9	-18	62	20	-63
	Diazinon	<LOQ	-	-92	<LOQ	-	-80	<LOQ	-	-79	<LOQ	-	-80	92	3	-4	74	25	-66	38	2	-18	90	8	-18
	Dichlofenthion	<LOQ	-	-92	<LOQ	-	-84	<LOQ	-	-97	<LOQ	-	-97	76	33	-5	139	33	-86	<LOQ	-	-93	<LOQ	-	-95
	Dimethoate	94	23	-73	<LOQ	-	-82	<LOQ	-	-90	<LOQ	-	-90	<LOQ	-	-93	143	17	-41	143	17	-41	94	23	-73
	Diuron	<LOQ	-	-90	<LOQ	-	-90	<LOQ	-	-90	<LOQ	-	-90	<LOQ	-	-80	<LOQ	-	-84	<LOQ	-	-90	<LOQ	-	-86
	DMA	<LOQ	-	-99	<LOQ	-	-99	<LOQ	-	-99	<LOQ	-	-99	<LOQ	-	-93	<LOQ	-	-90	<LOQ	-	-93	<LOQ	-	-93
	DMF	<LOQ	-	-82	<LOQ	-	-82	<LOQ	-	-82	<LOQ	-	-82	46	22	-55	<LOQ	-	-51	20	20	-59	141	5	-46
	DMPF	<LOQ	-	-93	<LOQ	-	-93	<LOQ	-	-93	<LOQ	-	-93	113	18	62	47	25	17	58	3	8	135	36	-3
	Ethion	<LOQ	-	-79	<LOQ	-	-79	<LOQ	-	-79	<LOQ	-	-79	41	34	-38	57	13	19	108	38	23	125	31	-86
	Etofenprox	<LOQ	-	-90	<LOQ	-	-99	<LOQ	-	-79	<LOQ	-	-80	<LOQ	-	-90	<LOQ	-	-86	<LOQ	-	-79	<LOQ	-	-84
	Fenthion	<LOQ	-	-89	<LOQ	-	-86	<LOQ	-	-97	<LOQ	-	-97	<LOQ	-	-89	<LOQ	-	-86	<LOQ	-	-90	45	28	-53
	Fenthion sulfone	<LOQ	-	-49	<LOQ	-	-21	<LOQ	-	-90	68	17	15	46	37	-67	97	7	141	<LOQ	-	-89	53	9	-4

	CECs	EXTRACTION SOLVENT TEST																					
		Procambarus clarkii												*Anodonta cygnea*									
		ACN + 0.1% FA			iPrOH/ACN (1:3) + 0.1% FA			H_2O:MeOH (7:3) + 1% F.A			MeOH			ACN + 0.1% FA			iPrOH/ACN (1:3) + 0.1% FA			H_2O:MeOH (7:3) + 1% F.A			MeOH		
		Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)
PESTICIDES	Fenthion sulfoxide	65	36	168	120	7	135	<LOQ	-	-98	<LOQ	-	-77	70	31	132	56	14	116	33	13	-35	39	22	9
	Fipronil	30	25	-87	133	9	140	63	25	-31	47	21	-75	31	22	-98	10	30	-68	45	30	3	140	2	-92
	Hexythiazox	49	19	-93	47	14	-90	141	10	-97	93	5	-95	58	20	-95	80	23	-97	151	3	-96	117	39	-97
	Imazalil	<LOQ	-	-96	<LOQ	-	-84	<LOQ	-	-99	34	0	-93	<LOQ	-	-87	<LOQ	-	-93	<LOQ	-	-99	99	0	-97
	Imidacloprid	54	41	48	76	10	140	67	5	3	85	14	13	86	10	139	53	14	105	102	11	31	73	10	86
	Isoproturon	54	32	38	117	7	141	<LOQ	-	-84	59	19	-47	55	9	-8	<LOQ	-	-8	33	35	-28	76	19	57
	Methiocarb	45	14	192	56	18	192	137	5	3	58	3	17	77	14	43	61	13	41	94	7	44	78	2	54
	Methoalachlor	36	15	169	100	10	32	<LOQ	-	-81	11	-	-66	117	26	-73	<LOQ	-	-13	153	-	-42	24	38	-40
	Molinate	100	17	-53	156	17	-75	<LOQ	-	-80	143	17	19	<LOQ	-	-95	139	-	-96	<LOQ	-	-76	61	4	134
	Omethoate	<LOQ	-	-92	<LOQ	-	-91	<LOQ	-	-84	98	16	-94	<LOQ	-	-96	<LOQ	-	-95	<LOQ	-	-92	62	7	-65
	Parathion-ethyl	<LOQ	-	-80	<LOQ	-	-95	<LOQ	-	-92	<LOQ	-	-84	<LOQ	-	-99	<LOQ	-	-89	<LOQ	-	-93	<LOQ	-	-72
	Prochloraz	<LOQ	-	-3	117	-	-79	<LOQ	-	-87	99	4	-97	<LOQ	-	-84	<LOQ	-	-90	<LOQ	-	-84	108	34	-97
	Propanil	<LOQ	-	-98	<LOQ	-	-86	<LOQ	-	-83	<LOQ	-	-92	<LOQ	-	-89	<LOQ	-	-81	<LOQ	-	-80	<LOQ	-	-89
	Propazine	56	41	68	113	20	12	46	39	-45	80	6	-30	105	11	-44	66	20	-16	62	7	52	71	20	14
	Pyriproxyfen	<LOQ	-	-84	<LOQ	-	-72	<LOQ	-	-95	<LOQ	-	-91	<LOQ	-	-93	<LOQ	-	-91	<LOQ	-	-82	<LOQ	-	-99
	Simazine	67	41	-33	106	9	-24	<LOQ	-	-97	<LOQ	-	-79	78	8	-75	<LOQ	-	-80	57	37	-70	48	7	-46
	Spinosad A	13	44	138	56	18	141	46	37	-61	19	1	-87	133	15	-95	41	18	-82	49	34	-84	24	5	-53
	Spinosad C	32	14	-98	95	15	129	105	17	137	113	1	-96	72	14	122	37	-	109	96	16	122	12	22	-74
	Spinosad D	74	38	133	58	12	137	132	12	-29	101	1	-97	<LOQ	-	-65	40	40	-38	64	9	35	100	1	-97
	Tebuconazole	26	6	-84	50	10	131	57	39	-39	48	11	2	80	5	-34	34	5	-13	72	13	37	63	10	45
	Terbumeton	46	35	-82	86	9	127	70	6	-30	59	4	27	106	38	-28	33	15	-5	73	9	78	63	23	30
	Terbumeton deethyl	69	13	141	84	19	134	52	6	53	88	2	-5	85	19	122	50	22	100	106	9	111	63	2	146
	Terbuthylazine	50	25	9	114	14	-35	34	20	-64	72	16	-51	97	9	-68	14	35	-56	53	29	29	73	36	-8
	Terbuthylazine-2OH Results	91	14	73	98	10	75	79	39	140	104	0.1	-38	96	20	118	75	11	120	<LOQ	-	-82	104	0.3	-36
	Terbuthylazine-deethyl	90	5	39	91	6	39	64	12	160	104	9	62	99	6	127	66	22	102	89	10	134	70	6	120
	Terbutryn	41	6	24	118	10	-30	58	24	-68	53	24	-46	124	17	-83	55	25	-77	59	10	20	70	36	-20
	Thiabendazole	66	15	136	73	17	140	60	6	61	85	1	17	58	4	90	59	12	94	99	4	82	71	1	125
	Thiametosan	54	31	-47	85	18	-25	74	17	-37	74	5	-24	58	22	125	45	15	85	114	7	-10	72	18	59
PhACs	5-methyl-1 H Benzotriazole	86	17	-6	55	5	-3	67	13	-21	73	4	-22	84	3	-7	67	15	-15	68	11	-5	65	3	-15
	Acetaminophen	32	19	-34	17	-	-60	0	-	-	10	3	-57	84	8	-2	60	13	-31	74	15	-46	73	4	-24
	Acridone	92	10	-10	58	3	-21	55	11	-27	68	1	-28	80	7	3	66	22	-17	57	15	-19	65	3	-18
	Alprazolam	94	9	55	59	0.2	45	70	7	37	67	2	52	74	7	18	65	20	19	65	10	57	71	1	38
	Amantadine	81	13	-35	57	5	-38	79	12	-60	56	11	-56	57	20	-28	63	15	-5	68	15	-34	60	2	-7
	Atenolol	54	11	-30	61	22	-53	68	9	-56	60	15	-58	46	8	45	60	18	49	70	9	4	66	2	29
	Atorvastatin	83	10	-16	60	7	-38	19	22	-26	141	2	-67	14	-	-76	0	-	-	45	3	-48	8	21	-66
	Benzotriazole	62	19	35	55	0.3	-4	42	8	8	39	5	18	78	4	4	61	21	1	50	11	12	33	2	68
	Benzoylecgonine	66	9	53	60	2	17	50	9	11	96	1	-15	52	6	32	58	18	61	69	13	10	70	2	53
	Bezafibrate	71	13	41	56	9	47	41	12	20	82	1	4	53	4	61	63	24	38	51	18	20	67	3	31
	Bisphenol-A	86	10	-24	47	11	-36	55	7	-36	62	2	-33	80	4	-20	73	12	-25	57	12	-3	67	1	-4
	Bromazepam	85	19	43	61	7	140	39	2	207	93	4	158	57	15	22	56	16	24	69	22	98	65	3	80
	Caffeine	101	10	129	70	21	110	73	1	70	111	4	30	69	3	16	65	19	27	65	5	7	65	7	23
	Carazolol	133	14	-45	71	8	-48	90	10	-33	41	7	-12	66	30	-52	59	11	-21	65	10	10	46	2	17
	Carbamazepine	92	9	7	56	7	-10	66	10	-25	67	1	-16	79	5	10	68	22	-8	64	13	-19	67	4	-3
	Carbamazepine-10,11-epoxid	100	4	22	57	5	58	59	14	35	79	1	16	74	3	24	64	19	38	68	9	32	67	3	42
	Chloramphenicol	93	7	-11	62	4	-6	69	10	-9	61	3	-1	83	1	0	65	22	4	59	9	19	68	4	-5
	Chlorpromazine	176	19	-65	137	21	-92	54	26	-28	39	2	-22	63	32	-62	69	32	-92	48	14	14	38	7	13
	Ciprofloxacin	3	22	12	31	19	12	40	13	-22	23	7	-26	23	13	-9	60	15	14	65	12	-1	6	23	35
	Citalopram	136	13	-57	70	9	-59	97	14	-46	43	5	-20	69	33	-57	61	10	-38	72	14	-7	56	4	-13
	Clarithromycin	104	12	-47	62	3	-47	107	9	-48	48	2	-21	69	24	-32	60	16	-15	73	15	-17	61	2	-26
	Clofibric acid	56	9	7	60	2	24	46	4	39	69	5	36	49	3	11	57	22	13	49	14	40	67	1	26
	Cocaethylene	103	10	-34	64	1	-34	87	14	-46	55	3	-33	70	21	-22	65	18	-11	74	13	-18	65	6	-14
	Cocaine	98	7	-25	59	5	-34	91	14	-57	62	3	-45	73	17	-10	65	21	-8	73	14	-28	65	6	-19
	Codeine	77	9	37	54	5	4	60	2	-32	79	6	-38	62	6	43	63	17	29	72	8	-6	61	1	32
	Cotinine	69	22	11	69	26	-26	75	11	-42	76	2	-49	64	6	15	59	21	-3	68	2	-35	72	7	-6
	Diazepam	94	10	-41	61	7	-54	58	10	-42	60	3	-34	78	12	-28	66	12	-36	62	14	-22	68	3	-26
	Diclofenac	87	2	61	103	11	38	89	2	33	83	3	30	82	2	-1	64	7	16	79	11	37	88	5	47
	Diltiazem	129	13	-51	71	8	-49	98	12	-41	49	6	-16	70	32	-52	61	13	-29	73	11	-7	61	4	-12
	Erythromycin	81	5	-18	61	0.4	-36	12	23	-51	765	7	-95	69	9	-6	69	20	-40	57	22	-88	66	3	-24
	Fenofibrate	159	28	-77	234	13	-94	2	12	-25	361	5	-93	67	31	-69	88	22	-90	39	19	-42	66	2	-15
	Fipronil Desulfynil	136	25	-49	95	4	-85	14	70	-15	164	2	-69	70	18	-51	62	9	-82	55	23	-23	79	3	-2
	Fipronil Sulfide	136	25	-49	95	4	-85	19	24	-15	164	2	-69	70	18	-51	62	9	-82	55	24	-23	79	3	-2
	Fipronil Sulfone	149	20	-46	105	6	-86	13	27	-2	197	8	-72	66	19	-48	64	5	-85	50	3	-38	75	5	12
	Fipronil	99	16	-27	74	7	-62	33	38	-14	90	3	-34	75	15	-28	67	4	-52	59	4	-13	71	4	-2
	Fluconazole	89	10	12	62	4	22	89	13	20	64	3	36	75	2	16	63	24	13	71	12	6	66	1	15
	Flumequine	17	7	30	51	11	17	64	11	5	31	10	23	67	2	19	66	22	4	57	11	24	38	4	18
	Fluoxetine	175	20	-66	136	25	-92	80	22	-33	34	3	-6	62	32	-63	76	14	-86	66	14	29	47	3	14
	Furazolidone	89	12	17	55	8	2	81	9	-62	30	7	-27	75	4	23	73	20	7	52	13	4	65	9	-25
	Furosemide	79	11	-1	57	6	-16	37	8	1	70	2	-9	52	16	2	50	15	-34	35	12	7	48	11	-16
	Hydrochlorothizade	84	24	-2	63	11	-13	48	7	-24	75	2	-37	79	1	10	61	22	3	54	9	7	59	2	-5
	Indomethacin	68	10	-17	63	6	-49	8	14	-34	146	5	-73	66	4	-3	62	11	-34	28	24	-34	62	3	-16
	Irgasan	155	70	-53	183	48	-80	0	-	9	180	78	-91	62	22	-13	89	30	-81	21	49	-70	56	5	104
	Ketoprofen	65	12	51	59	16	45	47	12	27	76	3	19	56	9	47	64	25	34	57	11	25	70	1	30
	Lamotrigine	90	13	-25	60	4	-61	58	11	-57	77	5	-61	77	11	-35	65	17	-44	66	11	-49	67	9	-47
	Loratadine	140	16	-63	161	17	-92	31	69	-25	80	4	-50	65	29	-63	82	17	-87	76	14	-9	63	4	-13
	Lorazepam	104	9	6	61	5	14	42	14	37	57	18	7	71	10	-18	61	16	-18	56	4	59	63	2	22
	Losartan potassium	71	13	19	56	5	-10	40	13	-26	106	3	-45	67	9	-18	63	10	-60	62	11	-50	65	2	-38
	Mefenamic acid	75	7	-9	69	3	-70	0	-	-16	389	2	-87	68	2	-12	70	1	-64	19	28	-47	56	6	14
	Metformin	20	27	-95	0	-	-	0	-	-	0	-	-	43	15	-84	58	4	-94	60	10	-95	63	12	-95
	Methadone	155	16	-69	77	6	-79	102	9	-45	38	7	-17	63	35	-67	56	5	-61	71	13	-5	57	4	-18
	Metoprolol	93	5	0	61	1	12	74	10	-11	66	4	-5	66	9	-1	58	18	34	75	10	27	64	3	34
	Midazolam	96	21	-39	69	7	-68	77	9	-40	58	5	-27	62	15	-49	55	7	-63	73	12	-15	58	3	-29
	Morphine	62	12	-28	55	14	-63	61	8	-74	65	4	-74	61	4	-15	49	32	-38	68	2	-54	58	12	-51
	N-acethyl SMX	60	8	8	61	5	29	58	16	11	72	2	9	61	4	17	62	23	16	70	12	25	68	3	14
	Nalidixic acid	9	14	6	51	9	-11	66	12	-23	28	11	-12	58	5	14	66	16	-7	62	14	-10	37	4	-4
	N-Desmethylcitalopram	140	13	-60	72	8	-67	107	14	-50	37	8	-19	67	32	-60	60	9	-46	71	14	-2	53	2	-13
	Norfluoxetine	186	18	-62	162	29	-91	66	30	2	33	3	28	72	30	-66	83	14	-86	65	13	110	44	4	52
	O-desmethylvenlafaxine	80	6	-11	58	3	-35	71	11	-55	71	6	-56	68	11	1	74	4	1	72	15	-38	68	3	-12
	Omeprazole	98	7	36	95	6	-68	0	-	-	0	1	-100	69	3	25	70	27	-68	0	-	-	65	3	99
	Oseltamivir phosphate	102	16	-27	66	9	96	84	9	139	58	3	187	66	16	-33	49	15	36	72	11	227	67	2	96
	Oseltamivir-CBX	6	20	6	60	4	40	60	7	40	79	3	27	14	14	17	52	15	25	70	13	65	64	5	48
	Oxazepam	98	9	-5	69	0.5	28	46	10	51	63	3	38	69	12	-15	59	19	-15	56	6	66	61	1	21
	Oxytetracycline	0	-	-	18	64	32	65	21	19	0	-	-	20	11	17	20	12	19	58	22	12	0	-	-
	Paroxetine	148	19	-50	131	27	-90	75	17	-9	26	4	34	63	29	-60	72	12	-82	57	15	73	36	2	49
	Pentobarbital	102	4	-15	66	2	-18	70	8	-21	76	2	-20	83	6	-3	87	15	-8	79	11	-12	86	3	-3
	Propyphenazone	93	9	-8	54	4	-24	74	11	-32	66	2	-23	80	2	6	66	21	-9	69	13	-15	70	3	-14
	Salbutamol	56	9	57	56	11	2	71	6	-29	65	5	-27	50	7	57	62	17	52	74	9	-1	65	2	40
	Salicylic acid	71	5	-6	59	3	-13	175	11	-13	35	5	57	55	2	-2	51	14	1	161	58	148	65	11	25
	Sertraline	142	18	-37	135	31	-88	43	39	7	51	7	-9	74	33	-41	104	11	-85	65	16	68	42	2	81
	Sitagliptin	104	8	-25	63	4	27	67	4	75	63	1	83	73	15	-22	58	19	7	65	8	122	56	5	29
	Sotalol	72	8	-17	59	5	-35	82	7	-55	61	6	-49	64	3	41	64	20	36	71	7	5	65	3	24
	Sulfadimethoxine	84	7	20	63	4	83	30	5	119	129	6	9	84	2	25	64	24	23	68	18	28	66	3	61
	Sulfamethazine	95	0	36	67	6	50	21	9	101	140	3	-8	87	0.2	10	67	23	13	76	20	-5	66	5	86
	Sulfamethoxazole	80	8	15	60	6	27	29	13	36	122	4	-30	82	2	13	65	24	8	71	19	-7	66	2	31
	Sulfapyridine	91	1	12	63	7	29	18	8	75	143	5	-26	78	1	14	64	25	1	73	18	-31	64	4	72
	Temazepam	101	8	92	60	3	81	58	9	84	63	2	90	77	8	65	66	18	53	57	13	87	66	4	76
	Tramadol	92	6	-19	60	6	-32	72	11	-50	68	3	-50	73	8	-3	65	22	-4	74	11	-30	65	4	-14
	Triclocarban	190	31	183	151	32	-65	0	-	-	4789	2	-96	66	33	153	61	20	-74	15	55	-17	38	3	842
	Trimethoprim	88	8	-15	60	0.3	-30	64	6	-43	74	1	-46	66	14	-9	61	20	-2	71	6	-26	61	1	-11
	Valsartan	29	14	93	49	11	85	26	15	49	131	4	-16	15	8	39	48	21	-4	53	15	-23	64	2	24
	Valsartan acid	5	33	-15	37	0.2	-28	38	6	-41	65	14	-48	4	7	8	42	16	-17	47	6	-16	62	3	-5
	Venlafaxine	97	9	-35	60	0.5	-37	83	13	-51	59	4	-42	71	19	-24	62	17	-9	77	10	-24	64	2	-15
	Verapamil	138	16	-52	77	14	-62	94	8	-21	47	3	8	67	34	-52	56	7	-34	71	12	23	59	3	4
	Warfarin	86	10	147	130	9	41	51	6	164	60	2	172	84	2	12	69	26	22	50	12	151	70	3	50
	Zolpidem	95	11	-13	61	0.4	-30	74	11	-35	70	2	-31	45	17	13	59	17	-14	75	12	-22	66	3	-16

*Nicotine — The nicotine standard was not available during the method development experiments, so it was only included in the validation.

Table S3. Solvent and clean-up test results

CECs		CLEAN-UP STEP TEST																	
		Procambarus clarkii									*Anodonta cygnea*								
		Z-Sep			Z-Sep+			Z-Sep/C18			Z-Sep			Z-Sep+			Z-Sep/C18		
		Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)
OPFRs	TPP	77	12	-65	98	0.3	-97	75	15	-72	100	0.1	-97	100	0.1	-97	63	14	-83
	TDBPP	104	1	-98	100	0.2	-97	6	18	-91	100	0.3	-97	100	0.6	-97	114	22	-71
	CDP	95	1	-52	100	0.5	-55	<LOQ	-	-93	100	0.8	-55	100	0.5	-55	<LOQ	-	-82
	TnBP	107	22	-88	99	0.2	-97	90	22	-85	100	0.1	-97	100	0.1	-97	95	9	-91
	TBEP	99	0.1	-97	100	0.01	-97	117	1	-96	100	0.02	-97	100	0.01	-97	36	17	-31
	TMPP	100	1	-97	100	0.1	-97	128	24	-97	100	0.1	-97	100	0.04	-97	110	25	-98
	TEHP	99	0.1	-97	100	0.2	-97	127	34	-54	100	0.03	-97	100	0.01	-97	139	34	-85
	TCEP	78	4	-39	53	22	-1	100	0.4	166	125	20	-15	52	21	-47	100	0.02	-80
	TClPP	64	35	1	49	18	-36	99	2	-46	32	26	-71	<LOQ	-	-99	100	0.3	-47
	TDClPP	55	21	-93	17	5	2	100	0.5	-97	17	0.7	-85	111	9	-83	100	0.5	-97
	TPhP	70	13	-86	12	-	-48	100	0.1	34	125	34	240	124	13	29	100	0.2	34
PFAS	PFPeA	18	31	141	98	2	-68	87	27	99	68	35	140	25	26	136	78	5	-1
	PFBS	<LOQ	-	-68	<LOQ	-	-92	75	24	-58	9	9	-67	<LOQ	-	-71	50	26	-62
	PFHpA	57	31	-26	71	24	-55	101	22	-3	72	9	12	36	13	20	58	4	12
	PFHpS	89	-	-10	62	12	-24	83	11	3	85	25	-64	74	27	-61	48	15	-22
	ipPFNA	81	25	-26	43	8	-15	91	13	-22	64	5	-19	11	24	-20	46	5	-4
	FOUEA	66	16	-17	43	3	-32	85	11	-10	73	4	-28	52	8	-44	69	5	-23
	ipPFNS	75	20	-51	64	53	-62	117	31	-69	106	22	-82	11	23	-72	39	20	-71
	PFDS	88	27	-72	<LOQ	-	-89	102	20	-96	67	28	-81	37	15	-92	28	22	-88
	PFTrDA	57	30	-74	70	16	-92	117	9	-89	100	3	-87	79	5	-94	81	21	-86
	PFTeDA	124	-	-91	<LOQ	-	-96	150	9	-97	<LOQ	-	-94	<LOQ	-	-96	96	10	-96
	PFHxDA	<LOQ	-	-96	<LOQ	-	-94	162	15	-96	<LOQ	-	-94	<LOQ	-	-88	<LOQ	-	-93
	PFODA	65	34	-66	93	1	-81	105	11	-82	81	15	-74	100	0	-82	102	12	-81
	PFBA	38	21	92	13	15	-37	61	13	108	60	9	123	62	19	80	76	11	107
	PFHxA	28	7	7	101	7	-68	125	15	-65	79	12	26	54	26	15	69	5	22
	PFHxS	80	17	-17	74	15	-2	76	13	19	66	12	-14	54	17	-17	59	3	-11
	PFOA	107	11	61	84	4	70	103	6	81	98	4	62	61	3	63	87	8	61
	PFOS	65	19	-71	<LOQ	-	-72	123	28	-72	61	20	-69	<LOQ	-	-56	62	15	-53
	PFNA	174	31	-82	61	-	-82	90	13	-32	<LOQ	-	-81	<LOQ	-	-86	46	5	-16
	PFDA	96	17	13	76	2	25	106	4	14	103	5	5	68	1	19	90	2	11
	PFUnDA	167	-	-89	<LOQ	-	-81	115	22	-78	<LOQ	-	-93	<LOQ	-	-83	49	22	-74
	PFDoDA	100	-	-81	<LOQ	-	-84	118	8	-93	22	10	-85	<LOQ	-	-96	32	29	-87
PESTICIDES	Acetamiprid	67	9	124	110	7	80	49	15	24	29	16	-10	23	7	-11	30	27	-47
	Alachlor	122	39	-52	<LOQ	-	-24	61	9	23	65	-	-20	117	14	-80	97	31	-1
	Atrazine	81	33	-20	80	7	42	71	15	-24	<LOQ	-	-63	14	6	-28	61	17	5
	Atrazine-Deethyl	51	28	63	54	29	-21	26	34	-25	24	15	-37	24	13	-29	32	28	-33
	Atrazine-Deisopropryl	54	19	43	36	22	-37	72	4	36	41	3	-37	44	25	-21	72	15	49
	Azinphos-ethyl	140	11	-76	106	21	-77	85	30	81	34	4	-61	45	17	-39	157	11	62
	Azinphos-Methyl	50	32	-19	40	23	4	69	28	-29	<LOQ	-	-82	<LOQ	-	-98	61	7	-18
	Buprofezine	<LOQ	-	-92	<LOQ	-	-93	84	15	-92	<LOQ	-	-93	<LOQ	-	-90	59	30	-81
	Carbendazim	52	4	121	56	29	42	56	25	-1	51	21	-11	38	6	17	50	13	-1
	Carbofuran	12	-	74	<LOQ	-	-15	15	22	-2	27	14	-68	<LOQ	-	-68	68	15	-26
	Carbofuran 3-OH	<LOQ	-	111	49	21	124	58	13	43	36	-	-11	96	-	-80	73	17	27
	Chlorfenvinphos	48	31	-53	7	39	115	81	27	-90	113	25	-66	133	36	-55	99	15	-91
PESTICIDES	Chlorpyriphos	55	33	-63	41	25	-87	<LOQ	-	-91	51	34	61	93	15	38	<LOQ	-	-95
	Chlothianidin	65	19	127	92	38	119	72	7	140	65	9	81	69	10	94	68	9	74
	Coumaphos	88	34	-89	44	33	-68	113	8	-92	45	2	-94	65	28	-67	55	28	-61
	Diazinon	60	30	-31	41	30	-13	102	10	-91	68	30	-65	80	36	-58	59	29	-78
	Dichlofenthion	<LOQ	-	-86	<LOQ	-	-84	54	17	-90	<LOQ	-	-81	<LOQ	-	-92	50	37	-89
	Dimethoate	48	13	-18	64	4	-75	58	11	36	<LOQ	-	-84	<LOQ	-	-83	61	5	-6
	Diuron	<LOQ	-	-87	<LOQ	-	-94	78	16	-22	<LOQ	-	-99	<LOQ	-	-93	85	35	95
	DMA	100	12	-97	99	1	-97	<LOQ	-	-91	80	1	-97	143	37	-97	<LOQ	-	-91
	DMF	65	29	-9	4	10	-30	62	13	-20	25	22	-84	22	12	-77	68	13	-28
	DMPF	38	18	136	60	11	24	50	3	19	70	18	7	45	9	15	51	11	31
	Ethion	128	12	-94	63	13	-95	<LOQ	-	-80	83	35	-88	121	12	-92	<LOQ	-	-74
	Etofenprox	<LOQ	-	-80	<LOQ	-	-81	73	33	-95	<LOQ	-	-99	<LOQ	-	-84	45	16	-91
	Fenthion	147	3	-94	53	24	-62	135	31	-81	131	8	-95	105	20	-91	74	10	-80
	Fenthion sulfone	64	26	136	63	29	140	72	21	70	65	14	18	71	26	16	74	17	14
	Fenthion sulfoxide	94	25	140	78	19	141	52	19	-13	50	27	107	20	-	5	48	17	-42
	Fipronil	50	32	-31	52	38	-39	97	29	161	61	13	-86	29	18	-56	52	9	114
	Hexythiazox	66	15	-96	57	8	-95	56	24	-92	128	4	-97	95	6	-97	86	30	-88
	Imazalil	99	1	-97	36	0	-93	73	13	-88	100	0.1	-97	100	-	-97	105	26	-79
	Imidacloprid	68	23	145	79	14	143	71	13	154	74	10	111	63	8	124	72	6	80
	Isoproturon	97	11	126	74	17	141	69	12	54	108	10	32	33	33	63	66	4	23
	Methiocarb	49	23	107	42	15	137	51	7	46	103	9	27	74	5	78	86	8	43
	Methoalachlor	35	8	179	50	18	125	60	28	-26	16	8	-72	22	22	-7	72	20	3
	Molinate	88	26	114	47	6	138	27	20	15	107	8	198	110	25	-4	58	-	-58
	Omethoate	61	17	-37	68	22	-62	52	7	-43	59	18	-61	47	13	-60	68	22	-63
	Parathion-ethyl	<LOQ	-	-99	<LOQ	-	-84	97	24	-75	<LOQ	-	-92	<LOQ	-	-84	61	43	-47
	Prochloraz	100	1	-97	67	1	-96	73	15	-87	136	6	-97	60	2	-96	79	34	-67
	Propanil	<LOQ	-	-87	<LOQ	-	-98	78	20	-40	<LOQ	-	-80	<LOQ	-	-89	78	13	-33
	Propazine	89	28	-7	77	14	141	75	30	-69	68	6	-9	56	19	74	64	9	-18
	Pyriproxyfen	<LOQ	-	-98	<LOQ	-	-82	102	17	-93	<LOQ	-	-83	<LOQ	-	-75	74	36	-84
	Simazine	60	24	-9	60	21	-34	36	9	-63	31	24	-52	24	36	-39	47	24	-67
	Spinosad A	23	1	-89	9	2	19	<LOQ	-	-85	20	1	-83	56	19	-89	<LOQ	-	-85
	Spinosad C	40	1	-91	43	19	-97	<LOQ	-	-91	97	30	-95	34	1	-97	<LOQ	-	-96
	Spinosad D	100	0	-97	96	1	-97	<LOQ	-	-72	100	0.1	-97	89	0.2	-97	<LOQ	-	-99
	Tebuconazole	35	4	53	10	31	140	115	30	-76	90	22	-58	32	4	53	58	37	-14
	Terbumeton	75	10	48	55	13	137	105	15	-64	70	20	-12	58	7	89	60	16	-3
	Terbumeton deethyl	78	20	137	70	12	99	73	10	109	75	4	138	57	7	104	76	13	108
	Terbuthylazine	89	20	-47	73	10	139	105	29	-79	65	21	-34	57	5	82	55	16	-28
	Terbuthylazine-2OH Results	100	0	-35	100	4	-35	<LOQ	-	-90	100	0.3	-35	100	0.2	-35	<LOQ	-	-84
	Terbuthylazine-deethyl	92	28	125	79	35	123	98	6	200	94	16	166	73	11	125	109	3	181
	Terbutryn	61	21	-52	48	17	135	49	11	-96	70	33	-79	62	14	42	26	20	-54
	Thiabendazole	65	27	46	50	39	113	72	9	18	121	15	-23	60	6	116	81	4	81
	Thiametosan	68	10	107	76	6	124	66	10	127	74	5	85	58	8	98	72	14	44
PhACs	5-methyl-1 H Benzotriazole	73	12	-30	74	37	-22	80	4	-30	64	3	-9	57	8	13	57	2	14
	Acetaminophen	33	52	-62	13	19	-62	15	2	-66	64	4	-38	53	7	-14	58	1	-35
	Acridone	79	10	-43	89	3	-41	87	3	-44	64	3	-28	51	4	-6	58	2	-13
	Alprazolam	70	10	19	48	11	28	71	3	18	71	4	1	37	5	10	53	3	48

CECs		CLEAN-UP STEP TEST																	
		Procambarus clarkii									*Anodonta cygnea*								
		Z-Sep			Z-Sep+			Z-Sep/C18			Z-Sep			Z-Sep+			Z-Sep/C18		
		Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)
PhACs	Amantadine	78	67	-49	29	4	-22	57	12	-54	56	3	-15	30	3	37	52	6	30
	Atenolol	35	2	-56	13	10	-47	42	4	-68	53	12	-1	36	3	17	59	2	18
	Atorvastatin	5	22	-0.2	1	9	7	20	6	16	0	-	-77	0	-	-27	0	-	-73
	Benzotriazole	76	8	-13	90	27	-13	83	7	-14	68	3	13	61	3	33	61	3	35
	Benzoylecgonine	22	16	-12	8	11	-14	42	7	-11	40	13	-5	16	4	10	57	3	18
	Bezafibrate	17	6	34	7	20	29	35	10	44	31	20	31	15	5	14	49	3	62
	Bisphenol-A	79	17	-6	67	5	3	100	9	-3	80	7	-17	46	6	7	63	9	9
	Bromazepam	82	20	-78	36	21	13	41	22	-62	119	13	-86	53	3	1	64	8	-4
	Caffeine	100	11	59	94	3	88	93	3	71	65	6	5	63	5	4	61	7	16
	Carazolol	78	31	-85	26	7	-34	107	23	-84	74	6	-70	27	5	0	62	2	-33
	Carbamazepine	77	11	-26	81	4	-25	85	5	-29	64	3	-18	47	5	1	59	0.3	1
	Carbamazepine-10,11-epoxide	78	1	-6	67	4	2	82	4	-3	65	3	-4	58	2	10	60	3	25
	Chloramphenicol	77	3	-25	56	7	-30	89	14	-33	60	12	-31	49	8	-21	64	8	-34
	Chlorpromazine	125	41	-95	24	18	-67	124	31	-93	134	5	-92	23	9	-24	48	5	-60
	Ciprofloxacin	0	-	-34	0	-	-22	0	-	-31	2	13	-65	1	38	-3	1	1	19
	Citalopram	78	33	-85	68	5	-35	100	29	-85	109	4	-81	51	6	-7	64	1	-45
	Clarithromycin	66	30	-79	17	9	-39	87	31	-80	113	5	-74	23	5	-12	55	5	-27
	Clofibric acid	14	4	-19	3	20	-27	30	5	-26	27	16	-31	9	11	-13	55	10	-22
	Cocaethylene	66	25	-53	69	4	-36	88	18	-62	103	4	-61	47	5	-8	55	2	-17
	Cocaine	66	16	-40	73	5	-34	80	10	-49	91	4	-50	48	4	-2	54	2	-9
	Codeine	54	17	-30	62	4	-36	62	6	-43	58	6	-5	51	2	14	55	4	11
	Cotinine	59	14	-36	58	7	-47	58	9	-38	61	3	-39	49	3	-23	56	5	-36
	Diazepam	80	23	-61	91	6	-40	89	11	-64	71	1	-49	57	5	-9	52	6	-30
	Diclofenac	44	10	-23	4	12	-18	53	11	-24	41	11	-28	6	16	-21	48	6	-21
	Diltiazem	72	38	-81	60	6	-35	104	31	-84	114	4	-79	48	6	-11	61	2	-40
	Erythromycin	60	10	-61	26	11	-40	78	7	-62	93	4	-62	34	5	-8	57	2	-18
	Fenofibrate	89	21	-84	66	19	-31	130	0.4	-81	112	20	-77	69	1	6	39	5	-45
	Fipronil Desulfynil	87	39	-51	50	17	16	106	19	-57	117	26	-58	74	23	26	57	3	18
	Fipronil Sulfide	82	34	-48	50	17	23	101	10	-54	111	19	-56	80	12	17	57	3	25
	Fipronil Sulfone	83	34	-53	51	17	14	111	25	-61	112	14	-62	78	25	12	55	5	5
	Fipronil	78	16	-45	62	5	-5	97	16	-41	100	8	-48	67	10	1	60	7	4
	Fluconazole	51	15	-16	16	10	-8	70	8	-14	49	8	1	16	9	11	53	3	30
	Flumequine	0.1	1	-7	0	-	-9	0.2	30	-7	1	25	-62	0	37	-17	2	8	18
	Fluoxetine	124	44	-96	52	9	-63	147	23	-94	130	20	-92	43	10	-19	61	1	-69
	Furazolidone	73	3	-13	91	6	-20	73	5	-9	56	2	7	53	7	8	51	2	19
	Furosemide	12	10	-25	16	9	-35	34	13	-31	17	17	-40	19	14	-21	35	9	-38
	Hydrochlorothizade	83	6	-23	86	7	-31	90	6	-27	62	2	-25	63	2	-17	61	4	-25
	Indomethacin	31	14	-16	0	-	-18	43	13	-20	38	27	-30	0	-	-14	49	1	-17
	Irgasan	59	4	89	29	1	287	93	5	-21	122	1	82	31	11	139	54	14	367
	Ketoprofen	30	19	61	3	19	57	37	0	72	38	14	50	5	11	15	50	3	45
	Lamotrigine	69	20	-54	97	4	-49	86	7	-60	64	2	-47	51	4	-11	53	4	-32
	Loratadine	91	14	-95	53	10	-65	115	17	-93	129	19	-91	46	5	-29	42	9	-74
	Lorazepam	41	16	-22	1	46	12	69	10	-17	61	8	-52	3	29	-22	52	5	-12
	Losartan potassium	60	13	75	53	10	117	66	12	75	58	4	-19	44	6	7	52	7	-18
	Mefenamic acid	12	25	-31	1	14	-16	21	11	-33	21	12	-49	1	10	-22	27	3	-18
	Metformin	11	2	-95	30	9	-93	19	25	-97	46	15	-91	31	7	-85	41	9	-92
	Methadone	61	9	-92	45	8	-35	118	35	-92	120	8	-89	36	10	-13	61	2	-62
	Metoprolol	61	9	-40	14	10	-28	73	7	-45	63	4	-26	20	4	3	58	2	2

CECs		CLEAN-UP STEP TEST																	
		Procambarus clarkii									*Anodonta cygnea*								
		Z-Sep			Z-Sep+			Z-Sep/C18			Z-Sep			Z-Sep+			Z-Sep/C18		
		Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)	Recovery (%)	RSD (%)	Matrix effect (%)
PhACs	Midazolam	71	55	-81	45	28	-41	65	12	-80	89	5	-79	35	4	-18	44	3	-46
	Morphine	31	6	-51	20	5	-54	56	5	-64	48	3	-55	24	9	-28	48	2	-55
	N-acethyl SMX	53	4	-17	44	7	-2	52	3	-16	63	5	-6	65	2	4	62	3	6
	Nalidixic acid	0	2	-29	0	3	-34	0	28	-34	1	7	-68	1	15	-25	1	8	-4
	N-Desmethylcitalopram	76	38	-87	69	4	-37	117	32	-88	99	6	-81	49	4	-4	65	2	-48
	Norfluoxetine	136	55	-96	51	9	-60	119	10	-92	122	14	-90	48	5	-13	61	4	-55
	O-desmethylvenlafaxine	60	10	-35	22	9	-37	63	3	-37	65	4	-31	64	1	-4	56	2	-5
	Omeprazole	67	9	-46	49	10	8	80	9	-37	61	13	-51	48	6	8	50	12	-18
	Oseltamivir phosphate	0	-	-11	0	-	4	0	-	-10	11	40	-3	22	7	10	37	3	14
	Oseltamivir-CBX	60	17	-55	17	4	41	70	12	-57	68	4	-35	46	2	14	61	4	14
	Oxazepam	35	22	-22	3	10	13	66	11	-20	53	14	-50	4	10	-21	52	1	-13
	Oxytetracycline	0	-	-37	0	-	53	0	-	0.3	0	-	1	0	-	4	0	-	46
	Paroxetine	0	-	-98	42	10	-54	141	4	-95	124	22	-90	41	8	-16	65	3	-62
	Pentobarbital	89	15	-17	78	5	-12	80	7	-9	58	12	-16	62	1	-4	62	5	-11
	Propyphenazone	79	10	-32	60	3	-34	83	3	-34	64	3	-19	32	8	1	55	2	-4
	Salbutamol	15	38	-17	5	19	-26	28	2	-17	32	17	2	19	5	27	51	4	19
	Salicylic acid	7	5	-42	0	-	-38	15	15	-39	9	3	-41	17	6	-31	36	18	-42
	Sertraline	128	60	-94	14	14	-75	120	25	-91	153	16	-87	20	4	-26	55	7	-38
	Sitagliptin	67	20	-46	65	5	-9	88	13	-52	68	3	-31	54	3	5	60	5	0
	Sotalol	58	12	-51	61	6	-49	69	2	-61	60	2	-6	53	0	10	57	4	15
	Sulfadimethoxine	70	3	-36	70	8	-21	71	4	-30	69	3	-32	69	4	7	63	6	-7
	Sulfamethazine	96	48	-32	70	9	-14	78	2	-29	66	3	-30	73	4	1	63	10	-5
	Sulfamethoxazole	69	8	-2	56	9	17	70	0.2	4	64	3	-5	72	4	15	61	6	18
	Sulfapyridine	71	5	-48	67	7	-26	80	7	-46	68	2	-28	68	5	12	64	15	-0.4
	Temazepam	44	23	18	3	20	33	75	6	18	58	9	-23	2	14	17	51	3	36
	Tramadol	67	10	-31	35	7	-24	75	5	-35	69	2	-22	31	5	12	55	2	10
	Triclocarban	146	33	217	36	43	200	45	2	44	212	59	120	106	32	867	51	40	708
	Trimethoprim	47	30	-49	71	3	-39	74	9	-52	64	4	-33	56	4	-10	58	4	-11
	Valsartan	1	55	77	2	50	74	5	18	102	9	55	-13	13	3	-16	30	11	8
	Valsartan acid	0	-	-43	0	-	-51	0	-	-44	0	-	-47	54	8	-26	28	12	-30
	Venlafaxine	63	21	-52	15	5	-30	73	18	-58	87	3	-52	16	6	0	56	3	-10
	Verapamil	87	38	-91	89	6	-36	132	34	-91	115	7	-86	60	6	-9	66	4	-54
	Warfarin	76	7	-7	25	1	-16	69	4	-0.2	58	9	-14	23	8	-22	59	8	-15
	Zolpidem	73	17	-46	85	5	-36	84	9	-50	86	2	-52	49	5	-8	54	2	-17

*Nicotine

Table S4. Validation parameters

	CECs	*Procambarus clarkii*								*Anodonta cygnea*							
		10 ng g^{-1}			100 ng g^{-1}			LOD (ng g^{-1})	LOQ (ng g^{-1})	10 ng g^{-1}			100 ng g^{-1}			LOD (ng g^{-1})	LOQ (ng g^{-1})
		Recovery (%)	RSD (%)	Matrix Effect (%)	Recovery (%)	RSD (%)	Matrix Effect (%)			Recovery (%)	RSD (%)	Matrix Effect (%)	Recovery (%)	RSD (%)	Matrix Effect (%)		
OPFRs	TPP	151	30	114	144	29	120	0.04	0.12	134	21	-89	144	24	-85	0.04	0.13
	TDBPP	105	79	146	92	82	152	0.19	0.57	28	15	-62	38	16	-60	0.71	2.14
	CDP	<LOQ	-	-	75	11	-86	4.00	12.00	<LOQ	-	-	72	17	-81	4.00	12.00
	TnBP	132	15.5	103	149	18	109	0.05	0.14	131	23.5	-84	141	19.5	-82	0.05	0.14
	TBEP	109	8	50	122	7	56	0.06	0.17	90	2	-43	100	6	-39	0.07	0.20
	TMPP	72	32	74	101	35	80	0.08	0.25	71	17	-92	81	13	-90	0.08	0.25
	TEHP	65	14	133	120	13	139	0.31	0.93	77	14	-67	87	17	-63	0.26	0.78
	TCEP	112	-0.2	148	113	3	154	0.05	0.16	103	3	171	113	2	173	0.06	0.18
	TClPP	100	12	60	113	11	66	0.40	1.20	103	3	-44	113	6	-40	0.39	1.17
	TDClPP	48	26	-78	80	29	-72	0.42	1.26	103	2	-92	113	2	-90	0.19	0.58
	TPhP	99	1	53	112	2	59	0.06	0.18	103	3	38	113	5	42	0.06	0.17
PFAS	PFPeA	123	45	103	136	14	146	1.5	4.4	75	16	152	88	8	195	2.4	7.2
	PFBS	89	31	-55	102	17	-12	0.3	0.8	98	23	-57	101	11	-14	0.2	0.7
	PFHpA	96	19	-3	109	11	40	0.1	0.3	86	24	37	99	12	80	0.3	1.0
	PFHpS	89	42	-20	102	12	23	0.1	0.2	73	30	-33	106	15	10	0.1	0.2
	ipPFNA	89	25	-28	102	14	15	0.7	2.0	79	17	-18	92	9	25	0.8	2.3
	FOUEA	99	20	-8	112	12	35	1.5	4.4	89	21	-6	92	11	37	2.0	6.0
	ipPFN	38	13	-44	71	18	-21	0.2	0.5	<LOQ	-	-	84	10	-41	4.1	12.3
	PFDS	47	18	-54	50	11	-41	2.9	8.7	76	27	-88	109	23	-45	1.8	5.4
	PFTrDA	79	17	-61	102	10	-38	0.1	0.2	87	9	-83	100	4	-40	0.0	0.1
	PFTeDA	<LOQ	-	-	87	18	-46	4.1	12.3	<LOQ	-	-	87	11	-46	4.5	13.5
	PFHxDA	69	8	-58	101	15	-45	0.1	0.3	<LOQ	-	-	89	14	-47	4.3	12.9
	PFODA	95	8	-63	98	15	-30	1.8	5.5	106	11	-75	99	5	-32	1.7	5.0
	PFBA	101	20	51	104	11	94	0.9	2.7	70	23	153	93	11	196	1.3	3.8
	PFHxA	81	44	-10	95	14	33	0.3	1.0	100	13	35	93	7	78	1.0	3.0
	PFHxS	120	16	-11	92	10	32	0.0	0.0	96	22	-0.3	99	11	43	0.0	0.0
	PFOA	115	12	55	105	17	98	0.1	0.2	99	8	86	92	4	129	0.1	0.2
	PFOS	120	24	-47	103	14	-4	0.3	0.8	81	32	-59	94	16	-16	0.4	1.2
	PFNA	89	25	-37	102	14	6	0.9	2.7	83	16	-16	96	8	27	1.0	2.9
	PFDA	94	17	14	107	10	57	0.1	0.2	99	10	3	94	5	46	0.1	0.2
	PFUnDA	47	11	-53	90	17	-30	0.1	0.4	72	22	-81	95	11	-38	0.1	0.2
	PFDoDA	<LOQ	-	-	84	19	-44	4.3	12.9	<LOQ	-	-	89	5.7	-46	4.3	12.9
PESTICIDES	Acetamiprid	59	29	148	104	24	155	1.0	3.0	83	35	2	89	36	8	2.5	7.5
	Alachlor	67	12	89	120	15	96	0.5	1.5	<LOQ	-	-86	62	15	-80	3.4	10.2
	Atrazine	41	3	56	68	8	63	1.0	5.0	146	36	-79	152	36	-73	0.6	1.9
	Atrazine-Deethyl	77	3.2	45	110	6.2	52	1.2	3.5	63	9.8	-20	69	10.8	-14	1.4	4.3
	Atrazine-Deisopropryl	81	29	32	108	15	39	1.1	3.3	70	15	5	76	16	11	1.3	3.8
	Azinphos-ethyl	<LOQ	-	-92	65	17	-85	5.0	15.0	<LOQ	-	-89	65	14	-83	5.0	15.0
	Azinphos-Methyl	33	32	121	51	17	128	3.1	9.2	<LOQ	-	-78	59	12	-72	4.0	12.0
	Buprofezine	<LOQ	-	-86	56	18	-79	5.0	15.0	<LOQ	-	-74	64	14	-68	5.2	15.6
	Carbendazim	61	5	81	108	10	88	0.6	1.8	68	27	0	74	28	6	0.5	1.6
	Carbofuran	35	-	67	45	19	74	2.0	6.0	51	13	-43	57	14	-37	1.2	3.5
	Carbofuran 3-OH	41	34	148	68	19	155	2.2	6.6	82	33	37	88	18	43	1.1	3.3
	Chlorfenvinphos	<LOQ	-	105	87	15	212	4.7	14.1	54	-	-83	60	12	-77	3.0	9.0
	Chlorpyriphos	73	5	12	102	10	19	2.2	6.6	103	26	142	100	27	148	1.1	3.4
	Chlothianidin	67	35	103	119	18	140	2.4	7.3	79	10	101	85	11	107	2.0	6.1
	Coumaphos	<LOQ	-	-9	62	15	-2	4.0	12.0	<LOQ	-	-	67	10	-71	4.0	12.0
PESTICIDES	Diazinon	<LOQ	-	-90	72	12	-83	3.8	11.4	<LOQ	-	-	73	13	-76	3.8	11.4
	Dichlofenthion	<LOQ	-	-83	75	13	-76	5.0	15.0	<LOQ	-	-	74	14	-79	5.0	15.0
	Dimethoate	66	7	-5	118	10	2	0.9	2.7	101	24	-66	107	25	-60	0.6	1.8
	Diuron	<LOQ	-	-73	86	15	-66	4.1	12.3	<LOQ	-	-	82	15	-77	4.1	12.3
	DMA	<LOQ	-	-86	73	18	-79	4.8	14.4	<LOQ	-	-	79	12	-86	4.8	14.4
	DMF	84	7	-58	119	12	-51	1.2	3.6	53	24	-48	59	25	-42	1.8	5.4
	DMPF	73	4	81	103	7	88	1.5	4.5	120	19	69	106	20	75	3.0	9.0
	Ethion	127	16	-21	125	21	-14	0.3	0.9	48	37	-31	54	17	-25	0.8	2.3
	Etofenprox	<LOQ	-	-91	87	17	-84	3.7	11.1	<LOQ	-	-	65	16	-77	3.7	11.1
	Fenthion	<LOQ	-	-8	85	10	-1	4.5	14.5	<LOQ	-	-	81	15	-76	4.5	14.5
	Fenthion sulfone	53	35	-60	92	22	-53	0.7	2.0	66	29	-42	72	20	-36	0.5	1.6
	Fenthion sulfoxide	72	34	175	87	19	182	0.7	2.1	77	34	139	83	14	145	0.6	1.9
	Fipronil	47	23	-80	109	18	-73	0.2	0.5	38	23	-91	44	24	-85	0.2	0.5
	Hexythiazox	56	17	-86	98	22	-79	0.9	2.7	65	22	-88	71	23	-82	0.8	2.3
	Imazalil	<LOQ	-	-89	88	22	-82	4.0	12.0	<LOQ	-		82	18	-74	4.5	13.5
	Imidacloprid	61	39	55	107	44	62	1.5	4.5	93	13	146	99	13	152	1.0	2.9
	Isoproturon	61	30	45	108	33	52	0.8	2.5	62	10	-1	68	11	5	0.8	2.4
	Methiocarb	52	12	119	90	15	206	1.2	3.5	84	15	50	90	16	56	0.7	2.1
	Methoalachlor	53	13	106	72	18	183	1.4	4.2	124	29	-66	100	19	-60	0.5	1.4
	Molinate	107	15	-46	98	18	-39	0.6	1.7	<LOQ	-	-	76	15	-82	3.5	10.5
	Omethoate	<LOQ	-	-85	72	15	-78	5.0	15.0	<LOQ	-	-	82	14	-83	5.0	15.0
	Parathion-ethyl	<LOQ	-	-73	78	12	-66	5.0	15.0	<LOQ	-	-	72	12	-86	5.0	15.0
	Prochloraz	<LOQ	-	4	76	10	11	4.0	12.0	<LOQ	-	-	79	10	-71	4.0	12.0
	Propanil	<LOQ	-	-91	73	18	-84	4.0	12.0	<LOQ	-	-	76	9	-76	4.0	12.0
	Propazine	63	39	75	69	17	82	0.8	2.4	112	13	-37	118	14	-31	0.4	1.3
	Pyriproxyfen	<LOQ	-	-77	71	18	-70	3.5	10.5	<LOQ	-	-	89	8	-80	3.5	10.5
	Simazine	74	39	-26	73	12	-19	0.8	2.4	85	11	-68	91	11	-62	0.7	2.1
	Spinosad A	40	42	105	55	10	152	2.0	6.0	100	16	-88	106	17	-82	0.4	1.3
	Spinosad C	39	12	-91	64	17	-84	1.5	4.6	79	16	129	85	17	135	0.8	2.3
	Spinosad D	81	36	110	102	19	147	0.7	2.2	72	12	34	80	8	-52	1.1	3.3
	Tebuconazole	53	4	-77	61	9	-70	1.8	5.5	87	8	-27	93	8	-21	0.7	2.1
	Terbumeton	53	33	-75	92	16	-68	0.1	0.3	113	39	-21	109	40	-15	0.1	0.2
	Terbumeton deethyl	76	11	118	108	16	155	0.1	0.2	92	21	129	98	22	135	0.1	0.2
	Terbuthylazine	57	23	16	100	12	23	0.1	0.3	104	10	-61	110	11	-55	0.1	0.2
	Terbuthylazine-2OH	98	12	80	103	17	87	0.6	1.8	103	23	125	109	23	131	0.6	1.7
	Terbuthylazine-deethyl	97	3	46	99	6	53	0.6	1.9	106	7	134	112	8	140	0.6	1.7
	Terbutryn	48	4	31	81	9	38	1.3	3.8	101	19	-76	107	20	-70	0.5	1.4
	Thiabendazole	73	13	103	101	16	150	0.8	2.5	65	5	97	71	6	103	0.9	2.8
	Thiametosan	61	29	-40	107	14	-33	2.0	5.9	85	24	132	91	25	138	1.4	4.2
PhACs	5-methyl-1H-Benzotriazole	99	1	-35	78	2	-53	0.30	1.02	94	5	-18	109	9	-29	0.05	0.16
	Acetaminophen	97	1	-82	79	1	-88	0.68	2.25	94	3	-34	104	3	-45	0.18	0.62
	Acridone	113	0.2	-38	87	1.8	-45	0.45	1.51	96	2.5	-16	114	3.6	-35	0.07	0.23
	Alprazolam	98	3	-23	99	4	-46	0.18	0.59	97	1	-36	118	7	-37	0.06	0.20
	Amantadine	89	12	-66	52	1	-52	0.57	1.91	80	3	60	116	23	22	0.20	0.68
	Atenolol	87	5	-64	77	0	-70	0.16	0.53	93	4	-10	113	4	-30	0.05	0.15
	Atorvastatin	<20	-	-100	<20	-	-100	0.48	1.60	<20	-	-100	<20	-	-67	-	-
	Benzotriazole	86	1	-36	63	1	-53	1.04	3.46	90	6	1	114	6	-22	0.15	0.49
	Benzoylecgonine	102	1	-46	78	2	-55	0.69	2.30	102	6	-20	115	2	-28	0.05	0.17

CECs		*Procambarus clarkii*								*Anodonta cygnea*							
		10 ng g^{-1}			100 ng g^{-1}			LOD (ng g^{-1})	LOQ (ng g^{-1})	10 ng g^{-1}			100 ng g^{-1}			LOD (ng g^{-1})	LOQ (ng g^{-1})
		Recovery (%)	RSD (%)	Matrix Effect (%)	Recovery (%)	RSD (%)	Matrix Effect (%)			Recovery (%)	RSD (%)	Matrix Effect (%)	Recovery (%)	RSD (%)	Matrix Effect (%)		
PhACs	Bezafibrate	91	2	64	97	3	27	0.14	0.48	94	4	9	116	15	-20	0.06	0.20
	Bisphenol-A	85	5	-20	85	6	-40	0.16	0.55	87	8	11	118	3	-26	0.04	0.13
	Bromazepam	92	4	300	115	2	136	0.56	1.88	113	1	157	82	12	18	0.09	0.29
	Caffeine	83	4	-40	71	2	-57	0.61	2.02	72	5	-7	109	6	-31	0.09	0.31
	Carazolol	114	0.2	-86	118	11.1	-91	0.57	1.88	83	0.7	-48	120	0.7	-62	0.29	0.96
	Carbamazepine	97	1	-33	86	1	-45	0.23	0.77	101	7	-21	110	8	-32	0.11	0.38
	CBZ-10,11-epoxide	91	1	-10	73	0	-24	0.12	0.38	97	9	-9	93	18	-29	0.13	0.45
	Chloramphenicol	94	1	-23	76	5	-19	0.88	2.94	96	9	-2	117	12	-22	0.13	0.44
	Chlorpromazine	<20	-	-77	81	38	-92	0.31	1.02	43	25	-96	110	60	-86	0.09	0.31
	Ciprofloxacin	<20	36	-7	<20	4	-76	0.48	1.60	<20	-	-93	<20	9	-73	0.01	0.05
	Citalopram	82	8	-84	87	9	-88	0.30	1.00	36	9	-17	98	5	-68	0.30	1.01
	Clarithromycin	73	10	-66	103	6	-77	0.46	1.52	28	2	-68	83	17	-45	0.07	0.23
	Clofibric acid	93	2	28	96	2	-5	0.15	0.50	61	12	7	96	9	-23	0.14	0.45
	Coca-ethylene	113	15	-62	71	2	-74	0.65	2.16	107	16	-58	111	1	-46	0.04	0.12
	Cocaine	98	34	-56	57	11	-61	0.28	0.92	93	3	-41	118	4	-39	0.06	0.19
	Codeine	<20	-	-100	67	1	-65	0.30	1.01	103	6	-23	99	13	-27	0.05	0.18
	Cotinine	97	4	-63	69	1	-70	0.24	0.81	106	1	-51	117	3	-57	0.18	0.59
	Diazepam	93	1	-49	104	7	-58	0.18	0.59	178	4	-45	174	0.4	-47	0.35	1.16
	Diclofenac	95	13	-12	56	14	-13	0.16	0.53	93	4	4	99	12	-17	0.09	0.30
	Diltiazem	67	0.8	-78	78	4.4	-85	0.28	0.92	40	0.1	-16	97	8.5	-64	0.21	0.70
	Erythromycin	90	12	-54	92	1	-57	0.23	0.78	109	14	-53	114	15	-43	0.06	0.21
	Fenofibrate	<20	-	-	55	26	264	0.50	1.68	<20	-	-	<20	-	-100	-	-
	Fipronil	98	5	-43	98	9	-39	0.44	1.48	98	7	-26	116	7	-32	0.02	0.06
	Fipronil Desulfynil	99	9	-51	76	16	-69	0.29	0.98	101	9	-40	119	12	-45	0.01	0.02
	Fipronil Sulfide	92	11	-48	56	14	-14	0.75	2.50	107	11	-49	105	27	-42	-	-
	Fipronil Sulfone	91	9	-36	59	15	-2	0.19	0.63	118	15	-48	100	27	-37	1.66	5.52
	Fluconazole	61	5	10	78	1	-52	0.07	0.23	89	8	-26	98	2	-36	0.05	0.17
	Flumequine	<20	11	-4	<20	2	-51	0.53	1.76	82	7	-97	<20	11	-66	0.03	0.09
	Fluoxetine	143	32	-90	97	7	-93	0.33	1.10	70	20	-94	117	35	-88	0.16	0.55
	Furazolidone	67	14	-45	37	1	-46	0.28	0.95	99	7	-6	117	8	-16	0.14	0.47
	Furosemide	100	10	-26	89	6	-36	0.39	1.30	93	9	-14	114	17	-26	0.11	0.38
	Hydrochlorothiazide	89	16	-36	74	1	-43	0.20	0.66	101	8	-4	106	4	-22	0.12	0.40
	Indomethacin	102	1	-13	84	1	-16	0.37	1.24	108	1	-19	115	4	-27	0.15	0.50
	Ketoprofen	98	8	33	96	2	-3	0.48	1.61	93	5	3	108	18	-23	0.06	0.19
	Lamotrigine	93	16	-79	92	1	-84	0.10	0.34	101	3	-41	118	0	-45	0.04	0.15
	Loratadine	78	8	-62	104	7	-75	0.29	0.96	<20	53	-45	63	28	-82	0.50	1.68
	Lorazepam	99	10	1	89	9	-28	0.56	1.88	101	4	-44	116	5	-43	0.43	1.44
	Losartan	90	4	19	101	5	0	0.32	1.06	96	12	-54	93	5	-53	0.17	0.56
	Mefenamic acid	81	4	-19	104	3	-36	0.20	0.67	55	8	-35	54	4	-31	0.09	0.31
	Metformin	<20	-	-100	87	14	-98	0.72	2.39	100	15	-93	113	5	-91	0.09	0.31
	Methadone	75	20	-89	74	1	-91	0.52	1.74	45	11	-89	92	15	-74	0.42	1.40
	Metoprolol	97	2	-53	84	2	-63	0.19	0.62	100	2	-19	112	2	-34	0.18	0.60
	Midazolam	54	13	-82	76	19	-87	0.24	0.80	115	1	-86	118	10	-69	0.02	0.06
	Morphine	104	14	-89	75	3	-86	0.52	1.74	110	15	-63	116	4	-59	0.05	0.17
	N-acethyl SMX	115	4	-20	80	0	-21	0.27	0.90	107	6	-25	126	1	-26	0.09	0.29
	Nalidixic acid	<20	8	-22	<20	2	-56	0.39	1.29	44	1	-96	<20	0	-55	0.02	0.08
	N-Desmethylcitalopram	98	5	-98	110	9	-93	0.40	1.32	80	8	-73	120	8	-68	0.02	0.05
	Nicotine	<20	-	-100	47	1	-34	0.04	0.14	69	1	2	88	6	205	0.03	0.10

CECs		*Procambarus clarkii*								*Anodonta cygnea*							
		10 ng g^{-1}			100 ng g^{-1}			LOD (ng g^{-1})	LOQ (ng g^{-1})	10 ng g^{-1}			100 ng g^{-1}			LOD (ng g^{-1})	LOQ (ng g^{-1})
		Recovery (%)	RSD (%)	Matrix Effect (%)	Recovery (%)	RSD (%)	Matrix Effect (%)			Recovery (%)	RSD (%)	Matrix Effect (%)	Recovery (%)	RSD (%)	Matrix Effect (%)		
PhACs	Norfluoxetine	<20	-	-94	91	13	-93	0.50	1.67	79	37	-94	110	56	-85	0.17	0.57
	O-desmethylvenlafaxine	114	7	-56	71	2	-65	0.25	0.82	81	4	-22	107	16	-36	0.14	0.48
	Omeprazole	116	25	-49	160	6	-77	0.15	0.49	66	19	-84	35	11	-82	0.10	0.32
	Oseltamivir	102	6	-64	100	5	-67	0.39	1.31	91	2	-19	118	4	-40	0.10	0.32
	Oseltamivir-CBX	69	12	-17	69	2	-32	0.07	0.23	46	2	29	51	13	-27	0.07	0.22
	Oxazepam	96	12	32	79	1	-6	0.20	0.65	98	6	-43	118	8	-40	0.07	0.22
	Oxytetracycline	<20	-	17	<20	-	192	0.31	1.05	<20	-	-100	<20	-	83	-	-
	Paroxetine	<20	-	-92	94	6	-92	0.35	1.16	96	17	-90	83	37	-86	0.02	0.07
	Pentobarbital	91	3	-14	83	3	-13	0.92	3.07	97	7	6	113	6	-10	0.08	0.25
	Propyphenazone	100	2	-28	97	2	-48	0.04	0.12	97	8	-10	105	7	-23	0.12	0.39
	Quetiapine	71	18.1	-83	112	9.4	-87	0.27	0.90	101	0.1	-81	111	0.5	-71	0.01	0.03
	Salbutamol	97	6	-59	63	2	-61	0.19	0.64	76	2	-13	105	10	-25	0.14	0.47
	Salicylic acid	<LOQ	-	31	82	0.3	-33	6.66	22.19	20	8	18	<20	20	-18	0.16	0.52
	Sertraline	<20	-	-100	39	20	-84	0.15	0.50	<20	-	-100	44	48	-76	0.01	0.04
	Sitagliptin	99	4	-53	97	5	-64	0.34	1.13	94	5	-28	113	5	-38	0.10	0.33
	Sotalol	112	6	-67	77	1	-73	0.09	0.31	87	4	-14	114	5	-32	0.09	0.30
	Sulfadimethoxine	90	1	15	85	0	-9	0.15	0.50	99	7	-19	109	8	-35	0.23	0.76
	Sulfamethazine	98	2	-28	75	0	-33	0.39	1.30	99	20	-14	99	18	-32	0.05	0.18
	Sulfamethoxazole	98	2	-17	74	3	-36	0.30	1.00	103	13	-9	117	14	-26	0.07	0.25
	Sulfapyridine	103	5	-48	74	4	-47	0.28	0.94	72	11	-15	93	26	-29	0.11	0.36
	Temazepam	105	9	39	101	2	-7	0.19	0.65	100	3	-22	117	1	-18	0.04	0.14
	Tramadol	97	3	-48	85	1	-61	0.48	1.62	102	3	-12	86	2	-7	0.07	0.22
	Triclocarban	59	5	130	58	17	-84	0.67	2.24	<20	-	-100	34	54	99	0.81	2.69
	Trimethoprim	112	4	-69	86	2	-77	0.43	1.43	100	6	-28	114	31	-39	0.04	0.14
	Valsartan	101	14	114	87	5	70	0.18	0.58	93	19	-26	91	9	-43	0.06	0.21
	Valsartan acid	35	21	-43	67	2	-48	0.16	0.53	<20	-	-22	105	11	-38	0.16	0.52
	Venlafaxine	98	24	-63	81	9	-76	0.20	0.65	101	15	-48	111	3	-39	0.08	0.25
	Verapamil	95	25	-89	111	13	-91	0.32	1.07	44	2	-85	117	20	-72	0.18	0.62
	Warfarin	91	4	121	84	2	102	1.52	5.08	90	6	13	115	8	-7	0.09	0.30
	Zolpidem	117	8	-49	103	6	-60	0.43	1.44	117	15	-57	111	4	-45	0.03	0.09

Table S4. Validation parameters

CECs		*Leopomis gibbosus*								*Corbicula fluminea*							
		10 ng g^{-1}			100 ng g^{-1}			LOD (ng g^{-1})	LOQ (ng g^{-1})	10 ng g^{-1}			100 ng g^{-1}			LOD (ng g^{-1})	LOQ (ng g^{-1})
		Recovery (%)	RSD (%)	Matrix Effect (%)	Recovery (%)	RSD (%)	Matrix Effect (%)			Recovery (%)	RSD (%)	Matrix Effect (%)	Recovery (%)	RSD (%)	Matrix Effect (%)		
OPFRs	TPP	70	11	140	76	14	142	0.09	0.26	70	9	-82	76	11	-76	0.09	0.26
	TDBPP	142	7	126	148	9	128	0.14	0.42	137	10	-1	143	10	5	0.15	0.44
	CDP	<LOQ	-	-	71	35	-84	4.00	12.00	<LOQ	-	-	69	13	-78	4.00	12.00
	TnBP	97	9.1	125	103	11.1	127	0.06	0.19	127	28.0	-85	133	28.0	-79	0.05	0.14
	TBEP	100	17	133	106	20	135	0.06	0.18	133	36	-73	139	38	-67	0.05	0.14
	TMPP	98	22	72	104	24	74	0.06	0.18	66	11	-89	72	11	-83	0.09	0.27
	TEHP	104	31	113	110	34	115	0.19	0.58	44	34	-66	50	36	-60	0.45	1.36
	TCEP	104	9	136	110	11	138	0.06	0.17	107	3	173	113	3	179	0.06	0.17
	TClPP	99	12	54	105	15	56	0.41	1.22	107	5	-40	113	7	-34	0.37	1.12
	TDClPP	83	38	-80	89	40	-78	0.24	0.72	107	3	-90	113	3	-84	0.19	0.56
	TPhP	105	2	52	111	5	54	0.06	0.17	107	5	41	113	7	47	0.06	0.17
PFAS	PFPeA	92	22	79	98	28	83	2.0	5.9	71	33	174	77	32	176	2.5	7.6
	PFBS	116	29	-59	122	34	-55	0.2	0.6	48	31	-43	54	30	-41	0.4	1.1
	PFHpA	72	6	2	78	12	6	0.4	1.2	60	26	36	66	25	38	0.5	1.5
	PFHpS	85	9	-28	91	14	-24	0.1	0.2	28	16	-54	34	15	-52	0.2	0.6
	ipPFNA	74	10	-53	80	16	-49	0.8	2.4	46	33	-39	52	32	-37	1.3	3.9
	FOUEA	87	26	-44	93	31	-40	1.2	3.6	77	3	-41	83	4	-39	1.0	3.0
	ipPFN	50	-	-95	56	18	-50	0.1	0.4	<LOQ	-	-88	78	11	-56	4.0	12.0
	PFDS	108	4	-88	114	9	-64	1.3	3.8	99	4	-30	105	5	-48	1.4	4.1
	PFTrDA	100	2	-83	106	4	-49	0.04	0.1	102	3	-35	108	4	-43	0.0	0.1
	PFTeDA	<LOQ	-	-	86	10	0.10	4.5	13.5	<LOQ	-	-85	89	-	-83	4.5	13.5
	PFHxDA	<LOQ	-	-	85	12	-55	4.3	12.9	<LOQ	-	-90	72	-	-88	4.3	12.9
	PFODA	107	7	-73	113	2	-69	1.6	4.9	106	4	-75	112	5	-73	1.7	5.0
	PFBA	85	16	50	91	22	54	1.1	3.2	71	31	133	77	30	135	1.3	3.8
	PFHxA	82	-	25	88	5	29	1.2	3.6	63	16	59	69	15	61	1.6	4.8
	PFHxS	77	9	-13	83	15	-9	0.01	0.0	61	18	-6	67	17	-4	0.0	0.0
	PFOA	90	26	51	96	31	55	0.1	0.2	90	11	72	96	10	74	0.1	0.2
	PFOS	52	35	-58	58	41	-54	0.6	1.9	38	12	-74	44	11	-72	0.8	2.5
	PFNA	69	13	-54	75	18	-50	1.2	3.5	54	17	-46	60	16	-44	1.5	4.5
	PFDA	97	5	-9	103	11	-5	0.1	0.2	100	2	-11	106	1	-9	0.1	0.2
	PFUnDA	77	7	-86	83	12	-62	0.1	0.2	63	3	-58	69	2	-86	0.1	0.3
	PFDoDA	<LOQ	-	-87	82	10.0	-83	4.3	12.9	<LOQ	-	-89	75	-	-87	4.0	12.0
PESTICIDES	Acetamiprid	118	28	117	104	28	123	2.3	7.0	41	21	-13	57	28	-7	2.4	7.2
	Alachlor	99	10	152	105	17	158	5.2	15.7	46	18	121	62	8	127	2.6	7.8
	Atrazine	114	63	10	120	23	16	0.8	2.4	48	32	-44	54	39	-38	2.9	8.7
	Atrazine-Deethyl	116	4.5	32	102	11.5	38	0.7	2.0	58	17.4	-17	64	7.4	-11	3.2	9.5
	Atrazine-Deisopropryl	119	32	7	125	22	13	0.8	2.3	57	26	-22	53	33	-16	1.9	5.8
	Azinphos-ethyl	<LOQ	-	-	<20	-	-70	5.0	15.0	107	25	-73	113	15	-67	0.4	1.2
	Azinphos-Methyl	112	4	148	118	4	154	0.9	2.7	<LOQ	-	-	95	-	-69	4.0	12.0
	Buprofezine	<LOQ	-	-	<20	-	-68	5.2	15.6	<LOQ	-	-	74	-	-83	5.2	15.6
	Carbendazim	125	4	35	101	4	41	0.3	0.9	48	12	8	54	19	14	0.8	2.3
	Carbofuran	79	12	74	85	19	80	0.8	2.3	<LOQ	-	-	72	-	-54	4.0	12.0
	Carbofuran 3-OH	113	3	147	119	3	153	0.8	2.4	59	25	124	65	32	130	1.5	4.6
	Chlorfenvinphos	76	-	148	82	15	154	2.1	6.4	147	18	-57	103	8	-51	1.1	3.3
	Chlorpyriphos	44	25	-51	50	25	-45	3.7	11.2	75	19	41	81	26	47	2.2	6.5
	Chlothianidin	86	12	103	92	19	109	1.9	5.7	69	12	70	75	2	76	2.4	7.1
	Coumaphos	108	14	-62	104	14	-56	0.6	1.8	64	4	-83	70	11	-77	1.4	4.2
PESTICIDES	Diazinon	<LOQ	-	-	74	10	-67	3.8	11.4	80	18	-20	86	8	-44	1.1	3.4
	Dichlofenthion	<LOQ	-	-	72	10	-86	5.0	15.0	<LOQ	-	-	91	12	-71	5.0	15.0
	Dimethoate	100	15	-34	106	22	-28	0.4	1.2	<LOQ	-	-	82	10	-72	4.1	12.3
	Diuron	<LOQ	-	-	82	12	-71	4.1	12.3	<LOQ	-	-	74	10	-69	4.8	14.4
	DMA	<LOQ	-	-	80	11	-77	4.8	14.4	125	20	-87	102	20	-81	2.9	8.6
	DMF	36	9	-64	42	9	-58	1.0	3.1	117	2	-39	103	9	-33	2.4	7.3
	DMPF	112	6	40	118	13	46	3.2	9.7	112	21	4	107	21	10	2.5	7.6
	Ethion	96	3	26	102	3	32	0.4	1.1	102	18	-79	102	15	-73	0.3	0.8
	Etofenprox	<LOQ	-	-	75	14	-86	3.7	11.1	<LOQ	-	-	78	20	-71	3.7	11.1
	Fenthion	<LOQ	-	-	104	11	-84	4.5	14.5	52	15	-46	58	12	-40	0.7	2.1
	Fenthion sulfone	104	5	148	110	12	154	0.3	1.0	75	22	22	81	12	28	0.5	1.4
	Fenthion sulfoxide	107	4	142	103	4	148	0.4	1.2	46	19	16	52	25	22	1.1	3.2
	Fipronil	100	7	147	106	14	153	0.0	0.1	107	7	-85	103	3	-79	0.0	0.1
	Hexythiazox	54	11	-83	60	11	-77	0.9	2.8	124	16	-90	100	13	-84	0.4	1.2
	Imazalil	<LOQ	-	-	78	15	-71	4.5	13.5	106	5	-90	92	5	-84	0.5	1.4
	Imidacloprid	83	7	147	89	7	153	1.1	3.2	80	7	93	86	14	99	1.1	3.4
	Isoproturon	124	5	148	120	12	154	0.4	1.2	83	14	64	89	14	70	0.6	1.8
	Methiocarb	63	16	199	69	23	205	0.9	2.8	85	7	61	91	3	67	0.7	2.1
	Methoalachlor	107	7	39	113	7	45	0.6	1.7	31	25	-33	37	12	-27	1.9	5.8
	Molinate	103	15	-68	109	22	-62	0.4	1.1	68	9	141	74	1	147	0.9	2.7
	Omethoate	<LOQ	-	-	72	17	-78	5.0	15.0	69	4	-28	75	11	-52	2.4	7.2
	Parathion-ethyl	<LOQ	-	-	79	16	-82	5.0	15.0	<LOQ	-	-	91	25	-59	5.0	15.0
	Prochloraz	124	12	-42	100	18	-66	2.8	8.4	115	21	-90	121	18	-84	1.5	4.4
	Propanil	<LOQ	-	-	<20	14	-73	4.0	12.0	<LOQ	-	-	72	24	-76	4.0	12.0
	Propazine	120	17	19	106	17	25	0.4	1.2	78	17	21	84	24	27	0.6	1.9
	Pyriproxyfen	<LOQ	-	-	<20	15	-59	3.5	10.5	<LOQ	-	-	70	12	-86	3.5	10.5
	Simazine	113	6	-17	119	6	-11	0.5	1.6	55	4	-39	61	11	-33	1.1	3.3
	Spinosad A	63	16	148	69	23	154	0.9	2.8	31	10	-46	67	13	-40	1.9	5.8
	Spinosad C	102	12	136	108	12	142	0.6	1.8	19	19	-67	55	16	-61	3.1	9.3
	Spinosad D	65	10	144	71	17	150	0.9	2.8	107	6	-90	113	4	-84	0.6	1.7
	Tebuconazole	57	7	138	63	7	144	1.1	3.2	70	7	52	76	14	58	0.9	2.6
	Terbumeton	93	7	134	99	14	140	0.1	0.2	70	21	37	76	18	43	0.1	0.3
	Terbumeton deethyl	91	16	141	97	16	147	0.1	0.2	70	1	153	76	6	159	0.1	0.3
	Terbuthylazine	121	12	-28	107	19	-22	0.0	0.1	80	21	-1	86	11	5	0.1	0.2
	Terbuthylazine-2OH	105	7	82	111	7	88	0.6	1.7	111	3	-29	117	4	-23	0.5	1.6
	Terbuthylazine-deethyl	98	4	46	104	11	52	0.6	1.8	77	11	127	83	1	133	0.8	2.3
	Terbutryn	125	7	-23	101	7	-17	0.5	1.4	77	23	-13	83	20	-7	0.8	2.3
	Thiabendazole	80	15	147	86	22	153	0.8	2.3	78	6	132	84	4	138	0.8	2.3
	Thiametosan	92	15	-18	98	15	-12	1.3	3.9	79	15	66	85	22	72	1.5	4.6
PhACs	5-methyl-1H-Benzotriazole	92	13	-39	94	10	-43	0.06	0.19	88	6	-39	92	5	-36	0.36	1.21
	Acetaminophen	69	10	-24	82	17	-43	0.17	0.57	87	5	-22	96	1	-30	0.42	1.42
	Acridone	101	11.5	-25	92	8.5	-41	0.07	0.24	93	2.8	-15	100	4.2	-18	0.25	0.83
	Alprazolam	96	22	10	97	10	-24	0.29	0.96	112	8	-12	96	15	-3	0.42	1.39
	Amantadine	108	17	77	109	8	144	0.21	0.69	120	8	96	117	4	265	0.28	0.95
	Atenolol	97	27	-5	99	7	-30	0.09	0.29	100	5	3	100	4	-23	0.08	0.28
	Atorvastatin	<20	-	-100	<20	-	-77	-	-	<20	-	-	<20	-	-	-	-
	Benzotriazole	98	14	-33	100	9	-40	0.60	2.01	90	7	-32	101	3	-32	0.18	0.60
	Benzoylecgonine	43	7	21	100	9	-7	0.34	1.13	78	3	14	117	2	2	0.33	1.10

CECs		*Leopomis gibbosus*								*Corbicula fluminea*							
		10 ng g^{-1}			100 ng g^{-1}			LOD (ng g^{-1})	LOQ (ng g^{-1})	10 ng g^{-1}			100 ng g^{-1}			LOD (ng g^{-1})	LOQ (ng g^{-1})
		Recovery (%)	RSD (%)	Matrix Effect (%)	Recovery (%)	RSD (%)	Matrix Effect (%)			Recovery (%)	RSD (%)	Matrix Effect (%)	Recovery (%)	RSD (%)	Matrix Effect (%)		
PhACs	Bezafibrate	99	21	14	98	6	-13	0.22	0.74	99	3	11	102	3	8	0.15	0.51
	Bisphenol-A	110	12	-42	97	17	-53	0.29	0.96	106	5	-31	99	9	-39	0.17	0.56
	Bromazepam	71	1	47	97	7	-2	0.44	1.48	100	11	-7	93	9	0	0.80	2.66
	Caffeine	86	16	44	97	7	15	0.41	1.37	92	7	60	100	4	32	0.30	1.01
	Carazolol	77	17.8	44	81	17.9	-4	0.10	0.34	87	10.0	92	87	18.1	48	0.29	0.97
	Carbamazepine	97	18	-1	101	7	-31	0.05	0.18	88	6	4	102	5	-11	0.30	0.99
	CBZ-10,11-epoxide	82	2	52	104	7	8	0.29	0.97	90	5	28	109	4	1	0.08	0.28
	Chloramphenicol	110	20	-1	118	10	-12	0.54	1.80	95	5	1	118	3	-15	0.25	0.83
	Chlorpromazine	86	7	300	81	5	213	0.16	0.54	100	16	300	97	20	300	0.10	0.33
	Ciprofloxacin	<20	-	-92	<20	20	-13	0.48	1.60	<20	-	31	<20	-	25	0.14	0.48
	Citalopram	119	19	-45	98	2	-40	0.49	1.65	89	10	-8	92	8	-7	0.13	0.42
	Clarithromycin	78	15	-45	80	14	-51	0.61	2.03	84	10	-11	80	18	-9	0.22	0.74
	Clofibric acid	89	19	-8	109	12	-10	0.28	0.92	94	4	-7	108	5	-5	0.19	0.64
	Coca-ethylene	117	5	-34	111	12	-31	0.40	1.32	97	10	-9	94	17	-9	0.33	1.09
	Cocaine	100	11	-16	98	6	-32	0.23	0.76	96	7	7	99	7	0	0.24	0.78
	Codeine	92	18	-23	100	8	-42	0.13	0.42	96	6	-41	98	4	-46	0.15	0.51
	Cotinine	78	12	-17	81	7	-34	0.25	0.84	91	5	-43	98	6	-44	0.16	0.54
	Diazepam	107	20	-2	110	11	-49	0.23	0.75	100	9	-13	87	20	-26	0.08	0.28
	Diclofenac	<20	-	-49	93	16	-45	0.13	0.43	83	16	-45	98	17	-35	0.16	0.54
	Diltiazem	91	12.8	48	89	3.6	-39	0.28	0.94	76	12.1	151	87	6.4	-9	0.06	0.19
	Erythromycin	97	28	-57	98	5	-66	0.05	0.16	95	5	-43	104	4	-34	0.21	0.70
	Fenofibrate	<20	-	80	99	3	5	0.49	1.63	<20	-	-100	103	1	119	0.32	1.07
	Fipronil	74	4	-29	99	1	61	0.28	0.93	78	5	1	100	9	150	0.13	0.42
	Fipronil Desulfynil	58	12	-12	119	7	-56	0.48	1.60	96	7	-13	105	3	-27	0.27	0.89
	Fipronil Sulfide	49	17	-31	117	0	-51	0.13	0.44	60	20	138	112	6	12	0.48	1.59
	Fipronil Sulfone	49	14	-31	107	1	-24	0.44	1.47	66	2	156	128	22	53	0.19	0.64
	Fluconazole	99	23	-7	98	7	-28	0.14	0.46	93	4	-8	99	6	-16	0.18	0.61
	Flumequine	54	11	-76	29	21	-25	0.54	1.80	28	7	-4	42	13	3	0.46	1.53
	Fluoxetine	101	9	300	102	21	175	0.12	0.40	95	7	300	102	7	293	0.19	0.65
	Furazolidone	62	3	300	73	5	-46	0.40	1.33	46	18	300	52	9	-17	0.07	0.23
	Furosemide	83	35	-39	89	19	-43	0.19	0.63	<20	-	-100	<20	-	-87	0.31	1.02
	Hydrochlorothiazide	65	16	-14	94	13	-24	0.24	0.81	80	8	-16	96	2	-21	0.06	0.20
	Indomethacin	<20	-	62	88	1	-62	0.28	0.93	<20	-	-44	109	12	-65	0.24	0.82
	Ketoprofen	93	2	0	95	6	-30	0.25	0.84	104	7	-4	103	2	-9	0.26	0.86
	Lamotrigine	116	2	-64	107	13	-70	0.43	1.44	96	10	-78	116	17	-74	0.23	0.78
	Loratadine	68	3	77	63	14	-7	0.51	1.70	62	9	70	62	6	27	0.95	3.15
	Lorazepam	71	9	39	101	16	-8	0.21	0.71	85	7	18	102	15	4	0.41	1.37
	Losartan	98	18	-78	74	12	-68	0.30	1.01	94	13	-94	89	2	-84	1.37	4.56
	Mefenamic acid	106	23	-65	115	18	-78	0.25	0.84	57	7	-54	98	15	-66	0.50	1.66
	Metformin	<20	-	-100	39	14	-98	0.25	0.84	<20	11	-94	92	3	-98	0.31	1.02
	Methadone	92	15	7	97	2	25	0.59	1.96	75	13	92	83	9	77	0.09	0.29
	Metoprolol	86	10	-2	98	8	-21	0.11	0.37	91	6	2	97	5	-2	0.14	0.46
	Midazolam	97	20	-61	86	3	-59	0.08	0.28	110	13	-71	104	19	-63	0.44	1.46
	Morphine	<20	-	-54	72	58	-66	0.26	0.86	<20	-	-83	80	2	-63	0.10	0.33
	N-acethyl SMX	92	20	10	105	11	-4	0.56	1.85	115	8	-2	118	7	-3	0.71	2.36
	Nalidixic acid	35	1	-79	<20	13	-36	0.76	2.53	<20	6	-22	32	6	-16	0.09	0.29
	N-Desmethylcitalopram	101	26	-48	88	2	-38	0.68	2.27	118	0	-28	92	0	-7	0.47	1.58
	Nicotine	<20	-	75	<20	6	300	0.52	1.74	47	9	66	110	5	300	0.21	0.70

CECs		*Leopomis gibbosus*								*Corbicula fluminea*							
		10 ng g^{-1}			100 ng g^{-1}			LOD (ng g^{-1})	LOQ (ng g^{-1})	10 ng g^{-1}			100 ng g^{-1}			LOD (ng g^{-1})	LOQ (ng g^{-1})
		Recovery (%)	RSD (%)	Matrix Effect (%)	Recovery (%)	RSD (%)	Matrix Effect (%)			Recovery (%)	RSD (%)	Matrix Effect (%)	Recovery (%)	RSD (%)	Matrix Effect (%)		
PhACs	Norfluoxetine	<20	-	221	99	13	97	0.13	0.43	85	11	300	98	12	286	0.13	0.45
	O-desmethylvenlafaxine	65	13	-24	91	6	-39	0.30	1.01	91	5	-16	114	4	-22	0.25	0.85
	Omeprazole	<20	-	-100	<20	-	-99	-	-	<20	-	-100	<20	-	-100	-	-
	Oseltamivir	73	0.2	50	103	12	1	0.18	0.60	101	6	32	97	8	15	0.13	0.44
	Oseltamivir-CBX	<20	-	12	51	14	-15	0.62	2.08	59	8	-4	76	2	-13	0.07	0.22
	Oxazepam	107	14	39	103	11	-12	0.51	1.71	91	6	11	100	14	-2	0.22	0.72
	Oxytetracycline	<20	-	-100	<20	-	-25	0.97	3.25	<20	-	-10	<20	-	5	0.54	1.80
	Paroxetine	103	12	267	109	6	82	0.12	0.38	111	8	231	95	15	160	0.16	0.53
	Pentobarbital	90	7	-25	100	7	-34	0.18	0.59	98	5	-13	101	3	-18	0.19	0.64
	Propyphenazone	93	15	18	97	6	-28	0.18	0.58	86	3	30	98	4	9	0.06	0.21
	Quetiapine	94	23.3	-48	99	8.7	-22	0.05	0.16	96	11.3	-19	100	2.8	11	0.05	0.16
	Salbutamol	64	33	-22	88	9	-35	0.24	0.81	76	3	-26	92	4	-29	0.44	1.45
	Salicylic acid	90	18	33	75	3	-13	0.47	1.56	90	16	89	79	8	8	0.34	1.12
	Sertraline	74	7	300	80	8	299	0.04	0.12	95	10	300	100	9	300	0.07	0.24
	Sitagliptin	73	21	-18	97	11	-30	0.07	0.23	92	7	-7	101	5	-8	0.10	0.32
	Sotalol	103	14	-16	96	7	-31	0.17	0.56	92	8	-12	99	5	-25	0.13	0.43
	Sulfadimethoxine	89	20	24	72	11	-4	0.13	0.44	79	10	-23	91	10	-26	0.62	2.07
	Sulfamethazine	51	9	34	101	14	-3	0.14	0.47	82	13	-1	99	14	-9	0.18	0.59
	Sulfamethoxazole	93	19	9	99	10	-15	0.09	0.32	86	7	12	107	10	-6	0.12	0.39
	Sulfapyridine	102	10	12	111	19	10	0.35	1.15	118	11	13	114	10	6	0.27	0.91
	Temazepam	89	21	60	97	10	2	0.13	0.44	95	5	60	104	8	33	0.09	0.30
	Tramadol	77	6	77	99	10	80	0.10	0.33	87	10	87	99	5	139	0.16	0.53
	Triclocarban	<20	-	201	104	7	300	1.47	4.91	53	5	300	118	7	300	0.43	1.43
	Trimethoprim	86	17	-34	97	10	-50	0.21	0.69	93	6	-34	96	4	-38	0.18	0.58
	Valsartan	67	27	-24	95	8	-35	0.09	0.31	94	7	-63	99	6	-60	0.26	0.85
	Valsartan acid	<20	-	-74	99	3	-69	0.43	1.42	<20	-	-76	109	14	-70	0.32	1.08
	Venlafaxine	95	18	6	84	12	0.5	0.09	0.31	92	4	35	100	13	32	0.10	0.34
	Verapamil	114	21	-44	90	20	-39	0.65	2.16	106	11	18	105	7	12	0.15	0.50
	Warfarin	99	16	209	100	12	109	0.28	0.93	90	5	160	101	15	64	0.42	1.41
	Zolpidem	101	14	-48	79	18	-41	0.33	1.10	90	15	-42	100	1	-31	0.71	2.37

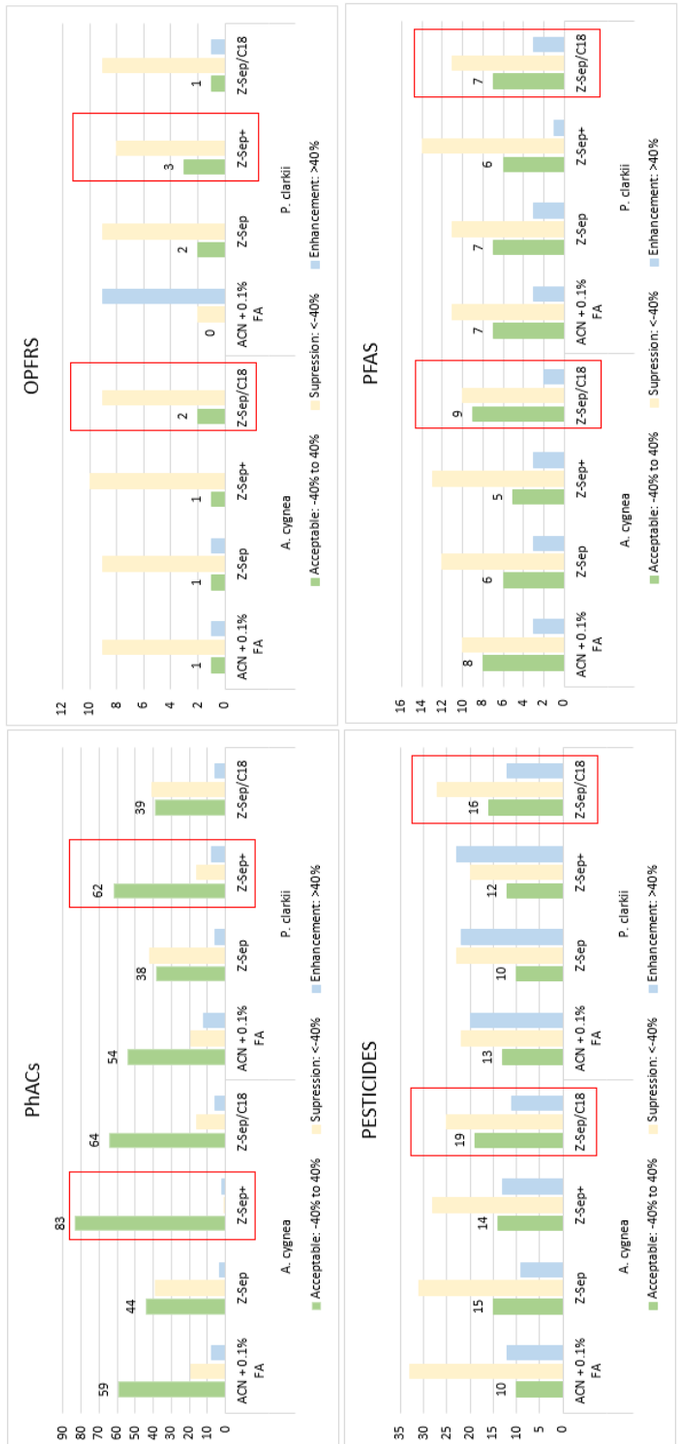

Fig. S1. Matrix effect before and after the clean-up step

3.3. Discusión

En esta sección se presenta una discusión general de los principales resultados tras la validación de los métodos analíticos desarrollados en esta tesis. En la Tabla 3.3 se resumen los parámetros de validación obtenidos por matriz y tipo de compuesto. Se observa que los valores de LOD y LOQ varían entre las diferentes matrices y especies. Por ejemplo, en los peces, el LOD y LOQ para los PhACs en el músculo tienen un rango de 0.01 a 30.9 ng g^{-1} y de 0.02 a 49.7 ng g^{-1} en el riñón, respectivamente. En comparación, los cangrejos de río y almejas tienen menor variabilidad y tienden a ser más bajos, indicando que las matrices pueden influir en la sensibilidad de detección. Los resultados del riñón pueden asociarse a que se trata de una matriz compleja, en la que el contenido de sangre puede interferir durante la extracción y posterior análisis.

La variabilidad entre los LOD y LOQ no solo está relacionada con la matriz y la especie, sino también con el tipo de compuesto. Los PFAS presentaron valores de LOD y LOQ más bajos en comparación con otros compuestos como los plaguicidas u OPFRs en la misma matriz. Así mismo el EM varió en función de la matriz y tipo de compuesto. Por ejemplo, en los peces, el efecto matriz para los PhACs en el corazón osciló entre -98% y 707%, mientras que, en la almeja asiática, el efecto matriz para los PFAS estuvo en el rango de -90% a 176%. Se podría deducir que las matrices que presentaron mayores interferencias, aun aplicando el paso de limpieza fueron el corazón e hígado, ya que presentaron un mayor número de compuestos con supresión de señal o con un aumento muy alto.

Cuatro de los métodos analíticos revisados e incluidos en la sección 3.1, también usaron como principio la combinación de la extracción basada en UAE y la técnica de limpieza con d-SPE. Se observó que esta combinación se ha realizado principalmente para el análisis de PhACs en músculo de peces (Mu et al., 2022; Peña-Herrera et al., 2020), para OPFRs en diferentes órganos de peces (Tang et al., 2019) y para el análisis de disruptores endocrinos, incluidos algunos compuestos de uso industrial como los bisfenoles en mejillones (García-Fernández et al., 2022). Sin embargo, el uso de esta combinación es limitada para el análisis de un mayor número de compuestos y matrices, como se ha abordado en las publicaciones 1 y 2.

Tabla 3.3. *Resumen de los parámetros de validación de los métodos desarrollados en esta tesis por matriz y tipo de CECs.*

Especie	Matriz	CECs	R%	LOD (ng g^{-1})	LOQ (ng g^{-1})	ME%
Peces	Músculo	PhACs	<20-105	0.01-1.56	0.02-30.9	-97-545
	Corazón		24-86	0.0004-3.85	0.001-12.8	-98-707
	Piel		<20-143	0.004-3.46	0.01-11.5	-100-298
	Hígado		<20-145	0.01-2.66	0.03-8.87	-98-415
	Riñón		21-140	0.01-14.9	0.02-49.7	-98-182
	Cerebro		<20-139	0.004-14.1	0.01-47	-99-155
	Percasol (entero)	PhACs	29-119	0.04-1.47	0.12-4.91	-100-300
		Plaguicidas	36-125	0.04-5.24	0.1-15.7	-86-205
		PFAS	50-122	0.01-1.97	0.02-5.90	-95-83
		OPFRs	70-148	0.06-4.00	0.17-12.0	-84-142
Crustáceos	Cangrejo de río rojo	PhACs	35-160	0.04-6.66	0.12-22.2	-100-300
		Plaguicidas	33-127	0.08-5.00	0.24-15.0	-92-212
		PFAS	38-136	0.01-2.89	0.02-8.67	-63-146
		OPFRs	48-151	0.04-4.00	0.12-12.0	-86-154
Bivalvos	Petxinot	PhACs	20-178	0.01-1.66	0.02-5.52	-100-205
		Plaguicidas	38-152	0.05-5.20	0.16-15.6	-91-152
		PFAS	70-109	0.01-2.40	0.02-7.21	-88-196
		OPFRs	28-144	0.04-4.00	0.13-12.0	-92-173
	Almeja Asiática	PhACs	28-128	0.05-1.37	0.16-4.56	-100-300
		Plaguicidas	19-147	0.04-5.20	0.12-15.6	-90-159
		PFAS	28-112	0.01-2.52	0.03-7.56	-90-176
		OPFRs	44-143	0.05-4.00	0.14-12.0	-90-179

R%: Porcentaje de recuperación

Tras realizar una comparación con los métodos propuestos por Peña-Herrera et al., (2020) y Mu et al., (2022), se observó que existe una variabilidad en la eficiencia de recuperación. Los estudios de Mu et al., (2022) y Peña-Herrera et al., (2020) mostraron un rango más amplio en las recuperaciones en contraste con los resultados presentados en la Publicación No. 1. Mientras que Mu et al., (2022) reportó recuperaciones entre 81% y 115% y Peña-Herrera et al., (2020) obtuvo recuperaciones entre 40% y 196%, nuestro método mostró recuperaciones entre 20% y 105%. Esta variabilidad podría estar relacionada con que el método de extracción desarrollado en la Publicación No.1 incluyó muchos más compuestos y por tanto abordó un rango de polaridades más amplio.

Respecto a la publicación No. 2, tan solo García-Fernández et al., (2022) aplicó la combinación de UAE y limpieza con d-SPE para el análisis de PhACs en mejillones, por lo cual solo se realiza la comparación de nuestros resultados con este método. En este caso, el método desarrollado por nosotros obtuvo LODs y LOQs en un rango más bajo (entre 0.05-1.37 ng g^{-1} y 0.16-4.56 ng g^{-1} respectivamente) en comparación

con los obtenidos por García-Fernández et al., (2022) (entre 0.2-1.5 ng g^{-1} y 1.0-4.0 ng g^{-1} respectivamente). Estos resultados podrían estar asociados al disolvente usado, en su caso, una mezcla de acetonitrilo con metanol, que podría haber afectado la recuperación de compuestos con un log K_{ow} más alto o con mayor cantidad de grasas extraídas debido a la presencia de metanol en la extracción con disolvente.

Aunque los métodos presentados en las Publicaciones 1 y 2 permitieron la recuperación de un amplio rango de analitos, algunos PhACs no fueron recuperados en ninguna de las matrices validadas (oxitetraciclina y ciprofloxacina). Estas tasas nulas de recuperación podrían estar relacionadas con la polaridad y degradabilidad de estos compuestos. Por ejemplo, la oxitetraciclina es el compuesto más polar del total de compuestos incluidos en la tesis, con un valor de log K_{ow} de -4.87 y de 0.28 para la ciprofloxacina. Durante las pruebas de disolventes y Z-Sep de limpieza, estos compuestos fueron recuperados con una eficiencia del 58% y 65% respectivamente en el petxinot, haciendo uso del disolvente que contenía una mezcla de agua/metanol, que claramente es a fin a su polaridad. No obstante, no se pudieron recuperar con el método finalmente optimizado, debido a que el disolvente elegido fue acetonitrilo acidificado, que obtuvo eficiencias de recuperación mayores para compuestos de diferente polaridad. Se han reportado recuperaciones mayores para estos compuestos en tejidos de peces, usando PLE y otros disolventes (Carmona et al., 2017; Lee et al., 2020).

Las recuperaciones obtenidas para estos compuestos utilizando diferentes disolventes de extracción en combinación con otras técnicas de limpieza evidencia que, en nuestro caso, la elección del disolvente de extracción implicó la pérdida de algunos analitos, con el objetivo de lograr abarcar un mayor número de familias químicas en un solo procedimiento. No obstante, para futuras aplicaciones del método en análisis dirigidos de rutina, la Tabla TS3 del material suplementario de la Publicación No. 2, constituye una excelente guía para elegir el disolvente más adecuado para una amplia lista de compuestos, lo cual puede ser de gran utilidad.

Teniendo en cuenta que el paso de limpieza fue una etapa crítica durante el desarrollo de los métodos analíticos publicados, a continuación se profundiza un poco más en este tema, debido a que posterior a la limpieza de los extractos se puede generar la pérdida de analitos. Tras las diferentes pruebas realizadas en la Publicación No. 2, se decidió hacer uso del acetonitrilo acidificado como disolvente durante la UAE, y del Z-Sep C_{18} para la limpieza de los extractos. En la Figura

3.3 se muestran los cambios en las eficiencias de recuperación obtenidas antes y después de aplicar el paso de limpieza.

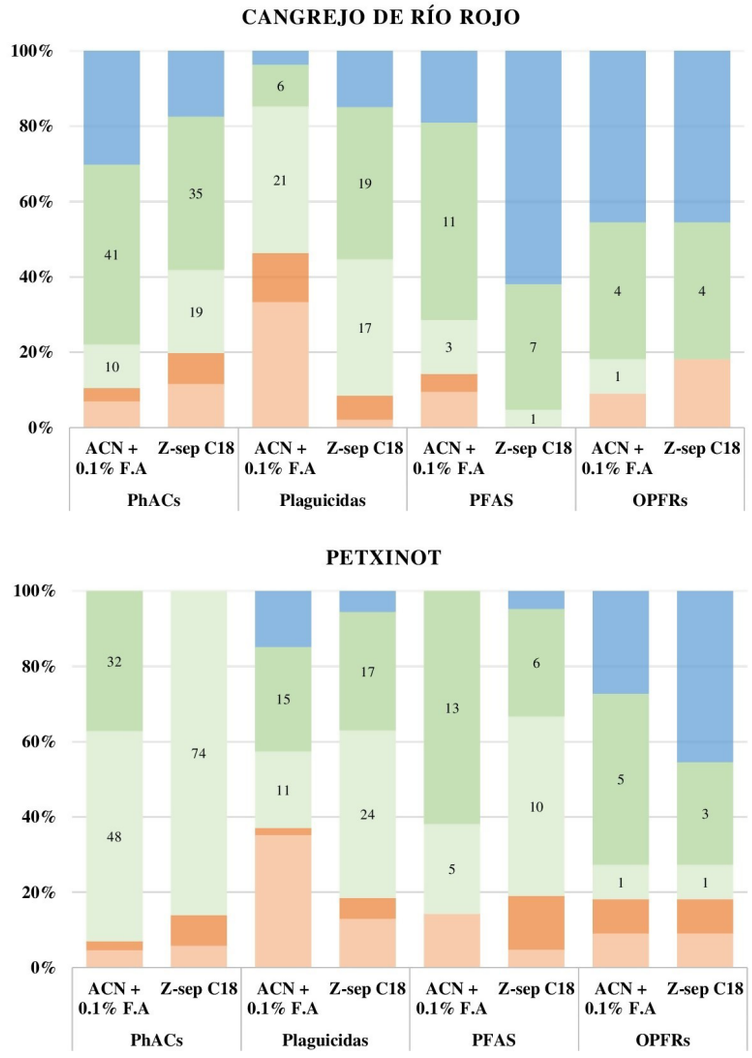

Figura 3.3. *Eficiencias de recuperación por grupo de compuestos antes y después de aplicar el paso de limpieza con Z-Sep C_{18}. Los números dentro de las franjas indican la cantidad de compuestos obtenidos dentro de ese rango de recuperación especifico.*

Los resultados obtenidos para el cangrejo de río rojo mostraron que en el caso de los PhACs se redujeron los porcentajes de recuperación, incrementando en un 10% el número de compuestos con recuperaciones por debajo del 40%. Así mismo, antes de la limpieza se lograron recuperaciones óptimas entre el 70 y 100% para 41 compuestos, mientras que tras la aplicación del Z-Sep, el número de compuestos

dentro de este rango se redujo a 35. Esto mismo ocurrió en el petxinot, donde inicialmente se recuperaron 32 PhACs con una eficiencia de extracción óptima, mientras que tras el paso de limpieza se redujo su recuperación, descendiendo al rango entre 40 y 70%. Un comportamiento similar se presentó para los OPFRs en las dos matrices de prueba. Sin embargo, se decidió mantener la fase de limpieza debido a que, pese a verse reducida la eficiencia de recuperación para algunos compuestos, estos se siguen manteniendo dentro de un rango aceptable y además la limpieza garantizó la estabilidad del equipo durante la adquisición de datos, manteniendo limpia la fuente de ionización y disminuyendo las interrupciones durante la secuencia.

Para los PFAS, la aplicación del Z-Sep tuvo los mismos efectos que para los PhACs en el petxinot, pero para el cangrejo de río se redujo el número de compuestos en un rango de recuperación entre 70 y 100% de 19 a 11, debido a que sus recuperaciones fueron mayores a 100%, lo cual se relaciona a que en este caso específico el adsorbente generó un efecto matriz aumentando la señal de los analitos.

Finalmente, los plaguicidas fueron el único grupo de compuestos que vieron incrementada la eficiencia de recuperación tras el paso de limpieza. El porcentaje de compuestos con recuperaciones inferiores al 40% se redujo del 46% al 7% en el cangrejo de río rojo y del 37% al 18% en el petxinot. El número de compuestos con recuperaciones entre 70 y 100% paso de 6 a 19 en el cangrejo de río rojo y de 15 a 17 para el petxinot, y para el rango entre 40 y 70% el número de compuestos incremento de 11 a 24.

La reducción de las eficiencias de extracción tras la aplicación de d-SPE es un problema ya conocido, por lo cual se han reportado varias estrategias para superarlo, incluidos pasos adicionales de limpieza antes o después de su aplicación (Tabla 3.2). Sin embargo, esto podría comprometer significativamente la simplicidad del método en términos de costo y tiempo. No obstante, los métodos aquí publicados se optimizaron para lograr una recuperación eficiente de diversos compuestos pertenecientes a diferentes familias químicas, en diversidad de matrices, obteniendo una alta sensibilidad, reproducibilidad y aplicabilidad.

Como oportunidad de mejora, que permita ampliar el alcance de los métodos desarrollados, se propone profundizar en la investigación de estrategias o técnicas de limpieza que no afecten la recuperación de lo analitos más polares en matrices

biológicas, y que sean compatibles con los disolventes de extracción utilizados. A modo de resumen, en la Fig. 3.4 se presenta un análisis FODA en donde se resaltan las principales características de los dos métodos publicados.

Fig. 3.4. *Análisis FODA de los métodos desarrollados.*

Una vez validados los métodos, se evaluó su aplicabilidad en muestras reales. En el caso de la Publicación No. 1 en muestras de órganos de cuatro peces salvajes capturados en el Río Llobregat (Barcelona, España) y en el caso de la Publicación No. 2 en muestras de especies invasoras recolectadas en el Parque Natural de L'Albufera (almeja asiática, pez percasol y cangrejo de río rojo) (Valencia, España). Como en la primera publicación sólo se incluyeron para su validación PhACs, la discusión se centra en la comparación de este grupo de compuestos. Siete de los PhACs analizados fueron detectados en las dos publicaciones, aunque al tratarse de matrices con tanta diferencia, las concentraciones variaron considerablemente entre órganos y especies.

La sertralina, por ejemplo, mostró las concentraciones más altas, de 5.70 a 957 ng g^{-1} en todos los órganos mientras que en la almeja asiática varió de 6.01 a 8.28 ng g^{-1}. El diltiazem se reportó entre 1.40 y 7.0 ng g^{-1} en cerebro, riñón, hígado y plasma,

pero fue <LOQ en la almeja asiática. La venlafaxina se detectó en concentraciones de 27.4 a 154 ng g^{-1} en varios órganos de peces y estuvo <LOQ en el cangrejo de río y la almeja asiática. La cafeína sólo se detectó en el riñón (50.2 ng g^{-1}), mientras que fue <LOQ en el percasol y la almeja asiática, alcanzando una concentración máxima de 24.5 ng g^{-1} en el cangrejo de río. El ketoprofeno presentó 1.10 ng g^{-1} en el corazón y 7.4 ng g^{-1} en el percasol. La fluoxetina varío de 5.20 a 22.9 ng g^{-1} en cerebro y riñón, y fue <LOQ en el cangrejo de río y alcanzó una concentración máxima de 0.72 ng g^{-1} en la almeja asiática.

Aunque las concentraciones no son directamente comparables debido a las diferencias entre las matrices, donde influyen factores como la afinidad de los PhACs por ciertos órganos/tejidos y el metabolismo específico de cada especie, estos resultados demuestran que los métodos desarrollados permiten evaluar eficientemente la presencia de PhACs en matrices de diversa naturaleza y procedentes de diferentes ecosistemas. Así mismo, se resalta la capacidad de los métodos analíticos desarrollados para detectar y cuantificar CECs en condiciones biológicas y ambientales variadas, convirtiéndose en una herramienta valiosa para el biomonitoreo de estos compuestos en estudios de evaluación ambiental.

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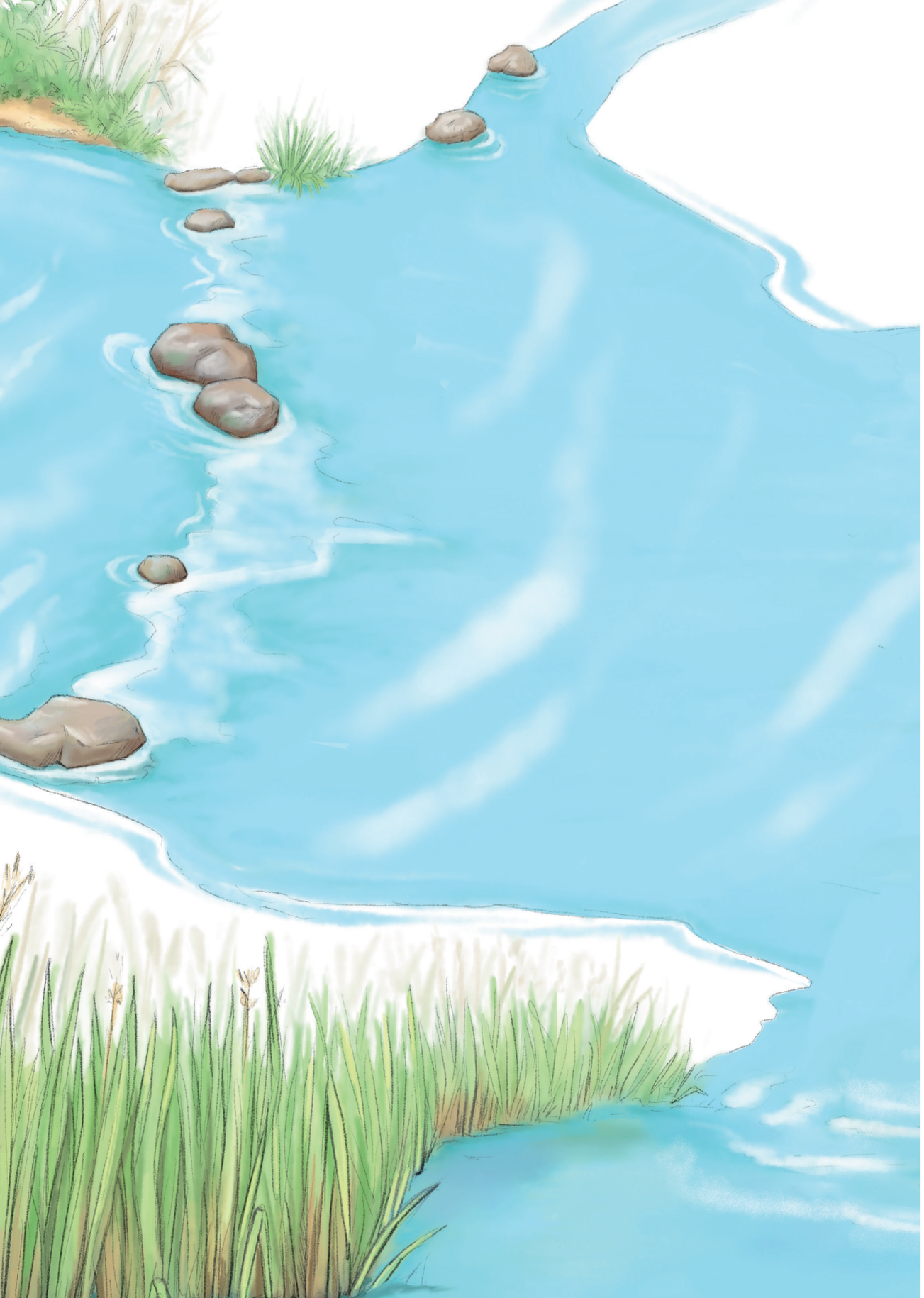

CAPÍTULO 4

DISTRIBUCIÓN DE CONTAMINANTES DE PREOCUPACIÓN EMERGENTE EN ÓRGANOS DE PECES Y EVALUCACIÓN DE SU RIESGO ECOLÓGICO

DISTRIBUCIÓN DE CONTAMINANTES DE PREOCUPACIÓN EMERGENTE EN ÓRGANOS DE PECES Y EVALUACIÓN DE SU RIESGO ECOLÓGICO

4.1. Introducción

En comparación con la evaluación de las concentraciones de CECs en muestras de pez entero homogenizado o únicamente en músculo, el análisis de estos compuestos a nivel de órganos de manera diferenciada, proporciona información más detallada sobre la exposición real de la biota acuática. Este enfoque permite determinar la bioacumulación específica de cada compuesto y evaluar los riesgos asociados. Por ello, tras validar los métodos analíticos para la determinación de los CECs incluidos en el capítulo 3, se realizó inicialmente un análisis enfocado en la distribución de PhACs en órganos y fluidos corporales de peces salvajes de agua dulce (hígado, riñón, corazón, cerebro, músculo, piel, plasma y bilis). A continuación, se presentan las cuatro especies de peces analizadas en la Publicación No. 3: barbo del Ebro (*Luciobarbus graellsii)*, lisa (*Liza ramada*), anguila europea (*Anguilla anguilla*) y carpa común (*Cyprinus carpio*).

El *barbo del Ebro* es una especie de pez de fondo endémica del Mediterráneo occidental y muy común en el río Ebro (Quirós et al., 2007), pero también se encuentra en los ríos Asón, Oria, Nervión, Artibai, Oca, Ter y Llobregat (MMA, 2024). Se trata de una especie litófila (afinidad por entornos rocosos) capaz de colonizar diversos tipos de hábitats, aunque muestra una predilección por los tramos medios y bajos de los ríos. Solo se desplaza a las partes altas durante la época de reproducción, eligiendo zonas con arena y grava para desovar. En general se alimenta tanto de algas como de macroinvertebrados dulceacuícolas. Esta especie prefiere áreas tranquilas con abundante vegetación y raíces de árboles que le proporcionan refugio (MMA, 2024).

La *lisa* es un pez catádromo (peces que viven en agua dulce la mayor parte de su vida pero que migran al mar para desovar) que realiza migraciones masivas a hábitats de agua dulce con fines alimentarios. El traslado a estos hábitats ribereños ocurre en primavera y verano, cuando el caudal del río es menor. No obstante, a pesar de su naturaleza catádroma, en algunos ríos una parte de la población puede permanecer en agua dulce durante todo el año (Pereira et al., 2021). Los adultos de esta especie se alimentan de algas epífitas, detritos, pequeños organismos bentónicos

y planctónicos, además de huevos pelágicos y larvas (Freyhof & Kottelat, 2007).

La *anguila* es un pez migratorio que se reproduce y pasa sus primeros años de vida en el mar, y luego migra a ríos y zonas húmedas hasta alcanzar la madurez sexual. Está presente en toda la península ibérica, tanto en ambientes lóticos como lénticos. A principios del siglo XX, era una de las especies más abundantes y ampliamente distribuidas en los ríos de la península y las albuferas de las islas Baleares. Sin embargo, muestreos a gran escala en los años 80 revelaron que se había extinguido en más del 80% de las cuencas y que solo era abundante en pequeños ríos costeros (Capoccioni et al., 2017).

La *carpa común* es probablemente la primera especie de pez cuya distribución se amplió significativamente debido a la intervención humana. Se introdujo intencionadamente como fuente de alimento, para pesca recreativa y como especie ornamental en aguas continentales templadas y actualmente está presente en más de 80 países, excepto en la Antártida. En regiones como Norteamérica, Australia y Nueva Zelanda se considera una plaga (MITECO, 2020). En España es dominante en áreas como la cuenca baja del Guadiana, el río Terri en Cataluña, y la Albufera de Valencia, siendo especialmente abundante en embalses y tramos medios y bajos de los ríos de la mayor parte del país (Doadrio, 2002). Suele vivir en ríos de corriente lenta, lagos y humedales con fondos fangosos y se alimenta de detritos, macroinvertebrados bentónicos, zooplancton, material vegetal (García-Berthou, 2001) y en ocasiones huevos y alevines de otros peces (Doadrio, 2002). Es una especie altamente fértil, capaz de producir hasta un millón de huevos por individuo cada temporada reproductiva, que generalmente ocurre en primavera o principios del verano. Su esperanza de vida es de 13 a 20 años (MITECO, 2020). La presencia de esta especie ha sido asociada con la degradación de ecosistemas nativos, al inducir un cambio hacia un estado turbio dominado por fitoplancton, lo que resulta en una notable pérdida de biodiversidad. La depredación de esta especie y sus efectos colaterales podrían ser los principales responsables de la reducción de la diversidad animal en las comunidades acuáticas invadidas (Kloskowski & Janusz, 2011).

Debido a los resultados obtenidos en la Publicación No. 3, en la cual la carpa común mostró una alta frecuencia de detección y altas concentraciones de PhACs, se decidió destacar su importancia en procesos de biomonitoreo ambiental. Por tal razón, a continuación, se amplía la información sobre esta especie en particular.

4.1.1. La carpa común para el biomonitoreo de CECs en función de su naturaleza invasora

La carpa común es una especie invasora de alto riesgo, incluida en el catálogo español de especies exóticas invasoras, regulado por el Real Decreto 630/2013 (BOE 2013). Sus actividades alimenticias degradan significativamente los hábitats acuáticos. Al remover el sedimento y arrancar la vegetación acuática, incrementa la turbidez del agua y re-suspenden nutrientes, lo que conduce a la proliferación de fitoplancton y a la pérdida de macrófitas esenciales para el ecosistema. Estos cambios transforman hábitats claros y oligotróficos en entornos turbios y eutróficos (Vilizzi, 2012).

Sus impactos incluyen la reducción de la biodiversidad de peces nativos y aves acuáticas debido a la competencia por alimento, la depredación de huevos y alevines, y la destrucción de zonas de cría. Además, su dieta centrada en invertebrados bentónicos disminuye la biomasa y diversidad de estos organismos, afectando negativamente a otras especies dependientes (Vilizzi, 2012).

La carpa común destaca por su notable tolerancia a la contaminación, siendo capaz de sobrevivir en ambientes con bajas concentraciones de oxígeno (0.3-0.5 mg L^{-1}). Es una especie muy resistente, lo que le permite adaptarse a una amplia variedad de hábitats, desde aguas dulces hasta salobres, y en condiciones ambientales adversas, como aguas turbias y contaminadas (Doadrio, 2002). Su presencia a menudo se asocia a hábitats degradados o afectados por aguas residuales o de escorrentía, donde supera a muchas especies nativas menos tolerantes. En España, a pesar de su impacto negativo comprobado en los ecosistemas, no se han implementado medidas de gestión efectivas para controlar su población (MITECO, 2020).

La presencia de esta especie ha sido reconocida como un buen indicador ecológico de contaminación ambiental en ecosistemas acuáticos, permitiendo además inferir actividades antropogénicas previas o actuales como la minería. Ha sido de especial interés para el biomonitoreo de contaminantes como metales pesados (Kebede & Geleta, 2023; Mancera-Rodríguez et al., 2023) y ha sido analizada previamente en estudios que tuvieron como objetivo evaluar la bioacumulación de PhACs (Castaño-Ortiz et al., 2024; J. Liu et al., 2018; Peña-Herrera et al., 2020a; Scott et al., 2016; Zhao et al., 2015), plaguicidas (Ray & Shaju, 2023) y OPFRs (Bekele et al., 2018; Tang et al., 2019).

En la Publicación No. 4, se analizó la distribución de CECs en la carpa común exclusivamente y adicional a esto, se aplicó un modelo PBPK con el objetivo de comparar los factores de bioconcentración (BCFs del inglés *bioconcentration factor*) previstos con los BAF calculados a partir de las concentraciones ambientales medidas. A continuación, se presenta una breve descripción de los fundamentos de este tipo de modelos.

4.1.2. Aplicación de un modelo farmacocinético de base fisiológica para evaluación de la distribución de CECs en peces

La modelización farmacocinética basada en fisiología se puede utilizar para estudiar la distribución de compuestos en organismos, incluidos los peces, permitiendo evaluar el comportamiento de contaminantes orgánicos a lo largo del tiempo (Bois et al., 2010). Este enfoque es útil para predecir la bioconcentración y el destino de contaminantes en diferentes órganos y tejidos de los peces y así comprender la toxicidad de estos compuestos en ecosistemas acuáticos.

Un modelo PBPK en peces simula la absorción, distribución, metabolismo y excreción de contaminantes orgánicos en el organismo (Wang et al., 2021), teniendo en cuenta características fisiológicas (tamaño, estructura de órganos, flujo sanguíneo) y propiedades fisicoquímicas del contaminante (coeficiente de partición, solubilidad, y vida media) (Salmina et al., 2016).

Este enfoque se ha desarrollado para la determinación de concentraciones internas de PhACs en peces como la trucha arcoíris (Salmina et al., 2016; Wang et al., 2021) y la carpa común (Zhang et al., 2019); COP en el pez de cabeza gorda (Stadnicka et al., 2012), y cuatro especies de peces de la familia cyprinidae (Zhou et al., 2023); PFAS en la trucha arcoíris (Mittal & Ng, 2018); PCBs en marsopas (Weijs et al., 2010); disruptores endocrinos en tilapia (Liu et al., 2022); y estabilizadores ultravioleta como el benzotriazol en pez cebra (Zhang et al., 2021).

Una vez realizado el análisis de distribución por órgano y teniendo claros los patrones de acumulación, generalmente se realiza un análisis de riesgo ecológico que permite identificar si las concentraciones obtenidas representan un peligro para la salud de los peces. El análisis de riesgo incluido en este capítulo se basa en la comparación de las concentraciones medidas con los valores de referencia propuestos por la NORMAN (PNECs), tal como se describió en el capítulo 1.

4.2. Metodología y resultados

Las publicaciones incluidas en este capítulo se centraron en la evaluación de la distribución de CECs, principalmente PhACs, en diferentes órganos de peces salvajes que habitan en un tramo del río expuesto a efluentes de EDAR. Los dos estudios se realizaron en la misma zona: la parte baja de la cuenca del río Llobregat, a la altura del municipio de Sant Joan Despí, en Barcelona (España). El primer muestreo se llevó a cabo durante la primavera del 2019 y el segundo en el 2021.

Durante el muestreo del año 2019 (Fig. 4.1), se capturaron doce peces de cuatro especies diferentes (carpa común, barbo del Ebro, anguila europea y lisa) para evaluar la presencia y distribución de 600 compuestos mediante análisis de sospechosos por HPLC-HRMS (Q-TOF-MS system X500R (SCIEX)). Los resultados se presentan en la Publicación No. 3: ***Manjarrés-López, D.P.,*** *Peña-Herrera, J. M., Benejam, L., Montemurro, N., & Pérez, S. (2023). Assessment of wastewater-borne pharmaceuticals in tissues and body fluids from riverine fish. Environmental Pollution, 324, 121374. https://doi.org/10.1016/J.ENVPOL.2023.121374*

Fig. 4.1. *Muestreo año 2019. Rio Llobregat (izquierda). Pesca eléctrica (derecha)*

Para llevar a cabo un análisis de riesgo ecólogico, se realizó una segunda campaña de muestreo en la primavera de 2021 (Fig. 4.2), en la que se capturaron diez individuos de carpa común. Es importante resaltar, que durante el muestreo, esta especie fue la única observada en el río, a diferencia del muestreo anterior, donde hubo mayor diversidad de especies. Su predominancia en este ecosistema cada vez más deteriorado ambientalmente, reforzó los resultados obtenidos en la Publicación No. 3, resaltando su tolerancia a la contaminación. Adicionalmente, se recolectaron muestras de agua y sedimentos para realizar una evaluación ambiental más exhaustiva.

Fig. 4.2. *Muestreo año 2021 Rio Llobregat. Pesca eléctrica (izquierda). Recolección de sedimentos con muestreador de cuchara Van Veen (derecha).*

Una vez capturadas las carpas comunes, para su análisis se incluyeron algunos compuestos que fueron detectados previamente en la publicación No. 3, incluso aquellos detectados con un nivel de confianza de 2.a en la escala de Schymanski et al., (2014) (para los que se disponian patrones de referencia), así como otros compuestos reportados previamente en la zona de estudio como plaguicidas y compuestos de uso industrial. El análisis se realizó mediante HPLC-HRMS/MS (Orbitrap Q-Exactive™). La evaluación ambiental se hizo multicompartimental (agua superficial, sedimento y biota), se calcularon factores de bioconcentración por órgano y para el pez entero, y se estableció el nivel de riesgo ecológico al que están expuestos los peces en este hábitat.

El análisis de los resultados se realizó en colaboración con el UFZ como fruto de la estancia de investigación predoctoral realizada dentro del Department of Exposure Science a cargo de la Dr. Annika Jahnke, la cual culminó con la Publicación No. 4: ***Manjarrés-López, Diana P.,*** *Montemurro, N., Ulrich, N., Ebert, R. U., Jahnke, A., & Pérez, S. (2024). Assessment, distribution, and ecological risk of contaminants of emerging concern in a surface water-sediment-fish system impacted by wastewater. Science of The Total Environment, 935, 173358. https://doi.org/10.1016/J.SCITOTENV.2024.173358*

4.2.1. PUBLICACIÓN No. 3

Assessment of wastewater-borne pharmaceuticals in tissues and body fluids from riverine fish

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Assessment of wastewater-borne pharmaceuticals in tissues and body fluids from riverine fish☆

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ABSTRACT

Riverine fish in densely populated areas is constantly exposed to wastewater-borne contaminants from effluent discharges. These can enter the organism through the skin, gills or by ingestion. Whereas most studies assessing the contaminant burden in exposed fish have focused either on muscle or a limited set of tissues. Here we set out to generate a more comprehensive overview of the distribution of pollutants across tissues by analyzing a panel of matrices including liver, kidney, skin, brain, muscle, heart, plasma and bile. To achieve a broad analyte coverage with a minimal bias towards a specific contaminant class, sample extracts from four fish species were analyzed by High-Performance Liquid Chromatography (HPLC) - high-resolution mass spectrometry (HRMS) for the presence of 600 wastewater-borne pharmaceutically active compounds (PhACs) with known environmental relevance in river water through a suspect-screening analysis. A total of 30 compounds were detected by suspect screening in at least one of the analyzed tissues with a clear prevalence of antidepressants. Of these, 15 were detected at confidence level 2.a (Schymanski scale), and 15 were detected at confidence level 1 following confirmation with authentic standards, which furthermore enabled their quantification. The detected PhACs confirmed with level 1 of confidence included acridone, acetaminophen, caffeine, clarithromycin, codeine, diazepam, diltiazem, fluoxetine, ketoprofen, loratadine, metoprolol, sertraline, sotalol, trimethoprim, and venlafaxine. Among these substances, sertraline stood out as it displayed the highest detection frequency. The values of tissue partition coefficients for sertraline in the liver, kidney, brain and muscle were correlated with its physicochemical properties. Based on inter-matrix comparison of detection frequencies, liver, kidney, skin and heart should be included in the biomonitoring studies of PhACs in riverine fish.

1. Introduction

Aquatic biota, such as wild fish living in surface waters, are continuously exposed to wastewater-borne contaminants discharged from wastewater treatment plants (WWTPs). They may be taken up through the gills, skin, or diet (Arnnok et al., 2017), transported by circulating blood and distributed to the various tissues according to their physicochemical properties. The extent of uptake of wastewater-borne PhACs into fish and their distribution within the organism has been addressed in a number of controlled laboratory experiments with exposure to (diluted) wastewater as well as in field studies, focusing on PhACs commonly detected in surface waters (Arnnok et al., 2017; Boulard et al., 2020; Duarte et al., 2023; Grabicová et al., 2020, 2018; 2017, 2014; Fernandez-Torres et al., 2011; Heynen et al., 2016; Lajeunesse et al., 2011; Liu et al., 2015; McCallum et al., 2017; Ondarza et al., 2019; Rojo et al., 2019; Tanoue et al., 2015; Valdés et al., 2016; Zhao et al., 2017, 2015). The principal parameter for comparing exposure of the species under investigation with the concentration of the surrounding medium is the bioconcentration factor (BCF), expressed as the ratio of concentration in organism to concentration in water (Liu et al., 2015). Depending on the approach chosen, the BCF has been calculated either on a whole-body basis (fish homogenate) or considering specific tissues or body fluids. By far the most common organs considered for assessing PhACs exposure are brain, liver, and muscle (*TS1*); levels in kidney, gill, and gonads have only been measured occasionally (*TS1*). For a given compound the calculated BCF has been shown to largely vary between

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matrices because its distribution from the blood stream into tissues and organs is governed by the affinity to blood plasma components on the one hand, and to specific tissue components on the other hand.

The aforementioned preference for analyzing brain, liver, and muscle – driven by the easy of harvesting samples and the relevance of flesh for human consumption – comes with the limitation that exposure in other organs is not addressed, thereby preventing the assessment of potential local effects induced by the interaction of PhACs with physiological processes taking place at the organ level. Considering that circulating blood transports analytes to the various organs and tissues, its analysis will allow to gain an understanding of the underlying mechanism of drug partitioning. In other words, the analysis of blood plasma as the non-cellular fraction of whole blood needs to be included. Hence, for a more comprehensive description of drug exposure in fish it is necessary to expand the scope of analysis in terms of matrices covered and compounds studied.

As far as the chemical analysis of wastewater-borne PhACs is concerned, traditionally, it has been performed in target mode with compound-specific acquisition using analytical standards, often resulting in a high cost to the laboratory (Lai et al., 2021). In recent years, suspect screening has been the subject of intense development aided by advances in High-Resolution Mass Spectrometers (HR-MS) such as Hybrid Quadrupole Time of Flight (Q-TOF) or Orbitrap-MS systems and in the development of new computational algorithms that have facilitated data analysis (Alygizakis et al., 2018; Lai et al., 2021). The main advantage of these screening techniques is to query discrete lists of substances potentially present in a sample without the need for authentic standards, which may not be available (Alygizakis et al., 2018; Sabater-Liesa et al., 2021).

In this regard, we set out to screen four wild riverine fish for wastewater-borne PhACs in six tissue types (skin, heart, muscle, kidney, liver, and brain) and two body fluids (plasma and bile) using a HR-MS suspect screening approach. The analysis was performed on a Q-TOF instrument using a Sequential Window Acquisition of All Theoretical MS (SWATH) acquisition methodology by Electrospray Ionization in positive mode (ESI+). Since this data acquisition allows for retrospective analysis, we performed a literature search and compiled a list of 600 potential analytes for detection in fish.

2. Materials and methods

2.1. Chemicals and standards

Standards and isotopically labeled standards (IS) > 99% purity used for this study were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). Sigma-Aldrich (St Louis, MA, USA). Santa Cruz Biotechnology (Dallas, TX, USA) and CDN Isotopes (Pointe-Claire, Quebec, Canada). Standard stock solutions (1000 mg L^{-1} and 100 mg L^{-1}) were prepared in MeOH, dimethyl sulfoxide (DMSO) or acetonitrile (MeCN) and stored at −20 °C. The list of standards stocks and IS used to prepare the calibration curve are shown in *TS2*. For sample preparation: HPLC water, acetonitrile ultra-gradient HPLC grade, isopropanol (iPrOH) HPLC grade, MeOH ultra-gradient HPLC grade from J.T.Baker. Fisher Scientific (Gliwice, Poland), formic acid (F.A.), chlorhydric acid (HCl) (ACS reagent from Sigma Aldrich) and ammonium acetate (NH_4OAc) from Merck (Darmstadt, Germany). For chromatographic separation and HR-MS analysis: water and MeCN with Optima LC/MS grade from Fisher Scientific (Geel, Belgium). F.A. ACS from Sigma Aldrich and NH_4OAc from Merck.

2.2. Sample collection

The samples were collected in Sant Joan Despí (UTM: 420,218.4,577,994) in the lower part of the Llobregat river because is near to Barcelona city. This sampling point was selected because it is heavily impacted by WWTPs' effluents, and the morphology of the easy-capturing of the fish in the shallow, low-flow stretch of the river. The sampling campaign was carried out in May 2019 following the technical requirements established by the Biodiversity and Animal Protection Service (Department of agriculture, livestock, fishing, food, and environment of the Regional Government Catalonia, Spain). Fish samples were collected by electrofishing (200–350 V. 2–3 A. fully rectified triphasic DC). Stunned fishes were collected with nets, classified and their fork length measured (*TS3*). Three individuals by species were captured: Ebro barbel (*Luciobarbus graellsii*), common carp (*Cyprinus carpio*), European eel (*Anguilla anguilla)*, and thinlip mullet (*Liza ramada)*. Fish samples were transported in a cooler (4 °C) to the lab and processed immediately upon arrival.

Ebro barbel and common carp are benthic omnivore that stirs the bottom to feed (García-Berthou, 2001; Benejam et al., 2009). The thinlip mullet is a catadromous fish that migrates to freshwater habitats for feeding purposes, where extended periods are spent in such environments (Pereira et al., 2021). Adults of thinlip mullet feed on epiphytic algae, detritus, small benthic or planktonic organisms, pelagic eggs and larvae (Freyhof & Kottelat, 2007). Eels migrate between the Sargasso Sea and European rivers, where it grows and matures sexually. During its life in the river, it uses benthic habitats (Cano-Barbacil et al., 2020). The eel has a wide range of diet, from detritus and benthic invertebrates to other fishes (Costa et al., 1992). Although all four species are categorized as tolerant, the thinlip mullet is especially recognized for being very tolerant to (Cano-Barbacil et al., 2020).

2.3. Sample preparation and extraction

Once caught the fish, 2-mL blood samples were immediately taken from each individual in the field and placed into heparin-coated tubes to avoid coagulation. Once in the laboratory, blood plasma was prepared by centrifugation of 1 mL aliquots for 10 min at 2500 rpm and 4 °C. The supernatant was transferred to a clean Eppendorf tube and centrifuged again for 10 min at 2500 rpm and 4 °C. Finally, 500 μL of the resulting plasma was stored at −80 °C until analysis. Fishes were dissected and organs/tissues were analyzed separately.

We decided to analyze the liver understanding it as the integration of the hepatopancreatic tissue and the liver, since in species of the order *Cypriniformes,* the pancreatic tissue is not concentrated in a separate organ but is diffused within the liver (Smallwood & Derrickson, 1933). As for the kidney, we have only considered the elongated portion corresponding to the caudal portion or renal trunk and the ventral lobe.

Samples were processed according to our previously published method (Manjarrés et al., 2022; Peña-Herrera et al., 2020). Briefly, 500 mg of fresh tissue was weighed into a 2–mL Eppendorf tube containing two stainless steel beads (4 mm Ø), spiked with 125 μL of 100 ng mL^{-1} of IS used as surrogate standards and homogenized using a TissueLyzer LT Sample Disruptor (Qiagen, Hilden, Germany) operating at 50 Hz (1.5 min). Samples were vortexed and allowed to settle for 15 min. Then 1000 μL of MeCN/iPrOH (3:1) + F.A 0.1% were added, and sonicated (Fisherbrand® FB15064, Waltham, MA, USA) for 10 min following centrifugation (14,000 rpm for 12 min at -2 °C), 750 μL of the supernatant were transferred into a 2-mL vial containing 50 mg Discovery® DSC-18 and 20 mg Z-Sep for clean-up (zirconium oxide and C18 dual bonded to silica; SupelTM QuE, Sigma-Aldrich, Steinheim, Germany). The mixture was vortexed and then centrifuged for 6 min. Finally, 500 μL of the supernatant was transferred to an HPLC vial, evaporated under a gentle stream of nitrogen, and reconstituted with 500 μL of 5 mM NH_4OH/MeCN (90:10) for further analysis by Ultra Performance Liquid Chromatography UPLC-HR-MS.

Bile samples were prepared by diluting 50 μL of bile with 325 μL of HCl (0.1 N), 112.5 μL of cold MeCN, and 12.5 μL of IS. The sample was vortexed and centrifuged (14,000 rpm for 10 min at 4 °C), 350 μL were recovered, 200 μL of acetone were added, evaporated to dryness, and reconstituted with 500 μL of 5 mM NH_4OH/MeCN (90:10) for UPLC-HR-MS analysis. Plasma was prepared by taking 100 μL of plasma, 387.5 μL

of cold MeCN, and 12.5 μL of IS (1 ng/μL). The sample was vortexed and centrifuged (8000 rpm for 10 min at 4 °C). Then, 500 μL of supernatant were separated, evaporated to dryness, and reconstituted with 500 μL of 5 mM NH_4OH/MeCN (90:10) for UPLC-HR-MS analysis.

2.4. HPLC-HR-MS analysis

First, a list of wastewater-borne PhACs was defined by combining the compound lists reported by Angeles et al. (2021) and Peña-Herrera et al. (2020), listing 600 compounds for suspect screening. The complete list is shown in *SI2* (SI2 Excel). Chromatographic separation and data acquisition were applied according to Peña-Herrera et al. (2020, 2019). Briefly, the UPLC Exion LC AD system (SCIEX, Framingham, MA, USA), connected to a hybrid Q-TOF-MS system X500R (SCIEX) was used. EVO C18 KINETEX (50 × 2.1 mm, 2.6 μm, Phenomenex, Torrance, CA) at 40 °C was used as chromatographic column. Mobile phases: A) NH_4OAc (5 mM) and B) MeCN, both acidified with F.A. (0.05%) at a flow rate of 0.8 mL min^{-1}. The injection volume was 10 μL. The elution gradient was performed as follows: B) started at 5% for 0.3 min and then increased to 25% until 5.9 min continued increment to 40% until 7.6 min and then to 98% until 8.9 min remain in this proportion until 9.9 min, to decrease to 5% at 10 min and remain at 5% until the final time (11 min). Compounds were ionized in ESI + mode. For TOF-MS and TOF-MS/MS data, the data acquisition technology SWATH was used. The data collection was divided into 11 experiments: the first experiment was a single TOF-MS over an *m/z* range from 100 to 1000 setup with a build-up time of 0.1 s. Then 10 MS/MS experiments with variable Q1 windows as follows: *m/z* (100–190, 189–280, 279–370, 369–460, 459–550, 549–640, 639–730, 729–820, 819–910, 909–1000).

For suspect screening analysis the SCIEX All-in-one High Resolution MS/MS Spectral Library was used. The criteria defined by Sabater-Liesa et al. (2021) for a positive identification (Library hit score >70%, fit >50%, rfit ≤100%, and purity ≤100%) were applied for suspect screening analysis obtaining a confidence level of 2.a based on the scale of Schymanski et al. (2014). Their presence was confirmed at level 1 and quantified for compounds for which a standard was available. The workflow schematic is shown in Fig.S1. Data analysis was performed using SCIEX OS V1.6 (SCIEX). For quantification purposes, a calibration curve was prepared in 5 mM NH_4OH/MeCN (90:10) with seven concentration points ranging from 0.5 ng mL^{-1} to 100 ng mL^{-1}, including an IS concentration at each point at 12.5 ng mL^{-1} for solids tissues and 25 ng mL^{-1} for body fluids. The calibration curve was constructed by plotting the ratio of the analyte signal to its surrogate standard signal against the analyte concentration. Calibration curve was constructed by linear weighted least-squares regression (1/x as weighting factor). Linearity was evaluated by calculating the coefficient of determination (r^2) for each analyte. The acceptance criterion was that the coefficient of correlation ≥0.99. The concentration results were corrected according to the recoveries, and matrix effects (ME) reported in *TS4*.

2.5. Quality assurance and quality control (QA/QC)

The stainless-steel beads used in the solid tissues' homogenization step were washed with ethanol and acetone and placed in an ultrasonic bath for 15 min for each solvent to avoid the contamination of samples. The other materials used during the extraction process were single-use. In the case of plasma, blood samples were extracted during the sampling with a syringe containing heparin to avoid clotting. They were transported at 4 °C to the laboratory, where the plasma was immediately extracted, as indicated in section 2.3. Quality control during chromatography was performed by introducing a mixture of reference standards containing the compounds described in *TS2* and analyzing every 5 samples during the analysis to evaluate the method's performance. Likewise, solvent blanks were incorporated to verify that there was no carryover effect on the column. A range of <0.03 min was established as an acceptable difference between the chromatographic retention times of the calibration curve and those of the samples, and a mass error of <5 ppm for compounds detected at confidence level 1. Calculated the recoveries, instrumental limits, and ME were reported in *TS4*.

3. Results and discussion

3.1. Compounds identification and detection frequency

As previously demonstrated for wastewater samples (Sabater-Liesa et al., 2021), HR-MS data acquired in SWATH mode can be queried using lists of suspect compounds without needing sample reinjection. Here, we processed the data corresponding to six tissues and two body fluids from four fish species to identify contaminants from the predefined list of 600 substances *(SI2)*. We tentatively identified 30 compounds in this suspect screening approach, 15 with confidence level 2.a according to Schymanski et al. (2014), based on the match between recorded and library MS/MS spectrum (Fig. 1, compound names in blue font) and 15 with a confidence level 1, i.e., its confirmation was achieved by comparing it with its standard (Fig. 1, compound names in green font). The fitting scores (Fit, Rev. Fit and Purity) used in the automated compound identification at confidence level 2.a are listed in *TS5*.

For compounds having reached confidence level 2.a the next level requires a reference standard that matches the retention time, thus confirming the identification of this unknown with level 1 confidence. The 15 compounds confirmed at level 1 were clarithromycin, codeine, diltiazem, fluoxetine, metoprolol, sertraline, sotalol, venlafaxine, acetaminophen, acridone, caffeine, diazepam, loratadine, trimethoprim, and ketoprofen. They were identified as level 1 using analytical reference standards and providing a retention time match within 0.03 min using an internal standard calibration approach. The results of quantification for this second group of compounds are presented in section 3.2.

Most of the compounds detected were PhACs except for triphenyl phosphate (flame retardant and plasticizer), fenpropimorph, and terbutryn (herbicides). The 30 compounds listed in Fig. 1 are unevenly distributed across ion classes, with a bias towards basic compounds (63%). Neutrals account for 31%, whereas acidic analytes constitute the remainder (6%). Similar trends were reported by other authors (Tanoue et al., 2020). This outcome can be attributed to several factors, including lower abundance in the river water, discrimination during sample preparation, and differences in ionization efficiency. The low detection of compounds with a negative charge at physiological pH can be associated with their high affinity to plasma albumin (Tanoue et al., 2017) which restricts their extensive distribution to peripheral organs.

Sertraline was the compound with the highest detection frequency with 77 positive hits, i.e., it was detected in all matrices except bile and in all individuals analyzed. It is followed by bisoprolol and amantadine with 49 and 43 hits, respectively. Sertraline is a selective serotonin reuptake inhibitor (SSRI) used to treat depression, anxiety, and obsessive-compulsive disorders. Due to its high detection frequency in all the matrices, a special section is dedicated to its distribution (*section 3.3*). Bisoprolol is a cardio-selective β1-adrenergic blocking agent used to treat high blood pressure. Amantadine is a primary amine that has both antiviral and dopaminergic activity and is used in the therapy of influenza A and the management of Parkinson's disease. The kidney and liver were the tissues where amantadine and bisoprolol were most frequently detected.

The species which detected the highest number of compounds was thinlip mullet (23 compounds). Twelve PhACs with a confidence level of 1 and 11 with a confidence level of 2a (Fig. 1). In this species, 129 reported hits. The compounds with the greatest detection frequency were sertraline (19 hits), bisoprolol (17 hits), and amantadine (15 hits). Liver (26 hits), skin (22 hits), kidney (21 hits), and plasma (19 hits) were the tissues with the most detection frequency for this specie. Seventeen compounds were detected in common carp. Eight with a confidence level of 1 and 9 with a confidence level of 2a. reported seventy-one hits. The largest hits were in the kidney (16 hits) and liver (12 hits). Sertraline

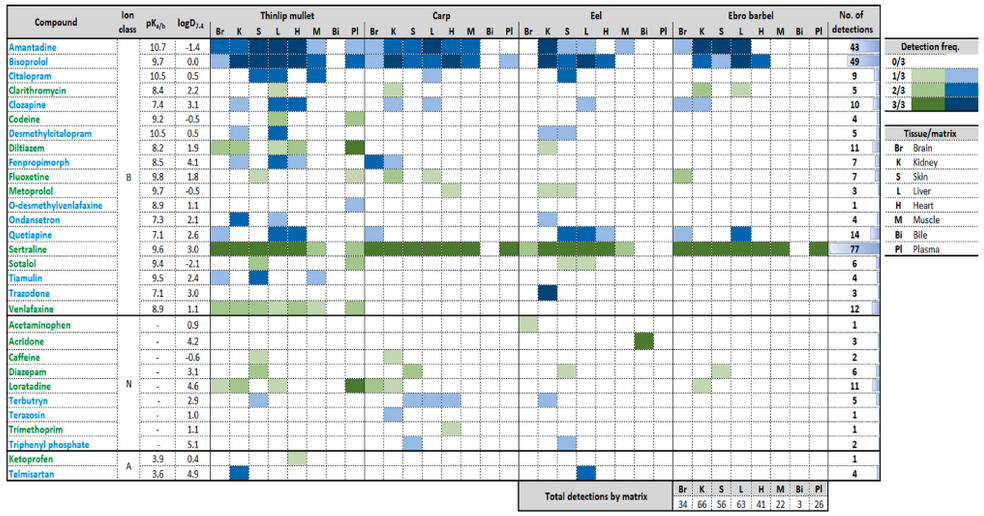

Fig. 1. Detection frequency of wastewater-borne contaminants analyzed with HR-MS in SWATH mode. **Ion class: B**: Basic, **N**: Neutral, **A**: Acid. Physicochemical properties $pK_{a/b}$ and $logD_{7.4}$ were calculated with Pipeline Pilot (Dassault Systèmes). **Blue**-shaded boxes: detection with a confidence level **2.a**. **Green**-shaded boxes: detection with a confidence level of **1** according to Schymanski et al. (2014). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

was detected in all tissues except bile (21 hits). Bisoprolol (14 hits) and amantadine (12 hits) also showed a high detection frequency. In the eel reported 60 hits. In their tissues, 18 compounds were detected (7 with a confidence level of 1 and 11 at 2a). Sertraline (16 hits) and bisoprolol (10 hits) were the compounds with the major number of detections. The kidney (17 hits), skin (15 hits), and liver (13 hits) were the tissues with the highest detection frequency. Finally, the species with the least number of compounds detected was Ebro barbel, with 9 PhACs. Five have a confidence level of 1 and 4 with a confidence level of 2a. Sertraline (21 hits) and amantadine (10 hits) were de compounds with the highest detection frequency. Kidney and liver had the largest hits in this specie (12 hits each).

The high pollution tolerance of thinlip mullet (Tancioni et al., 2015) compared to the other species, and its catadromous nature may explain why it had a higher number of hits. Likewise, the fact that it is a euryhaline species may be related to the hits presented in the plasma. In the thinlip mullet plasma, 10 compounds were detected (19 hits), while the plasma obtained the lowest detection frequency in the other species. The

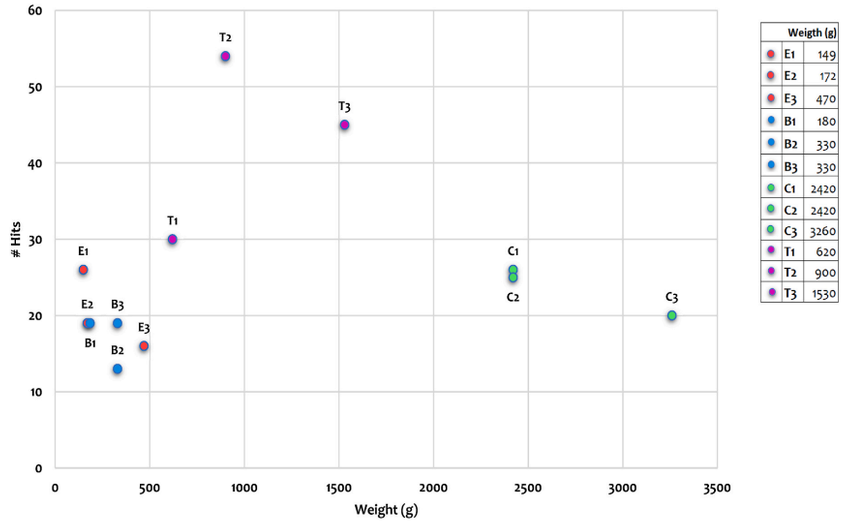

Fig. 2. Hits reported for each individual vs weight. E (eel); B (Ebro barbel); C (carp); T (thinlip mullet).

thinlip mullet can adapt without showing significant alterations in plasma osmotic pressure and plasma electrolytes (Lasserre & Gallis, 1975). When it is found in freshwater, it is hyper-osmotic and hyper-ionic, which induces an osmotic water gain. That can facilitate the compounds absorption, especially the most polar compounds found in the surrounding water. This is reflected in the logD value of the most frequently detected compounds in the plasma of this species (Fig. 1).

Pearson's correlation coefficient ($p = 0.13$), calculated for the 12 individuals captured independently of the species, showed a negligible correlation between weight and hits reported. However, species such as carp and eel observed that heavier individuals had lower hits (Fig. 2). This could be related to these species' metabolism and elimination rates, which may condition the amount of compound available for storage. It has been reported that the lipid content of the organism, body size, and life stage influences differences in accumulation for different species caught in the same area (Miller et al., 2018).

Miller et al. (2018) reported that in fish, diet generally shows a low influence on the accumulation of PhACs. Organisms belonging to lower trophic positions are the main recipients of PhACs, and it has been noted that trophic transfer to higher levels is limited (Bowen Du et al., 2014; Lagesson et al., 2016; Xie et al., 2017). Therefore, we have not performed an analysis of the number of hits reported as a function of this variable.

3.2. Occurrence and concentrations of compounds by tissue/body fluids

Genuine analytical standards were available for 16 compounds. Fifteen compounds were confirmed and quantified using a calibration curve. The recoveries of these compounds and their detection and quantification limits were reported previously by Manjarrés et al. (2022). *TS2* shows the MS parameters and the IS associated for each analyte. The results are summarized in Fig. 3. The 15 compounds confirmed with a confidence level of 1 were PhACs. According to Grabicova et al. (2018), it is not enough to define only the detection frequency to analyze this type of compound in biological matrices; the concentration in each tissue must also be determined to assess the bio-accumulation capacity. Likewise, the tissues with the highest average concentrations are the ones on which attention should be focused. For this reason, the liver and kidney are particularly interested in this study since they presented the highest average concentrations and detection frequency.

Of the 15 compounds confirmed at confidence level 1, sertraline had the highest average concentrations in the liver and kidney of thinlip mullet, carp and ebro barbel (Fig. 3). In the case of eel, the highest concentration was reported for acridone in bile, being the only compound detected in this body fluid.

Sixty-three hits were reported in the **liver** fish samples. The highest average concentrations were reported for sertraline (795 ng g^{-1} in thinlip mullet, 618 ng g^{-1} in the carp, 278 ng g^{-1} in Ebro barbel, and 30 ng g^{-1} in the eel) (Fig. 3). Studies of PhACs analysis in wild fish and fish exposed to different concentrations of PhACs have shown that the liver tends to have a higher accumulation of compounds compared to other tissues (Grabicová et al., 2020, 2017; Vaclavik et al., 2020; Xie et al., 2015; Yin et al., 2021). This could be related to the liver being the main metabolizing organ in fish (Yin et al., 2021). Here the detoxification processes are led (Miller et al., 2018) because it is the leading site where the cytochrome P450 (CYP450) enzymes that metabolize PhACs in fish are produced.

In the **kidney**, reported 66 hits. The highest mean concentration was for sertraline (810 ng g^{-1} in the carp, 346 ng g^{-1} in thinlip mullet, 211 ng g^{-1} in ebro barbel, and 121 ng g^{-1} in the eel). These values are above the maximum concentrations reported in other studies (Table 1). In recent studies about the presence of PhACs in fish tissues, the kidney has only been analyzed sporadically (*TS1*). One of the reasons could be that this matrix has a high ME, especially in suppressing the signal of the analytes. Grabicova et al. (2018) reported that tissues with higher lipid

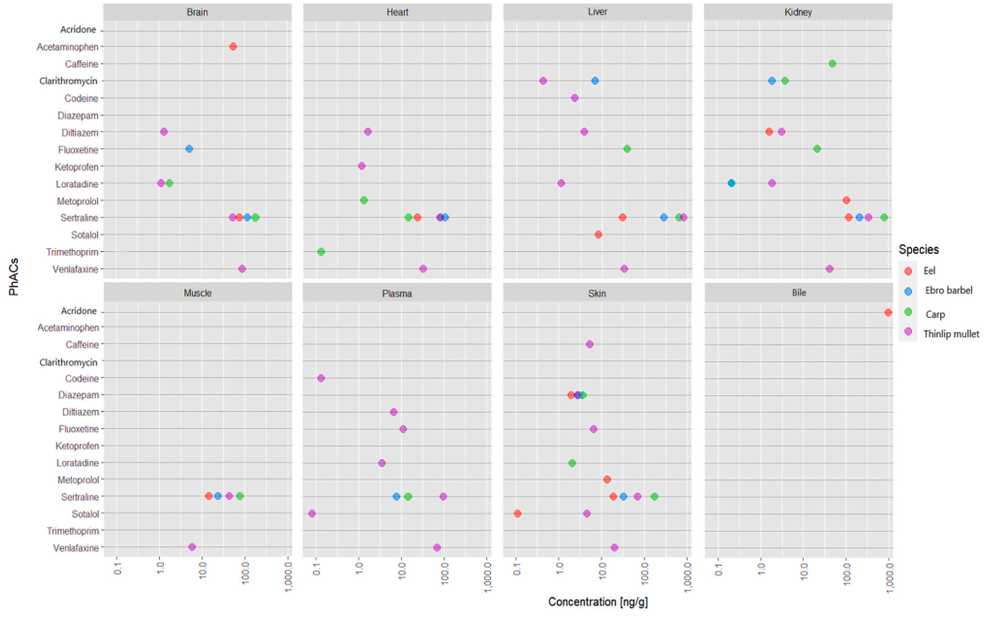

Fig. 3. Average concentrations of PhACs confirmed with level 1 of confidence according to Schymanski et al. (2014) by tissue and specie. Plasma and bile concentrations are expressed in ng mL^{-1}.

Table 1
Compounds detected and quantified by tissue.

Matrix	Compound	This study		Other studies
		Conc. tissue (ng g^{-1}) Min - Max	Conc. tissue (ng g^{-1})	Ref.
Bile[1]	Acridone	643.5–4319.8	–	–
Brain	Acetaminophen	55.7	10	Mijangos et al. (2019)
	Amantadine	2.a	–	–
	Bisoprolol	2.a	–	–
	Clozapine	2.a	–	–
	Diltiazem	1.3	0.2–1.5	(McCallum et al., 2017; Tanoue et al., 2015)
	Fenpromimorph	2.a	–	–
	Fluoxetine	5.2	0.08–1.58	(Brooks et al., 2005; Lajeunesse et al., 2011; Schultz et al., 2010)
	Loratadine	1.1–2.7	–	–
	Quetiapine	2.a	–	–
	Sertraline	<LOQ-384.8	0.21–4411	(Brooks et al., 2005; Grabicová et al., 2020; Lajeunesse et al., 2011; Schultz et al., 2010; Tanoue et al., 2014; Vaclavik et al., 2020; Xie et al., 2015)
	Tiamulin	2.a	–	–
	Venlafaxine	23.4–153.3	0.43–13	(Duarte et al., 2023; Grabicová et al., 2020, 2014; Lajeunesse et al., 2011; McCallum et al., 2017; Schultz et al., 2010)
Heart	Amantadine	2.a	–	–
	Bisoprolol	2.a	–	–
	Clozapine	2.a	–	–
	Diltiazem	1.3–1.9	–	–
	Fenpromimorph	2.a		–
	Ketoprofen	1.1	–	–
	Metoprolol	1.3	–	–
	Quetiapine	2.a	–	–
	Sertraline	5.1–148.7	–	–
	Terbutryn	2.a	–	–
	Trimethoprim	<LOQ	–	–
	Venlafaxine	14.8–48.6	–	–
Kidney	Amantadine	2.a	–	–
	Bisoprolol	2.a	14	Grabicova et al. (2018)
	Caffeine	50.2	–	–
	Clarithromycin	1.4–3.9	–	–
	Clozapine	2.a	–	–
	Desmetylcitalopram	2.a	13	Grabicová et al. (2020)
	Diltiazem	1.7–3.5	1.9	Tanoue et al. (2015)
	Fenpromimorph	2.a		–
	Fluoxetine	21.5–22.9	–	–
	Loratadine	<LOQ-2.6	–	–
	Metoprolol	105.5	0.5	Grabicova et al. (2018)
	Ondansetron	2.a	–	–
	Sertraline	44.2–956.8	1–44	(Grabicová et al., 2020, 2018, 2017, 2014; Tanoue et al., 2015; Vaclavik et al., 2020)
	Terazosin	2.a	–	–
	Terbutryn	2.a	–	–
	Telmisartan	2.a	0.1–8.7	(Grabicová et al., 2020, 2018)
	Trazodone	2.a	–	–
	Triphenyl phosphate	2.a	–	–
	Venlafaxine	32.6–53.6	2.2–5.3	(Grabicová et al., 2020, 2018, 2017)
Liver	Amantadine	2.a	–	–
	Bisoprolol	2.a	–	–
	Citalopram	2.a	3.0–31	(Grabicová et al., 2020, 2018, 2017, 2014; Zhao et al., 2017)
	Clarithromycin	6.8	–	–
	Clozapine	2.a	–	–
	Codeine	2.2–2.3	0.5–1.7	Duarte et al. (2023)
	Desmethylcitalopram	2.a	2.6	Grabicova et al. (2018)
	Diltiazem	3.8	0.5–1.0	(B. Du et al., 2012; McCallum et al., 2017; Ramirez et al., 2009; Tanoue et al., 2015)
	Fenpropimorph	2.a	–	–
	Fluoxetine	38.4	0.2–86	(B. Du et al., 2012; Brooks et al., 2005; Lajeunesse et al., 2011)
	Ondansetron	2.a	–	–
	Loratadine	<LOQ	–	–
	Quetiapine	2.a	–	–
	Sertraline	5.8–1420	0.29–7250	(B. Du et al., 2012; Brooks et al., 2005; Grabicová et al., 2020, 2018, 2017, 2014; Lajeunesse et al., 2011; Liu et al., 2018, 2014; Ramirez et al., 2009; Tanoue et al., 2015; Vaclavik et al., 2020; Xie et al., 2015)
	Sotalol	<LOQ-7.9	–	–
	Terbutryn	2.a	–	–
	Telmisartan	2.a	1.6–6.2	(Grabicová et al., 2020, 2018)
	Venlafaxine	32.7	0.9–20.8	(Arnnok et al., 2017; Duarte et al., 2023; Grabicová et al., 2020, 2018, 2017, 2014; Lajeunesse et al., 2011; McCallum et al., 2017; Moreno-González et al., 2016)
Muscle	Amantadine	2.a	–	–
	Bisoprolol	2.a	–	–
	Citalopram	2.a	0.95–1.3	(Grabicová et al., 2020, 2018)
	Sertraline	8.0–86.6	0.12–535	(Brooks et al., 2005; Grabicová et al., 2020, 2018; Lajeunesse et al., 2011; Ramirez et al., 2009; Tanoue et al., 2015; Xie et al., 2015)

(*continued on next page*)

Table 1 (*continued*)

Matrix	Compound	This study		Other studies
		Conc. tissue (ng g^{-1}) Min - Max	Conc. tissue (ng g^{-1})	Ref.
	Tiamulin	2.a	–	–
	Venlafaxine	5.9	0.034–2.5	Duarte et al., 2023; Grabicová et al., 2020, 2018; Lajeunesse et al., 2011; McCallum et al., 2017; Rojo et al., 2019)
Plasma[1]	Amantadine	2.a	–	–
	Bisoprolol	2.a	–	–
	Codeine	<LOQ	–	–
	Diltiazem	6.2–7.0	0.05–42.73	(Bean et al., 2018; McCallum et al., 2017; Nozaki et al., 2023; Scott et al., 2016; Tanoue et al., 2015, 2020)
	Fluoxetine	11.1	0.33–7130	(Bean et al., 2018; Chen et al., 2015; David et al., 2018; Muir et al., 2017; Nozaki et al., 2023)
	Loratadine	3.0–4.6	–	–
	O-Desmethylvenlafaxine	2.a	0.16–0.2	(Grabicová et al., 2020, 2018)
	Sertraline	4.4–142.0	0.11–2260	(Bean et al., 2018; Chen et al., 2015; David et al., 2018; Muir et al., 2017; Nozaki et al., 2023; Tanoue et al., 2015, 2014, 2020)
	Sotalol	<LOQ	–	–
	Venlafaxine	50.7–87.2	0.03–3.3	(David et al., 2018; Grabicová et al., 2020, 2018, 2014; McCallum et al., 2017; Muir et al., 2017; Tanoue et al., 2020)
Skin	Amantadine	2.a	–	–
	Bisoprolol	2.a	–	–
	Caffeine	5.3	–	–
	Citalopram	2.a	–	–
	Desmethylcitalopram	2.a	–	–
	Diazepam	1.9–3.5	–	–
	Fluoxetine	6.4	–	–
	Metoprolol	13.6	–	–
	Quetiapine	2.a	–	–
	Sertraline	8.0	–	–
	Sotalol	<LOQ-4.7	–	–
	Terbutryn	2.a	–	–
	Tiamulin	2.a	–	–
	Triphenyl phosphate	2.a	–	–
	Venlafaxine	12.8–27.4	–	–

Compounds detected in each of the matrices analyzed (n = 12). The calculated minimum and maximum concentration are indicated. [1]Concentrations expressed in ng mL^{-1}. All concentrations are expressed in wet weight. **<LOQ**: Less than the quantification limit. **2.a**: level of confidence according to Schymanski et al. (2014).

content, such as the kidney, show a more significant matrix influence. In their study, 70% of the ions analyzed showed signal suppression in this tissue, with ME values ranging from −1616% to 51%. Similarly, Manjarrés et al. (2022) reported that more than 40% of the compounds validated for the kidney presented a strong suppression of the analytes signal, i.e., ME values of less than −40% were obtained. However, it is essential to increase the analysis of the kidney for biomonitoring and distribution studies because of higher detection frequency and average concentration than other tissues. In addition, compounds such as clarithromycin and terazosin were only detected in this tissue.

Glomerular filtration, renal tubular secretion, or reabsorption could be why the average concentration of compounds in the kidney is higher than in other tissues (Steinbach et al., 2016). Analyzing the kidney is important because its primary function is the elimination of metabolic residues and osmoregulation. Therefore, in this matrix there is a greater probability of detecting compounds that fish do not metabolize. Another important reason for its analysis is that some non-steroidal anti-inflammatory drugs (NSAIDs) reduce the production of prostaglandins, which regulate blood circulation, especially in the kidney. Reducing these substances could produce renal disorders in fish (Tanoue et al., 2015). Studies have previously reported that the kidney is one of the tissues with the highest concentration when exposing fish to different concentrations of PhACs (Grabicová et al., 2020; Vaclavik et al., 2020).

In **skin** and **heart**, 56 and 41 hits were reported, respectively. According to the literature consulted (*TS5*), there are no reports in which these fish tissues have been analyzed. Here concentrations of PhACs in the skin and heart of wild fish are reported for the first time. The maximum average concentration in skin and heart was for sertraline (71 ng g^{-1} and 79 ng g^{-1} in thinlip mullet; 36 ng g^{-1} and 19 ng g^{-1} in the carp; 33 ng g^{-1} and 103 ng g^{-1} in the ebro barbel; 19 ng g^{-1} and 23 ng g^{-1} in the eel, respectively) (Fig. 3).

The high lipid content, which hinders the homogenization of the sample during the **skin** extraction process, could be related to its null analysis rate. The constant exposure of the skin to the surrounding water could reveal the presence of compounds that, although they fail to accumulate in the other fish tissues, could be precursors of metabolites that require further attention. Our results showed the presence of citalopram in the skin of eel and thinlip mullet. Still, its metabolite desmethylcitalopram was not detected in the skin but in other tissues such as the kidney and liver. Toxicokinetic studies have shown that pharmaceutical metabolites such as benzodiazepines tend to accumulate more than the compounds from which they are generated (Miller et al., 2018). This premise is essential in the case of diazepam, which was detected exclusively in the skin of the 4 species analyzed and in 50% of the individuals. Its presence implies that its metabolites are highly likely to be detected in other tissues.

As mentioned above, the liver is the most important organ concerning CPY450. However, a study by Van Veld et al. (1997) showed that CYP1A enzymes belonging to this cytochrome, which metabolizes exogenous compounds in fish (Goksøyr & Husøy, 1998), were also expressed in other tissues such as the **heart** when mummichog (*Fundulud heteroclitus*) was exposed to aqueous benzo [a]pyrene. Although the test was with polycyclic aromatic hydrocarbons (PAHs), we consider that the analysis of organs such as the fish heart should be deepened since it reveals sensitive information against other groups of pollutants. Additionally, the presence of PhACs such as ketoprofen and trimethoprim, which were not detected in any other tissue in this study, was reported in this organ.

Forty percent of the detected compounds have the **brain** as their target organ. They are mainly antidepressants or anxiolytics (fluoxetine, sertraline, venlafaxine, citalopram, trazodone, desmethylcitalopram, O-desmethylvenlafaxine, diazepam), antipsychotics (clozapine,

quetiapine), nervous system stimulants (caffeine) or sedatives (codeine). Of the 34 hits reported in the brain, 56% correspond to this kind of compound (Fig. 1). In this sense, it would be expected that the highest concentrations for these compounds would be in the brain, as previously reported (Grabicová et al., 2020, 2014; Lajeunesse et al., 2011; McCallum et al., 2017; Tanoue et al., 2015; Yin et al., 2021). However, our results do not follow this trend, only fulfilled for venlafaxine in the thinlip mullet, which a mean concentration in the brain of 88 ng g^{-1}, was the highest reported in our manuscript. Ninety-seven percent of the compounds detected in the brain were also detected in other tissues. This may occur because compounds such as antidepressants generally undergo first-pass metabolism in the human liver (Thompson & Vijayan, 2022). These conditions could be emulated if they enter into the fish organism. Thus, the accumulation of these compounds in tissues other than those for which they were designed could occur. This reinforces our theory that to cover a wider range of contaminants that may affect fish, other tissues should also be analyzed, not only the target organs for which the PhACs were designed.

Plasma ranks third in the literature (*TS1*) as one of the most analyzed matrices in fish studies. The reasons include its ease of extraction because it can do it with acidified solvent and centrifugation (Grabicova et al., 2018; Manjarrés et al., 2022; McCallum et al., 2017); and its low ME. Generally, in plasma, there are enhancement of the analytes (Grabicova et al., 2018; Manjarrés et al., 2022). In this matrix, 26 detections were reported. All compounds detected with a confidence level of 1 (7 compounds) in the plasma samples (Fig. 3) were quantified in the thinlip mullet, except for sertraline, which was also detected in carp and ebro barbel. Thinlip mullet obtained a higher number of hits compared to the other species for the plasma. We addressed this discussion in section 3.1.

Plasma analysis helps determine potential effects in the fish related to human therapeutic plasma concentrations (HTPCs). This is done by the fish plasma model proposed by Huggett et al. (2010). Sumpter & Margiotta-Casaluci (2022) concluded that compounds such as sertraline (96 ng mL^{-1}), fluoxetine (11 ng mL^{-1}), and loratadine (3.5 ng mL^{-1}) (Fig. 3), are classified as a high pharmacological risk to freshwater fish. I.e., they may reach plasma concentrations high enough to cause pharmacological effects comparable to those observed in humans in a clinical setting. The values calculated here, however, are well below the reported HTPCs.

The low detection frequency in plasma could be explained by the specific accumulation of compounds in other tissues, i.e., the partition coefficient per tissue is higher than in the blood (Miller et al., 2018). Even though it is essential to determine the presence of compounds in the plasma, higher concentrations in tissue relative to plasma do not allow conclusions on local effects (see section 3.2).

Muscle is the most analyzed matrix in the literature about the presence of contaminants in fish. One of the main reasons for studying contaminants in fish muscle, is the possibility that the presence of these types of compounds may affect human health after consuming fish fillets. In addition, the treatment of muscle samples is usually efficient since during the dissection of the fish, it is easier to obtain, and a sufficient amount of sample is guaranteed for analysis. In analytical terms, muscle has a lower ME than other tissues, such as the kidney or liver, because it contains a lower percentage of lipids (Grabicova et al., 2018). Muscle was also reported to be the tissue for which the most significant number of compounds with an acceptable ME (−40%–40%) was calculated (Manjarrés et al., 2022).

In this study, muscle is the tissue with the lowest number of hits reported (22). Of the 6 compounds detected, only sertraline and venlafaxine were confirmed at level 1. The mean concentrations calculated in muscle were the lowest compared to the other tissues. Similar results have been obtained by Grabicova et al. (2018) by simultaneously evaluating the liver, kidney, brain, and plasma. Also, the BCF reported for muscle is lower than other tissues (Grabicová et al., 2020; McCallum et al., 2017). The exclusive analysis of this matrix represents a limitation for distribution and biomonitoring studies of risk factors in fish, as PhACs that tend to have a higher accumulation by a specific tissue may still cause harmful effects. Risk assessments based on accumulation factors only in fish muscle may not accurately describe the risks and avoid clarity on the extent of effects that PhACs may have (Miller et al., 2018).

Finally, we report the presence of acridone in the **bile** of eel, the only species in which it was detected with an average concentration of 1928 ng mL^{-1}. Bile analysis should be prioritized for metabolite screening because contaminants are potentially bio-transformed in fish liver and the metabolites formed are excreted into the intestine through bile (Ondarza et al., 2019), increasing the probability of detection. However, bile is one of the least analyzed matrices in studies of contaminants in fish, maybe due to the problems of obtaining it. Usually, the liver produces bile that is stored in the gallbladder until a bolus passes through the stomach when the bile is expelled into the intestine. Bile salts' intestinal release through the feces results from feeding and digestion. Its main function is to contribute to the digestion of fats. Still, it also allows you to eliminate some waste substances and by-products of various metabolic reactions through the feces (Buchinger et al., 2014). Therefore, it may be that at the time of capture of the fish, the gallbladder is empty, and it is impossible to obtain bile in all individuals.

In summary, Fig. 4 shows the distribution of hits per tissue/fluid highlighting that the highest number of hits are in the kidney, liver, skin and, heart. The results indicate that 72% of all hits were in these four tissues. We emphasize that if the study focuses solely on the analysis of fish muscle or plasma, compounds such as clarithromycin, caffeine, metoprolol, and diazepam detected in one of these four tissues would be out of the discussion. We also highlight the importance of skin analysis as a sentinel organ for detecting compounds with accumulation potential.

We show that it is necessary to extend the analysis to organs that, due to their metabolic or absorption kinetic characteristics, facilitate the accumulation of a wide range of PhACs. Deepening the research in these tissues would make it possible to define a higher number of compounds that can generate effects in fish.

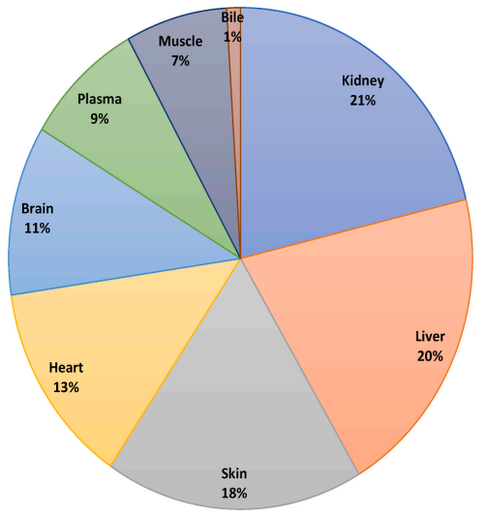

Fig. 4. Summary of the distribution by tissue. Calculated based on the total number of hits reported.

3.3. Tissue distribution of sertraline

In controlled uptake studies with fish exposed to sertraline-containing test medium as well as in samples collected from wastewater-impacted rivers, quantitative analysis of sertraline in liver, kidney and brain indicated high bioaccumulation factors (BAF) in chub (Grabicová et al., 2020) and rainbow trout (Grabicova et al., 2014). In the former species the BAF were 69–310 (kidney) and 44–330 (liver), while in the latter they are 85 (liver) and 180 (brain). Unfortunately, no plasma concentrations were reported in the aforementioned studies, either for not being quantifiable or this biofluid being beyond the scope of the work, which precluded the assessment of the extent of drug distribution from the systemic compartment into tissues. From the point of view of pharmacokinetic analysis and modeling, tissue distribution is generally referenced to the drug concentration in plasma (or blood). Given the high detection frequency in the present study of sertraline across species and tissues – only amantadine and bisoprolol were measurable with similar regularity in the suspect screening (Fig. 1) – an ample dataset could be generated, including plasma concentrations, enabling a more detailed description of the fate of sertraline in the investigated species. Normalizing the tissue concentrations to that of plasma allowed to calculate relevant partition coefficients: 31.2 for liver, 25.9 for kidney, 9.0 for brain, and 2.8 for muscle. These values, which reflect the interaction of sertraline with tissue components at the molecular level, are intimately linked to its physicochemical properties: a lipophilic drug of moderate size with a strongly basic nitrogen atom that is protonated at physiological pH. While on the one hand the lipophilicity facilitates rapid diffusion across cell membranes and thus efficient tissue penetration (Xie et al., 2015), non-specific binding of the positively charged molecule to the phospholipid bilayer of cell membranes results in tissue/plasma concentration ratios above unity. A plausible reason for the rank order of liver ~ kidney > brain > muscle is the expected difference in tissue-specific phospholipid composition (Murakami & Yumoto, 2011). As demonstrated for laboratory animals, the former two tissues contain higher amounts of anionic phosphatidylserine which enhances the non-specific binding of basic drugs. By contrast, muscle tissue contains lower concentrations of phospholipids and thus commonly displays lower partition coefficients for basic molecules. As far as the distribution of sertraline across the blood-brain barrier (BBB) is concerned, its relatively small size (molecular weight: 306 Da) and a low polarity-driving heteroatom count ($C_{12}H_{17}Cl_2N$) translate into small polar surface area with which ultimately favor efficient crossing of the BBB. The lipophilic character of sertraline then contributes to extensive binding to brain fat. Overall, the measured partition coefficients of sertraline in the studied fish species closely mirror the behavior of basic lipophilic drugs in higher species (Björkman, 2002; Jansson et al., 2008). From a toxicological perspective, the observation of high tissue concentration of contaminants like PhACs in fish species is not a warning signal *per se*. In line with the aforementioned explanation, if a large fraction of basic drugs is immobilized through non-specific binding to tissue components, it cannot exert any on-target or off-target effects.

4. Conclusions

We successfully performed the detection of 30 compounds by suspect screening in different tissues and body fluids of fish captured in its natural habitat. Fifteen compounds were quantified with authentic standards in at least one of the matrices. We define additional wild fish tissues in which the most significant number of contaminants from wastewater, especially PhACs, can be detected, such as kidney, liver, skin and heart. By contrast, muscle, which is a frequently chosen matrix due to its ready accessibility and relevance for human consumption, has proven a less sensitive matrix in our screening. As regards the compounds to be included in prioritization lists, sertraline, amantadine, and bisoprolol were detected in almost all the matrices with high detection frequency. From a risk assessment perspective, analyte concentrations in a given matrix should not be interpreted solely based on total abundance, but should consider that a considerable fraction of the contaminant is present in bound form in non-target tissues as has been discussed for sertraline.

Credit author statement

Diana P. Manjarrés-López: Methodology, lab work, writing - Original Draft, **Juan Manuel Peña Herrera:** lab work, Validation, **Lluís Benejam**: Conceptualization, sampling, **Nicola Montemurro:** Methodology. Validation and writing, **Sandra Pérez:** Conceptualization, Supervision, Project administration, Writing - Review & Editing, 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.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.envpol.2023.121374.

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INFORMACIÓN COMPLEMENTARIA
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Assessment of wastewater-borne pharmaceuticals in tissues and body fluids from riverine fish

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Figure S1. Workflow for suspect screening analysis

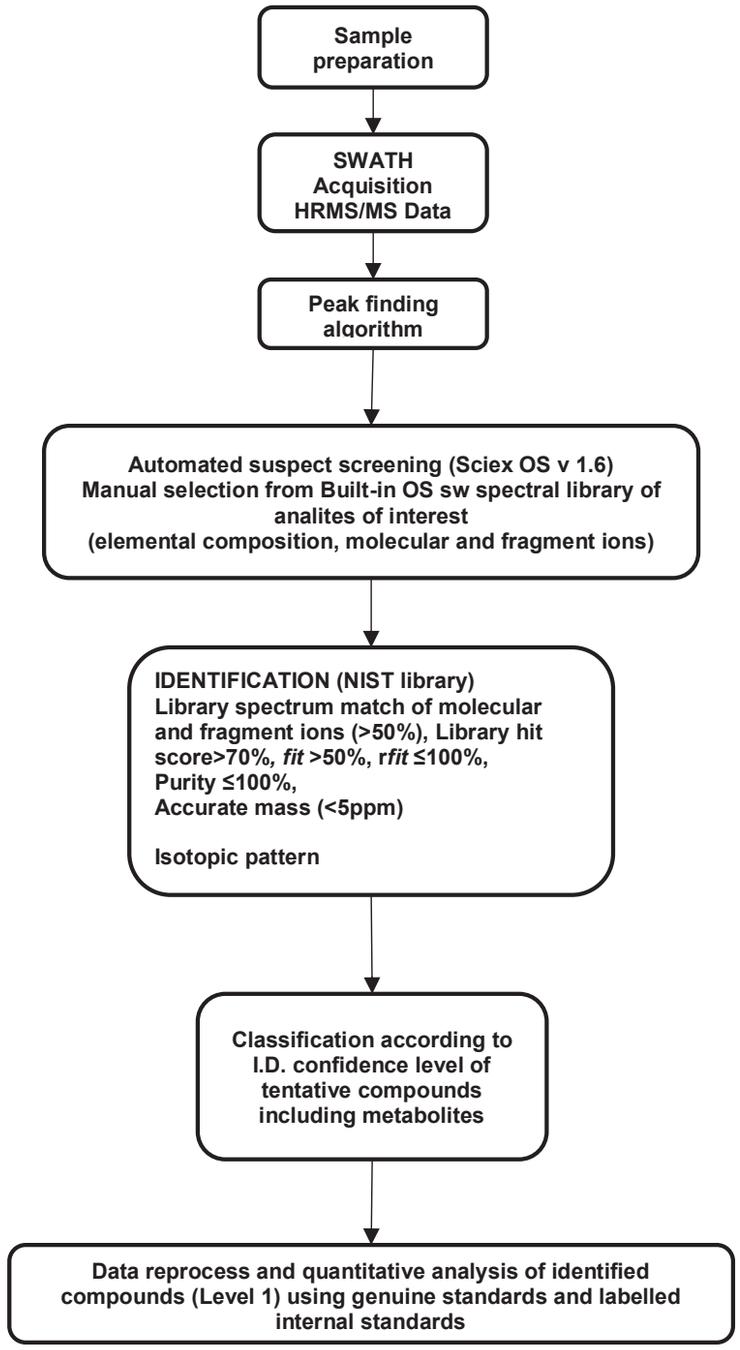

Table S1. The most common PhACs detected in fish tissues and body fluids

Matrix	Compound	Concentration (ng g^{-1})	Ref.
Bile*	Acetaminophen	39	[1]
	Bezafibrate	10.8	[1]
	Bisphenol A	0.89	[2]
	Butylparaben	55	[1]
	Carbamazepine	5	[1]
	Ciprofloxacin	51.3	[3]
	Diclofenac	3.38	[2]
	Enrofloxacin	334	[3]
	Irbesartan	24	[1]
	Lincomycin	58.6	[3]
	Methocarbamol	180	[4]
	Norfloxacin	83.9	[3]
	Ofloxacin	37.8	[3]
	Rosuvastatin	20.2	[4]
	Sulfamethazine	49.4	[3]
	Sulfamethoxazole	<2.20-20.4	[4][3]
	Temazepam	4940	[4]
	Trimethoprim	4.01	[3]
Brain (d)	Acetaminophen	10	[1]
	Amitriptyline	58	[1]
	2-hidroxi-carbamazepine	218	[5]
	Bezafibrate	20	[1]
	Bupropion	1.6	[6]
	Butylparaben	12	[1]
	Caffeine	278.6	[6]
	Carbamazepine	1.6-3182	[1][6][5]
	Ciprofloxacin	36.4	[6]
	Citalopram	3.3	[6]
	Diphenhydramine	7.3	[6]
	Irbesartan	28	[1]
	Iopamidol	1058.4	[6]
	Norfluoxetine	33.9	[6]
	Norsertraline	400.1	[6]
	Phenytoin (3ilantin)	17.2	[6]
	Sertraline	16.9	[6]
	Sulfadiazine	6	[1]

Brain (w)	17-α-ethinylestradiol	23.4	[7]
	2-hidroxi-carbamazepine	48	[5]
	Amitriptyline	7.5	[8]
	Azithromycin	1.2	[9]
	Bezafibrate	0.10	[10]
	Bupropion	22	[11]
	Caffeine	5.2-9.7	[12][13]
	Carbamazepine	0.1-701	[14][7][9][11][12][5][10][13]
	Citalopram	<1-4.6	[8][9][15][12]
	Codeine	0.5-1.7	[13]
	Clofibric acid	0.13	[10]
	Clotrimazole	7.2	[11]
	Crotamiton	1.7	[10]
	Desmethylsertraline	2.08-15.6	[16][17]
	Diclofenac	0.32-3.6	[7][10][14]
	Diltiazem	0.2-1.5	[14][11]
	Diphenhydramine	6.2-25.7	[10][14][11]
	Donezepil	0.14	[9]
	Duloxetine	1.6	[13]
	Ethenzamide	0.09	[10]
	Erythromycin	3.7	[7]
	Fenofibric acid	0.26	[10]
	Fluoxetine	0.08-5.7	[16][17][8]
	Haloperidol	0.3	[14]
	Ibuprofen	1.5	[10]
	Indomethacin	0.35-4.4	[10][14][18]
	Losartan	0.05	[10]
	Mefenamic acid	0.21-0.9	[10][14]
	Methocarbamol	1.48	[4]
	Mirtazapine	0.7	[12]
	N-Desmethylcitalopram	1.0	[12]
	Norclozapine	9.4	[8]
	Nordiazepam	<1	[8]
	Norfluoxetine	8.86-14	[8][17]
	Norsertraline	7.8-459	[8][10][12][14]
	N.N-diethyl-3- toluamide	3.2	[10]
	Oxazepam	73-147	[19]
	Paroxetine	0.19-1	[16][8]
	Propranolol	0.1	[7]
	Risperidone	0.1-0.3	[13]
	Roxithromycin	28.1	[7]
	S-citalopram	0.18	[16]

	Sertraline	0.21-21	[8][10][20][16][17][9]
	Sulfamethoxazole	7.22	[4]
	Telmisartan	0.35	[9]
	Temazepam	15.1	[4]
	Topiramate	1.3-207	[13]
	Tramadol	8.9-200	[12][21]
	Triclosan	0.51-1.3	[10]
	Trihexyphenidyl	0.11-0.14	[13]
	Venlafaxine	0.43-13	[8][9][15][11][16][13]
Gills (d)	2-hidroxi-carbamazepine	243	[5]
	Acetaminophen	9	[1]
	Amitriptyline	16	[1]
	Bezafibrate	28	[1]
	Butylparaben	73	[1]
	Carbamazepine	14-1731	[1][5]
	Epoxi-carbamazepine	175	[5]
	Irbesartan	35	[1]
Gills (w)	Benzoylecgonine	1.6	[22]
	Bezafibrate	0.2	[14]
	Caffeine	6.7	[22]
	Carbamazepine	0.1-407	[18][7][14][5]
	Crotamiton	2.4	[14]
	Diclofenac	1.5-6.67	[7][4][14]
	Diltiazem	0.07	[14]
	Diphenhydramine	3.0	[14]
	Epoxi-carbamazepine	41	[5]
	Erythromycin	2.0-25.7	[22][7][23]
	Haloperidol	0.3	[14]
	Ibuprofen	100.7	[24]
	Indomethacin	21.0	[14]
	Methocarbamol	1.1	[4]
	Norfluoxetine	3.3	[22]
	Propranolol	0.1	[7]
	Rosuvastatin	0.53	[4]
	Roxithromycin	6.1	[7]
	Sulfamethoxazole	1.05-1.9	[4][22]
	Sulfathiazole	2.1	[22]
	Temazepam	5.81	[4]
	Trimethoprim	5.5	[22]
	17-α-ethinylestradiol	11.4	[7]

Gonads (d)	Acetaminophen	4.7	[6] [6]
	Bupropion	13.5	[6]
	Caffeine	140.1	[6]
	Carbamazepine	6.6	[6]
	Citalopram	1.7	[6]
	Diphenhydramine	2.4	[6]
	Erythromycin	28.7	[6]
	Iopamidol	749.8	[6]
	Norfluoxetine	12.7	[6]
	Norsertraline	42.2	[6]
	Phenytoin (6ilantin)	0.4	[6]
	Sertraline	6.7	[6]
	Venlafaxine	5.5	[6]
Gonads (w)	Bupropion	0.8	[11]
	Carbamazepine	1.3	[11]
	Diltiazem	1.3	[11]
	Diphenhydramine	8.2	[11]
	Donepezil	0.5	[11]
	Flecainide	0.2	[11]
	Venlafaxine	1.9	[11]
Gut (d)	2-hidroxi-carbamazepine	221	[5]
	Carbamazepine	1655	[5]
Gut (w)	Carbamazepine	372	[5]
	Methocarbamol	3.95	[4]
	Rosuvastatin	4.94	[4]
	Sulfamethoxazole	7.61	[4]
	Temazepam	139.5	[4]
Kidney (w)	Azithromycin	11.0	[9]
	Bezafibrate	10.2-12	[18][12]
	Bisoprolol	14	[12]
	Carbamazepine	0.2-0.91	[9][14][12]
	Citalopram	3.1-110	[9][12][25]
	Clarithromycin	0.6	[12]
	Clindamycin	0.6	[12]
	Clomipramine	0.4-1.8	[12][25]
	Crotamiton	2.9	[14]
	Diclofenac	1.6-5.6	[14][12]
	Diiltiazem	1.9	[14]
	Diphenhydramine	58.9	[14]
	Donepezil	0.3-0.13	[12][9]
	Epoxi-carbamazepine	0.2	[12]

Kidney (w)	Erythromycin	1.7	[18]
	Gemfibrozil	1.2	[18]
	Haloperidol	0.17-9.5	[9][14][25]
	Hydroxyzine	0.34	[25]
	Ibuprofen	142.3	[24]
	Indomethacin	11.3	[14]
	Levamisole	11	[12]
	Levomepromazine	8.1	[25]
	Mefenamic acid	7.2	[14]
	Metoprolol	0.5	[12]
	Metoprolol acid	15.0	[9]
	Mianserin	3.8-14	[25][12]
	Mirtazapine	0.3-95	[12][25]
	N-Desmethylcitalopram	13.0	[9]
	Norsertraline	61.6	[14]
	O-desmethyelvenlafaxine	1.5-1.6	[9][12]
	Paroxetine	9.5	[25]
	Propranolol	1.2	[18]
	Sertraline	1-44	[20][12][9][14][25]
	Telmisartan	0.1-8.7	[12][9]
	Tramadol	2.6-6.3	[9][25][12]
	Venlafaxine	2.2-5.3	[9][25][12]
	Verapamil	0.96	[9]
Liver (d)	2-hidroxi-carbamazepine	335	[5]
	Acetaminophen	2	[1]
	Amitriptyline	18	[1]
	Bezafibrate	41	[1]
	Bupropion	1.0	[6]
	Butylparaben	214	[1]
	Caffeine	869.7	[6]
	Carbamazepine	2.6-2154	[1][5][26]
	Citalopram	3.7	[6]
	Clopidogrel	1.2	[27]
	Diclofenac	22-47	[27][26]
	Diphenhydramine	3.8-7.8	[6][27]
	Erythromycin	46.9	[6]
	Flecainide	18	[27]
	Irbesartan	53	[1]
	Levamisole	0.5	[26]
	Lidocaine	3.6	[27]
	Metformin	1.5	[6]
	Norlidocaine	15.3	[27]

	Norsertraline	647.3	[6]
	Sulfadiazine	0.9	[1]
	Tramadol	15	[27]
	Venlafaxine	56.8	[6][26]
Liver (w)	17-α-ethinylestradiol	57.1	[7]
	2-Hidroxi-carbamazepine	107	[5]
	Amitriptyline	0.29	[16]
	Atenolol	0.2-1.9	[9][12]
	Azithromycin	4.4-169.8	[12][9][28]
	Benzoylecgonine	0.5	[22]
	Bezafibrate	0.10	[10]
	Bupropion	0.1-0.4	[11][13]
	Caffeine	3.4-13	[12][22][13]
	Carbamazepine	0.11-688	[29][30][9][10][7][14][12][5][27]
	Cetirizine	0.1	[12]
	Chlortetracycline	326	[3]
	Ciprofloxacin	2.44	[3]
	Citalopram	3.0-31	[9][12][15][25]
	Clindamycin	0.1	[12]
	Clofibric acid	0.13	[10]
	Clomipramine	0.2-1.2	[12][25]
	Clotrimazole	1.0	[11]
	Codeine	0.6-1.6	[13]
	Crotamiton	4.2-4.3	[10][14]
	Desmethyl-citalopram	2.6	[12]
	Desmethylsertraline	10.29-600	[16][17][30]
	Diazepam	10	[30]
	Diclofenac	0.32-8.6	[7][10][14]
	Diltiazem	0.5-1.0	[30][11][14]
	Diphenhydramine	6.5-64	[10][11][30][14]
	Donezepil	0.21	[9]
	Duloxetine	1.7-3.0	[13]
	Enrofloxacin	56.7	[3]
	Erythrohydrobupropion	73.7	[28]
	Erythromycin	5.6-2390	[22][7][3]
	Ethenzamide	0.09	[10]
	Fenofibric acid	0.26	[10]
	Fipronil	9.41	[31]
	Fipronil sulfone	19.91	[31]
	Fluoxetine	0.20-86	[17][30][16]
	Gemfibrozil	34	[30]
	Haloperidol	0.10-6.8	[9][25][12][14][13]
	Hydroxyzine	1.2-1.4	[25][13]
	Ibuprofen	1.5-114.2	[10][24]
	Indomethacin	1.3-5.4	[10][14]
	Levamisole	0.2	[12]
	Levomepromazine	11	[25]
	Lincomycin	114.1-1384	[28][3]
	Loperamide	1.8	[12]
	Lorazepam	5.3	[13]
	Losartan	0.05	[10]
	Mefenamic acid	4.9-19.2	[10][14]
	Methocarbamol	2.63	[4]
	Metoprolic acid	12	[12]
	Metoprolol	0.4	[12]
	Metoprolol acid	8.1	[9]
	Mianserin	2	[13]
	Mirtazapine	0.3-4.7	[12][25]
	N-Desmethylcitalopram	1.2	[9]
	N.N-diethyl-3- toluamide	9.2	[10]
	Norfloxacin	7.67	[3]
	Norfluoxetine	0.63-110	[16][22][17][30]
	Norsertraline	6.4-54	[10][14]
	O-Desmethyelvenlafaxine	0.74-1.9	[9][12]
	Ofloxacin	9.85	[3]
	Orphenadrine	0.3	[12]
	Oxazepam	6.7-439	[19]-[13]
	Paroxetine	0.35-13	[16][25][30]
	Propranolol	0.4-1.84	[7][27]
	Risperidone	0.1-0.6	[13]
	Rosuvastatin	0.67	[4]
	Roxithromycin	9.94-41.2	[7][23]
	S-citalopram	0.41	[16]
	Sertraline	0.29-110	[20][9][10][12][16][17][18][15][14][25][30]
	Sulfamethazine	0.1-3.12	[12][3]
	Sulfamethoxazole	0.4-41.7	[12][22][4][28][3]
	Sulfathiazole	2.4	[22]
	Telmisartan	1.6-6.2	[9][12]
	Temazepam	25.5	[4]
	Tramadol	1.2-3.2	[9][12][25]
	Triclosan	110-910	[10]
	Triclocarban	1.1-4.3	[10]
	Trihexyphenidyl	0.11-0.12	[13]
	Trimethoprim	0.4-2.8	[12][22][3]
	Venlafaxine	0.69-20.8	[9][12][25][11][15][16][13]
	Verapamil	0.2-0.77	[9][12]
	2-hidroxi-carbamazepine	0.3-283	[5][26]
	10,11-EpoxyCBZ	0.2	[26]

Muscle (d)	Acetaminophen	3	[1]
	Amitriptyline	7.1	[1]
	Azithromycin	0.05	[32]
	Bezafibrate	6.2	[1]
	Bupropion	<LOQ	[6]
	Butylparaben	73	[1]
	Caffeine	60.7-69	[6]
	Carazolol	1.7	[26]
	Carbamazepine	3.8-1775	[1][33][6][5][26]
	Citalopram	0.4-2.4	[33][6]
	Clarithromycin	0.23	[32]
	Clopidogrel	0.2-8.0	[33][27][26]
	Diazepam	1.91	[33][26]
	Diclofenac	0.7-12	[27][33][26]
	Diphenhydramine	0.5-3.9	[6][27]
	Epoxi-carbamazepine	246	[5]
	Erythromycin	2.9	[6]
	Flecainide	3.4	[27]
	Hydrochlorothiazide	1.1-10.5	[33][26]
	Irbesartan	14	[1]
	Lidocaine	3.9	[27]
	Metoprolol	0.7	[26]
	Nadolol	0.6-2.3	[33][26]
	Norfluoxetine	1.8	[6]
	Norlidocaine	9.6	[27]
	Norsertraline	66.5	[6]
	OH-carbamazepine	2.5	[33]
	Phenytoin (dilantin)	1.2	[6]
	Propranolol	0.4-0.5	[33][26]
	Roxithromycin	1.12	[32]
	Salbutamol	0.6-6.5	[33][26]
	Sertraline	17.1	[33]
	Sotalol	37.5	[33]
	Tetracycline	5.63	[32]
	Tilmicosin	0.42	[32]
	Tramadol	8.6	[27]
	Trimethoprim	0.7	[6]
	Tylosin	0.24	[32]
	Sulfadimethoxine	0.02	[32]
	Sulfamethoxazole	0.12	[32]
	Sulfisoxazole	0.09	[32]
	Venlafaxine	0.4-22.9	[6][33]

Muscle (w)	10.11-epoxyCBZ	0.63	[34]
	17-α-ethinylestradiol	2.4	[7]
	17-β-ethinylestradiol	0.31 - 0.91	[35]
	2-Hidroxi-carbamazepine	69	[5]
	2-HydroxyCBZ	10.2	[34]
	Acetaminophen	2.88	[36]
	Amoxicillin	0.32 - 1.23	[35]
	Ampicillin	<LOD	[37]
	Atenolol	0.61	[34]
	Bezafibrate	<LOQ	[38]
	Benzophenone	1.28	[36]
	Benzoylecgonine	0.9	[22]
	Bisphenol A	0.92-8.94	[35][2]
	Bupropion	-0.10.2	[11][13]
	Caffeine	4.58-69	[22][38][36][13]
	Carazolol	0.21	[34]
	Carbamazepine	0.2-430	[7][30][34][39][14][5][9]_[38]
	Chlortetracycline	580	[37]
	Citalopram	0.95-1.3	[12][9]
	Ciprofloxacin	1.26	[3]
	Clarithromycin	0.1	[12]_[38]
	Clopidogrel	0.51	[34]
	Codeine	0.7-1.1	[34]
	Desmethyl-sertraline	0.42-12	[16][17][30]
	Diazepam	0.19	[30][34]
	Diclofenac	0.5-10.76	[7][4][14][35][23]
	Diltiazem	34	[30][39][14][11][38]
	Diphenhydramine	0.31-1.32	[30][11][39][14]
	Enoxacin	4.5	[40]
	Enrofloxacin	6.96	[3]
	Epoxi-carbamazepine	60	[5]
	Erythromycin	1.9-10	[7][22][40][3]
	Estrone	0.05-1.18	[2]
	Fipronil	0.13	[31]
	Fipronil-desulfinyl	0.07	[31]
	Fipronil sulfone	4.05	[31]
	Flecainide	0.2	[11]
	Flumequine	9	[40]
	Fluoxetine	0.09-0.11	[16][17]
	Furazolidone	49	[38]
	Galaxolide	0.71	[36]
	Haloperidol	0.05	[14]
	Hydrochlorothiazide	5.98	[34]
	Ibuprofen	21.1	[24]

Muscle (w)	Indomethacin	1.4	[14]
	Ketoprofen	<LOQ	[38]
	Ketoconazole	5.0-6.57	[7][23]
	Lincomycin	26.2	[3]
	Lorazepam	0.73	[34]
	Mefenamic acid	0.09	[14]
	Methocarbamol	0.95	[4]
	Metoprolol	2.4	[34]
	Mianserin	1.1	[13]
	Nadolol	0.49	[34]
	Naproxen	1.29	[2]
	N-desmethylcitalopram	0.21-0.3	[12][9]
	Norfluoxetine	3.9-0.27	[16][39][17][22]
	Norsertraline	11.8	[14]
	Ofloxacin	16.8	[3]
	Oxazepam	47-192	[19]
	Paroxetine	<MDL	[16][30]
	Primidone	0.45 - 1.05	[35]
	Progesterone	0.81 - 1.90	[35]
	Propranolol	1.9	[7][34]
	Risperidone	0.1-0.4	[13]
	Roxithromycin	3.9	[7]
	Salbutamol	0.28	[34]
	Sertraline	0.08-0.69	[16][17][12][14][9]
	Sotalol	0.15	[34]
	Sulfadiazine	20-50	[37][40]
	Sulfamerazine	<LOD	[37]
	Sulfamethazine	8.0	[3]
	Sulfamethoxazole	0.27-1.9	[4][22][35][3]
	Sulfathiazole	2.1	[22]
	Sulfapyridine	9.7	[38]
	Temazepam	5.69	[4]
	Terbinafine	0.1	[12]
	Testosterone	0.33 - 0.70	[35]
	Triclosan	0.73-50	[2][41]
	Trihexyphenidyl	0.11-0.14	[13]
	Trimethoprim	0.39-26	[22][42][38][40][3]
	Verapamil	35	[38]
	Venlafaxine	0.034-2.5	[12][34][11][9][13]
	Acetaminophen	< MDL	[1]
	Amitriptyline	0.15–15	[1][8][43]
	Amlodipine	<LOQ-0.20	[44]
	Aripiprazole	<LOD-0.087	[44]

Plasma	Benztropine	0.41	[43]
	Bezafibrate	0.14-57	[1][10]
	Bisphenol A	6.21-1760	[2][45]
	Bupropion	3.8	[11]
	Butylparaben	27	[1]
	Caffeine	2.2-5.05	[12][43]
	Carbamazepine	0.0089-18	[1][9][12][14][45][10][44]
	Chlorpheniramine	<LOD-0.15	[44]
	Chlopromazine	<LOD-0.050	[44]
	Citalopram	0.16-8.0	[9][12][11][8][43]
	Ciprofloxacin	<4.55	[3]
	Clarithromycin	0.09	[12]
	Clindamycin	0.1	[12]
	Clofibric acid	0.13	[10]
	Clopidogrel	0.08	[27]
	Clozapine	< 0.2	[8]
	Crotamiton	1.5-1.6	[10][14]
	DEET	1.15	[43]
	Diazepam	33.7	[43]
	Diclofenac	0.15-25.20	[10][12][14][2][45][46][27][44]
	Diltiazem	0.05-42.73	[14][11][47]
	Diphenhydramine	0.19-148.9	[10][14][11][27][47][48][43][44]
	Donepezil	4.2	[11]
	Enrofloxacin	34.7	[3]
	Erythromycin	0.79	[43]
	Estrone	7.52	[2]
	Ethenzamide	0.03	[10]
	Fenofibric acid	0.03	[10]
	Fexofenadine	<LOD-0.14	[44]
	Flecainide	0.2-0.63	[11][27]
	Flumequine	1.37	[43]
	Fluoxetine	0.33-7.13	[45][43]
	Gemfibrozil	0.028-235	[2][45][8][43][44]
	Haloperidol	0.011-0.04	[14][44]
	Ibuprofen	0.24-314.8	[10][2][24][45][43][44]
	Indomethacin	0.83-5.7	[10][14][44]
	Iopamidol	20.9	[43]
	Irbesartan	88	[1]
	Lincomycin	567	[3]
	Losartan	0.021	[10]

Plasma	Lomefloxacin	<5.30	[3]
	Mefenamic acid	0.13-1.2	[10][14][44]
	Metoprolic acid	0.1	[12]
	Metoprolol	0.1	[12]
	Metoprolol acid	0.15	[9]
	Naproxen	0.23-279	[2][45][46]
	N-desmethylcitalopram	0.1	[12]
	N.N-diethyl-3- toluamide	1.7	[10]
	Noramitriptyline	0.3	[8]
	Norclozapine	< 0.33	[8]
	Nordiazepam	0.55	[8]
	Norfluoxetine	0.43–0.83	[8]
	Norlidocaine	< 0.90	[27]
	Norsertraline	0.57-15	[8][10][14][44]
	O-desmethyelvenlafaxine	0.16-0.2	[9][12]
	Ofloxacin	11.9	[3]
	Oxazepam	18-313	[19]
	Paroxetine	0.17	[8]
	Phenytoin	<LOD-0.048	[44]
	Quetiapine	0.1	[8]
	Risperidone	0.65	[45]
	Salbutamol	0.09	[46]
	Sertraline	0.11-2.26	[8][10][14][45][43][44]
	Sulfamethazine	0.17-144	[43][3]
	Sulfamethoxazole	0.061-5.50	[3][44]
	Telmisartan	0.086-1.7	[9][12][44]
	Tramadol	0.038-46	[12][9][21][44]
	Triclocarban	0.12–3.35	[10][43][44]
	Triclosan	0.47-110	[10][2][45][44]
	Trimethoprim	0.17-613	[27][46][3]
	Valsartan	1.23	[43]
	Venlafaxine	0.03-3.3	[8][9][12][11][15][43]
	Zotepine	<LOD-0.10	[44]
Plasma*	Carbamazepine	1.8	[49]
	Desmethylsertraline	7.2	[49]
	Diclofenac	<MDL	[49]
	Diltiazem	0.15	[49]
	Dyphenidramine	25	[49]
	Methocarbamol	1.25	[4]
	Norfluoxetine	13	[49]
	Rosuvastatin	2.15	[4]
	Temazepam	7.92	[4]
Viscera	Chlortetracycline	590	[37]
	N^4-acetylsulfamethazine	20	[37]
	Oxytetracycline	50	[37]
	Sulfadiazine	190	[37]
	Sulfamerazine	70	[37]

concentration are in ng mL^{-1}. (w): wet weight. (d): dry weight.

*Concentration in µg Kg^{-1}

Table S2. Compound MS parameters for the quantification in SWATH mode

PhACs	Molecular formula	MW	$(M+H)^+$	Confirming product ion $(M+H)^+$	RT (min)	Labelled Internal Standard (IS)	Coefficient of determination (r^2)
Acetaminophen	$C_8H_9NO_2$	151.163	152.07060	110.0598	0.48	Acetaminophen-d4	0.9963
Acridone	$C_{13}H_9NO$	195.217	196.07572	167.07340	4.28	Carbamazepine-d10	0.9972
Caffeine	$C_8H_{10}N_4O_2$	194.191	195.08770	138.06550	1.13	Trimethoprim-d3	0.9931
Carbamazepine	$C_{15}H_{12}N_2O$	236.269	237.10226	194.09490	5.31	Carbamazepine-d10	0.9980
Clarithromycin	$C_{38}H_{69}NO_{13}$	747.953	748.48417	158.11740	6.87	Diazepam-d5	0.9933
Codeine	$C_{18}H_{21}NO_3$	299.364	300.15943	215.10670	0.63	Codeine-d3	0.9976
Diazepam	$C_{16}H_{13}ClN_2O$	284.740	285.07896	154.04130	7.30	Diazepam-d5	0.9989
Diltiazem	$C_{22}H_{26}N_2O_4S$	414.518	415.16862	178.03050	5.53	Carbamazepine-d10	0.9957
Fluoxetine	$C_{17}H_{18}F_3NO$	309.326	310.14136	148.11180	6.29	Diazepam-d5	0.9961
Ketoprofen	$C_{16}H_{14}O_3$	254.281	255.10160	105.03280	7.05	Diazepam-d5	0.9956
Loratadine	$C_{22}H_{23}ClN_2O_2$	382.883	383.15211	337.11150	7.80	Bezafibrate-d4	0.9905
Metoprolol	$C_{15}H_{25}NO_3$	267.364	268.19074	133.06570	2.23	Metoprolol-d7	0.9974
Sertraline	$C_{17}H_{17}Cl_2N$	306.230	306.08109	158.97650	6.76	Diazepam-d5	0.9946
Sotalol	$C_{12}H_{20}N_2O_3S$	272.364	273.12678	133.07660	0.34	Codeine-d3	0.9976
Trimethoprim	$C_{14}H_{18}N_4O_3$	290.318	291.14518	230.11690	1.32	Trimethoprim-d3	0.9975
Venlafaxine	$C_{17}H_{27}NO_2$	277.402	278.21149	58.06560	3.32	Venlafaxine-d6	0.9987

Table S3. General information about fish samples

Species	*Anguilla anguilla*			*Liza ramada*			*Cyprinus carpio*			*Luciobarbus graellsi*		
Sample	**3**	**2**	**1**	**2**	**1**	**3**	**3**	**1**	**2**	**3**	**2**	**1**
Length (cm)	58	46	48	41.5	36.5	49	54	53	51	27	28	23
Weight (g)	470	172	149	900	620	1530	3260	2420	2420	330	330	180
Skin (g)	10.05	4.87	6.61	7.10	5.45	10.22	23.59	19.89	14.66	4.02	4.00	2.93
Gills (g)	1.36	0.35	0.73	5.42	3.79	6.78	19.61	10.35	12.92	1.78	2.02	0.88
Plasma (µL)	500	500	500	270	500	500	500	500	500	500	500	500
Muscle (g)	24.00	19.62	23.45	15.86	13.24	14.65	20.65	14.86	21.68	18.46	17.95	14.58
Liver (g)	7.93	3.94	2.10	18.71	19.28	21.91	34.04	27.58	15.74	7.25	4.29	4.46
Kidney (g)	1.79	1.23	1.16	1.18	1.61	2.59	4.30	2.98	3.46	1.73	0.87	1.16
Brain (g)	0.48	0.13	0.17	1.44	1.19	3.16	7.25	6.23	3.78	0.87	0.63	0.46
Bile (µL)	600	500	400	500	500	500	300	600	1000	500	250	200
Heart (g)	0.34	0.34	0.29	1.53	1.38	2.33	6.85	7.81	7.53	0.38	0.41	0.27
Age (years)	8-10	7-9	7-9	4-5	4-5	6-7	7-8	7-8	7-8	7-8	7-8	6-7

Table S4. Validation parameters by tissue

PhACs	Recovery (ME) (%)						iLOD (ng g^{-1})	iLOQ (ng g^{-1})
	Liver	Kidney	Skin	Brain	Heart	Muscle	Bile	Plasma		
Acetaminophen	145 (-80)	32 (-88)	51 (-66)	35 (-19)	31 (-71)	27 (-12)	98 (-83)	113 (-30)	0.6 - 1.7	1.9 - 5.2
Acridone	70 (-46)	76 (-68)	75 (-30)	71 (-18)	57 (-56)	70 (-47)	82 (-47)	105 (-13)	0.004 - 0.8	0.01 - 2.3
Caffeine	75 (67)	68 (45)	77 (83)	74 (62)	70 (-41)	73 (-40)	100 (-58)	108 (52)	0.1 - 1.7	0.3 - 5.3
Clarithromycin	66 (-78)	109 (-84)	86 (-88)	60 (-28)	63 (-68)	70 (-67)	81 (-96)	108 (-35)	0.01 - 1.9	0.03 - 5.8
Codeine	60 (-27)	52 (-56)	71 (-52)	63 (7)	61 (-57)	68 (-45)	101 (-52)	111 (4)	0.1 - 1.7	0.2 - 5.3
Diazepam	64 (-76)	93 (-81)	74 (-85)	86 (-53)	55 (-54)	68 (-69)	101 (-48)	104 (-27)	0.01 - 2.3	0.03 - 6.9
Diltiazem	51 (-83)	80 (-81)	82 (-85)	58 (-18)	45 (-57)	69 (-63)	<20 (-100)	109 (-10)	0.03 - 0.6	0.1 - 2
Fluoxetine	48 (-97)	70 (-94)	118 (-93)	42 (-69)	46 (-89)	62 (-86)	79 (-70)	89 (6)	0.1 - 1.9	0.3 - 5.8
Ketoprofen	69 (59)	76 (-61)	81 (-62)	67 (84)	59 (-43)	66 (-45)	99 (-5)	109 (70)	0.1 - 1.7	0.2 - 5
Loratadine	34 (-96)	61 (-24)	91 (-97)	<20 (-80)	59 (-88)	58 (-88)	101 (-14)	115 (-36)	0.1 - 3.6	0.4 - 10.8
Metoprolol	69 (15)	75 (-9)	75 (-64)	72 (24)	72 (-42)	75 (-47)	104 (-78)	111 (17)	0.1 - 1.3	0.2 - 3.9
Sertraline	64 (-53)	48 (-86)	102 (-98)	66 (-38)	59 (-87)	99 (-83)	98 (109)	105 (147)	0.03 - 2.8	0.1 - 8.5
Sotalol	64 (-22)	76 (-55)	75 (-47)	76 (11)	72 (-58)	72 (-51)	124 (-71)	116 (2)	0.1 - 1.1	0.4 - 3.4
Trimethoprim	62 (-52)	77 (-76)	73 (-75)	70 (-11)	64 (-62)	72 (-59)	134 (-74)	106 (-12)	0.1 - 1.8	0.3 - 5.5
Venlafaxine	58 (-43)	87 (-55)	74 (-52)	57 (-1)	65 (-53)	71 (-66)	67 (-100)	120 (-22)	0.02 - 1.2	0.1 - 3.7

Table S5. Confirmation values for compounds with a confidence level of 2.a

CASRN	Name	RT	Specie	Tissue	Library Score	Fit	Rev. Fit	Purity
768-94-5	Amantadine	1.1	*Cyprinus carpio*	Brain	91.1	94.2	100	94.2
				Kidney	91.5	98.2	100	98.2
				Skin	90.5	74.3	99.2	73.3
				Liver	94.2	98.4	100	98.4
				Heart	95.2	88.3	100	88.3
				Muscle	90.9	98.5	98.5	96.5
			Chelon ramada	Brain	92.9	94.6	100	94.6
				Kidney	92.7	98.3	100	98.3
				Skin	90.4	84.0	100	84
				Liver	91.2	93.5	99.5	93.1
				Heart	93.4	98.3	97.9	95.5
				Muscle	93.5	93.4	98.4	89.6
				Plasma	95.1	87.0	94.9	71.3
			Anguilla anguilla	Kidney	98.1	67	100	67
				Skin	95.6	66.3	100	66.3
				Liver	94.2	85.5	100	85.5
				Muscle	94.4	67.0	100	67
			Barbus graellsii	Brain	94.1	84.3	96.7	72.8
				Kidney	93.1	98.4	100	98.4
				Skin	97.6	68.8	100	68.8
				Liver	93.4	98.4	100	98.4
93413-62-8	O-Desmethylvenlafaxine	1.7	*Chelon ramada*	Plasma	88.8	95.8	68.0	52.1
99614-02-5	Ondansetron	3.1	*Chelon ramada*	Kidney	96.9	96.8	97.3	92.4
				Liver	90.6	96.6	96.2	90.3
			Anguilla anguilla	Kidney	90.1	87.3	86.8	54.5
63590-64-7	Terazosin	3.3	*Cyprinus carpio*	Kidney	95.6	100	96.5	96.5
66722-44-9	Bisoprolol	3.6	*Cyprinus carpio*	Brain	84.7	81.1	100	81.1
				Kidney	93.3	99.5	100	99.5
				Skin	96.1	99.5	99.8	99.3
				Liver	96.2	100	98.2	98.2
				Heart	90.8	98.2	100	98.2
				Muscle	84.0	98.6	100	98.6
				Plasma	96.5	98.3	99.3	97.3
			Chelon ramada	Brain	83.2	99.6	98.3	97.7
				Kidney	97.7	99.4	99.7	99.1
				Skin	87.7	99.5	100	99.5
				Liver	95.2	99.5	100	99.5
				Heart	90.7	99.5	99.4	98.9
				Muscle	87.9	98.4	100	98.4
				Plasma	90.8	99.5	100	99.5
			Anguilla anguilla	Kidney	97.9	100	94.1	94.1
				Skin	94.2	100	90.5	90.5
				Liver	94.7	98.5	100	98.5
				Heart	92.0	99.4	100	99.4
				Muscle	91.1	98.5	99.2	97.5
			Barbus graellsii	Kidney	83.9	99.4	100	99.4
				Skin	94.5	80.6	97.6	71.1
				Liver	90.6	98.6	100	98.6
				Heart	98.9	74.9	100	74.9
19794-93-5	Trazodone	4.0	*Anguilla anguilla*	Kidney	95.6	100	97.9	97.9
5786-21-0	Clozapine	4.6	*Cyprinus carpio*	Kidney	90.1	99.3	93.3	91.6
				Liver	90.9	100	86.1	86.1

CASRN	Name	RT	Specie	Tissue	Library Score	Fit	Rev. Fit	Purity
			Chelon ramada	Kidney	97.0	94.8	93.7	82.6
				Liver	94.7	100	92.3	92.3
				Heart	92.8	90.7	93.6	74.4
			Anguilla anguilla	Liver	98.2	91	98.4	86.9
			Barbus graellsii	Brain	94.9	89.3	92.9	70.2
				Kidney	94.9	89.7	88.1	61.3
111974-69-7	Quetiapine	4.9	*Cyprinus carpio*	Brain	95.3	98.4	100	98.4
			Liza ramada	Brain	78.7	58.3	100	58.3
				Liver	89.5	85.4	98.6	80.4
				Heart	84.4	94.2	98.4	90.8
			Anguilla anguilla	Skin	92.1	59.3	100	59.3
				Pancreas	95.0	93.5	100	93.5
				Heart	81.5	56.2	99.6	53.9
			Barbus graellsii	Brain	96.2	85.3	100	85.3
				Liver	87.3	76.4	98.2	68.2
59729-33-8	Citalopram	5.1	*Cyprinus carpio*	Liver	94.2	98.9	57.5	51.9
			Chelon Ramada	Skin	91.2	100	100	100
				Liver	96.5	100	98.5	98.5
				Muscle	94.9	90.6	100	90.6
			Anguilla anguilla	Skin	93.7	94.7	96.3	87.2
62498-67-3	Desmethylcitalopram	5.1	*Liza ramada*	Kidney	96.5	97.7	100	97.7
				Liver	91.4	97.8	99.1	96.5
			Anguilla anguilla	Kidney	83.1	93.0	100	93
				Skin	70.0	85.8	98.3	79.9
55297-95-5	Tiamulin	6.5	*Liza ramada*	Brain	99.3	93.2	90.4	72.9
				Skin	94.5	100	80.7	80.7
				Muscle	88.4	97	82.2	71
144701-48-4	Telmisartan	7.5	*Liza ramada*	Kidney	94.5	100	100	100
			Anguilla anguilla	Liver	96.7	100	100	100
886-50-0	Terbutryn	8.3	*Cyprinus carpio*	Heart	95.5	100	56.7	56.7
				Liver	97.7	99.7	79.5	78
				Skin	92.9	100	97.9	97.9
			Liza ramada	Skin	88.2	98.5	100	98.5
			Anguilla anguilla	kidney	96.7	100	70.5	70.5
115-86-6	Triphenyl phosphate	8.5	*Cyprinus carpio*	Skin	95.3	97.8	100	97.8
			Anguilla anguilla	Skin	86.1	89.6	95.6	77.3
67564-91-4	(2R.6S)-Fenpropimorph	8.6	*Cyprinus carpio*	Brain	90.7	94.6	100	94.6
				kidney	94.4	96.8	100	96.8
			Liza ramada	Heart	98.0	78.1	100	78.1
				kidney	95.2	91.4	100	91.4
			Liza ramada	Liver	95.8	90.9	100	90.9

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4.2.2. PUBLICACIÓN No. 4

Assessment, distribution, and ecological risk of contaminants of emerging concern in a surface water-sediment-fish system impacted by wastewater

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Assessment, distribution, and ecological risk of contaminants of emerging concern in a surface water-sediment-fish system impacted by wastewater

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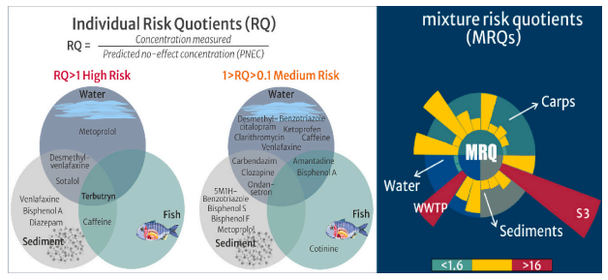
HIGHLIGHTS

- The ecological risk of CECs was assessed using real concentrations in wild fish.
- Caffeine, terbutryn, sotalol, desmethylvenlafaxine, and diazepam pose ecological risks.
- Amantadine, terbutryn and diazepam have bioaccumulation potential in fish organs.
- BCFs from the PBPK model varied 2 log units from experimental BCFs.

GRAPHICAL ABSTRACT



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ABSTRACT

The presence of contaminants of emerging concern in aquatic ecosystems represents an ever-increasing environmental problem. Aquatic biota is exposed to these contaminants, which can be absorbed and distributed to their organs. This study focused on the assessment, distribution, and ecological risk of 32 CECs in a Spanish river impacted by effluents from a wastewater treatment plant, analyzing the organs and plasma of common carp. Environmental concentrations in water and sediment were examined at sites upstream and downstream of the wastewater treatment plant. The two downstream sites showed 15 times higher total concentrations (12.4 μg L^{-1} and 30.1 μg L^{-1}) than the two upstream sites (2.08 μg L^{-1} and 1.66 μg L^{-1}). Half of the CECs were detected in fish organs, with amantadine having the highest concentrations in the kidney (158 ng g^{-1} w.w.) and liver (93 ng g^{-1} w.w.), followed by terbutryn, diazepam, and bisphenol F in the brain (50.2, 3.82 and 1.18 ng g^{-1} w.w.). The experimental bioaccumulation factors per organ were compared with the bioconcentration factors predicted by a

Abbreviations: CECs, Contaminants of emerging concern; WWTP, Wastewater treatment plant; WRS, Water regeneration station; BAFs, Experimental bioaccumulation factors; BCFs, Predicted bioconcentration factors; PhACs, pharmaceutically active compounds; PBPK, Physiologically based pharmacokinetic model; RQ, Risk quotient; MRQ, Mixed risk quotient; PNEC, Predicted no-effect concentrations; DWTP, Drinking water treatment plant; UAE, Ultrasound-assisted extraction; PPT, Poorly perfused tissues; RPT, Richly perfused tissues; LSER, Linear solvation energy relationships.

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physiologically based pharmacokinetic model, obtaining differences of one to two logarithmic units for most compounds. Risk quotients indicated a low risk for 38 % of the contaminants. However, caffeine and terbutryn showed an elevated risk for fish. The mixed risk quotient revealed a medium risk for most of the samples in the three environmental compartments: surface water, sediment, and fish.

1. Introduction

The occurrence of contaminants of emerging concern (CECs) in aquatic ecosystems, such as pharmaceutically active compounds (PhACs), industrial compounds, and pesticides, is an established reality. In particular, the persistence and mobility of pesticides in various environmental compartments have led authorities to restrict their use and impose increasingly stringent toxicological criteria (Manjarres-López et al., 2021). Despite advancements in technologies for removing PhACs from wastewater, their widespread implementation in wastewater treatment plant (WWTP) processes remains limited globally. Consequently, the presence of PhACs continues to be prevalent in natural surface waters (Kumar et al., 2023).

Due to their continuous emission to receiving water bodies, aquatic biota is constantly exposed to them. Organisms such as fish can absorb these compounds through the skin, gill respiration, or ingestion, followed by distribution in their organs through blood circulation. Once distributed within the organs, these compounds can accumulate, hence freshwater fish serve as bioindicators for assessing the environmental quality of aquatic ecosystems (Barbieri et al., 2019; Bean et al., 2018; Kebede and Geleta, 2023; Kim et al., 2023; Lorenzo et al., 2018; Mancera-Rodríguez et al., 2023; Manjarrés-López et al., 2023; Muir et al., 2017; Omar et al., 2019; Peña-Herrera et al., 2020; Pignotti et al., 2017; Ramirez et al., 2007, 2009; Schultz et al., 2010; Tang et al., 2019; Tanoue et al., 2017; Zhao et al., 2015; Zheng et al., 2022).

The bioconcentration factor (BCF) stands out as the most widely used metric for expressing the bioaccumulation potential of environmental contaminants in fish. Although BCF is commonly determined in exposure tests in controlled environments, these tests are often expensive, time-consuming, limited to single contaminants, and in some cases difficult to extrapolate to real environmental conditions (Lo et al., 2016). An alternative to these experiments is the use of physiologically based pharmacokinetic (PBPK) models, which provide estimates of chemical concentrations in specific organs until equilibrium conditions are reached. These models are based on the processes of absorption, distribution, metabolism, and excretion in fish. Despite their advantages, fish PBPK models are limited to some specific species, such as rainbow trout, due to the availability of input parameters (Wang et al., 2021). Consequently, empirical data on bioaccumulation factors (BAF) in other fish species are still lacking, especially about PhACs (Matthee et al., 2023).

Furthermore, a limitation has been identified concerning the availability of data related to ecological risk assessments in fish, particularly regarding the distribution of compounds within their organs. Frequently, adverse effects of environmental contaminants are assessed based on external exposure concentrations (Zhang et al., 2019), thereby overlooking critical information about a compound's bioavailability, distribution patterns, potential metabolization, and preferred sites of accumulation (Salmina et al., 2016). However, for a more precise comprehension of toxicological effects and to carry out more comprehensive risk assessments, it is more relevant to consider concentrations in specific target organs (Escher and Hermens, 2002; Manjarrés-López et al., 2023).

Based on the above and considering that several CECs, such as PhACs, lack information on ecological risk assessments (Matthee et al., 2023), this study aims to: i) assess the distribution of 32 detected CECs, compromising 24 PhACs, five industrial compounds, and three pesticides, across abiotic compartments such as surface water and sediments, as well as in biota, specifically the species *Cyprinus carpio* (common carp) in a river influenced by treated wastewater inputs and adjacent to agricultural areas; ii) determine the potential bioaccumulation of these compounds in organs (skin, muscle, brain, kidney, liver, heart) and plasma of common carp by calculating experimental BAFs and predicted BCFs with a PBPK model; and iii) to perform an ecological risk assessment in the three compartments to ascertain whether the detected environmental concentrations of the compounds pose a risk. Our findings may provide new insights into assessing ecological risks caused by CECs on wildlife at real environmental concentrations.

2. Materials and methods

2.1. Chemical and reagents

The 32 analytes were selected for target analysis according to their previous presence reported in the study area (Manjarrés-López et al., 2023). Their physicochemical properties are shown in ***Table S1*** in the supplementary information (SI). Information about the preparation of native standards and labelled internal standard (IS) solutions is given in our previous study (Manjarrés et al., 2022). For the preparation of matrix-matched calibrations in the case of fish samples, common carps were provided by Llacunats Dinàmics (Mallorca, Spain) with concentrations below the detection limit of the target analytes. Additional information is shown in ***Text S1***.

2.2. Sample collection

The Llobregat River, the second-largest river in Catalonia, receives effluents from seven WWTPs in the lower part of its basin, servicing the sewage network of 40 municipalities within the Barcelona metropolitan area. The Sant Feliu de Llobregat WWTP is one of the major treatment plants of the network, with a treatment capacity of 64,000 m^3 day^{-1}. In 2021, this plant processed 17,527,993 m^3 of wastewater (Aguas de Barcelona, 2021). The treated water, if not repurposed for agricultural or recreational purposes such as golf course irrigation, is discharged into the Llobregat River via the "Tub del Governador" channel. This discharge occurs downstream of the catchment area for the Sant Joan Despí drinking water treatment plant (DWTP) so as not to compromise the supply (Fig. 1). Additionally, this channel accommodates the flow from the Rubí River, a segment of the Anoia River, and rainwater from the freeway channel, all of which are mixed in the chamber (AMB, 2023).

In this area, we selected five sampling sites, covering 6.8 km of the river. Two of the sampling sites were located upstream of the WWTP (sampling sites 1 y 2) and two downstream (sampling sites 3 y 4). Water (W) and sediment (S) samples were taken at each site. A sample of the treated effluent water before leaving the WWTP was also taken. At sampling site 3, two sediment samples were collected, one anoxic near the fish capture site (S3) and another sample near the river bank (S3.1). The map with the sampling sites is shown in Fig. 1.

Surface water samples were collected in 1 L-amber polyethylene terephthalate (PET) bottles after previously rinsed with water from the sampling site. Once in the laboratory, the water was filtered with a 0.7 μm GF/F glass microfiber filter from Whatman (UK) and stored in the dark at −20 °C until sample extraction. At each sampling site, a sediment sample (approximately 250 g) was collected from the river bank or a deeper area depending on the conditions at each site. The collection was done with a stainless-steel shovel or Van Veen grab sampler (0.5 L capacity) and stored in refrigerated PET containers. Once in the

laboratory, sediment samples were frozen at −40 °C for subsequent freeze-drying. The coordinates of the sampling sites, basic quality parameters including pH, dissolved oxygen, electrical conductivity, dissolved solids of water samples, and description of sediment sampling are shown in Fig. 1.

Fish were captured following the technical requirements established by the Biodiversity and Animal Protection Service (Ministry of Agriculture, Livestock, Fisheries, Food and Environment of the Generalitat of Catalonia, Spain). Carps were only captured in sampling site 3, positioned 260 m downstream of the WWTP discharge site (Fig. 1). This site was distinguished by a higher water temperature (19 °C) compared to the other sampling sites (14 °C) and coincided with the discharge from the Prat water regeneration station (WRS). This discharge is directed into this segment of the river to sustain its ecological flow. The fish were captured by electric fishing (200–350 V. 2–3 A. DC three-phase fully rectified). Only common carp were caught ($n = 10$), which was our target species according to the results obtained in our previous study (Manjarrés-López et al., 2023). One female and nine males were captured, i.e. there was no sex balance. The carps were weighed, measured and scale samples were taken to determine their age (Aikawa and Katō, 1938). The biological characteristics of the fish are detailed in ***Table S2***. Blood samples (2 mL) were drawn from the caudal vein with a heparinized syringe. To avoid any possible contamination, each fish was individually wrapped in aluminum foil and then stored in polyethylene bags in the field. All samples were transported in coolers at 4 °C and collected in April 2021.

Once in the laboratory, the fish were dissected immediately in accordance with Directive 2010/63/EU (EC, 2010) and RD 53/2013 (BOE, 2013). Six organs were collected for individual analysis (muscle, skin, brain, kidney, liver, and heart) and stored at −40 °C until their extraction. Plasma was obtained from the blood by centrifuging 1.0 mL aliquots for 10 min at 2500 rpm and 4 °C. The supernatant was then subjected to a second centrifugation for 10 min at 2500 rpm and 4 °C to yield 500 μL of plasma, which was stored at −80 °C until analysis.

2.3. Sample preparation and extraction

All samples (water, fish organs, plasma, and sediment) were spiked before extraction with an IS mixture (1000 ng mL^{-1}) containing the IS described in ***Text S2***. Surface water samples were spiked with 12.5 μL of the IS mix and extracted according to Gómez-Navarro et al. (2023) by solid phase extraction, passing 500 mL of water through previously cleaned and conditioned multilayer cartridges (Oasis HLB sorbent (200 mg) at the top, and a mixture of the Bond Elut PPL (150 mg), WAX (100 mg) and WCX (100 mg) sorbents at the bottom). After loading, the cartridges were air dried and then eluted using a 5 % solution of ammonia in methanol/ethyl-acetate followed by 2 % of formic acid in the same mixture and finally with methanol/ethyl-acetate alone. The resulting eluate was evaporated to dryness under a stream of nitrogen, and the residue was reconstituted in a mixture of 500 μL water/methanol (95:5 v/v).

Sediment samples were lyophilized with a Telstar LyoAlfa (Telstar Technologies, Terrassa, Spain) at −85 °C for 48 h. Then, the lyophilized sediment was sieved to collect the <250 μm fraction. Extraction was done according to Pignotti et al. (2017). Briefly, 1.0 g of dried sediment was taken, spiked with 25 μL of an IS mix, and left under the fume hood for 20 min. Subsequently, 10 mL of methanol was added and the sediments were extracted by ultrasound-assisted extraction (UAE) for 45

Water samples	Coordinates	pH	%OD	DO (ppm)	Conductivity (μS cm^{-1})	Dissolved Solids	Sediment samples	Description
W1	41°23'22.0"N 2°01'17.6"E	8.05	47.4	4.29	2705	1353	S1	Collected from the bridge (Gual del Riu Llobregat) with Van Veen Grab
W2	41°22'37.8"N 2°01'47.0"E	8.09	62.3	5.18	2708	1354	S2	Shovel-collected riverbank sediment
WWTP	41°22'43.5"N 2°01'59.5"E	7.56	39.2	3.4	4179	2089	-	NO
W3	41°20'58.6"N 2°02'48.9"E	7.77	46.9	3.82	3601	1800	S3-S3.1	S3. Sample of anoxic sediment S3.1. Sediment sample from the shore
W4	41°20'53.6"N 2°03'10.7"E	7.91	40.5	3.37	3388	1693	S4	Shovel-collected near the shore

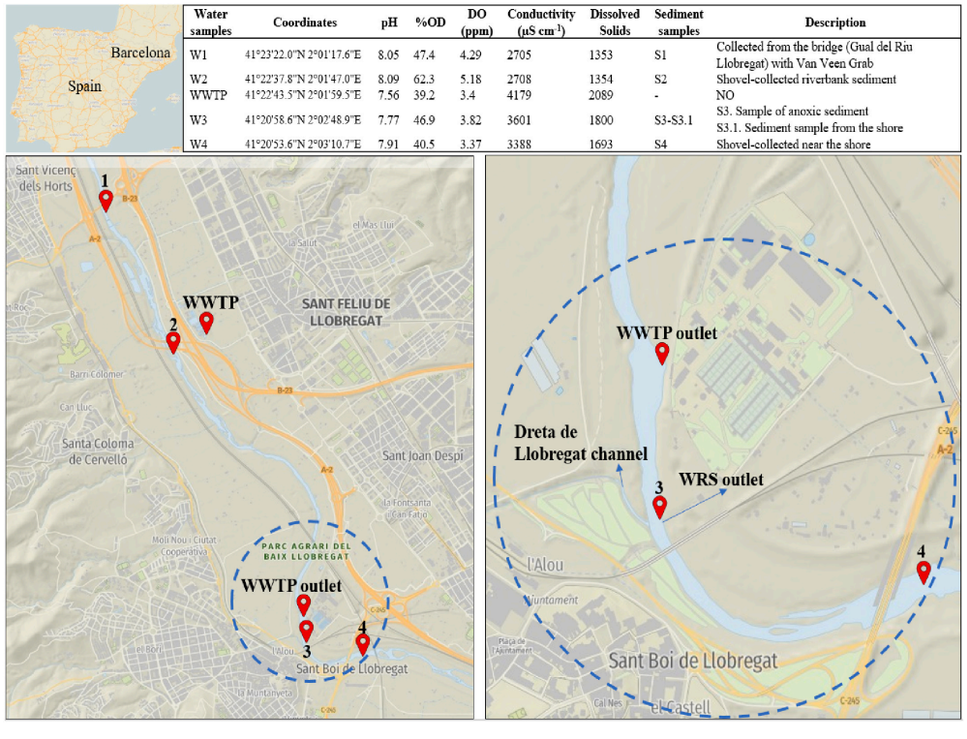

Fig. 1. Description of sampling sites.

min. The extracts were then centrifuged for 10 min at 2500 rpm at 17 °C. Five mL of the supernatant was evaporated to dryness and reconstituted in 500 μL of a water/methanol mixture (9:1 v/v).

The extraction method for organs and plasma was carefully detailed in our previous work (Manjarrés et al., 2022). Briefly, 500 mg of each organ was spiked with 25 μL of an IS mix and homogenized in a TissueLyzer LT. Disruptor (Qiagen, Hilden, Germany). Extraction was performed by UAE with 1.0 mL of acetonitrile plus formic acid 0.1 % followed by a clean-up step with Discovery® DSC-18. The mixture was shaken and then centrifuged at 14000 rpm for 6 min. Finally, 500 μL of the supernatant was evaporated to dryness and reconstituted in a mixture of 5 mM ammonium acetate water/acetonitrile (9:1 v/v).

2.4. Quantitative analysis by HPLC-HRMS/MS

Chromatographic separation and detection of the analytes were carried out using a Waters Acquity UPLC system (Waters. Milford. MA), coupled with an Orbitrap Q-Exactive™ mass spectrometer (Thermo Fischer Scientific, San Jose, CA, USA). An EVO C_{18} KINETEX EVO column (100 × 2.1 mm; 2.6 μm. Phenomenex) was used for chromatographic separation of the target analytes. As mobile phases, 5 mM ammonium acetate water plus 0.1 % formic acid and acetonitrile for electrospray ionization ESI (+), and 2 mM ammonium fluoride water and acetonitrile for ESI (−) were used. The acquisition mode was data-independent acquisition (DIA). Information about chromatographic separation and the detection system is described elsewhere (Manjarrés et al., 2022). Quantification was performed using matrix-matched calibration and following the internal standard method with Trace Finder 5.1 software (Thermo Fisher Scientific). During calculation, plotting, and analysis, for concentrations of individual compounds <MLOD (less than method limit of detection) or <MLOQ (less than method limit of quantification) half of the value of the MLOD or MLOQ was used. The MLOD, MLOQ values, and recoveries are available at Gómez-Navarro et al. (2023) and Manjarrés et al. (2022). Statistical analyses were performed using Origin 2022 (OriginLab Corp., USA) and Microsoft Excel.

2.5. Quality assurance/quality control

To quantify the analytes in organs, matrix-matched calibration curves were prepared following the procedure outlined in Section 2.3. The reference and IS standards were spiked after extraction to cover a range from 0.10 ng g^{-1} to 500 ng g^{-1}, using organ pools from clean carps. The samples contained non-detectable levels of the target compounds. For surface water, sediment, and plasma samples the calibration curve was prepared in Milli-Q water in the range of 0.05 ng L^{-1} to 1000 ng L^{-1}. During acquisition, as quality control (QC), clean matrix extracts spiked with reference standards at a concentration of 50 ng mL^{-1} were analyzed after every fifth sample to evaluate the method's performance. Likewise, solvent blanks (methanol) were analyzed after each QC to confirm the absence of any carryover effect on the column. The R^2 for all compounds was >0.99 for each calibration curve. Target compounds in the samples were identified based on their exact *m/z* (error < 5 ppm), by comparing retention times with those of native standards (within ±0.5 min) and confirmed using the most abundant fragment.

2.6. Organ/plasma partition coefficients ($K_{organ/plasma}$)

$K_{organ/plasma}$ were calculated individually by dividing the concentration in each organ by the concentration in plasma as shown in Eq. (1):

$$K_{organ/plasma} = \frac{Cf_i}{Cp_i} \quad (1)$$

Where, Cf_i concentration organ fish A (ng g^{-1} w.w.), and Cp_i: concentration plasma fish A (ng mL^{-1}).

2.7. Experimental BAF and predicted BCF

$BAF_{experimental}$ was determined for both whole fish and for each organ. For the determination of BAF in whole fish, first the total concentration of each compound and fish was obtained according to Eq. (2):

$$Concentration_{Whole\ fish}(C_{wf}) = \frac{\sum_i (W_i \times C_i)}{\sum_i W_i} \quad (2)$$

Where, *C*: concentration (ng g^{-1} w.w. and ng mL^{-1} in the case of plasma), and *W*: weight of each organ (g w.w.). The blood concentration was included considering that bony fish have between 2 and 4 mL of blood per 100 g and assuming a blood density of 1.0 g mL^{-1} (Padmavathi, 2018).

The BAF was then determined for each fish according to Eq. (3):

$$BAF_{experimental} = \frac{C_{wf}}{C_w}\ x\ 1000 \quad (3)$$

Where C_w: mean river water concentration (ng L^{-1}).

For the $BAF_{experimental}$ per organ, Eq. (3) was used by substituting the numerator for the mean concentration of each compound per organ ($n = 10$).

To predict BCFs, a physiologically based pharmacokinetic (PBPK) model adapted from Salmina et al. (2016) was used. The model describes fish as an open five-compartment system that continuously interacts with the exposure medium at constant concentration and the BCF is determined at steady-state. The compartments defined are the organs (1) kidney and (2) liver which are treated as individual compartments, (3) adipose or fat tissue, (4) poorly perfused tissues (PPT), and (5) richly perfused tissues (RPT) (***Fig. S1***). PPT comprises skin, muscle, and skeleton, and RPT comprises all other organs.

The model is designed in the form of a Microsoft Excel spreadsheet that employs Visual Basic programming tools to run the simulation. The input parameters we included in the model were body weight (Kg), water temperature (19 °C), dissolved oxygen (3.82 mg O^2 L^{-1}), concentration of the compound in water (g L^{-1}), weight of the compounds (mol), linear solvation energy relationships (LSER descriptors) and, half-life of the compound (days) (***Table S3***). The model was applied to predict the BCF in whole fish, liver, and kidney using the mean weight of the fish caught (2.35 Kg) ($n = 10$). The half-lives used in the model were obtained from ChemProp 7.1.1 software (Department of Ecological Chemistry Helmholtz Centre for Environmental Research - UFZ) using the Arnot model (Arnot et al., 2008). LSER descriptors for the calculation of partition coefficients and predicted BCFs in the PBPK model were obtained from Ulrich et al. (2017).

2.8. Ecological risk assessment

The potential risks of the target compounds on the different environmental compartments (whole fish, water, and sediment) were assessed by individual risk quotients (RQ) comparing the measured concentration with their predicted no-effect concentration (PNEC), as shown in Eq. (4):

$$RQ = \frac{Measured\ environmental\ concentration\ per\ compound}{PNEC} \quad (4)$$

To be conservative, the lowest PNECs proposed by the NORMAN Ecotoxicology Database were used (***Table S4***). The lowest PNECs in the database were primarily determined using experimental ecotoxicity data. In instances where such data were unavailable, QSAR predictions were employed to estimate provisional P-PNEC values. These lower PNECs were predominantly calculated for freshwater (*fw*) environments. In cases where experimental values were lacking for other matrices such as sediments and biota (fish), alternative calculation procedures were employed by NORMAN, as depicted in Eqs. (5) and (6)

(NORMAN, 2023):

$$\text{Lowest PNEC sediments (d.w.)} = Lowest\ PNECfw\ x\ 2.6\ x(0.615 + 0.019\ x\ K_{oc}) \quad (5)$$

$$\text{Lowest PNEC fish (w.w)} = \frac{PNECfw*BCF}{10} \quad (6)$$

In the present study, the potential risk was classified as high if RQ ≥ 1 and medium if 1 > RQ ≥ 0.1. Additionally, the mixed risk quotient (MRQ) was calculated as the sum of the individual RQs (Nozaki et al., 2023; Zheng et al., 2022). In the case of fish, the RQ and MRQ were calculated for each individual, and for surface water and sediment, they were calculated at each sampling site.

3. Results and discussion

3.1. Concentrations in surface water and sediments

All 32 target analytes were detected in at least one surface water or sediment sample. The concentrations of target compounds in surface water and sediment samples are shown in ***Table S5*** and summarized in ***Fig. S2***. The highest concentrations were found in the WWTP effluents: sotalol (41.1 μg L^{-1}), amantadine (19.8 μg L^{-1}), metoprolol (9.00 μg L^{-1}), 5-methyl-1H-bezotriazole (4.01 μg L^{-1}), hydrochlorothiazide (3.24 μg L^{-1}), *O*-desmethylvenlafaxine (2.76 μg L^{-1}) and at sampling site 4: sotalol (11.1 μg L^{-1}), amantadine (5.71 μg L^{-1}) and benzotriazole (3.71 μg L^{-1}). Previous studies in the Llobregat river basin have reported mean concentrations for eleven of the compounds detected in the surface water samples of this research: acetaminophen (1.31–420 ng L^{-1}), clarithromycin (0.17–102 ng L^{-1}), diazepam (1.87–15.0 ng L^{-1}), ketoprofen (29–790 ng L^{-1}), trimethoprim (8.02–140 ng L^{-1}), codeine (48.6–123 ng L^{-1}), citalopram (2.97–3.24 ng L^{-1}), hydrochlorothiazide (47–210 ng L^{-1}), venlafaxine (3.90–21.2 ng L^{-1}), metoprolol (10–738 ng L^{-1}), and sotalol (3.98–570 ng L^{-1}) (Ginebreda et al., 2010; López-Roldán et al., 2010; Osorio et al., 2012, 2016). According to the data shown in ***Table S5***, concentrations of acetaminophen and clarithromycin fall within the previously reported ranges. Sampling sites W1 and W2 exhibited concentrations below the mean (5.40 and 3.84 ng L^{-1}) for ketoprofen. Trimethoprim was detected at a concentration above the mean at sampling site W3 (294 ng L^{-1}), while hydrochlorothiazide was found at concentrations above the mean range at points downstream of the WWTP (3501 and 1783 ng L^{-1}). Concentrations of codeine were below the reported means at all sampling points, whereas citalopram and venlafaxine were above. For diazepam, metoprolol, and sotalol, concentrations at sampling site W4 were above the mean (27.9, 2085, and 11,064 ng L^{-1}, respectively).

Total concentrations were six and fifteen times higher for the sampling sites downstream of the WWTP (sampling site 3: 12.4 μg L^{-1} and sampling site 4: 30.1 μg L^{-1}) compared to those upstream (sampling site 1: 2.08 μg L^{-1} and sampling site 2: 1.66 μg L^{-1}). Usually, the concentrations of compounds detected downstream of WWTPs tend to be lower than those reported in the WWTP effluent due to the dilution effect. In this regard, because sampling site 4 was farther away from the WWTP (990 m), concentrations would be expected to be lower than at sampling site 3, which was closer to the WWTP (260 m) (Fig. 1). However, an unusual rise in the concentrations of compounds such as amantadine, benzotriazole, caffeine, metoprolol carbendazim, and ondansetron at sampling site 4 compared to sampling site 3 (***Table S5***) could be linked to the discharge of effluent from the "Dreta de Llobregat channel", situated 30 m downstream of site 3 (Fig. 1). This channel, approximately 5.67 km long, receives inputs from tributaries, especially from the Can Soler River micro-basin, and traverses cultivated areas. Additionally, the convergence with the effluent from the "ERA del Prat" may account for the elevated concentrations observed in the water body sampled at site 4.

Depending on their physicochemical properties, PhACs can bind to suspended particles and accumulate in sediments (Osorio et al., 2012). In our study, sediment sample S3, taken from the anoxic sediment at site 3 presented the highest total concentration (2.37 μg g^{-1} d.w.).

The differences in concentration reported for the S3 sample compared to the others may be attributed to its higher organic carbon and organic matter content (3.87 % and 6.68 % d.w., respectively) (see ***Table S6***). Within the compounds detected, the most notable levels were found for bisoprolol (0.93 μg g^{-1} d.w.), metoprolol (0.51 μg g^{-1} d.w.), and sotalol (0.23 μg g^{-1} d.w.). These three compounds are part of the beta-blocker group and become protonated at a pH of 9.3 to 13.9 due to their amino group. The positive charge of this group enhances their sorption affinity. This affinity tends towards negatively charged sites in the natural organic matter (NOM) or clay minerals with negative charges due to isomorphic substitution in the mineral structure (Ramil et al., 2010). Studies report the adsorption of sotalol in the interlayer space of clay minerals, presumably due to ion exchange and ion-dipole interactions, exhibiting maximum adsorption capacity at pH 7 (Sánchez-Camazano et al., 1987).

We calculated sediment-water partition coefficients (log K_d) for each sampling site by dividing the reported sediment concentration (μg Kg^{-1}) by the water concentration (μg L^{-1}) (Koba et al., 2018). The results were summarized in ***Table S5***. Compounds with log K_d values <2 are considered mobile, with insignificant sorption capacity that keeps them predominantly in the aqueous phase. On the other hand, those with log K_d values ≥2 tend to be easily adsorbed and are only mobilized if there are sediment disturbances (Castaño-Ortiz et al., 2024).

Compounds that showed log $K_d \geq 2$ values at most sampling sites and exhibited continuous partitioning to sediment included bisphenol A, bisphenol F, loratadine, metoprolol, quetiapine, telmisartan, and terbutryn. Site 3, where we captured carp and where they apparently feed, was the site with the highest number of compounds with log $K_d \geq 2$ (47 %) and the highest values. Among these compounds were bisoprolol, bisphenol A, bisphenol F, caffeine, diazepam, metoprolol, sotalol, and terbutryn, which were also detected in fish organs (see Section 3.2). Carps typically forage on light sandy and gravelly bottoms, where their diet primarily consists of crustaceans, insect nymphs, aquatic worms, and vegetation debris. Furthermore, carps typically feed from below, ingesting food sources and sifting through the gravel or mud at the bottom, resulting in a cloud of mud. Consequently, sediment disturbance during foraging could facilitate the ingestion of compounds with high log K_d values, while still acknowledging the significance of water as a route of exposure.

3.2. Concentrations and distribution in fish

Sixteen compounds were detected in the carps. The concentrations detected per whole fish (***Fig. S3***) and the mean distribution of each compound per organ and plasma are summarized in Fig. 2 and listed in ***Tables S7*** and ***S8***. The Pearson's correlation coefficient (r) values (−0.10, 0.03, and − 0.18) indicated that there was no relationship between the total concentration of PhACs in whole fish and the weights, ages, and lengths of carp, respectively. However, when analyzing the r by compound, it was observed that 5-methyl-1H-benzotriazole exhibited a strong negative linear correlation ($r = -0.73$) with carp age, while bisphenol S, caffeine, cotinine, and hydrochlorothiazide showed a moderate negative correlation ($-0.7 < r < -0.3$) with fish weight and length. These findings suggest that as the size and weight of carp increase, the concentrations of these compounds tend to decrease. This decrease could be attributed to higher metabolic rates in larger fish or a more uniform distribution capacity of PhACs, resulting in a reduction of their concentration in organs (Manjarrés-López et al., 2023; Miller et al., 2018). On the other hand, amantadine, bisoprolol, metoprolol, and sotalol, appear to have a moderate positive correlation, with r values between 0.30 and 0.46. This suggests that elimination rates in captured carp may vary according to the physicochemical characteristics of the

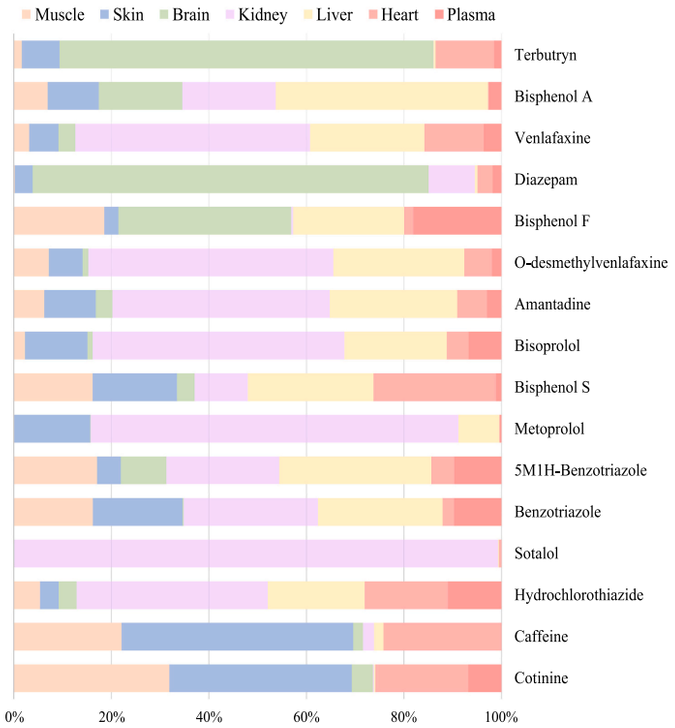

Fig. 2. Distribution of the compounds detected per organ and plasma, organized from highest to lowest log K_{ow} from top to bottom. The distribution was calculated with the mean concentrations obtained in each organ ($n = 10$). Compounds with concentrations <MLOD or < MLOQ were replaced by MLOD/2 and MLOQ/2.

accumulated compounds. In this case, a similar trend was observed for compounds belonging to the beta-blocker family.

Amantadine showed the highest concentrations in whole fish (5.55 ng g^{-1} to 51.6 ng g^{-1}) (***Fig. S3***). This compound was detected in all individuals and all organs, presenting a maximum mean concentration of 158 ng g^{-1} in the kidney. It was followed by 5-methyl-1H-benzotriazole (4.09 ng g^{-1} to 7.95 ng g^{-1} in whole fish) and a maximum mean concentration of 11.9 ng g^{-1} in the liver. Caffeine, detected in 9/10 fish in a range of 0.44 ng g^{-1} to 24.5 ng g^{-1} in whole fish, showed a maximum mean concentration of 14.7 ng g^{-1} in the skin.

The sum of mean concentrations per organ ($n = 10$) showed that the kidney had the highest concentrations (219 ng g^{-1}), followed by the liver (127 ng g^{-1}), brain (76.4 ng g^{-1}), skin (72.2 ng g^{-1}), muscle (45.6 ng g^{-1}), heart (44.3 ng g^{-1}), and plasma (20.6 ng mL^{-1}). Amantadine showed a contribution rate of >50 % in muscle, skin, heart, and plasma and up to 70 % in kidney and liver. The extended detection of amantadine in various fish organs could be attributed to a combination of their physicochemical characteristics and the specific absorption processes of freshwater fish. Fish are susceptible to gill absorption of PhACs with low molecular weight and moderate lipophilicity. The absorption of ionizable compounds is also influenced by the pKa of the compound, as well as by the pH of the water and body fluids (Matthee et al., 2023). Additionally, pH adjustments resulting from the fish's ability to modify the chemistry of the water they breathe by extracting oxygen and ions while releasing carbon dioxide and ammonia might favor the uptake of the ionizable compounds (Matthee et al., 2023). In this case, the absorption of amantadine through the gills could be facilitated due to its physicochemical properties such as its low molecular weight (151 g mol^{-1}), log K*ow* (2.2), half-life (32 h, the longest of the compounds detected in fish in this study), its protonation at physiological pH (***Table S1***), and its log K_d, which obtained a maximum value of 1.85 (***Table S5***).

The highest contribution rates in the brain were found for terbutryn, with a mean concentration of 50.2 ng g^{-1}, diazepam (3.82 ng g^{-1}), and bisphenol F (1.18 ng g^{-1}). These compounds are classified as lipophilic compounds with a log K*ow* of 3.4, 2.9, and 2.7 respectively, and at pH 7, they are neutral. These characteristics favor their distribution into lipids-rich tissues, thereby increasing their capacity for absorption and accumulation in the organism. The high concentrations in the brain could be attributed to the greater affinity of terbutryn and diazepam for this organ, given its lipid content (Goss et al., 2018). Additionally, the brain is richly perfused, which enhances accumulation within it (Fig. 3), and considering the brain as the target organ for the antidepressant diazepam, its presence in this organ was to be expected. On the other hand, based on the previously presented log K_d results, we can infer that one of the primary routes of access for these compounds to the fish organism is through ingestion.

Trace-level concentrations of terbutryn were reported in trout muscle in Italy (<10 ng g^{-1}) (Barbieri et al., 2019), bivalves in China (<2 ng g^{-1}) (Yu et al., 2021), and shellfish, algae, and trout in South Korea (mean concentration 2 ng g^{-1}) (Kim et al., 2023). The use of terbutryn as a plant protection product in Europe has been restricted per Annex I of Commission Regulation No. 2076/2002 (EC, 2002) and it is part of the list of priority substances regulated by Directive 2013/39/EU (EC, 2013). It is also included in the EU list of possible endocrine disruptors in category 1 (The European Chemicals Agency (ECHA)) (Barbieri et al., 2019), so the control over its emission sources should be extended.

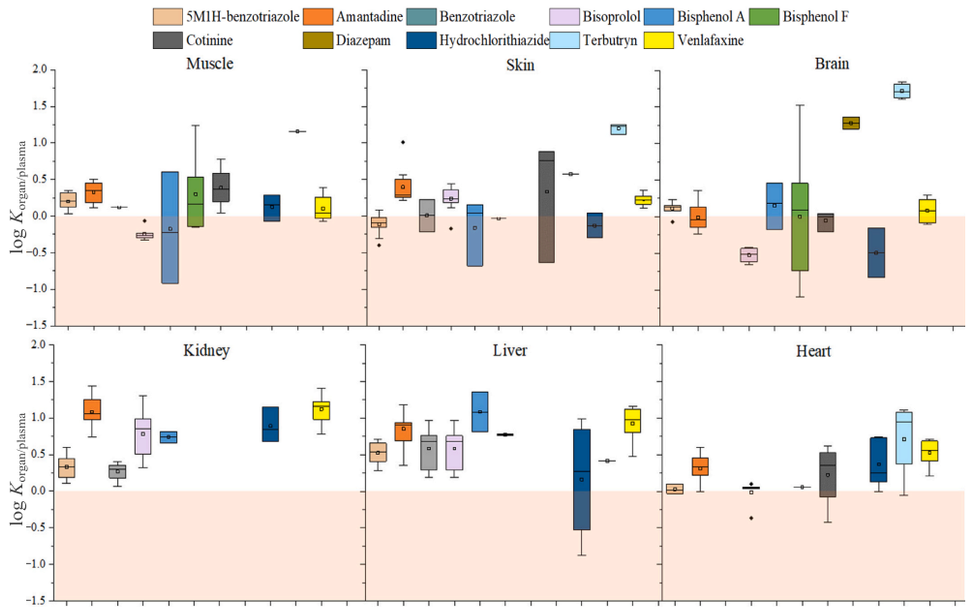

Fig. 3. Estimated log $K_{organ/plasma}$ partition coefficients in carps ($n = 10$). The box plot shows the 25th (bottom edge of box) and 75th (top edge of box) percentiles. Interquartile range (IQR) = 1.5. The horizontal lines and squares in the boxes represent the median and mean, respectively. The small squares are outliers. The orange bars represent the values where the concentration in plasma was higher than in organ.

3.2.1. $K_{organ/plasma}$

The relative exposure of the compounds in the different organs of the carps was assessed by calculating organ/plasma partition coefficients ($K_{organ/plasma}$). We only considered compounds with a detection frequency higher than 60 % in plasma. Therefore, bisphenol S, caffeine, metoprolol, *o*-desmethylvenlafaxine, and sotalol were excluded from this analysis. The $K_{organ/plasma}$ are shown in Fig. 3 and summarized in ***Table S9***.

The highest mean $K_{organ/plasma}$ were found for terbutryn (53.1) and diazepam (19.5) in the brain, followed by terbutryn in the skin (15.9), bisphenol A in liver (14.6), amantadine in kidney (13.6) and terbutryn in heart (7.31). According to Matthee et al. (2023), the absence of proteins analogous to albumin, such as α1-acid glycoprotein (AGP), in fish may have significant consequences for PhACs interactions compared to mammals. In mammals, AGP plays an essential role in the binding and transport of various drugs with lipophilic, basic, and neutral properties. The lack of AGP in fish plasma could result in a larger free fraction of these, facilitating their distribution to organs. This physiological peculiarity could have implications, especially on the mobility of neutral compounds, such as terbutryn, diazepam, and bisphenol A, through the plasma, facilitating their distribution.

The partition coefficients indicated that, for the most of compounds, the mean concentration of the analytes in the organs exceeded the mean plasma concentration (boxes above the orange band in Fig. 3). This may occur because the distribution of organic chemicals in organs is mainly influenced by passive diffusion into lipid compartments, with blood being regarded more as a transfer or transport medium than as a matrix in itself (Tang et al., 2019). This underscores the significance of distribution models in attaining a more comprehensive analysis of the effects of CECs on aquatic species.

3.3. Experimental BAF and predicted BCF by the PBPK Model

Due to the ability of fish to move along the river, we calculated the BAFs and BCFs considering the average water concentration from the four sampled sites, even though fish capture was only possible at site 3. The BAFs and BCFs were computed only for compounds detected simultaneously in fish and surface water, and they were calculated for whole fish as well as individual organs.

Cotinine exhibited the highest mean experimental BAF in whole fish (47.1), followed by caffeine (41.6) and terbutryn (35.5). All compounds obtained a BAF below 2000 (***Table S10***), indicating that they do not bioaccumulate (Goss et al., 2018) when considering their total concentration in whole fish. However, when calculating the BAF based on the mean concentrations of each analyte per organ, as proposed by Grabicova et al. (2017), terbutryn was found to bioaccumulate in the brain of carp, as its BAF exceeded 500 (1041). Other potentially bioaccumulative compounds included diazepam in the brain (396) and amantadine in the kidney (104), which, although they did not surpass a BAF of 500, showed high values for these specific organs compared to the others (***Table S11***).

This greater potential for bioaccumulation in a specific organ is correlated with the analyte's affinity for that organ based on its physicochemical properties, its ability to move easily through the bloodstream, and whether it serves as a target organ for which the compound was designed. For instance, diazepam, which directly affects the central nervous system, demonstrates this pattern. Previous studies showed that antipsychotic and/or antidepressant compounds such as sertraline, norsertraline, carbamazepine, and fluoxetine had a high potential for bioaccumulation in the fish brain (Arnnok et al., 2017; Grabicova et al., 2017; Tanoue et al., 2015; Valdés et al., 2016; Zheng et al., 2022). It has also been shown that the liver and kidney usually present higher BAF values for different classes of PhACs due to their metabolic activity (Grabicova et al., 2017; Xie et al., 2015, 2016).

The experimental BAFs for whole fish, kidney, and liver were compared with the predicted BCFs from the PBPK model. The results are shown in Fig. 4. The experimental BAFs and predicted BCFs for most compounds showed a variance of one to two log units. The experimental BAFs and predicted BCFs for the liver presented a higher correlation (r, 0.57) than in the kidney and whole fish (r, 0.19 and 0.23 respectively). The differences presented between the experimental BAFs and predicted BCFs for the kidney may be attributed to the compound recoveries, as this organ tends to exhibit lower recoveries due to its higher matrix effect (Manjarrés et al., 2022). The model underestimated the predicted BCFs for caffeine in the three compartments analyzed. The same occurred for the beta-blockers sotalol, bisoprolol, and metoprolol in the kidney, and cotinine in the whole fish. The predicted BCFs for terbutryn and bisphenol F in the kidney were overestimated by the model.

It has been established that partition coefficients are among the most influential variables in PBPK models for predicting BCF (Zhang et al., 2019). PBPK models are often used to forecast the behavior of neutral chemicals in controlled environments. For compounds such as amantadine, venlafaxine, cotinine, and sotalol, which are protonated at physiological pH, the use of LSER descriptors in their neutral state could have impacted the reported BCF predictions. Regarding of caffeine, which remains in its neutral state at physiological pH, the underestimation of its predicted BCF could be related to its fast biotransformation rate included in the model (0.01 h), leading to rapid attainment of the equilibrium state in the system.

Experimental BAF and predicted BCF data can vary between and within studies (Matthee et al., 2023). To reduce the uncertainty of concentrations predicted by PBPK models, Wang et al. (2021) developed a generalized PBPK model that allowed the inclusion of ionizable PhACs in different fish species. Estimates for ionizable substances were more accurate than those of most specific PBPK models, however, model accuracy varied from compound to compound, with 3- to 10-fold differences between measurements. Likewise, Zhang et al. (2019) obtained differences of up to one log unit for 10 PhACs and personal care products modeled for *Cyprinus carpio*. This may be because there is more than one determinant influencing the prediction results.

In the PBPK model applied in this study, we evidenced that, by including the biotransformation rates of each compound, the predicted BCF values were closer to the experimental BAFs (Tang et al., 2019; Wang et al., 2021). Our model considers ambient water temperature as an input variable. Zhang et al. (2019) showed that water temperature is a determinant factor in PBPK models for wild common carp. It can influence its metabolic capacity because it is a species that varies its body temperature as a function of ambient temperature. Some physiological processes in carp are critically dependent upon temperature such as oxygen consumption, immune competence, growth, and metabolism (Metz et al., 2003). To evaluate the temperature dependence of our PBPK model, we compared the BCFs for whole fish at three different temperatures: 11 °C, 19 °C, and 25 °C. After performing a two-tailed *t*-test assuming equal variances with a significance of 0.05, we obtained values ranging from 0.83 to 0.96. These results indicated that there was no statistically significant difference between the calculated BCFs. Only for compounds with a log BCF small at 11 °C such as caffeine (−1.34), cotinine (−0.62), and hydrochlorothiazide (−0.4), there was temperature dependence, as it increased between 0.21 and 0.34 log units at 25 °C. This can be attributed to the fact that the applied model primarily relies on the use of partition coefficients predicted based on LSER descriptors.

Based on the obtained results, it can be inferred that while temperature can indeed influence the bioconcentration of PhACs in fish, this effect varies depending on the properties of each compound. Previous findings have demonstrated that in perch, exposure to a temperature increase from 10 °C to 20 °C resulted in a lower bioconcentration of temazepam, while the bioconcentration of oxazepam increased (Cerveny et al., 2021). Additionally, increases in BCF values have been observed for chlorinated benzenes with rising water temperatures (Opperhulzen et al., 1988).

Water concentration was a variable that did not influence the predicted BCF values, but could be related to the differences between the BCF and BAF obtained. The predicted BCF did not change with varying water concentrations because the partition coefficients and the rate constants (half-life) determine the overall BCF in the predictions, and

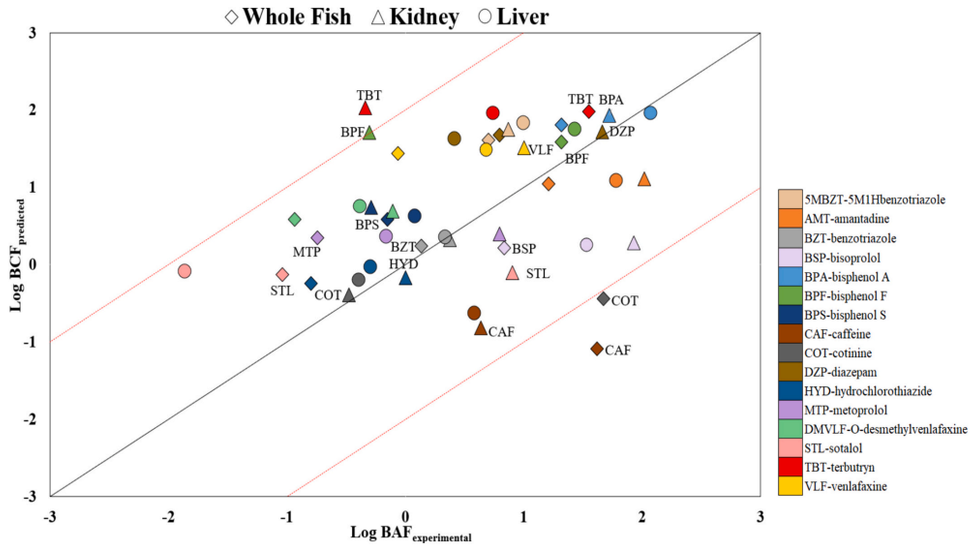

Fig. 4. Comparison of predicted log BCFs in whole fish, liver, and kidney by PBPK model vs. experimental log BAFs. The solid line represents an exact match between experimental and predicted values. Red lines indicate deviations of two logarithmic units.

these parameters did not change. When a lower water concentration was used as input for the model, the concentrations in the corresponding compartments changed relatively according to the water concentration used. Thus, the BCF itself (at equilibrium) was not impacted. However, for the experimental data, this is different as the BAF increased with decreasing water concentration. This discrepancy in experimental BAF values could be attributed to higher uncertainties, stemming from fish movement and concentration fluctuations over time and across sampling locations.

3.4. Ecological risk assessment

The individual RQ values calculated as the ratios between the measured environmental concentrations in each compartment (whole fish, surface water, and sediment) and the PNECs from the NORMAN database are summarized in Fig. 5. It was shown that 69 % of the compounds detected in common carp did not represent a risk (RQ < 0.1). However, caffeine and terbutryn were deemed compounds of particular interest for common carp due to their high RQ values (ranging from 1.09 to 10.9 and 1.48 to 9.70) observed in 6 and 4 fish, respectively, and medium RQ values (ranging from 0.19 to 0.76 and 0.38 to 0.65) observed in 3 and 6 fish, respectively. In the case of terbutryn, the main risk in fish is associated with its potential bioaccumulation in the brain.

Velisek et al. (2012) demonstrated that chronic exposure to terbutryn in the early life stages of common carp affected their survival, early ontogeny, and histology at concentrations higher than 20,0 μg L^{-1}. However, the growth rate was impaired in fish exposed to the lowest concentration tested (20 ng L^{-1}). In another experiment of exposure to terbutryn for 96 h, morphological abnormalities, increased mortality, reduced body length and eye size, and vitelline sac edema were induced in zebrafish larvae (Min et al., 2023).

Although the most severe effects of terbutryn are usually manifested during the early stages of carp's growth, effects have been demonstrated in one-year-old carps. Among the effects were alterations in the biochemical and hematological profiles of internal organs. An increase in the formation of reactive oxygen species that induced oxidative damage to lipids and proteins and inhibition of antioxidant capacities were observed (Velisek et al., 2011). The effects occurred when carps were exposed to environmental concentrations recorded in Czech rivers, ranging from 200 to 2000 ng L^{-1} which are well above those reported in this study.

In the case of caffeine, the risk is high in fish despite its low bioaccumulation potential. This is because its PNEC is one of the lowest (2.25 μg Kg^{-1} w.w.) considering the continuous and high exposure rate of aquatic organisms to this compound. The PNEC value is related to studies that have shown that the lowest observed behavioral effect concentration in zebrafish (1.00 μg L^{-1}), was 40 times lower than the maximum concentration detected in European surface waters near a WWTP (40 μg L^{-1}) (Zhou et al., 2019a, 2019b). Caffeine is a common ingredient of food products, drinks, PhACs, and illegal drugs, and is one of the most frequently detected PhACs in surface waters (Li et al., 2020). Approximately 5 % of ingested caffeine is excreted in human urine and eventually reaches water bodies (Vieira et al., 2022). Caffeine residues in aquatic ecosystems are extremely stable, with a half-life between 100 and 240 d (Hillebrand et al., 2012).

A significant increase in biotransformation enzyme activity (ethoxyresorufin O-desethylase) has been reported in clam digestive glands at caffeine concentrations >15.0 μg L^{-1} and effects on biomarkers of phase I and II metabolism at 0.10 μg L^{-1} (Aguirre-Martínez et al., 2015). In zebrafish larvae, locomotor behavior was altered after embryonic exposure to 0.10 μg L^{-1} caffeine in early developmental stages, and erratic movement was accelerated from 1.00 μg L^{-1} (Zhou et al., 2019a, 2019b). Although studies have been conducted to evaluate the effects of

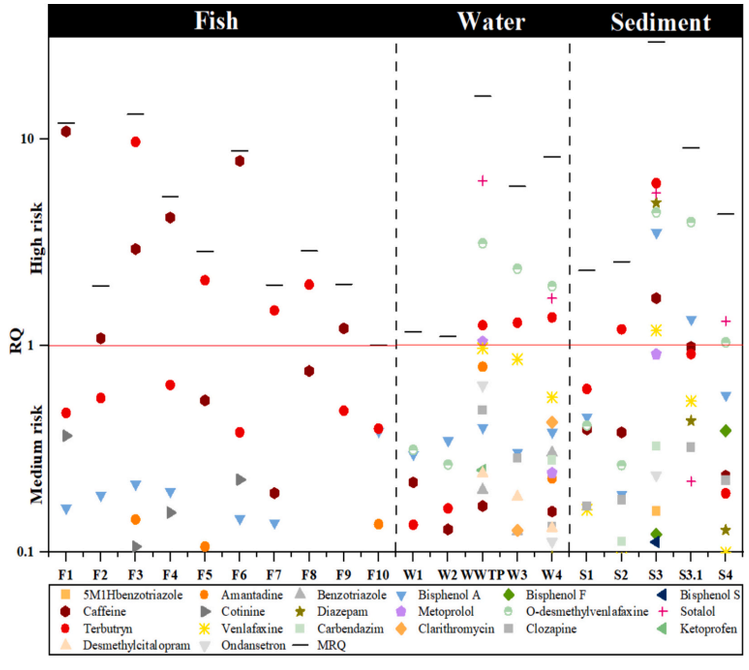

Fig. 5. Individual RQ for compounds with medium risk (1 > RQ ≥ 0.1) and high risk (RQ ≥ 1) and MRQ for each environmental compartment.

caffeine at the laboratory scale, information about the effects it may have on wild fish is still limited (Cerveny et al., 2022).

In the case of water and sediment samples, the risk was found to be higher at the WWTP effluent (MRQ, 16.2), followed by W4 (MRQ, 8.21) and W3 (MRQ, 5.90) located downstream. The concentrations of eleven compounds showed a medium risk ($1 > RQ \geq 0.1$) in at least one water or sediment sample (5-methyl-1H-bezotriazole, amantadine, benzotriazole, bisphenol F, bisphenol S, carbendazim, clarithromycin, clozapine, ketoprofen, desmethylcitalopram, and ondansetron). Seven compounds had concentrations implying a high risk ($RQ \geq 1$) in sediments: terbutryn (RQmax, 6.11), sotalol (RQmax, 5.49), diazepam (RQmax, 4.93), *o*-desmethylvenlafaxine (RQmax, 4.41), bisphenol A (RQmax, 3.52), caffeine (RQmax, 1.70), and venlafaxine (RQmax, 1.19). In water samples taken upstream of the WWTP, no compound was found to be high risk, while the WWTP effluent had RQ values >1 for sotalol (6.31), *o*-desmethylvenlafaxine (3.13), terbutryn (1.26) and metoprolol (1.05). Downstream sites (W3 and W4) had high RQ values for *o*-desmethylvenlafaxine (2.36 and 1.94), terbutryn (1.29 and 1.37), and for sotalol only in sample W4 (1.69).

Caffeine and venlafaxine are on the list of the 45 most frequently analyzed PhACs in Europe, with mean surface water concentrations of 885 ng L^{-1} and 131 ng L^{-1} respectively (Zhou et al., 2019a, 2019b). At the risk analysis level, they have been proposed as priority substances according to the optimized risk quotient (RQ_f) developed by Zhou et al. (2019a, 2019b), as high risk (venlafaxine, RQ_f 6.67) and moderate risk (caffeine, RQ_f 0.77). These results are aligned with ours, except that in this study caffeine was prioritized due to its high detection frequency in fish organs, and venlafaxine due to its detection frequency and concentrations in surface water and sediment samples.

Venlafaxine and *o*-desmethylvenlafaxine are included in the watch list of substances for monitoring purposes at the European Union level in the realm of water politics (EC, 2020). The concentrations reported by this study for *o*-desmethylvenlafaxine were 2077 and 1711 ng L^{-1} at the sampling sites located downstream of the WWTP. These concentrations are above their PNECs in freshwater (880 ng L^{-1}), so we suggest implementing corrective actions that involve reducing the release of the substance to the environment or implementing mitigation measures.

A previous study evaluated the hazard quotient (HQ) for fish, daphnia, and algae in the Llobregat River basin (Ginebreda et al., 2010). The percentage contribution of metoprolol in HQ was above 40 % for fish and 10 % for daphnia and algae at most sampling sites. While the detection frequency of metoprolol and sotalol in fish organs was not high in this study, the calculation of the RQ for sotalol in surface water revealed a substantial ecological risk for aquatic organisms of lower trophic levels at sampling site 4 (1.69) (Fig. 5).

Bisphenols are endocrine disruptors and their effects on aquatic biota have been demonstrated (Tsai, 2023). In this study, detection frequencies and concentrations in carp organs were low for bisphenol S and bisphenol F, there was no tendency for bioaccumulation and the RQ in fish was not significant. However, bisphenol A did have a high detection frequency and a medium environmental risk in 70 % of the carps. This is because bisphenol A is one of the most widely used bisphenols as a raw material for industrial products.

Bisphenols tend to accumulate in the solid phase, such as sludge and sediments, to a greater extent than in the aqueous phase. This is mainly because they are more persistent in sediments (Liu et al., 2021). Bisphenol A and bisphenol S have a half-life of 37.5 d in water, while in sediments it is 338 d. Bisphenol F, on the other hand, has a half-life of 15.0 d in water and 135 d in sediments. This explains the high RQ in S3 for bisphenol A and medium RQ in S3 for bisphenol F and bisphenol S.

In the EU, USA, Canada, China, and other countries, regulations have been enacted to restrict or prohibit the use of bisphenol A. Their main substitutes have been bisphenol F and bisphenol S. However, having the same main structure, they can generate the same effects as bisphenol a. After collecting data from China, Japan, and South Korea, fish were most at risk when exposed to bisphenol F, followed by bisphenol A and bisphenol S (Liu et al., 2021). This is an indication that by increasing the use of bisphenol F and bisphenol S, concentrations such as those reported in the present study could be increased and higher values obtained during risk analysis.

Since CECs are found in rivers as mixtures of multiple components, the MRQ was assessed as the sum of the individual RQs. Consequently, 9/10 common carps had medium risk (MRQ between 1.97 and 13.2), as did the WWTP effluent and samples W3 and W4 (MRQ between 5.90 and 16.2). Sample S3 obtained the highest MRQ with a value of 29.5. This was because the anoxic sediment had accumulated a greater number of contaminants due to its high percentage of NOM.

4. Conclusions

This study conducted a comprehensive assessment of CECs across three environmental compartments of a wastewater-impacted river, highlighting the presence and potential for bioaccumulation of compounds like amantadine, terbutryn, and diazepam within the kidney, liver, and brain of common carps. The kinetics and distribution of the compounds in the various analyzed organs can be extrapolated to fish of the *Cyprinidae* family, laying the groundwork for exposure studies under real environmental conditions. While the correlation between experimental BAFs and predicted BCFs using the PBPK model fell within acceptable parameters, there is a call for the development of more complex PBPK models that allow improved predictions of ionized compounds at physiological pH.

Measurement of actual environmental concentrations confirmed significant risks associated with compounds such as caffeine and terbutryn in fish, particularly at sites downstream of the WWTP within the lower part of the Llobregat River basin. Despite some compounds showed low risk to fish, such as *o*-desmethylvenlafaxine and sotalol, ecological risk analysis indicated hazards to lower trophic level aquatic organisms due to the concentrations at which they were detected exceeding the PNECs established for surface waters. The importance of assessing risks in various environmental compartments and at the multi-organ level highlights the need to understand the actual stress levels in aquatic ecosystems. Ultimately, the study allowed the prioritization of compounds such as terbutryn, caffeine, *o*-desmethylvenlafaxine, sotalol, metoprolol, venlafaxine, bisphenol A, and diazepam, because they were detected in concentrations above the reference values, posing an important risk to this aquatic ecosystem.

CRediT authorship contribution statement

Diana P. Manjarrés-López: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Nicola Montemurro:** Writing – review & editing, Supervision, Funding acquisition. **Nadin Ulrich:** Writing – review & editing, Visualization, Methodology. **Ralf-Uwe Ebert:** Validation, Software. **Annika Jahnke:** Writing – review & editing, Validation, Supervision, Conceptualization. **Sandra Pérez:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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Ethical approval

Ethics approval was not required for this research.

Statement on animal welfare

This research was carried out following Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and the Spanish legislation (Royal Decree 53/2013, from the 1st of February, which includes that "Euthanasia of animals is not considered a procedure when performed for the sole purpose of using their organs or tissues".

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.173358.

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INFORMACIÓN COMPLEMENTARIA
PUBLICACIÓN No. 4

Assessment, distribution, and ecological risk of contaminants of emerging concern in a surface water-sediment-fish system impacted by wastewater

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SUPPLEMENTARY INFORMATION

Assessment, distribution, and ecological risk of contaminants of emerging concern in a surface water-sediment-fish system impacted by wastewater

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Table S1. Physicochemical properties of target compounds with the abbreviation (Abbr) used, their use, the corresponding CAS number, molecular formula, molecular weight (MW), and the octanol-water partition coefficients in forms of log K_{ow}, and log D at different pH values as well as the half-life

Compound	Use	CAS	Molecular formula	MW (g mol^{-1})	log K*ow*	logD (pH5)	logD (pH7)	pKa (base)	pKa (acid)	Half-life (h)
5-methyl-1H-Benzotriazole	TP[a] BZT	136-85-6	$C_7H_7N_3$	133.15	1.60	1.60	1.59	1.70	8.50	7.75
Acetaminophen	Analgesic	103-90-2	$C_8H_9NO_2$	151.16	0.34	0.34	0.34	1.70	9.90	0.35
Amantadine	Antiviral	768-94-5	$C_{10}H_{17}N$	151.14	2.22	-0.88	-0.79	10.8	-	32.1
Benzotriazole	Industrial compound	95-14-7	$C_6H_5N_3$	119.12	1.14	1.14	1.12	1.50	8.40	9.02
Bisoprolol	β-blocker agent	66722-44-9	$C_{18}H_{31}NO_4$	325.40	2.14	-0.94	-0.29	9.40	13.9	2.20
Bisphenol A	ED[b]	80-05-7	$C_{15}H_{16}O_2$	228.29	3.43	3.43	3.43	-	10.3	8.15
Bisphenol F	ED[b]	620-92-8	$C_{13}H_{12}O_2$	200.23	2.73	2.73	2.73	-	9.90	3.75
Bisphenol S	ED[b]	46765-03-1	$C_{12}H_{10}O_4S$	250.27	1.83	1.83	1.80	-	8.20	1.33
Caffeine	Stimulant	58-08-2	$C_8H_{10}N_4O_2$	194.19	-0.13	-0.13	-0.13	0.50	-	0.01
Carbendazim	Pesticide	10605-21-7	$C_9H_9N_3O_2$	191.19	1.52	1.37	1.52	4.30	7.80	0.81
Citalopram	Antidepressant	59729-33-8	$C_{20}H_{21}FN_2O$	324.16	2.51	-0.58	0.05	9.60	-	4.36
Clarithromycin	Antibacterial	81103-11-9	$C_{38}H_{69}NO_{13}$	747.95	3.16	0.38	2.07	8.20	13.0	0.58
Clozapine	antipsychotic	5786-21-0	$C_{18}H_{19}ClN_4$	326.80	2.36	0.79	2.22	6.70	-	2.81
Cocaine	Stimulant	50-36-2	$C_{17}H_{21}NO_4$	303.35	3.08	0.08	1.41	8.70	-	0.14
Codeine	Analgesic	76-57-3	$C_{18}H_{21}NO_3$	299.36	1.20	-1.67	-0.08	8.20	13.7	0.94
Cotinine	Metabolite nicotine	486-56-6	$C_{10}H_{12}N_2O$	176.22	-0.23	-0.41	-0.23	4.70	-	0.01
Desmethylcitalopram	Metabolite CTL	62498-67-3	$C_{12}H_{13}N_3O_4S$	295.32	3.25	0.15	0.29	10.5	-	12.2
Diazepam	Antidepressant	439-14-5	$C_{16}H_{13}ClN_2O$	284.74	2.91	2.90	2.91	3.40	-	20.5
Hydrochlorothiazide	Diuretics	58-93-5	$C_7H_8ClN_3O_4S_2$	297.74	-0.07	-0.07	-0.07	-3.80	9.30	0.13
Ketoprofen	NSAID[c]	22071-15-4	$C_{16}H_{14}O_3$	254.28	2.81	1.97	0.08	-	4.20	16.7
Loratadine	Antihistamine	79794-75-5	$C_{22}H_{23}ClN_2O_2$	382.90	5.94	5.88	5.94	4.30	-	0.22
Metoprolol	β-blocker agent	37350-58-6	$C_{15}H_{25}NO_3$	267.36	1.79	-1.27	-0.63	9.40	13.9	3.01
O-desmethylvenlafaxine	Antidepressant	93413-62-8	$C_{16}H_{25}NO_2$	263.19	2.26	-0.80	0.15	9.30	10.0	0.52
Ondansetron	Receptor Antagonist	99614-02-5	$C_{18}H_{19}N_3O$	293.40	2.07	-0.14	1.45	7.50	-	2.66
Pentobarbital	Sedative	76-74-4	$C_{11}H_{18}N_2O_3$	226.28	2.05	2.05	2.00	-	7.90	0.06
Quetiapine	Antipsychotic	111974-69-7	$C_{21}H_{25}N_3O_2S$	383.50	1.57	0.00	1.43	6.50	14.4	1.30

Compound	Use	CAS	Molecular formula	MW (g mol^{-1})	log *Kow*	logD (pH5)	logD (pH7)	pKa (base)	pKa (acid)	Half-life (h)
Sotalol	β-blocker agent	3930-20-9	$C_{12}H_{20}N_2O_3S$	272.36	0.32	-2.75	-1.94	9.30	8.50	3.33
Tebuconazole	Pesticide	107534-96-3	$C_{16}H_{22}ClN_3O$	307.82	3.58	3.58	3.58	2.50	13.7	121
Telmisartan	Hypertension	144701-48-4	$C_{33}H_{30}N_4O_2$	514.60	7.73	6.12	4.90	5.20	3.80	289
Terbutryn	Pesticide	886-50-0	$C_{10}H_{19}N_5S$	241.36	3.44	3.35	3.44	4.00	-	2.92
Trimethoprim	Antibacterial	738-70-5	$C_{14}H_{18}N_4O_3$	290.32	0.79	-1.03	0.53	6.90	-	0.61
Venlafaxine	Antidepressant	93413-69-5	$C_{17}H_{27}NO_2$	277.40	2.91	-0.15	0.82	9.30	14.8	2.67

[a] Transformation product. [b] Endocrine disruptor. [c] Non-steroidal anti-inflammatory drugs. Octanol/water partition coefficients (log *Kow*) for neutral form, pH-dependent distribution coefficients (log D), and pKa were obtained from ACD Percepta software (Advanced Chemistry Development, Inc).

Table S2. Biological characteristics of fish

Fish	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Long (mm)	406	414	448	464	475	486	505	565	554	535
Weight (g)	1305	1699	1821	1927	2402	2496	2577	2824	3149	3298
Gender	Male	Male	Male	Female	Male	Male	Male	Male	Male	Male
Muscle (g)	514	669	717	759	946	983	1015	1113	1241	1299
Skin (g)	45	59	63	67	83	87	89	98	109	114
Brain (g)	4	6	7	8	8	8	9	11	8	9
Kidney (g)	5	12	10	5	10	11	17	16	17	20
Liver (g)	12	15	11	7	11	8	11	14	17	12
Heart (g)	3	3	4	2	4	5	7	9	6	8
Plasma (mL)	52	68	73	77	96	100	103	113	126	132
Age (years) using fish length from Flix reservoir	>9	>9	>10	>10	>10	>10	>10	>11	>11	>11
Age (years) using fish length from several sites	>5	6	>6	>7	>7	>7	>7	>8	>8	8
Age (years) using fish scales	>7	>7	(>5)*	>9	>9	(>3)*	(>3)*	(>4)*	>8	(>6)*
Approx. Age (years)	**>7**	**>7**	**>8**	**>9**	**>9**	**>9**	**>9**	**>8**	**>8**	**>8**

* The scales have no marks in the central part

Table S3. Input parameters for the PBPK model of the compounds detected in fish

Compound	Molecular weight	LSER descriptors E	S	A	B	V	Half-life (d)
5M1Hbenzotriazole	133.15	1.25	0.88	0.90	0.20	1.0051	0.3228
Amantadine	151.14	0.78	0.97	0.20	0.58	1.2916	1.3401
Benzotriazole	119.12	1.47	1.46	0.67	0.48	0.8642	0.3757
Bisoprolol	325.40	0.83	1.50	0.30	2.19	2.7418	0.0916
Bisphenol A	228.29	1.61	1.56	0.99	0.91	1.8643	0.3395
Bisphenol F	200.23	1.56	1.78	1.06	0.66	1.5825	0.1564
Bisphenol S	250.27	1.93	2.74	1.06	1.09	1.7225	0.0553
Caffeine	194.19	1.50	1.82	0.08	1.25	1.3632	0.0004
Cotinine	176.22	1.02	1.75	0.00	1.36	1.3867	0.0004
Diazepam	284.74	2.17	1.78	0.00	1.27	2.0739	0.8538
Hydrochlorothiazide	297.74	2.19	2.90	0.96	1.39	1.7323	0.0056
Metoprolol	267.36	1.17	1.33	0.17	1.76	2.2604	0.1254
O-desmethylvenlafaxine	263.19	1.42	1.66	0.84	1.58	2.2340	0.0217
Sotalol	272.36	1.47	1.55	0.68	2.06	2.1010	0.1387
Terbutryn	241.36	1.43	1.23	0.12	0.99	1.9425	0.1218
Venlafaxine	277.40	1.31	1.30	0.32	1.52	2.3749	0.1112

E: excess molar refraction, ***S:*** polarizability/dipolarity, ***A*****:** solute hydrogen bond acidity, ***B*****:** solute hydrogen bond basicity, and ***V:*** McGowan characteristic volume [1].

Table S4. Lowest PNECs used for individual RQ calculation

Comp.	Lowest PNEC Biota (Fish) (µg Kg^{-1} w.w.)	Lowest PNEC Freshwater (µg L^{-1})	Lowest PNEC Sediments (µg Kg^{-1} d.w.)
5M1Hbenzotriazole	489	150	705
Acetaminophen	532	46	182
Amantadine	357	25	2781
Benzotriazole	-	12.2	-
Bisoprolol	4338	92	17850
Bisphenol A	10.5	0.24	17.4
Bisphenol F	260	4.9	149
Bisphenol S	40.9	12.9	353
Caffeine	2.25	1.2	5.61
Carbendazim	1.1	0.44	5.57
Citalopram	323	16	1923
Clarithromycin	0.49	0.12	269
Clozapine	26.3	0.18	5.02
Cocaine	17.2	2.46	143
Codeine	114	7.19	69580
Cotinine	30.5	10	55.3
Diazepam	10.8	0.29	11.6
Hydrochlorothiazide	216	100	420
Ketoprofen	6.35	2.1	75.4
Loratadine	657	5.3	1127
Metoprolol	148	8.6	557
Desmethylcitalopram	0.92	0.5	24
O-desmethylvenlafaxine	14.1	0.88	13.5
Ondansetron	19	0.99	28.4
Pentobarbital	117	49.5	392
Quetiapine	102	10	573
Sotalol	13.3	6.52	41.7
Tebuconazole	58.5	0.24	12.2
Telmisartan	2208	49	311142
Terbutryn	0.94	0.065	2.38
Trimethoprim	5670	120	872
Venlafaxine	14.3	0.88	29.8

Lowes PNECs obtained from NORMAN [2].

Table S5. Water and sediment concentrations

Compound	Water samples (ng L^{-1})					Sediment samples (ng g^{-1} d.w.)					Log K_D (L Kg^{-1})				
	W1	W2	WWTP	W3	W4	S1	S2	S3	S3.1	S4	1	2	3	3.1	4
5M1Hbenzotriazole	485	522	4009	1937	1837	5.72	7.32	112	29.6	7.22	1.07	1.15	1.76	1.18	0.59
Acetaminophen	36.9	47.6	32.0	49.0	28.8	0.83	<LOQ	3.01	<LOQ	<LOD	1.35	-0.28	1.79	-0.29	-0.76
Amantadine	223	15.8	19800	131	5708	<LOD	1.12	5.28	<LOQ	2.77	-1.17	1.85	1.61	-0.38	-0.31
Benzotriazole	191	218	2447	1540	3708	1.81	2.22	27.8	10.6	3.78	0.98	1.01	1.26	0.84	0.01
Bisoprolol	133	15.0	n.a	232	n.a	0.92	4.72	932	22.9	61.9	0.84	2.50	3.60	1.99	-
Bisphenol A	71.7	83.5	96.7	73.4	91.6	7.89	3.33	61.3	23.4	10.0	2.04	1.60	2.92	2.50	2.04
Bisphenol F	4.75	4.57	46.4	64.0	39.0	1.07	0.39	18.3	7.16	57.8	2.35	1.93	2.46	2.05	3.17
Bisphenol S	36.9	31.7	93.1	716	288	1.41	0.46	39.7	10.7	4.20	1.58	1.16	1.74	1.17	1.16
Caffeine	262	155	202	34.9	189	2.21	2.14	9.53	5.54	1.30	0.93	1.14	2.44	2.20	0.84
Carbendazim	12.8	14.4	19.5	10.0	123	0.14	0.63	1.82	0.21	0.25	1.04	1.64	2.26	1.32	0.31
Citalopram	9.10	10.5	258	142	180	0.73	1.98	4.04	0.33	0.25	1.90	2.27	1.45	0.37	0.14
Clarithromycin	<LOD	<LOD	<LOD	15.4	51.3	<LOD	<LOD	3.43	<LOD	<LOD	-	-	2.35	-0.49	-1.01
Clozapine	3.55	3.28	88.0	51.4	23.9	0.84	0.90	<LOD	1.62	1.12	2.37	2.44	-0.53	1.50	1.67
Cocaine	3.56	2.91	6.25	<LOQ	3.53	0.03	0.08	1.53	<LOD	<LOD	0.93	1.44	3.75	0.74	-0.37
Codeine	5.80	6.63	18.3	119	65.9	<LOD	<LOD	<LOD	<LOD	<LOD	-0.06	-0.12	-1.38	-1.38	-1.12
Cotinine	51.7	53.5	216	78.2	79.6	0.20	0.26	3.09	1.43	0.31	0.61	0.69	1.60	1.26	0.59
Desmethylcitalopram	<LOD	<LOD	121	93.1	65.6	0.67	0.68	2.03	<LOD	<LOD	5.13	5.13	1.34	-1.27	-1.12
Diazepam	1.65	1.62	11.6	7.47	27.9	0.15	0.15	57.2	5.02	1.49	1.96	1.97	3.88	2.83	1.73
Hydrochlorothiazide	106	103	3235	3501	1783	0.22	0.10	15.0	7.71	1.53	0.32	-0.01	0.63	0.34	-0.07
Ketoprofen	5.40	3.84	525	37.0	97.3	<LOD	<LOD	<LOD	<LOQ	<LOD	-0.03	0.11	-0.87	-0.39	-1.29
Loratadine	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	23.2	1.70	<LOD	3.30	3.30	6.67	5.53	-
Metoprolol	8.26	5.62	9004	38.3	2085	1.43	2.74	507	13.4	22.0	2.24	2.69	4.12	2.54	1.02
O-desmethylvenlafaxine	276	234	2758	2077	1711	5.55	3.57	59.6	53.6	14.0	1.30	1.18	1.46	1.41	0.91
Ondansetron	<LOD	<LOD	638	<LOD	112	<LOD	<LOD	6.72	1.16	<LOD	-	-	6.13	5.37	-1.35
Pentobarbital	2.25	2.01	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	1.12	1.17	-	-	2.04

Compound	Water samples (ng L^{-1})					Sediment samples (ng g^{-1} d.w.)					Log K_D (L Kg^{-1})				
	W1	W2	WWTP	W3	W4	S1	S2	S3	S3.1	S4	1	2	3	3.1	4
Quetiapine	3.00	3.47	<LOD	10.7	12.7	0.30	0.40	7.63	1.39	0.62	2.00	2.06	2.85	2.11	1.69
Sotalol	37.1	11.5	41134	229	11064	<LOQ	1.86	228	9.20	54.9	-0.09	2.21	3.00	1.60	0.70
Tebuconazole	<LOD	3.00	8.96	13.7	<LOD	0.13	0.14	<LOD	0.59	0.12	4.72	1.67	-0.74	1.64	4.68
Telmisartan	9.11	5.58	83.9	33.8	30.5	3.34	6.08	192	13.4	2.75	2.56	3.04	3.75	2.60	1.95
Terbutryn	8.85	10.6	81.7	84.1	89.2	1.47	2.86	14.6	2.17	0.46	2.22	2.43	2.24	1.41	0.71
Trimethoprim	7.10	6.96	130	294	144	<LOD	0.11	3.32	7.80	<LOD	-0.85	1.20	1.05	1.42	-2.16
Venlafaxine	80.7	80.5	855	758	495	4.80	2.80	35.4	16.1	2.98	1.77	1.54	1.67	1.33	0.78

<LOD: Less than limit of detection. <LOQ: Less than limit of quantification. n.a: Data not available. Log K_d: Sediment-water partition coefficient calculated by dividing the sediment concentration at each site (µg Kg^{-1} d.w) by the water concentration reported at the same sampling site (µg L^{-1}). For sediment sampling sites S3 and S3.1, the water concentration at site W3 was used. Compounds highlighted in yellow (log $K_d \geq 2$) represent compounds that accumulate more in the solid phase than in the aqueous phase.

Table S6. Organic carbon and organic matter content in sediment samples

Sample	Organic carbon (% on dry weight)	Organic matter (% on dry weight)
S1	0.74	1.28
S2	0.53	0.91
S3	3.87	6.68
S3.1	2.12	3.66
S5	0.87	1.5

Table S7. Concentrations per whole fish

Compound	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 6	Fish 7	Fish 8	Fish 9	Fish 10
5M1Hbenzotriazole	6.70	7.43	5.95	5.40	5.65	5.19	4.09	6.22	7.95	5.64
Amantadine	10.3	5.55	51.6	7.26	38.1	23.7	23.3	15.7	22.6	48.9
Benzotriazole	0.40	2.26	5.04	2.14	1.05	2.97	2.14	0.38	2.78	0.12
Bisoprolol	0.94	0.51	0.77	0.45	0.37	1.41	1.28	1.27	0.35	1.31
Bisphenol A	1.72	1.99	2.25	2.07	0.61	1.53	1.46	0.25	0.65	4.05
Bisphenol F	0.78	0.71	0.83	0.92	0.02	0.10	0.77	0.15	0.10	1.44
Bisphenol S	0.67	0.05	0.19	0.29	0.01	0.52	0.05	0.01	0.05	0.04
Caffeine	24.5	2.45	6.61	9.40	1.22	17.6	0.44	1.70	2.73	0.05
Cotinine	11.2	1.10	3.25	4.75	0.71	6.86	0.45	0.87	1.18	0.59
Diazepam	0.04	0.05	0.05	0.03	0.13	0.05	0.01	0.13	0.09	0.01
Hydrochlorothiazide	0.24	0.32	0.38	0.28	0.06	0.16	0.13	0.12	0.40	0.11
Metoprolol	0.06	0.03	0.19	0.02	0.14	0.20	0.09	0.06	0.05	0.13
O-desmethylvenlafaxine	0.16	0.19	0.18	0.30	0.01	0.02	0.03	0.02	0.33	0.01
Sotalol	0.03	0.01	0.36	0.04	0.06	0.63	0.69	0.14	0.49	0.15
Terbutryn	0.45	0.53	9.12	0.61	1.95	0.36	1.39	1.86	0.46	0.37
Venlafaxine	0.13	0.40	0.52	0.49	0.39	0.34	0.09	0.13	0.44	0.15
TOTAL	**58.4**	**23.6**	**87.3**	**34.5**	**50.5**	**61.7**	**36.4**	**29.0**	**40.7**	**63.1**

Concentrations in ng g^{-1} (w.w.) ordered from the lowest to the highest fish weight. To calculate the concentrations in whole fish we used the following formula: $\sum \frac{(C1*P1)+(C2*P2)+\cdots}{\sum P}$ Where: C: Concentration of each compound (ng g^{-1}), and P: weight of each organ (g).

Table S8. Concentrations per organ (ng g^{-1} w.w.) and plasma (ng mL^{-1})

	Muscle			Skin			Brain			Kidney			Liver			Heart			Plasma		
Comp	Mean	Median	DF	Mean	Median	DF	Mean	Median	DF	Mean	Median	DF	Mean	Median	DF	Mean	Median	DF	Mean	Median	DF
5M1Hbenzotriazole	6.54	6.41	100%	1.86	1.24	90%	3.56	4.90	70%	8.84	8.93	100%	11.9	13.3	100%	1.80	<LOQ	100%	3.70	3.95	90%
Amantadine	22.4	19.9	100%	37.5	20.1	100%	12.1	8.44	100%	158	147	100%	93.0	67.2	100%	21.6	18.1	100%	10.6	10.7	100%
Benzotriazole	1.97	2.35	60%	2.24	0.57	50%	0.03	<LOD	-	3.35	3.36	90%	3.10	1.96	50%	0.29	<LOD	40%	1.18	0.72	100%
Bisoprolol	0.48	0.61	60%	2.67	2.77	100%	0.22	0.04	50%	10.7	10.8	100%	4.36	4.47	90%	0.93	0.94	100%	1.40	1.35	100%
Bisphenol A	1.53	1.43	90%	2.29	2.75	90%	3.77	1.90	90%	4.20	1.58	100%	9.56	9.84	60%	0.04	0.04	50%	0.57	<LOQ	70%
Bisphenol F	0.62	0.79	90%	0.10	<LOD	30%	1.18	0.37	90%	0.01	<LOD	10%	0.76	<LOD	40%	0.06	0.02	50%	0.60	0.55	100%
Bisphenol S	0.20	<LOQ	80%	0.22	<LOQ	70%	0.05	<LOQ	90%	0.14	<LOD	40%	0.33	<LOQ	70%	0.32	<LOQ	60%	0.01	<LOD	20%
Caffeine	6.85	2.68	80%	14.7	<LOQ	80%	0.62	0.38	50%	0.69	0.09	80%	0.61	<LOQ	70%	7.46	4.57	90%	0.04	<LOD	10%
Cotinine	3.37	1.24	100%	3.96	<LOQ	90%	0.46	0.43	80%	0.02	<LOD	20%	0.03	<LOD	30%	2.02	1.20	100%	0.72	<LOQ	70%
Diazepam	0.01	<LOD	-	0.17	<LOD	70%	3.82	5.22	70%	0.45	<LOD	10%	0.03	<LOD	-	0.15	<LOD	10%	0.09	<LOD	30%
Hydrochlorothiazide	0.19	0.15	80%	0.13	<LOQ	60%	0.13	0.16	100%	1.37	1.37	100%	0.70	0.48	100%	0.60	0.49	90%	0.39	0.38	100%
Metoprolol	0.01	<LOD	-	0.69	0.38	50%	0.01	<LOD	-	3.33	3.31	90%	0.37	<LOD	30%	0.01	<LOD	-	0.02	<LOD	-
O-desmethylvenlafaxine	0.12	0.09	50%	0.12	0.06	60%	0.02	<LOD	10%	0.84	0.73	100%	0.45	0.36	80%	0.09	<LOD	50%	0.03	<LOQ	60%
Sotalol	0.01	<LOD	-	0.02	<LOD	20%	0.01	<LOD	-	22.8	11.3	100%	0.04	<LOQ	60%	0.11	<LOD	40%	0.02	<LOD	30%
Terbutryn	1.08	<LOD	30%	5.07	<LOQ	50%	50.2	51.8	100%	0.02	<LOD	20%	0.27	<LOD	30%	7.88	7.16	100%	0.98	0.90	100%
Venlafaxine	0.23	0.31	60%	0.44	0.46	90%	0.25	0.28	80%	3.55	3.84	100%	1.73	1.98	80%	0.90	0.89	100%	0.27	0.28	100%

Table S9. $K_{organ/plasma}$

Compound	Muscle			Skin			Brain			Kidney			Liver			Heart		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Amantadine	2.21	2.22	0.68	3.02	1.96	2.64	1.07	0.92	0.52	13.6	11.6	6.77	8.11	8.10	4.04	2.22	2.19	0.91
Bisoprolol	0.58	0.54	0.14	1.85	1.73	0.64	0.30	0.31	0.07	7.71	7.22	5.60	4.50	4.84	2.63	1.02	1.11	0.30
Venlafaxine	1.37	1.09	0.63	1.85	1.83	0.36	1.29	1.22	0.48	14.7	14.6	6.59	9.54	9.55	4.35	3.61	3.68	1.29
5M1Hbenzotriazole	1.64	1.59	0.43	0.81	0.82	0.26	1.32	1.36	0.29	2.27	2.17	0.84	3.53	3.50	1.17	1.07	1.03	0.17
Benzotriazole	1.32	1.32	0.00	1.15	1.15	0.76	-	-	-	1.92	1.99	0.57	3.69	3.69	0.74	-	-	-
Bisphenol A	1.59	0.60	2.14	0.92	1.10	0.64	1.70	1.52	1.13	5.55	5.55	1.40	14.6	14.6	11.4	-	-	-
Bisphenol F	4.27	1.64	6.59	0.92	0.92	-	5.24	1.26	11.3	-	-	-	5.93	5.93	0.13	1.14	1.14	-
Cotinine	2.95	2.33	2.13	4.51	5.75	3.81	0.91	1.00	0.25	-	-	-	-	-	-	2.28	2.27	1.60
Diazepam	-	-	-	3.74	3.74	-	19.5	19.5	5.18	-	-	-	-	-	-	-	-	-
Hydrochlorothiazide	1.41	1.43	0.54	0.80	0.80	0.42	0.61	0.42	0.34	7.41	5.89	4.69	3.91	2.92	4.45	3.00	1.79	2.24
Terbutryn	14.3	14.3	-	15.9	16.8	2.50	53.1	51.5	11.5	-	-	-	2.60	2.60	-	7.31	8.95	4.91

Compounds ordered from basic to neutral

Table S10. $BAF_{experimental}$ per whole fish

Comp.	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	Mean
5M1Hbenzotriazole	5.60	6.22	4.98	4.51	4.73	4.34	3.43	5.21	6.66	4.72	**5.04**
Amantadine	6.80	3.65	33.9	4.78	25.1	15.6	15.3	10.3	14.9	32.2	**16.3**
Benzotriazole	0.28	1.60	3.56	1.51	0.74	2.10	1.52	0.27	1.97	0.09	**1.36**
Bisoprolol	7.40	4.03	6.08	3.56	2.92	11.2	10.1	10.0	2.77	10.3	**6.83**
Bisphenol A	21.5	24.8	28.1	25.9	7.64	19.2	18.3	3.14	8.17	50.6	**20.7**
Bisphenol F	27.7	25.3	29.4	32.7	0.56	3.54	27.3	5.44	3.70	51.2	**20.7**
Bisphenol S	2.49	0.17	0.71	1.09	0.04	1.96	0.17	0.03	0.20	0.16	**0.70**
Caffeine	153	15.2	41.2	58.6	7.63	110	2.72	10.6	17.0	0.30	**41.6**
Cotinine	170	16.7	49.5	72.3	10.8	104	6.87	13.2	17.9	8.97	**47.1**
Diazepam	4.65	5.14	5.46	3.51	14.0	4.73	1.10	13.4	9.12	1.06	**6.22**
Hydrochlorothiazide	0.18	0.23	0.28	0.21	0.04	0.12	0.10	0.09	0.29	0.08	**0.16**
Metoprolol	0.11	0.06	0.36	0.03	0.27	0.38	0.17	0.11	0.09	0.25	**0.18**
O-desmethylvenlafaxine	0.15	0.18	0.16	0.28	0.01	0.02	0.02	0.02	0.31	0.01	**0.12**
Sotalol	0.01	<0.01	0.13	0.01	0.02	0.22	0.24	0.05	0.17	0.05	**0.09**
Terbutryn	9.24	10.9	189	12.6	40.5	7.44	28.9	38.6	9.46	7.75	**35.5**
Venlafaxine	0.38	1.12	1.46	1.39	1.09	0.97	0.26	0.36	1.25	0.41	**0.87**

Table S11. $BAF_{experimental}$ per organ fish

Comp.	Muscle	Skin	Brain	Kidney	Liver	Heart	Plasma
5M1Hbenzotriazole	5.47	1.56	2.98	7.40	9.97	1.51	3.09
Amantadine	14.7	24.7	7.99	104	61.2	14.2	6.98
Benzotriazole	1.39	1.58	0.02	2.37	2.19	0.21	0.83
Bisoprolol	3.79	21.1	1.73	84.8	34.4	7.38	11.1
Bisphenol A	19.1	28.6	47.1	52.4	119	0.50	7.12
Bisphenol F	22.1	3.46	42.2	0.50	27.1	2.23	21.5
Bisphenol S	0.76	0.82	0.17	0.51	1.22	1.19	0.05
Caffeine	42.7	91.8	3.85	4.33	3.83	46.5	0.23
Cotinine	51.3	60.2	7.02	0.33	0.41	30.7	10.9
Diazepam	1.04	17.8	396	46.2	2.59	15.2	8.86
Hydrochlorothiazide	0.14	0.10	0.09	1.00	0.51	0.44	0.28
Metoprolol	0.01	1.29	0.01	6.23	0.69	0.01	0.03
O-desmethylvenlafaxine	0.11	0.11	0.02	0.79	0.42	0.09	0.03
Sotalol	0.00	0.01	0.00	8.06	0.01	0.04	0.01
Terbutryn	22.5	105	1041	0.46	5.54	164	20.3
Venlafaxine	0.66	1.25	0.72	10.0	4.88	2.53	0.76

Highlighted values show potentially bioaccumulative compounds BAF ≥500.

Text S1. Chemicals and reagents

The chemical standards as well as the isotopically labeled standards (IS) were purchased from Sigma-Aldrich (St. Louis, MO, USA), Sigma-Aldrich (Steinheim, Germany), Toronto Chemicals Research (Toronto, Ontario, Canada), CDN Isotopes (Pointe-Claire, Quebec, Canada), Santa Cruz Biotechnology (Dallas, TX), Wellington (Ontario, Canada), and Cymit Quimica S.L. (Barcelona, Spain). The organic reagents, including LC-MS grade acetonitrile (≥ 99.9%); methanol (≥ 99.9%) and ethyl acetate (≥ 99.5%) were obtained from J.T.Baker (Fisher Scientifc SL, Madrid, Spain) and J.T. Backer (Deventer, The Netherlands). Formic acid (≥ 96%, ACS reagent) was obtained from Sigma-Aldrich. The d-SPE sorbents Discovery® DSC-18 was obtained from SupelTM QuE, Sigma-Aldrich, Steinheim, Germany). The solid phase extraction (SPE) cartridges were prepared using Oasis HLB, WAX, and WCX purchased from Waters Corp., (Milford, MA, USA), while Bond Elut PPL was purchased from Agilent Technologies Inc. (Santa Clara, CA, USA). MeCN, water (Optima™ LCMS Grade), ammonium acetate and ammonium fluoride used for the mobile phases were obtained from Sigma-Aldrich and Fisher Chemical (Fisher Scientifc SL, Madrid, Spain).

Text S2. IS mixture (1000 ng mL^{-1})

Acetaminophen-d4, alprazolam-d5, atenolol-d7, benzotriazole-d4, bezafibrate-d4, bisphenol A-d8, bromazepam-d4, caffeine-13C3, carbamazepine-d10, cocaine-d5, codeine-d3, cotinine-d3, hydrocholorothiazide-13CD2, ketoprofen-d3, lorazepam-d4, metoprolol-d7, midazolam13C6, erytrhromycin-13CD3, fenofibrate-d6, fipronil-13C3, fluconazole-13C3, lamotrigine-13C3, oxazepam-d5, paroxetine-d4, pentobarbital-d5, sitagliptin-d4, sotalol-d6, tramadol-13C3, trimethoprim-d9, valsartan-d3, valsartan acid-d4, venlafaxine-d6, ibersartan-d6, 2,4 DC phenoxy-d3, atrazine-d5, carbendazim-d4, tebuconazole-d9, terbutryn-d5.

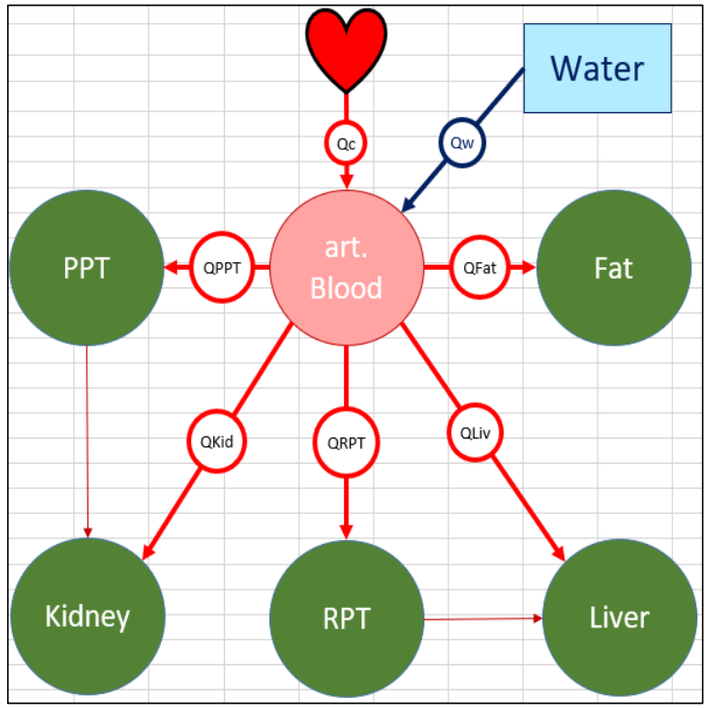

Figure S1. Compartments of PBPK model. PPT: poorly perfused tissue. RPT: richly perfused tissue [3]

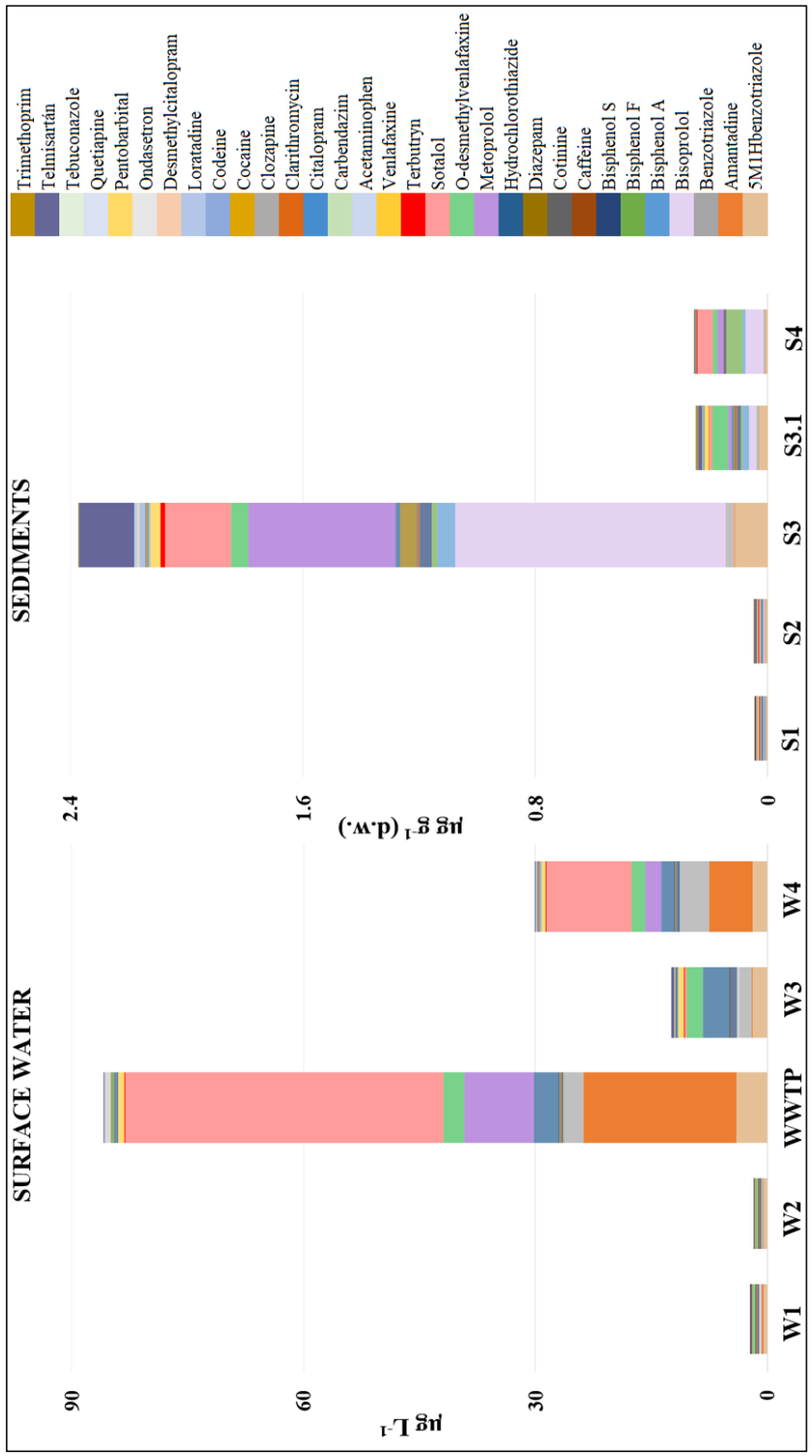

Figure S2. Total concentrations per surface water samples (a) and sediments (b) in each sampling site.

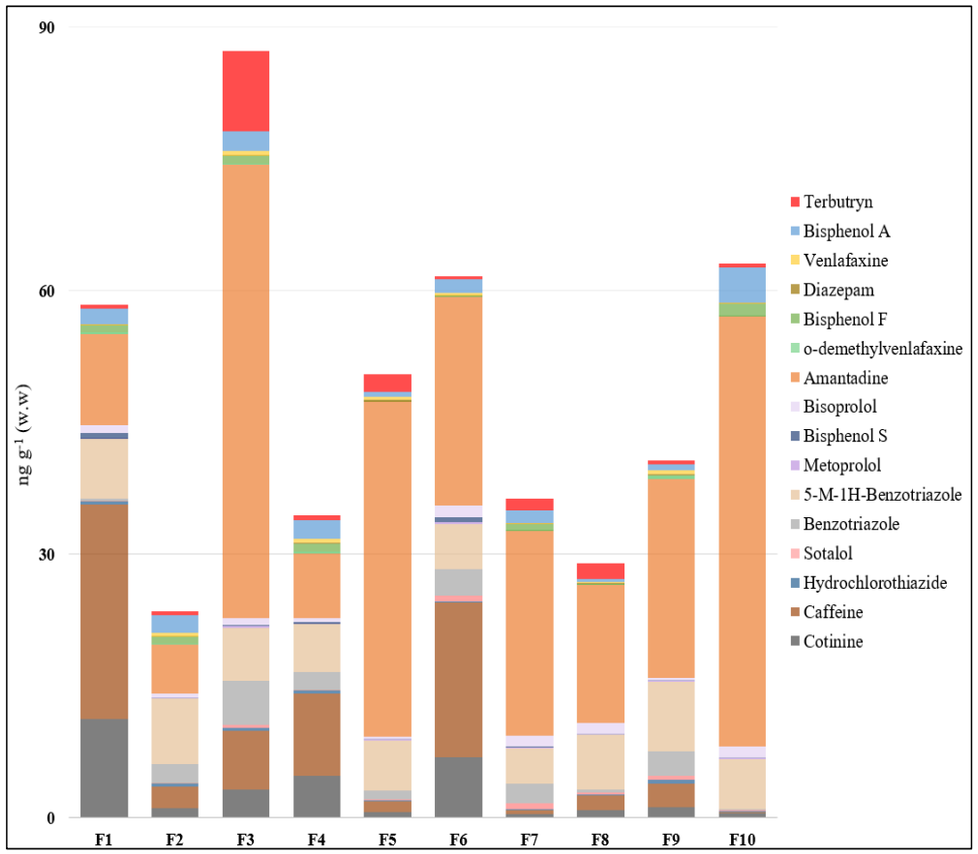

Figure S3. Concentrations per fish ordered from lowest to highest fish weight.

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[2] NORMAN, "NORMAN Ecotoxicology Database - Lowest PNECs." https://www.norman-network.com/nds/ecotox/lowestPnecsIndex.php?checkSelect=0.

[3] Ralph Kühne, "Breaking down the wall between human health and environmental testing of endocrine disrupters: EndocRine Guideline Optimisation. Deliverable Report," 2020.

4.3. Discusión

En esta discusión se destaca la importancia de monitorear múltiples órganos para una evaluación integral del impacto de los CECs en los ecosistemas acuáticos. En las publicaciones No. 3 y No. 4 el análisis de la presencia de contaminantes a nivel de órgano en los peces permitió dar una aproximación más real de los riesgos asociados a su exposición. Se recalca que si bien la acumulación específica de PhACs depende, entre otras características, del órgano objetivo para el que fue diseñado, la acumulación de los compuestos tienden a centrarse principalmente en el hígado y riñón, lo que los convierte en matrices importantes para el biomonitoreo de la contaminación ambiental. La piel y el cerebro también sobresalieron como órganos relevantes para evaluar la exposición a contaminantes, especialmente aquellos con potencial neurotóxico y disruptor endocrino.

En el estudio presentado en la publicación No. 4 se amplió la cantidad de compuestos a analizar mediante análisis dirigido, de tal forma que se pudieron obtener concentraciones para compuestos que sólo pudieron detectarse a un nivel de confianza 2.a en la primera campaña de muestreo. Debido a que en la publicación No. 3 la carpa común destacó para el biomonitoreo ambiental, en esta sección se discuten principalmente los resultados de este especie. En la Tabla 4.1, se presenta un resumen de las concentraciones reportadas en las dos publicaciones.

Compuestos como la amantadina y el bisoprolol, que presentaron altas frecuencias de detección en las muestras de la publicación No. 3 con un nivel de confianza 2.a, fueron nuevamente detectados y cuantificados en las muestras de la publicación No. 4. Para la amantadina en particular, se reportaron concentraciones medias altas en el riñón y el hígado. Estos resultados destacan la importancia del uso del análisis de sospechosos como una primera aproximación para identificar una amplia gama de compuestos presentes en la biota acuática. Este enfoque permitió detectar compuestos que podrían haber pasado desapercibidos en un análisis dirigido inicial. Posteriormente, la cuantificación específica de estos compuestos mediante un análisis dirigido proporcionó datos más precisos sobre sus concentraciones y sus riesgos potenciales, lo que refuerza la necesidad de integrar estos métodos para una evaluación ambiental completa y efectiva.

Por otro lado, durante el segundo muestreo, se cuantificaron varios compuestos en órganos de la carpa común que no fueron detectados en el primer muestreo,

Tabla 4.1. *Comparativa de las concentraciones medias (ng g^{-1}) de los compuestos detectados en la carpa común en las dos campañas de muestreo y otros estudios en peces.*

Órganos	Compuestos	Pub. No. 3	Pub. No. 4	Otros Estudios	Referencia
Músculo	5-M1HBenzotriazol	N.a	6.54	--	--
	Amantadina	2.a	22.4	--	--
	Benzotriazol	N.a	1.97	--	--
	Bisoprolol	2.a	0.48	--	--
	Bisfenol A	N.a	1.53	0.9 –8.94	(Serra-Compte et al., 2017; Yu & Wu, 2014)
	Bisfenol F	N.a	0.62	--	--
	Bisfenol S	N.a	0.20	--	--
	Cafeína	-	6.85	4.58-69.0	(Baduel et al., 2015; Duarte et al., 2023; Ondarza et al., 2019; Peña-Herrera et al., 2020b)
	Cotinina	-	3.37	--	--
	Hidroclorotiazida	N.a	0.19	--	--
	O-desmetilvenlafaxina	-	0.12	--	--
	Sertralina	81.1	N.a	0.08-0.69	(Brooks et al., 2005; Grabicova et al., 2018, 2020; Tanoue et al., 2015)
	Terbutrina	-	1.08	<10	(Barbieri et al., 2019)
	Venlafaxina	-	0.23	0.034-2.50	(Duarte et al., 2023; Grabicova et al., 2018, 2020; McCallum et al., 2017; Rojo et al., 2019)
Piel	5-M1HBenzotriazol	N.a	1.86	--	--
	Amantadina	2.a	37.5	--	--
	Benzotriazol	N.a	2.24	--	--
	Bisoprolol	2.a	2.67	--	--
	Bisfenol A	N.a	2.29	--	--
	Bisfenol S	N.a	0.22	--	--
	Cafeína	-	14.7	--	--
	Cotinina	-	3.96	--	--
	Diazepam	2.98	0.17	--	--
	Hidroclorotiazida	N.a	<LOQ	--	--
	Metoprolol	-	0.69	--	--
	O-desmetilvenlafaxina	-	0.12	--	--
	Sertralina	35.7	N.a	--	--
	Terbutrina	2.a	5.07	--	--
	Venlafaxina	-	0.44	--	--
Cerebro	5-M1HBenzotriazol	N.a	3.56	--	--
	Amantadina	2.a	12.1	--	--
	Bisfenol S	N.a	<LOQ	--	--
	Bisoprolol	2.a	0.22	--	--
	Bisfenol A	N.a	3.77	--	--
	Bisfenol F	N.a	1.18	--	--
	Cafeína	-	0.62	5.20-9.70	(Duarte et al., 2023; Grabicova et al., 2018)
	Cotinina	-	0.46	--	--
	Diazepam	-	3.81	--	--
	Fenpropimorf	2.a	N.a	--	--
	Hidroclorotiazida	N.a	<LOQ	--	--
	Loratadina	2.00	-	--	--
	Quetiapina	2.a	-	--	--
	Sertralina	175	N.a	0.21-21	(Brooks et al., 2005; David et al., 2018; Grabicová et al., 2020; Lajeunesse et al., 2011; Tanoue et al., 2014; Vaclavik et al., 2020.)
	Terbutrina	-	50.2		
	Venlafaxina	-	0.25	0.43-13	(David et al., 2018; Duarte et al., 2023; Grabicova et al., 2014, 2020;

Tabla 4.1. *Continuación.*

Órganos	Compuestos	Pub. No. 3	Pub. No. 4	Otros Estudios	Referencia
					Lajeunesse et al., 2011; McCallum et al., 2017)
Riñón	5-M1HBenzotriazol	N.a	8.84	--	--
	Amantadina	2.a	158	--	--
	Benzotriazol	N.a	3.35	--	--
	Bisoprolol	2.a	10.7	14	(Grabicova et al., 2018)
	Bisfenol A	N.a	4.2	--	--
	Cafeína	50.2	0.69	870*	(Arnnok et al., 2017)
	Claritromicina	3.9	-	0.60	(Grabicova et al., 2018)
	Clozapina	2.a	-	--	--
	Fenpropimorf	2.a	N.a	--	--
	Fluoxetina	22.2	N.a	--	--
	Hidroclorotiazida	N.a	1.37	--	--
	Loratadina	0.2	-	--	--
	Metoprolol	-	3.33	0.50	(Grabicova et al., 2018)
	o-desmetilvenlafaxina	-	0.84	1.5-1.60	(Grabicova et al., 2018, 2020)
	Sertralina	809.7	N.a	1.00-44	(Grabicova et al., 2017, 2018, 2020; Tanoue et al., 2015; Vaclavik et al., 2020)
	Sotalol	-	22.9	--	--
	Terazosin	2.a	N.a	--	--
	Terbutrina	2.a	<LOQ	--	--
	Venlafaxina	-	3.55	2.2-5.30	(Grabicova et al., 2017, 2018, 2020)
Hígado	5-M1HBenzotriazol	N.a	11.9	--	--
	Amantadina	2.a	93.0	--	--
	Benzotriazol	N.a	3.10	--	--
	Bisoprolol	2.a	4.36	--	--
	Bisfenol A	N.a	9.56	--	--
	Bisfenol F	N.a	0.76	--	--
	Bisfenol S	N.a	0.33	--	--
	Cafeína	-	0.61	3.4-13.0	(Duarte et al., 2023; Grabicova et al., 2018; Ondarza et al., 2019)
	Citalopram	2.a	-	3.0-31.0	(Grabicova et al., 2014, 2017, 2018, 2020)
	Clozapina	2.a	-	--	--
	Fluoxetina	38.4	N.a	0.20-86	(Brooks et al., 2005; Du et al., 2012; Lajeunesse et al., 2011)
	Hidroclorotiazida	N.a	0.70	--	--
	Metoprolol	-	0.37	0.40	(Grabicova et al., 2018)
	O-demetilvenlafaxina	-	0.45	0.74-1.90	(Grabicova et al., 2018, 2020)
	Sertralina	618.4	N.a	0.29-110	(Brooks et al., 2005; Du et al., 2012; Grabicova et al., 2017, 2018, 2020; Lajeunesse et al., 2011; Liu et al., 2018; Tanoue et al., 2014, 2015; Vaclavik et al., 2020)
	Sotalol	-	<LOQ	--	--
	Terbutrina	2.a	0.27		
	Venlafaxina	-	1.73	0.69-20.8	(Duarte et al., 2023; Grabicova et al., 2014, 2017, 2018, 2020; Lajeunesse et al., 2011; McCallum et al., 2017)
Corazón	5-M1HBenzotriazol	N.a	1.80	--	--
	Amantadina	2.a	21.6	--	--
	Benzotriazol	N.a	0.29	--	--
	Bisoprolol	2.a	0.93	--	--
	Bisfenol A	N.a	<LOQ	--	--
	Bisfenol F	N.a	0.06	--	--
	Bisfenol S	N.a	0.32	--	--

Tabla 4.1. *Continuación.*

Órganos	Compuestos	Pub. No. 3	Pub. No. 4	Otros Estudios	Referencia
	Cafeína	-	7.46	--	--
	Cotinina	-	2.02	--	--
	Hidroclorotiazida	N.a	0.60	--	--
	Metoprolol	1.3	<LOD	--	--
	O-demetilvenlafaxina	-	0.09	--	--
	Sertralina	19.1	N.a	--	--
	Sotalol	-	0.11	--	--
	Terbutrina	-	7.88	--	--
	Trimetoprima	<LOQ	-	--	--
	Venlafaxina	-	0.90	--	--
Plasma**	5-M1HBenzotriazol	N.a	3.70	--	--
	Amantadina		10.6	--	--
	Benzotriazol	N.a	1.18	--	--
	Bisoprolol	2.a	1.40	--	--
	Bisfenol A	N.a	0.57	6.21-1760	(Chen et al., 2015; Yu & Wu, 2014)
	Bisfenol F	N.a	0.60	--	--
	Cotinina	-	0.72	--	--
	Hidroclorotiazida	N.a	0.39	--	--
	O-demetilvenlafaxina	-	<LOQ	0.16-0.2	(Grabicova et al., 2018, 2020)
	Terbutrina	-	0.98		
	Sertralina	30.7	N.a	0.11-2.26	(Chen et al., 2015; David et al., 2018; Muir et al., 2017; Tanoue et al., 2014, 2015, 2020)
	Venlafaxina	-	0.27	0.03-3.3	(David et al., 2018; Grabicova et al., 2014, 2018, 2020; McCallum et al., 2017; Muir et al., 2017)

** Peso seco; ** ng mL^{-1}; Pub: Publicación; N.a: No analizado, -: No detectado. --: Sin referencia. 2.a: Compuestos evaluados mediante análisis de sospechosos a los que se les confirmó su estructura y hubo coincidencia con el espectro de la biblioteca, pero no se cuantificaron por falta de patrón de referencia.*

como la cafeína, cotinina, terbutrina, o-desmetilvenlafaxina y venlafaxina en músculo, piel y cerebro. Un caso particularmente relevante, fue el de la terbutrina, que se detectó en el cerebro y que fue reportado en la publicación No. 4 categorizándola como bioacumulativa, con un valor BAF de 1041, y que adicionalmente, reportó un riesgo alto en cuatro de los diez peces analizados. Otro ejemplo notable fue el de la cafeína, que, aunque no se clasificó como bioacumulativa en ningún órgano específico, se encontró en concentraciones que representaron un riesgo elevado cuando se consideró el pez entero en seis de las carpas analizadas.

Esta variación en los resultados puede deberse a varios factores. En primer lugar, el tamaño reducido de la muestra en el primer muestreo, donde sólo se capturaron tres peces para esta especie, podría haber limitado la representatividad de los datos. Además, las fluctuaciones en las concentraciones de los compuestos en el agua superficial y las tasas de absorción de los mismos por los peces, que están directamente relacionadas con su disponibilidad en el medio acuático, también

podrían haber influido en los resultados. Estos hallazgos subrayan la importancia del biomonitoreo continuo de los CECs en diferentes órganos y en distintas campañas de muestreo. La variabilidad en la detección de estos compuestos señala la necesidad de realizar un monitoreo regular y de emplear tanto análisis iniciales de sospechosos como análisis dirigidos para garantizar una comprensión exhaustiva de la bioacumulación y los riesgos asociados en la biota acuática.

Por otro lado, pese a que la protección y el bienestar animal son consideraciones fundamentales en la investigación científica, es crucial seguir utilizando una combinación de enfoques que incluyan tanto el análisis de peces salvajes como el desarrollo de modelos predictivos como los PBPK. Los modelos PBPK, como el presentado en la publicación No. 4, ofrecen una herramienta prometedora para estimar la acumulación y distribución de CECs en los órganos de peces, permitiendo reducir la dependencia del uso de animales en estudios de biomonitoreo y ampliando la capacidad para incluir un mayor número de especies y compuestos.

Sin embargo, a pesar de los avances en la precisión de estos modelos para predecir la bioacumulación de CECs en el organismo de los peces (Michel et al., 2016; Wang et al., 2021; Zhang et al., 2019), todavía existen desafíos para emular de manera satisfactoria las condiciones ambientales. Uno de los principales retos es lograr una descripción precisa de la fisiología de los peces, incluyendo aspectos críticos como las rutas de excreción, el flujo sanguíneo y la producción de huevos, además de integrar el impacto del crecimiento y la temperatura en los modelos y el mecanismo de partición para compuestos que se ionizan a pH fisiológico.

Para avanzar en este campo, es importante considerar la contribución relativa de las branquias, en comparación con el intestino, hígado y riñón, en los procesos ADME, así como incorporar factores ambientales y las características del ciclo de vida de los peces (Grech et al., 2019). Los esfuerzos también deben aunarse para permitir la inclusión de un mayor número de órganos en los modelos, ya que como se ha abordado durante todo el capítulo, la distribución de los CECs es variable. La integración de estos parámetros permitirá desarrollar modelos más robustos y representativos, mejorando así la evaluación del riesgo y la protección de los ecosistemas acuáticos.

Teniendo en cuenta las limitaciones actuales de los modelos PBPK, el análisis de peces salvajes sigue siendo necesario, pero un enfoque combinado, no solo permitiría avanzar hacia estudios de biomonitoreo más éticos y sostenibles, sino

que también aseguraría una evaluación integral manteniendo un equilibrio entre la innovación científica y la conservación de la biodiversidad.

Respecto al análisis de riesgo ecológico, los resultados de la publicación No. 4 permitieron identificar y priorizar compuestos como la cafeína y la terbutrina, que se encontraron en concentraciones superiores al PNEC propuesto por la red NORMAN para peces. Por otro lado, en la publicación No. 3, se reportó que la sertralina fue el compuesto detectado con mayor frecuencia y en las concentraciones más altas en la carpa común, lo que subraya su relevancia en términos de contaminación y riesgo ecológico potencial. Sin embargo, durante el análisis de las muestras de las carpas muestreadas en el 2021, no fue posible incluir la sertralina debido a que no se contaba con su patrón de referencia, ya que la autorización para su uso, al tratarse de una sustancia controlada, sufrió retrasos. Esto impidió realizar un análisis comparativo actualizado de este compuesto.

A pesar de esta limitación, aquí se presenta el cálculo de los cocientes de riesgo (RQ del inglés *risk quotient*) de la sertralina, basados en las concentraciones medias reportadas en el 2019 para las tres carpas analizadas, utilizando las fórmulas presentadas en la publicación No. 4. Los valores obtenidos fueron significativamente altos (61, 62 y 72), siendo aproximadamente siete veces mayores que los valores de RQ reportados para la terbutrina y la cafeína en el 2021. Esto sugiere que, aunque no se pudo analizar directamente en el 2021, la sertralina representa un riesgo ecológico considerablemente mayor.

Para explorar más a fondo este riesgo, se realizó un estudio de colaboración en el que se expusieron larvas de peces cebra a concentraciones de sertralina ambientalmente relevantes, desde 0.01 a 10 µg L^{-1}. El objetivo fue evaluar las posibles afectaciones de este antidepresivo sobre el desarrollo de las larvas. Los resultados indicaron que, a partir de una concentración tan baja como 0.1 µg L^{-1} se indujo hipo-locomoción y un retraso en el proceso de aprendizaje (Faria et al., 2022), lo que subraya la necesidad de continuar monitoreando este compuesto en futuras investigaciones.

4.4. Bibliografía

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CAPÍTULO 5

POTENCIAL DE USO DE LAS ESPECIES INVASORAS PARA EL BIOMONITOREO DE CONTAMINANTES DE PREOCUPACIÓN EMERGENTE EN ECOSISTEMAS ACUÁTICOS

POTENCIAL DE USO DE LAS ESPECIES INVASORAS PARA EL BIOMONITOREO DE CONTAMINANTES DE PREOCUPACIÓN EMERGENTE EN ECOSISTEMAS ACUÁTICOS

5.1. Introducción

Los resultados de biomonitoreo de CECs en peces presentados en el capítulo 4, mostraron que la especie *Cyprinus carpio* fue la especie con las tasas de acumulación más elevadas. Este resultado, se asoció a su naturaleza invasora en el ecosistema evaluado, que le otorgaría una mayor resistencia a la contaminación. Bajo esta premisa, en este capítulo ampliaremos el conocimiento respecto al uso de especies invasoras en estudios de biomonitoreo de CECs, analizando las especies invasoras presentes en el Parque Natural de L'Albufera (Valencia, España).

El empleo de estas especies permite evaluar de manera eficaz la acumulación de CECs en un ecosistema sometido a diversas presiones antropogénicas, aprovechando su capacidad para tolerar y bioacumular compuestos químicos. Con este enfoque, buscamos obtener una visión más completa del impacto de los CECs en las especies acuáticas y la calidad ambiental del parque.

A continuación, se explicará brevemente el origen, propagación, impactos y la inclusión en estudios de biomonitoreo de las tres especies acuáticas invasoras objeto de estudio: cangrejo de río rojo americano (*Procambarus clarkii*), almeja asiática (*Corbicula fluminea)* y el pez percasol (*Lepomis gibbossus*).

5.1.1. Cangrejo de río rojo americano

Origen: es originario de Luisiana, criado y cosechado en arrozales inundados en gran parte de su área de distribución nativa, particularmente en el sur de los Estados Unidos. Al contrario de otros invertebrados cuya introducción fue principalmente accidental, *P. clarkii* se introdujo intencionadamente por su valor alimentario. En Europa, las primeras introducciones se produjeron en España desde 1973 para la acuicultura. Mientras que en Europa meridional la introducción se centró en la producción y cosecha del cangrejo. En Europa central su presencia está asociada principalmente con liberaciones accidentales por el comercio de alimentos y acuarios, especialmente a partir de la década de 1980 (Souty-Grosset et al., 2016).

Propagación: Esta especie puede habitar en pantanos terrestres, tanto naturales como seminaturales, y en zonas húmedas terrestres, como praderas o áreas gestionadas para el cultivo de arroz (Souty-Grosset et al., 2016). *P. clarkii* fue introducido en el Parque Natural L'Albufera en 1976, y ha logrado sobrevivir y prosperar debido a las condiciones ambientales favorables, como la humedad constante de la región y la abundancia de arrozales, que proporcionan un hábitat adecuado para su desarrollo (Martín-Torrijos et al., 2021).

El creciente comercio a través de internet ha incrementado significativamente el riesgo de transportar cangrejos vivos, ya sean autóctonos o exóticos de un país a otro. En ferias de toda Europa se venden peces de acuario e invertebrados sin que existan controles efectivos en las fronteras. En particular, el auge de la afición por los invertebrados entre los amantes de los acuarios alemanes desde principios de la década de 2000, ha generado una gran preocupación. Esto ha facilitado la importación de más de 120 especies de cangrejos no autóctonos, de las cuales aproximadamente 20 están disponibles de manera regular en el mercado, incluido el cangrejo de río rojo (Chucholl, 2013).

Impacto: es una de las cinco especies de crustáceos decápodos de agua dulce incluidas en la lista de especies exóticas invasoras de preocupación para la Unión Europea, conforme al Reglamento de Ejecución (UE) 2016/1141 de la Comisión (CE., 2016), debido a su alarmante y creciente impacto sobre la flora y fauna de los ecosistemas europeos. Además, ésta especie se clasifica como ampliamente extendida en Europa y se considera imposible de erradicar de manera rentable, según lo establecido en el Reglamento (UE) 1143/2014 del Parlamento Europeo y del Consejo (CE., 2014).

Debido a su alta plasticidad ecológica, el cangrejo de río rojo ha logrado colonizar la mayoría de los tipos de masas de agua. Además de su condición omnívora, esta especie invasora es un importante depredador de vertebrados acuáticos como anfibios y peces, e invertebrados, y es responsable del declive y extinciones locales de muchas especies nativas (Martín-Torrijos et al., 2021).

Entre las 27 principales especies animales exóticas introducidas en Europa por la acuicultura y actividades relacionadas, esta especie ha demostrado tener el mayor grado de impacto, que va desde la competencia con especies nativas hasta la alteración de la red alimentaria y la estructura del hábitat (Savini et al., 2010).

Como especie omnívora, afecta significativamente la estructura trófica en aguas dulces y estuarios invadidos, interactuando con diferentes niveles y alterando el funcionamiento del ecosistema (Souty-Grosset et al., 2016). Además, provoca la resuspensión de sedimentos, lo que puede transformar rápidamente lagos de aguas claras dominados por macrófitos en lagos turbios dominados por fitoplancton (Gallardo et al., 2016). El aumento de los sólidos suspendidos incrementa la turbidez de los hábitats acuáticos y su actividad excavadora en humedales costeros no solo altera el ecosistema, sino que también reduce la protección natural contra tormentas y el aumento del nivel del mar (Souty-Grosset et al., 2016).

Desde el punto de vista económico, el cangrejo de río rojo americano se considera responsable de la disminución de la productividad en los arrozales de Europa, una pérdida que a menudo supera los beneficios de la cosecha. Infestaciones experimentales con densidades de hasta un cangrejo de río por m^2 pueden reducir la producción de arroz en un 41,6% (Anastácio et al., 2005). En Portugal durante el 2004, las asociaciones de productores de arroz estimaron una pérdida económica directa promedio de aproximadamente 43 € por hectárea, debido a la presencia de esta especie en sus plantaciones (Souty-Grosset et al., 2016).

Biomonitoreo: se ha considerado para el biomonitoreo de la contaminación por metales pesados en ecosistemas de agua dulce (Berthet, 2015) en países como España (Alcorlo et al., 2006; Suárez-Serrano et al., 2010), China (Anandkumar et al., 2020) y Egipto (Abbas et al., 2023; Soliman et al., 2016). Su uso en este tipo de estudios se relaciona con su supervivencia en diversas circunstancias y su tolerancia al estrés ambiental (Bian H. et al., 2023).

También se ha usado para la detección de CECs, aunque en menor medida, para compuestos como PhACs (Kazakova et al., 2018, 2021), pesticidas organoclorados (Schilderman et al., 1999) y PFAS, especialmente en zonas dónde su consumo es común como en Italia (Marin et al., 2024), China (Bian J. et al., 2024) y el delta del Ebro en España (Fernández-Sanjuan et al., 2010) (Tabla 5.1).

5.1.2. Pez percasol

Origen: conocido también como pez luna de agua dulce, es originario del este y centro de América del Norte. Su distribución natural abarca desde la cuenca del río Misisipi, al este de las Grandes Llanuras, hasta los estados costeros, desde Carolina del Sur hasta Nueva Inglaterra, extendiéndose hacia el norte hasta Ontario

y Quebec (Yavno et al., 2020). A finales del siglo XIX, fue introducido por primera vez en Europa con fines recreativos de pesca y como pez ornamental para acuarios y estanques de jardín (Van Kleef et al., 2008). El primer registro confirmado de esta especie en la Península Ibérica, específicamente en el Lago de Banyoles (Cataluña, España), data de entre 1910 y 1913. Aunque hasta la década de 1980 su población estuvo controlada, desde entonces se ha expandido significativamente, colonizando numerosas cuencas hidrográficas en la Península (Oliva-Paterna et al., 2005). Actualmente existen poblaciones introducidas de *L. gibbossus* en más de 30 países de Europa, el norte de África y América Central y del Sur (Yavno et al., 2020).

Propagación: es posible que los ejemplares del pez percasol que se han establecido en España y Portugal se hayan propagado mediante dispersión natural, a través de las cuencas ibéricas adyacentes utilizando los sistemas interconectados de embalses y ríos de toda la región. Sin embargo, también hay pruebas de que el transporte por seres humanos facilitó su propagación, usándolo como pez ornamental o para acuicultura (Bhagat et al., 2011).

La distribución del percasol en Europa abarca una variedad de ecosistemas acuáticos, incluidos masas de agua en llanuras aluviales de grandes ríos, pequeños arroyos, estanques, lagos y masas de agua lénticas. En algunos casos, su expansión en humedales se facilitó por actividades humanas, como la eliminación de plantas invasoras para favorecer el crecimiento de especies vegetales nativas (Almeida et al., 2014; Yavno et al., 2020). El aumento de la temperatura podría favorecer la propagación del percasol, ya que el clima cálido promueve tanto su densidad poblacional como su capacidad de dispersión (Copp et al., 2004).

Impacto: se encuentra entre las diez principales especies de peces introducidas con efectos ecológicos adversos. Se ha informado que es responsable de la disminución de otras especies de peces (Copp et al., 2004), así como de gasterópodos. En particular, la abundancia promedio de macroinvertebrados en estanques donde ésta especie está presente es un 83% menor en comparación con aquellos estanques donde la especie no ha sido introducida (Van Kleef et al., 2008).

Su presencia en un pequeño afluente del río Ouse (Sussex, Inglaterra) sugiere que, en las zonas donde esta especie alcanzaba altas densidades, se observaron cambios significativos en la estructura de la red trófica. Estos cambios incluyeron variaciones en la proporción de los peces presentes y en la composición de la comunidad de

arañas ribereñas (Copp et al., 2010). Otro estudio realizado en un humedal ibérico relacionó su presencia con la reducción en el tamaño y la abundancia del zooplancton del hábitat, lo que provocó un aumento en la turbidez del agua, así como mayores niveles de clorofila α, fósforo total y nitrógeno (Angeler et al., 2002).

Además de los efectos ecológicos, la interferencia en el comportamiento constituye un impacto adverso de los peces invasores sobre las especies nativas. La agresión es el principal mecanismo de esta interacción, provocando la alteración de procesos clave como la alimentación, la reproducción o la búsqueda de refugio de la fauna autóctona (Almeida et al., 2014).

Biomonitoreo: los estudios de biomonitoreo con percasol son menos frecuentes en comparación con las otras dos especies incluidas en esta tesis, y se han centrado principalmente en la detección de metales pesados. En Turquía, se usó para comparar las concentraciones de metales en una zona contaminada y una no contaminada de un lago (Yilmaz et al., 2007). Además, se ha utilizado para evaluar la calidad del agua de un arroyo y la distribución de algunos metales pesados en tres órganos diferentes (branquias, músculo e hígado) (Koca et al., 2005). Respecto al biomonitoreo de CECs, cinco individuos de esta especie fueron incluidos en un estudio para evaluar la bioacumulación de PFAS en una red alimentaria de Canadá (Munoz et al., 2022).

5.1.3. Almeja asiática

Origen: es originaria del sur y este de Asia, Australia, el norte de África y el sureste de Rusia. Durante el siglo XX se dispersó rápidamente como especie invasora en América. En Europa fue descrita por primera vez por Mouthon, (1981) en el río Tajo (Portugal) y en el río Dordoña (Francia) en 1980. Posteriormente, se registró su presencia en los Países Bajos. Actualmente, está ampliamente distribuida por Europa continental, habiéndose propagado a numerosas cuencas hidrográficas, como las del Rin, el Ródano, el Elba, el Danubio en Bulgaria, el delta del Danubio en Ucrania y Rumanía, así como la cuenca del río Prut en Moldavia (Minchin & Mcdonald, 2014).

Propagación: es una de las especies invasoras de agua dulce más eficientes del mundo. Desde su primer registro en la Península Ibérica, la distribución de la almeja asiática ha aumentado de manera exponencial hasta 2008, estando presente en al menos seis grandes cuencas ibéricas. Su expansión está asociada a sitios lénticos

de agua dulce modificados por actividades humanas, como embalses, y a hábitats lóticos con caudal permanente, como canales de riego, muelles, así como grandes ríos con zonas profundas y estables (Pérez-Quintero, 2008). Se considera que el agua de lastre ha sido una de las principales vías de introducción de bivalvos asiáticos, incluida *C. fluminea*, que ha invadido ecosistemas de agua dulce en América del Sur desde el 2000 (Havel et al., 2015).

El éxito invasivo de la almeja asiática se puede atribuir a sus características biológicas, como su condición de hermafrodita auto-fecundante, crecimiento rápido, maduración sexual temprana (de 3 a 6 meses), vida corta, alta fecundidad, elevadas tasas de filtración, amplias capacidades de dispersión, capacidad para habitar diferentes tipos de sustrato, competitividad frente a especies nativas, e interacción con actividades humanas (Gomes et al., 2016). Adicionalmente, los juveniles de la almeja asiática se adhieren a algas filamentosas, facilitando su transporte pasivo a través de las patas o plumas de aves acuáticas, contribuyendo a su dispersión (McMahon, 2002).

Impacto: las poblaciones de esta especie pueden alcanzar altas densidades y biomasa y pueden ser consumidas por niveles tróficos superiores alterando la red alimentaria. Respecto a efectos no tróficos, su principal impacto está relacionado con sus actividades de "ingeniería de ecosistemas", las cuales pueden provocar cambios significativos en la claridad del agua, la bioturbación de sedimentos, el ciclo de nutrientes y la colonización del sustrato, principalmente por la acumulación masiva de conchas (Gallardo et al., 2016; Gomes et al., 2016).

La presencia de esta especie puede incrementar la abundancia y diversidad de invertebrados bentónicos y macrófitos, mientras que reducen la de las comunidades planctónicas. Los mecanismos detrás de estos cambios incluyen una combinación de filtración directa y la alteración indirecta de las condiciones del hábitat. La almeja asiática puede filtrar grandes cantidades de partículas de la columna de agua y disminuir la concentración de fósforo, un nutriente clave para la producción de fitoplancton en ecosistemas de agua dulce. La mayor claridad del agua resultante favorece el crecimiento de macrófitos acuáticos, con estudios que reportan aumentos de hasta tres veces su biomasa (Gallardo et al., 2016).

La presencia de esta especie puede afectar negativamente a las poblaciones de almejas de agua dulce en la Comunidad Valenciana (España). Se han encontrado

ejemplares de esta especie en el río Júcar, donde comparten hábitat con bivalvos autóctonos, lo que podría desencadenar una competencia por recursos como el alimento e incluso por el espacio disponible. Su expansión a lo largo del Júcar y su llegada al Parque Natural de L'Albufera representan una seria amenaza para las poblaciones de *Anodonta cygnea* (petxinot), que están presentes en esta área y podrían verse desplazadas por la competencia directa (Conselleria d'infraestructures territori i medi ambient, 2014).

Biomonitoreo: para esta especie el monitoreo de campo ha indicado que las concentraciones de metales pesados son a menudo varios órdenes de magnitud mayores que las del agua superficial o la de los sedimentos (Li et al., 2023; Reis, Pedro A. et al., 2014). Estudios de bioacumulación de metales, así como de bifenilos policlorados, plaguicidas organoclorados, diclorodifenil-tricloroetano, hidrocarburos aromáticos policíclicos y compuestos órgano-estánicos en la almeja asiática, confirman la viabilidad de utilizar estas almejas como organismos centinela (Guo & Feng, 2018). En cuanto a su uso para el biomonitoreo de CECs, se ha utilizado para evaluar la presencia de PhACs (Burket et al., 2019; Du et al., 2014), plaguicidas organoclorados (Colombo et al., 1995; Takabe et al., 2010), PFAS (Koban et al., 2024) y OPFRs (La Guardia et al., 2012).

Tabla 5.1. *CECs detectados en las especies invasoras objeto de estudio*

Especie	*Compuestos*	*Concentraciones (ng g^{-1} w.w.)*	*Referencia*
Cangrejo de río rojo americano	Carbamazepina	<0.9-20.8	(Kazakova et al., 2021)
	Sulfametoxazol	<0.3	
	Flumequina	<1.1-34.2	
	Ciprofloxacina	<1.0	
	Ibuprofeno	<1.5	
	Sulfametoxazol	<LOQ	(Kazakova et al., 2018)
	Flumequina	<LOQ	
	Atrazina, diazinón, clorfenvinfos, fentión, metlacloro, paratión-metilo, pronamida,	<LOD	(Marin et al., 2024)
	PFBA, PFHxS, PFUnDA, PFOA, PFNA, PFODA	<LOD	
	PFBA	<LOQ-2.47	(Bian J. et al., 2024)
	PFPeA	<LOQ-2.62	
	PFTeDA	<LOQ-1.15	
	PFDA	<LOQ-0.51	
	PFDS	<LOQ-0.43	
	PFNA	<LOQ-0.62	
	PFHxS	<LOQ-2.00	
	PFOA	<LOQ-4.39	
	PFOS	<LOQ-0.45	
	PFBS	0.14-0.58	
	PFHxA	<LOQ-8.82	
	PFOS	0.23-0.63	(Fernández-Sanjuan et al., 2010)
	PFOA	0.14-0.40	
	PFNA	0.07-0.11	
	TnBP	0.66-3.96	(Peng et al., 2024)
	TCEP	0.51-0.93	
	TEP	2.95-4.38	
	TPHP	1.78-8.45	
	TPP	3.70-29.0	
	TBEP	0.19-0.49	
	TCPP	3.02-8.53	
Almeja asiática	Difenhidramina	1.6-2.8	(Du et al., 2014)
	Carbamazepina	1.2-2.9	
	Norfluoxetina	<LOD	
	Sertralina	56-140	
	Fluoxetina	10.0-12.0	
	Desmetil-sertralina	67.0-86.0	
	Celecoxib	22.0-26.0	
	Diclofenaco	18.0-33.0	
	Diltiazem	<LOD	
	Fluoxetina	5.45-8.42	(Burket et al., 2019)
	Nor-fluoxetina	0.65-0.98	
	Sertralina	158-226	
	Aldrin	<LOD	(Takabe et al., 2010)
	Dieldrin	0.012-1.10	
	Endrin	<LOD-0.047	
	PFBA	<LOD	(Koban et al., 2024)
	PFBS	<LOD	
	PFPeA	<LOD	
	PFHxA	2.40-9.96	
	PFHxS	<LOD	
	PFHpA	0.18-0.94	
	PFOA	0.19-2.53	

Tabla 5.1. *Continuación.*

Especie	*Compuestos*	*Concentraciones (ng g^{-1} w.w.)*	*Referencia*
	PFDoA	0.14-2.21	
	PFTeDA	0.02-0.78	
	PFPeS	0.09-1.63	
	PFOS	<LOD	
	PFDA	0.06-2.01	
	TBB	2220*	(La Guardia et al., 2012)
	TBPH	37-1370*	
	BTBPE	112-153*	

*Concentraciones expresadas como % de lípidos basado en su peso seco

Como se puede observar, la mayoría de los estudios de biomonitoreo en los que se han usado las especies invasoras incluidas en esta tesis, se enfocan en un número limitado de compuestos o en un grupo de compuestos químicamente relacionados, principalmente inorgánicos como metales pesados, y suelen utilizar una sola especie. Sin embargo, en un mismo ecosistema puede haber múltiples especies invasoras, y su análisis conjunto permitiría obtener una visión más amplia sobre las mezclas de contaminantes presentes y sus posibles efectos.

5.2. Metodología y resultados

Este capítulo aborda el uso de especies invasoras para el biomonitoreo de CECs en un ecosistema de alto valor ecológico como lo es el Parque Natural de L'Albufera. Este enfoque busca ampliar el conocimiento sobre la efectividad de las especies invasoras como herramientas para evaluar la calidad ambiental en ecosistemas sometidos a presión antropogénica, minimizando el impacto sobre las especies autóctonas y proporcionando una comprensión más profunda de la bioacumulación de CECs en ambientes acuáticos.

Para alcanzar este objetivo, se llevó a cabo un experimento de exposición en condiciones ambientales reales, tras el despliegue de ejemplares en diez sitios estratégicos dentro del parque. En estos sitios, se ubicaron en jaulas las tres especies invasoras objeto de estudio (*Procambarus clarkii, Lepomis gibbosus y Corbicula fluminea*), junto con una especie nativa protegida (*Anodonta cygnea)*, durante catorce días. En la Fig. 5.1 se muestran las jaulas en las que se introdujeron los ejemplares para llevar a cabo el experimento. Una vez finalizado, se evaluó la presencia y acumulación de 171 CECs de acuerdo al método analítico presentado en la Publicación No. 2.

El desarrollo de este estudio permitió la evaluación de la presencia y

bioacumulación de CECs, la comparación de los patrones de acumulación entre las diferentes especies y la priorización de aquellas especies que brindaron mayor información sobre la presión química que enfrenta este ecosistema acuático, reduciendo así la carga de monitoreo sobre la especie autóctona. Los resultados se presentan en la Publicación No. 5: ***Manjarrés-López, D.P.,*** *Martínez-Megías, C., Vitale, D., Picó, Y., Rico, A., & Pérez, S. (2024). Occurrence, bioaccumulation, and ecological risk assessment of contaminants of emerging concern using native and invasive species as biomonitors (Enviado).*

Fig. 5.1*. Jaulas en las que se depositaron los ejemplares de la especie L. gibbosus (izquierda) y P. clarkii (derecha) durante el experimento de exposición.*

5.2.1. PUBLICACIÓN No. 5

Occurrence, bioaccumulation, and ecological risk assessment of contaminants of emerging concern using native and invasive species as biomonitors

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Occurrence, bioaccumulation, and ecological risk assessment of contaminants of emerging concern using native and invasive species as biomonitors

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ABSTRACT

Traditionally, contaminants of emerging concern (CECs) monitoring has focused on assessing their occurrence in abiotic compartments and in native fish species. The use of non-native species in environmental quality studies has recently proven to be a key tool for evaluating the CECs exposure and their ecological risks. In this study, the use of different native and invasive species was proposed to assess the environmental quality of a coastal Mediterranean wetland. A biomonitoring assessment was conducted at ten locations within the Albufera Natural Park (Spain), including irrigation channels, the lake, and the artificial wetland. The bioaccumulation of 171 CECs was evaluated in the Asian clam, the American red swamp crayfish, and the pumpkinseed sunfish, which are primary invasive species in this ecosystem. Furthermore, a comparative analysis was done with the native clam to verify whether invasive species could provide equivalent information. A total of 35 CECs were detected in at least one of the species analyzed. The Asian clam exhibited the highest number of detected compounds (23), as well as the highest chemical concentrations, particularly for pharmaceuticals. The ecological risk assessment performed with internal concentrations of CECs also pointed at the Asian clam as the most suitable species for chemical biomonitoring in this area. The compounds that had the highest contribution to the calculated ecological risk were sertraline, fluoxetine, terbuthylazine, caffeine, and oseltamivir. This study shows that the analysis of chemical concentrations in invasive species can be considered a complementary tool to determine the ecological status of coastal wetlands.

1. Introduction

Freshwater ecosystems, including wetlands, are continuously exposed to contaminants of emerging concern (CECs) such as organophosphate flame retardants (OPFRs), per- and poly-fluoroalkyl substances (PFAS), pesticides, and pharmaceutically active compounds (PhACs) (Martínez-Megías & Rico, 2022). The biomonitoring of CECs in selected aquatic species provides crucial insights into the levels of contaminant exposure and bioaccumulation in aquatic biota, which can be better linked to chemical-related effects in aquatic ecosystems (Álvarez-Ruiz & Picó, 2020; Manjarrés-López et al., 2024; Miller et al., 2018; Pantelaki & Voutsa, 2019). Invasive aquatic species could serve as effective bioindicators due to their greater tolerance to environmental stress and their capacity to bioaccumulate CECs. Using invasive species in biomonitoring can also reduce the pressure on native species, as invasive populations are typically larger and more abundant so that their use in environmental quality assessments minimizes ecosystem disturbances (Berthet, 2015; Koban et al., 2024). However, most biomonitoring studies of CECs in freshwater ecosystems have focused on the assessment of a narrow range of compounds in native fish species, neglecting the value of invasive species for environmental quality studies (Manjarrés-López., et al., 2023; Pemberthy M et al., 2020; Pittura et al., 2022; Zhao et al., 2015).

L'Albufera Natural Park (ANP) in Valencia, Spain, is a strategic aquatic ecosystem that faces several

anthropogenic pressures (Martínez-Megías & Rico, 2022), including the colonization by invasive species such as the American crayfish, *Procambarus clarkii* (native to northeastern Mexico and the southern U.S.) (Anandkumar et al., 2020), the pumpkinseed sunfish, *Lepomis gibbosus* (an ornamental fish introduced for sport fishing from eastern North America) (Copp et al., 2004), and the Asian clam, *Corbicula fluminea* (one of the world's most widespread invasive clams from East Asia) (Rong et al., 2021). These species compete with endangered endemic species, such as *Anodonta cygnea*, a clam whose abundance has declined significantly in Mediterranean coastal wetlands and that currently is under special protection (Conselleria d'infraestructures territori i medi ambient, 2014).

Due to the proximity of the ANP to the city of Valencia and the various economic activities in the region, the Park is continuously subjected to significant pressure from CECs, including OPFRs and PFAS from urban and industrial sources; pesticides from rice and citrus cultivation in the vicinity of the lake, and PhACs from WWTPs effluents that are commonly used for rice field irrigation (Martínez-Megías et al., 2024; Soriano et al., 2023, 2024). Despite the number of studies pointing at complex CEC mixtures as one of the main drivers of biodiversity loss in the ANP, there have been no studies aimed at assessing their bioaccumulation potential and their ecotoxicological risks in sentinel species representative of this ecosystem.

In this study we comparatively assessed the bioaccumulation potential of CECs in three common invasive species, and a protected native species representative of the ANP and determined their capacity to be used as sentinel organisms to assess the environmental quality of Mediterranean coastal wetlands. The specific objectives of this study were: i) to evaluate the occurrence and bioaccumulation of 49 pesticides, 86 PhACs, 36 industrial compounds including 11 OPFRs, 20 PFAS, and 5 other industrial compounds in different strategic areas of the ANP, ii) to determine differences in CECs bioaccumulation potential between invasive and native species, and iii) to perform an ecological risk assessment using invasive and native species as biomonitors of ecosystem quality.

1. Materials and methods

2.1. Chemicals and reagents

The complete list of reference standards used in this study can be found in Table S1, while its provenance and their isotopically labeled standards (IS) are detailed in Text S1. The solvents and materials used for the extraction method and chromatographic separation are specified in Text S2 and Text S3. Calibration curves were generated using standard mixtures prepared through serial dilutions from a 2-µg mL^{-1} stock solution. A matrix-matched calibration curve was created by spiking the extracts to achieve ten concentration levels, ranging from 0.1 to 500 ng g^{-1} (wet weight, w.w.).

2.2. Study area and sampling design

The study was carried out in the ANP (Valencia, Spain), a protected ecosystem since 1986 due to its ecological relevance as a key habitat for threatened endemic and migratory species (Soriano et al., 2023). The area spans 21,120 hectares, including wetlands, lagoons, marshes, dunes, and rice fields (Soriano et al., 2024), with the Albufera lake (23.7 km^2), at its center, surrounded by rice fields (223 km^2), as the dominant crop (Soria & Vicente, 2002; Soriano et al., 2024). The ANP features an extensive network of irrigation ditches and channels that supply water to the rice fields and transport drainage waters to the Albufera lake (Soriano et al., 2023). The ANP receives effluents from wastewater treatment plants (WWTPs), such as the Pinedo 2 WWTP, which are used for irrigation of rice fields in the northern part of the ANP and that end up in the lake (Soria & E. Vicente, 2002; Martínez-Megías et al., 2024). The middle and southern parts are irrigated with waters from the Turia and Júcar River basins, transported through a 59.7 km network of artificial channels. Overall, rice fields contribute about 40% of the ANP total water flow (Soria & E. Vicente, 2002).

The specimens used in the study were captured from ditches and ponds within the ANP, and local experimental facilities. After capture, they underwent a depuration period of 14 days in 26 L tanks with a closed-circuit system of clean water and a filtration module at El Palmar Fish Research Center (Valencian Government). Some individuals from each species were sacrificed post-depuration and used as reference biological material.

To assess the CEC bioaccumulation potential of each species, exposures were conducted at ten sampling sites within the ANP, covering three distinct habitats. Two sites were located in the lake (one in the north and one in the south), and two in the artificial wetland that receives effluents from the Albufera Sur WWTP (one at the entry and one at the exit). Additionally, four sites were selected at the irrigation and drainage channels of the rice fields (channels of Overa, Alqueresia, Font Nova, and Comú), along with two points at channels that also receive effluents from WWTPs (channels of Campets and Tancaeta). Two control points were included to monitor species mortality: one at a fish research Centre of Natural

Park (Control 1) and another at the channel Real del Júcar, which brings clean waters for orchards and rice field irrigation (Control 2) (Fig. 1). The physicochemical parameters of the water measured at the different exposure sites and control points are shown in Table S2.

Cages containing individuals of each species were placed at the field on September 21, 2020, and were exposed to the surface water medium for 14 days. *C. fluminea* and *A. cygnea* were placed in three cages per site with a sandy substrate inside the cage, with eight *C. fluminea* and one *A. cygnea* per cage. *P. clarki* were placed in three cages per site, with each cage containing four individuals, some local vegetation and PVC tubes serving as refuges. Four cages of *L. gibbosus* were placed per site, with each cage containing one individual. The cages allowed the water to circulate through them and food to be obtained from the exposure medium. Examples of the cages used are shown in Fig. S1. The cages were tied next to each other and sunk in the water column at an approximate distance of 30-40 cm from the sediment. At the end of the exposure period, the fish were sacrificed by asphyxiation, while the other species were sacrificed via freezing. Text S4 briefly describes the pretreatment of the biotic samples and surface water sampled at each exposure site.

2.3. Extraction method

The method for extracting CECs from invasive and native species has been carefully detailed in a previous study (Manjarrés-López et al., 2023) and is briefly described in Text S5. Surface water samples were extracted by Solid Phase Extraction (SPE) according to the method described by Soriano et al., (2023), which is briefly summarized in Text S6.

2.4. Quantitative analysis by LC-HRMS/MS

Details about the chromatographic separation, mobile phases used for ionization modes, elution gradient, source conditions, and other parameters related to the HRMS/MS acquisition method for tissue sample analysis are summarized in Text S7 (Manjarrés-López et al., 2023). Information regarding the chromatographic separation and analytical method for each group of compounds analyzed in the surface water were previously published (Gómez-Navarro et al., 2023; Soriano et al., 2023).

2.5. Quality control

The survival rate of the exposed species was 98%, including the ten sampling sites and the two control sites selected for mortality monitoring. During acquisition, matrix blanks spiked with reference standards at a concentration of 50 ng mL^{-1} were analyzed after every five samples to assess method performance.

Fig. 1. Map showing the locations where CECs were monitored and where the cages were located (Control sites, channels, lake, artificial wetland). The locations are presented in higher resolution on the right-hand side of the figure

Additionally, solvent blanks (methanol) were analyzed to confirm the absence of any carryover in the column. The R^2 for all compounds was greater than 0.99. The recoveries, linearity, and limits of detection and quantification of the method for each compound and per matrix were previously detailed (Manjarrés-López et al., 2023).

2.6. Data analysis

For the analysis of CECs in the species, median concentrations were calculated for compounds detected in at least two of the triplicate samples per species (>50%) and present in at least one sampling site. For samples meeting these criteria, values below the limit of detection (LOD) or limit of quantification (LOQ) were replaced with LOD/2 or LOQ/2 as appropriate. To estimate the accumulated concentration over the 14-day exposure period, the concentrations measured in the blanks analyzed after the depuration process were subtracted. Most blanks showed undetectable concentrations, except for: TDBPP (0.60 ng g^{-1}), and caffeine (94.2 ng g^{-1}) in *P. clarkii*; caffeine (0.51 ng g^{-1}), carbafuran -3-hydroxy (<LOQ), TDBPP (0.55 ng g^{-1}), TMPP (1.33 ng g^{-1}), cotinine (21.4 ng g^{-1}), fluoxetine (0.33 ng g^{-1}), o-desmethylvenlafaxine (0.13 ng g^{-1}), salbutamol (6.40 ng g^{-1}), tramadol (1.16 ng g^{-1}) and venlafaxine (0.17 ng g^{-1}) in *C. fluminea*; TDBPP (0.50 ng g^{-1}), TPP (1.06 ng g^{-1}), bisphenol A (0.48 ng g^{-1}), salbutamol (0.41 ng g^{-1}), and tramadol (0.10 ng g^{-1}) in *L. gibbosus*; TDBPP (2.12 ng g^{-1}), TMPP (0.92 ng g^{-1}), benzotriazole (0.25 ng g^{-1}), citalopram (0.60 ng g^{-1}), cotinine (10.2 ng g^{-1}), fluoxetine (0.60 ng g^{-1}), methadone (18.1 ng g^{-1}), and tramadol (0.11 ng g^{-1}) in *A. cygnea*.

Statistical analyses were performed using Origin 2022 (OriginLab Corp., USA). Values <LOQ were incorporated as LOQ/2. The Shapiro-Wilk test indicated non-normality of the data (p-value < 0.05), so the Kruskal-Wallis test was applied to determine significant differences between the CEC concentrations measured in the four species.

To relate the concentrations detected in surface water with those found in the monitored species and to assess differences among sampling sites, bioaccumulation factors (BAFs) were calculated by dividing the mean concentration per compound and species at each site by the mean concentration of the same compound in surface water, according to Eq. (1). The mean concentrations for surface water were based on the two measurements taken at the start of the exposure period and at the end of the exposure period (i.e., with a 14-d time interval), while the internal concentrations in the monitored species corresponded to those measured at the end of the exposure period.

$$BAF = \frac{Mean\ concentration\ C_i\ Sp_i (ng\ g^{-1})}{Mean\ concentration\ surface\ water\ Site_i\ (ng\ L^{-1})}\ x\ 1000 \quad (1)$$

Where C_i represents each compound, Sp_i each species, and $Site_i$ each sampling site.

Finally, to assess the risks of the measured concentrations for the different species, risk quotients (RQ) were calculated by comparing the measured environmental concentration (MEC) with the predicted no-effect concentration (PNEC) proposed by the NORMAN ecotoxicology database (NORMAN, 2023) according to Eq. (2). The database includes specific PNECs for internal concentrations in freshwater fish and mollusks, but none are available for crustaceans. Therefore, in this study, the PNECs for mollusks were also applied to the crab *P. clarkii*, as both species crabs and clams, fall under the category of aquatic invertebrates, ensuring similar reference levels.

$$RQ = \frac{MEC}{PNEC} \quad (2)$$

The RQ calculation was performed by compound and species at each of the exposure sites. The results were classified as posing high risk (RQ ≥ 1), medium risk (0.1 ≤ RQ < 1), and no risk (RQ < 0.1). Finally, a Hazard Index was calculated as the sum of the RQs for a given species in a given sampling site, assuming additive effects of the measured contaminant mixture.

3. Results and discussion

3.1. Occurrence of CECs in surface waters and monitoring species

In total, the presence of 63 PhACs, 26 pesticides, 14 PFAS, 7 OPFRs and 2 other industrial compounds was confirmed in at least one of the surfawater samples (Table S3). The sampling site located at the inlet of the artificial wetland (Wet. Entry) had the highest accumulated mean concentration of CECs (18343 ng L^{-1}), followed by Camptes (53216 ng L^{-1}). The high concentrations at Wet. Entry was expected as it receives effluents from the Albufera Sur WWTP (Martínez-Megías et al., 2024), which also influences Camptes due to its proximity. The detection of compounds varied across species. Out of the 171 compounds studied, 35 were detected in the monitoring species. Fig. 2 shows the species in which each of these compounds was detected. The species with the highest number of detected compounds (23) and the

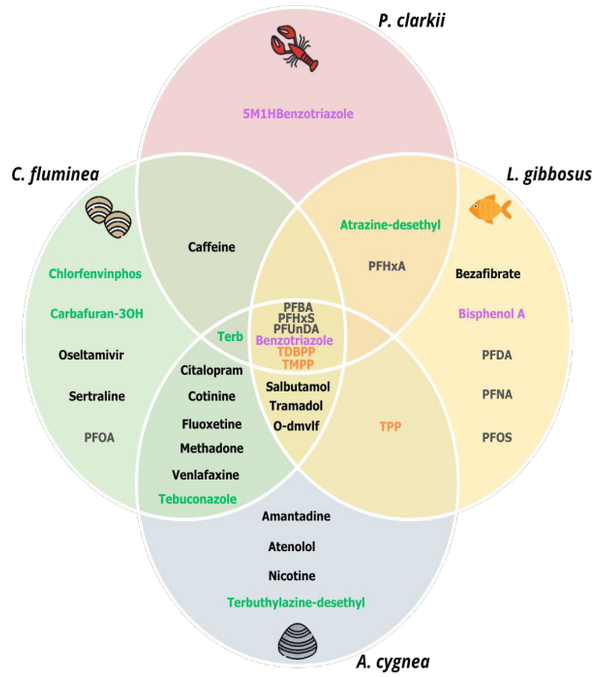

Fig. 2. Venn diagram of the 35 CECs detected in the invasive species analyzed (*P. clarkii, C. fluminea, L. gibbosus*) and the native species (*A. cygnea*) in at least one exposure site. The detected compounds were classified as PhACs (black), pesticides (green), PFAS (gray), OPFRs (orange) and other compounds of industrial use (purple). Overlapping areas indicate compounds that have been detected in more than one species. O-dmvlf: O-desmethylvenlafaxine. Terb: Terbuthylazine.

highest reported concentrations was the clam *C. fluminea* (Fig. 3).

Other Industrial compounds: In surface water, the highest concentrations were observed for benzotriazole, categorized under other industrial compounds, with levels ranging from 19.9 to 12650 ng L^{-1} (Fig. S2). Among the compounds included in this category, it was the only one detected in all species. The highest median concentration was reported in *L. gibbosus*, with 70.2 ng g^{-1} in Wet Entry. In *C. fluminea*, was detected at 57.7 ng g^{-1} at Alqueresia, while in *A. cygnea*, it was 48.9 ng g^{-1} at Camptes, and in *P. clarkii*, the highest median concentration was 32.2 ng g^{-1} at Wet Entry.

PhACs: Bezafibrate showed the highest mean concentration in surface water, and it was detected at all exposure sites at levels ranging from 83.0 to 2741 ng L^{-1}, followed by ibuprofen, which ranged from 49.8 to 657 ng L^{-1}. These concentrations exceed those reported previously (Sadutto et al., 2021), where bezafibrate levels were between 1.00 and 79.0 ng L^{-1}, and ibuprofen levels ranged from 20 to 217 ng L^{-1} in the same study area. In contrast, the concentrations of atenolol (0.18–4.33 ng L^{-1}) and tramadol (3.14–377 ng L^{-1}) detected in our study were lower than those previously reported, which were 52–221 ng L^{-1} for atenolol and 100–1260 ng L^{-1} for tramadol (Sadutto et al., 2021).

In the species the highest concentration was for sertraline (413 ng g^{-1}) at the Alqueresia site, followed by Wet Entry (344 ng g^{-1}) in *C. fluminea.* Oseltamivir had the highest detection frequency in *C. fluminea*, found at all exposure sites except Lake South, with concentrations ranging from 27.6 ng g^{-1} (Lake North) to 134 ng g^{-1} (Campets). The site with the highest cumulative median concentration for this species was Wet. Entry (1032 ng g^{-1}), followed by Alqueresia (618 ng g^{-1}).

Antidepressants such as sertraline and fluoxetine have previously been reported in *C. fluminea* at concentrations lower than those found in this study. Previous studies reported levels ranging from 56 to 226 ng g^{-1} for sertraline and from 5.45 to 12.0 ng g^{-1} for fluoxetine (Burket et al., 2019; Du et al., 2014). Although the detection frequency in these studies was higher, in the present study, the presence of these compounds is primarily associated with the Wet. Entry exposure site,

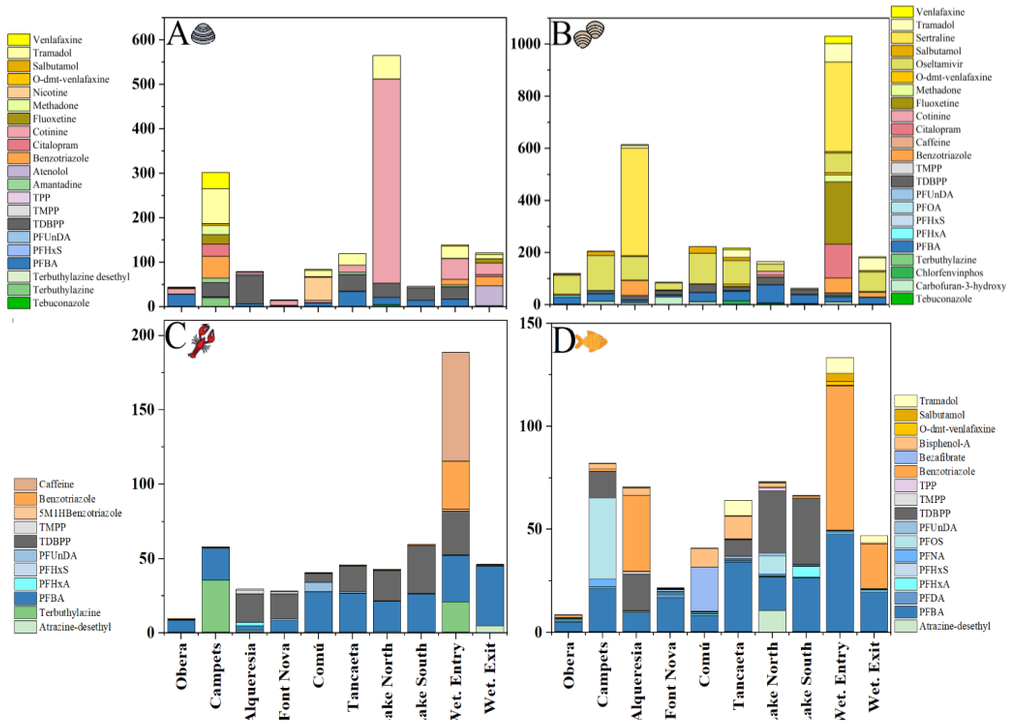

Fig. 3. Distribution of CECs by species and sampling site. **A:** *A. cygenea*; **B:** *C. fluminea*; **C:** *P. clarkii*; **D:** *L. gibbossus*. The figure shows median concentrations in ng g^{-1}(w.w) calculated for each compound on the y-axis, and the exposure sites organized by channels, lake, and artificial wetland on the x-axis. The legend of each box shows the compounds detected in each of the species.

where higher contributions of effluents from the WWTP are received, which may explain the differences observed

PFAS: PFOS had the highest mean concentrations in water: 10.2 ng L^{-1} at Lake North and 8.10 ng L^{-1} at Lake South. The mean concentrations reported for the PFAS group in this study are lower than those previously recorded in the study area. Lorenzo et al., (2019) reported mean concentrations of 31.6, 16.1, and 9.70 ng L^{-1} for PFOS, PFPeA, and PFOA, respectively; while the concentrations obtained in our study were below 5 ng L^{-1} for PFPeA and PFOA across all exposure sites.

PFAS were reported in all species and at all exposure sites, with *L. gibbosus* showing the highest detection frequency for these compounds. PFBA had the highest median concentration across all species: 69.7 ng g^{-1} in *C. fluminea*, 47.5 ng g^{-1} in *L. gibbosus*, 40.0 ng g^{-1} in *P. clarkii*, and 33.2 ng g^{-1} in *A. cygnea*. PFOS followed in *L. gibbosus* with 39.2 ng g^{-1}. The other detected PFAS were reported at concentrations below 8.00 ng g^{-1}. The presence of PFAS, such as PFBA, has previously been reported in the muscle tissue of *P. clarkii,* with concentrations <2.47 ng g^{-1} (Bian et al., 2024), which are lower than those reported in this study. In contrast, PFHxS concentrations reached up to 2.00 ng g^{-1}, slightly higher than those observed in our findings.

In crayfish from the Ebro Delta (Spain), no PFAS were detected in the muscle tissue of *P. clarkii*. However, when analyzing the whole body, including the head, concentrations of PFOS and PFOA, and, to a lesser extent, PFNA were reported, ranging from 0.07 to 0.63 ng g^{-1} (Fernández-Sanjuan et al., 2010). Nevertheless, in ANP, a similar ecosystem, these compounds were not detected in *P. clarkii* but were found in *C. fluminea* and *L. gibbosus*, except for PFNA.

OPFRs: TClPP had the highest mean concentration in surface water, with a value of 740 ng L^{-1} at Wet Entry. The mean concentrations of OPFRs obtained in this study are higher than those previously reported, but only at the entry of constructed wetlands. Compounds such as TCEP, TDCIPP, and TCIP showed higher mean concentrations (146, 67.0, and 740 ng L^{-1}

respectively), compared to 6.40, 17.4, and 70.4 ng L^{-1} reported by Lorenzo et al., (2019).

TDBPP and TMPP were the compounds most frequently detected across all species. TDBPP showed the highest median concentrations in the invasive species, with values of 32.3 ng g^{-1} in *P. clarkii* and 31.9 ng g^{-1} in *L. gibbosus* at Lake South, and 32.2 ng g^{-1} in *C. fluminea* at C. Comú. The absorption of OPFRs through gills is more common than their absorption via the food chain (Hou et al., 2016). This could explain why TDBPP and TMPP were detected in all species analyzed, regardless of their diet. Research on the presence of OPFRs in the species examined in this study is limited. However, some reports indicate the presence of compounds such as TCPP and TPP in perch, with concentrations ranging from 170 to 770 ng g^{-1} and 21 to 180 ng g^{-1}, respectively (Sundkvist et al., 2010).

Pesticides: They exhibited higher concentrations in surface water and thus, a higher percentage of contribution to the total sum at the sampling sites located at the lake (both north and south), and in the Comú (1343, 1486, and 1425 ng L^{-1}, respectively). The elevated pesticide concentrations at the lake could be explained by the confluence of water from irrigation channels, which carry pesticides applied in the surrounding agricultural areas (Martínez-Megías et al., 2024). Furthermore, the high concentrations obtained were mainly due to the presence of the fungicide azoxystrobin, which exceeded 1300 ng L^{-1} at the three mentioned sites, being the main contributor to the total concentrations. This fungicide, widely used in wheat crops to prevent and treat blast infestations, has previously been detected in similar concentrations in water samples from ANP, as it is used in the region's rice crops at the end of the summer period (Soriano et al., 2024).

However, this group of compounds obtained the lowest detection frequency among the species studied. No compound was detected simultaneously in all four species. For *P. clarkii* and *A. cygnea*, the compound with the highest median concentration was terbuthylazine, with 34.8 ng g^{-1} and 18.9 ng g^{-1} respectively, at Camptes. For *C. fluminea*, the compound with the highest concentration was the carbofuran-3-hydroxy metabolite (28.6 ng g^{-1}) in C. Font Nova, while for *L. gibbosus* it was atrazine-desethyl (10.6 ng g^{-1}) in Lake North.

Despite the restrictions imposed on their use (Comisi et al., 2021; EC, 2013), the presence of triazines continues to be reported in aquatic ecosystems because they are historically among the most widely used and persistent pesticides. Their presence in this ecosystem could be explained, in addition to the residues existing from previous use, by the contributions made by the Turia River to the ANP, since these types of compounds have been previously reported in this river (Canccapa et al., 2016). As far as we know, no studies have reported the presence of the specific pesticides included in this research in *P. clarkii*, *L. gibbosus*, or *A. cygnea*. Therefore, we could not conduct a comparative analysis of the concentrations presented here.

3.2. Patterns and species differences in the accumulation of CECs

A Kruskal-Wallis test was performed to assess statistical differences in CEC concentrations across species by compounds category. As shown in Fig. 4, no

Compound Categories	Vs	Vs	Vs	Vs	Vs	Vs
OPFRs						
Fungicides						
Herbicides						
Insecticides						
Metabolites Pesticides						
Antidepressant						
Antivirals						
Stimulants						
Opiods						
β blocker agents						
Others PhACs						
Metabolites PhACs						
Industrial compounds						
PFAS						

Fig.4. Statistically significant differences in CECs accumulation between the species analyzed according to the Kruskall-Wallis test. Blank boxes indicate no significant differences between species pairs, while colored boxes indicate a significant difference (p-value <0.05). Each color represents the species with the highest concentration for a specific category of compounds: green for *C. fluminea*, red for *P. clarkii*, yellow for *L. gibbosus*, and grey for *A. cygnea*

statistically significant differences were observed for OPFRs and PFAS groups, indicating similar concentrations across species. This suggests no particular susceptibility to accumulation in any single species, with observed variations likely due to natural fluctuations.

When comparing the native clam *A. cygnea* with the invasive species, it was observed that *A. cygnea* accumulated higher concentrations of a greater number of compounds compared to *P. clarkii* and *L. gibbosus,* except in the case of pesticide metabolites and stimulants, to which *P. clarkii* may provide more relevant information, and for industrial compounds, to which *L. gibbosus* showed greater accumulation. However, when compared to the invasive species *C. fluminea,* the significant differences were smaller, likely because both species belong to the same taxonomic group and share similar characteristics and accumulation patterns. These differences in accumulation could be related to the water filtration capacity of *C. fluminea*, making it more efficient at absorbing contaminants from the aquatic environment. It has been estimated that *C. fluminea* can reach a filtration rate of 3000 mL h^{-1} per individual, which is ten times higher than that of similarly sized native species, whose rates range between 150- and 300 mL h^{-1} per individual (Pouil et al., 2021).

Differences in accumulation patterns between the species analyzed can be attributed to factors such as diet and morphological and physiological traits. Studies have shown that body size, surface area, and volume can influence the bioaccumulation of organic contaminants, with bioaccumulation being inversely proportional to the volume or weight of different species (Rubach et al., 2011). In this study, *A. cygnea* and *C. fluminea* are the smallest species, with average sizes of 40–45 mm and 25 mm, respectively. In contrast, the crayfish *P. clarkii* has an average size of 18 cm, while *L. gibbosus* measures around 8 cm. The smaller size and larger surface-area-to-volume ratio of filtering tissue of *C. fluminea* could have contributed to higher concentrations for most of the compound categories.

The high concentrations in clams, particularly in *C. fluminea*, could also be attributed to species-specific biotransformation processes. It has been shown that bivalves have less efficient enzymatic systems for the biotransformation of chemical contaminants compared to fish. Bivalves can accumulate contaminants more readily, but they metabolize and eliminate them more slowly than vertebrates (Johnson et al., 2013). This slower rate of metabolism contributes to the greater retention of contaminants in their tissues.

The nature of species' body surfaces can affect the diffusion rates of contaminants through direct water contact (Boudou et al., 1991). Among the analyzed species, there are differences in how their body surfaces interact with the aquatic environment. For example, *P. clarkii* possesses a semi-permeable chitin exoskeleton, which acts as a protective barrier and may reduce the absorption of less lipophilic contaminants (Gu¨ & Vogt, 2018). In contrast, fish, like *L. gibbosus*, particularly juveniles and larvae, tend to have higher dermal absorption rates, primarily due to the thinner skin and lack of fully developed scales, which increases as they grow. This allows for more direct contaminant absorption through the skin during the early stages of life, but it decreases as they mature and their protective layers develop (Rombough, 2002; Tierney et al., 2013).

On the other hand, clams such as *A. cygnea* and *C. fluminea* have a soft body that is much more exposed when they initiate the filtration process, increasing the contact time with the surrounding water and enhancing the accumulation of some compounds, mainly weak bases. It has been shown that some weak bases may experience increased bioaccumulation due to the attraction of the positively charged cation to the cell membrane in clams (Ismail et al., 2014). This feature could explain the significant differences for antidepressants such as venlafaxine and its metabolite o-desmethylvenlafaxine, sertraline, fluoxetine and, citalopram; the b-blocker tramadol; the antiviral oseltamivir; the opioid methadone and compounds such as salbutamol and cotinine, which were detected mainly in *C. fluminea,* and with higher concentrations.

3.3. Bioaccumulation factors (BAFs) and the influence of exposure sites

BAFs were calculated for 22 compounds with concentrations >LOQ that were detected simultaneously in at least one of the species and at least one of the exposure sites (Fig. 5). A compound was considered bioaccumulative if its BAF was equal to or greater than 1000 (Koban et al., 2024). The site with the highest BAFs was Wet Entry, where BAF values greater than 1000 were recorded for twelve cases: PFBA and cotinine in *A. cygnea*; PFBA, citalopram, fluoxetine, methadone, and salbutamol in *C. fluminea*; terbuthylazine, PFBA, and caffeine in *P. clarkii*; and PFBA and o-desmethylvenlafaxine in *L. gibbosus*. In second place, the exposure site Camptes recorded BAFs>1000 for seven cases.

The high BAF values observed at Wet. Entry can be attributed to its role as the inlet of the Milia artificial wetland, a former rice field adjacent to ANP that has been converted into a constructed wetland or green filter for water treatment before it reaches the lake (Hernández-Crespo et al., 2017).

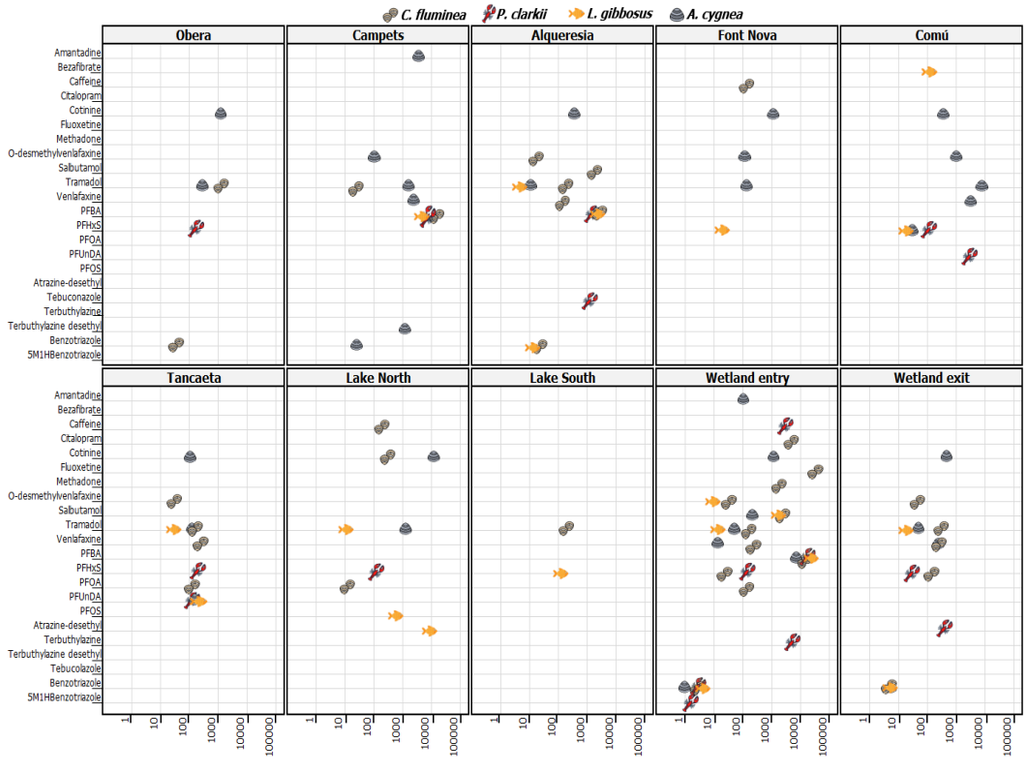

Fig. 5. BAFs per species and exposure site for CECs with mean concentrations >LOQ

This artificial wetland receives treated water from the Albufera Sur WWTP located 10 kilometers away (Soriano et al., 2023). On the other hand, there is clear evidence of a reduction in the concentrations of compounds in the effluent treated by the artificial wetland, as reflected by the fact that no BAF values above 1000 were observed in the species exposed at Wet Exit. This suggests that the wetland's treatment process effectively reduces contaminants' bioavailability, lowering their accumulation in aquatic organisms downstream of the constructed wetland.

The highest BAFs were calculated for fluoxetine in *C. fluminea* (52795); PFBA in *L. gibbosus* (from 5842 to 29113), *P. clarkii* (from 2174 to 19046), and *C. fluminea* (from 5646 to 11796) and; for cotinine in *A. cygnea* (from 153 to 15718). The species with the highest number of BAF values exceeding 1000 was *A. cygnea*, with 14 cases (each case represents the BAF value of a compound at a specific exposure site). Notably, *A. cygnea* showed elevated BAF values for compounds such as cotinine, and tramadol (from 16 to 2245), and for terbuthylazine desethyl, PFBA, amantadine, o-desmethylvenlafaxine, and venlafaxine.

Following this, *C. fluminea* exhibited BAF>1000 in nine cases, particularly for compounds like PFBA, citalopram, fluoxetine, methadone, and salbutamol. *P. clarkii* had seven cases, primarily for terbuthylazine, PFBA, PFUnDA, and caffeine. Lastly, *L. gibbosus* showed five cases, specifically for atrazine-desethyl, PFBA, and salbutamol. This variation in bioaccumulation could be attributed to species-specific physiological traits and their interaction with environmental factors at the various exposure sites.

The bioaccumulation capacity of antidepressants in *C. fluminea* has been previously reported by Burket et al., (2019), who exposed this species downstream of a WWTP in Pecan Creek Lake, USA. After 42 days of exposure, they reported BAF values ranging from 454 to 702 for fluoxetine and from 3361 to 6485 for sertraline. While the BAF for fluoxetine observed in this study is lower than the values we report, both studies highlight *C. fluminea's* strong bioaccumulation potential for this class of compounds. Unfortunately, we were unable to

calculate a BAF for sertraline, as the compound was not quantified in water samples; however, its concentration in the tissue was among the highest recorded (344 to 413 ng g^{-1}).

The elevated BAF values observed in clams, particularly in *A. cygnea*, are primarily attributed to their role as filter-feeders. Bivalves are capable of filtering large volumes of water, processing microalgae, bacteria, and organic matter while also capturing particles from both, the water column and the sediments. This filtering capacity allows them to ingest contaminants present in the environment, especially from sediments, which is one of the main pathways through which pollutants accumulate in their tissues (Bjork, 1995; Ismail et al., 2014). These characteristics grant bivalves a higher absorption and accumulation potential compared to the other species analyzed in this study.

Regarding the differences between the two-clam species, *A. cygnea* appears to have a higher bioaccumulation efficiency for certain contaminants, likely due to its physiological traits, including protein-binding capacity and lipid content. *A. cygnea* has a lipid percentage of 2.2%, compared to *C. fluminea*, which has only 0.7% (Manjarrés-López et al., 2023). This difference in lipid content could explain why contaminant concentrations tend to be higher in *A. cygnea*, as compounds with a higher affinity for lipids or proteins may accumulate more efficiently in species with higher fat reserves.

Given the lipid content of *P. clarkii* (3.0%) (Manjarrés-López, et al., 2023), higher BAFs, especially for nonpolar compounds, would be expected. However, its bioaccumulation capacity may have been limited by exposure conditions, where access to detritus, macrophytes, mollusks, insects, annelids, tadpoles, and small fish—its typical diet—was restricted due to confinement to water and pelagic organisms only. Additionally, compared to clams, it filters smaller volumes of water and its exoskeleton is impermeable (Anandkumar et al., 2020; Loureiro et al., 2015).

A variable that can influence the bioaccumulation of CECs in aquatic organisms is the physicochemical properties of the compounds. The higher the log K_{ow} value the greater the affinity the compound tends to have for lipid-rich tissues, which could favor its accumulation and limit its partitioning into water. Based on this, we performed an analysis on the influence of log K_{ow} on the BAFs calculated in each of the species (Fig. S3). In this analysis, it was observed that bioaccumulation values are not always directly correlated with a high log K_{ow}, suggesting that affinity for lipid-rich tissues is not the only determining factor.

In the case of *A. cygnea,* the accumulation of cotinine, a polar compound with a low log K_{ow} (-0.23) that is generally not expected to bioaccumulate in large amounts, stood out. However, its ability to bioaccumulate in this species appears to be influenced by its physiology as a filter-feeding organism, which allows it to retain a wide range of compounds through ingestion of particles and sediments, regardless of the lipophilicity of the contaminant. This condition may suggest that this species lacks the capacity to biotransform and/or eliminate cotinine. However, to confirm this, further studies on its metabolism in this species are needed.

C. fluminea and *P. clarkii*, on the other hand, showed a greater tendency to bioaccumulate compounds with a higher log K_{ow} (>4) such as PFHxS, which is consistent with the theory that more lipophilic compounds tend to accumulate more readily in fat-rich tissues. In particular, these results are consistent with the idea that apolar compounds show a higher affinity for organisms with higher lipid content, as is the case of *P. clarkii*, which has a lipid percentage of 3%, the highest concerning the other study species. Finally, in *L. gibbosus*, although high BAFs were also observed for some compounds with intermediate log K_{ow} values, such as PFUnDA and PFBA, the overall accumulation seems to be less influenced by the compounds' lipophilicity compared to other species, suggesting that other factors, such as metabolism and the specific physiology of this species, also play a relevant role.

3.4. Ecological risk assessment

In this section, to evaluate the risk of each detected compound, risk quotients (RQ) were calculated (Fig. 6). Henceforth, to facilitate the understanding of the discussion, the combination of species, compound, and exposure site will be referred to as a "case". The highest RQs were observed for sertraline in *C. fluminea* at the Alqueresia and Wet. Entry sites, with values of 1251 and 1043, respectively. *C. fluminea* presented medium RQ values in 36 cases and high RQs in 29 cases. Notably, median concentrations of oseltamivir exceeded the PNEC at all exposure sites except Lake South, with RQs ranging between 4.12 and 19.8. Measured concentrations in *A. cygnea* exceeded the PNEC for 17 compounds, reaching medium risk in 26 cases and high risk in 20 cases. The highest RQs for this species were calculated for cotinine (66.5) at Lake North, terbuthylazine (59.2) and its metabolite terbuthylazine desethyl (56.2) at Camptes, and atenolol (11.3) at Wet. Exit.

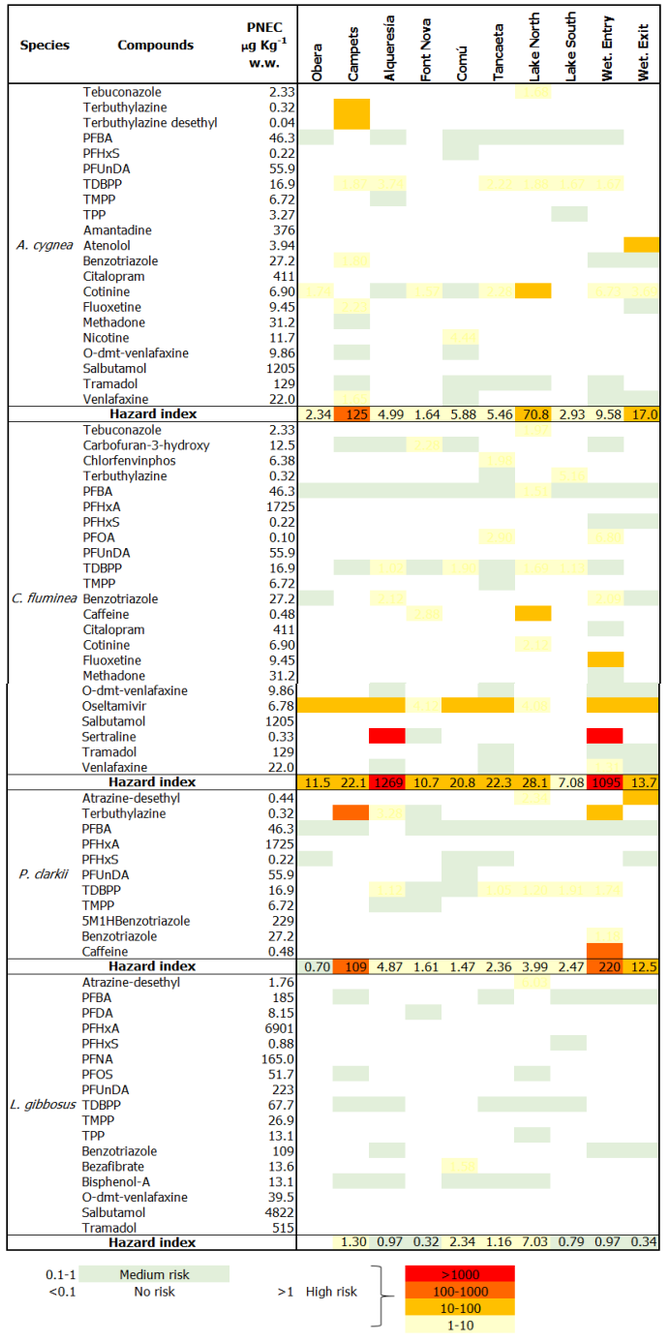

Fig. 6. Calculated risk quotient for each single compound at each site, and calculated hazard index for each species

The effects of oseltamivir have been demonstrated in *Mytilus galloprovincialis* (mussels) and *Ruditapes philippinarum* (clams). In mussels, exposure to oseltamivir caused a significant increase in glutathione reductase (GR) activity in the gills, with no marked antioxidant response observed in the digestive gland. In addition, lipid peroxidation (LPO) levels were significantly reduced in mussels, indicating possible protective effects against oxidative damage. No response in gill antioxidant defenses was evident in clams, but LPO levels were significantly elevated, suggesting increased oxidative stress (Dallarés et al., 2019).

P. clarkii exhibited RQs greater than 1.00 in 12 cases, and between 0.10 and 1.00 in 19 cases. The highest RQs for this species were recorded for caffeine (152) at Wet. Entry, terbuthylazine (109) at Camptes, and atrazine-desethyl at Wet. Exit. Previous studies have shown that crayfish exposed to atrazine displayed impaired movement, which indicates potential neurological effects. Additionally, exposure to terbuthylazine has been associated with histological damage, oxidative stress, and alterations in antioxidant biomarkers (Belanger et al., 2017).

The species with the fewest cases where RQ values exceeded 1.00 was *L. gibbosus*, which surpassed this threshold in only two cases: for atrazine desethyl (6.03) at Lake North and bezafibrate (1.58) at Comú. In the group of PFAS, RQs were reported below 7.00, with the highest values being for PFOA in *C. fluminea* (6.80) and PFBA in *A. cygnea* (1.51). Regarding OPFRs, RQs remained below 4.00, with the highest values reported for TDBPP in *A. cygnea* (from 1.67 to 2.22).

According to the hazard index (HI), which evaluates the level of exposure to a mixture of substances per site for each species, *C. fuminea* presented a high risk in most of the exposure sites, with HI values ranging from 11.5 in Obera to 1269 in Alqueresia. It was observed that the risk of mixing substances at each exposure site varied by species, with *L. gibbosus* having the lowest HI. However, at most sites, HI values posed a high risk, indicating strong toxic pressure exerted by the CECs mixture for both native and invasive species.

4. Conclusions

This study revealed the presence of 35 CECs, primarily PhACs and carboxylic acids PFAS, in invasive and native freshwater species of the Albufera Natural Park. The Asian clam, *Corbicula fluminea*, exhibited the highest number of detected compounds and concentrations, particularly for PhACs such as sertraline and oseltamivir. The highest concentrations and BAFs for invasive species were recorded at the inlet of the artificial wetland within the park, while the native species (*Anodonta cygnea*) showed the highest bioaccumulation at North Lake, mainly for cotinine.

Bivalves, *A. cygnea* and *C. fluminea*, accumulated a broader range of compounds, with fluoxetine and cotinine showing the highest BAFs. This accumulation pattern is linked to their morphological traits, such as their ability to filter large volumes of water and deposit feeding, which differentiate them from the other species included in the study. The bioaccumulation patterns varied based on the intrinsic properties of the compounds, such as their log K_{ow}, with *A. cygnea* showing a greater tendency to accumulate compounds with lower log K_{ow} values compared to *C. fluminea* and *P. clarkii*, which tended to accumulate more lipophilic compounds.

The study also demonstrated significant statistical differences in the accumulation of various compound categories between invasive species. *C. fluminea* was found to accumulate a wider variety of compounds, making it a suitable bioindicator for this ecosystem. No statistically significant differences were observed between *C. fluminea* and *A. cygnea* in most compound categories, indicating that *C. fluminea* can effectively reveal contaminant presence similarly to native species.

Based on the risk assessment, it is inferred that in the Albufera Natural Park, the monitored species are predominantly exposed to and have a higher probability of being affected by the presence of PhACs, particularly the antidepressant sertraline and the antiviral oseltamivir, followed by some herbicides and their transformation products. *C. fluminea* is the species that most exceeded the reference values, as it showed the highest internal concentrations which may be an indicator of greater resistance. *P. clarkii* and *A. cygnea* also had high RQ values, though to a lesser extent. Follow-up studies are recommended to evaluate the long-term toxicological effects of the CEC mixtures identified in this study on these species.

The use of invasive species as biomonitors provided crucial insights into the chemical exposure in specific areas of the Albufera Natural Park, highlighting compounds such as sertraline, fluoxetine, terbuthylazine, caffeine, and oseltamivir as priority CECs based on the calculated risk quotients. This research demonstrates that invasive species can serve as effective tools for biomonitoring, reducing the pressure on native species while providing valuable information on chemical stressors in the ecosystem. Their use could improve conservation efforts by lessening the competition that native species face from invasive populations.

CRediT authorship contribution statement

Diana P. Manjarrés-López: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Claudia Martínez-Megías:** Methodology. **Dyana Vitale:** Methodology, Data curation. **Yolanda Picó:** Validation, supervision. **Andreu Rico:** Writing – review & editing, Validation, Supervision, Conceptualization. **Sandra Pérez:** Writing – review & editing, 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.

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Data availability

Data will be made available on request.

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INFORMACIÓN COMPLEMENTARIA
PUBLICACIÓN No. 5

Occurrence, bioaccumulation, and ecological risk assessment of contaminants of emerging concern using native and invasive species as biomonitors

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Environmental Research (2024)

Enviado

Supporting information

Occurrence, bioaccumulation, and ecological risk assessment of contaminants of emerging concern using native and invasive species as biomonitors

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Text S1. **Chemicals and list of isotopically labeled standards (IS)**

The 11 OPFRs, along with their IS, were obtained from Cambridge Isotope Laboratories (Andover, MA, USA), while the 20 PFAS and their IS were sourced from Wellington Laboratories Inc. (Canada). The 49 pesticides were purchased from Sigma-Aldrich, with their IS supplied by LGC Standards (Barcelona, Spain). The 86 PhACs, together with their IS, as well as five industrial compounds, were acquired from various suppliers, including Sigma-Aldrich (St. Louis, MO, USA; Steinheim, Germany), Toronto Research Chemicals (Toronto, Ontario, Canada), CDN Isotopes (Pointe-Claire, Quebec, Canada), Santa Cruz Biotechnology (Dallas, TX, USA), and Cymit Quimica S.L. (Barcelona, Spain).

TCEP-d12, TPhP-d15, TClPP-d18, TDCIPP-d15, chlorfenvinphos-d10, chlorpyrifos-d10, perfluoro-n-[1,2,3,4-$^{13}C_4$] butanoic acid, perfluoro-n-[1,2-$^{13}C_2$] hexanoic acid, perfluoro-n-[1,2,3,4-$^{13}C_4$] octanoic acid, perfluoro-n-[1,2,3,4,5-$^{13}C_5$] nonanoic acid, perfluoro-n-[1,2-$^{13}C_2$] decanoic acid, perfluoro-n-[1,2-$^{13}C_2$] undecanoic acid, perfluoro-1-[$^{18}O_2$] hexane sulphonic acid, perfluoro-1-[1,2,3,4-$^{13}C_4$] octane sulphonic acid, acetaminophen-d4, alprazolam-d5, atenolol-d7, benzotriazole-d4, bezafibrate-d4, bisphenol-a-d8, bromazepam-d4, caffeine-13c3, carbamazepine-d10, cocaine-d5, codeine-d3, cotinine-d3, diclofenac-13c6, erythromycin-13cd3, fenofibrate-d6, fluconazole-13c3, fluoxetine-d5, friponil-13c3, furosemide-d5, hydrochlorothiazide-13cd2, ibuprofen-d3, indomethacin-d4, irbesartan-d6, ketoprofen-d3, lamotrigine-13cd3, lorazepam-d4, metformin-d6, metropolol-d7, midazolam-13c6, morphine-d3, nicotine-d4, norfluoxetine-d6, oseltamivir-d3, oxazepam-d5, paroxetine-d4, pentobarbital-d5, sertraline-d3, sotalol-d6, sulfamethazine-d4, sulfamethoxazole-13c6, tramadol-13cd3, trimethoprim-d9, valsartan acid-d4, valsartan-d3, venlafaxine-d6.

Text S2. **Solvents and materials used for the extraction method and chromatographic separation of fresh tissues**

LC-MS grade MeCN (≥ 99.9%) used as the extraction solvent was sourced from J.T. Baker (Fisher Scientific SL, Madrid, Spain), while formic acid (≥ 96%, ACS reagent) was obtained from Sigma-Aldrich. The d-SPE sorbent Z-Sep/C18 was supplied by Supel TM QuE (Supelco/Sigma-Aldrich, St. Louis, MO, USA). MeCN and water (Optima™ LCMS Grade) for mobile phase preparation, ammonium acetate, and ammonium fluoride were sourced from Sigma-Aldrich and Fisher Chemical (Fisher Scientific SL, Madrid, Spain). HPLC-grade methanol (MeOH ≥ 99.8% purity) was obtained from VWR Chemicals® (Radnor, PA, USA), and ammonium formate was purchased from Alfa Aesar (Karlsruhe, Germany).

Text S3. **Solvents and materials used for the extraction method and chromatographic separation for freshwater**

Solvents used included HPLC grade methanol (MeOH) ≥ 99.8% purity were purchased from VWR Chemicals® (Radnor, PA, USA) and ammonium formate , formic acid, ammonium fluoride, and acetone were purchased from Alfa Aesar (Karlsruhe, Germany). Acetonitrile ≥ 99.9% purity was from Merck (Darmstadt, Germany). High purity water was obtained using a Milli-Q water purification system (Millipore, Milford, MA, USA). The 15 mL and 50 mL polypropylene centrifuge Falcon tubes were from VWR International Eurolab (Barcelona, Spain).

Text S4. **Pretreatment of soft tissue samples from the species and surface water samples**

Soft tissues were collected from each species: shells were removed from clams, the exoskeleton from crayfish, and the entire specimen was used in the case of fish. The tissues were homogenized using a blade mill with a stainless-steel grinding chamber (Grindomix GM 200, Retsch GmbH, Haan, Germany) and stored at -40 °C until extraction. Three replicates of *P. clarkii* samples and two replicates of the rest of the species were obtained at each sampling site. Additionally, surface water samples were collected at each sampling site the day the cages were placed in the environment and the day they were retrieved. Surface water samples were collected in 1.5 L polyethylene terephthalate (PET) bottles and refrigerated during transport. Once in the laboratory, they were filtered with a 0.45-µm glass fiber filter (Advantec MFS, Dublin, CA, USA) and frozen at -20 °C in the dark until extraction for analysis.

Text S5. **Extraction method of CECs in invasive species**

Briefly, 0.5 g of soft tissue was used, to which 25 µL of an IS mixture (1 ppm) was added. The extraction was performed via UAE (ultrasound assisted solvent extraction) with 1.0 mL of acetonitrile with 0.1% formic acid, followed by a clean-up step using d-SPE (dispersive solid phase extraction) with Discovery® DSC-18. The mixture was centrifuged at 14,000 rpm for 6 minutes. Finally, 500 µL of the supernatant was evaporated to dryness and reconstituted in a water/acetonitrile solution with 5 mM ammonium acetate (9:1 v/v) [1].

Text S6. **Extraction method of CECs in surface water samples**

SPE cartridges (Phenomenex Strata-X-33 µm Polymeric Reversed Phase) were preconditioned with methanol and ultrapure water. A 250 mL sample was passed through the cartridges under vacuum at a controlled flow rate, then the cartridges were washed, dried, and the analytes eluted with methanol and a methanol-dichloromethane mixture. The extract was evaporated under nitrogen at 40ºC, redissolved in methanol, and prepared for analysis through vortexing and sonication. The extract was divided for separate analyses: one portion was filtered for pesticide analysis, while the other was reconstituted and filtered for PPCPs, PFAS, and OPFRs analysis. All extracts were stored at -20ºC until analysis [2].

Text S7. **Chromatographic and analytical conditions by group of compounds**

Invasive species

For **PhACs**, chromatographic separation was carried out on a Waters Acquity UPLC System (Waters, Milford, MA) with an EVO C18 KINETEX column (100 × 2.1 mm; 2.6 µm. Phenomenex). Water 5 Mm ammonium acetate + 0.1% formic acid as aqueous phase (A) for ESI ionization (+) and water with 2Mm ammonium fluoride for ESI ionization (-) were used as mobile phases. Acetonitrile (B) was used as the organic phase. The elution gradient was 95% of (A) until minute 10, then 70% of (A) until minute 13.3, decreasing to 0% from minute 15.5 with a flow rate of 0.2 mL min^{-1}. Detection of the analytes was performed on an Orbitrap Q-Exactive™ mass spectrometer (Thermo Fischer Scientific, San Jose, CA, USA) by Data independent acquisition described in [3].

For **OPFRs** and **pesticides**, the chromatographic separation was performed with Thermo Scientific™ Vanquish™ UHPLC system. The KINETEX XB-C18 column (50.0 × 2.1 mm. 1.7 µm. Phenomenex. Torrance, USA) was used for OPFRs and the Luna C18 column (15.0 cm × 0.21 cm, 3 µm, Phenomenex. Torrance, USA) for pesticides. The elution gradient for the OPFRs started with 70% of (A) (water 0.1% formic acid), increasing to 79% at minute 0.5 and decreasing to 2% from minute 12. The organic mobile phase used was methanol 0.1% formic acid, maintaining a flow rate of 0.4 mL min^{-1}.

The elution gradient for the **pesticides** started with 50% of the aqueous phase (5Mm ammonium formate water + 0.1% formic acid), which decreased to 17% at minute 10, and reduced to 2% at minute 12.5. The organic aqueous phase used for the separation was 5Mm ammonium methanol formate + 0.1% formic acid at a flow rate of 0.4 mL min^{-1}. Mass analysis for both pesticides and OPFRs was performed on the Thermo Scientific™ Orbitrap Exploris 120™ mass spectrometry instrument according to the acquisition methods described in Lorenzo et al., (2019) and Álvarez-Ruiz et al., (2021).

Finally, for **PFAS** analysis a Kinetex® EVO C18 KINETEX column (100 × 2.1 mm; 2.6 µm, Phenomenex. Torrance, USA) was used and as mobile phases, 4 Mm ammonium fluoride water was used as aqueous phase (A) and 4Mm ammonium fluoride methanol as organic phase (B) for ESI ionization (-) in an Agilent 1260 HPLC UHPLC system (degasser, quaternary pump, and column oven). The elution gradient started with 95% of (A), decreasing at minute 0.5 to 70% and down to 5% from minute 12 with a flow rate of 0.3 mL min^{-1}. Detection of the analytes was performed on the Sciex TRIPLE QUAD 6500+ mass instrument in multiple selection of monitored reactions mode (SRM) (Sciex, Concord, Ontario, Canada) as described in [6].

Quantification was carried out using matrix-matched calibration by spiking the extracts to create a ten-point curve, ranging from 0.1 to 500 ng g^{-1} (wet weight, w.w.) for solid tissues. For surface water samples, the calibration curve was prepared in Milli-Q water in the range of 0.05 ng L^{-1} to 1000 ng L^{-1}. The internal standard method was applied using Trace Finder 5.1 software (Thermo Fisher Scientific). The precursor ion's presence was confirmed based on the following criteria: a signal-to-noise ratio greater than 10, mass accuracy within 5 ppm, retention time within 30 seconds of the reference standard, and detection of the product-ion with the highest relative abundance in the MS/MS spectrum.

Surface water

Chromatographic and analytical conditions of PFAS, OPFRs and pesticides for the analysis of surface water is described in detail by [2]. For PhACs, chromatographic separation was carried out with a Waters Acquity UPLC system equipped with a reverse-phase EVO C18 KINETEX column (100 × 2.1 mm, 2.6 µm). The mobile phases used for ESI ionization (+) and (-) were (A) 5 mM ammonium acetate and 0.1% formic acid in water and (B) 100% acetonitrile, and (A) 2 mM ammonium fluoride in water and (B) 100% acetonitrile, respectively. The flow rate was 0.2 mL min^{-1} ; the chromatographic run was completed in 19 min. The elution gradient was as follows: 5% A (0 min), 30% A (10 min), 65% A (13.30 min), 100% A (15.50 min) and 5% A (17.70 min). The column temperature was adjusted to 40 °C and the injection volume was 10 µL. Detection was performed using an Orbitrap Q-Exactive™ mass spectrometer and the mode of acquisition was by targeted-Data Independent Acquisition according to [7].

Table S1. Reference standards and target compounds

Category	Compounds	CAS number	Chemical formula
Pesticides			
Fungicides	Carbendazim	10605-21-7	$C_9H_9N_3O_2$
	Imazalil	35554-44-0	$C_{14}H_{14}Cl_2N_2O$
	Prochloraz	67747-09-5	$C_{15}H_{16}Cl_3N_3O_2$
	Tebuconazole	107534-96-3	$C_{16}H_{22}ClN_3O$
	Thiabendazole	148-79-8	$C_{10}H_7N_3S$
Herbicides	Alachlor	15972-60-8	$C_{14}H_{20}ClNO_2$
	Atrazine	1912-24-9	$C_8H_{14}ClN_5$
	Diuron	330-54-1	$C_9H_{10}Cl_2N_2O$
	Isoproturon	34123-59-6	$C_{12}H_{18}N_2O$
	Metoalachlor	51218-45-2	$C_{15}H_{22}ClNO_2$
	Molinate	2212-67-1	$C_9H_{17}NOS$
	Propanil	709-98-8	$C_9H_9Cl_2NO$
	Propazine	139-40-2	$C_9H_{16}ClN_5$
	Simazine	122-34-9	$C_7H_{12}ClN_5$
	Terbumeton	33693-04-8	$C_{10}H_{19}N_5O$
	Terbuthylazine	5915-41-3	$C_9H_{16}ClN_5$
	Terbutryn	886-50-0	$C_{10}H_{19}N_5S$
Insecticides	Acetamiprid	135410-20-7	$C_{10}H_{11}ClN_4$
	Azinphos-ethyl	2642-71-9	$C_{12}H_{16}N_3O_3PS_2$
	Azinphos-metyl	86-50-0	$C_{10}H_{12}N_3O_3PS_2$
	Buprofezine	69327-76-0	$C_{16}H_{23}N_3OS$
	Carbofuran	1563-66-2	$C_{12}H_{15}NO_3$
	Chlorfenvinphos	470-90-6	$C_{12}H_{14}Cl_3O_4P$
	Chlorpyrifos	5598-13-0	$C_7H_7Cl_3NO_3PS$
	Chlothianidin	210880-92-5	$C_6H_8ClN_5O_2S$

Category	Compounds	CAS number	Chemical formula
	Coumaphos	56-72-4	$C_{14}H_{16}ClO_5PS$
	Diazinon	333-41-5	$C_{12}H_{21}N_2O_3PS$
	Dichlofenthion	97-17-6	$C_{10}H_{13}Cl_2O_3PS$
	Dimethoate	60-51-5	$C_5H_{12}NO_3PS_2$
	Ethion	563-12-2	$C_9H_{22}O_4P_2S_4$
	Etofenprox	80844-07-1	$C_{25}H_{28}O_3$
	Fenthion	55-38-9	$C_{10}H_{15}O_3PS_2$
	Hexythiazox	78587-05-0	$C_{17}H_{21}ClN_2O_2S$
	Imidacloprid	138261-41-3	$C_9H_{10}ClN_5O_2$
	Methiocarb	2032-65-7	$C_{11}H_{15}NO_2S$
	Omethoathe	1113-02-6	$C_5H_{12}NO_4PS$
	Parathion ethyl	56-38-2	$C_{10}H_{14}NO_5PS$
	Pyriproxyfen	95737-68-1	$C_{20}H_{19}NO_3$
	Spinosad A	131929-60-7	$C_{41}H_{65}NO_{10}$
	Spinosad D	131929-63-0	$C_{42}H_{67}NO_{10}$
	Thiamethoxam	153719-23-4	$C_{18}H_{10}ClN_5O_3S$
Metabolites pesticides	Atrazine-deethyl	6190-65-4	$C_6H_{10}ClN_5$
	Atrazine-desisopropyl	1007-28-9	$C_5H_8ClN_5$
	Carbofuran-3-OH	16655-82-6	$C_{12}H_{15}NO_4$
	Fenthion sulfone	3761-42-0	$C_{10}H_{15}O_5PS_2$
	Fenthion sulfoxide	3761-41-9	$C_{10}H_{15}O_4PS_2$
	Terbumeton-deethyl	30125-64-5	$C_8H_{15}N_5O$
	Terbuthylazine-2-OH	66753-07-9	$C_9H_{17}N_5O$
	Terbuthylazine-deethyl	30125-63-4	$C_7H_{12}ClN_5$
Flame retardants			
OPFRs	Cresyl diphenyl phosphate (CDP)	26444-49-5	$C_{19}H_{17}O_4P$
	Tributyl phosphate (TnBP)	126-73-8	$C_{12}H_{27}O_4P$

Category	Compounds	CAS number	Chemical formula
	Tri-n-propylphosphate (TPP)	513-08-6	$C_9H_{21}O_4P$
	Triphenyl phosphate (TPhP)	115-86-6	$C_{18}H_{15}O_4P$
	Tris(1.3-dichloro-2-propyl) phosphate (TDCIPP)	13674-87-8	$C_9H_{15}Cl_6O_4P$
	Tris(2.3-dibromopropyl) phosphate (TDBPP)	126-72-7	$C_9H_{15}Br_6O_4P$
	Tris(2-Butoxyethyl) phosphate (TBEP)	78-51-3	$C_{18}H_{39}O_7P$
	Tris(2-chloroethyl) phosphate (TCEP)	115-96-8	$C_6H_{12}Cl_3O_4P$
	Tris(2-chloroisopropyl) phosphate (TCIPP)	13674-84-5	$C_9H_{18}Cl_3O_4P$
	Tris-(2-ethylhexyl) phosphate (TEHP)	78-42-2	$C_{24}H_{51}O_4P$
	Tris(methylphenyl) phosphate (TMPP)	1330-78-5	$C_{21}H_{21}O_4P$
Perfluoroalkyl substances			
Perfluoroalkyl carboxylic acids	Perfluorobutanoic acid (PFBA)	375-22-4	C_4F_7COOH
	Perfluoro-7-methyloctanoic acid (ipPFNA)	Not available	-
	Perfluorodecanoic acid (PFDA)	335-76-2	$C_{10}F_{19}COOH$
	Perfluorododecanoic acid (PFDoA)	307-55-1	$C_{12}F_{23}COOH$
	Perfluorohexadecanoic acid (PFHxDA)	67905-19-5	C_5F_9COOH
	Perfluorononanoic acid (PFNA)	375-95-1	$C_9F_{17}COOH$
	Perfluorooctadecanoic acid (PFODA)	16517-11-6	$C_6F_{11}COOH$
	Perfluorooctanoic acid (PFOA)	335-67-1	$C_8F_{15}COOH$
	Perfluoropentenoic acid (PFPeA)	2706-90-3	C_5F_9COOH
	Perfluorotetradecanoic acid (PFTeDA)	376-06-7	C_4F_7COOH
	Perfluorotridecanoic acid (PFTrDA)	72629-94-8	$C_{13}F_{25}COOH$
	Perfluoroundecanoic acid (PFUnDA)	2058-94-8	$C_{11}F_{21}COOH$
	Perfluroheptanoic acid (PFHpA)	375-85-9	$C_7F_{13}COOH$
	Perflurohexanoic acid (PFHxA)	307-24-4	$C_6F_{11}COOH$
Perfluoroalkyl sulfonic acids	Perfluorobutane sulfonic acid (PFBS)	29420-49-3	$C_4HF_9SO_3$
	Perfluorodecane sulfonic acid (PFDS)	335-77-3	$C_{10}HF_{21}SO_3$
	Perfluoroheptane sulfonic acid (PFHpS)	375-92-8	$C_7HF_{15}SO_3$

Category	Compounds	CAS number	Chemical formula
	Perfluorooctane sulfonic acid (PFOS)	4021-47-0	$C_8HF_{17}SO_3$
	Perflurohexane sulfonic acid (PFHxS)	82382-12-5	$C_6HF_{13}SO_3$
	Perfluoro-7-methyloctane sulfonate (ipPFNS)	-	-
Other industrial compounds			
	1H Benzotriazole	2592-95-2	$C_6H_5N_3O$
	5-methyl 1H benzotriazole	136-85-6	$C_7H_7N_3$
	Bisphenol A	80-05-7	$C_{15}H_{16}O_2$
	Bisphenol S	80-09-1	$C_{12}H_{10}O_4S$
	Bisphenol F	620-92-8	$C_{13}H_{12}O_2$
Pharmaceuticals active compounds			
Antidepressants/anxiolytics/sedatives /hypnotics	Alprazolam	2898-97-7	$C_{17}H_{13}ClN_4$
	Bromazepam	1812-30-2	$C_{14}H_{10}BrN_3O$
	Citalopram	59729-33-8	$C_{20}H_{21}FN_2O$
	Clozapine	5786-21-0	$C_{18}H_{19}ClN_4$
	Diazepam	439-14-5	$C_{16}H_{13}ClN_2O$
	Fluoxetine	54910-89-3	$C_{17}H_{18}F_3NO$
	Lorazepam	846-49-1	$C_{15}H_{10}Cl_2N_2O_2$
	Midazolam	59467-70-8	$C_{18}H_{13}ClFN_3$
	Oxazepam	604-75-1	$C_{15}H_{11}ClN_2O_2$
	Paroxetine	61869-08-7	$C_{19}H_{20}FNO_3$
	Pentobarbital	76-74-4	$C_{11}H_{18}N_2O_3$
	Quetiapine	111974-69-7	$C_{21}H_{25}N_3O_2S$
	Sertraline	79617-96-2	$C_{17}H_{17}C_{l2}N$
	Temazepam	846-50-4	$C_{16}H_{13}ClN_2O_2$
	Venlafaxine	93413-69-5	$C_{17}H_{27}NO_2$
	Zolpidem	82626-48-0	$C_{19}H_{21}N_3O$
Analgesics/anti-inflammatories	Acetaminophen	103-90-2	$C_8H_9NO_2$

Category	Compounds	CAS number	Chemical formula
	Codeine	76-57-3	$C_{18}H_{21}NO_3$
	Diclofenac	5307-86-5	$C_{14}H_{11}Cl_2NO_2$
	Flufenamic acid	30-78-9	$C_{14}H_{10}F_3NO_2$
	Ibuprofen	31121-93-4	$C_{13}H_{18}O_2$
	Ketoprofen	22071-15-4	$C_{16}H_{14}O_3$
	Mefenamic acid	61-68-7	$C_{15}H_{15}NO_2$
	Propyphenazone	479-92-5	$C_{14}H_{18}N_2O$
	Salicylic acid	69-72-7	$C_7H_6O_3$
Antibacterials	Clarithromycin	81103-11-9	$C_{38}H_{69}NO_{13}$
	Irgasan - Triclosan	3380-34-5	$C_{12}H_7Cl_3O_2$
	Oxytetracycline	79-57-2	$C_{22}H_{24}N_2O_9$
	Triclocarban	101-20-2	$C_{13}H_9Cl_3N_2O$
	Trimethoprim	738-70-5	$C_{14}H_{18}N_4O_3$
Antibiotics	Chloramphenicol	56-75-7	$C_{11}H_{12}Cl_2N_2O_5$
	Ciprofloxacin	85721-33-1	$C_{17}H_{18}FN_3O_3$
	Erythromycin	114-07-8	$C_{37}H_{67}NO_{13}$
	Flumequine	42835-25-6	$C_{14}H1_2FNO_3$
	Furazolidone	67-45-8	$C_8H_7N_3O_5$
	Nalidixic acid	389-08-2	$C_{12}H_{12}N_2O_3$
	Sulfadimethoxine	122-11-2	$C_{12}H_{14}N_4O_4S$
	Sulfamethazine	57-68-1	$C_{12}H_{14}N_4O_2S$
	Sulfamethoxazole	723-46-6	$C_{10}H_{11}N_3O_3S$
	Sulfapyridine	144-83-2	$C_{11}H_{11}N_3O_2S$
Anticonvulsants	Acridone	578-95-0	$C_{13}H_9NO$
	Carbamazepine	298-46-4	$C_{15}H_{12}N_2O$
	Lamotrigine	84057-84-1	$C_9H_7Cl_2N_5$
Antidiabetics	Metformin	657-24-9	$C_4H_{11}N_5$

Category	Compounds	CAS number	Chemical formula
	Sitagliptin	486460-32-6	$C_{16}H_{15}F_6N_5O$
Anti-hypertensives	Diltiazem	34933-06-7	$C_{22}H_{26}N_2O_4S$
	Losartan	114798-26-4	$C_{22}H_{23}ClN_6O$
	Telmisartan	144701-48-4	$C_{33}H_{30}N_4O_2$
	Valsartan	137862-53-4	$C_{24}H_{29}N_5O_3$
Antivirals	Amantadine	768-94-5	$C_{10}H_{17}N$
	Oseltamivir	196618-13-0	$C_{16}H_{28}N_2O_4$
Diuretics	Furosemide	54-31-9	$C_{12}H_{11}ClN_2O_5S$
	Hydrochlorothiazide	58-93-5	$C_7H_8ClN_3O_4S_2$
Lipid regulators	Atorvastatin	134523-00-5	$C_{33}H_{35}FN_2O_5$
	Bezafibrate	41859-67-0	$C_{19}H_{20}ClNO_4$
	Clofibric acid	882-09-7	$C_{10}H_{11}ClO_3$
Metabolites/Transformation products	Benzoylecgonine	519-09-5	$C_{16}H_{19}NO_4$
	Carbamazepine-10,11-epoxide	36507-30-9	$C_{15}H_{12}N_2O_2$
	Cocaethylene	529-38-4	$C_{18}H_{23}NO_4$
	Cotinine	486-56-6	$C_{10}H_{12}N_2O$
	Fipronil Desulfinyl	205650-65-3	$C_{12}H_4Cl_2F_6N_4$
	Fipronil sulfide	120067-83-6	$C_{12}H_4Cl_2F_6N_4S$
	Fipronil sulfone	120068-36-2	$C_{12}H4Cl_2F_6N_4O_2S$
	N-acetylsulfamethoxazole	18607-98-2	$C_{12}H_{13}N_3O_4S$
	N-Desmethylcitalopram	62498-67-3	$C_{19}H_{19}FN_2O$
	Norfluoxetine	83891-03-6	$C_{16}H_{16}F_3NO$
	O-desmethylvenlafaxine	93413-62-8	$C_{16}H_{25}NO_2$
	Oseltamivir carboxylate	Not available	$C_{14}H_{24}N_2O_4$
	Valsartan acid	164265-78-5	$C_{14}H_{10}N_4O_2$
Opioids	Methadone	76-99-3	$C_{21}H_{27}NO$
	Morphine	57-27-2	$C_{17}H_{19}NO_3$

Category	Compounds	CAS number	Chemical formula
Stimulants	Caffeine	58-08-2	$C_8H_{10}N_4O_2$
	Cocaine	50-36-2	$C_{17}H_{21}NO_4$
βblocker agents	Atenolol	29122-68-7	$C_{14}H_{22}N_2O_3$
	Bisoprolol	66722-44-9	$C_{18}H_{31}NO_4$
	Metoprolol	37350-58-6	$C_{15}H_{25}NO_3$
	Sotalol	3930-20-9	$C_{12}H_{20}N_2O_3S$
	Tramadol	27203-92-5	$C_{16}H_{25}NO_2$
Other PhACs	Bezafibrate	41859-67-0	$C_{19}H_{20}ClNO_4$
	Carazolol	57775-29-8	$C_{18}H_{22}N_2O_2$
	Fipronil	120068-37-3	$C_{12}H_4C_{12}F_6N_4OS$
	Fluconazole	86386-73-4	$C_{13}H12_2F_2N_6O$
	Loratadine	79794-75-5	$C_{22}H_{23}ClN_2O_2$
	Omeprazole	73590-58-6	$C_{17}H_{19}N3O_3S$
	Ondansetron	99614-02-5	$C_{18}H_{19}N_3O$
	Salbutamol	18559-94-9	$C_{13}H_{21}NO_3$
	Verapamil	52-53-9	$C_{27}H_{38}N_2O_4$
	Warfarin	81-81-2	$C_{19}H_{16}O_4$

Table S2. **Physicochemical water parameters measured at the different exposure sites and control points**. DO: dissolved oxygen; C.: channel; Wet.: wetland

Site code	pH	Temperature (°C)	Conductivity (µS/cm)	Salinity (PSU)	DO (%)	DO (ppm)
C. Obera	7.61± 0.1	20.0±1.7	1465±20	0.74±0.01	69.5±6.7	6.3±0.8
C. Camptes	7.60± 0.1	19.7±1.8	1589±140	0.80±0.1	68.0±7.8	6.2±0.9
C. Alqueresía	7.58± 0.1	21.0±1.9	1393±50	0.70±0.03	89.4±8.7	8.0±0.9
C. Font Nova	7.58 ± 1.77	20.8±1.77	1698±16.41	0.86±0.01	64.4±15.77	5.8±1.57
C. Comú	7.63 ± 0.07	20.1±2.21	1781±59.73	0.91±0.03	70.5±8.44	6.4±0.89
C. Tancaeta	7.58 ± 0.05	20.6±2.42	1865±36.23	0.95±0.07	58.6±19.14	5.2±1.44
Lake North	8.38 ± 0.1	20.5±2.15	2031±36.23	1.04±0.02	122.8±6.01	10.9±1.16
Lake South	8.32 ± 0.15	20.6±2.14	1840±90.88	0.95±0.03	126.9±8.75	11.4±0.98
Wet. Entry	7.43± 0.1	23.2±1.3	2060±70	1.05±0.04	20.4±4.2	1.7±0.4
Wet. Exit	8.14± 0.1	22.5±3	3064±50	1.60±0.03	119±26.1	10.4±2.1
Control 1	7.97± 0.1	19.1±2.8	1519±60	0.77±0.03	87.3±1.2	8.1±1.2
Control 2	8.22 ± 0.13	22.1±2.17	1067±7.59	0.53±0	114.8±3.68	10.1±0.33

Table S3. Mean concentration of CECs in surface water at each exposure site. *Concentrations in ng L^{-1}. D0: detected only on the first sampling day (day 0, at the beginning of the exposure experiment). D14: detected only on the final sampling day (after 14 days of exposure). <LOQ: below the limit of quantification. N.d: not detected in any sample*

Category	Compound	C. Obera	C. Campets	C. Alqueresia	C. Font Nova	C. Común	C. Tancaeta	Lake North	Lake south	Wet. Entry	Wet. Exit
Pesticides	Acetamiprid	7.16	4.07	6.45	3.52	2.91	9.43	21.0	20.8	63.4	18.1
	Acrinathrin_aduct	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d
	Atrazine	0.60	N.d	0.90^{D14}	N.d	N.d	0.5^{D0}	0.50^{D0}	1.30^{D0}	0.68	1.10
	Atrazine-Desethyl	4.01	4.30	7.91	4.10	7.51	1.10	1.17	1.82	123	10.0
	Atrazine-Desisopropyl	N.d	2.53	N.d	5.05	5.0^{D14}	N.d	N.d	N.d	2.90^{D14}	N.d
	Azoxystrobin	560	399	24.8	25.5	1388	220	1314	1458	150	38.0
	Buprofezine	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d
	Carbendazim	0.73	0.92	1.52	0.66	1.34	9.81	2.09	1.70	12.5	4.59
	Carbofuran-3-OH	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d
	Chlorpyriphos	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d
	Diazinon	N.d	8.40^{D0}	0.80^{D14}	N.d	N.d	N.d	N.d	N.d	N.d	N.d
	Dimethoate	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	11.3	1.80^{D14}
	Diuron	5.34	N.d	7.32	N.d	N.d	N.d	N.d	32.1^{D0}	N.d	N.d
	DMPF	N.d	17.9^{D14}	N.d	N.d	15.3^{D14}	42.2^{D14}	N.d	N.d	N.d	N.d
	Ethion	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d
	Fenthion sulfoxide	N.d	4.50^{D14}	0.50^{D14}	N.d	N.d	N.d	N.d	N.d	4.73	N.d
	Fipronil	1.20^{D0}	0.80	0.94	0.60^{D0}	0.50^{D0}	1.52	0.60^{D0}	0.50^{D0}	4.34	0.74
	Fluvalinate	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d
	Hexythiazox	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d
	Imazalil	4.14	N.d	3.00^{D0}	N.d	N.d	N.d	N.d	15.0^{D0}	N.d	2.72

Category	Compound	C. Obera	C. Campets	C. Alqueresia	C. Font Nova	C. Común	C. Tancaeta	Lake North	Lake south	Wet. Entry	Wet. Exit
	Imidacloprid	6.35	5.75	9.58	0.50	5.50	13.0	1.66	0.50	42.4	6.94
	Isoproturon	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d
	Metolachlor	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d
	Prochloraz	0.50^{D0}	N.d	7.45	5.05	5.90^{D0}	5.10^{D0}	3.0^{D0}	0.6^{D0}	3.00	18.6
	Propanil	N.d	N.d	8.97	N.d	N.d	N.d	N.d	N.d	N.d	N.d
	Simazine	2.30	1.40	1.90^{D0}	3.26	2.21	1.20^{D0}	N.d	0.71	4.32	0.50^{D0}
	Spinosad A	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d
	Spinosad D	0.60^{D0}	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d
	Tebuconazole	2.96	N.d	48.9	98.5	127^{D0}	6.60^{D0}	5.10^{D0}	3.70^{D0}	9.67	98.2
	Terbumeton	0.78	N.d	0.50^{D0}	0.50^{D0}	0.50^{D0}	0.56^{D0}	0.41^{D0}	2.86^{D0}	1.07	0.94
	Terbumeton desethyl	14.9	0.48	4.05	11.3	11.3^{D0}	18.9^{D0}	17.5^{D0}	30.3^{D0}	60.2	32.8
	Terbuthylazine	1.12	N.d	0.71	0.61	0.50^{D0}	0.90^{D0}	0.80^{D0}	2.40^{D0}	2.92	0.81
	Terbuthylazine-2OH	2.87	0.50	5.81	9.13	7.20^{D0}	2.00^{D0}	1.70^{D0}	5.60^{D0}	5.65	8.25
	Terbuthylazine-desethyl	12.6	1.60	4.13	7.09	6.10^{D0}	16.7^{D0}	13.5^{D0}	20.8^{D0}	50.6	23.8
	Terbutryn	1.05	N.d	3.35	0.86	N.d	N.d	0.40^{D0}	6.70^{D0}	0.55	0.50^{D14}
	Thiabendazole	1.81	5.43	2.25	1.70	18.4	10.8	3.12	2.84	9.58	3.28
Other industrial compounds	1H_Benzotriazole	83.4	1120	1487	19.9	20.2	141^{D0}	211	133	12650	3177
	5-methyl-1H_Benzotriazole	14.5	50.2	57.6	23.8	22.4	222	92.5	61.8	719	275
PhACs	Acetaminophen	5.72	3.89	3.76	3.50	4.50	42.3	4.60	3.68	6.80	7.40^{D0}
	4-Hydroxydiclofenac	N.d	0.90^{D0}	138	N.d	N.d	N.d	N.d	N.d	141	N.d
	Acridone	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	1.64^{D14}
	Alprazolam	N.d	0.58^{D0}	N.d	N.d	N.d	0.58	N.d	0.58	0.58	0.58^{D0}
	Amantadine	N.d	1.59	3.12	N.d	N.d	17.0^{D0}	3.52	1.22	33.0	16.4
	Atenolol	0.18^{D14}	1.02	3.72	N.d	N.d	4.33	0.62^{D0}	0.40	12.0	N.d
	Atorvastatin	2.41	3.32^{D0}	1.93	0.95^{D0}	0.95^{D14}	0.95^{D14}	1.64^{D14}	N.d	N.d	1.60^{D14}
	Benzoylecgonine	0.90	0.94	0.42	0.30	1.24	67.0	2.50	1.50	0.74	0.46^{D14}
	Bezafibrate	289	2741	114	183	142	117	80.5	83.0	248	179
	Caffeine	11.3	10.7	3.12	7.66	19.0	385	31.5	26.2	22.4	20.1
	Carbamazepine	0.40	3.18	3.92	0.46	0.55	22.2	7.32	5.12	28.3	19.7
	CBZ-10,11-epoxide	N.d	0.68^{D0}	2.10	N.d	N.d	6.12	1.56	1.64^{D0}	18.4	6.88
	Chloramphenicol	0.84	0.58	0.46	0.90	0.76	0.96	0.70	0.80	0.60	0.84
	Ciprofloxacin	0.96^{D14}	N.d	0.96^{D0}	N.d	N.d	N.d	N.d	N.d	0.96	N.d
	Citalopram	N.d	0.82^{D0}	2.41	N.d	N.d	0.82	N.d	N.d	18.8	N.d
	Clarithromycin	N.d	10.0^{D0}	10.40	N.d	N.d	N.d	N.d	N.d	19.4	N.d
	Coca-ethylene	N.d	N.d	N.d	N.d	0.66^{D14}	N.d	N.d	N.d	N.d	N.d
	Cocaine	N.d	N.d	N.d	0.73^{D0}	N.d	3.29	N.d	N.d	N.d	N.d
	Codeine	0.90^{D14}	0.91	2.36	N.d	N.d	0.90^{D0}	N.d	N.d	9.12	0.90^{D0}
	Cotinine	7.60	11.1	8.42	5.76	7.52	103	29.2	25.5	36.6	31.1
	Diazepam	0.61^{D0}	2.20	2.30	0.61^{D14}	N.d	5.00	10.1	2.06	6.38	4.28
	Diclofenac	1.54	7.78	17.9	1.14	1.10	6.50	1.08^{D0}	1.24^{D0}	43.0	4.50
	Diltiazem	N.d	25.4^{D0}	N.d	N.d	N.d	N.d	N.d	N.d	27.6^{D0}	N.d

Category	Compound	C. Obera	C. Campets	C. Alqueresia	C. Font Nova	C. Común	C. Tancaeta	Lake North	Lake south	Wet. Entry	Wet. Exit
	Erythromycin	1.64^{D0}	1.64	0.76	N.d	N.d	N.d	N.d	N.d	5.86	N.d
	Fluconazole	N.d	2.38	4.10	N.d	N.d	20.1	14.36	10.6	29.2	36.2
	Flufenamic acid	N.d	N.d	7.10^{D14}	N.d	N.d	17.1	N.d	N.d	18.88	N.d
	Fluoxetine	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	4.52	N.d
	Furosemide	1.54	3.52	14.7	0.59	N.d	2.75	N.d	N.d	23.8	N.d
	Hydrochlorothiazide	3.52	38.3	70.0	2.50	1.86	68.1	1.32	0.74	392	12.2
	Ibuprofen	158	657	75.7	105	76.4	290	54.0	49.8	152	101
	Indomethacin	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	0.37	N.d
	Ketoprofen	N.d	4.16^{D0}	12.5	N.d	N.d	N.d	N.d	N.d	27.9	N.d
	Lamotrigine	1.92	11.8	18.6	3.12	1.28	114	37.8	26.5	127.4	86.6
	Lorazepam	N.d	3.48^{D0}	3.40	N.d	N.d	12.7	2.56^{D0}	N.d	22.6	6.56
	Losartan	0.54^{D0}	9.10	18.9	N.d	N.d	20.4	N.d	N.d	104	7.56
	Mefenamic acid	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	0.65^{D0}	N.d
	Metformin	N.d	N.d	0.20^{D14}	N.d	N.d	6.59	0.20^{D0}	N.d	2.12	N.d
	Methadone	N.d	0.76^{D0}	1.00	N.d	N.d	N.d	N.d	N.d	7.80	0.20^{D0}
	Metoprolol	N.d	N.d	0.61^{D0}	N.d	N.d	1.25	N.d	N.d	0.61	N.d
	N-acethyl_SMX	N.d	0.64^{D0}	0.64	N.d	N.d	0.64^{D0}	N.d	N.d	3.34	N.d
	Nalidixin_acid	0.84^{D14}	N.d	N.d	0.84	0.84	0.84	N.d	N.d	N.d	N.d
	N-desmethylcitalopram	N.d	N.d	1.94	N.d	N.d	0.50^{D0}	N.d	N.d	20.8	N.d
	Nicotine	12.7	32.9^{D14}	3.72	15.3	16.2^{D0}	42.2	17.5	17.9	193	19.3
	O-desmethylvenlafaxine	1.78	20.5	39.9	3.24	1.10	184	5.16	2.82	200	38.2
	Omeprazole	0.20^{D0}	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d
	Oseltamivir-CBX	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	0.76^{D0}	N.d
	Oxazepam	N.d	3.64	5.70	N.d	N.d	20.9	3.76	0.79^{D14}	36.7	10.2
	Propyphenazone	N.d	N.d	N.d	N.d	0.79^{D0}	1.22	0.60	0.38	N.d	N.d
	Quetiapine	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d
	Salbutamol	N.d	N.d	1.00	N.d	N.d	N.d	N.d	N.d	1.86	1.00^{D0}
	Salicylic acid	23.7	28.3	9.92	19.9	22.1	27.2	14.6	18.5	22.0	22.5
	Sitagliptin	1.92^{D0}	15.4	33.1	1.81	N.d	99.2	3.92^{D0}	0.99^{D0}	243	21.2
	Sotalol	N.d	N.d	1.64	N.d	N.d	3.95	N.d	N.d	5.08	N.d
	Sulfamethazine	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	11.2^{D14}	0.58^{D14}
	Sulfamethoxazole	1.44	5.96	7.26	1.10	N.d	12.1	2.44	1.68	61.5	12.5
	Sulfapyridine	N.d	2.84	4.08	N.d	N.d	N.d	N.d	N.d	45.8	2.86
	Temazepam	2.00	6.18	8.48	2.00^{D14}	2.00^{D0}	24.4	7.10	5.42	44.1	19.0
	Tramadol	3.14	35.1	52.9	3.76	1.48	162	27.8	17.8	377	119
	Triclocarban	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d
	Trimethoprim	N.d	0.69^{D0}	0.72	N.d	N.d	N.d	N.d	N.d	2.62	N.d
	Valsartan	6.82	33.4	82.2	3.20	3.04^{D0}	321	57.9	42.4	198	34.9
	Valsartan acid	3.52	41.6	39.7	0.77	0.77^{D0}	209	74.7	119	270	427
	Venlafaxine	1.02	9.44	16.2	1.60	0.48	25.9	1.94	1.12	124	10.7
	Verapamil	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d

Category	Compound	C. Obera	C. Campet	C. Alqueres	C. Font Nov	C. Común	C. Tancaet	Lake Nortl	Lake soutl	Wet. Entry	Wet. Exit
	Warfarin	0.91	0.91^{D14}	0.91^{D14}	0.60^{D0}	0.73	0.77	0.87	0.91	0.91	0.83
	Zolpidem	N.d	N.d	N.d	N.d	N.d	N.d	N.d	N.d	0.20	N.d
PFAS	PFBA	N.d	2.37	1.57	N.d	N.d	N.d	N.d	N.d	1.63	N.d
	PFBS	N.d	0.28	N.d	1.35	0.25	0.37	0.40	0.26	0.30	0.47
	PFDA	N.d	N.d	N.d	N.d	N.d	2.23	1.39	N.d	N.d	N.d
	PFDS	1.15	<LOQ	<LOQ	1.16	<LOQ	0.18	<LOQ	<LOQ	<LOQ	<LOQ
	PFHpA	N.d	N.d	N.d	0.62	N.d	0.83	1.53	1.12	0.50	1.20
	PFHpS	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.10	0.46	<LOQ	<LOQ	<LOQ
	PFHxS	0.51	0.32	0.34	0.65	0.90	0.72	0.88	0.61	0.79	0.76
	PFNA	0.90	<LOQ	<LOQ	<LOQ	<LOQ	2.91	2.48	<LOQ	<LOQ	<LOQ
	PFOA	1.42	2.40	2.11	2.08	2.38	2.52	3.23	2.05	4.52	3.55
	PFODA	N.d	N.d	N.d	0.64	N.d	N.d	<LOQ	<LOQ	N.d	0.72
	PFOS	N.d	N.d	N.d	N.d	N.d	N.d	10.2	8.10	N.d	N.d
	PFPeA	N.d	N.d	3.63	1.76	N.d	1.95	4.15	3.07	N.d	N.d
	PFTrDA	<LOQ	1.49	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	1.37	<LOQ
	PFUnDA	<LOQ	<LOQ	<LOQ	<LOQ	2.09	3.04	<LOQ	2.02	4.08	<LOQ
OPFRs	TCEP	<LOQ	<LOQ	<LOQ	26.6	<LOQ	<LOQ	<LOQ	<LOQ	146	62.2
	TBEP	<LOQ	0.70	4.26	<LOQ	<LOQ	18.7	<LOQ	<LOQ	13.3	1.28
	TClPP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	54.5	<LOQ	<LOQ	740	<LOQ
	TDClPP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	67.0	<LOQ
	TnBP	<LOQ	<LOQ	0.11	4.28	1.45	150	2.69	1.45	17.4	3.68
	TPhP	<LOQ	<LOQ	<LOQ	<LOQ	2.77	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	TPP	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.73	<LOQ	<LOQ	1.85	1.11

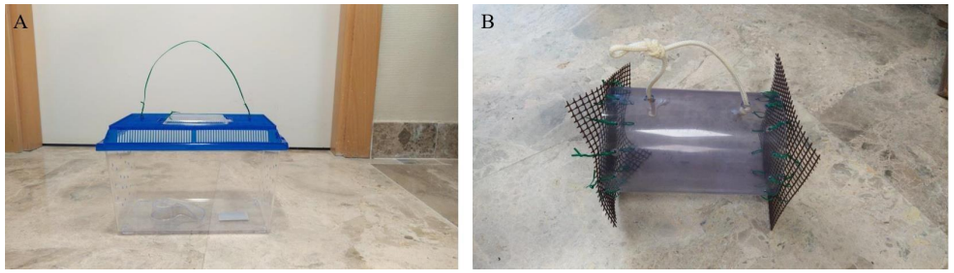
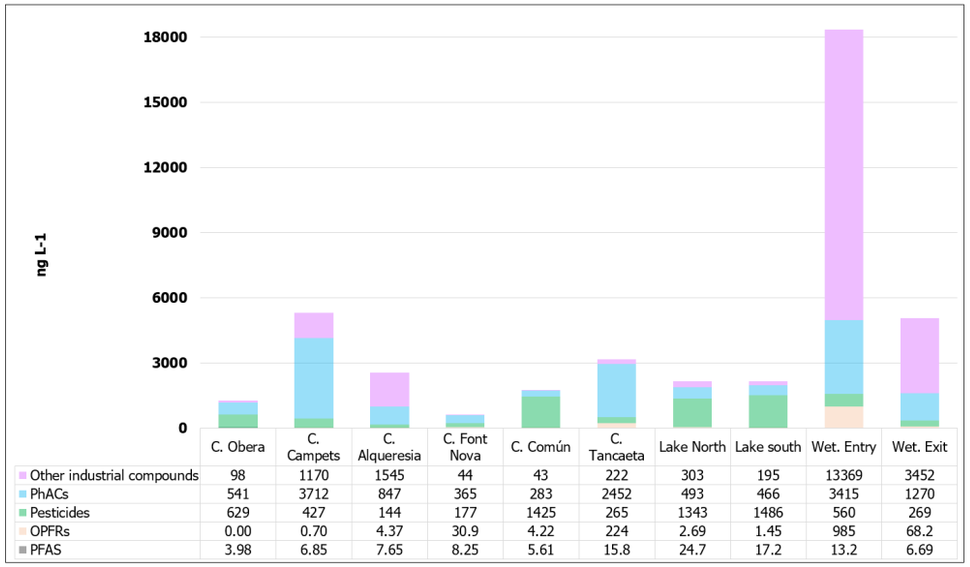


Fig. S1. *Cage for A) P. clarkii, A. cygnea. and C. fluminea, B) L. gibbosus.*



	C. Obera	C. Campets	C. Alqueresia	C. Font Nova	C. Común	C. Tancaeta	Lake North	Lake south	Wet. Entry	Wet. Exit
Other industrial compounds	98	1170	1545	44	43	222	303	195	13369	3452
PhACs	541	3712	847	365	283	2452	493	466	3415	1270
Pesticides	629	427	144	177	1425	265	1343	1486	560	269
OPFRs	0.00	0.70	4.37	30.9	4.22	224	2.69	1.45	985	68.2
PFAS	3.98	6.85	7.65	8.25	5.61	15.8	24.7	17.2	13.2	6.69

Fig. S2. *Mean surface water concentrations by CEC group for compounds detected in the two samplings and with values >LOQ.*

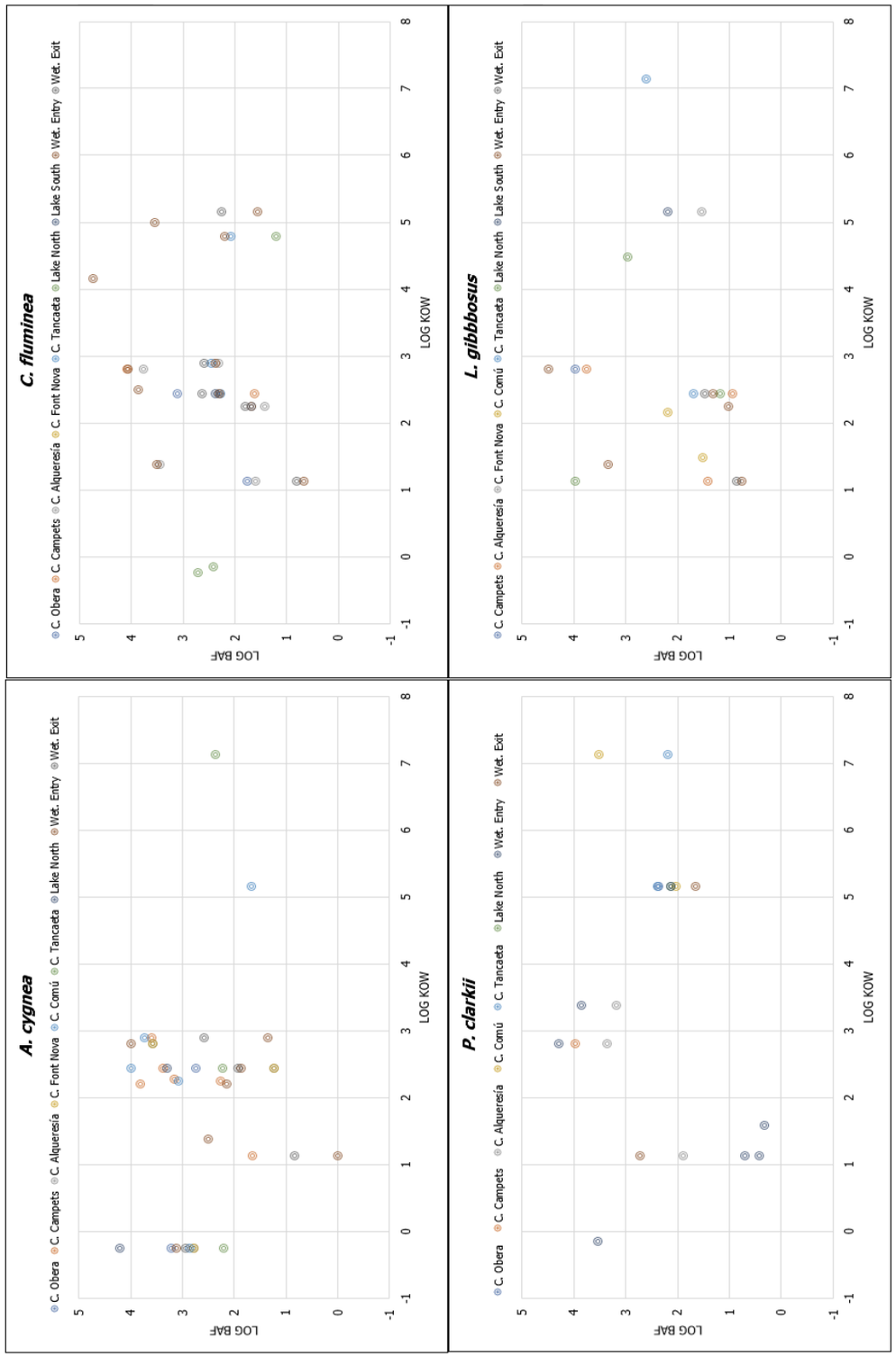


Fig. S3. *Influence of log K_{ow} on log BAF calculated by species and exposure site.*

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5.3. Discusión

De acuerdo a los resultados obtenidos en la Publicación No. 5, se observaron variaciones en el porcentaje de compuestos detectados por especie. Teniendo en cuenta el total de compuestos analizados: 72 PhACs, 14 metabolitos de PhACs, 20 PFAS, 42 plaguicidas, 7 metabolitos de plaguicidas, 11 OPFRs y 5 compuestos catalogados como de otro uso industrial, el mayor porcentaje de detección se presentó para el grupo de otros compuestos de uso industrial (40%) en *L. gibbosus* y *P. clarkii*, seguido por el 50% de los PFAS detectados en *L. gibbosus*. En la Fig. 5.2 se muestra la representatividad de cada grupo de compuestos por especie.

Al comparar las concentraciones de los compuestos detectados en la Publicación No. 5 con las obtenidas en especies invasoras muestreadas al azar presentadas en la Publicación No. 2 en la misma zona de estudio, se evidenciaron variaciones principalmente en las concentraciones de PhACs. En la Publicación No. 2 se obtuvieron concentraciones inferiores a 10 ng g^{-1}, mientras que en la Publicación No. 5 fueron más altas para compuestos como la sertralina en *C. fluminea* (entre 344 y 413 ng g^{-1}), cafeína en *P. clarkii* y *C. fluminea* (entre 1,38 y 73,2 ng g^{-1}), y tramadol (entre 0,44 y 70,0 ng g^{-1}). Además, se detectaron PhACs que no se reportaron en la Publicación No. 2, como oseltamivir, bezafibrato, amantadina, atenolol y metformina.

Estas diferencias podrían estar relacionadas con el método de muestreo. En la Publicación No. 2, las muestras se recogieron aleatoriamente de individuos en libertad localizados en zonas que podrían considerarse más limpias, mientras que, en la Publicación No. 5, las especies estuvieron expuestas durante un periodo definido en sitios con mayor probabilidad de estar contaminados.

Durante el análisis del potencial de bioacumulación desarrollado en la Publicación No. 5, no fue posible calcular los BAFs de determinados compuestos debido a que no fueron detectados en ninguna de las muestras de agua superficial. Este fue el caso de los OPFRs, TMPP y TDBPP, que se detectaron en las cuatro especies analizadas, y en al menos un sitio de exposición. Su ausencia en el compartimento acuático puede estar relacionado con sus valores de log K_{ow}: 6,35 para el TMPP y 3,65 para el TDBPP, que los clasifica como compuestos lipofílicos. Esta propiedad les confiere una mayor afinidad para bioacumularse en tejidos adiposos. Hou et al., (2016), reportaron valores de BAFs en peces de agua dulce para cinco de los

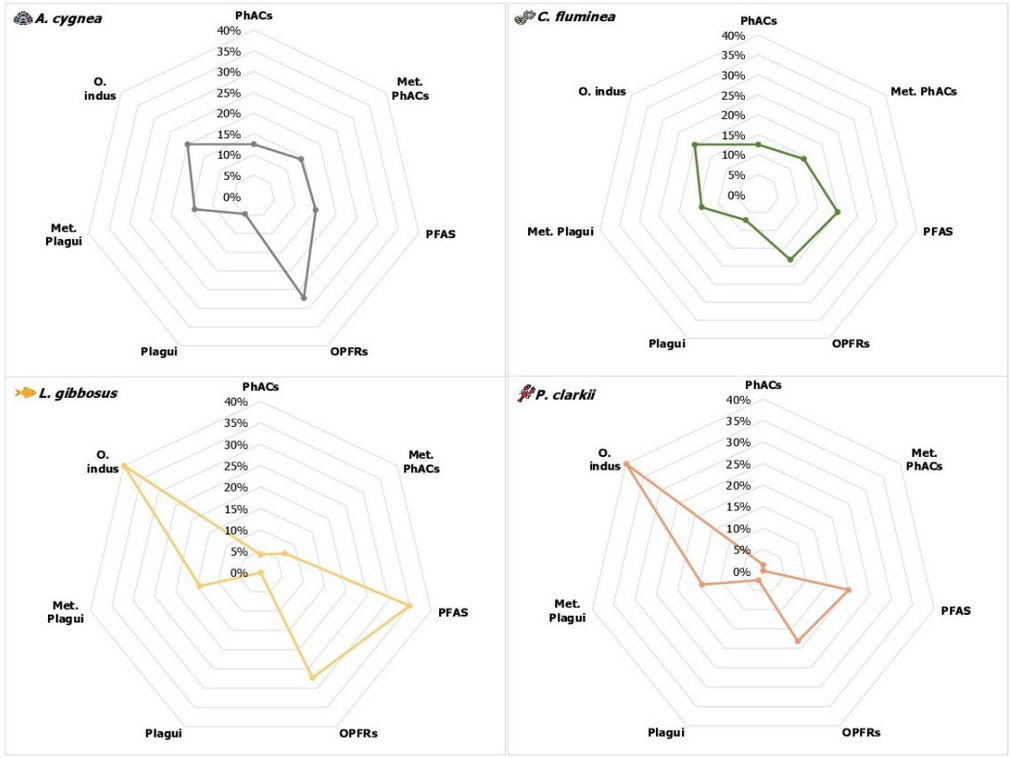

Fig. 5.2. *Porcentaje de compuestos detectados por especie en función del número total de compuestos analizados*

compuestos analizados en la Publicación No. 5: TCIPP (0,8-4,6), TCEP (0,6-5,1), TDCIPP (0,3-113), THEP (250) y TPhP (110-300), demostrando que existe una alta variabilidad en el potencial de bioacumulación para los compuestos de esta familia química.

Otro compuesto detectado en las tres especies invasoras, pero no confirmado en las aguas superficiales fue el PFHxA (log K_{ow} 3,48). El sitio de exposición en el que fue reportado varió en función de la especie, y estuvo presente en concentraciones inferiores a 8,00 ng g^{-1}. La variación de su presencia en diferentes sitios de exposición por especie, podrían estar relacionadas con fluctuaciones de su disponibilidad dentro del Parque Natural de L'Albufera. La variabilidad de la presencia de CECs en el agua superficial, particularmente de PFAS, y OPFRs, ha sido previamente reportada por Soriano et al., (2023), quienes señalaron que parámetros ambientales como el pH y el oxígeno disuelto pueden influir en la dinámica de compuestos como PFOA, PFHpA, PFOS, PFNA, y PFHxDA dentro de la zona estudio. Además, demostraron que la salinidad, la conductividad y la temperatura, afectan al comportamiento de

los OPFR, en particular el TDCIPP y el TCIPP. Estos factores desempeñan un papel importante en la determinación del destino ambiental y la biodisponibilidad de estos contaminantes en los ecosistemas acuáticos, lo que repercute en su distribución y persistencia en diferentes lugares del parque y por ende en su posible bioacumulación por parte de los organismos acuáticos.

Respecto a la bioacumulación de compuestos como los PFAS, la longitud de su cadena es un factor clave (Ma et al., 2022). Por lo general los organismo acuáticos tienden a bioacumular en mayor medida los PFAS de cadena larga (es decir aquellos con más de siete átomos de carbono), porque estos compuestos presentan una mayor afinidad por las proteínas y se eliminan del organismo a un ritmo más lento en comparación con los PFAS de cadena corta (Brendel et al., 2018). Sin embargo, en la Publicación No. 5 se observó una alta frecuencia de detección del PFBA, un compuesto de cadena corta. Este compuesto se detectó en todas las especies y sitios de exposición, a pesar de que solo se detectó en tres muestras de agua superficial.

La presencia generalizada del PFBA en las muestras de biota podría atribuirse a su papel como producto de descomposición de otros PFAS (MDH, 2022). Esto significa que su concentración puede ser mayor en el medio ambiente en comparación con los compuestos que aún no se han degradado. Además, su valor log K_{ow} (2,82) lo clasifica como moderadamente polar. Esto indica que, aunque es soluble en agua, también puede tener afinidad por los tejidos adiposos, lo que le permite bioacumularse en los organismos a pesar de ser un compuesto de cadena corta.

El PFBA ha sido reportado previamente en otros organismos como cochinillas y caracoles (Groffen et al., 2023); y en un lago urbano en el este de China, los valores de BAFs variaron de 0.22 a 1.88 para *P. clarkii* y de 0.51 a 0.62 para *C. fluminea* (Chu et al., 2022). Su principal vía de absorción es probablemente a través de la dieta, ya que tiende a acumularse en plantas que luego son ingeridas por animales, o a través de partículas suspendidas que son filtradas por especies como los bivalvos, o removidas para alimentación en el caso de algunos peces (Chu et al., 2022; Groffen et al., 2023).

Burkhard,. (2021) reportó BAFs para varios PFAS en diferentes bivalvos de agua dulce: PFHpA (2.43), PFOA (1.68), PFNA (2.43), PFDA (2.47), PFUnDA (2.47), PFHxS (3.44) y PFOS (2.98). Estos valores son inferiores a los presentados en la Publicación No. 5 para las almejas analizadas. Estas diferencias podrían deberse

a los procesos de absorción y eliminación de PFAS, que involucran tanto difusión pasiva como mecanismos de transporte activo. Mientras que la difusión pasiva no depende de la concentración, los mecanismos de transporte activo, como la depuración renal y los procesos de biotransformación, desempeñan un papel crucial en la eliminación de estos compuestos (Burkhard, 2021), los cuales pueden variar entre especies y condiciones ambientales.

El estudio realizado reveló que las especies invasoras son eficientes para el biomoniotoreo de CECs, evidenciando la presencia de compuestos que no podrían haber sido identificados si tan sólo se analizaran los compartimentos abióticos como el agua superficial (TMPP, TDBPP y PFHxA). También permitió identificar compuestos que no hubieran sido reportados si solo se hubiera analizado la especie autóctona, como la sertralina, oseltamivir y clorfenvinfos detectados solo en *C. fluminea*, el 5M-1H benzotriazol detectado sólo en *P. clarkii* y el bisfenol A, bezafibrato, PFOS PFNA y PFDA detectados solo en *L. gibbossus*.

El análisis de riesgo ecológico realizado respaldó la teoría de que las especies invasoras son más resistentes a la contaminación y, por lo tanto, permiten obtener una visión más amplia sobre los CECs que pueden representar un peligro para la fauna acuática nativa. Esto se evidenció en que la especie invasora *C. fluminea* obtuvo los valores de RQ más altos, lo cual indica que las concentraciones detectadas en esta especie superaron en mayor medida los valores de referencia (PNECs) a partir de los cuales se prevén efectos ecológicos adversos. A pesar de haber acumulado mayores concentraciones de contaminantes, *C. fluminea* no mostró efectos negativos en su supervivencia, y obtuvo las concentraciones acumuladas más altas en comparación con las otras especies, pese a que todas estuvieron expuestas bajo las mismas condiciones.

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CAPÍTULO 6

CONCLUSIONES

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This thesis evaluated the presence and distribution of contaminants of emerging concern in freshwater ecosystems, using native and invasive species as biomonitors. To achieve this goal, studies on different fish organs to prioritize matrices other than muscle have been conducted. Additionally, comparisons were made between different types of invasive species, revealing differentiated accumulation patterns. Through this approach, biomonitoring with invasive species has been demonstrated to be a valuable tool for ecological risk assessment, offering a new perspective on monitoring contaminants of emerging concern in aquatic environments.

The most relevant conclusions, addressing both methodological aspects and the results obtained and their implications for future research, are presented below.

In relation to analytical methodologies, in this thesis two methods were developed to enable the simultaneous analysis of contaminants of emerging concern from various chemical classes in a wide range of biological matrices. These methods, characterized by their simplicity, cost-effectiveness, and low environmental impact, proved to be effective and reproducible in extracting contaminants from different fish organs (brain, heart, liver, kidney, muscle, and skin), as well as from the soft tissues of crayfish, freshwater clams, and whole small fish. The combination of TissueLyser homogenization and ultrasound-assisted extraction reduced processing time to 10 minutes per cycle using acetonitrile acidified with 0.1% formic acid while maintaining satisfactory recovery efficiency. In contrast to previous studies, where clean-up via dispersive solid-phase extraction required additional steps, this work implemented a single clean-up step using Z-Sep C_{18} adsorbent, maintaining method simplicity without compromising analytical quality parameters. Additionally, the use of a reduced solvent volume (1.0 mL) improved compatibility with extraction and clean-up steps, optimizing resource consumption and analytical efficiency.

The application of suspect screening approaches with high-resolution mass spectrometry was crucial in identifying a large number of contaminants that might have been overlooked in an initial targeted analysis. This methodology, followed by specific quantification through targeted analysis, provided detailed information on the concentrations and potential risks of contaminants of emerging concern, reinforcing the importance of integrating these two data acquisition approaches in environmental biomonitoring.

The analysis of distribution of contaminants of emerging concern in different fish organs facilitated the detection of compounds that would not have been identified if the study had been limited to muscle, which is the commonly used matrix. These results allowed for the prioritization of organs such as the liver, kidney, skin, and brain as key sentinel matrices for biomonitoring these compounds in wild river fish. The liver and kidney, in particular, stood out due to their high mean concentrations and detection frequency, which is related with the role of the liver as the main metabolizing organ and the function of the kidney as a filtration system for waste excretion and osmoregulation. Additionally, the skin and brain proved useful for assessing contaminant exposure, especially for more lipophilic compounds. Although skin is not a commonly studied matrix, its analysis enabled the detection of compounds that had not been previously reported in freshwater fish, such as amantadine and quetiapine.

The determination of experimental bioaccumulation factors allowed the identification of compounds with specific affinities for certain organs in fish and different macroinvertebrates. In common carp, high bioaccumulation potential was observed for diazepam and terbutryn in the brain and for amantadine in the kidney. In macroinvertebrates, selective bioaccumulation was observed depending on the species: the native clam petxinot showed high BAFs for cotinine and tramadol, and to a lesser extent for desethylterbuthylazine, venlafaxine, and its metabolite O-desmethylvenlafaxine. The invasive Asian clam exhibited high bioaccumulation potential for salbutamol, tramadol, fluoxetine, and methadone, while the red swamp crayfish mainly bioaccumulated pesticides such as tebuconazole and terbuthylazine, as well as caffeine and perfluoroundecanoic acid (PFuNDA). In *Lepomis gibbosus*, salbutamol and atrazine were the most accumulated compounds. An important finding was perfluorobutanoic acid (PFBA), which recorded BAFs above 1,000 in all species, which highlighted its persistence in the Albufera Natural Park (Valencia, Sapin).

The comparison of experimentally determined bioaccumulation factors for different fish organs with those predicted by the applied physiologically based pharmacokinetic model identified opportunities for improvement in the modeling. These include incorporating a greater number of organs in the models and considering partition coefficients for compounds that are protonated at physiological pH, which could optimize the accuracy and applicability of the results in environmental assessments.

This doctoral research established that invasive species are effective biomonitors in various ecosystems under anthropogenic pressure, such as the Llobregat River and the Albufera Natural Park, both affected by wastewater treatment plant effluents, agricultural activities, and pollutants associated with proximity to large urban areas like Barcelona and Valencia. In the river ecosystem dominated by the Llobregat River, common carp, an invasive species in this environment, stood out among the fish species analyzed—not only for its ability to persist despite deteriorating of water quality but also for exhibiting high concentrations of contaminants of emerging concern in most of its organs. On the other hand, in the coastal wetland of Albufera Natural Park, bivalves demonstrated a notable capacity for accumulating various contaminants of emerging concern, attributed to their absorption, distribution, metabolism, and excretion processes, particularly their ability to filter large volumes of water and feed on sediment deposits. When comparing the native clam petxinot with the invasive Asian clam, the latter showed higher mean concentrations of contaminants and exceeded reference levels established by NORMAN for freshwater mollusks. These findings highlight the invasive Asian clam and common carp as valuable species for biomonitoring, given their ability to reflect a wide range of compounds, emphasizing their utility in ecological risk assessment.

This research expanded knowledge on the accumulation patterns of contaminants of emerging concern in invasive species that have been understudied in environmental assessments of organic contaminants, such as the red swamp crayfish (*P. clarkii*) and Pumpkinseed (*L. gibbosus*), as well as in native species like "*petxinot"* clam. The findings contribute to a deeper understanding of the role of these species in contamination dynamics within ecologically significant aquatic ecosystems.

Furthermore, the species analyzed in this thesis allowed the detection of compounds that had been rarely studied in these specific species. These included 5-methyl-1H-benzotriazole, amantadine, benzotriazole, cotinine, and hydrochlorothiazide in common carp; 5-methyl-1H-benzotriazole in the red swamp crayfish; and bezafibrate, bisphenol A, perfluorononanoic sulfonate (PFDS), perfluorononanoic acid (PFNA), and perfluorooctane sulfonate (PFOS) in Pumpkinseed. These findings suggest that Pumpkinseed could be used in biomonitoring studies where it represents a dominant invasive species. Additionally, compounds such as amantadine, atenolol, nicotine, desethyl-terbuthylazine, and cotinine were detected in petxinot clam a native species, providing insights for developing management strategies to ensure its protection.

The ecological risk assessment conducted using invasive species exposed to real environmental concentrations prioritized certain high-risk compounds, such as the antidepressant sertraline and the stimulant caffeine, in both ecosystems. Moreover, specific contaminants were prioritized based on individual risk quotients in each ecosystem. In the Llobregat River, the herbicide terbutryn, the antiviral amantadine, and the metabolite cotinine were prioritized, while in the Albufera Natural Park, terbuthylazine, fluoxetine, oseltamivir, and the organophosphate flame-retardant tris(2,3-dibromopropyl) phosphate (TDBPP) stood out.

In summary, this thesis highlights the potential of invasive species, particularly the Asian clam and common carp, for biomonitoring contaminants of emerging concern in aquatic ecosystems, thereby reducing pressure on native species. Their use in biomonitoring studies is crucial for identifying and assessing ecological risks, providing accurate data for developing more effective environmental management strategies. Strengthening research and implementation of biomonitoring programs with these species is recommended to improve the understanding of the long-term impacts of these contaminants on the health of aquatic ecosystems.