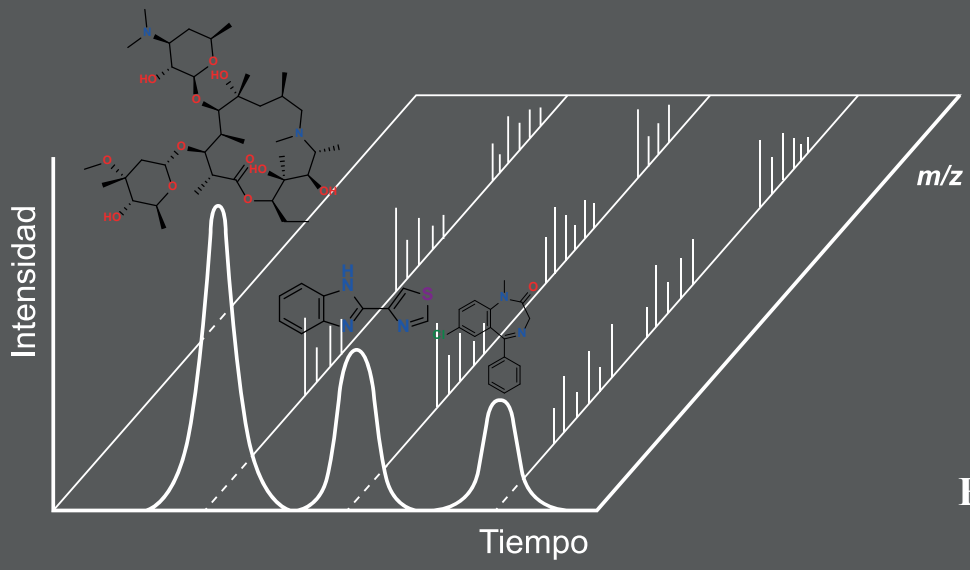
UNIVERSITAT JAUME·I

INVESTIGACIÓN DE RESIDUOS DE PLAGUICIDAS Y FÁRMACOS EN AGUAS MEDIOAMBIENTALES. ESTUDIO DE LA ELIMINACIÓN DE FÁRMACOS EN UNA ESTACIÓN CONVENCIONAL DE TRATAMIENTO DE AGUAS RESIDUALES

Tesis Doctoral



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I) Eddie Fonseca, Arianna Renau-Pruñonosa, María Ibáñez, Emma Gracia-Lor, Teodoro Estrela, Sara Jiménez, Miguel Ángel Pérez-Martín, Francisco González, Félix Hernández, Ignacio Morell. Investigation of pesticides and their transformation products in the Júcar River Hydrographical Basin (Spain) by wide-scope high-resolution mass spectrometry screening. *Environmental Research* 177 (2019) 108570. https://doi.org/10.1016/j.envres. 2019.108570. Factor de impacto: 5.200

II) Lubertus Bijlsma, Elena Pitarch, Félix Hernández, Eddie Fonseca, José M. Marín, María Ibáñez, Tania Portolés, Andreu Rico. Ecological risk assessment of pesticides in the Mijares River (eastern Spain) impacted by citrus production using wide-scope screening and target quantitative analysis. *Journal of Hazardous Materials* 412 (2021) 125277. https://doi.org/10.1016/j.jhazmat.2021.125277. Factor de impacto: 9.038

III) Eddie Fonseca, Félix Hernández, María Ibáñez, Andreu Rico, Elena Pitarch, Lubertus Bijlsma. Occurrence and ecological risks of pharmaceuticals in a Mediterranean river in Eastern Spain. *Environment International* 144 (2020) 106004. https://doi.org/10.1016/ j.envint.2020.106004. Factor de impacto: 7.577

IV) Elena Pitarch, Lubertus Bijlsma, Eddie Fonseca, María Ibáñez, Ana María Botero, Javier Claros, Laura Pastor, Félix Hernández. Investigation of pharmaceuticals in a conventional wastewater treatment plant: removal efficiency, seasonal variation and impact of a nearby hospital. *Journal of Environmental Chemical Engineering* 9 (2021) 105548. https://doi.org/10.1016/j.jece.2021.105548. Factor de impacto: 4.02

"*Esta tesis dispone de la aceptación de los coautores de las publicaciones que el doctorando presenta como tesis y su renuncia expresa a presentarlas como parte de otra tesis doctoral*"

A mis padres y hermanos

"Triste del país que no tome a las ciencias por guía en sus empresas y trabajos. Se quedará postergado, vendrá a ser tributario de los demás y su ruina será infalible, porque en la situación actual de las sociedades modernas la que emplea más sagacidad y saber, debe obtener ventajas seguras sobre las otras"

Dr. José María Castro Madriz
Discurso inaugural de la apertura de la Universidad de Santo Tomás, 1844

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Resumen

La contaminación del medio ambiente está estrechamente ligada a las actividades cotidianas del ser humano. Si bien algunos fenómenos naturales pueden llegar a ser una causa importante de contaminación, no se comparan, ni de lejos, con la contaminación que generan las actividades antropogénicas. En la agricultura, por ejemplo, el afán de proteger los cultivos de plagas y enfermedades lleva al ser humano a aplicar cantidades considerables de plaguicidas, que, irremediablemente, terminan llegando al aire, suelo y agua. Aunque quizás menos estudiadas, las estaciones depuradoras de aguas residuales (EDAR) también pueden llegar a ser fuentes de contaminación de plaguicidas si industrias que utilizan estos compuestos descargan sus efluentes a la EDAR (p.e industrias de tratamiento poscosecha cosecha). De igual forma, para prevenir o curar enfermedades o aliviar síntomas, se recurre a los fármacos, los cuales una vez consumidos son excretados y pueden llegar a las EDAR, donde muchas veces no se eliminan completamente y pueden llegar a las aguas receptoras con la descarga de efluente de las aguas residuales.

Los plaguicidas y los fármacos, así como sus productos de transformación (TPs)/metabolitos, pueden encontrarse en el medio acuático, normalmente a bajos niveles de concentración (ng/L-µg/L). Para medir estos niveles es necesario utilizar metodologías analíticas avanzadas, capaces de detectar, identificar y cuantificar estos compuestos de manera fiable. La instrumentación analítica utilizada resulta fundamental para alcanzar los niveles de concentración requeridos en el análisis de contaminantes en el medioambiente.

En la presente tesis doctoral se ha estudiado el potencial y la aplicabilidad del acoplamiento de cromatografía de líquidos (LC) y de gases (GC) a espectrometría de masas (MS) con analizadores de baja (triple cuadrupolo, QqQ) y alta (cuadrupolo-tiempo de vuelo, QTOF) resolución. El objetivo principal es la investigación de residuos de plaguicidas, fármacos y sus TPs/metabolitos en muestras ambientales (aguas superficial y subterránea) y en aguas residuales, y la evaluación del riesgo toxicológico que estos productos suponen para el medio acuático.

La primera parte de la tesis consta de una introducción general (**capítulo 1**) sobre la problemática ambiental del medio acuático, aspectos relacionados con la legislación vigente

en la Unión Europea, y una breve introducción a la determinación analítica de plaguicidas y fármacos en aguas.

La segunda parte **(capítulo 2)** incluye el trabajo experimental, el cual se estructuró en tres secciones diferenciadas El **capítulo 2** se inicia con la **sección 2.1.2,** que comprende el **artículo científico I**, en el cual se exploró el potencial del acoplamiento LC-QTOF MS para investigar la presencia de plaguicidas y TPs en la cuenca hidrográfica del Júcar. Se desarrolló una metodología de screening para detectar e identificar 500 compuestos, 20 de los cuales fueron sometidos a una validación cualitativa en aguas naturales, aplicando un tratamiento de muestra consistente en extracción en fase sólida (SPE). Tras analizar 8 muestras de aguas superficiales y 11 muestras de aguas subterráneas, se encontraron 33 compuestos (27 plaguicidas y 6 TPs). Los más detectados en las aguas subterráneas fueron los herbicidas triazínicos atrazina, simazina, terbutilazina y los TPs atrazina-desetil, terbumetón-desetil y terbutilazina-desetil. En las aguas superficiales, los más detectados fueron los fungicidas carbendazim, tiabendazol e imazalil, el herbicida terbutrina y el TP terbumetón-desetil.

En **sección 2.1.3**, que comprende el **artículo científico II**, se investiga el potencial del screening combinado mediante LC-QTOF MS y GC-QTOF MS, y del acoplamiento LC con masas en tándem (LC-MS/MS) con analizador QqQ para investigar la presencia de plaguicidas y TPs en las aguas del río Mijares. De igual forma, se buscó establecer el riesgo que estos compuestos representan para el medio acuático. El screening preliminar, mediante la combinación de LC-QTOF MS y GC-QTOF MS, permitió identificar compuestos relevantes presentes en las aguas superficiales objeto de estudio. Las bases de datos utilizadas incluyeron alrededor de 550 compuestos para LC y 425 para GC. Una vez realizado el cribado por HRMS, se estableció una lista de 19 plaguicidas y 5 TPs, que fueron incluidos en la metodología cuantitativa mediante LC-MS/MS QqQ. Un total de 57 muestras de agua superficial tomadas en tres épocas diferentes del año, fueron sometidas a la metodología cuantitativa sin ningún tratamiento previo de muestra (inyección directa). Los plaguicidas que, en alguna ocasión, excedieron el valor de 0.1 µg/L fueron 2,4-D, imidacloprid, tiabendazol e imazalil. Imidacloprid se encuentra en la lista de observación de sustancias a efectos de seguimiento a nivel de la Unión en el ámbito de la política de aguas (Watch List). Tiabendazol e imazalil son compuestos que se aplican ampliamente en la Comunidad Valenciana en tratamientos

poscosecha. Con los resultados obtenidos en el análisis cuantitativo de las muestras del río Mijares, se llevó a cabo la evaluación de los riesgos para el medio ambiente acuático utilizando dos aproximaciones, la de unidades de toxicidad (UT) y la fracción potencialmente afectada de múltiples sustancias (msPAF). Mediante la aproximación UT, los riesgos de toxicidad de mezcla fueron bajos o insignificantes para los productores primarios. En los puntos de muestreo 17 y 18, que tienen en sus cercanías una EDAR, la toxicidad crónica de la mezcla para los vertebrados fue alta en septiembre, con $\Sigma UT_{máx} = 1.7$ (punto 18). La aproximación msPAF permitió detectar riesgos moderados para los productores primarios a partir de mezclas de herbicidas diurón, simazina y 2,4-D, y se calcularon riesgos moderados a altos para especies de invertebrados y vertebrados debido a la exposición al fungicida tiabendazol.

La **sección 2.2** incluye el **artículo científico III**, en el cual se exploró el potencial de los acoplamientos UHPLC-QTOF MS y UHPLC-MS/MS QqQ para investigar la presencia de fármacos y sus metabolitos en las aguas recogidas en el río Mijares y realizar una evaluación del riesgo ecológico que estas mezclas de compuestos representan para el medio acuático. Las 57 muestras de agua se sometieron a una metodología cuantitativa mediante UHPLC-MS/MS QqQ para la determinación de 40 fármacos, de los cuales acetaminofén (67%), gabapentina (42%) y venlafaxina (40%) fueron los compuestos de mayor frecuencia de detección. Por su parte, los compuestos que presentaron las mayores concentraciones fueron la fenazona (1.98 µg/L), el tramadol (1.94 µg/L) y la gabapentina (1.92 µg/L), pertenecientes a los grupos terapéuticos de antibióticos, antiinflamatorios y antihipertensivos. En general, no se encontraron tendencias claras en función de la temporada de muestreo, aunque en la tercera campaña (invierno) se observó un ligero aumento en la concentración de antibióticos, probablemente porque en esa época se incrementa su uso para tratar infecciones respiratorias. Para complementar los resultados obtenidos por UHPLC-MS/MS QqQ, y tras extracción de muestra por SPE, se llevó a cabo un screening por UHPLC-HRMS, usando una base de datos de alrededor de 900 compuestos, entre fármacos y metabolitos. En total, se detectaron 50 compuestos (41 fármacos y 9 metabolitos). Los fármacos que presentaron la mayor frecuencia de detección fueron el acetaminofén y la venlafaxina. Los metabolitos más detectados fueron 4-acetilaminoantipirina (4-AAA) y 4-formilaminoantipirina (4-FAA), ambos metabolitos del metamizol. Los resultados obtenidos en el análisis cuantitativo se utilizaron para la evaluación

del riesgo ecológico, encontrando que los compuestos que ejercían mayor toxicidad sobre los ecosistemas acuáticos fueron los analgésicos/antiinflamatorios y los antibióticos; en concreto, fenazona > azitromicina > diclofenaco, y, en menor medida, norfloxacino > ciprofloxacino > claritromicina. También se encontró que cinco de los antibióticos detectados excedían los umbrales de resistencia a los antibióticos (ciprofloxacino, azitromicina, norfloxacino, trimetoprima y claritromicina).

En la tercera y última parte experimental, de la cual resultó el **artículo científico IV** (**sección 2.3, capítulo 2**), se exploró el potencial de los acoplamientos UHPLC-QTOF MS y UHPLC-MS/MS QqQ para investigar la presencia de fármacos en las aguas residuales de entrada (IWW) y de salida (EWW) de una EDAR con tratamiento convencional a lo largo de tres campañas de muestreo. La EDAR estudiada también recibe aguas de un hospital cercano, las cuales fueron recogidas durante la primera campaña para su estudio. En una etapa preliminar, se llevó a cabo un screening por UHPLC-QTOF MS para evaluar los compuestos más relevantes presentes en los diferentes tipos de agua. A partir de los resultados del screening, se elaboró una lista de 40 compuestos que se incluyeron en la metodología cuantitativa mediante UHPLC-MS/MS QqQ. En el análisis cuantitativo de las muestras IWW y de la descarga hospitalaria se pudo cuantificar 28 de los 40 fármacos en estudio, de los cuales 24 estuvieron presentes en ambos tipos de muestras, aunque la concentración fue, en general, superior en las muestras hospitalarias, indicando una contribución importante de fármacos de la descarga del hospital a la EDAR. A partir de los resultados obtenidos para las 21 muestras de IWW y EWW, que fueron recogidas en tres campañas de muestreo diferentes, se estimó la eficiencia de eliminación (RE) de los fármacos detectados. Un 34% de los compuestos encontrados fueron eliminados casi completamente (RE >75%), mientras que un 9% adicional se eliminó de forma parcial (50% < RE <75%) y el 18% no se eliminó en absoluto. En relación con la variación estacional, la temporada con menor concentración de familias farmacéuticas fue el invierno, excepto por los antibióticos, cuyos valores aumentaron notablemente en ese período.

En el **capítulo 3** se indican las conclusiones más relevantes del estudio llevado a cabo y algunas sugerencias para trabajos futuros, finalmente, en el **capítulo 4** se encuentra la lista de la bibliografía más relevante usada en la tesis doctoral.

Summary

Environmental pollution is closely linked to everyday human activities. While some natural phenomena can be a major cause of pollution, they are not generally considered as serious as the pollution generated by anthropogenic activities. In agriculture, for instance, the desire to protect crops from pests and diseases leads humans to apply considerable quantities of pesticides, which inevitably end up in the air, soil and water. Wastewater treatment plants (WWTPs) can also become sources (maybe less studied) of pesticide contamination when industries using these compounds discharge their effluents to the WWTP (e.g. post-harvest treatment industries). Similarly, pharmaceuticals are compounds used to prevent or cure diseases or alleviate symptoms and once consumed are excreted and can reach the WWTP, where they are often not completely eliminated and can reach the receiving water with the discharge of wastewater effluent.

Pesticides and pharmaceuticals, as well as their transformation products (TPs)/metabolites, can be found in the aquatic environment, usually at low concentration levels (ng/L-µg/L). In order to measure these levels, it is necessary to use advanced analytical methodologies capable of reliably detecting, identifying, and quantifying these compounds. The analytical instrumentation used is essential to achieve the concentration levels required in the analysis of pollutants in the environment.

The present PhD thesis has studied the potential and applicability of coupling liquid chromatography (LC) and gas chromatography (GC) to mass spectrometry (MS) with low (triple quadrupole, QqQ) and high (quadrupole-time-of-flight, QTOF) resolution analyzers. The main objective is the investigation of pesticide residues, pharmaceuticals and their TPs/metabolites in environmental samples (surface and groundwater) and in wastewater, and the evaluation of the toxicological risk that these products pose to the aquatic environment.

The first part of the thesis consists of a general introduction (**Chapter 1**) about environmental concerns of the aquatic environment, aspects related to current legislation in the European Union, and a brief introduction to the analytical determination of pesticides and pharmaceuticals in water.

The second part (**Chapter 2**) includes experimental work, which was structured into three distinct sections. **Chapter 2** begins with **Section 2.1.2**, comprising **Scientific Paper I,** which explores the potential of LC-QTOF MS coupling to investigate the presence of pesticides and TPs in the Júcar River basin. A screening methodology was developed to detect and identify 500 compounds, 20 of which were subjected to qualitative validation in natural waters, applying a sample treatment of solid phase extraction (SPE). After analyzing 8 surface water samples and 11 groundwater samples, 33 compounds were found (27 pesticides and 6 TPs). The most detected analytes in groundwater were the triazine herbicides atrazine, simazine, terbuthylazine and the TPs atrazine-desethyl, terbumeton-desethyl and terbuthylazine-desethyl. In surface waters, the most detected compounds were the fungicides carbendazim, thiabendazole and imazalil, the herbicide terbutryn and the TP terbumeton-desethyl.

Section 2.1.3, which comprises **Scientific Paper II**, investigates the potential of combined screening by LC-QTOF MS and GC-QTOF MS, and LC coupling with tandem mass (LC-MS/MS) with a QqQ analyzer to investigate the presence of pesticides and TPs in the Mijares River. Likewise, the aim was to establish the risk that these compounds pose to the aquatic environment. Preliminary screening, using a combination of LC-QTOF MS and GC-QTOF MS, allowed the identification of relevant compounds present in the surface waters under study. The databases used included about 550 compounds for LC and 425 for GC. After HRMS screening, a list of 19 pesticides and 5 TPs was established and included in the quantitative methodology by LC-MS/MS QqQ. A total of 57 surface water samples taken at three different times of the year were subjected to the quantitative methodology without any sample pretreatment (direct injection). The pesticides that exceeded 0.1 µg/L were 2,4-D, imidacloprid, thiabendazole and imazalil. Imidacloprid is on the Watch List of substances for monitoring purposes at the EU level in the field of water policy. Thiabendazole and imazalil are compounds that are widely applied in the Valencian Community in post-harvest treatments. With the results obtained in the quantitative analysis of the samples from the Mijares River, the risk assessment for the aquatic environment was undertaken using two approaches, the toxicity units (TUs) and the multi-substance potentially affected fraction (msPAF). Using the UT approach, the toxicity risks of the mixture were low or negligible for the primary producers. At sampling points 17 and 18, which have a nearby WWTP, chronic toxicity of the mixture for

vertebrates was high in September, with ΣUT_{max} = 1.7 (point 18). The msPAF approach detected moderate risks to primary producers from the herbicide mixtures of diuron, simazine and 2,4-D, and moderate to high risks were calculated for invertebrate and vertebrate species due to exposure to the fungicide thiabendazole.

Section 2.2 includes **Scientific Paper III**, which explored the potential of UHPLC-QTOF MS and UHPLC-MS/MS QqQ couplings to investigate the presence of pharmaceuticals and their metabolites in water collected from the Mijares River and to perform an assessment of the ecological risk that these mixtures of compounds pose to the aquatic environment. The 57 water samples were subjected to a quantitative analysis using UHPLC-MS/MS QqQ for the determination of 40 pharmaceuticals, of which acetaminophen (67%), gabapentin (42%) and venlafaxine (40%) were the compounds with the highest frequency of detection. On the other hand, the compounds with the highest concentrations were phenazone (1.98 µg/L), tramadol (1.94 µg/L) and gabapentin (1.92 µg/L), belonging to the antibiotic, anti-inflammatory and antihypertensive therapeutic groups. In general, no clear trends were found according to the sampling season, although in the third campaign (winter) a slight increase in the concentration of antibiotics was observed, probably because at that time their use to treat respiratory infections increases. To complement the results obtained by UHPLC-MS/MS QqQ, and after the sample extraction by SPE, a screening by UHPLC-HRMS was carried out, using a database of around 900 compounds, including pharmaceuticals and metabolites. In total, 50 compounds were detected (41 pharmaceuticals and 9 metabolites). The pharmaceuticals with the highest frequency of detection were acetaminophen and venlafaxine. The most frequently detected metabolites were 4-acetylaminoantipyrine (4-AAA) and 4-formylaminoantipyrine (4-FAA), both metabolites of metamizole. The results obtained in the quantitative analysis for ecological risk assessment found that the compounds that exert the highest toxic pressure on aquatic ecosystems were analgesics/anti-inflammatory pharmaceuticals and antibiotics; specifically, phenazone > azithromycin > diclofenac, and, to a lesser extent, norfloxacin > ciprofloxacin > clarithromycin. Five of the antibiotics detected were also found to exceed antibiotic resistance thresholds (ciprofloxacin, azithromycin, norfloxacin, trimethoprim, and clarithromycin).

The third and final experimental part comprises **Scientific Paper IV (Section 2.3, Chapter 2)**. This paper explored the potential of UHPLC-QTOF MS and UHPLC-MS/MS QqQ

couplings to investigate the presence of pharmaceuticals in influent (IWW) and effluent (EWW) wastewaters of a conventionally treated WWTP over three sampling campaigns. The WWTP studied also receives water from a nearby hospital, which was collected during the first campaign for study. In a preliminary stage, a screening by UHPLC-QTOF MS was carried out to evaluate the most relevant compounds present in the different types of water. From the screening results, a list of 40 compounds was prepared and included in the quantitative methodology by UHPLC-MS/MS QqQ. In the quantitative analysis of the IWW and the hospital discharge samples, 28 of the 40 pharmaceuticals under study were quantified, of which 24 were present in both types of samples. The fact that concentrations were generally higher in the hospital samples, indicated a significant contribution of pharmaceuticals from the hospital discharge to the WWTP. From the results obtained for the 21 IWW and EWW samples, which were collected in three different sampling campaigns, the removal efficiency (RE) of the detected pharmaceuticals was estimated. Thirty-four percent of the compounds found were almost completely eliminated (RE >75%), while 9% were partially eliminated (50% < RE <75%) and 18% were not eliminated at all. In relation to seasonal variation, winter was the season with the lowest concentration of pharmaceutical families, except for antibiotics, whose values increased noticeably in that period.

Chapter 3 elaborates on the most relevant conclusions of the study undertaken and some suggestions for future work. Finally, **Chapter 4** consists of the list of the most relevant bibliography consulted for this doctoral thesis.

ÍNDICE GENERAL

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Lista de acrónimos

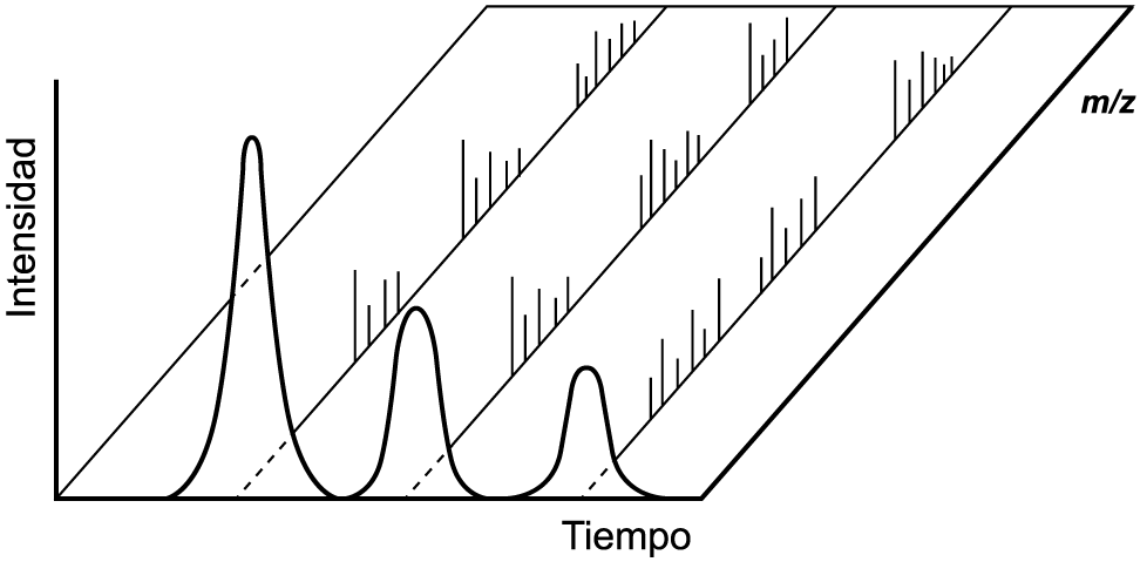
APCI	Atmospheric pressure chemical ionization Ionización química a presión atmosférica
CID	Collision-induced dissociation Disociación inducida por colisión
DDD	Defined daily dose Dosis diarias definidas
DHD	Defined daily doses per 1000 inhabitants per day Dosis diarias definidas por cada mil habitantes y día
DI	Direct injection Inyección directa
DIA	Data independent acquisition Adquisición independiente de datos
EI	Electron ionization Ionización electrónica
ESI	Electrospray ionization Ionización por electrospray
EWW	Effluent wastewater Efluente de agua residual
FAO	Food and Agriculture Organization Organización de las Naciones Unidas de la Alimentación y la Agricultura
GC	Gas chromatography Cromatografía de gases
GC-HRMS	Gas chromatography couple to high resolution mass spectrometry Cromatografía de gases acoplada a espectrometría de masas de alta resolución
GC-MS	Gas chromatography coupled to mass spectrometry Cromatografía de gases acoplada a espectrometría de masas
GC-MS/MS	Gas chromatography tandem mass spectrometry Cromatografía de gases acoplada a espectrometría de masas en tándem
GUS	Groundwater ubiquity score Puntuación de ubicuidad en aguas subterráneas

GW	Groundwater Agua subterránea
HE	High energy function Función de alta energía
HPLC	High performance liquid chromatography Cromatografía de líquidos de alta resolución
HRMS	High resolution mass spectrometry Espectrometría de masas de alta resolución
ILIS	Isotope labeled internal standard Estándar interno marcado isotópicamente
IUPAC	International Union of Pure and Applied Chemistry Unión Internacional de Química Pura y Aplicada
IWW	Influent wastewater Influente de agua residual
Kow	Octanol/water partition coefficient Coeficiente de reparto octanol/agua
LC	Liquid chromatography Cromatografía de líquidos
LC-HRMS	Liquid chromatography coupled to high-resolution mass spectrometry Cromatografía de líquidos acoplada a espectrometría de masas de alta resolución
LC-MS	Liquid chromatography coupled to mass spectrometry Cromatografía de líquidos acoplada a espectrometría de masas
LC-MS/MS	Liquid chromatography tandem mass spectrometry Cromatografía de líquidos acoplada a espectrometría de masas en tándem
LE	Low energy function Función de baja energía
$[M+H]^+$	Protonated molecule Molécula protonada
$[M-H]^-$	Deprotonated molecule Molécula desprotonada
m/z	Mass/charge ratio Relación masa/carga

$M^{+\cdot}$	Molecular ion Ion molecular
MS	Mass spectrometry Espectrometría de masas
MS^E	DIA mode for Waters Corporation Modo DIA de Waters Corporation
msPAF	Multiple substances potentially affected fraction Fracción potencialmente afectada de múltiples sustancias
NPLC	Normal-phase liquid chromatography Cromatografía de líquidos en fase normal
PNEC	Predicted no effect concentration Concentración prevista sin efecto
Q	Quadrupole mass analyser Cuadrupolo simple
q/Q	Ion ratio Relación de iones
QC	Quality control Control de calidad
QqQ	Triple quadrupole mass analyser Analizador de masas de triple cuadrupolo
QTOF	Hybrid quadrupole time-of-flight mass analyser Analizador de masas híbrido cuadrupolo-tiempo de vuelo
RE	Removal efficiency Eficiencia de eliminación
RF	Radio frequency voltage Voltaje de radiofrecuencia
RPLC	Reversed phase liquid chromatography Cromatografía de líquidos en fase reversa
RQ	Risk Quotient Cociente de riesgo
RSD	Relative standard deviation Desviación estándar relativa
S/N	Signal-to-noise ratio Relación señal/ruido

SIM	Selected Ion Monitoring Monitoreo de ion seleccionado
SPE	Solid phase extraction Extracción en fase sólida
SRM	Selected reaction monitoring Monitorización de reacción seleccionada
SSD	Species sensitivity distributions Distribución de sensibilidad de las especies
SW	Surface water Agua superficial
TP	Transformation product Producto de transformación
TU	Toxicity units Unidades de toxicidad
UHPLC	Ultra-high-performance liquid chromatography Cromatografía de líquidos de ultra alta resolución
XIC	Extracted ion chromatogram Cromatograma del ion extraído

OBJETIVOS



Objetivos

El **objetivo principal** de la presente tesis doctoral es explorar las capacidades analíticas de la cromatografía de líquidos de ultra-alta resolución (UHPLC) acoplada a la espectrometría de masas en tándem (MS/MS) con analizador de triple cuadrupolo (QqQ) y analizador híbrido cuadrupolo-tiempo de vuelo (QTOF), y de la cromatografía de gases acoplada a la espectrometría de masas con analizador híbrido cuadrupolo-tiempo de vuelo (GC-QTOF), para investigar la presencia de plaguicidas, fármacos y metabolitos/TPs en aguas continentales (superficial y subterránea) y en aguas residuales provenientes de una estación depuradora de aguas residuales (EDAR).

Para alcanzar el objetivo principal, se establecen los siguientes **objetivos específicos**:

1- Realizar una validación cualitativa del screening basado en UHPLC-QTOF MS, previa extracción en fase sólida (SPE) de la muestra, para la identificación de 20 plaguicidas y TPs seleccionados en aguas superficiales de la cuenca del Júcar.

2- Aplicar una metodología de amplio screening cualitativo, basado en el uso combinado de SPE-UHPLC-QTOF MS y SPE-GC-QTOF MS, para investigar la presencia de los plaguicidas y/o fármacos relevantes presentes en diferentes tipos de aguas (superficial, subterránea y residual).

3- Aplicar una metodología de screening cualitativo mediante SPE-UHPLC-QTOF MS para la identificación de fármacos y metabolitos en aguas superficiales, llevando a cabo diferentes campañas de muestreo con el objetivo de complementar los resultados obtenidos mediante metodologías analíticas target.

4- Determinar los niveles de concentración de plaguicidas, fármacos y TPs presentes en las aguas superficiales del río Mijares, llevando a cabo varias campañas de muestreo, y aplicando metodología analítica basada en la inyección directa y posterior determinación por UHPLC-MS/MS QqQ.

5- Evaluar los riesgos derivados de la presencia de plaguicidas y fármacos en el río Mijares utilizando dos aproximaciones (Unidades de toxicidad y distribución de sensibilidad de especies).

6- Investigar la presencia de fármacos en las aguas residuales de una EDAR convencional, llevando a cabo diferentes campañas de muestreo y aplicando metodología analítica basada en la inyección directa y posterior determinación cuantitativa por UHPLC-MS/MS QqQ, con el fin de:

   a. Estudiar la contribución de las aguas residuales de un hospital a la EDAR.
   b. Estimar la eficacia de la eliminación de los fármacos presentes en las aguas residuales de la EDAR tras el tratamiento biológico aplicado.
   c. Estudiar la variación estacional de fármacos de las aguas residuales de la EDAR en tres campañas de muestreo distribuidas a lo largo de un año de control.

Objetives

The **main objective** of this doctoral thesis is to explore the analytical capabilities of ultra-high performance liquid chromatography (UHPLC) coupled to tandem mass spectrometry (MS/MS) with a triple quadrupole analyzer (QqQ) and a hybrid quadrupole-time-of-flight (QTOF) analyzer, and of gas chromatography coupled to mass spectrometry with a hybrid quadrupole-time-of-flight analyzer (GC-QTOF), to investigate the presence of pesticides, pharmaceuticals and metabolites/TPs in inland water (surface and groundwater) and wastewater from a wastewater treatment plant (WWTP).

To achieve the main objective, the following **specific objectives** were established:

1- Develoment of a qualitative validation of the screening based on UHPLC-QTOF MS, with a previous sample treatment by solid phase extraction (SPE), for the identification of 20 selected pesticides and TPs in surface water of the Júcar basin.

2- Application of a broad qualitative screening methodology, based on the combined use of SPE-UHPLC-QTOF MS and SPE-GC-QTOF MS, to investigate the presence of the relevant pesticides and/or pharmaceuticals present in different types of water (surface, ground and wastewater).

3- Application of a qualitative screening methodology using SPE-UHPLC-QTOF MS for the identification of pharmaceuticals and metabolites in surface waters, collected from different campaigns, to complement the results obtained using target analytical methodologies.

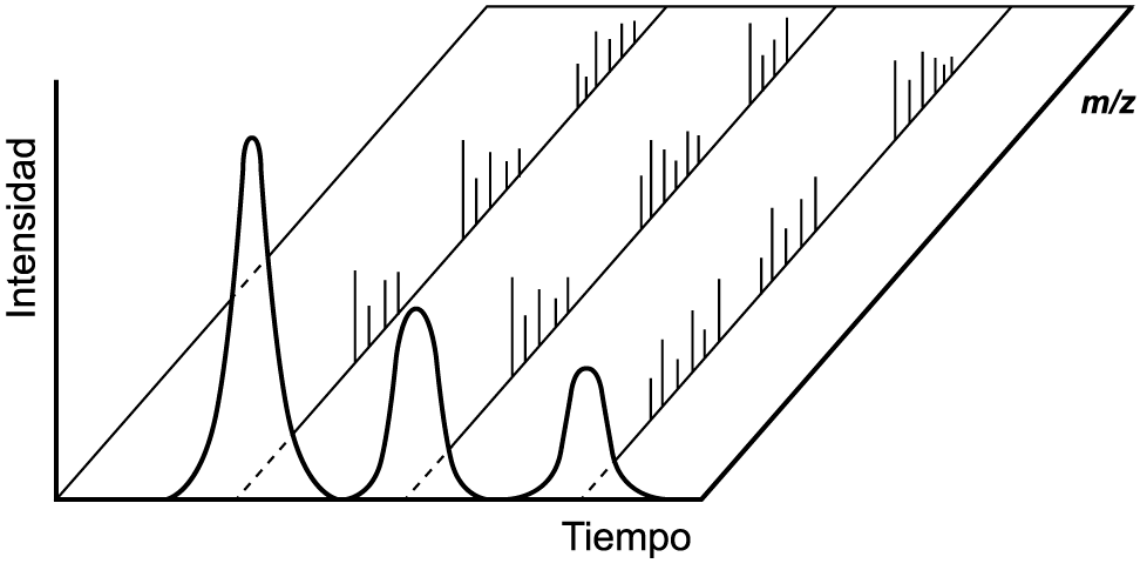
4- Application of the analytical methodology based on direct injection and determination by UHPLC-MS/MS QqQ for the quantitative analysis of pesticides, pharmaceuticals and TPs in the surface waters of the Mijares River sampled in different campaigns.

5- Ecotoxicological risk assessment of pesticides and pharmaceuticals in the Mijares River using two approaches (Toxicity Units and Species Sensitivity Distribution).

6- Implementation of an analytical methodology based on direct injection and quantitative determination by UHPLC-MS/MS QqQ for the identification of pharmaceuticals in wastewater samples from a conventional WWTP. In this section, the following components were included:

   a. Study the contribution of wastewater from a hospital to the WWTP.
   b. Estimate the removal efficiency of the pharmaceuticals present in the WWTP wastewater after the biological treatment applied.
   c. Study of the seasonal variation of pharmaceuticals in the wastewater from the WWTP in three sampling campaigns distributed over one year.

CAPÍTULO 1
INTRODUCCIÓN GENERAL



Capítulo 1. INTRODUCCIÓN GENERAL

1.1. Problemática ambiental de la contaminación del agua

El agua es un recurso natural indispensable, no solo para los organismos vivos, sino para un sinnúmero de actividades humanas: domésticas, agrícolas e industriales, entre otras. El problema de la contaminación del agua, de origen natural o antropogénico, se remonta a la Antigüedad y, probablemente, se agudiza más en la actualidad. Cada sustancia, natural o sintética, que llega al medioambiente deja su huella en los ecosistemas naturales, en particular en los sistemas acuáticos. El crecimiento poblacional y las exigencias tecnológicas son, sin duda, factores que han contribuido a un consumo, cada vez mayor, de productos y sustancias que son nocivas tanto para el medio ambiente como para la salud humana.

A lo largo de los últimos cincuenta años, en Europa se han realizado esfuerzos considerables por regular muchas de las sustancias que contaminan el medio ambiente. El avance de las técnicas analíticas, ha permitido la determinación de muchas más sustancias en diferentes tipos de matrices. Algunas de estas sustancias se han utilizado por mucho tiempo y no se sospechaba el impacto que podían causar en las aguas. Se trata de los contaminantes emergentes definidos como contaminantes que actualmente no están incluidos en los programas de seguimiento de la Unión Europea pero que pueden suponer un riesgo importante, de ahí que haya que regularlos y medir sus posibles efectos ecotoxicológicos y toxicológicos. De estos contaminantes, los fármacos están entre los que más preocupación han generado en los últimos treinta años.

Ya que la presente tesis doctoral trata sobre la contaminación de aguas por plaguicidas y fármacos, la problemática ambiental presentada a continuación se centrará en estos dos tipos de compuestos.

1.1.1. Plaguicidas

La Unión Internacional de Química Pura y Aplicada (IUPAC) define plaguicida como “En sentido estricto, [un plaguicida es] cualquier sustancia destinada a matar plagas; en el uso común [un plaguicida es] cualquier sustancia que se utilice para controlar, prevenir o destruir plagas animales, microbiológicas o vegetales” [1]. El término incluye “aquellas sustancias que se usan como reguladores del crecimiento de las plantas, los defoliantes, los desecantes y los

agentes que se utilizan para eliminar los frutos pequeños o para prevenir su caída prematura, así como las sustancias que se aplican a los cultivos antes o después de la cosecha para proteger el bien contra su deterioro durante el almacenamiento y el transporte" [2].

Son muchos los productos que se han diseñado para combatir las plagas vegetales, y son, ciertamente, una forma efectiva de prevenir el deterioro de los cultivos, mejorar el rendimiento del suelo y aumentar la calidad y cantidad de los productos que se consumen diariamente, como las frutas y las hortalizas. Dado el aumento poblacional, su uso parece ventajoso; sin embargo, una cantidad considerable de plaguicidas sintéticos afectan de manera negativa la salud y el medio ambiente. Es por esto que se ha promovido el uso de bioplaguicidas, que muestran una toxicidad efectiva incluso a dosis muy bajas y que, además, tienen menor persistencia en el medio ambiente.

En 2014, el uso de bioplaguicidas mostraba un crecimiento de un 16% anual, mientras que los plaguicidas sintéticos crecían a un 5.5% [3]. No obstante, pese a los esfuerzos que se han realizado por disminuir su consumo, los plaguicidas sintéticos continúan a la vanguardia del mercado. La necesidad de aumentar el rendimiento y de minimizar las pérdidas en los diferentes tipos de cultivos hace que cada año se utilicen aproximadamente 2 millones de toneladas de plaguicidas convencionales en todo el mundo. Si bien se ha señalado que esa cifra podría alcanzar los 3.5 millones de toneladas en 2020 [4], otras estimaciones indican que para el periodo 2020-2025 el consumo global de plaguicidas podría experimentar una tasa de crecimiento anual de un 4.7% [5].

Como se observa en la **Figura 1.1**, en 2017, Europa ocupó el tercer lugar del mundo en uso de plaguicidas en el campo agrícola **(Fig. 1.1**a). España, por su parte, ocupó el segundo lugar en la Unión Europea (**Fig. 1.1**b).

De las diferentes familias de plaguicidas que se emplean en agricultura a nivel mundial, en 2018 destacaron, por orden de importancia, los herbicidas (29.5%), fungicidas (12.9%) e insecticidas (9.7%) [7].

En 2012, la cantidad mundial estimada de ingrediente activo de plaguicida utilizada por productor, en el caso de los herbicidas y los reguladores de crecimiento vegetal (RCV), era de un 49% (**Fig. 1.2**a), mientras que en España era de un 29.1% (**Fig. 1.2**b**).** En 2017, si bien se

observó una disminución en el uso de reguladores de crecimiento vegetal, de insecticidas y de herbicidas, que mostraron valores de 0.3%, 10.9% y 26.4%, respectivamente, el porcentaje de uso de fungicidas y bactericidas alcanzó la cifra de un 62.4% [6].

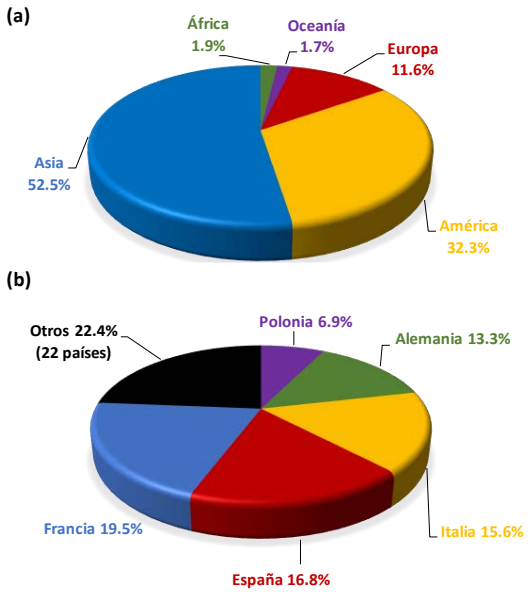


Figura 1.1. Consumo de plaguicidas a nivel (a) continental, (b) Unión Europea. Fuente: Elaborada con base en FAO, 2019 [6].

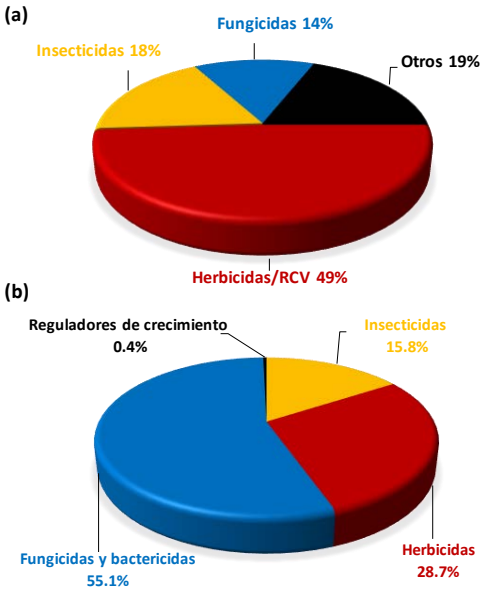


Figura 1.2. Consumo de plaguicidas a nivel (a) mundial, (b) España. Fuente: Elaborado con base en FAO, 2019 [6].

La persistencia y la movilidad son características que determinan el comportamiento de un plaguicida en el medio ambiente y dependen de la matriz ambiental y de las propiedades fisicoquímicas del compuesto (ver **Tabla A1** en el anexo). La persistencia se refiere al tiempo de residencia de una especie química, el cual está sujeto a la degradación o a la remoción física, sea en el suelo, en un cultivo, en un animal o en cualquier otro compartimento ambiental. El parámetro que lo caracteriza es la vida media y se expresa en unidades de tiempo. La movilidad describe la capacidad del compuesto de transportarse en el suelo o sedimento, y se mide a través del coeficiente de adsorción. Estas dos características, junto con el volumen de uso y la toxicidad del compuesto en los organismos que no son el blanco de control, están relacionadas con el riesgo que representa un plaguicida en el medio ambiente [2].

A pesar de los beneficios económicos, de protección de la salud humana y de preservación de los ecosistemas que ofrecen los plaguicidas, la afectación que ocasionan a los recursos hídricos está más que probada. En efecto, los plaguicidas que se aplican en la agricultura, la industria y la salud pública se desplazan por diversos compartimentos ambientales llegando finalmente a alcanzar las aguas superficiales y subterráneas (**Figura 1.3**).

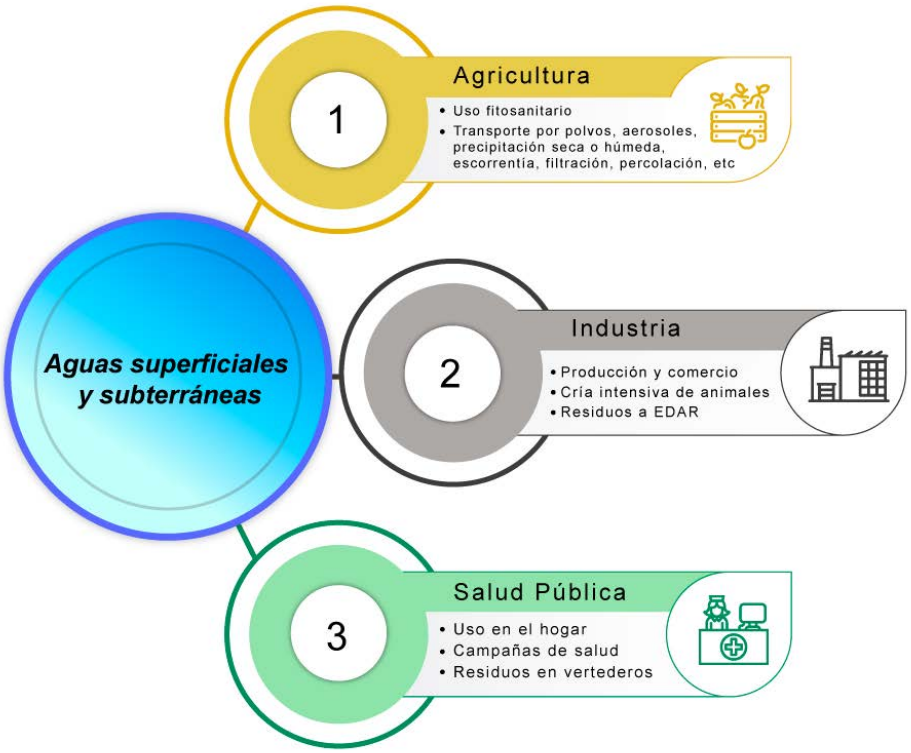


Figura 1.3. Origen de los plaguicidas que afectan el medio acuático.

Aunque la escorrentía superficial de las áreas agrícolas es la principal fuente de contaminación del medio acuático, las EDAR deben considerarse como otra posible fuente de contaminación, quizás menos estudiada, pues sus efluentes aportan una mezcla compleja de plaguicidas y otras sustancias que podrían afectar seriamente a los organismos acuáticos [8]. Y es que los tratamientos convencionales de aguas residuales no siempre eliminan las sustancias tóxicas por completo [9]. Esa remoción pobre, o incluso negativa, puede deberse a la desconjugación de los metabolitos y productos de transformación, así como a la hidrólisis y desorción de los analitos de las partículas orgánicas que tiene lugar durante el tratamiento de las aguas [10].

En cuanto a las aguas subterráneas, los plaguicidas también pueden alcanzar los acuíferos y contaminarlos. La movilización puede producirse desde la superficie, por escorrentía, y alcanzar las aguas subterráneas por drenajes internos y por percolación. Para evaluar el poder contaminante de un compuesto normalmente se utiliza una aproximación empírica o Índice de Riesgo Potencial de Contaminación (GUS), que relaciona el coeficiente de adsorción (criterio de movilidad) y la vida media de los compuestos (criterio de persistencia). Este indicador experimental permite clasificar el potencial de lixiviación de los plaguicidas en las aguas subterráneas [11,12].

La transformación o degradación de los plaguicidas puede seguir procesos bióticos, llevados a cabo por microorganismos o plantas, como procesos abióticos, como la temperatura y reacciones químicas y fotoquímicas. Estos procesos degradan los plaguicidas o los metabolitos y los transforman en uno o más TPs, que podrían ser inofensivos, tanto para los organismos objetivo como para el medio ambiente, o bien, ser más tóxicos y más persistentes que los propios compuestos originales [13].

Son numerosos los trabajos que reportan la presencia de plaguicidas y TPs en el medio acuático. Cabe destacar algunas revisiones recientes que reportan datos de plaguicidas estudiados en aguas continentales de diferentes países. El neocotinoide imidacloprid se ha encontrado en aguas superficiales en concentraciones entre 1.1–105 ng/L [14], y acetamiprid en Texas a un nivel elevado de hasta 225 μg/L [15]. Otros ejemplos encontrados en aguas superficiales serían los herbicidas 2,4-D detectado en España entre 62–207 ng/L [16], atrazina en China ente los 21.28–1726 ng/L [17], y metolaclor a 56 μg/L en Hungría [18]. Por lo que respecta a TPs, en España se han detectado los correspondientes a atrazina (-desetil y -desisopropil) en

un rango de concentración de 0.40–1.33 y 7.33–36.89 ng/L, respectivamente [19]. En el caso de las aguas subterráneas, diazinon se ha detectado en Irán a altos niveles de concentración (0.572 μg/L) [20], metolaclor en Croacia entre 4–100 ng/L [21], bentazona en Nueva Zelanda a 6 μg/L [22], y simazina y clorpirifos en la India, a niveles de 40 μg/L y 0.104–0.623 μg/L, respectivamente [23].

1.1.2. Fármacos

Los fármacos son sustancias que también contribuyen a la contaminación de los recursos hídricos. Un fármaco es una sustancia química de origen natural o sintético que se utiliza para el diagnóstico, cura, mitigación, tratamiento o prevención de enfermedades en el ser humano o en otros animales. Estas sustancias se introducen continuamente al medio ambiente, principalmente a los ecosistemas acuáticos, y a pesar de estar presentes a bajas concentraciones, pueden afectar la calidad del agua y potencialmente al suministro de agua potable, los ecosistemas y la salud humana.

En la **Figura 1.4**, se indica el consumo de medicamentos en España expresado en dosis diarias por cada mil habitantes y por día (DHD). Estos datos arrojan información sobre patrones de uso de medicamentos y tendencias a lo largo del tiempo, y permiten, también, comparar las cifras con datos de otros países. Se observa la tendencia creciente, a lo largo del tiempo, de la DHD, excepto en el caso de los antiinflamatorios no esteroideos y de los antibióticos (**Fig. 1.4**d), donde se observa una clara tendencia decreciente. El caso de los antibióticos es de especial interés, pues la disminución en la tasa de consumo responde a la implementación del primer Plan Nacional frente a la Resistencia a los Antibióticos (PRAN), que se lleva a cabo en España desde 2014. En el mismo período se observa una tendencia decreciente en las ventas de antibióticos veterinarios. Los datos registrados en el PRAN muestran una disminución del 7.2% en el consumo de antibióticos para el período 2015-2018 y una reducción de un 32.4% en las ventas de antibióticos veterinarios para el período 2014-2017, lo cual es alentador, ya que España es uno de los países que más consume antibióticos en la Unión Europea, tanto en el ámbito humano como en el veterinario [24,25].

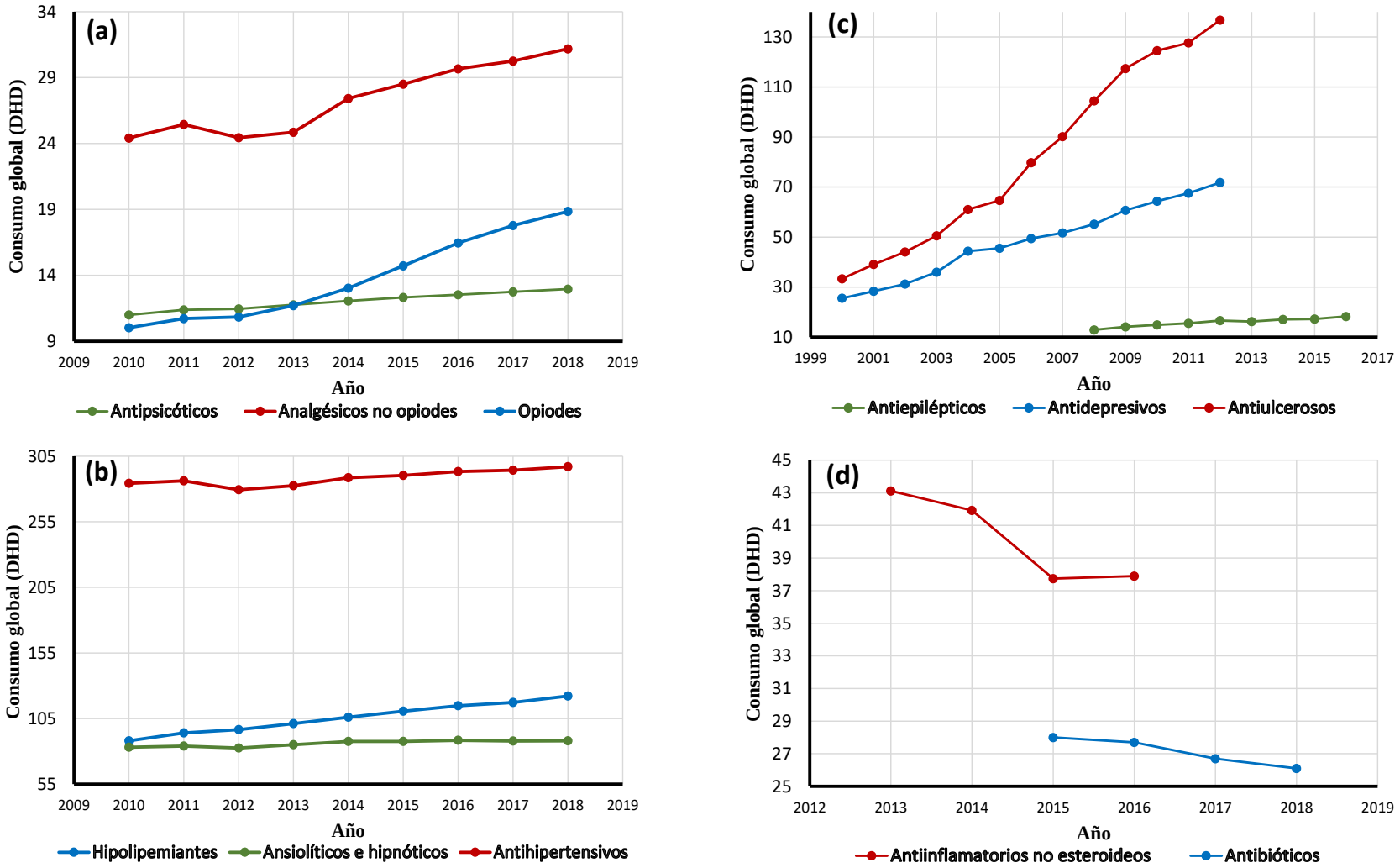


Figura 1.4. Consumo de fármacos en España (a) antipsicóticos, analgésicos no opioides, opioides, b) hipolipemiantes, ansiolíticos e hipnóticos, antihipertensivos, (c) antiepilépticos, antidepresivos, antiulcerosos y (d) antiinflamatorios no esteroideos y antibióticos. Fuente: Elaborada con base en AEMPS, 2019 [24].

Una vez consumido un fármaco, este se excreta a través de las heces y la orina, como una mezcla de metabolitos y la sustancia inalterada, que llega al sistema de alcantarillado y a la EDAR, pudiendo llegar finalmente al medio acuático a través del vertido de los efluentes tratados, si el sistema de tratamiento empleado no es capaz de eliminar completamente estos compuestos. Las aguas residuales se consideran la principal ruta de introducción de los fármacos al medio ambiente donde a su vez pueden sufrir procesos abióticos de degradación, como la fotólisis y la hidrólisis. Otras vías de entrada de fármacos al medio acuático son a través de las aguas de las industrias, de los hospitales y del sector agrario, como se indica en la **Figura 1.5**. Los residuos orgánicos, como el estiércol y los lodos de las depuradoras, se pueden reutilizar como materia prima de fertilizantes o como fuentes de nitrógeno, fósforo y potasio en los campos agrícolas, pero al pasar al suelo contribuyen al esparcimiento de los contaminantes, que pueden ser absorbidos por las plantas y llegar a las aguas superficiales por medio de la escorrentía, o bien hasta los acuíferos subterráneos por percolación [26].

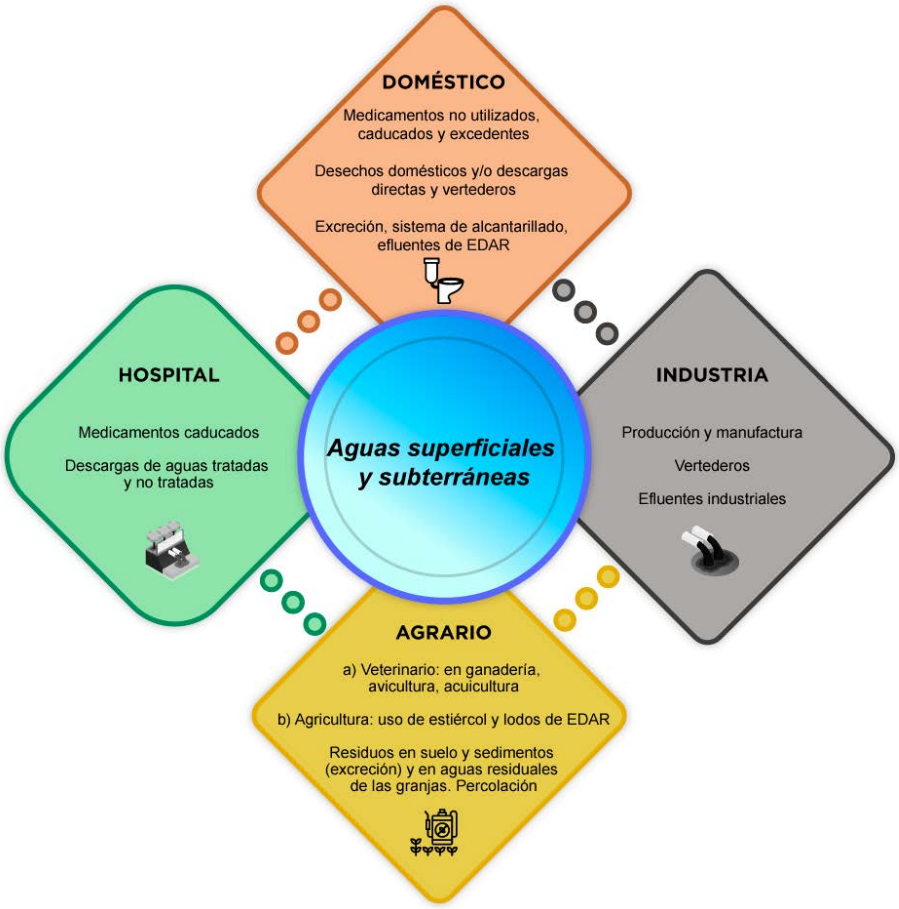


Figura 1.5. Origen de los fármacos que afectan el medio acuático.

Una vez alcanzan las aguas superficiales, los fármacos y sus metabolitos/TPs se pueden incorporar a la cadena alimentaria y tener efectos nocivos en los seres vivos. En general, este tipo de contaminantes tiene una vida media inferior a la de los xenobióticos clásicos, son de naturaleza polar y poco volátiles (ver **Tabla A2** en el anexo), por lo que su persistencia en el medio ambiente no es muy elevada; sin embargo, su uso cotidiano hace que se viertan continuamente al medio ambiente, por lo que algunos autores los consideran contaminantes "pseudo-persistentes" [27–29]. Las concentraciones de fármacos en el medio acuático se encuentran en el orden de los ng/L o µg/L; sin embargo, aún a esos niveles, sus efectos tóxicos pueden ser notables. Los antibióticos, por ejemplo, pueden producir cambios en las bacterias, provocar resistencias y, con ello, problemas de salud pública.

Incluso se han encontrado fármacos en el agua potable. La función de las estaciones de tratamiento de agua potable (ETAP) es eliminar materia orgánica natural y microorganismos, pero los fármacos, al ser moléculas de pequeño tamaño y polares, son muy móviles y difícil de eliminar, por lo que pueden convertirse en un producto de consumo involuntario para el ser humano [30]. A modo de ejemplo, se puede citar que, en una zona de abastecimiento de agua en Francia, se encontró diclofenaco en concentraciones de 56 ng/L, y en análisis de agua mineral realizados en Valencia, España, se encontraron valores de 25 ng/L [31]. Está claro que, ante una exposición crónica, este fármaco puede crear un problema medioambiental para las especies no diana e incluso para los seres humanos.

Numerosos trabajos reportan la presencia de fármacos y sus metabolitos/TPs en el medio acuático. Un estudio llevado a cabo en aguas superficiales de España detectó la presencia de antibióticos de diferentes familias (fluoroquinolonas, macrólidos, nitroimidazoles, quinolonas, sulfonamidas, tetraciclinas y derivados de la trimetoxibenzilpirimidina) a diferentes niveles de concentración entre 3-1195.5 ng/L [32]. En Corea del Sur se llevó a cabo una investigación sobre dos fármacos, el agente betabloqueante atenol y el agente de diagnóstico iopromida, encontrándose a concentraciones de 83 ng/L y de 1013 ng/L, respectivamente [33]. También se han detectado en la India los antiinflamatorios no esteroideos (AINE) diclofenaco, naproxeno, ketoprofeno e ibuprofeno en concentraciones de hasta 0.66 µg/L [34]. En algunos países del Mediterráneo se detectaron los hipolipemiantes atorvastatina, bezafibrato y gemfibrozilo en concentraciones de hasta 27, 15060 y 7780 ng/L, respectivamente [35]. Metabolitos como el

ácido carboxílico de atenolol y de valsartán se encontraron en Alemania en concentraciones de 0.20 y 2.1 μg/L, respectivamente [36].

1.1.3. Legislación

Dada la necesidad de proteger los recursos hídricos y reconociendo el aumento en su demanda, así como la importancia de proporcionar una calidad aceptable del agua y de asegurar que pueda llegar a quienes la necesiten, surgió la obligación de consolidar las acciones relacionadas con la gestión del agua en la Unión Europea. Así, el 23 de octubre de 2000 entró en vigor la ***Directiva 2000/60/CE*** del Parlamento Europeo y del Consejo de la Unión Europea, conocida como la Directiva Marco del Agua (DMA), la cual establece un marco comunitario de actuación en el ámbito de la política de aguas, que busca proteger las aguas superficiales continentales, las aguas de transición, las aguas costeras y las aguas subterráneas. Las medidas anotadas apuntan a garantizar el suministro suficiente de agua superficial o subterránea en buen estado, a reducir de forma significativa la contaminación de las aguas subterráneas, a proteger las aguas territoriales y marinas, y a alcanzar los objetivos de los acuerdos internacionales, incluidos aquellos cuya finalidad es prevenir y erradicar la contaminación del medio ambiente marino.

El 20 de noviembre de 2001 se emitió la ***Decisión 2455/2001/CE*** del Consejo por la que se aprobó una lista de sustancias prioritarias en el ámbito de la política de aguas y por la que se modificó la Directiva 2000/60/CE. La lista la formaron 33 sustancias clasificadas como sustancias prioritarias y como sustancias peligrosas prioritarias, según sus características de toxicidad, persistencia, bioacumulación y una evaluación específica basada en los riesgos y dirigida solamente a la ecotoxicidad acuática y a la toxicidad humana a través del medio acuático.

La ***Directiva 2006/118/CE*** del Parlamento Europeo y del Consejo de 12 de diciembre de 2006 relativa a la protección de las aguas subterráneas contra la contaminación y el deterioro busca establecer normas de calidad y criterios para la evaluación del estado químico de las masas de agua subterránea. Así, las normas de calidad de las aguas subterráneas que se recogen en el Anexo I establecen, para las sustancias activas de los plaguicidas, incluidos los metabolitos y

los productos de degradación y reacción que sean pertinentes, un valor de 0.1 µg/L referido a cada sustancia, y de 0.5 µg/L en total (referido a la suma de todos los plaguicidas).

La aprobación de la ***Directiva 2008/105/CE*** del Consejo de 16 de diciembre de 2008, relativa a las normas de calidad ambiental en el ámbito de la política de aguas, ha supuesto un avance importante en el manejo integral de las aguas. La Directiva establece normas de calidad ambiental (NCA) para las sustancias prioritarias y para otros contaminantes a fin de mejorar el estado químico de las aguas superficiales.

Con la publicación de la ***Directiva 2013/39/CE*** del Consejo de 12 de agosto de 2013, por la que se modifican las Directivas 2000/60/CE y 2008/105/CE, se ha actualizado la lista de sustancias prioritarias existente, dado que se identificaron nuevas sustancias, se establecieron NCA para las sustancias identificadas recientemente, se revisaron las NCA para algunas sustancias existentes en función del progreso científico y se establecieron NCA de la biota para algunas sustancias prioritarias existentes y para las sustancias identificadas recientemente. Finalmente, la lista consta de 45 sustancias o grupos de sustancias (European Parliament and the Council of the European Union, 2008), de las cuales casi la mitad son plaguicidas. En la **Tabla A3** del anexo de la tesis doctoral se muestran los plaguicidas incluidos en la lista de sustancias prioritarias objeto de estudio de la presente tesis doctoral.

Posteriormente, la Comisión Europea se ha encargado de redactar una lista de observación (Watch List) con las sustancias prioritarias que pueden presentar un riesgo significativo para el medio acuático o a través de él, y sobre las cuales deben recabarse datos de seguimiento en toda la Unión Europea. La primera lista de observación se divulgó en la Decisión de Ejecución (UE) 2015/495 de la Comisión, de 20 de marzo de 2015. Durante el primer año de seguimiento de las sustancias recogidas en la primera lista de observación, se procedió a retirar los plaguicidas trialato y oxadiazón, y, en 2018, se incorporó a la nueva lista el insecticida metaflumizona. En el mes de agosto de 2020, se actualizó la lista de observación, sustituyendo los plaguicidas de la lista anterior, excepto el insecticida metaflumizona, e incluyendo siete plaguicidas azólicos y los fungicidas dimoxistrobina y famoxadona, tal y como se observa en la **Tabla 1.1**.

Tabla 1.1. Lista de observación de sustancias a efectos de seguimiento a nivel de la Unión Europea, de conformidad con el artículo 8 ter de la Directiva 2000/60/CE.

Decisión de Ejecución de la Comisión Europea[a]	
2018/840	**2020/1161**
Nombre de la sustancia/grupo de sustancias,	
Plaguicidas	
Metaflumizona Metiocarb	Metaflumizona Dimoxistrobina Famoxadona
Neonicotinoides: Acetamiprid Clotianidina Imidacloprid Tiacloprid Tiametoxam	Compuestos azólicos: Imazalil Ipconazol Metconazol Penconazol Procloraz Tebuconazol Tetraconazol
Fármacos	
Amoxicilina Azitromicina[b] Ciprofloxacino Claritromicina[b] Eritromicina[b]	Amoxicilina Ciprofloxacino Clotrimazol[c] Fluconazol[c] Miconazol[c] Sulfametoxazol Trimetoprim Venlafaxina y O-desmetilvenlafaxina[d]

[a] Se muestran los plaguicidas y fármacos incluidos en la Decisión de Ejecución (UE) 2018/840 y 2020/1161.
[b] Antibiótico macrólido
[c] Compuesto azólico
[d] Metabolito

En cuanto a los fármacos, en la última actualización de 2020 se han retirado de la lista de observación los tres antibióticos macrólidos, y se han incluido los antibióticos sulfametoxazol y trimetoprim, el antidepresivo venlafaxina y su metabolito O-desmetilvenlafaxina, y un grupo de tres productos farmacéuticos azólicos. Esta es la primera ocasión en que se incluye un metabolito en una lista de observación, lo cual refleja la importancia de estudiar este tipo de sustancias en el medio acuático.

A pesar de la gran cantidad de estudios sobre la presencia de fármacos en las aguas y sus efectos sobre diferentes organismos, la legislación actual apenas los contempla y no se han establecido niveles máximos de concentración.

A modo de resumen, se presenta la **Tabla 1.2,** donde se indican las normativas relacionadas con los recursos hídricos, que se han descrito en esta sección.

Tabla 1.2. Legislación de aplicación a los recursos hídricos en el Marco Normativo de la Unión Europea.

Año	Título
2000	Directiva 2000/60/CE del Parlamento Europeo y del Consejo de 23 de octubre de 2000 por la que se establece un marco comunitario de actuación en el ámbito de la política de aguas [37]
2001	Decisión No 2455/2001/CE del Parlamento Europeo y del Consejo de 20 de noviembre de 2001 por la que se aprueba la lista de sustancias prioritarias en el ámbito de la política de aguas, y por la que se modifica la Directiva 2000/60/CE [38]
2006	Directiva 2006/118/CE del Parlamento Europeo y del Consejo de 12 de diciembre de 2006 relativa a la protección de las aguas subterráneas contra la contaminación y el deterioro [39]
2008	Directiva 2008/105/CE deL Parlamento Europeo y del Consejo de 16 de diciembre de 2008 relativa a las normas de calidad ambiental en el ámbito de la política de aguas, por la que se modifican y derogan ulteriormente las Directivas 82/176/CEE, 83/513/CEE, 84/156/CEE, 84/491/CEE y 86/280/CEE del Consejo, y por la que se modifica la Directiva 2000/60/CE [40]
2013	Directiva 2013/39/UE del Parlamento Europeo y del Consejo de 12 de agosto de 2013 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 [41]
2015	Decisión de Ejecución (UE) 2015/495 de la Comisión de 20 de marzo de 2015 por la que se establece una lista de observación de sustancias a efectos de seguimiento a nivel de la Unión en el ámbito de la política de aguas, de conformidad con la Directiva 2008/105/CE del Parlamento Europeo y del Consejo [42]
2018	Decisión de Ejecución (UE) 2018/840 de la Comisión de 5 de junio de 2018 por la que se establece una lista de observación de sustancias a efectos de seguimiento a nivel de la Unión en el ámbito de la política de aguas, de conformidad con la Directiva 2008/105/CE del Parlamento Europeo y del Consejo, y se deroga la Decisión de Ejecución (UE) 2015/495 de la Comisión [43]
2020	Decisión de Ejecución (UE) 2020/1161 de la Comisión de 4 de agosto de 2020 por la que se establece una lista de observación de sustancias a efectos de seguimiento a nivel de la Unión en el ámbito de la política de aguas, de conformidad con la Directiva 2008/105/CE del Parlamento Europeo y del Consejo [44]

1.2. Determinación de plaguicidas y fármacos en aguas

Una gestión adecuada de la problemática ambiental de aguas implica, además de buenas políticas medioambientales, la aplicación de metodologías analíticas capaces de detectar, identificar y cuantificar la presencia de contaminantes de modo fiable.

Dado que los plaguicidas, los fármacos y sus TPs/metabolitos generalmente se encuentran en concentraciones muy bajas en el medio ambiente -del orden de µg/L o ng/L, y algunas sustancias incluso a niveles aún más bajos-, es necesario desarrollar metodologías analíticas avanzadas, lo suficientemente sensibles y selectivas. El diseño de un método capaz de producir resultados fiables en un análisis multirresidual no es una tarea fácil, debido a las diferencias de las propiedades fisicoquímicas de los compuestos y de la variabilidad en la composición de las muestras. Consecuentemente, la determinación de residuos de plaguicidas y fármacos en los diferentes compartimentos ambientales es absolutamente necesaria para tener una visión más completa de la situación, y entender el destino y comportamiento de estos compuestos en el medio ambiente, y proceder a su control periódico.

1.2.1. Tratamiento de muestra

En el diseño de metodología analítica, la etapa de preparación de muestra es crucial para eliminar interferencias y obtener extractos limpios y compatibles con los sistemas instrumentales. Aunque se pueden utilizar diferentes técnicas de extracción en la determinación de plaguicidas y fármacos en muestras acuosas, en esta sección sólo se van a describir la extracción en fase sólida (SPE) y la inyección directa (DI), puesto que son las que se emplearon en los diferentes trabajos experimentales de la presente tesis doctoral.

La extracción en fase sólida (SPE) es una técnica de preparación de muestras que permite separar los analitos de la matriz (gas, sólido o líquido), pudiéndose llevar a cabo en línea (automatizado) o fuera de línea (manual). Esta última es la forma más utilizada y es la que se empleó en esta tesis.

En un procedimiento SPE, la muestra (líquida o gaseosa) se pasa a través de una fase sólida (adsorbente) donde quedan retenidos los analitos. Posteriormente, se añade un disolvente para eluir dichos analitos del cartucho. En ocasiones, también se aplica una etapa de lavado

asegurando que no se pierden los analitos en la misma, y finalmente el eluato obtenido se inyecta en el sistema cromatográfico, bien directamente o bien (generalmente) previa evaporación/pre-concentración y cambio de disolvente. Por lo general, el adsorbente se encuentra en un tubo o cartucho de polipropileno que se encuentra confinado entre dos discos sinterizados o fritas y que tiene un tamaño de poro menor al tamaño de partícula del absorbente. Además, a nivel comercial se dispone de una amplia gama de adsorbentes con diferentes estructuras químicas, lo que permite trabajar con diferentes mecanismos de extracción.

Los materiales adsorbentes pueden ser hidrofóbicos, hidrofílicos, mixtos, de intercambio iónico y de afinidad. Los materiales clásicos disponibles para SPE incluyen C18, carbón negro grafitizado (GCB), copolímeros de N-vinilpirrolidona-divinilbenceno (Oasis HLB), modo mixto/ intercambio catiónico (MCX), modo mixto/intercambio aniónico (MAX), intercambio aniónico débil (WAX) entre otros [45]. Los materiales más comúnmente utilizados en la SPE para el análisis de plaguicidas y fármacos en aguas son los de polímero de balance hidrófilo-lipófilo (HLB), de intercambio aniónico débil (WAX) y de tipo C18 [46,47].

En el caso del cartucho Oasis HLB, se han realizado numerosos estudios en diferentes tipos de aguas. Por ejemplo, en aguas residuales, tanto de influente como de efluente, en un estudio de 44 fármacos de diferentes grupos terapéuticos se obtuvieron valores de recuperación aceptables y límites de cuantificación entre 0.01-30.3 ng/L [48]. De igual forma, en aguas superficiales, el método aplicado para 63 fármacos y metabolitos proporcionó porcentajes de recuperación entre 68% y 134% y una precisión intermedia de 14%. Este tipo de fase polimérica permitió retener compuestos polares y apolares en las muestras. La utilización de Oasis HLB permitió obtener rangos de recuperación aceptables (entre 75% y 125%) en 54% de los compuestos, mientras que con el modo mixto/intercambio catiónico las recuperaciones aceptables alcanzaron solo al 43% de los compuestos [49].

En el caso de los plaguicidas, el desarrollo de un método de screening cuantitativo en aguas superficiales para 252 plaguicidas y sus respectivos TPs/metabolitos confirmó la utilidad de este tipo de fase polimérica [50]. También, se desarrolló y optimizó un método analítico multirresidual para analizar, de manera simultánea, insecticidas, fungicidas y herbicidas, tanto en aguas superficiales como subterráneas. Se obtuvieron mejores resultados de recuperación

con el cartucho HLB que con el cartucho C18 [51]. En aguas residuales, la SPE con cartucho HLB también se utilizó satisfactoriamente para el screening de 279 plaguicidas relevantes [52].

Los cartuchos Oasis HLB se ha usado frecuentemente en metodologías de amplio screening basadas en el uso de la espectrometría de masas de alta resolución, usando de forma complementaria la cromatografía de líquidos acoplada a espectrometría de masas con analizador de tiempo de vuelo y la cromatografía de gases con fuente de ionización a presión atmosférica acoplada a la espectrometría de masas con analizador de tiempo de vuelo, intentando avanzar hacia el deseado, pero inalcanzable, screening universal [53].

Una buena opción para la determinación de plaguicidas y fármacos en aguas usando los modernos instrumentos como los cromatógrafos de líquidos acoplados a espectrometría de masas con analizador de triple cuadrupolo es la inyección directa (DI) de la muestra, lo cual es posible dada la alta sensibilidad y selectividad de los equipos instrumentales actuales. A lo sumo, sólo es necesaria la filtración, centrifugación o un ajuste del pH, antes de inyectar la muestra. En el caso de aguas residuales con alto contenido de carga orgánica, conviene aplicar un paso adicional de centrifugación y dilución. Con la DI, se consume poco o ningún disolvente orgánico, no se necesitan cartuchos de SPE y el tratamiento de la muestra es mínimo, por lo que las pérdidas son pocas y el rendimiento de preparación de muestras muy superior [54–56].

1.3. Técnicas cromatográficas

La calidad de las aguas y su nivel de contaminación han sido objeto de preocupación en los últimos años. Prueba de ello es la proliferación de metodologías analíticas que buscan determinar, de forma fiable y precisa, la presencia de contaminantes en aguas, y evaluar las consecuencias en el medio ambiente y en la salud humana. Entre estas metodologías, destacan la cromatografía de gases (GC) y la cromatografía de líquidos (LC), con distintos sistemas de detección, destacando su acoplamiento a espectrometría de masas. La IUPAC define la cromatografía como un método físico de separación en el cual los componentes a separar se distribuyen entre dos fases, una que permanece estacionaria (fase estacionaria) y otra que se mueve en una dirección definida (fase móvil) [57].

En **GC**, los analitos se separan y se distribuyen entre una fase estacionaria, sólida o líquida, inmóvil e inerte, y una fase móvil, que pasa por la fase estacionaria. La fase móvil o gas portador es la responsable de transportar los analitos a través de la columna cromatográfica y no participa en el mecanismo de retención, siempre que el tamaño de la muestra sea pequeño, la presión de la columna sea baja y se utilicen gases de baja masa molecular [58]. En cuanto a las columnas cromatográficas, en GC normalmente se utilizan columnas capilares abiertas, que suelen tener un diámetro interno de 0.10 mm a 0.53 mm y una longitud de 15 m a 100 m; la fase estacionaria que recubre la pared interna puede ser de diferente naturaleza y polaridad [59].

La cromatografía de gases permite separar los compuestos volátiles y semivolátiles, generalmente de baja polaridad, y hacer análisis cualitativos y cuantitativos, pero los compuestos deben ser termoestables, ya que la muestra se introduce en una cámara de vaporización que generalmente se encuentra entre 230 ºC y 275 ºC. Una vez que la cámara de vaporización ha cumplido su función, independientemente de la forma en que se haya inyectado la muestra, el proceso debe desarrollarse lo más rápidamente posible, sin discriminar compuestos para que la muestra llegue a la columna con la menor dispersión (variabilidad) posible.

En **LC**, la separación física de los compuestos presentes en una muestra se produce en función de su interacción con una fase móvil líquida que fluye a través de una columna, y una fase sólida, que permanece estacionaria. Esta técnica sirve de complemento a la cromatografía de gases, pues, a diferencia de la GC, permite separar compuestos polares, de baja volatilidad y termolábiles.

Según sea la naturaleza de la fase estacionaria, la cromatografía líquida se puede clasificar en fase normal (NPLC), cuando la fase estacionaria es polar y la fase móvil es apolar, o en fase reversa (RPLC), cuando es a la inversa. Normalmente, se trabaja en fase reversa, debido a la diversidad de fases estacionarias, que favorece las interacciones selectivas de los analitos, y a la volatilidad de los disolventes orgánicos que se usan como fases móviles en el acoplamiento de la cromatografía de líquidos con la espectrometría de masas (LC-MS).

La LC también se puede clasificar según el tamaño de partícula de la fase estacionaria. Si se trabaja con tamaños de 3.5 µm a 5 µm, la técnica suele denominarse cromatografía líquida de

alta resolución (HPLC), pero si se trabaja con tamaños inferiores a 2 µm, se le denomina cromatografía líquida de ultra-alta resolución (UHPLC). Cuanto menor sea el tamaño de la partícula, mayores serán la eficacia de la separación, aunque aumenta considerablemente la presión del sistema cromatográfico. Los tiempos de análisis se reducen considerablemente y se produce un aumento notable de la sensibilidad, debido a que los picos cromatográficos son más altos y más estrechos [60].

1.4. La espectrometría de masas

La espectrometría de masas es una técnica que permite ionizar átomos o moléculas que se encuentran en estado gaseoso y separarlos según su relación masa/carga (*m/z*). Esta herramienta brinda información sobre la composición elemental de los analitos, la estructura de las moléculas y las relaciones isotópicas de los átomos.

Los componentes principales de un espectrómetro de masas son la fuente de ionización, el analizador de masas y el detector, como se ilustra en la **Figura 1.6**. Seguidamente, se comentarán algunas de las características más relevantes de las fuentes de ionización y de los analizadores de masas utilizados en la presente tesis doctoral.

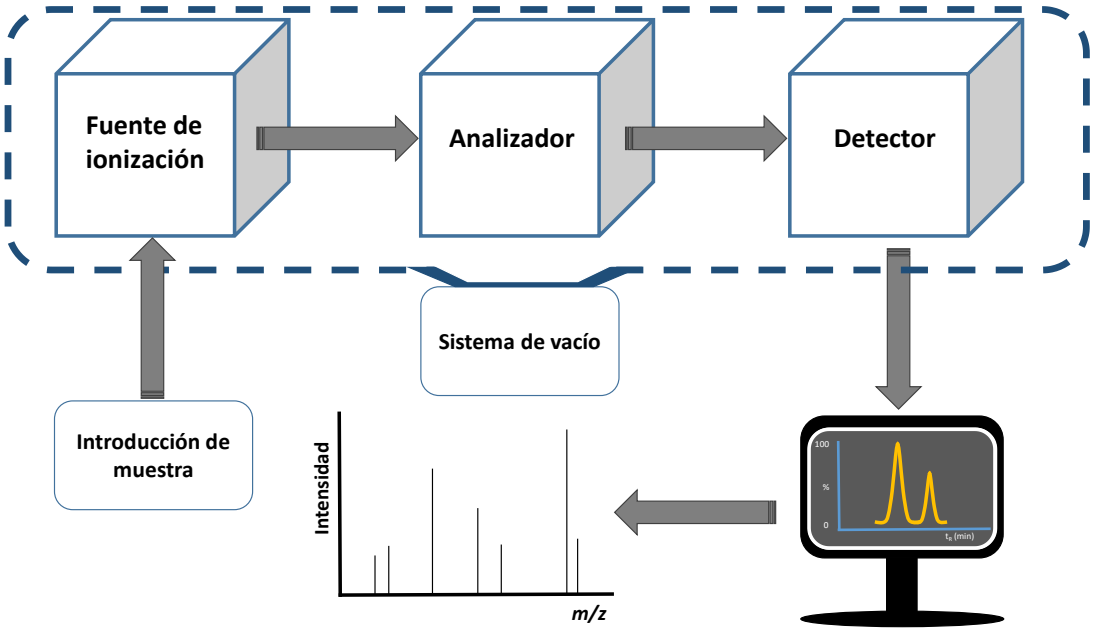


Figura 1.6. Componentes de un espectrómetro de masas.

1.4.1. Fuentes de ionización

Una de las fuentes de ionización más comunes para GC es la ionización electrónica (EI), la cual posee un filamento que emite electrones, los cuales son acelerados —por una diferencia de voltaje eléctrico— hasta adquirir un grado de energía tal (70 eV) que hace que colisionen con las moléculas neutras, que se encuentran en estado gaseoso, para formar iones [61]. La EI ha venido considerándose la fuente de ionización por excelencia en GC por su universalidad, reproducibilidad y por disponer de librerías de espectros comerciales. Sin embargo, con el progreso de la instrumentación analítica, han aparecido otras fuentes de ionización más suaves, de baja energía, como la de ionización química a presión atmosférica (APCI).

En APCI, el gas reactivo (nitrógeno) se ioniza mediante una descarga eléctrica que se produce en una aguja (*pin*) y por efecto corona se genera un plasma. El plasma ioniza los analitos a través de dos mecanismos que ocurren de manera simultánea y que dan lugar a una molécula protonada ($[M+H]^+$) y a un ion molecular ($M^{+\cdot}$) en el espectro de masas [62]. El primero se desencadena por protonación y es un proceso que se ve favorecido por la presencia de humedad o de modificadores próticos en la cámara de ionización; el segundo ocurre por transferencia de cargas, como puede observarse en la **Figura 1.7**.

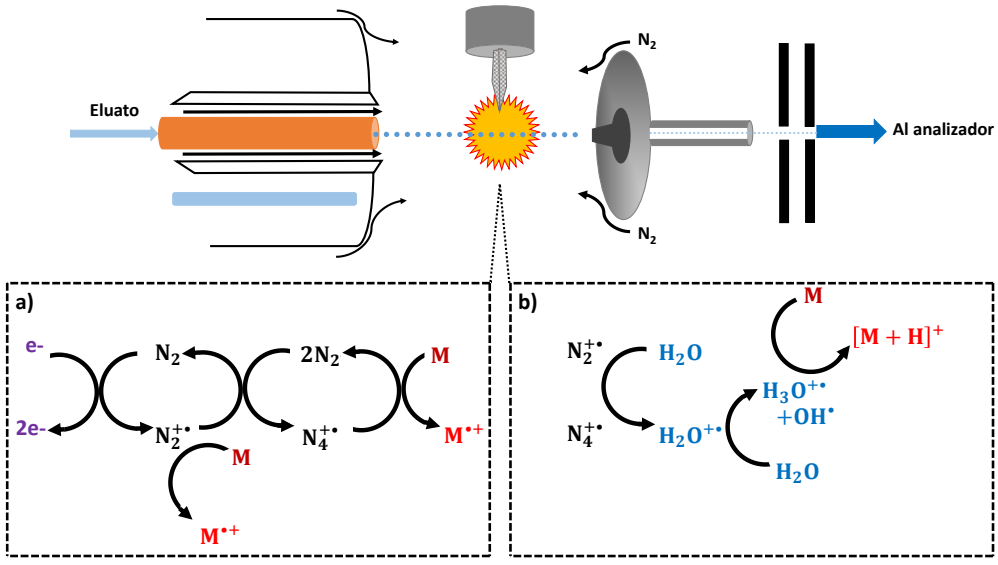


Figura 1.7. Diseño esquemático de una fuente de ionización APCI. En la parte superior las flechas negras indican los diferentes flujos de nitrógeno. En la parte inferior se ilustran los mecanismos de ionización por a) transferencia de carga y b) transferencia de protón. Fuente: Adaptado de Niu et al., 2020 [63] y Portolés et al., 2010 [64].

Aunque en APCI no se dispone de librerías de espectros, resulta una técnica muy sensible, siendo una alternativa para aplicaciones medioambientales de GC, como se verá en el capítulo 2, sección 2.1 de esta tesis doctoral.

En el caso de LC, se suele utilizar la ionización por electrospray, una técnica de ionización suave a presión atmosférica [65]. El efluente de la columna cromatográfica que contiene el analito o una disolución introducida por infusión directa pasa a través de un capilar e ingresa a la cámara de ionización a un voltaje de unos pocos kilovoltios con respecto a las paredes de la cámara. El voltaje crea un campo eléctrico que a su vez provoca la acumulación de cargas sobre las gotas que salen del capilar, dispersándolas, mediante fuerzas de Coulomb, en gotas más pequeñas y con carga (nebulización) (ver **Figura 1.8**). Mediante la aplicación de un gas inerte (generalmente nitrógeno) a alta temperatura, las gotas pierden el disolvente, lo que hace que su diámetro se reduzca y aumente la densidad de carga en la superficie, al punto en que la superficie de la gota no puede soportar más la carga y se alcanza el límite de Rayleigh, donde la repulsión de Coulomb (la fuerza repulsiva) llega a ser del mismo orden que la tensión superficial (la fuerza cohesiva). A la inestabilidad que se produce en la gota debido a su carga se le conoce como la "explosión de Coulomb" y corresponde al momento en que la gota se rompe, produciendo gotas más pequeñas que siguen perdiendo disolvente. En el proceso, las gotas son dirigidas hacia el cono de muestreo de la cámara de ionización aplicando un campo eléctrico. Esta secuencia de sucesos se repite hasta que el radio de curvatura de las gotas llega a ser tan pequeño y el campo eléctrico tan fuerte (por la densidad de carga superficial) que se produce la desorción de los iones de la gota en el gas inerte [66].

Según sea el modo de polaridad seleccionado (positivo o negativo) en la ESI, los espectros de masas serán regidos, ya sea por una molécula protonada o una molécula desprotonada, lo que le otorga al método gran versatilidad para analizar moléculas de un amplio rango de masas moleculares. Además, en los espectros de masas es usual que se formen aductos, que, en general, son poco reproducibles [67]; los más frecuentes son los de sodio, potasio y amonio en modo positivo, y los de acetato, formiato y trifluoroacetato en modo negativo [68]. Todos los trabajos (capítulo 2) de LC-MS que se presentan en esta tesis se realizaron utilizando esta fuente de ionización.

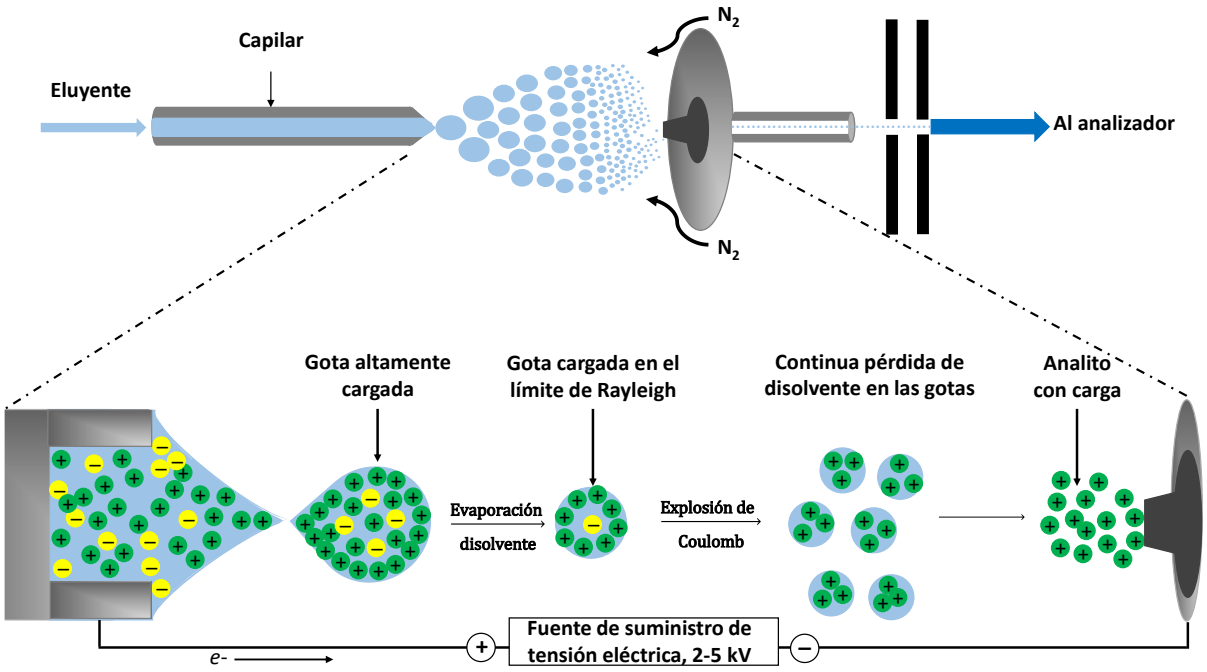


Figura 1.8. Diagrama de una fuente de ionización mediante electrospray. Fuente: Adaptado de Banerjee and Mazumdar, 2012 [69].

1.4.2. Los analizadores de masas

Una vez generados los iones en la fuente de ionización, éstos son dirigidos al analizador de masas que se encarga de su separación en función de su relación *m/z*. Hay diferentes tipos de analizadores, cada uno con sus propias fortalezas y debilidades. A continuación, se van a describir las características más relevantes de los analizadores utilizados en la presente tesis.

1.4.2.1. Triple cuadrupolo (QqQ)

Un analizador QqQ consta de tres cuadrupolos consecutivos: dos cuadrupolos de transmisión, separados por un tercer cuadrupolo en modo de radiofrecuencia (RF), que actúa como una celda de colisión y que normalmente es un hexapolo o un octapolo. Los iones que provienen de la fuente de ionización se seleccionan en el primer cuadrupolo (Q_1), se fragmentan en el segundo (q_2), y los iones producto generados se analizan en el tercero (Q_3), siguiendo una disposición en tándem [70] (**Figura 1.9**).

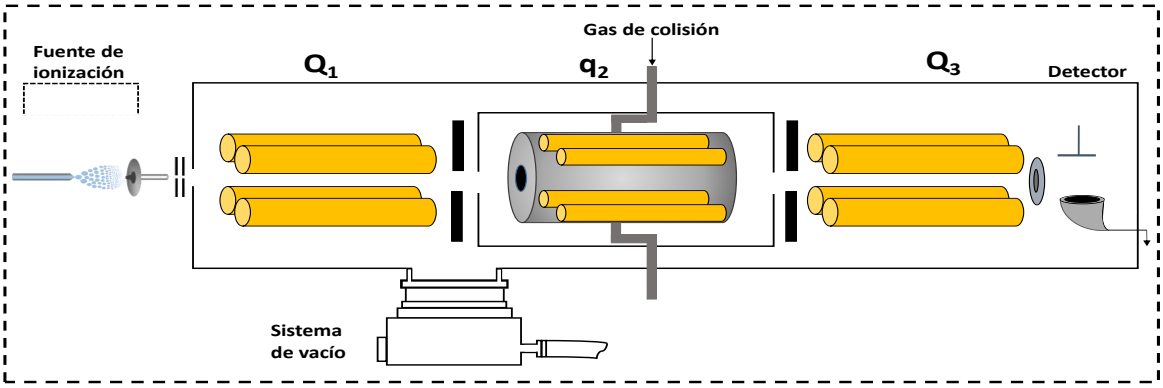


Figura 1.9. Diagrama de un analizador de triple cuadrupolo. Fuente: Adaptado de Picó et al., 2004 [71].

La fragmentación que tiene lugar en q_2 se conoce como disociación inducida por colisión (CID) y es el resultado de la colisión de los iones con un gas inerte, por lo general argón, helio o nitrógeno. En este proceso, los iones precursores originados en la fuente de ionización pasan por el primer quadrupolo (Q_1) cargados de energía cinética, chocan con las moléculas del gas inerte que se encuentra en la celda de colisión y, durante el choque, se produce una transferencia de energía. Parte de esta energía se transforma en energía interna que genera una ruptura y un reordenamiento de enlaces químicos y la consiguiente fragmentación del ion precursor. A estos fragmentos se les llama iones producto. Estos iones pasan al tercer cuadrupolo (Q_3) hasta llegar al detector [72].

El analizador QqQ ofrece la posibilidad, entre otras, de trabajar en modo SRM (selected reaction monitoring), altamente adecuado para análisis cuantitativo debido a su elevada sensibilidad y selectividad [73]. Para optimizar las transiciones SRM, que consisten en pares predefinidos de iones precursores e iones producto, se llevan a cabo experiencias como las presentadas en la **Figura 1.10**. En primer lugar, se realiza un barrido (*full scan*) donde los iones procedentes de la fuente de ionización atraviesan los dos primeros cuadrupolos (transmisión total de iones). Q_1 y q_2 deben estar en modo RF para que no se produzca una disociación inducida por colisión (CID). Todos los iones que se producen en la fuente de ionización se dejan pasar por un intervalo específico al Q_3 hasta llegar al detector. Durante este proceso se puede optimizar el voltaje del cono de muestreo variando el voltaje eléctrico. El escaneo total revela un espectro de masas en tres dimensiones: tiempo de retención, relación *m/z* y

abundancia. A partir de esa información se selecciona el ion precursor, que en LC corresponde generalmente a la molécula protonada, $[M+H]^+$, o a la molécula desprotonada $[M-H]^-$.

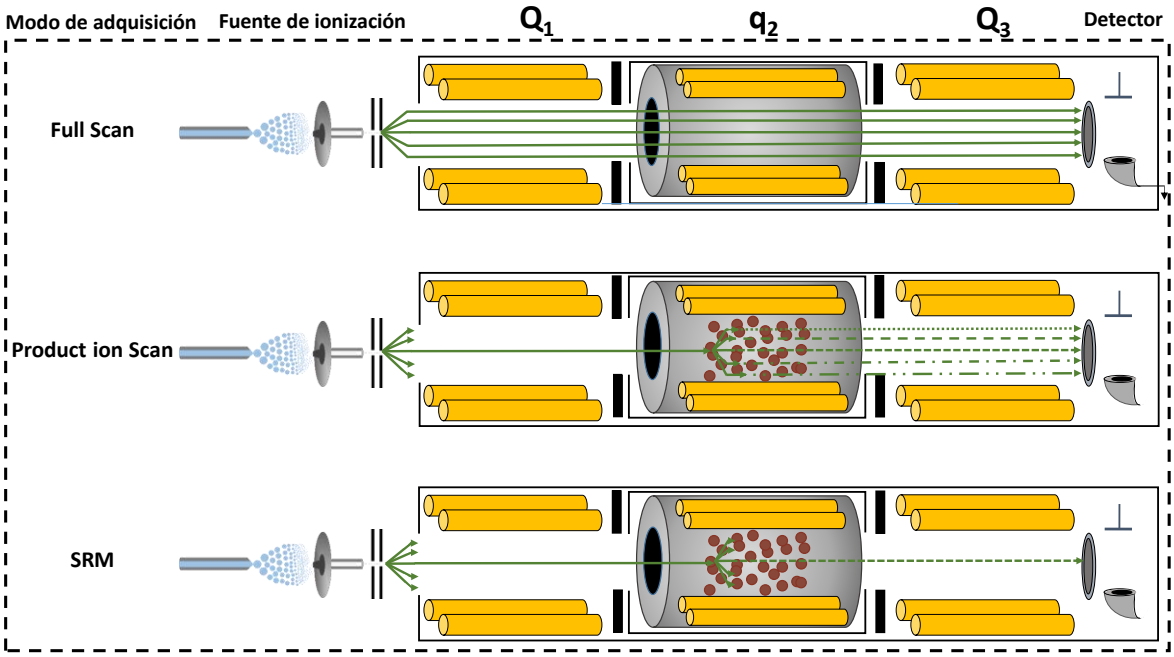


Figura 1.10. Representación esquemática de varios tipos de experimentos de espectrometría de masas en tándem. Fuente: Adaptada de de Hoffmann, 1996 [74].

A continuación, se realiza una segunda experiencia en modo escaneo de iones producto (*product ion scan*). En este caso, solo los iones precursores previamente seleccionados se dejan pasar al Q_1, donde se trabaja en modo SIM; en el q_2, se trabaja en modo CID y a diferentes energías de colisión, y en el Q_3, se trabaja en modo escaneo total y en un intervalo de masas previamente definido. Los fragmentos que se producen a partir de los iones precursores son los iones producto. Así se obtiene el típico espectro de fragmentación de masas en tándem (MS/MS). Por lo general, se seleccionan de 2 a 3 iones producto (con su respectiva energía de colisión), de preferencia los más abundantes y/o selectivos, para lograr una mayor sensibilidad y especificidad.

En modo de trabajo SRM, se elige, por tanto, el ion precursor para cada analito seleccionado en modo SIM en Q_1, los 2 o 3 iones producto, seleccionados en Q_3 en modo SIM, y la energía de colisión en q_2 para cada par. A cada par de iones específico con un valor de *m/z* asociado a los respectivos iones precursor y producto se le suele denominar “transición”.

Una de las ventajas de trabajar en modo SRM, con respecto al modo SIM clásico, es la especificidad y mayor sensibilidad, ya que se reducen considerablemente las interferencias y se incrementa la relación señal/ruido (S/N). El modo de trabajo SRM es el preferido para el análisis cuantitativo, por su sensibilidad, su amplio intervalo de respuesta lineal y los bajos límites de detección. Además, tiene muy buena selectividad, derivada de la selección de varias "transiciones". Esto facilita la confirmación de la identidad de los analitos de las muestras ambientales o de otras áreas de trabajo; sin embargo, tiene la limitación de que solo se pueden medir las "transiciones" previamente seleccionadas (en modo *target*), lo que deja por fuera cualquier otro compuesto para el que no se hayan adquirido sus transiciones y que también podría estar presente en las muestras. En los artículos científicos II, III y IV de esta tesis, se empleó el analizador de triple cuadrupolo para la determinación cuantitativa de los contaminantes considerados.

1.4.2.2. Cuadrupolo – tiempo de vuelo (QTOF)

El analizador de masas QTOF es un sistema híbrido compuesto por dos analizadores, un cuadrupolo (Q) y un tiempo de vuelo (TOF), tal y como se muestra en la **Figura 1.11**. El TOF es un analizador que mide el tiempo que tardan los iones en pasar por un tubo de vuelo de longitud conocida que se encuentra al vacío (~10^{-5} Pa). En un TOF con simetría ortogonal (o-TOF) los iones provenientes de la fuente de ionización ingresan al analizador acelerados ortogonalmente mediante la aplicación de un pulso de voltaje eléctrico [75,76]. Todos los iones salen al mismo tiempo y poseen la misma energía cinética. Como no todos los iones llegan al mismo tiempo, hay que aplicar varios pulsos de voltaje para 'obligar' al siguiente paquete de iones a llegar de forma sincrónica al detector [77].

Además del pulsador de iones, los sistemas o-TOF tienen reflectrones o "espejos iónicos", situados en el tubo de vuelo. Este dispositivo está compuesto por una serie de electrodos que producen un campo eléctrico para compensar la propagación de la energía inicial de los iones [78]. Los iones con mayor energía viajan proporcionalmente más adentro del reflectrón antes de reflejarse y la distancia recorrida hasta el detector es ligeramente mayor. A los iones con menos energía les ocurre el efecto contrario. Por lo tanto, en general, el tiempo de llegada de los iones al detector muestra una mayor tolerancia a los efectos de su propagación de energía inicial, lo

que permite compensar las diferencias de velocidad causadas por la diferencia de energía restante entre los iones acelerados.

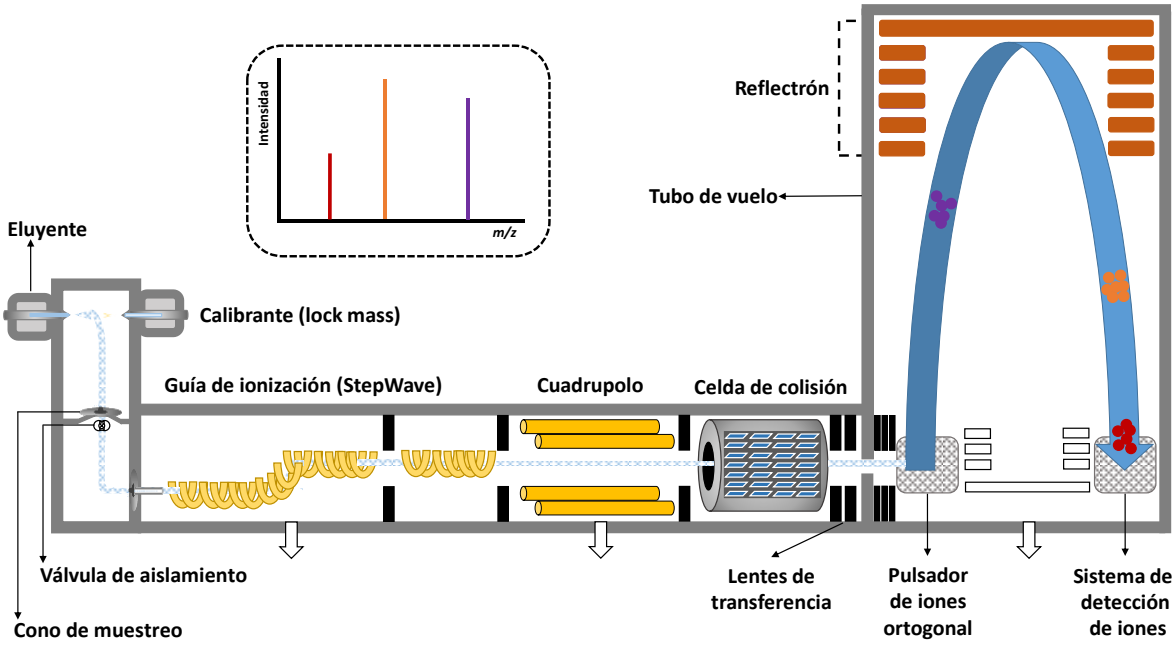


Figura 1.11. Esquema de un sistema híbrido QTOF. Fuente: Adaptado de Wang Alelyunas et al., 2015 [79].

El analizador híbrido QTOF ofrece la ventaja de trabajar en modo *data independent acquisition* (DIA), que en equipos como los utilizados en esta tesis, del fabricante Waters, se conoce como MS^E. Considerando que el modo de adquisición del TOF siempre es mediante barrido de iones (*full spectrum*), las diferentes maneras de adquisición dependerán de la forma en que se configuren el modo de trabajo del cuadrupolo y el de la celda de colisión. Cuando el cuadrupolo y la celda de colisión permiten la transmisión de todos los iones, la adquisición se hace mediante un barrido de iones (*full spectrum*). La elevada exactitud de masa, que reduce considerablemente la cantidad de posibles combinaciones de composiciones elementales, sumada a la alta resolución que tiene el instrumento, hacen que esta forma de adquisición sea muy utilizada en el análisis no dirigido (*non target*).

Finalmente, en el modo de adquisición MS^E, el cuadrupolo se utiliza en modo RF para la transmisión de los iones precursores, y en la celda de colisión se pueden transmitir los iones (función de baja energía, LE) y, a su vez, aplicar distintos valores de voltaje (función de alta

energía, HE) [80]. En el primer caso, al aplicarse baja energía, apenas existe fragmentación de los iones, con lo que en el espectro LE predomina la molécula (des)protonada, y, en ocasiones, algún aducto, mientras que en el segundo se favorece la fragmentación originando diversos iones fragmento, lo que es de gran utilidad para la identificación de los compuestos. Esta forma de obtención de información es de gran utilidad en estrategias de screening y además permite realizar análisis retrospectivo, pudiendo evaluar los datos obtenidos en cualquier momento, incluso años después de haber realizado los análisis, sin tener que volver a medir las muestras.

En los todos artículos científicos de esta tesis se utilizó el analizador QTOF en modo de adquisición MS^E.

1.4.3. Acoplamiento cromatografía- espectrometría de masas

El acoplamiento de las técnicas cromatográficas con la espectrometría de masas (GC-MS y LC-MS) ha mostrado ser de gran utilidad en los métodos analíticos desarrollados para la determinación de contaminantes en muestras acuáticas. El uso de analizadores como QqQ permite, sin lugar a dudas, una excelente sensibilidad para la cuantificación de compuestos target. Sin embargo, muchas veces resulta necesario ampliar el alcance de la detección a otros compuestos que no han sido incluidos en los métodos target iniciales y tener así un reflejo más fiel de la realidad ambiental. En este sentido, la utilización de la espectrometría de masas de alta resolución (HRMS) ofrece la posibilidad de adquirir un espectro de masas completo con medidas de masa exacta, tiene buena sensibilidad y no hace falta seleccionar los analitos previamente a la medida analítica [81].

El acoplamiento LC-HRMS y GC-HRMS con analizador híbrido QTOF ha demostrado ser una herramienta avanzada que permite investigar un gran número de compuestos en una única inyección y permite realizar análisis retrospectivos, es decir, buscar otros compuestos en cualquier momento, sin tener que volver a inyectar las muestras. También se pueden hacer búsquedas de contaminantes sin tener que acudir a los estándares de referencia, logrando una identificación tentativa del analito altamente fiable sobre la base de la información contenida en el espectro de masas de alta resolución. Se pueden establecer amplias listas de compuestos a investigar (*suspect screening*), lo cual facilita las búsquedas, usando bases de datos

elaboradas por el propio laboratorio (home-made) tanto para métodos basados en GC-HRMS como en LC-HRMS. La aplicación de métodos de amplio screening facilita la identificación de muchos más compuestos en las muestras, lo que resulta muy útil para definir compuestos prioritarios que luego se pueden incorporar a metodologías target del tipo GC- o LC-MS/MS QqQ. Por ello, HRMS cumple un papel significativo en la detección de contaminantes emergentes en campos como la contaminación de las aguas medioambientales [53].

El acoplamiento cromatografía-espectrometría de masas es actualmente la aproximación más poderosa para la investigación de plaguicidas, fármacos y TPs/metabolitos en aguas. Por ejemplo, un estudio en la cuenca baja del río Llobregat, en Barcelona, España, empleó las técnicas de GC-MS y LC-MS/MS para determinar la presencia de plaguicidas y TPs tanto en aguas superficiales como subterráneas y residuales. Los resultados arrojaron la presencia de 102 analitos [82].

De igual forma, se buscó detectar, de manera sistemática la presencia de plaguicidas y otros contaminantes orgánicos en 35 muestras de agua superficial y 28 de aguas residuales (14 de influente y 14 de efluente). Esta tarea se completó combinando LC-MS/MS QqQ y LC-QTOF MS. El uso de HRMS permitió detectar 5 plaguicidas y 3 productos de degradación diferentes a los seleccionados en el método por triple cuadrupolo. La combinación de LC-MS/MS QqQ y UHPLC-QTOF MS demostró ser una forma factible y eficiente para la determinación sistemática de los analitos estudiados [83].

Este tipo de acoplamiento (LC-HRMS) también se ha utilizado para detectar fármacos y TPs/metabolitos en muestras de aguas superficiales en España y en muestras de aguas residuales (de influente y efluente) en España e Italia. La instrumentación utilizada permitió detectar, y confirmar, numerosos contaminantes presentes a bajas concentraciones. En total, se detectaron e identificaron 28 compuestos, entre los cuales el irbesartán y el valsartán fueron los más comúnmente encontrados en todas las muestras [84].

1.5. Criterios de confirmación de la identidad de los compuestos detectados

La identificación fiable de los analitos es esencial para evitar falsos positivos y falsos negativos. La guía europea de procedimientos de control de la calidad analítica y de validación para análisis de residuos de plaguicidas en alimentos y piensos indica [85], en el apéndice E, que "las técnicas de espectrometría de masas son a menudo el enfoque más práctico y menos equívoco para la confirmación". La identificación se basa en la selección correcta de iones. Los requisitos que se aplican para confirmar la identidad de un compuesto mediante HRMS establecen que deben observarse, como mínimo, dos iones, con una exactitud de masa menor o igual a 5 ppm, incluyendo, preferiblemente, el ion molecular, la molécula protonada y/o desprotonada, o un aducto, y al menos un ion fragmento. Además, según la guía, "la relación señal/ruido debe ser mayor o igual a 3, y los picos de los iones precursores y/o producto se deben superponer completamente en el cromatograma del ion extraído (XIC)".
Cuando se utiliza el analizador de triple cuadrupolo en modo SRM, se necesitan al menos dos iones producto, lo que implica la adqusición de, como mínimo, dos transiciones MS/MS. Si se obtiene suficiente sensibilidad y selectividad para ambos iones, y las respuestas se encuentran dentro del ámbito lineal y son concluyentes, la relación iónica de los analitos de la muestra debe estar dentro de ±30%, calculado a partir del promedio de los estándares de calibración de la misma secuencia de inyección. En caso de utilizar estándares en matriz, en lugar de disolvente, para calcular el valor de la relación iónica de referencia, es imprescindible eliminar cualquier interferencia, pues un nivel de tolerancia alto puede conducir a la obtención de falsos positivos y un nivel de tolerancia bajo a la obtención de falsos negativos.

El único requerimiento cromatográfico, tanto para GC como para LC, es que el tiempo de retención del analito en el extracto debe corresponder al del estándar de calibración, con una tolerancia de ±0.1 min. Una desviación mayor es aceptable, si el tiempo de retención y la forma del pico coinciden con los de un estándar interno marcado isotópicamente (ILIS) o se dispone de pruebas documentadas de estudios previos de validación de la metodología.

1.6. Evaluación del riesgo ecológico

Como se ha mencionado anteriormente, el uso frecuente de plaguicidas y fármacos ocasiona efectos no deseados en los compartimentos ambientales, pues estos productos pueden resultar perjudiciales para los organismos o diferentes especies que los habitan, y pueden provocar un riesgo ecológico. Se entiende como daño medioambiental a los "los daños a las especies y hábitats naturales protegidos, es decir, cualquier daño que produzca efectos adversos significativos en la posibilidad de alcanzar o de mantener el estado favorable de conservación de dichos hábitats o especies" [86].

La existencia de daños medioambientales o la amenaza inminente de que se produzcan, hace necesaria la realización de estudios de evaluación del riesgo ecológico, cuyo objetivo calcular la probabilidad de que se den efectos adversos para las comunidades de especies en lugares potencialmente expuestos a contaminantes y otras sustancias [87].

Algunos de los métodos para la caracterización de los riesgos ambientales en muestras de agua se basan en el cálculo de los cocientes de riesgo (RQ) [88], las unidades de toxicidad (TU), y las distribuciones de sensibilidad de las especies (SSD) [89].

El RQ es la relación entre la estimación de exposición y la estimación puntual de efectos. La Agencia Europea de Medicamentos (EMA), establece en la guía de evaluación del riesgo medioambiental por medicamentos de uso humano que el RQ se calcula como sigue [90]:

$$\mathrm{RQ} = \frac{\mathrm{PEC}}{\mathrm{PNEC}} \qquad (1)$$

donde el PEC se refiere a la concentración esperada en el ambiente y el PNEC es la concentración esperada sin efecto o que se considera segura en el medio ambiente. Una relación PEC/PNEC < 1 indica que no se espera ningún riesgo ambiental [91].

La TU se basa en la suma de la contribución tóxica individual ejercida por cada compuesto en una especie de prueba estándar determinada, asumiendo un modelo de concentraciones aditivas [92]. La TU_i calculada para una especie en una muestra determinada se define como la relación

entre la concentración medida (c_i) de un compuesto i en la muestra y su toxicidad aguda ($EC50_i$) [93]. Bajo el supuesto del modelo de concentraciones aditivas, la medida de toxicidad agregada se obtiene mediante la adición de los diferentes compuestos presentes en la muestra. Así, las TU totales para la mezcla de compuestos en la muestra se calcula a partir de la siguiente ecuación [94]:

$$\mathrm{TU}_{Total} = \sum_{i=1}^{n} \frac{c_i}{\mathrm{EC50}_i} \qquad (2)$$

La SSD, por su parte, describe la variación en la sensibilidad de las especies a una sustancia tóxica en particular. Se trata de una curva que ajusta los datos de toxicidad de ciertas especies relevantes a una distribución de probabilidad acumulada. El concepto de SSD se puede usar en la evaluación de riesgos para calcular la fracción de especies que podrían resultar afectadas (PAF) por la exposición a un contaminante ambiental y también para derivar estándares de calidad ambiental (EQS), como el HC5, que corresponde a la concentración de no-efecto para el 95% de las especies o, dicho de otra manera, la concentración a la cual un 5% de las especies podrían estar en peligro. La SSD se recomienda para evaluar los riesgos de toxicidad de sustancias individuales, pero en el medio ambiente las sustancias normalmente se encuentran formando mezclas. La evaluación del riesgo, entonces, debe tener en cuenta la toxicidad conjunta de los compuestos [95].

1.7. Trabajo realizado en esta tesis doctoral

En la presente tesis doctoral se ha desarrollado metodología analítica para la determinación de contaminantes ambientales (fármacos, plaguicidas y TPs) en diferentes tipos de aguas (superficial, subterránea y residual). Después de un tratamiento de muestra basado en SPE y/o DI, la determinación analítica ha sido llevada a cabo utilizando diferentes acoplamientos cromatografía-MS con analizadores de baja resolución (LC-MS/MS (QqQ)), así como analizadores de alta resolución (LC-QTOF MS y GC-QTOF MS).

En primer lugar, se llevó a cabo una investigación de los posibles plaguicidas y TPs presentes en la Cuenca Hidrográfica del río Júcar (capítulo 2, sección 2.1.2). Los resultados obtenidos de las muestras analizadas mostraron que algunos compuestos presentaban niveles de concentración superiores a 0.1 μg/L. Si bien estos resultados se basan en la detección de amplio alcance por LC-QTOF MS, para una identificación fiable fue necesario confirmar los niveles de concentración mediante una técnica cuantitativa. El trabajo realizado en esta parte de la tesis permitió la publicación del **artículo científico I**.

Seguidamente, se efectuaron tres campañas de muestreo (entre junio 2018 y febrero 2019) en el río Mijares, situado en la zona Mediterránea del Este de España, para investigar la presencia de plaguicidas y TPs (capítulo 2, sección 2.1.3). Después de un cribado (*screening*) preliminar para identificar los plaguicidas más relevantes, las muestras de agua superficial se analizaron cuantitativamente mediante LC-MS/MS (QqQ) para la determinación de un número determinado de plaguicidas. Este trabajo ha permitido la redacción del **artículo científico II** en colaboración con Dr. Andreu Rico (IMDEA Water Institute, Madrid, España) que ha participado activamente en la evaluación del riesgo en el ecosistema acuático de los plaguicidas encontrados en las muestras analizadas.

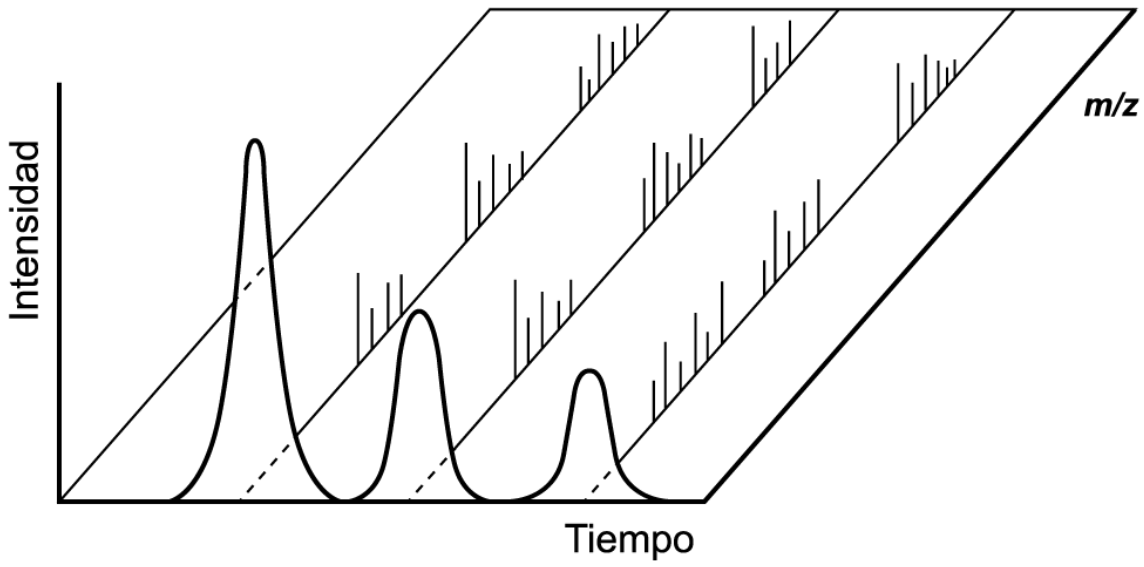
A partir de las muestras tomadas del río Mijares, también se hizo un estudio sobre la presencia de productos farmacéuticos y los riesgos ecológicos que esto conlleva (capítulo 2, sección 2.2.2). Se investigó una amplia lista de fármacos utilizando un método cuantitativo dirigido (tipo *target*) y se complementó mediante un cribado para identificar la presencia de otros fármacos no estudiados, así como sus principales metabolitos. Las experiencias de este trabajo se reflejan en el **artículo científico III**, en el que también se ha contado con la colaboración del Dr. Andreu Rico quien ha evaluado los riesgos ecológicos que suponen estas mezclas farmacéuticas utilizando el modelo de fracción potencialmente afectada por múltiples sustancias (ms-PAF).

La tesis doctoral finaliza con un estudio en el que se evaluó la presencia de residuos farmacéuticos en una EDAR convencional situada en Asturias (capítulo 2, sección 2.3.2). Se recolectaron muestras compuestas de 24 h durante una semana, tanto del influente como del efluente de la estación depuradora, a lo largo de tres campañas de monitoreo distribuidas a lo largo de un año. Además, en el primer muestreo se recolectaron 7 muestras de descarga de un

hospital cercano a la EDAR. El objetivo fue el estudio de la eficiencia de eliminación y la variación estacional de los fármacos encontrados, así como el impacto de la entrada de las aguas residuales de un hospital. La calidad de los efluentes de las plantas de tratamiento de aguas residuales es vital, ya que estas aguas son una de las principales fuentes de contaminación del medio ambiente acuático. El trabajo realizado en esta parte de la tesis permitió la publicación del **artículo científico IV**.

CAPÍTULO 2

INVESTIGACIÓN DE PLAGUICIDAS, FÁRMACOS Y PRODUCTOS DE DEGRADACIÓN EN AGUAS SUPERFICIALES, SUBTERRÁNEAS Y RESIDUALES MEDIANTE EL USO DE ESPECTROMETRÍA DE MASAS CON ANALIZADOR HÍBRIDO CUADRUPOLO-TIEMPO DE VUELO Y TRIPLE CUADRUPOLO



2.1. INVESTIGACIÓN DE PLAGUICIDAS Y PRODUCTOS DE TRANSFORMACIÓN EN AGUAS SUPERFICIALES Y SUBTERRÁNEAS MEDIANTE LC-QTOF MS, GC-QTOF MS Y LC-MS/MS QqQ

2.1.1. Introducción

2.1.2. Artículo científico I

Investigation of pesticides and their transformation products in the Júcar River Hydrographical Basin (Spain) by wide-scope high-resolution mass spectrometry screening

Environmental Research, 177 (2019) 108570

2.1.3. Artículo científico II

Ecological risk assessment of pesticides in the Mijares River (eastern Spain) impacted by citrus production using wide-scope screening and target quantitative analysis.

Journal of Hazardous Materials 412 (2021) 125277

2.1.4. Discusión de resultados

2.1.1. Introducción

Tal y como se mencionó en la introducción general de esta tesis, el uso de plaguicidas trae consigo numerosas ventajas socioeconómicas, pero también puede afectar seriamente al medio ambiente y a la salud humana. Los plaguicidas sirven, sobre todo, para prevenir el crecimiento de organismos no deseados y la propagación de plagas agrícolas.

Ahora bien, cuando se desarrolla un plaguicida se busca combatir ciertos organismos específicos, pero muchas veces se termina afectando a otros organismos, incluidos los seres humanos. Los reportes de problemas de salud y de fallecimientos, producto de malas prácticas ocupacionales, así como de envenenamientos accidentales o intencionales, son un reflejo de los efectos tóxicos, agudos o crónicos, de muchos plaguicidas [96,97].

También importa señalar que el uso de plaguicidas se ha venido incrementando paulatinamente, conforme aumenta la demanda mundial de alimentos y conforme aumenta, también, el interés de los productores por disminuir las pérdidas y mejorar las utilidades.

En esta sección se reúnen los resultados de un primer estudio realizado de masas de aguas pertenecientes a tres comunidades autónomas. En concreto, en el primer estudio se recolectaron 8 muestras de agua superficial y 11 de agua subterránea pertenecientes a la Comunidad Valenciana y de Castilla-La Mancha, y en el segundo estudio 57 muestras del río Mijares. La Comunidad Valenciana, región española situada en el litoral del mar Mediterráneo y principal área de estudio de la presente tesis, está recorrida por varios ríos y tiene una superficie total de cultivo y aprovechamiento del suelo de 2.326.198 ha [98]. Cabe mencionar que parte del área de estudio se encuentra fuera de esta comunidad pues también se muestreó la fuente del río Mijares. En efecto, este río recorre dos comunidades autónomas, pues nace en la Sierra de Gudár, en la provincia de Teruel, en la comunidad autónoma de Aragón y desemboca en la provincia de Castellón, ciudad al norte de la Comunidad Valenciana [99]. De los ríos investigados, los que corresponden a la Comunidad Valenciana presentan una relevancia mayor en cuanto a plaguicidas por cuanto esta comunidad tiene una superficie total de cultivo y aprovechamiento del suelo de 2.326.198 ha [98]. Según la Encuesta sobre superficies y rendimientos de cultivos en 2019 la Comunidad Valenciana presentaba un total de tierra cultivada, por secano, regadío o invernadero, de 640.982 ha. Los principales cultivos fueron:

cítricos (naranjo, mandarino, limonero), frutales no cítricos (granado, melocotonero, albaricoquero), viñedos para producir uvas para vino de mesa y pasas, y olivares de aceituna de almazara y de mesa [100]. Tal y como se observa en la **Figura 2.1**, la mayor parte de la superficie (49.2%) la ocuparon los cítricos y los frutales no cítricos [98].

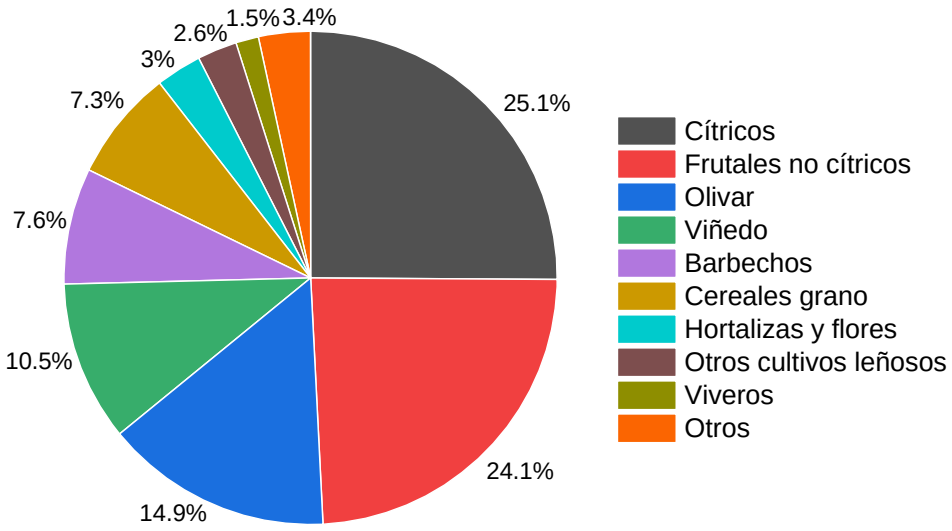


Figura 2.1. Distribución relativa de la superficie de cultivo en la Comunidad Valenciana en 2019. Fuente: Elaborada con base en ESYRCE, 2019 [98].

La intensa actividad agrícola que tiene lugar en esta comunidad y la diversidad de productos que se comercializan parecen ir de la mano con el uso de plaguicidas. Así, en el caso de los cítricos, se suelen aplicar compuestos como acetamiprid, deltametrina, fosetil-aluminio, lambda cihalotrina y piriproxifeno (todos debidamente autorizados) y plaguicidas para el tratamiento poscosecha, como imazalil, tiabendazol, metil-tiofanato, pirimetanilo, tebuconazol y 2-fenilfenol [101].

Una vez en el suelo, los plaguicidas pueden trasladarse por arrastre superficial (escurrimiento), volatilización o erosión. Además, cuando se encuentran en la parte superficial de la corteza terrestre, pueden trasladarse mediante procesos como adsorción, desorción, percolación y vaporización. Las propiedades fisicoquímicas del plaguicida son determinantes, pues, dependiendo de las características de cada sustancia, pueden llegar a las aguas subterráneas. Las aguas superficiales, por su parte, pueden verse afectadas por lo que se denomina

contaminación difusa (deriva, erosión y escorrentía) o por acciones puntuales, como la disposición inapropiada de los envases vacíos de plaguicidas, las aguas sobrantes del lavado de los equipos (pulverizadores y atomizadores) y por el manejo incorrecto de productos agrícolas que contienen residuos tóxicos [2]. La mayoría de los plaguicidas se transforman, mediante procesos físicos, químicos y biológicos, en uno o más productos de transformación (TPs) y estas sustancias se distribuyen a través del suelo, de las aguas continentales, de los sedimentos y del aire. Tanto los plaguicidas como los TPs pueden ser tóxicos para los organismos presentes en cada compartimento ambiental [102].

Con el fin de velar por la calidad del agua, la Directiva Marco del Agua dicta una serie de medidas que buscan proteger las aguas continentales [37]. El buen estado de las aguas superficiales se define en base a su estado ecológico y químico, mientras que en el caso de las aguas subterráneas se valora su estado cuantitativo y químico. El estado ecológico de una masa de agua superficial depende de una serie de indicadores de calidad de tipo biológico, hidromorfológico, químico y fisicoquímico que se deben evaluar para realizar su debida clasificación. El estado químico de las aguas superficiales, por su parte, se define en función del grado de cumplimiento de una serie de normas de calidad ambiental, y el de las aguas subterráneas, de acuerdo a parámetros de conductividad y concentración de contaminantes.

La legislación española respecto a la presencia de plaguicidas en aguas establece que los estándares de calidad para aguas superficiales varían según la sustancia [103], mientras que para las aguas subterráneas el umbral de concentración máxima admisible es de 0.1 μg/L para cada sustancia individual y de 0.5 μg/L para la suma de todos los plaguicidas o TPs detectados. Los valores de concentración de plaguicidas presentes en estos dos tipos de agua deben vigilarse puesto que podrían llegar al agua de consumo humano, donde los valores paramétricos de los plaguicidas son los mismos que para aguas subterráneas, excepto para los organoclorados persistentes con valores individuales de 0.03 μg/L [104].

Para detectar y cuantificar los plaguicidas presentes en aguas, generalmente a niveles de concentración muy bajos, es necesario trabajar con instrumentos de alta tecnología y tratar las muestras de forma adecuada. El acoplamiento de las técnicas cromatográficas y la espectrometría de masas es una de las técnicas que más se utilizan. El uso de cuadrupolos sencillos o analizadores QqQ en modo de adquisición SRM [80,105] permite trabajar con métodos

pre-target, en el que los analitos se seleccionan a priori. A pesar de la sensibilidad y selectividad de las metodologías de QqQ, por lo general sólo se detecta un número limitado de analitos, lo que reduce las posibilidades de analizar otros contaminantes que podrían estar presentes en la muestra. El acoplamiento con instrumentación HRMS puede ser una buena solución puesto que permite realizar un amplio barrido de analitos sin necesidad de definir los compuestos previamente.

La combinación de técnicas complementarias como la GC-HRMS y la LC-HRMS es una herramienta poderosa para detectar plaguicidas de volatilidad y polaridad diferentes. La excelente sensibilidad y poder de resolución de la instrumentación QTOF en modo barrido de iones completo (*full scan*), resulta altamente satisfactoria para el análisis cualitativo o de screening, además permite trabajar en modo MS^E y obtener espectros completos con medidas de masa exacta. Posteriormente, se pueden seleccionar ciertos analitos previamente determinados (método pre-target) y estudiarlos cuantitativamente con el analizador QqQ.

Una buena opción para determinar la presencia de plaguicidas en aguas es la inyección directa de la muestra, pues se evita el pretratamiento. Dependiendo del tipo de muestra podrían ser necesarios la centrifugación previa y la dilución, para reducir interferentes. Esta metodología resulta apropiada cuando se usan analizadores QqQ; sin embargo, en las metodologías con espectrómetros de masas de alta resolución, de menor sensibilidad que los de triple cuadrupolo [106,107], es recomendable hacer una preconcentración de la muestra, especialmente cuando se quiere detectar analitos que se encuentran a muy bajas concentraciones. La extracción en fase sólida (SPE) suele ser el tratamiento elegido, ya que también se logran reducir las interferencias presentes en la matriz.

Para analizar muestras ambientales de agua, generalmente se usan cartuchos Oasis® HLB [108]. El relleno del cartucho comercializado por Waters es un copolímero de fase reversa que permite analizar analitos con un amplio ámbito de polaridad. Al poseer un monómero hidrofóbico (divinilbenceno) y un monómero hidrofílico (n-vinilpirrolidona) se equilibran las propiedades hidrofílicas y lipofílicas del relleno, lo que resulta ideal para la fase reversa SPE. Este tipo de adsorbente tiene un área superficial de 810 m^2/g, lo cual provoca un aumento en la capacidad de retención sin necesidad de utilizar grandes cantidades de relleno. Además,

posee un diámetro de poro de 80 Å, lo que favorece la separación de analitos de baja masa molecular [109].

La presencia de plaguicidas en los compartimentos ambientales puede resultar nociva para la vida en general; de ahí la importancia de complementar la investigación sobre su presencia en aguas con la evaluación del riesgo ambiental.

Para estudios de toxicidad de plaguicidas en muestras ambientales es primordial disponer de valores de concentraciones. Los datos de toxicidad acuática para plaguicidas que superan un umbral determinado se pueden obtener en diferentes bases de datos, como la ECOTOX de la Agencia de Protección Ambiental (EPA) de los Estados Unidos o la de Propiedades de Plaguicidas (PPDB), desarrollada por la Unidad de Investigación de Agricultura y Medio Ambiente (AERU) de la Universidad de Hertfordshire en Reino Unido. La toxicidad de una sustancia se evalúa mediante protocolos de laboratorio estandarizados que arrojan entre otros parámetros toxicológicos, los valores de concentración que: 1) afectan el 50% de los organismos (EC50); 2) que no afectan significativamente a los individuos expuestos (NOEC); 3) que matan al 50% de los organismos (LC50). Los valores de los parámetros toxicológicos (EC50, NOEC, LC50) se usan para calcular las unidades de toxicidad (TU) para obtener así una medida de la toxicidad agregada de los plaguicidas encontrados en las muestras. La suma de las TU individuales de cada compuesto presente en las muestras, suponiendo la aditividad de los compuestos medidos, permite determinar la toxicidad de la mezcla. Al aumentar la toxicidad también aumenta el valor numérico de la TU. Así, el riesgo acuático se clasifica como bajo cuando la $TU < 0.1$, moderado si $0.1 \leq TU \leq 1$ y alto si $TU > 1$.

Por otra parte, la sensibilidad del sistema también se puede calcular estadísticamente. Para ello, se puede calcular la media geométrica o la distribución de la sensibilidad de las especies (SSD). Si el número de datos de toxicidad es menor a 5 para peces, e inferior a 8 para invertebrados y productores primarios, se sugiere utilizar la media geométrica, y cuando ese número es superior, la SSD [110]. La media geométrica se calcula como la raíz del producto de una cantidad arbitraria de números.

Ccanccapa et al. [88] realizaron un estudio en la cuenca del río Ebro para establecer la ocurrencia, distribución espacial y transporte de plaguicidas y evaluar el riesgo ecotoxicológico en tres niveles tróficos (algas, dafnias y peces). La suma de las UT en cada punto de muestreo fue <1

en todos los casos muestreados, lo que indica que no había un riesgo asociado a la contaminación del medio acuático.

En otro estudio, realizado en Alemania por Le et al. [111] se evaluó la contribución de las EDAR a la toxicidad de los plaguicidas en pequeños ríos agrícolas, utilizando datos de seguimiento de plaguicidas. Se comparó la toxicidad de los plaguicidas en términos de logaritmo de unidad tóxica máxima (log mTU) en los puntos de muestreo. La diferencia obtenida entre los sitios con EDAR y sin EDAR, no se debió a diferencias en el número de compuestos analizados en ambos sitios, sino a la presencia de herbicidas en los sitios con EDAR. Entre ellos, el diurón, fue uno de los que más contribuyó a la toxicidad total de los ríos que reciben las descargas de estaciones depuradoras.

Con el fin de estimar el impacto de la mezcla de plaguicidas en aguas superficiales, Silvia et al. [112] combinaron modelos de distribución de sensibilidad de especies (SSD) y modelos de toxicidad de mezcla. Así, observaron que en las aguas superficiales de todas las cuencas fluviales el valor promedio de la msPAF para los productores primarios y los artrópodos excedió el 5%, porcentaje que representa el valor de corte utilizado en el enfoque prospectivo de SSD para derivar estándares individuales de calidad ambiental. La variabilidad en los resultados de msPAF se explica por la alta frecuencia de detección de plaguicidas encontrados en las muestras, donde los herbicidas fueron detectados con mayor frecuencia.

2.1.2. Artículo científico I

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Investigation of pesticides and their transformation products in the Júcar River Hydrographical Basin (Spain) by wide-scope high-resolution mass spectrometry screening

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ABSTRACT

The Water Framework Directive 2000/60/EC implemented by the European Union established as the main objectives to achieve a "good ecological and chemical status" of the surface water and a "good quantitative and chemical status" of groundwater bodies. One of the major pressures affecting water bodies comes from the use of pesticides and their potential presence in the water ecosystems. For this purpose, the reliable determination of pesticides and their transformation products (TPs) in natural waters (both surface and groundwater) is required. The high number of compounds potentially reaching the aquatic environment makes extraordinary difficult, if not impossible, to investigate all these compounds even using the most powerful analytical techniques. Among these, liquid chromatography coupled to high-resolution mass spectrometry is emphasized due to its strong potential for detection and identification of many organic contaminants thanks to the accurate-mass full spectrum acquisition data. This work focuses on wide-scope screening of many pesticides and their TPs in surface water and groundwater samples, collected between March and May 2017, in the Júcar River Hydrographical Basin, Spain. For this purpose, a home-made database containing more than 500 pesticides and TPs was employed. Analyses performed by liquid chromatography coupled to quadrupole-time of flight mass spectrometry (LC-QTOF MS) allowed the identification of up to 27 pesticides and 6 TPs. The most detected compounds in groundwater were the herbicides atrazine, simazine, terbuthylazine, and their TPs (atrazine-desethyl, terbumeton-desethyl and terbuthylazine-desethyl). Regarding surface water, the fungicides carbendazim, thiabendazole and imazalil, the herbicide terbutryn and the TP terbumeton-desethyl were also detected. These results illustrate the wide use of these compounds (in the present or in the recent past) in the area under study and the vulnerability of the water bodies, and are in accordance with previous findings in other water bodies of the different Spanish Hydrographic systems.

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Investigation of pesticides and their transformation products in the Júcar River Hydrographical Basin (Spain) by wide-scope high-resolution mass spectrometry screening

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Highlights

- High resolution mass spectrometry wide-scope screening of pesticides in water.
- 27 pesticides and 6 transformation products were found in water samples.
- Some compounds not included in routine target quantitative methods were identified.
- Screening methodology was validated for 20 compounds commonly found in water.
- Triazines and transformation products were the most frequently identified.

Abstract

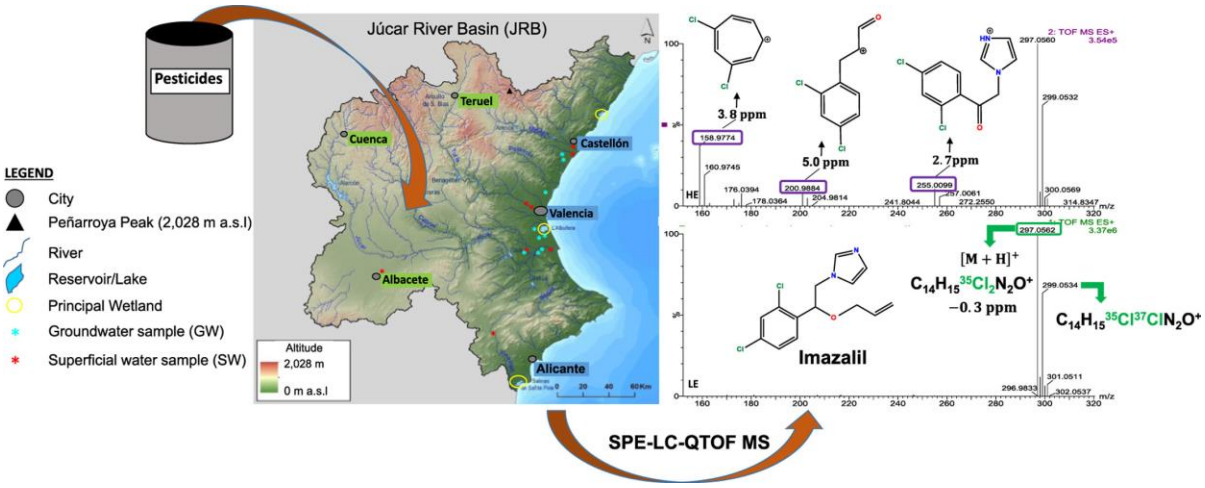
The Water Framework Directive 2000/60/EC implemented by the European Union established as the main objectives to achieve a "good ecological and chemical status" of the surface water and a "good quantitative and chemical status" of groundwater bodies. One of the major pressures affecting water bodies comes from the use of pesticides and their potential presence in the water ecosystems. For this purpose, the reliable determination of pesticides and their transformation products (TPs) in natural waters (both surface and groundwater) is required. The high number of compounds potentially reaching the aquatic environment makes extraordinary difficult, if not impossible, to investigate all these compounds even using the most powerful analytical techniques. Among these, liquid chromatography coupled to high-resolution mass spectrometry is emphasized due to its strong potential for detection and identification of many organic contaminants thanks to the accurate-mass full spectrum acquisition data. This work focuses on wide-scope screening of many pesticides and their TPs in surface water and groundwater samples, collected between March and May 2017, in the Júcar River Hydrographical Basin, Spain. For this purpose, a home-made database containing more than 500 pesticides and TPs was employed. Analyses performed by liquid chromatography coupled to quadrupole-time of flight mass spectrometry (LC-QTOF MS) allowed the identification of up to 27 pesticides and 6 TPs. The most detected compounds in groundwater were the herbicides atrazine, simazine, terbuthylazine, and their TPs (atrazine-desethyl, terbumeton-desethyl and terbuthylazine-desethyl). Regarding surface water, the fungicides carbendazim, thiabendazole and imazalil, the herbicide terbutryn and the TP terbumeton-desethyl were also detected. These results illustrate the wide use of these compounds (in the present or in the recent past) in the area under study and the vulnerability

of the water bodies, and are in accordance with previous findings in other water bodies of the different Spanish Hydrographic systems.

Keywords

Pesticides; transformation products; water; screening; high resolution mass spectrometry.

Graphical abstract



1. Introduction

Pesticides are compounds used worldwide, in different sectors, but especially in the agricultural field. These substances can be degraded by living organisms (generating metabolites) and in the atmosphere, soil and/or water (transformation products, TPs). In all these cases, TPs (including also metabolites) can reach concentration levels higher than the parent substances and be even more toxic (Richardson and Ternes, 2014). As a result of their massive use around the world, pesticides and TPs move through the different environmental compartments, leading to a consequent potential risk to living organisms (including humans) or/and environment.

In the last years, it has been observed a general trend towards the use of more effective pesticides with lower environmental impact/persistence (Dumas et al., 2017; Sannino et al., 2004) and a shift from "traditional cultivation techniques" to "modern agricultural practices" (Pascual Aguilar et al., 2017). Although most pesticides cataloged as very persistent (Sousa et al., 2018) have been banned, and many compounds have been withdrawn after implementation of Directive 91/414/EEC (The Council of the European Communities, 1991), it is still possible to find products forbidden some years ago in groundwater (GW) and in soil. This is the case, among others, of atrazine (triazine herbicide) and its TPs, which 10 years after its prohibition, remain detected in GW (Pitarch et al., 2016; Talja et al., 2008; Tappe et al., 2002). So, despite the decrease in pesticides use, a comprehensive monitoring, including compounds currently used as well as those banned, is needed in order to evaluate their presence in surface water (SW) and GW (Hernández et al., 2008). In addition, the pesticides used in agriculture are constantly changing, according to the increasingly restrictive regulations and the advances in the control of pests and weeds in the chemical industries.

In order to reach an improved water status, the European Parliament established the Water Framework Directive in 2000, including as the main objective to achieve a "good ecological and chemical status" for all European Union water bodies by 2015 (Directive, 2000/60/EC; European Community, 2000). This Directive stipulates that GW bodies must "register and invert any significant and sustained upward trend in the concentration of any contaminant". The Article 17 of the Directive 2006/118/EC (European Parliament and Council of European Union, 2006), announces several strategies for the prevention and control of GW

pollution. This Directive shows the criteria for considering a GW body to have a "good chemical status" and to reverse the trend of increasing contaminant concentrations. Regarding SW bodies, the Spanish ordinance (Ministerio de Agricultura Alimentación y Medio Ambiente, 2015) establishes the threshold values for concentrations of pollutants (Menchen et al., 2017).

To this aim, it is necessary to go in depth on the knowledge of occurrence and concentration levels of priority substances (some of them pesticides). The first studies of the River Basin Management Plans in Europe (EU) showed that more than half of Europe's SW bodies are in worse conditions than the "good ecological status" (Werner et al., 2012); therefore, monitoring water sources is a priority issue and needs to be periodically performed to guarantee the appropriate quality.

In general, the water bodies of the Iberian Peninsula do not present a better state than the rest of European ones ("Eurostat, Pesticide sales statistics.," 2014). von der Ohe et al. (2011) analyzed waters collected in four European rivers (including the Llobregat Spanish River), concluding that pesticides were among the substances more frequently detected and presented high or very high risk. Spain is among the European countries with highest use of pesticides as a consequence of the intensive agricultural activity (Parris, 2011). The quality standards established in Spain for SW contaminants vary depending on each substance (Royal Decree 817/2015, Annex IV and V) (Ministerio de Agricultura Alimentación y Medio Ambiente, 2015). In GW, the maximum allowable concentration threshold of active substance (pesticides, TPs and metabolites) is 0.1 μg L^{-1} for each individual substance, and 0.5 μg L^{-1} for the sum of all detected pesticides, TPs and metabolites (Royal Decree 1514/2009) (*Ministerio de Medio Ambiente, y Medio Rural y Marino, RD 1514/2009, de 2 de octubre, por el que se regula la protección de las aguas subterráneas contra la contaminación y el deterioro*, 2009). Currently, the River Basin (RB) Authorities are the organizations responsible for ruling on non-compliance based on current legislation.

The Júcar River Basin (JRB) District shows a very fragile balance between available resources (3400 hm^3/year) and demands for water (3200 hm^3/year), with more than 80% destined to irrigation. Almost 30% of the territory of the JRB District is occupied by agricultural land (either rainfed or irrigated), being agriculture the main source of diffuse pollution by an excess

of fertilizers and/or pesticides. It is also relevant the high use of groundwater, which is on the order of half of total water withdrawals. Although the first studies on the presence of pesticides in JRB District were performed several years ago (Hernández et al., 2008; Marín et al., 2006), recent data have shown the continued presence of pesticides in environmental waters (Belenguer et al., 2014; Ccanccapa et al., 2016a; Menchen et al., 2017; Pascual Aguilar et al., 2017; Rousis et al., 2017) illustrating the impact of the agricultural activities, mainly, on water resources. Until now, most of those studies have focused on a limited number of pesticides, based on data reported by target analysis. This is the most common situation, where a small number of analytes is included in the target list of the analytical method. Only in a few cases, TPs have been included in the analysis (e.g. Rousis et al., 2017; von der Ohe et al., 2011).

For a comprehensive monitoring, where a large number of compounds is investigated, the application of powerful techniques, such as high-resolution mass spectrometry (HRMS), is required. The potential of ultra-high performance liquid chromatography (UHPLC) coupled to HRMS for screening of pesticides in environmental waters has already been demonstrated (Hernández et al., 2015a, 2015b; Pitarch et al., 2016; Soulier et al., 2016; Wille et al., 2011). The most commonly used analysers, Orbitrap™ (Ruff et al., 2015) and time-of-flight (TOF) (Masiá et al., 2014), provide high mass accuracy and high resolution in full acquisition mode (Cotton et al., 2016). These characteristics make these instruments very useful for the detection and identification of a huge number of pesticides as well as their TPs in a single analysis, even without reference standards (tentative identification) (Ibáñez et al., 2017).

The main objective of this work was to investigate the occurrence of a large number of compounds in GW and SW from the JRB District, based on wide-scope screening by LC-QTOF MS. An important number of TPs have also been included, in order to fill the gap of the little information available on the presence of these compounds in waters. In total, more than 500 compounds have been investigated using a generic sample treatment based on solid phase extraction (SPE) with polymeric cartridges, followed by LC- QTOF MS analysis.

2. Material and methods

2.1. Chemicals

Atrazine, atrazine-desethyl (DEA), atrazine-desisopropyl (DIA), bromacil, carbendazim, chlorpyrifos, diuron, imazalil, linuron, metolachlor, prometryn, simazine, terbumeton, terbumeton-desethyl (TED), terbuthylazine, terbuthylazine-desethyl (TD), terbutryn, thiabendazole, 3,4-dichloroaniline (3,4- DCA), 3,5,6-trichloro-2-pyridinol (TCP) were purchased from Sigma-Aldrich (Madrid, Spain), with a purity higher than 97%. The list of pesticides and TPs available for this study, as well as some physicochemical properties, are listed in Supplementary Material (**Table SM1**).
HPLC-grade methanol (MeOH), acetone (pesticide residue analysis quality) and formic acid (98–100%, LC-MS grade), were supplied by Scharlau S.L (Barcelona, Spain). HPLC-grade water (resistivity of 18 MΩ cm) was obtained by purifying demineralised water (Millipore Ltd., Bedford, MA, USA).

Standard stock solutions (500 mg/L) of each compound (except carbendazim) were prepared by dissolving 25 mg (± 0.01 mg) of each solid compound in 50 mL of acetone considering the purity of each solid standard. Standard stock solution of carbendazim was prepared in MeOH. Individual stock solutions and working standard solutions were stored at −20 and 4 °C in the dark, respectively.

2.2. General site description

The JRB Authority is a Spanish public institution, responsible, among other functions, of monitoring water resources, quality and quantity monitoring network, in the JRB District. The JRB District is an inter-regional river basin located within Spanish territory, at the Eastern part of the Iberian Peninsula, in south Europe, bordering with the Mediterranean Sea (**Fig. 1**).

The area occupied by the JRB District with respect to the Spanish territory is 8.5%, this is, 42.851 km^2 ("Confederación Hidrográfica del Júcar, O.A," 2018). The two main rivers located in this area are the Júcar and the Turia River, with a length of 509 and 250 km, respectively. In the East sector, it can be found the largest shallow coastal lagoon (23.94 km^2), named Albufera, within the Valencia Natural Park (223 km^2), which has a great environmental value.

According to the definition of GW bodies carried out by the Water Framework Directive (WFD), the JRB District is made up of 90 GW bodies (Menchen et al., 2017). For more information about geology and hydrogeology see Supplementary Material.

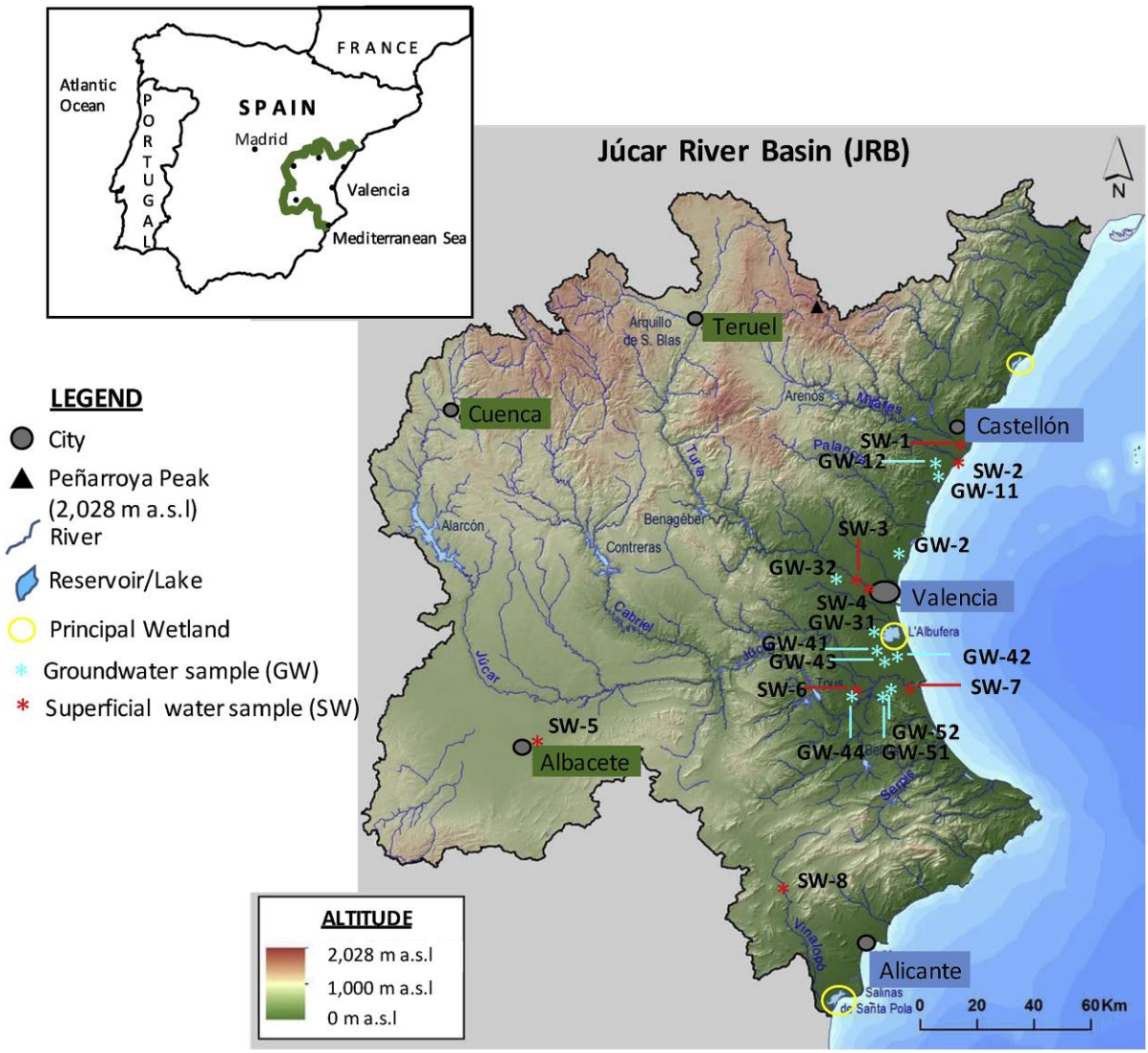


Fig. 1. Location, topography and main cities of Júcar River Basin (JRB) and monitoring network location.

Regarding land use in the JRB District, it is worth mentioning that 50.5% corresponds to forest and semi-natural areas, 35.5% are rainfed areas, 10.5% corresponds to irrigated agricultural areas, 2.8% to urban and industrial areas and finally 0.7% are SW (including wetlands). The

permanent population located in the JRB District in the year 2009 were 5,162,163 inhabitants according to the Spanish National Institute of Statistics ("Instituto Nacional de Estadística," 2009). In addition, it is estimated that the seasonal population (summer) increases approximately in 500,000 inhabitants ("Confederación Hidrográfica del Júcar, O.A," 2018).

The eastern sector is formed by a succession of coastal plains in which an intense agricultural activity is developed, mainly of citrus cultivation, which represents an important sector of the export economy of the Valencian Community. The 342,000 ha of citrus are irrigated mainly by drip systems, with an annual demand of around 2050 hm^3. In the internal zones, the predominant crops are corn and cereals, with a global demand of around 1500 hm^3/year. The use of pesticides is widespread in these crops (Belenguer et al., 2014; Cotton et al., 2016).

The study area is under typical Mediterranean weather, with marked seasonality. The summer is warm and dry (July and August) and winters are relatively wet and mild. Mean annual precipitation is around 500 mm, and varies depending on the topography (inlands and coast). In addition, torrential rains are concentrated in few days in the year, usually in months of autumn and spring. It is not uncommon to observe months with no rainfall.

2.3. Monitoring network and sampling

The monitoring network was designed based on previous detection of pesticides in SW and GW from this area ("Confederación Hidrográfica del Júcar, O.A." 2018). The selected sampling points belong to the JRB Authority quality-monitoring network, and all presented relatively high concentrations of pesticides in previous campaigns, including some non-compliances according to the current regulation (Ministerio de Agricultura Alimentación y Medio Ambiente, 2015; *Ministerio de Medio Ambiente, y Medio Rural y Marino, RD 1514/2009, de 2 de octubre, por el que se regula la protección de las aguas subterráneas contra la contaminación y el deterioro*, 2009). The monitoring network consisted of 19 samples: 8 corresponded to 8 SW bodies and the remaining 11 to 5 GW bodies (**Fig. 1, Table SM2**). GW samples were collected from March-April 2017, whereas SW samples were taken in May 2017.

Polyethylene bottles (1 L volume) used for collection of samples were previously rinsed with the same water. For collection of GW samples, the wells were pumped until the pH and conductivity (EC) parameters were stabilized. Immediately after sampling, the sample bottles were placed into isothermal bags with ice to keep them cold, and shipped to the laboratory to arrive within 24 h.

2.4. Qualitative validation

The analytical methodology applied was validated to evaluate its capability to detect and identify 20 compounds selected as a model, on the basis of their frequent detection in previous campaigns ("Confederación Hidrográfica del Júcar, O.A," 2018). The samples were spiked at two concentration levels (0.01 and 0.1 μg/L), and three sample volumes (100, 250 and 500 mL) were tested (n = 3 each one) in the SPE procedure applied. The validation was qualitative, i.e. the objective was to evaluate whether the compounds were correctly detected and identified by LC-QTOF MS. A river sample was used as "blank" for preparation of spiked samples.

2.5. Sample treatment

Samples were thawed at room temperature before being processed. SPE cartridges (Oasis® HLB, 200 mg sorbent/6 mL, Waters Corp., Milford, MA, USA) were conditioned by washing with MeOH (2 x 2.5 mL) followed by rinsing with ultra-pure Milli-Q water (2 x 2.5 mL). Water samples (500 mL) were pumped through the cartridge and dried under vacuum for approximately 20 min. The analytes were eluted by gravity with MeOH (2 x 2.5 mL) and collected in glass tubes. The eluate was evaporated to dryness at 40 °C under a gentle stream of nitrogen and reconstituted in 0.5 mL MeOH:water (10:90, v/v). Analysis was performed by injecting 20 μL of the final extract in the UHPLC-HRMS system. The criteria used for confirmation of the identity are indicated in the following section. **Fig. 2** shows the overall scheme of both sample treatment and data processing.

2.6. Instrumentation

UHPLC-HRMS analysis was performed using a Waters ACQUITY UPLC ultra-high performance liquid chromatography (UHPLC) system (Waters, Milford, MA, USA) interfaced to a hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (XEVO G2 QTOF,

Waters Micromass, Manchester, UK), using an orthogonal Z-spray electrospray ionization (ESI) interface operating in both positive and negative ionisation modes.

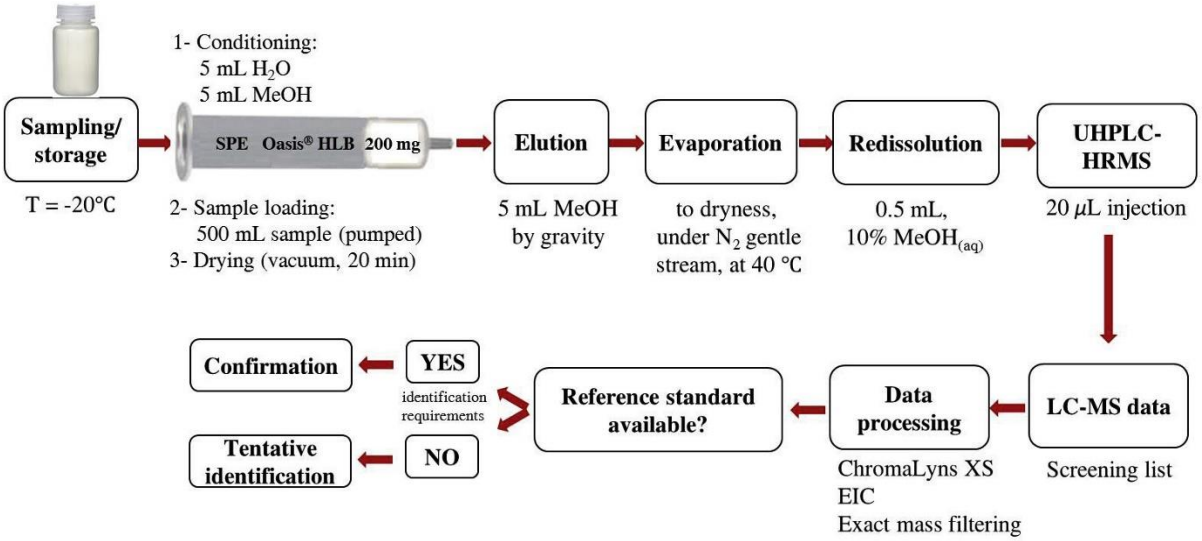


Fig. 2. Overall scheme of the screening procedure applied (sample treatment + HRMS analysis and data processing).

The UHPLC separation was performed using an CORTECS C18 column (2.1 i.d. × 100 mm length, 2.7 µm particle size, Waters), at a flow rate of 300 µL/min. The mobile phases were (A) H_2O with 0.01% HCOOH and (B) MeOH with 0.01% HCOOH. The mobile phase gradient was: 10% B at 0 min, 90% B at 14 min linearly increased, 90% B at 16 min, and finally 10% B at 18 min in order to return to initial conditions. The injection volume was 20 µL.

MS data were acquired over a *m/z* range of 50–1000. Nitrogen was used as drying and nebulizing gas. The gas flow was set at 1000 L/h. TOF-MS resolution was approximately 18,000 at full width half maximum (FWHM) at *m/z* 556. Capillary voltages of 0.7 kV and 3.0 kV were used in positive and negative ionisation modes, respectively. A cone voltage of 20 V was selected for both ionisation modes. Collision gas was argon 99.995% (Praxair, Valencia, Spain). The desolvation temperature was 600 °C and the source temperature 130 °C. For MS^E experiments, two acquisition functions with different collision energies were used: the low energy (LE), selecting a collision energy of 4 eV in order to obtain information about

the protonated molecule and adducts (if present), and the high energy (HE) function, with a collision energy ramp ranging from 15 to 40 eV, in order to obtain a greater range of fragment ions. The LE and HE functions settings were for both a scan time of 0.3 s. Data were automatically processed by ChromaLynx XS software (MassLynx v 4.1, Waters).

2.7. Criteria for detection/identification used in the UHPLC-HRMS screening

Pesticides and TPs included in a large home-made database were investigated in the samples for a qualitative screening (i.e. detection and identification) based on mass accuracy, isotopic pattern and retention time deviation and the presence of fragment ions (European Commission, 2017). **Tables SM3-SM6** show the empirical formula of all pesticides included in the database and the exact mass corresponding to the (de)protonated molecule. For those compounds included in the reference standard mix, retention time as well as the 2–3 most important fragment ions are also showed. The extracted ion chromatograms (EIC) corresponding to (de)protonated molecules and fragment ions were automatically obtained using ChromaLynx XS. The following detection settings were applied: ± 5 ppm for mass accuracy and ± 0.1 min for retention time deviation. The classification of the different analytes as detected, identified/confirmed or tentatively identified depended on the fulfillment of the criteria indicated in **Table 1**, as well as on the availability of the reference standards. A compound was considered as "detected" when the most abundant ion (typically the (de)protonated molecule) was observed at the appropriate retention time (tolerance of ± 0.1 min respect to a reference standard), measured at accurate mass (mass error lower than 5 ppm). For reliable "identification", the presence of the (de)protonated molecule (or any adduct, if more favorable) and, at least, one fragment ion was required, both with mass errors below 5 ppm at the expected Rt in comparison with the reference standard (± 0.1 min).

When only the elemental composition was included in the database (i.e., the reference standard was not available in the laboratory), a suspect screening was carried out (Ibáñez et al., 2017). "Tentative identification" occurred when an expected ion was found with mass error below 5 ppm, together with its characteristic isotopic pattern (if exists), and one or more fragment ions were in agreement with data reported and compatible with the chemical structure of the candidate (mass error < 5 ppm). Although not applied in the present work, the use of retention time (Rt) prediction models can be useful for suspect compounds whose reference standards

are not available, because it allows to discard false positives focusing the identification in those peaks that fall into the predicted Rt window (Bade et al., 2015b). Rt prediction based on artificial neural networks (ANN) will be applied in future screening (Bade et al., 2015a). For those compounds tentatively identified, the acquisition of the reference standard would be required for confirmation of their identity.

Table 1. Criteria used in the UHPLC-HRMS screening for detection/identification of compounds.

	Reference standard		
	Available		Not available
	Detected	Confirmed/ Identified	Tentatively identified
Accurate mass protonated molecule $[M+H]^+$	< 5 ppm	< 5 ppm	< 5 ppm
Accurate mass fragment ion		< 5 ppm	< 5 ppm[a]
Deviation ($[M+H]^+$) respect to the reference standard	± 0.1 min	± 0.1 min	

ppm: mass accuracy expressed as a relative value in parts per million
[a] at least one fragment ion should be justified on the basis of the structure or on existing literature

3. Results and discussion

3.1. Qualitative validation

Table SM7 shows data obtained after testing different sample volumes (100, 250 and 500 mL) at the two concentration levels (0.01 and 0.1 μg/L) in a water sample subjected (in triplicate) to the overall SPE-LC-QTOF MS procedure.

As it can be seen, the best results were obtained when 500 mL water was loaded, as all compounds (except for chlorpyrifos) were detected and confirmed in all the 3 replicates, even at the lowest concentration level assayed (0.01 μg/L).

Although the screening was validated for only 20 compounds -selected because of their frequent occurrence in the aquatic environment of the area under study-, the accurate-mass full-spectrum acquired in the QTOF MS analysis allowed us to widen the screening to a large list of more than 500 pesticides and TPs. Under these circumstances, the positives detected in

the wide-scope screening should be taken as unequivocal when the reference standard was available and allowed its full confirmation, even in the case that the compound was not subjected to validation. However, potential false negatives might occur for compounds not previously validated, especially when their physico chemical characteristics (mainly polarity) differed notably from the model compounds.

3.2. Results obtained in the wiede-scope screening

Table 2 shows the results obtained in the screening of GW samples. Up to 17 compounds were confirmed with reference standards: 12 pesticides (atrazine, simazine, terbumeton, terbuthylazine, bromacil, carbendazim, imazalil, thiabendazole, bentazone, imidacloprid, metalaxyl, and terbacil) and 5 TPs (DEA, DIA, TED, terbuthylazine-2-hydroxy (T2H), and TD). One more compound (metolachlor oxanilic acid (MOA)) was just tentatively identified, as the reference standard was not available at our lab.

The herbicides atrazine and simazine were found in all GW samples analysed (**Table 2**). Terbuthylazine was present in 10 out of the 11 samples (90%), and terbumeton and bromacil in 7 (63%). Regarding TPs, DEA, TD and TED were found in all GWs samples. Other TPs were DIA (72%), T2H (54%) and MOA (36%). Some other herbicides, such as diuron, bentazone and terbacil, and the insecticide neonicotinoid imidacloprid were found at least once. The fungicides thiabendazol, metalaxyl and carbendazim were present in 3 and 4 samples, respectively, whereas the post-harvest fungicide imazalil was positive in just one water sample.

Table 2. Results obtained in GW samples by UHPLC-QTOF MS screening. Samples collected between March and April 2017.

	Compounds	GW11	GW12	GW2	GW31	GW32	GW41	GW42	GW43	GW44	GW51	GW52	Detection frequency (%)
Subjected to validation	Atrazine	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100
	Atrazine-desethyl (DEA)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100
	Atrazine-desisopropyl (DIA)	✓		✓		✓	✓	✓	✓		✓	✓	72
	Bromacil	✓	✓	✓		✓			✓		✓	✓	63
	Carbendazim	✓				✓			✓				27
	Diuron					✓					✓		18
	Imazalil					✓							9
	Simazine	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100
	Terbumeton				✓	✓		✓	✓	✓	✓	✓	63
	Terbumeton-desethyl (TED)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100
	Terbuthylazine	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	90
	Terbuthylazine-desethyl (TD)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100
	Thiabendazole		✓	✓					✓				27
No subjected to validation	Terbuthylazine-2-hydroxy(T2H)				✓	✓		✓	✓		✓	✓	54
	Bentazone					✓							9
	Imidacloprid				✓								9
	Metalaxyl				✓	✓			✓		✓		26
	Metolachloroxanilic acid (MOA)				t	t			t		t		36
	Terbacil	✓									✓		18

✓: confirmed ((de)protonated molecule and at least one fragment ion were observed at the expected retention time).
t: tentative identification ((de)protonated molecule was observed and at least one ion fragment was justified).

Regarding SW, the results are shown in **Table 3**. The number of compounds found in the samples increased up to 24, all confirmed with reference standards (11 of them were in fact included in the qualitative validation). Another two compounds (nicosulfuron and MOA), where tentatively identified as reference standards were not available.

Table 3. Results obtained in SW samples by UHPLC-QTOF MS screening. SW samples collected in May 2017.

	Compounds	**SW1**	**SW2**	**SW3**	**SW4**	**SW5**	**SW6**	**SW7**	**SW8**	Detection frequency (%)
Subjected to validation	Carbendazim	✓	✓	✓	✓	✓	✓	✓	✓	100
	Imazalil	✓	✓		✓		✓	✓		62
	Linuron								✓	12
	Metolachlor								✓	12
	Simazine		✓					✓	✓	37
	Terbumeton		✓					✓	✓	37
	Terbumeton-desethyl (TED)	✓	✓	✓	✓	✓	✓	✓		87
	Terbuthylazine	✓	✓					✓	✓	50
	Terbuthylazine-desethyl (TD)		✓					✓		25
	Terbutryn	✓		✓		✓	✓		✓	62
	Thiabendazole	✓	✓	✓	✓	✓	✓	✓	✓	100
No subjected to validation	Acetamiprid					✓	✓			25
	Azoxystrobin								✓	12
	Diazinon		✓				✓			25
	Imidacloprid								✓	12
	Isoproturon								✓	12
	MCPA			✓						12
	Metalaxyl		✓		✓		✓		✓	50
	Metolachlor oxanilic acid (MOA)		t		t				t	37
	Nicosulfuron	t								12
	Paclobutrazol						✓			12
	Propamocarb	✓							✓	25
	Propiconazole	✓					✓	✓	✓	50
	Tebuconazole	✓			✓				✓	37
	Tetraconazole								✓	12
	Terbuthylazine-2-hydroxy (T2H)		✓		✓		✓	✓	✓	62

✓: confirmed ((de)protonated molecule and at least one fragment ion were observed at the expected retention time).
t: tentative identification ((de)protonated molecule was observed and at least one ion fragment was justified).

Similarly to GW, the majority of compounds in SW were herbicides (13 compounds): simazine, terbumeton, terbuthylazine, terbutryn and the TPs TED, TD and T2H (triazines); nicosulfuron, linuron, metolachlor, isoproturon, and MCPA; and the TP metolachlor oxanilic acid. An important number of fungicides were also detected, specifically benzimidazoles (carbendazim and thiabendazole), triazoles (propiconazole, tetraconazole and tebuconazole), carbamates (propamocarb), strobilurins (azoxystrobin), acylalanines (metalaxyl), and imidazols (imazalil). The presence of three insecticides (acetamiprid, imidacloprid and diazinon) and the plant growth regulator paclobutrazol was also confirmed (**Table 3**).

Fig. 3 shows a summary of the results obtained, grouped by families. As observed, triazine herbicides were the most frequently detected, representing 34% of the findings in GW and 19% in SW, followed by benzimidazoles (5% and 20%, respectively). The other families were below 11%. The TPs represented 46% of the positives in GW and 22% in SW. The occurrence of TPs in GW and SW was rather similar in terms of detection frequency for TED, MOA and T2H. However, TD was detected in all GW samples but was present in only 25% of the SW analysed. DEA and DIA were found in 100% and 73% of the GW samples, but not in SW.

A rough semi-quantitative estimation was performed by comparing the area of the spiked samples (0.1 $\mu g\,L^{-1}$) used for validation of the screening with the responses of samples. Based on these data, two compounds seemed to be present at a concentration higher than 0.1 $\mu g\,L^{-1}$ in GW: the herbicide bromacil (3 out samples) and the main TP of terbumeton, TED (4 samples). In SW, the two compounds exceeding the 0.1 $\mu g\,L^{-1}$ level were the fungicides imazalil (2 samples) and thiabendazole (1 sample). Although these data should be confirmed by a validated quantitative method, it seems clear that these compounds may become a problem from a regulatory point of view if appropriate measurements are not taken.

As an illustrative example of the potential of QTOF MS, **Fig. 4** shows the tentative identification of the herbicide nicosulfuron in a SW sample (reference standard non-available). The LE spectrum of the chromatographic peak at 7.51 min in ESI positive mode, showed an abundant signal at *m/z* 411.1087 (**Fig. 4**b**,** bottom). It might correspond to the protonated molecule of nicosulfuron ($C_{15}H_{19}N_6O_6S^+$, expressed as protonated molecule), with a mass error of 0 ppm in relation with its theoretical exact mass.

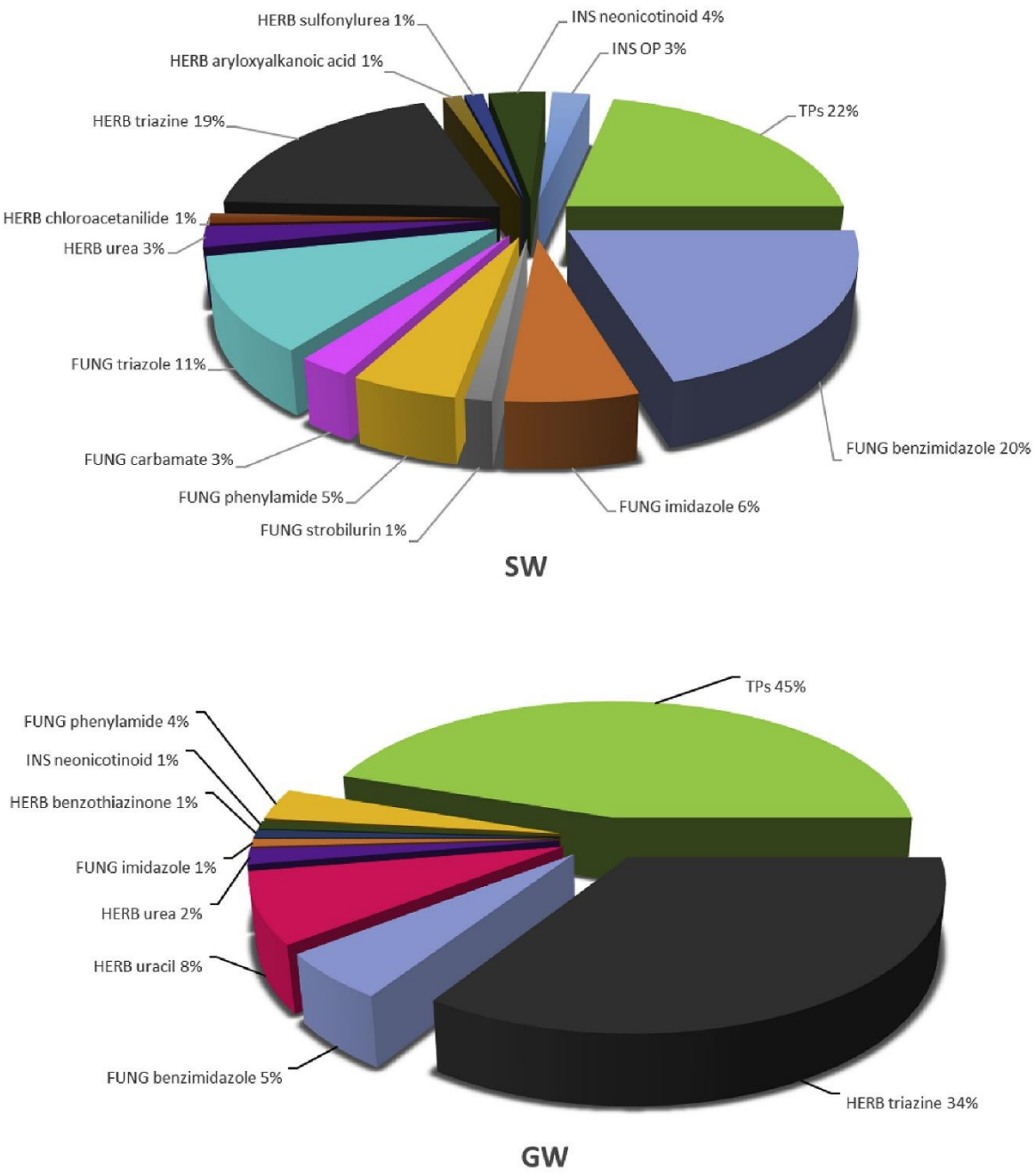


Fig. 3. Percentages of the different families of pesticides identified in surface water (top) and groundwater samples (bottom). FUNG: fungicide; HERB: herbicide; INS: insecticide; OP: organophosphorus; TPs: transformation products.

The peak corresponding to the sodium adduct was also observed at *m/z* 433.0908. The LE spectrum also showed 3 fragment ions at *m/z* 366.0510 ($C_{13}H_{12}N_5O_6S^+$, corresponding to the neutral loss of dimethylamine), *m/z* 213.0337 ($C_8H_9N_2O_3S^+$) and 182.0568 ($C_7H_8N_3O_3^+$), all with mass errors below 2 ppm. In addition, the HE spectrum (**Fig. 4**b**,** top) showed another

fragment ion at *m/z* 106.0300 ($C_6H_4NO^+$). The structure of all fragment ions was justified based on their measured accurate masses, and all were compatible with the structure of the candidate. Moreover, the 4 fragment ions were in accordance with the scientific literature (Massbank, 2006). Although all these data strongly supported the tentative identification of the compound as nicosulfuron, the injection of the corresponding reference standard should be required for a definitive unequivocal confirmation.

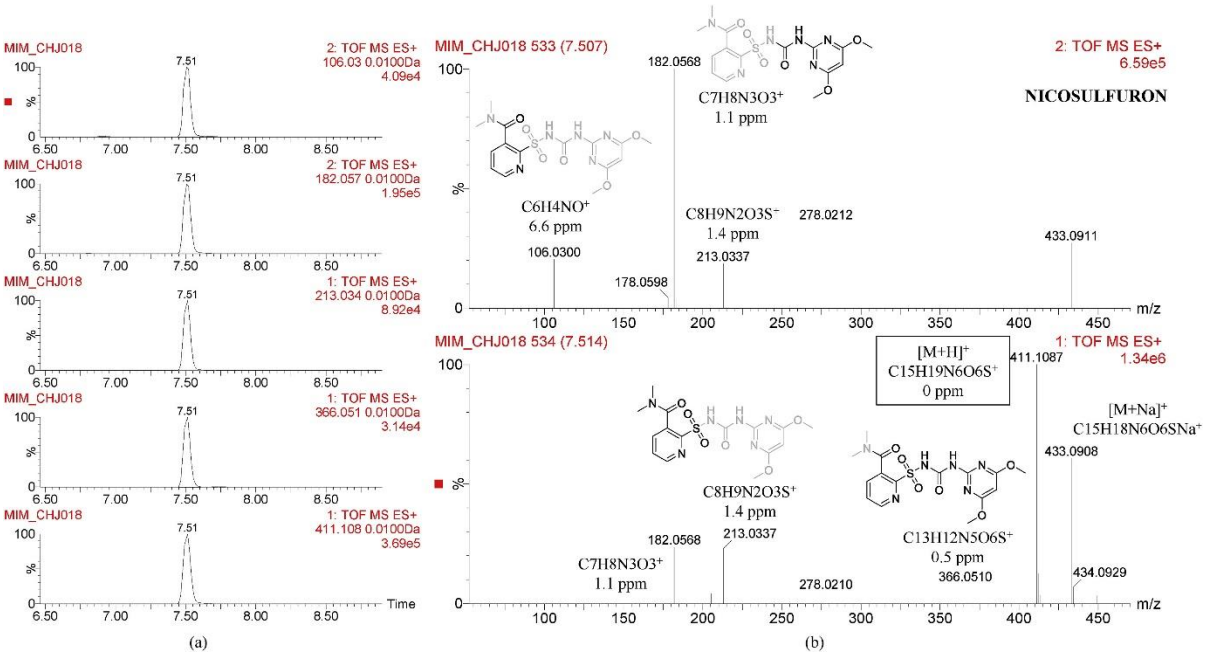


Fig. 4. Detection and tentative identification of nicosulfuron in a SW sample. (a) nw-XICs at 0.01 Da mass window for $[M+H]^+$ in LE function and main fragments in LE/HE functions. (b) Low energy (bottom) and high energy (top) TOF mass spectra obtained for the chromatographic peak at 7.51 min.

In a similar way, **Fig. 5** shows the tentative identification of the metolachlor oxanilic acid in a GW sample. The LE spectrum of the chromatographic peak at 9.18 min (**Fig. 5**b**,** bottom), showed an abundant signal at *m/z* 280.1545, that might correspond to the protonated molecule ($C_{15}H_{22}NO_4^+$, −1.4 ppm), and at *m/z* 302.1370, corresponding to its sodium adduct. Two fragment ions were also observed in the LE spectrum, at *m/z* 248.1287 ($C_{14}H_{18}NO_3^+$, from a methanol neutral loss) and *m/z* 220.1343 ($C_{13}H_{18}NO_2^+$), both with mass errors below 1 ppm. The HE spectrum (**Fig. 5**b**,** top) showed an additional fragment ion at *m/z* 160.1127 ($C_{11}H_{14}N$).

The fragment ions were in accordance with the reported information (Massbank, 2006); however, a plausible structure could not be proposed for fragment at *m/z* 220. So, it would be necessary to acquire the reference standard to finally confirm or discard the identity of the compound.

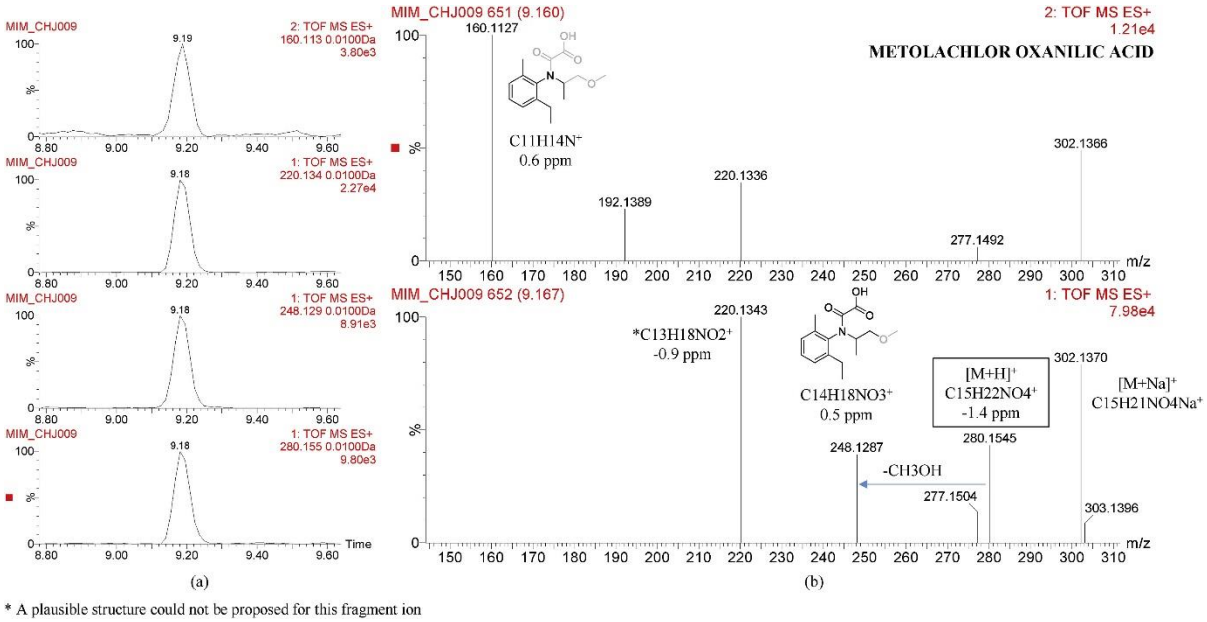


Fig. 5. Detection and tentative identification of metolachlor oxanilic acid in a GW sample. (a) nw-XICs at 0.01 Da mass window for $[M+H]^+$ in LE function and main fragments in LE/HE functions. (b) Low energy (bottom) and high energy (top) TOF mass spectra obtained for the chromatographic peak at 9.18 min.

3.2.1. Comparison of the results obtained with the legislation

In the present study, 33 compounds were detected (**Table 2, and 3**). Among them, terbuthylazine, metolachlor, simazine and atrazine have been regularly monitored by a quantitative method since 1999 in GW (see **Table SM8**). Among these, only terbuthylazine is allowed to be used at present. In the case of metolachlor, its enantiomer S-metolachlor was allowed until July 2018 (*Commission Implementing Regulation (EU) 2017/841*, 2017). Regarding SW, diazinon has also been regularly controlled since 1999, and metolachlor, simazine, terbuthylazine, and isoproturon since 2002.

Although our data can only be considered as semi-quantitative, bromacil and TED seemed to be present in several GW samples at concentrations around or even above 0.1 μg/L. This herbicide was widely used for many years until its prohibition in 2003 (*Commission Regulation (EC) No* 2076*/2002*, 2002; Resolution of 30 June 2003 of the General Directorate of Agriculture, Ministerio de Agricultura Pesca y Alimentación, 2003). Historical data reported by JRB District showed two cases of 0.1 μg/L exceedance for bromacil along the period 2010–2015, but a progressive reduction of the concentration has been observed along this period of time. The last quantitative data reported for bromacil in GW in the regular monitoring performed by JRB District corresponded to the 2017 campaign, where this herbicide was the only compound exceeding 0.1 $\mu g L^{-1}$ (27% of the GW samples analyzed). However, it was not found in SW. In case of TED, it is analysed in control samples since 2013. Historically, it has had defaults in 3 GW bodies. In the current sampling, the reference value of 0.1 $\mu g\ L^{-1}$ seems to have been exceeded in nearly 40% of the samples.

Although the cases of non-compliance with the regulation are scarce in GW, they always correspond to these two compounds, with the peculiarity that bromacil was withdrawn from the market more than 10 years ago. The fact that this herbicide is still regularly found in GW is a subject of concern, although its concentration seems to be in progressive decrease in the last few years.

In previous campaigns ("Confederación Hidrográfica del Júcar, O.A," 2018) performed in SW (since 2004 for imazalil, and since 2013 for thiabendazole), imazalil and thiabendazole were found above 0.1 μg/L in several samples. Both are authorized post-harvest fungicides widely used at present. They are frequently found in SW samples from this area, but there is not a maximum allowable concentration established (RD 817/2015, (Ministerio de Agricultura Alimentación y Medio Ambiente, 2015)).

For the other analytes detected, which were submitted to validation in this study, a high frequency of detection was in general observed for most of the triazines in GW and for benzimidazoles in SW (**Table 2 and 3**). The levels did not seem to exceed the maximum concentrations set up by legislation (GW and SW), even though many of these compounds have been detected historically in different geographical areas where an intensive agricultural activity takes place.

Several compounds detected in the screening applied in this work had not been included until now in the regular monitoring performed by JRB Authority. These compounds were acetamiprid, azoxystrobin, diazinon, isoproturon, MCPA, metalaxyl, nicosulfuron, paclobutrazol, propamocarb, propiconazole, tebuconazole, tetraconazole and the TPs MOA and T2H in SW, and bentazone, carbendazim, imidacloprid, metalaxyl, terbacil, and the TPs MOA and T2H in GW. Among them, the use of terbumeton, terbacil and carbendazim was unauthorized in 2003, 2007 and 2011, respectively. This illustrates the relevance of performing large scope screening to widen the list of compounds monitored, with the aim of detecting compounds not included in the regular target quantitative analysis.

3.2.2. Pesticides detected in other geographical areas

The presence of pesticides in each RB District has been periodically monitored by the RB Authorities. Previous results in SW and GW revealed the presence of a notable number of pesticides in the studied areas, illustrating the impact of these compounds in our country, where agriculture plays an important role. **Table 4** shows the compounds, among those found in the present work, also found in other geographical areas. Although a comparison of results between areas is complicated because of the different analytical methodologies applied and different compounds included in the methods, it can be observed that many of the pesticides found in other Spanish RB Districts were also present in the water bodies of the JRB District, particularly in SW. However, some pesticides/TPs frequently detected in other areas were not found in SWs of the JRB District, such as chlorfenvinphos, dichlofenthion, prochloraz, pyriproxyfen, phenoxon sulfoxide (Belenguer et al., 2014; Ccanccapa et al., 2016a).

Table 4. Pesticides detected in different geographical areas.

River Hydrographical Basin	Type water	Pesticides detected[a]	Reference
Duero	SW	metalaxyl, metolachlor, simazine, terbuthylazine	Hildebrandt et al., (2008)
	GW	atrazine, DEA, DIA, metalaxyl, simazine, terbuthylazine	
Ebro	SW	carbendazim, diazinon, imazalil, imidacloprid, T2H, thiabendazole	Ccanccapa et al., (2016b)
Guadalquivir	SW	simazine, terbuthylazine	Hermosin et al., (2013)
	GW	atrazine, DEA, DIA, diuron, simazine, terbuthylazine	
Guadiana	SW	isoproturon, MCPA, terbuthylazine	Palma et al., (2014)
Miño-Sil	SW	terbutylazine	Dagnac et al., (2012)
Segura	SW	diazinon, simazine, TD, terbumeton, terbuthylazine, terbutryn	Moreno-González et al., (2013)
Tajo	SW	diazinon, imazalil, metolachlor, terbutryn, terbutylazine	Gómez et al., (2012)
Catalonia (Internal Basin)	GW	atrazine, bentazone, DEA, DIA, diuron, imazalil, simazine, terbuthylazine	(Cabeza et al., 2012) (Köck-Schulmeyer et al., 2014)
	SW	acetamiprid, imidacloprid, isoproturon, MCPA, simazine	(Kuster et al., 2008) (Rubirola et al., 2017)
Júcar	SW	carbendazim, diazinon, imidacloprid, imazalil, isoproturon, metalaxyl, metolachlor, propiconazole, simazine, TED, T2H, terbuthylazine, terbutryn, thiabendazole	(Ccanccapa et al., 2016a) (Rousis et al., 2017) (Masiá et al., 2013) (Benvenuto et al., 2010) (Kuzmanović et al., 2015)
	GW	atrazine, bentazone, bromacil, carbendazim, DIA, DEA, diuron, imazalil, imidacloprid, simazine, TED, TD, T2H, terbacil, terbumeton, terbuthylazine	(Pitarch et al., 2016) (Hernández et al., 2008) (Menchen et al., 2017) (Portolés et al., 2014)

[a]: only those found in this study have been included.

In relation to GW, it is remarkable that triazine herbicides and some of their TPs are the predominant compounds, a fact that can be explained by their wide use in agricultural activities (Köck-Schulmeyer et al., 2014) and illustrates that most GW in Spain are vulnerable to this type of compounds. Therefore, the strict control of triazines and TPs is highly recommended. Another compound of concern is bromacil, an herbicide widely used in citrus crops until it was banned 15 years ago (Menchen et al., 2017). Its high water solubility (815 mg/L, at 20 °C) and its potential leaching index (GUS = 3.44) may explain the pollution of GW by this compound ("IUPAC, Pesticide properties database," 2007). This is also because it is relatively persistent and poorly adsorbed onto the soil (Zhu and Li, 2002).

3.2.3. Hydrogeological issues

In general, more pesticides were detected in SW than GW samples (**Table 2 and 3**). This fact seems to be related with the dominant mechanisms of propagation of pollutants. The arrival of pesticides to SW bodies can be produced by surface runoff in periods of intense precipitation (SW2 sample) or by dragging abandoned waste (Sancho et al., 2004). Frequently, discharges to natural watercourses of effluents from water treatment plants, which treat wastewater from agro-food industries, are the focus of contamination in the studied area. This seems to be the case of SW3, SW4 and SW6 samples, as imazalil and thiabendazole are frequently used in the post-harvest treatment of citrus fruits. In fact, imazalil was detected in 67% of the SW samples and Thiabendazole in 100% (**Table 3**).

In contrast, fewer pesticides, usually at lower concentration levels, were detected in GW. In this case, the origin was associated to the direct use in the crop fields. The transport mechanism from the surface to the groundwater is the percolation through the unsaturated zone, favored by rainfall or, in some cases, by intensive irrigation (flooding), which is still used in some areas.

The two main factors that govern the movement of pesticides are the thickness and nature of the unsaturated zone. Moreover, the reactivity of each compound is responsible for its leaching potential, which depends on its solubility and half-life which, in turn, is a function of its degradability and its tendency to adsorption (Morell and Candela, 1998).

In this work, however, the most significant factor seems to be the nature of the unsaturated zone. Thus, GW31, GW32 and GW51 samples are located in karstic aquifers, in which the transit of pesticides, in general, is faster and the attenuation reactions are of less intensity than in detrital aquifers. These kind of aquifers have high intrinsic vulnerability and GW is reached more easily by pesticides (Rodríguez et al., 2018).

Conversely, in detrital aquifers the attenuation processes are more intense and the leaching of pollutants is lower (Brusseau, 1995). Thus, in GW12, GW41, GW42 and GW44 samples, which correspond to detrital aquifers, the number of pesticides found was lower.

Around 30% of the GW samples with more than 10 detected compounds (**Tables SM2 and 2**) were associated with detrital materials, whereas the remaining 70% with karst materials.

4. Conclusions

In this work, a wide-scope screening of pesticides has been applied to surface water and groundwater samples from the Júcar River Basin District, an important geographical area in Spain where agriculture plays a relevant role with predominance of citric crops. Groundwater quality is a key issue, as irrigation by GW is about half of total water abstractions, and is the main source of drinking water supply in the area under study. Therefore, it is essential to protect GW from pesticide pollution. After LC-HRMS screening of water samples from this area, a total of 27 pesticides and 6 TPs were identified. The most frequent compounds in GW were triazine herbicides and several of their TPs, whereas in the SW were mainly fungicides, such as carbendazim, thiabendazole and imazalil, as well as the herbicide triazine and terbutryn, and the TP terbumeton-desethyl.

Several of the compounds identified in the present screening had not been included in monitoring campaigns periodically performed in the area (e.g. metalaxyl, propiconazole, tebuconazole, carbendazim, MOA, T2H), where target quantitative methods were applied for a limited number of compounds. This fact highlights that the commonly applied quantitative target methods (although are sensitive, reliable and accurate) are restricted to the target list of compounds with the consequence that several compounds present in the samples may be ignored. Our data are useful to update the information gathered by River Basin Authorities.

This is especially relevant for those compounds that are found in water bodies despite their use is not allowed according to the current legislation.

This work illustrates the need of widening the scope of the methods making use of HRMS in order to identify the most problematic compounds, including transformation products. This allows focusing subsequent quantitative analysis on those compounds actually present in the water environment. The notable number of pesticides and TPs found in water demonstrates the vulnerability of this area and the impact of the use of pesticides. The information obtained in this study should be supported by concentration data obtained in future monitoring campaigns periodically applied to waters collected in the JRB District applying quantitative methods with an updated list of target compounds.

The combination of the next three elements will allow a comprehensive overview of the pesticides impact in SW and GW in a river basin: 1) searching data of the pesticides use in the study area, 2) qualitative and quantitative assessment of pesticides occurrence in waters, and 3) development of a conceptual model, and also a mathematical one if possible, to establish the relationship between pesticides application and their presence in SW and GW.

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SUPPLEMENTARY MATERIAL

Investigation of pesticides and their transformation products in the Júcar River Hydrographical Basin (Spain) by wide-scope high-resolution mass spectrometry screening

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Materials and methods

Table SM1. Class, common name, CAS number, biocide action, molecular weight, chemical structure, chemical formula and some physicochemical properties of the compounds studied (IUPAC, Pesticide properties database., 2007) (Conrad et al., 2006).

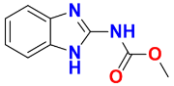
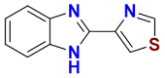
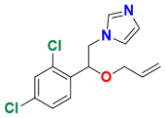
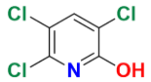
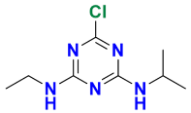
Pesticide name (CAS number)	Biocide action	$[M+H]^+$	Chemical structure[a]	Chemical formula	Water solubility (mg L^{-1} at 20 °C)	p*K*a (25 °C)	log P (pH 7, 20 °C)
Benzimidazole							
Carbendazim (10605-21-7)	Fungicide	192.0773		$C_9H_9N_3O_2$	8.0	4.2	1.48
Thiabendazole (148-79-8)	Fungicide	202.0439		$C_{10}H_7N_3S$	30	4.73	2.39
Imidazole							
Imazalil (35554-44-0)	Fungicide	297.0561		$C_{14}H_{14}Cl_2N_2O$	184	6.49	2.56
Organophosphorus							
Chlorpyrifos (2921-88-2)	Insecticide	349.9341		$C_9H_{11}Cl_3NO_3PS$	1.05	NA	4.7
3,5,6-trichloro-2-pyridinol (TCP) (6515-38-4)	TP	197.9280		$C_5H_2Cl_3NO$	80.9	NA	3.21
Triazine							
Atrazine (1912-24-9)	Herbicide	216.1016		$C_8H_{14}ClN_5$	35	1.7	2.7

Table SM1 (cont.). Class, common name, CAS number, biocide action, molecular weight, chemical structure, chemical formula and some physicochemical properties of the compounds studied (IUPAC, Pesticide properties database., 2007) (Conrad et al., 2006).

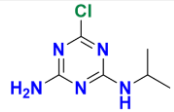
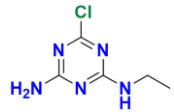
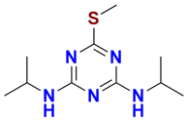
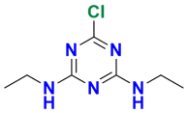
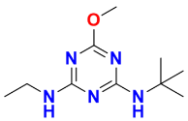
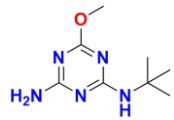
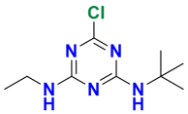
Pesticide name (CAS number)	Biocide action	$[M+H]^+$	Chemical structure[a]	Chemical formula	Water solubility (mg L^{-1} at 20 °C)	p*K*a (25 °C)	log P (pH 7, 20 °C)
Atrazine-desethyl (DEA) (6190-65-4)	TP	188.0703		$C_6H_{10}ClN_5$	2700	NA	1.51
Atrazine-desisopropyl (DIA) (1007-28-9)	TP	174.0546		$C_5H_8ClN_5$	980	NA	1.15
Prometryn (7287-19-6)	Herbicide	242.1439		$C_{10}H_{19}N_5S$	33	4.1	3.34
Simazine (122-34-9)	Herbicide	202.0859		$C_7H_{12}ClN_5$	5	1.62	2.3
Terbumeton (33693-04-8)	Herbicide	226.1668		$C_{10}H_{19}N_5O$	130	NA	3.04
Terbumeton-desethyl (30125-64-5)	TP	198.1355		$C_8H_{15}N_5O$	NA	4.39	1.93
Terbuthylazine (5915-41-3)	Herbicide	230.1172		$C_9H_{16}ClN_5$	6.6	1.9	3.4

Table SM1 (cont.). Class, common name, CAS number, biocide action, molecular weight, chemical structure, chemical formula and some physicochemical properties of the compounds studied (IUPAC, Pesticide properties database., 2007) (Conrad et al., 2006).

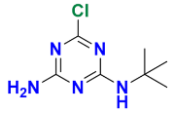
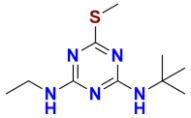
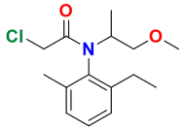
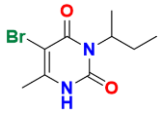
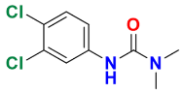
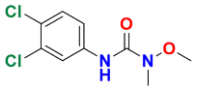
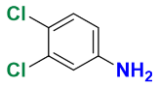
Pesticide name (CAS number)	Biocide action	$[M+H]^+$	Chemical structure[a]	Chemical formula	Water solubility (mg L^{-1} at 20 °C)	p*K*a (25 °C)	log P (pH 7, 20 °C)
Terbuthylazine-desethyl (30125-63-4)	TP	202.0859		$C_7H_{12}ClN_5$	327.1	NA	2.3
Terbutryn (886-50-0)	Herbicide	242.1439		$C_{10}H_{19}N_5S$	25	4.3	3.66
Chloroacetanilide							
Metolachlor (51218-45-2)	Herbicide	284.1417		$C_{15}H_{22}ClNO_2$	530	NA	3.4
Uracil							
Bromacil (314-40-9)	Herbicide	261.0239		$C_9H_{13}BrN_2O_2$	815	9.27	1.88
Urea							
Diuron (330-54-1)	Herbicide	233.0248		$C_9H_{10}Cl_2N_2O$	35.6	NA	2.87
Linuron (330-55-2)	Herbicide	249.0198		$C_9H_{10}Cl_2N_2O_2$	63.8	NA	3.0

Table SM1 (cont.). Class, common name, CAS number, biocide action, molecular weight, chemical structure, chemical formula and some physicochemical properties of the compounds studied (IUPAC, Pesticide properties database., 2007) (Conrad et al., 2006).

Pesticide name (CAS number)	Biocide action	$[M+H]^+$	Chemical structure[a]	Chemical formula	Water solubility (mg L^{-1} at 20 °C)	p*K*a (25 °C)	log P (pH 7, 20 °C)
Others							
3,4-dichloroaniline (95-76-1)	TP	161.9877		$C_6H_5Cl_2N$	580	2.97	2.69

[a]Chemical structures created using ChemDraw Professional, Version 16.0, PerkinElmer Informatics
TP = Transformation Product
NA = Not applicable

General site description

Geological and hydrogeological characterization of the study area

The topographic, geological and hydrogeological description of the study area is complicated due to its extension and heterogeneity. The altitude varies between sea level in the coast (Mediterranean sea) and 2,000 m above sea level (a.s.l.) inland (Iberian System, Peñarroya Peak 2,024 m a.s.l) (**Figure 1**). In the coastal zone, some coastal plains are located, in which wetlands of great environmental and hydrogeological value are present (or they have been in the past) (**Figure 1**).

From a structural point of view, three sectors are differentiated. In the south, structures with predominance of the Betic Mountain range orientation (NE-SW) are located. In the central sector the predominance of the Iberian Mountain range is clear in the structures but its continuity, in the North sector, is interrupted by a series of structures of Catalan coastal chain (NNE-SSW) ("Confederación Hidrográfica del Júcar, O.A," 2018).

From a geological and hydrogeological point of view, it is difficult to describe the materials that define the JRB District. Three general domains can be distinguished. A clear predominance of carbonated materials is observed; in the southwestern sector and in the half northern area, limestones and dolomites with karstification are found, which provide an important aquiferous character. On the coast (coastal plains), in the banks of the main rivers (Júcar and Turia River) and in the valleys of the mountain ranges (piedmont materials), detrital heterogeneous materials are found, with non-uniform distribution, few development (depth) and generally high permeability. The third domain corresponds to the sedimentary rocks, the sandstone, which present a greater or lesser fracture and therefore the degree of water storage is highly variable. They are located in disseminated way throughout the central sector of the Basin, with the exception of a sector close to the coast in the northern half. Even so, the surface occupied by materials (sandstone) is a small percentage compared to the rest of the River Basin District ("Confederación Hidrográfica del Júcar, O.A," 2018).

The hydrological and hydrogeological regime works in a different way. The first one is affected by floods and drought events. On the other hand, the GW flow, although it is difficult to describe due to the large number of aquifers and the heterogeneous nature, usually flows from

inland to the coast. The hydric resources have been deeply altered due to human activities, and water resources are under increasing pressure (Ccanccapa et al., 2016).

Coastal aquifers, where the most intensive agriculture is developed, are permeable by primary porosity and have a low thickness of the unsaturated zone, which is why they have a high intrinsic vulnerability to contamination. Also, the internal aquifers, mostly of karstic type and with little coating, are very vulnerable. The polluting processes put at risk the guarantee of supply for human consumption that, in many cases, is carried out with GW.

Monitoring network and sampling

Table SM2. Monitoring network location (UTM), type of water, and ID.

SW bodies					
ID	**Station Code***	**Water Origin**	**UTM X**	**UTM Y**	**Observations**
SW1	10.13	SW	751984	4423832	River
SW2	11.01	SW	752052	4418431	Delta
SW3	15.17	SW	714520	4377235	River
SW4	15.18	SW	719768	4373908	River
SW5	18.14.01.06	SW	604662	4320653	Canal (artificial)
SW6	18.32.01.11	SW	716047	4342718	River
SW7	18.36	SW	736034	4339868	River
SW8	31.04	SW	687430	4271216	River

Table SM2 (cont.). Monitoring network location (UTM), type of water, and ID.

GW bodies					
ID	**Station Code***	**Water Origin**	**UTM X**	**UTM Y**	**Observations**
GW11	08.127.CA003	GW	744463	4416684	Detrital aquifer
GW12	08.127.CA593	GW	746556	4421004	Detrital aquifer
GW2	08.131.CA004	GW	728572	4385259	Karstic aquifer
GW31	08.140.CA002	GW	721682	4354894	Karstic aquifer
GW32	08.140.CA142	GW	706550	4370310	Karstic aquifer
GW41	08.142.CA003	GW	723552	4347207	Detrital aquifer
GW42	08.142.CA006	GW	725044	4352583	Detrital aquifer
GW43	08.142.CA008	GW	718899	4350861	Detrital aquifer
GW44	08.142.CA188	GW	714860	4339814	Detrital aquifer
GW51	08.149.CA001	GW	721553	4333742	Karstic aquifer
GW52	08.149.CA004	GW	723273	4336604	Karstic aquifer

UTM: Universal Transverse Mercator

* by JRB Authority

Table SM3. Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
2-hydroxy-atrazine	C8H15N5O	4.04	198.1355
2-hydroxy-atrazine F1	C5H9N5O	4.04	156.0885
2-hydroxy-atrazine F2	C4H7N3O	4.04	114.0667
2-hydroxy-simazine	C7H13N5O	2.86	184.1198
2-hydroxy-simazine F1	C4H7N3O	2.86	114.0667
2-hydroxy-simazine F2	C2H3N3O	2.86	86.0354
2-hydroxy-terbuthylazine	C9H17N5O	5.28	212.1511
2-hydroxy-terbuthylazine F1	C5H9N5O	5.28	156.0885
2-hydroxy-terbuthylazine F2	C2H3N3O	5.28	86.0354
Acetamiprid	C10H11N4Cl	5.4	223.0750
Acetamiprid (Na)	C10H10N4ClNa	5.4	245.0570
Acetamiprid F1	C6H4NCl	5.4	126.0111
Alachlor	C14H20ClNO2	11.6	270.1261
Alachlor (Na)	C14H19NaClNO2	11.6	292.1080
Alachlor F1	C13H16NOCl	11.6	238.0999
Alachlor F2	C11H15N	11.6	162.1283
Aldicarb	C7H14N2O2S	6.61	191.0854
Aldicarb (Na)	C7H13NaN2O2S	6.62	213.0674
Aldicarb F1	C5H9NS	6.61	116.0534
Aldicarb F2	C4H8S	6.62	89.0425
Aldicarb sulfone	C7H14N2O4S	2.92	223.0753
Aldicarb sulfone (Na)	C7H13NaN2O4S	2.92	245.0572
Aldicarb sulfone F1	C4H7NO	2.92	86.0606
Aldicarb sulfoxide	C7H14N2O3S	2.62	207.0803
Aldicarb sulfoxide (Na)	C7H13NaN2O3S	2.62	229.0623
Aldicarb sulfoxide F1	C4H8S	2.62	89.0425
Aldicarb sulfoxide F2	C5H9NOS	2.62	132.0483
Atrazine	C8H14N5Cl	9.18	216.1016
Atrazine F1	C5H8N5Cl	9.18	174.0546
Atrazine F2	C2H2N3Cl	9.18	104.0015
Atrazine F3	C4H5N3	9.18	96.0562
Azaconazol	C12H11Cl2N3O2	9.72	300.0307
Azaconazol F1	C7H4Cl2	9.72	158.9768
Azaconazol F2	C10H8O2Cl2	9.72	230.998

Table SM3 (cont.). Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
Azinphos-ethyl	C12H16N3O3PS2	11.42	346.0449
Azinphos-ethyl F1	C9H11N3O2PS2	11.42	289.0109
Azinphos-ethyl F2	C7H7N3O2PS2	11.42	260.9796
Azinphos-ethyl F3	C8H5NO	11.42	132.0449
Azinphos-methyl	C10H12N3O3PS2	9.96	318.0136
Azinphos-methyl (Na)	C10H11N3O3PS2Na	9.96	339.9955
Azinphos-methyl F1	C8H5N3O	9.96	160.0511
Azinphos-methyl F2	C8H5NO	9.96	132.0449
Azoxystrobin	C22H17N3O5	10.47	404.1246
Azoxystrobin (Na)	C22H16NaN3O5	10.47	426.1066
Azoxystrobin F1	C21H13N3O4	10.47	372.0984
Azoxystrobin F2	C19H10N3O3	10.47	329.0800
Bensulide	C14H24NO4PS3	12.07	398.0683
Bensulide (Na)	C14H23NO4PS3Na	12.07	420.0503
Bensulide F1	C6H7NO2S	12.07	158.0276
Bensulide F2	C6H4O2S	12.07	141.0010
Bifenazate	C17H20N2O3	11.35	301.1552
Bifenazate (Na)	C17H19N2O3Na	11.35	323.1372
Bifenazate F1	C13H11NO	11.35	198.0919
Bifenazate F2	C12H11N	11.35	170.0970
Bixafen	C18H12Cl2F3N3O	12.13	414.0388
Bixafen (Na)	C18H11Cl2F3N3ONa	12.13	436.0207
Bixafen F1	C13H6NFCl2	12.13	265.9940
Bixafen F2	C18H10N3OFCl2	12.13	374.0263
Boscalid	C18H12Cl2N2O	10.8	343.0405
Boscalid F1	C18H11N2OCl	10.8	307.0638
Boscalid F2	C18H11N2O	10.8	272.0950
Bromacil	C9H13BrN2O2	7.78	261.0239
Bromacil (Na)	C9H12NaBrN2O2	7.78	283.0058
Bromacil F1	C5H5N2O2Br	7.78	204.9613
Bromophos ethyl	C10H12BrCl2O3PS	14.95	392.8883
Bromophos methyl	C8H8BrCl2O3PS	13.99	364.8570
Bromophos methyl F1	C6H4OCl2	13.99	162.9717
Bromophos methyl F2	C6H5O3PSCl2Br	13.99	337.8336

Table SM3 (cont.). Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
Bromuconazol 1	C13H12N3OCl2Br	11.2	375.9619
Bromuconazol 1 F1	C7H4Cl2	11.2	158.9768
Bromuconazol 2	C13H12N3OCl2Br	12.11	375.9619
Bromuconazol 2 F1	C7H4Cl2	12.11	158.9768
Buprofezin	C16H23N3OS	13.5	306.1640
Buprofezin F1	C7H7N	13.5	106.0657
Buprofezin F2	C9H16N2OS	13.5	201.1062
Buprofezin F3	C4H7NO	13.5	86.0606
Butachlor	C17H26ClNO2	13.85	312.1730
Butachlor (Na)	C17H25ClNO2Na	13.85	334.1550
Butachlor F1	C13H16NOCl	13.85	238.0999
Butachlor F2	C11H15N	13.85	162.1283
Butocarboxym	C7H14N2O2S	6.61	191.0854
Butocarboxym (Na)	C7H13N2O2SNa	6.61	213.0674
Butocarboxym F1	C3H6S	6.61	75.0268
Cadusafos	C10H23O2PS2	12.97	271.0955
Cadusafos F1	C6H15O2PS2	12.97	215.0329
Cadusafos F2	H3O2PS2	12.97	130.9390
Cadusafos F4	HO2PS	12.97	96.9513
Carbaryl	C12H11NO2	8.44	202.0868
Carbaryl (Na)	C12H10NaNO2	8.44	224.0687
Carbaryl F1	C10H8O	8.44	145.0653
Carbaryl F2	C10H6	8.44	127.0548
Carbendazim	C9H9N3O2	3.39	192.0773
Carbendazim F1	C8H5N3O	3.39	160.0511
Carbendazim F2	C7H5N3	3.39	132.0562
Carbetamide	C12H16N2O3	7.27	237.1239
Carbetamide (Na)	C12H15N2O3Na	7.27	259.1059
Carbetamide F1	C7H5NO	7.27	120.0449
Carbofuran	C12H15NO3	7.97	222.1130
Carbofuran (Na)	C12H14NaNO3	7.97	244.0950
Carbofuran F1	C10H12O2	7.97	165.0916
Carbofuran F2	C7H6O2	7.97	123.0446
Carbofuran-3-OH	C12H15NO4	5.37	238.1079

Table SM3 (cont.). Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
Carbofuran-3-OH (Na)	C12H14NaNO4	5.37	260.0899
Carbofuran-3-OH F1	C12H13NO3	5.37	220.0974
Carbofuran-3-OH F2	C10H10O2	5.37	163.0759
Carfentrazone ethyl	C15H14Cl2F3N3O3	12.12	412.0443
Carfentrazone ethyl (Na)	C15H13Cl2F3N3O3Na	12.12	434.0262
Carfentrazone ethyl F1	C13H7N3O2F2Cl2	12.12	345.9962
Carfentrazone ethyl F2	C13H8N3O2F3Cl2	12.12	366.0024
Chlorfenvinphos	C12H14Cl3O4P	12.5	358.9774
Chlorfenvinphos (Na)	C12H13NaCl3O4P	12.5	380.9593
Chlorfenvinphos F1	C2H7O4P	12.5	127.0160
Chlorfenvinphos F2	C4H11O4P	12.5	155.0473
Chloridazon	C10H8N3OCl	5.33	222.0434
Chloridazon (Na)	C10H7N3OClNa	5.33	244.0254
Chloridazon F1	C7H5N	5.33	104.0500
Chloridazon F2	C6H5N	5.33	92.0500
Chlorpropham	C10H12ClNO2	10.99	214.0635
Chlorpropham (Na)	C10H11ClNO2Na	10.99	236.0455
Chlorpropham F1	C7H6NO2Cl	10.99	172.0165
Chlorpyrifos	C9H11Cl3NO3PS	14.06	349.9341
Clorpyrifos (Na)	C9H10NaCl3NO3PS	14.06	371.9161
Chlorpyrifos F1	C5H2NOCl3	14.06	197.9280
Chlorpyrifos F2	C2H5O2PS	14.06	124.9826
Chlorpyrifos-methyl	C7H7Cl3NO3PS	12.94	321.9028
Chlorpyrifos-methyl F1	C2H5O2PS	12.94	124.9826
Chlorpyrifos-methyl F2	C6H3NO2PSCl3	12.94	289.8766
Chlorsulfuron	C12H12N5O4SCl	8.61	358.0377
Chlorsulfuron (Na)	C12H11N5O4SClNa	8.61	380.0196
Chlorsulfuron F1	C6H6N4O2	8.61	167.0569
Chlorsulfuron F2	C5H8N4O	8.61	141.0776
Clodinafop-Propargyl	C17H13ClFNO4	12.13	350.0595
Clodinafop-Propargyl F1	C13H9NO2FCl	12.13	266.0384
Clodinafop-Propargyl F2	C11H5NOFCl	12.13	222.0122
Clomazone	C12H14ClNO2	10.05	240.0791
Clomazone (Na)	C12H13ClNO2Na	10.05	262.0611

Table SM3 (cont.). Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
Clomazone F1	C7H5Cl	10.05	125.0158
Clothianidin	C6H8ClN5O2S	4.76	250.0165
Clothianidin (Na)	C6H7ClN5O2SNa	4.76	271.9985
Clothianidin F1	C6H8N4S	4.76	169.0548
Clothianidin F2	C4H2NSCl	4.76	131.9675
Coumaphos	C14H16ClO5PS	12.36	363.0223
Coumaphos F1	C10H8O5PSCl	12.36	306.9597
Coumaphos F2	C10H7O2SCl	12.36	226.9934
Cyproconazol 1	C15H18ClN3O	11.39	292.1217
Cyproconazol 1 F1	C7H5Cl	11.39	125.0158
Cyproconazol 1 F2	C2H3N3	11.39	70.0405
Cyproconazol 2	C15H18ClN3O	11.06	292.1217
Cyproconazol 2 F1	C7H5Cl	11.06	125.0158
Cyproconazol 2 F2	C2H3N3	11.06	70.0405
Cyprodinil	C14H15N3	11.43	226.1344
Cyprodinil F1	C13H11N3	11.43	210.1031
Cyprodinil F2	C7H9N	11.43	108.0813
Dichlorvos	C4H7Cl2O4P	7.75	220.9537
Dichlorvos (Na)	C4H6Cl2O4PNa	7.75	242.9357
Deethyl terbuthylazine	C7H12N5Cl	8.36	202.0859
Deethyl terbuthylazine F1	C3H4N5Cl	8.36	146.0233
Deethyl terbuthylazine F2	C3H3N5	8.36	110.0467
Deethylatrazine	C6H10N5Cl	5.9	188.0703
Deethylatrazine F1	C3H4N5Cl	5.9	146.0233
Deethylatrazine F1	C2H2N3Cl	5.9	104.0015
Deethylterbumeton	C8H15N5O	6.22	198.1355
Deethylterbumeton F1	C4H7N5O	6.22	142.0729
Deethylterbumeton F2	C2H3N3O	6.22	86.0354
Deisopropyl-2-hydroxy-atrazine	C5H9N5O	0.99	156.0885
Deisopropyl-2-hydroxy-atrazine	C2N2O	0.99	69.0089
Deisopropyl-2-hydroxy-atrazine	C2H3N3O	0.99	86.0354
Deisopropylatrazine (DIA)	C5H8N5Cl	4.22	174.0546
DIA F2	C4H6N3Cl	4.22	132.0329
DIA F3	C2H2N3Cl	4.22	104.0015

Table SM3 (cont.). Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
DIA F4	C4H5N3	4.22	96.0562
Diazinon	C12H21N2O3PS	12.39	305.1089
Diazinon F1	C8H12N2S	12.39	169.0799
Diazinon F2	C8H12N2O	12.39	153.1028
Dichlofenthion	C10H13Cl2O3PS	13.95	314.9778
Dichlofenthion (Na)	C10H12NaCl2O3PS	13.95	336.9598
Dichlofenthion F1	C8H9O3PSCl2	13.95	286.9465
Dichlofenthion F2	C6H5O3PSCl2	13.95	258.9152
Dicrotophos	C8H16NO5P	4.49	238.0844
Dicrotophos (Na)	C8H15NO5PNa	4.49	260.0664
Dicrotophos F1	C2H7O4P	4.49	127.0160
Dicrotophos F2	C6H9NO	4.49	112.0762
Difenoconazole	C19H17Cl2N3O3	13.08	406.0725
Difenoconazole F1	C13H8OCl2	13.08	251.0030
Difenoconazole F2	C17H14O3Cl2	13.08	337.0398
Diflubenzuron	C14H9ClF2N2O2	11.93	311.0399
Diflubenzuron (Na)	C14H8ClF2N2O2Na	11.93	333.0218
Diflubenzuron F1	C7H5NOF2	11.93	158.0417
Diflubenzuron F2	C7H2OF2	11.93	141.0152
Diflufenican	C19H11F5N2O2	13.18	395.0819
Diflufenican F1	C13H6NO2F3	13.18	266.0429
Diflufenican F2	C13H5NO2F2	13.18	246.0367
Dimetachlor	C13H18ClNO2	9.81	256.1104
Dimetachlor (Na)	C13H17ClNO2Na	9.81	278.0924
Dimetachlor F1	C12H14NOCl	9.81	224.0842
Dimetachlor F2	C10H13N	9.81	148.1126
Dimethoate	C5H12NO3PS2	5.19	230.0074
Dimethoate (Na)	C5H11NaNO3PS2	5.19	251.9894
Dimethoate F1	C2H5O2PS	5.19	124.9826
Dimethomorph 1	C21H22NO4Cl	10.67	388.1316
Dimethomorph 1 (Na)	C21H21NO4ClNa	10.67	410.1135
Dimetomorph 1 F1	C17H13O3Cl	10.67	301.0631
Dimetomorph 1 F2	C9NO	10.67	139.0058
Dimethomorph 2	C21H22NO4Cl	11.08	388.1316

Table SM3 (cont.). Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
Dimethomorph 2 (Na)	C21H21NO4ClNa	11.08	410.1135
Dimetomorph 2 F1	C17H13O3Cl	11.08	301.0631
Dimetomorph 2 F2	C9NO	11.08	139.0058
Diuron	C9H10N2OCl2	9.56	233.0248
Diuron F1	C3H5NO	9.56	72.0449
Ethiofencarb	C11H15NO2S	8.71	226.0902
Ethiofencarb (Na)	C11H14NaNO2S	8.71	248.0721
Ethiofencarb F1	C9H9NO2	8.71	164.0712
Ethiofencarb F2	C7H6O	8.71	107.0497
Ethiofencarb sulfone	C11H15NO4S	4.51	258.0800
Ethiofencarb sulfone (Na)	C11H14NaNO4S	4.51	280.0619
Ethiofencarb sulfone F1	C7H6	4.51	91.0548
Ethiofencarb sulfone F2	C7H6O	4.51	107.0497
Ethiofencarb sulfoxide	C11H15NO3S	4.71	242.0851
Ethiofencarb sulfoxide (Na)	C11H14NaNO3S	4.71	264.0670
Ethiofencarb sulfoxide F1	C7H6O	4.71	107.0497
Ethion	C9H22O4P2S4	13.89	384.9954
Ethion (Na)	C9H21NaO4P2S4	13.89	406.9774
Ethion F1	C5H11O2PS2	13.89	199.0016
Ethion F2	CH3O2PS2	13.89	142.9390
Ethofumesate	C13H18O5S	10.45	287.0953
Ethofumesate (Na)	C13H17NaO5S	10.45	309.0773
Ethofumesate F1	C11H12O4S	10.45	241.0535
Ethofumesate F2	C9H8O	10.45	133.0653
Ethoxyquin	C14H19NO	9.27	218.1545
Ethoxyquin dimer	C28H36N2O2	16.17	433.2855
Ethoxyquin dimer F2	C14H18N2O	16.17	231.1497
Ethoxyquin dimer F3	C24H26N2O2	16.17	375.2037
Ethoxyquin F1	C13H15NO	9.27	202.1232
Ethoxyquin F3	C11H11NO	9.27	174.0919
Fenamiphos	C13H22NO3PS	11.91	304.1136
Fenamiphos (Na)	C13H21NO3PSNa	11.91	326.0956
Fenamiphos F1	C8H9O3PS	11.91	217.0088
Fenamiphos F2	C7H6O3PS	11.91	201.9854

Table SM3 (cont.). Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
Fenhexamid	C14H17Cl2NO2	11.51	302.0715
Fenhexamid (Na)	C14H16Cl2NO2Na	11.51	324.0534
Fenhexamid F1	C7H12	11.51	97.1017
Fenitrothion	C9H12NO5PS	11.12	278.0238
Fenitrothion F5	C2H5O2PS	11.12	124.9826
Fenoxaprop	C16H12ClNO5	12.34	334.0482
Fenoxaprop (Na)	C16H11NaClNO5	12.34	356.0302
Fenoxaprop F1	C16H12NO5Cl	12.34	334.0482
Fenoxaprop F2	C15H10NO3Cl	12.34	288.0427
Fenoxycarb	C17H19NO4	12.04	302.1392
Fenoxycarb (Na)	C17H18NaNO4	12.04	324.1212
Fenoxycarb F1	C3H5NO2	12.04	88.0399
Fenpropimorph	C20H33NO	9.6	304.2640
Fenpropimorph F1	C11H14	9.6	147.1174
Fenpropimorph F2	C10H11	9.6	132.0939
Fenpropimorph F3	C7H15NO	9.6	130.1232
Fenpropimorph F4	C6H11N	9.6	98.0970
Fenthion	C10H15O3PS2	12.28	279.0278
Fenthion F1	C9H11O2S2P	12.28	247.0016
Fenthion F2	C8H9S	12.28	138.0503
Fluazifop-P-butyl	C19H20F3NO4	13.58	384.1423
Fluazifop-P-butyl (Na)	C19H19NaF3NO4	13.58	406.1242
Fluazifop-P-butyl F1	C15H12NO4F3	13.58	328.0797
Fluazifop-P-butyl F2	C14H10NO2F3	13.58	282.0742
Fludioxonil	C12H6F2N2O2	10.81	249.0476
Fludioxonil (Na)	C12H5NaF2N2O2	10.81	271.0295
Fludioxonil F1	C12H5N2O2F	10.81	229.0413
Flufenoxuron	C21H11ClF6N2O3	14.46	489.0441
Flufenoxuron (Na)	C21H10ClF6N2O3Na	14.46	511.0260
Flufenoxuron F1	C7H5NOF2	14.46	158.0417
Flufenoxuron F2	C7H2OF2	14.46	141.0152
Fluquinconazole	C16H8Cl2FN5O	11.39	376.0168
Fluquinconazole F1	C15H7N4OFCl2	11.39	349.0059
Fluquinconazole F2	C14H5N2OFCl2	11.39	306.9841

Table SM3 (cont.). Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
Fluroxypyr	C7H5Cl2FN2O3	7.34	254.9740
Fluroxypyr (Na)	C7H4Cl2FN2O3Na	7.34	276.9559
Fluroxypyr F1	C6H3N2OFCl2	7.34	208.9685
Fluroxypyr F2	C5H3N2FCl2	7.34	180.9736
Flutriazole	C16H13F2N3O	9.39	302.1105
Flutriazole F1	C7H3OF	9.39	123.0246
Flutriazole F2	C2H3N3	9.39	70.0405
Haloxyfop-2-ethoxyethyl	C19H19ClF3NO5	13.55	434.0982
Haloxyfop-2-ethoxyethyl (Na)	C19H18ClF3NO5Na	13.55	456.0802
Haloxyfop-2-ethoxyethyl F1	C14H9NO2F3Cl	13.55	316.0352
Haloxyfop-methyl	C16H13ClF3NO4	13.08	376.0563
Haloxyfop-methyl (Na)	C16H12NaClF3NO4	13.08	398.0383
Haloxyfop-methyl F1	C14H9NO2F3Cl	13.08	316.0352
Hexythiazox	C17H21ClN2O2S	14.11	353.1091
Hexythiazox (Na)	C17H20NaClN2O2S	14.11	375.0910
Hexythiazox F1	C10H10NOSCl	14.11	228.0250
Hexythiazox F2	C9H10NCl	14.11	168.0580
Imazalil	C14H14Cl2N2O	8.48	297.0561
Imazalil F1	C11H8N2OCl2	8.48	255.0092
Imazalil F2	C9H6OCl2	8.48	200.9874
Imazalil F3	C7H4Cl2	8.48	158.9768
Imidacloprid	C9H10ClN5O2	4.72	256.0601
Imidacloprid (Na)	C9H9NaClN5O2	4.72	278.0421
Imidacloprid F1	C9H9N4Cl	4.72	209.0594
Imidacloprid F2	C9H10N4	4.72	175.0984
Indoxacarb	C22H17ClF3N3O7	13.18	528.0785
Indoxacarb (Na)	C22H16NaClF3N3O7	13.18	550.0605
Indoxacarb F1	C13H9N2O4Cl	13.18	293.0324
Iprodione	C13H13Cl2N3O3	11.9	330.0412
Iprodione (Na)	C13H12Cl2N3O3Na	11.9	352.0232
Iprodione F1	C9H6N2O2Cl2	11.9	224.9885
Iprodione F2	C6H5NCl2	11.9	161.9877
Iprovalicarb	C18H28N2O3	11.37	321.2178
Iprovalicarb F1	C9H18N2O3	11.37	203.1396

Table SM3 (cont.). Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
Iprovalicarb F3	C9H10	11.37	119.0861
Isoproturon	C12H18N2O	9.43	207.1497
Isoproturon (Na)	C12H17NaN2O	9.43	229.1317
Isoproturon F1	C3H5NO	9.43	72.0449
Isopyrazam	C20H23F2N3O	13.08	360.1887
Isopyrazam F1	C13H10N3OF	13.08	244.0886
Isopyrazam F2	C20H21N3O	13.08	320.1763
Isoxaben	C18H24N2O4	10.95	333.1814
Isoxaben F1	C9H8O3	10.95	165.0552
Isoxaben F2	C8H5O3	10.95	150.0317
Linuron	C9H10Cl2N2O2	10.4	249.0198
Linuron F1	C8H6N2OCl	10.4	182.0247
Linuron F2	C6H3NCl2	10.4	159.9721
Lufenuron	C17H8Cl2F8N2O3	13.99	510.9862
Lufenuron (Na)	C17H7Cl2F8N2O3Na	13.99	532.9682
Lufenuron F1	C7H2OF2	13.99	141.0152
Lufenuron F2	C7H5NOF2	13.99	158.0417
Malaoxon	C10H19O7PS	8.18	315.0667
Malaoxon (Na)	C10H18O7PSNa	8.18	337.0487
Malaoxon F1	C4H2O3	8.18	99.0082
Malaoxon F2	C6H6O3	8.18	127.0395
Malathion	C10H19O6PS2	10.93	331.0439
Malathion (Na)	C10H18NaO6PS2	10.93	353.0258
Malathion F1	C2H5O2PS	10.93	124.9826
Malathion F2	CH3O2P	10.93	78.9949
Mepanipyrim	C14H13N3	11.26	224.1188
Mepanipyrim F1	C7H7N	11.26	106.0657
Mepanipyrim F2	C7H5N	11.26	104.0500
Mephosfolan	C8H16NO3PS2	7.79	270.0387
Mephosfolan (Na)	C8H15NO3PS2Na	7.79	292.0207
Mephosfolan F1	CH2NO3SP	7.79	139.9571
Mephosfolan F2	C3H6NO3SP	7.79	167.9884
Metalaxyl	C15H21NO4	9.54	280.1549
Metalaxyl (Na)	C15H20NaNO4	9.54	302.1368

Table SM3 (cont.). Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
Metalaxyl F1	C14H17NO3	9.54	248.1287
Metalaxyl F2	C13H17NO2	9.54	220.1338
Metalaxyl F4	C11H13N	9.54	160.1126
Metconazole	C17H22N3OCl	12.69	320.1530
Metconazole F1	C2H3N3	12.69	70.0405
Metconazole F2	C7H5Cl	12.69	125.0158
Methabenzthiazuron	C10H11N3OS	9.15	222.0701
Methabenzthiazuron F1	C8H8N2S	9.15	165.0486
Methabenzthiazuron F2	C7H5N2S	9.15	150.0252
Methamidophos	C2H8NO2PS	1.12	142.0092
Methamidophos F1	C2H5O2PS	1.12	124.9826
Methidathion	C6H11N2O4PS3	9.73	302.9697
Methidathion (Na)	C6H10N2O4PS3Na	9.73	324.9516
Methidathion F1	C4H4N2O2S	9.73	145.0072
Methidathion F2	C3H4N2O	9.73	85.0402
Methiocarb	C11H15NO2S	8.71	226.0902
Methiocarb (Na)	C11H14NaNO2S	8.71	248.0721
Methiocarb F1	C9H12OS	8.71	169.0687
Methiocarb F2	C7H6O	8.71	107.0497
Methiocarb sulfone	C11H15NO4S	4.51	258.0800
Methiocarb sulfone (Na)	C11H14NO4SNa	4.51	280.0619
Methiocarb sulfone F1	C9H12O3S	4.51	201.0585
Methiocarb sulfone F2	C7H6O	4.51	107.0497
Methiocarb sulfoxide	C11H15NO3S	5.12	242.0851
Methiocarb sulfoxide (Na)	C11H14NO3SNa	5.12	264.0670
Methiocarb sulfoxide F1	C9H12O2S	5.12	185.0636
Methiocarb sulfoxide F2	C8H9O2S	5.12	170.0402
Methiocarb sulfoxide F3	C8H9O	5.12	122.0732
Methomyl	C5H10N2O2S	3.42	163.0541
Methomyl (Na)	C5H9N2O2SNa	3.42	185.0361
Methomyl F1	C3H7NOS	3.42	106.0327
Metolachlor	C15H22ClNO2	11.69	284.1417
Metolachlor (Na)	C15H21NaClNO2	11.69	306.1237
Metolachlor F1	C14H18NOCl	11.69	252.1155

Table SM3 (cont.). Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
Metolachlor F2	C13H17N	11.69	188.1439
Metrafenone	C19H21BrO5	12.82	409.0651
Metrafenone F1	C11H12O4	12.82	209.0814
Metrafenone F2	C9H7O2Br	12.82	226.9708
Mevinphos 1	C7H13O6P	5.32	225.0528
Mevinphos 1 (Na)	C7H12O6PNa	5.32	247.0347
Mevinphos 1 F1	C6H9O5P	5.32	193.0266
Mevinphos 1 F2	C2H7O4P	5.32	127.0160
Mevinphos 2	C7H13O6P	6.28	225.0528
Mevinphos 2 (Na)	C7H12O6PNa	6.28	247.0347
Mevinphos 2 F1	C6H9O5P	6.28	193.0266
Mevinphos 2 F2	C2H7O4P	6.28	127.0160
Monocrotophos	C7H14NO5P	4.01	224.0688
Monocrotophos (Na)	C7H13NaNO5P	4.01	246.0507
Monocrotophos F1	C6H9O5P	4.01	193.0266
Monocrotophos F2	C5H11O4P	4.01	167.0473
Omethoate	C5H12NO4PS	2.34	214.0303
Omethoate (Na)	C5H11NaNO4PS	2.34	236.0122
Omethoate F1	C3H7O3PS	2.34	154.9932
Omethoate F2	C2H5O2PS	2.34	124.9826
Oxadixyl	C14H18N2O4	7.3	279.1345
Oxadixyl (Na)	C14H17N2O4Na	7.3	301.1164
Oxadixyl F1	C12H14N2O2	7.3	219.1134
Oxadixyl F2	C9H9N	7.3	132.0813
Oxamyl	C7H13N3O3S	3.1	220.0756
Oxamyl (Na)	C7H12N3O3SNa	3.1	242.0575
Oxamyl F1	C3H5NO	3.1	72.0449
Oxydemeton-methyl	C6H15O4PS2	3.47	247.0228
Oxydemeton-methyl F1	C4H9O3SP	3.47	169.0088
Oxydemeton-methyl F2	C2H7O3SP	3.47	142.9932
Oxydemeton-methyl F3	C2H7O4P	3.47	127.0160
Oxyfluorfen	C15H11NO4F3Cl	13.68	362.0407
Oxyfluorfen F1	C14H9NO2F3Cl	13.68	316.0352
Paclobutrazol	C15H20N3OCl	10.91	294.1373

Table SM3 (cont.). Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
Paclobutrazol F1	C7H5Cl	10.91	125.0158
Paclobutrazol F2	C2H3N3	10.91	70.0405
Parathion-ethyl	C10H14NO5PS	12.01	292.0409
Parathion-ethyl F1	C8H10NO5PS	12.01	264.0096
Parathion-ethyl F2	C6H6NO5PS	12.01	235.9783
Parathion-ethyl F3	C6H5O	12.01	94.0419
Parathion-methyl	C8H10NO5PS	10.34	264.0096
Pendimethalin	C13H19N3O4	14.13	282.1454
Pendimethalin F1	C8H9N3O4	14.13	212.0671
Phosmet	C11H12NO4PS2	10.06	318.0024
Phosmet (Na)	C11H11NaNO4PS2	10.06	339.9843
Phosmet F1	C9H5NO2	10.06	160.0399
Phosmet F2	C8H4O2	10.06	133.0290
Pirimicarb	C11H18N4O2	6.95	239.1508
Pirimicarb (Na)	C11H17NaN4O2	6.95	261.1327
Pirimicarb F1	C9H15N3O	6.95	182.1293
Pirimicarb F2	C3H5NO	6.95	72.0449
Pirimiphos-methyl	C11H20N3O3PS	12.5	306.1041
Pirimiphos-methyl F1	C9H13N3	12.5	164.1188
Pirimiphos-methyl F2	C7H9N3	12.5	136.0875
Pirimiphos-methyl F3	C5H5N3	12.5	108.0562
Prochloraz	C15H16Cl3N3O2	12.44	376.0386
Prochloraz F1	C12H12NO2Cl3	12.44	308.0012
Prochloraz F2	C4H7N	12.44	70.0657
Profenofos	C11H15BrClO3PS	13.51	372.9430
Profenofos F1	C9H11O3SClBrP	13.51	344.9117
Profenofos F2	C6H5O3SClBrP	13.51	302.8647
Promecarb	C12H17NO2	10.86	208.1338
Promecarb (Na)	C12H16NaNO2	10.86	230.1157
Promecarb F1	C7H8O	10.86	109.0653
Promecarb F2	C6H5O	10.86	94.0419
Propamocarb	C9H20N2O2	1.22	189.1603
Propamocarb F1	C4H7NO2	1.22	102.0555
Propanil	C9H9NOCl2	10.47	218.0139

Table SM3 (cont.). Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
Propanil F1	C6H5NCl2	10.47	161.9877
Propazine	C9H16ClN5	10.38	230.1172
Propazine F1	C6H10N5Cl	10.38	188.0703
Propazine F2	C3H5N5Cl	10.38	147.0312
Propazine F3	C3H3N5	10.38	110.0467
Propham	C10H13NO2	9.07	180.1025
Propham (Na)	C10H12NO2Na	9.07	202.0844
Propham F1	C7H7NO2	9.07	138.0555
Propiconazole	C15H17Cl2N3O2	12.54	342.0776
Propiconazole F1	C7H4Cl2	12.54	158.9768
Prosulfocarb	C14H21NOS	13.33	252.1422
Prosulfocarb (Na)	C14H20NOSNa	13.33	274.1242
Prosulfocarb F1	C7H13NO	13.33	128.1075
Prosulfocarb F2	C7H6	13.33	91.0548
Prothioconazole	C14H15Cl2N3OS	12.58	344.0391
Prothioconazole (Na)	C14H14Cl2N3OSNa	12.58	366.0211
Prothioconazole F1	C14H13N3SCl2	12.58	326.0285
Prothioconazole F2	C12H9	12.58	154.0783
Pymethrozine	C10H11N5O	1.12	218.1042
Pymethrozine (Na)	C10H10N5ONa	1.12	240.0861
Pymethrozine F1	C6H4N2	1.12	105.0453
Pyraclostrobin	C19H18ClN3O4	12.61	388.1064
Pyraclostrobin (Na)	C19H17NaClN3O4	12.61	410.0884
Pyraclostrobin F1	C9H8NO2	12.61	163.0633
Pyraclostrobin F2	C8H6NO2	12.61	149.0477
Pyridaphenthion	C14H17N2O4PS	11.2	341.0725
Pyridaphenthion (Na)	C14H16N2O4PSNa	11.2	363.0544
Pyridaphenthion F1	C10H8N2O2	11.2	189.0664
Pyridaphenthion F2	C10H8N2OS	11.2	205.0436
Pyrifenox isomer 1 F1	C14H12Cl2N2O	10.53	295.0405
Pyrifenox isomer 1 F1	C6H6N	10.53	93.0578
Pyrifenox isomer 2 F1	C14H12Cl2N2O	10.84	295.0405
Pyrifenox isomer 2 F1	C6H6N	10.84	93.0578
Pyriproxyfen	C20H19NO3	13.89	322.1443

Table SM3 (cont.). Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
Pyriproxyfen F1	C12H8O2	13.89	185.0603
Pyriproxyfen F2	C5H5NO	13.89	96.0449
Pyriproxyfen F3	C8H6O	13.89	119.0497
Quinalphos	C12H15N2O3PS	12.01	299.0619
Quinalphos F1	HO2PS	12.01	96.9513
Quinalphos F2	C8H7N2O3PS	12.01	242.9993
Quinalphos F3	C8H6N2O	12.01	147.0558
Quizalofop-ethyl	C19H17ClN2O4	13.46	373.0955
Quizalofop-ethyl F1	C16H11N2O2Cl	13.46	299.0587
Quizalofop-ethyl F2	C14H7N2OCl	13.46	255.0325
Simazine	C7H12ClN5	7.76	202.0859
Simazine F1	C6H9N3	7.76	124.0875
Simazine F2	C4H6N3Cl	7.76	132.0328
Simazine F3	C4H5N3	7.76	96.0562
Spiroxamine	C18H35NO2	9.98	298.2746
Spiroxamine F1	C8H17NO	9.98	144.1388
Spiroxamine F2	C6H13N	9.98	100.1126
tau-Fluvalinate	C26H22ClF3N2O3	15.18	503.1349
tau-Fluvalinate F1	C14H9NO	15.18	208.0762
tau-Fluvalinate F2	C13H8O	15.18	181.0653
Tebuconazole	C16H22ClN3O	12.33	308.1530
Tebuconazole F1	C9H7Cl	12.33	151.0315
Tebuconazole F2	C7H5Cl	12.33	125.0158
Tebuconazole F3	C2H3N3	12.33	70.0405
Tebufenozide	C22H28N2O2	11.99	353.2226
Tebufenozide (2M+Na)	C44H55N4O4Na	11.99	727.4199
Tebufenozide (Na)	C22H27N2O2Na	11.99	375.2048
Tebufenozide F1	C18H20N2O2	11.99	297.1603
Tebufenozide F2	C9H8O	11.99	133.0653
Tebufenpyrad	C18H24ClN3O	13.68	334.1686
Tebufenpyrad (Na)	C18H23ClN3ONa	13.68	356.1506
Tebufenpyrad F1	C6H9N2Cl	13.68	145.0533
Tebufenpyrad F2	C4H5N2Cl	13.68	117.022
Tepraloxydim	C17H24ClNO4	8.64	342.1472

Table SM3 (cont.). Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
Tepraloxydim F1	C14H19NO3	11.33	250.1443
Tepraloxydim F2	C9H11NO2	11.33	166.0868
Terbufos	C9H21O2PS3	13.63	289.0520
Terbufos (Na)	C9H20O2PS3Na	13.63	311.0339
Terbufos F1	HO2PS	13.63	96.9513
Terbufos F2	H3O2PS2	13.63	130.939
Terbumeton	C10H19N5O	8.51	226.1668
Terbumeton F1	C6H11N5O	8.51	170.1042
Terbumeton F2	C4H7N5O	8.51	142.0729
Terbumeton F3	C4H7N3O	8.51	114.0667
Terbumeton F4	C3H5N3O	8.51	100.0511
Terbuthylazine	C9H16ClN5	10.6	230.1172
Terbuthylazine F1	C5H8N5Cl	10.6	174.0546
Terbuthylazine F2	C4H5N3	10.6	96.0562
Terbutryn	C10H19N5S	10.51	242.1439
Terbutryn F1	C6H11N5S	10.51	186.0813
Terbutryn F3	C2H6N2S	10.51	91.0330
Tetraconazole	C13H11Cl2F4N3O	11.68	372.0294
Tetraconazole F1	C7H4Cl2	11.68	158.9768
Tetraconazole F2	C2H3N3	11.68	70.0405
Thiabendazole	C10H7N3S	4.11	202.0439
Thiabendazole F1	C9H6N2S	4.11	175.0330
Thiabendazole F2	C8H6N2	4.11	131.0609
Thiacloprid	C10H9ClN4S	4.11	253.0315
Thiacloprid (Na)	C10H8ClN4SNa	6.13	275.0134
Thiacloprid F1	C6H4NCl	6.13	126.0111
Thiamethoxam	C8H10ClN5O3S	3.72	292.0271
Thiamethoxam (Na)	C8H9ClN5O3SNa	3.72	314.0091
Thiamethoxam F1	C8H10N4OS	3.72	211.0654
Thiamethoxam F2	C8H9N4OS	3.72	210.0575
Thiobencarb	C12H16ClNOS	12.81	258.0719
Thiobencarb (Na)	C12H15NaClNOS	12.81	280.0539
Thiobencarb F1	C7H5Cl	12.81	125.0158
Thiodicarb	C10H18N4O4S3	8.85	355.0568

Table SM3 (cont.). Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
Thiodicarb (Na)	C10H17NaN4O4S3	8.85	377.0388
Thiodicarb F1	C3H5NS	8.85	88.0221
Thiophanate-methyl	C12H14N4O4S2	7.87	343.0535
Thiophanate-methyl (Na)	C12H13N4O4S2Na	7.87	365.0354
Thiophanate-methyl F1	C11H10N4O3S2	7.87	311.0273
Thiophanate-methyl F2	C7H6N2S	7.87	151.0330
Thiram	C6H12N2S4	9.96	240.9962
Thiram (Na)	C6H11N2S4Na	9.96	262.9781
Thiram F1	C4H5NS4	9.96	195.9383
Thiram F2	C3H5NS2	9.96	119.9942
Tolclofos-methyl	C9H11Cl2O3PS	12.69	300.9622
Tolclofos-methyl F1	C7H4OCl2	12.69	174.9717
Tolclofos-methyl F2	C2H5O2PS	12.69	124.9826
Tolyfluanid	C10H13Cl2FN2O2S2	12.27	346.9858
Tolyfluanid (Na)	C10H12Cl2FN2O2S2Na	12.27	368.9677
Tolyfluanid F1	C8H6NSCl2F	12.27	237.966
Tolyfluanid F2	C7H6NS	12.27	137.0299
Triadimefon	C14H16N3O2Cl	11.08	294.1009
Triadimefon F1	C11H13OCl	11.08	197.0733
Triadimefon F2	C5H8	11.08	69.0704
Triadimenol	C14H18N3O2Cl	11.46	296.1166
Triadimenol (Na)	C14H17N3O2ClNa	11.46	318.0985
Triadimenol F1	C12H15O2Cl	11.46	227.0839
Triadimenol F2	C6H10O	11.46	99.0810
Triadimenol F3	C2H3N3	11.46	70.0405
Trichlorfon	C4H8Cl3O4P	5.13	256.9304
Trichlorfon (Na)	C4H7NaCl3O4P	5.13	278.9123
Trichlorfon F1	C2H5O3P	5.13	109.0055
Tridemorph	C19H39NO	11.51	298.3110
Tridemorph F1	C18H37NO	11.51	284.2953
Triflumizole	C15H15ClF3N3O	13.18	346.0934
Triflumizole F1	C12H11NOF3Cl	13.18	278.0560
Triflumizole F2	C8H3NF3Cl	13.18	205.9984
Triforine	C10H14Cl6N4O2	10.09	432.9326

Table SM3 (cont.). Pesticides included in the reference standard mix (ESI positive).

Compound	Elemental composition	Retention time (min)	$[M+H]^+$
Triforine (Na)	C10H13Cl6N4O2Na	10.09	454.9146
Triforine F1	C9H11N3OCl6	10.09	387.9112
Triforine F2	C5H9N2	10.09	98.0844

Table SM4. Pesticides not included in the reference standard mix (ESI positive).

Compound	Elemental composition	$[M+H]^+$
Fenarimol	C17H12Cl2N2O	331.0405
1-Naphtalene acetamide	C12H11NO	186.0919
1-Naphthaleneacetic acid	C12H10O2	187.0759
2,4,5-T	C8H5Cl3O3	254.9383
2,4,5-T Isopropyl ester	C11H11Cl3O3	296.9852
2,4-D	C8H6Cl2O3	220.9772
2,4-D Butyl ester	C12H14O3Cl2	277.0398
2,4-D isopropyl ester	C11H12Cl2O3	263.0242
2,4-D methyl ester	C9H8Cl2O3	234.9929
2-aminobenzimidazol	C7H5N2O	134.0480
2-Naphtoxyacetic acid	C12H10O3	203.0708
2-phenoxypropionic acid	C9H10O3	167.0708
3,4,5-trimethacarb	C11N15NO2	389.0468
3,4-Dichloraniline	C6H5Cl2N	161.9877
3,5,6-trichloro-2-pyridinol	C5H2Cl3NO	197.9280
3-hydroxy-carbofuran	C12H15NO4	238.1079
5-hydroxy-imidacloprid	C9H10ClN5O3	272.0550
5-OH-clethodim-sulfon	C17H26NO6SCl	408.1248
6-chloro-4-hydroxy-3-	C10H7N2OCl	207.0325
8-hydroxyquinoline	C9H7NO	146.0606
Absidic acid	C15H20O4	265.1440
ACC	C4H7NO2	102.0555
Acephate	C4H10NO3PS	184.0197
Acequinocyl	C24H32O4	385.2379

Table SM4 (cont.). Pesticides not included in the reference standard mix (ESI positive).

Compound	Elemental composition	$[M+H]^+$
Acetate	C13H12O2	201.0916
Acetochlor	C14H20ClNO2	270.1261
Acibenzolar-S-methyl	C8H6N2OS2	211.0000
Aclonifen	C12H9ClN2O3	265.0380
Acrinathrin	C26H21F6NO5	542.1402
Alanycarb	C17H25N3O4S2	400.1364
Albendazole	C12H15N3O4S	298.0862
Albendazole sulfone	C12H15N3O2S	266.0963
Albendazole sulfoxide	C12H15N3O3S	282.0912
Ametryn	C9H17N5S	228.1283
Amidosulfuron	C9H15N5O7S2	370.0491
Amidosulfuron (Na)	C9H14N5O7S2Na	392.0311
Aminocarb	C11H16N2O2	209.1290
Amitraz	C19H23N3	294.1970
Anilazine	C9H5N4Cl3	274.9658
Anilofos	C13H19ClNO3PS2	368.0311
ANTU	C11H10N2S	203.0643
Atrazine desethyl desisopropyl	C3H4N5Cl	146.0233
Atrazine mercapturate	C13H22N6O3S	343.1552
Avermectin B1a (abamectin)	C48H72O14	873.5000
Avermectin B1b (abamectin)	C47H70O14	859.4844
Azaconazole	C12H11Cl2N3O2	300.0307
Azamethiphos	C9H10N2O5PSCl	324.9815
Azaridachtin	C35H44O16	721.2708
Benalaxyl	C20H23NO3	326.1756
Bendiocarb	C11H13NO4	224.0923
Bendiocarb (Na)	C11H12NaNO4	246.0742
Benfluralin	C13H16F3N3O4	336.1171
Benfuracarb	C20H30N2O5S	411.1594
Benomyl	C14H18N4O3	291.1457
Benoxacor	C11H11Cl2NO2	260.0245
Bensulfuron-methyl	C16H18N4O7S	411.0974
Bensultap	C17H21NO4S4	432.0432
Benthiavalicarb-isopropyl	C18H24FN3O2S	366.1652

Table SM4 (cont.). Pesticides not included in the reference standard mix (ESI positive).

Compound	Elemental composition	$[M+H]^+$
Benzoximate	C18H18ClNO5	364.0952
Bifenox	C14H9NO5Cl2	341.9936
Bifenthrin	C23H22ClF3O2	423.1339
Bitertanol	C20H23N3O2	338.1869
Bromoxynil	C7H3Br2NO	275.8660
Bromuconazole	C13H12N3OCl2Br	375.9619
Bupirimate	C13H24N4O3S	317.1647
Butoxycarboxym	C7H14N2O4S	223.0753
Butoxycarboxym (Na)	C7H13NaN2O4S	245.0572
Buturon	C12H13ClN2O	237.0795
Cambendazole	C14H14N4O2S	303.0916
Captan	C9H8NO2SCl3	299.9420
Carbetamide	C12H16N2O3	237.1239
Carbophenotion	C11H16ClO2PS3	342.9817
Carbosulfan	C20H32N2O3S	381.2212
Carboxin	C12H13NO2S	236.0745
Chlorantraniliprole	C18H14BrCl2N5O2	481.9786
Chlorbromuron	C9H10N2O2BrCl	292.9692
Chlorfenapyr	C15H11BrClF3N2O	406.9774
Chlorfenvinphos-Met	C10H10Cl3O4P	330.9461
Chlorfluazuron	C20H9Cl3F5N3O3	539.9708
Chlorophenoxyacetic acid	C8H7ClO3	187.0162
Chloropicrin	CCl3NO2	163.9073
Chlorotoluron	C10H13ClN2O	213.0795
Chloroxuron	C15H15ClN2O2	291.0900
Chromafenozide	C24H30N2O3	395.2335
Cinosulfuron	C15H19N5O7S	414.1083
Clethodim	C17H26NO3SCl	360.1400
Clethodim-imin-sulfon	C14H23NO4S	302.1426
Clethodim-imin-sulfoxide	C14H23NO3S	286.1477
Clethodim-sulfon	C17H26NO5SCl	392.1298
Clethodim-sulfoxid	C17H26NO4SCl	376.1349
Clodinafop-propargyl	C17H13ClFNO4	350.0595
Clofentezine	C14H8Cl2N4	303.0204

Table SM4 (cont.). Pesticides not included in the reference standard mix (ESI positive).

Compound	Elemental composition	$[M+H]^+$
Clopyralid	C6H3Cl2NO2	191.9619
Cloquintocet-mexyl	C18H22ClNO3	336.1366
Coroxon	C9H10N2O2BrCl	292.9692
Cyanazine	C9H13ClN6	241.0968
Cyanazine acid	C9H14ClN5O2	260.0914
Cyanazine amide	C9H15N6OCl	259.1074
Cyanofenphos	C15H14NO2PS	304.0561
Cyanofenphos oxygen	C15H14NO3P	288.0790
Cyazofamid	C13H13ClN4O2S	325.0526
Cycloate	C11H21NOS	216.1422
cycloheximide	C15H23NO4	282.1705
Cycloxydim	C17H27NO3S	326.1790
Cyfluthrin	C22H18Cl2FNO3	434.0726
Cymoxanil	C7H10N4O3	199.0831
Cypermethrin	C22H19NO3Cl	381.1132
Cyproconazole	C15H18ClN3O	292.1217
Cyromazine	C6H10N6	167.1045
Dacthal	C10H6Cl4O4	330.9098
Daminozide	C6H12N2O3	161.0926
Dazomet	C5H10N2S2	163.0364
Deethyl ametryn	C7H13N5S	200.0970
Deethyl cyanazine	C7H9ClN6	213.0655
Deethyl cyanazine acid	C7H10N5O2Cl	232.0601
Deethyl cyanazine amide	C7H11N6OCl	231.0761
Deethyl-2-hydroxy-	C7H13N5O	184.1198
Deethylhydroxyatrazine	C6H11N5O	170.1042
Deethylsymetrine	C6H11N5S	186.0813
Deethylterbutryn	C4H7N5S	158.0500
Deisopropylprometryne	C7H13N5S	200.0970
Deltamethrin	C22H19NO3Br2	503.9810
Demethyl fluometuron	C9H9F3N2O	219.0745
Demethyl isoproturon	C11H16N2O	193.1341
Demethyl monuron	C8H9ClN2O	185.0482
Demeton-S-methyl	C6H15O3PS2	231.0278

Table SM4 (cont.). Pesticides not included in the reference standard mix (ESI positive).

Compound	Elemental composition	$[M+H]^+$
Demeton-S-methyl sulfon	C6H15O5PS2	263.0177
Demeton-S-methyl sulfoxide	C6H15O4PS2	247.0228
Desmedipham	C16H16N2O4	301.1188
Desmedipham (NH4)	C16H19N3O4	318.1454
Desmethylpirimicarb	C10H16N4O2	225.1352
Desmethylpirimicarb (Na)	C10H15NaN4O2	247.1171
Desmetryn	C8H15N5S	214.1126
Diafenthiuron	C23H32N2OS	385.2314
Dialifos	C14H17ClNO4PS2	394.0103
Diallate	C10H17Cl2NOS	270.0486
Dibenzylamine	C14H15N	198.1283
Dichlofluanid	C9H11Cl2FN2O2S2	332.9701
Dichlone	C10H4Cl2O2	226.9667
Dichlorprop	C9H8Cl2O3	234.9929
Diclobutrazol	C15H19Cl2N3O	328.0983
Diclofop-methyl	C16H14Cl2O4	341.0347
Dicloran	C6H4Cl2N2O2	206.9728
Dicryl	C10H9Cl2NO	230.0139
Dieldrin	C12H8Cl6O	378.8785
Diethofencarb	C14H21NO4	268.1549
Difenoconazole	C19H17Cl2N3O3	406.0725
Difenoxuron	C16H18N2O3	287.1396
Dimefuron	C15H19ClN4O3	339.1224
Dimethachlor	C13H18NO2Cl	256.1104
Dimethylvinphos	C10H10Cl3O4P	330.9461
Dimoxystrobin	C19H22N2O3	327.1709
Diniconazole	C15H17Cl2N3O	326.0827
Dinocap	C18H24N2O6	365.1713
Diphacinone	C23H16O3	341.1178
Diphenylamine	C12H11N	170.0970
Diquat dibromide	C12H14N2Br2O	360.9551
Disulfoton	C8H19O2PS3	275.0363
Disulfoton (Na)	C8H18O2PS3Na	297.0182
Disulfoton -sulfon	C8H19O4PS3	307.0261

Table SM4 (cont.). Pesticides not included in the reference standard mix (ESI positive).

Compound	Elemental composition	$[M+H]^+$
Disulfoton -sulfoxide	C8H19O3PS3	291.0312
Dixanthogen	C6H10O2S4	242.9642
DMSA	C8H12N2O2S	201.0698
DMST	C9H14N2O2S	215.0854
Dodemorph	C18H35NO	282.2797
Dodine*	C15H33N3O2	288.2651
Edifenphos	C14H15O2PS2	311.0329
Endrin	C12H8Cl6O	378.8785
EPN	C14H14NO4PS	324.0459
Epoxiconazole	C17H13ClFN3O	330.0809
EPTC	C9H19NOS	190.1266
Ethephon	C2H6ClO3P	144.9821
Ethiofencarbsulfon	C11H15NO4S	258.0800
Ethiofencarbsulfon (Na)	C11H14NaNO4S	280.0619
Ethiprole	C13H9Cl2F3N4OS	396.9904
Ethofenprox	C25H28O3	377.2117
Ethoprophos	C8H19O2PS2	243.0642
Ethoxazole	C21H23F2NO2	360.1775
Etrimfos	C10H17N2O4PS	293.0725
Famoxadone	C22H18N2O4	375.1345
Fenamidone	C17H17N3OS	312.1171
Fenamiphos-sulfon	C13H22NO5PS	336.1035
Fenamiphos-sulfoxide	C13H22NO4PS	320.1085
Fenazaquin	C20H22N2O	307.1810
Fenbendazole	C15H13N3O2S	300.0807
Fenbuconazole	C19H17N4Cl	337.1220
Fenfuram	C12H11NO2	202.0868
Fenoxaprop-ethyl	C18H16NO5Cl	362.0795
Fenpiclonil	C11H6Cl2N2	236.9986
Fenpropathrin	C22H23NO3	350.1756
Fenpropidin	C19H31N	274.2535
Fenpropimorph	C20H33NO	304.2640
Fenpyroximate	C24H27N3O4	422.2080
Fensulfothion	C11H17O4PS2	309.0384

Table SM4 (cont.). Pesticides not included in the reference standard mix (ESI positive).

Compound	Elemental composition	$[M+H]^+$
Fensulfothion-sulfone	C11H17O5PS2	325.0333
Fenthion oxon	C10H15O4PS	263.0507
Fenthion sulfoxide	C10H15O4PS2	295.0228
Fenthion sulphone	C10H15O5PS2	311.0177
Fenuron	C9H12N2O	165.1028
Flamprop	C16H13NO3FCl	322.0646
Flamprop isopropyl	C19H19ClFNO3	364.1116
Flamprop methyl	C17H15ClFNO3	336.0803
Flazasulfuron	C13H12N5O5SF3	408.0589
Flonicamid	C9H6F3N3O	230.0541
Florasulam	C12H8F3N5O3S	360.0378
Fluacrypyrim	C20H21F3N2O5	427.1481
Fluazifop acid	C15H12F3NO4	328.0797
Fluazinam	C13H4Cl2F6N4O4	464.9592
Flucythrinate	C26H23F2NO4	452.1673
Flufenacet	C14H13F4N3O2S	364.0743
Fluometuron	C10H11F3N2O	233.0902
Fluoxastrobin	C21H16N4O5FCl	459.0872
Fluquinconazole	C16H8Cl2FN5O	376.0168
Fluridone	C19H14F3NO	330.1106
Flurtamone	C18H14F3NO2	334.1055
Flusilazole	C16H15N3F2Si	316.1082
Folpet	C9H4Cl3NO2S	295.9107
Fonofos	C10H15OPS2	247.0380
Forchlorfenuron	C12H10ClN3O	248.0591
Formetanate	C11H15N3O2	222.1243
Fosthiazate	C9H18NO3PS2	284.0544
Fuberidazole	C11H8N2O	185.0715
Furathiocarb	C18H26N2O5S	383.1641
Furathiocarb (Na)	C18H25NaN2O5S	405.1460
Gibberellic acid	C19H24O6	349.1651
Haloxyfop-ethoxyethylester	C19H19ClF3NO4	418.1033
Haloxyfop-etotyl	C19H19F3NO5Cl	434.0982
Haloxyfop-P	C15H11ClF3NO4	362.0407

Table SM4 (cont.). Pesticides not included in the reference standard mix (ESI positive).

Compound	Elemental composition	$[M+H]^+$
Heptenophos	C9H12ClO4P	251.0240
Hexaconazole	C14H17Cl2N3O	314.0827
Hexazinone	C12H20N4O2	253.1665
Hormodin	C12H13NO2	204.1025
Imazameth	C14H17N3O3	276.1348
Imazamethabenz-methyl	C16H20N2O3	289.1552
Indoxacarb	C22H17ClF3N3O7	528.0785
Iodosulfuron-methyl	C14H13IN5O6S	506.9709
Iprodione desisopropyl	C10H7Cl2N3O3	287.9943
Iprovalicarb	C18H28N2O3	321.2178
Isazofos	C19H17ClN3O3PS	434.0495
Isofenphos	C15H24NO4PS	346.1242
Isopyrazam	C20H23F2N3O	360.1887
Isoxaflutole	C12H15NO4SF3	327.0752
Isoxathion	C13H16NO4PS	314.0616
Kresoxim-methyl	C18H19NO4	314.1392
Lenacil	C13H18N2O2	235.1447
Malathion dicarboxylic acid	C6H11O6PS2	274.9813
Malathion monocarboxylic	C8H15O6PS2	303.0126
MCPA methylester	C10H11ClO3	215.0475
Mecarbam	C10H20NO5PS2	330.0599
Mefenpyr-diethyl	C16H18Cl2N2O4	373.0722
Mepanipyrim	C14H13N3	224.1188
Merphos	C12H27PS3	299.1091
Mesotrione	C14H13NO7S	340.0491
Metamitron	C10H10N4O	203.0933
Metazachlor	C14H16N3OCl	278.1060
Metazachlor (Na)	C14H15N3OClNa	300.0880
Metconazole	C17H22N3OCl	320.1530
Methabenxthiazuron	C10H11N3OS	222.0701
Methacrifos	C7H13O5PS	241.0300
Methfuroxam	C14H15NO2	230.1181
Methoxyfenozide	C22H28N2O3	369.2178
Metobromuron	C9H11N2O2Br	259.0082

Table SM4 (cont.). Pesticides not included in the reference standard mix (ESI positive).

Compound	Elemental composition	$[M+H]^+$
Metolachlor ethanesulfonic acid	C15H23NO5S	330.1375
Metolachlor oxanilic acid	C15H21NO4	280.1549
Metolcarb	C9H11NO2	166.0868
Metosulam	C14H13N5O4SCl2	418.0144
Metoxuron	C10H13ClN2O2	229.0744
Metrafenone	C19H21BrO5	409.0651
Metribuzin	C8H14N4OS	215.0967
Metsulfuron	C14H15N5O6S	382.0821
Molinate	C9H17NOS	188.1109
Monolinuron	C9H11ClN2O2	215.0587
Monuron	C9H11ClN2O	199.0638
Myclobutanil	C15H17N4Cl	289.1220
Naled	C4H7Br2Cl2O4P	378.7904
Napropamide	C17H21NO2	272.1651
Neburon	C12H16Cl2N2O	275.0718
Nicosulfuron	C15H18N6O6S	411.1087
Nitempyram	C11H15ClN4O2	271.0962
N-m-Tolylphthalamic acid	C15H13NO3	256.0974
Norflurazone	C12H9F3N3OCl	304.0464
Nuarimol	C17H12FN2OCl	315.0700
Ofurace	C14H16ClNO3	282.0819
Oxamide	C15H15N2O3Cl	307.0849
Oxycarboxin	C12H13NO4S	268.0644
Oxydemeton-methyl	C6H15O4PS2	247.0228
Oxygen	C11H15Cl2O3PS	328.9935
Paraoxon	C10H14NO6P	276.0637
Paraoxon-methyl	C8H10NO6P	248.0324
Paraquat dichloride	C12H14N2Cl2	257.0612
Parathion	C10H14NO5PS	292.0409
Pebulate	C10H21NOS	204.1422
Penconazole	C13H15Cl2N3	284.0721
Pencycuron	C19H21ClN2O	329.1421
Penxonazole	C13H15Cl2N3	284.0721
Permethrin	C21H20Cl2O3	391.0868

Table SM4 (cont.). Pesticides not included in the reference standard mix (ESI positive).

Compound	Elemental composition	$[M+H]^+$
Phenmedipham	C16H16N2O4	301.1188
Phenothrin	C23H26O3	351.1960
Phenoxyacetic acid	C8H8O3	153.0552
Phenthoate	C23H26O3	351.1960
Phenyl mercuric acetate	C8H8HgO2	333.0261
Phorate	C7H17O2PS3	261.0207
Phorate oxygen analogue	C7H17O3PS2	245.0435
Phorate sulfoxide	C7H17O4PS2	261.0384
Phorate-sulfon	C7H17O5PS2	277.0333
Phosalone	C12H15NO4PS2Cl	367.9947
Phosfon	C20H34Cl2P	376.1853
Phosphamidon	C10H19NO5PCl	300.0768
Phoxim	C12H15N2O3PS	299.0619
Picolinafen	C19H12F4N2O2	377.0913
Picoxystrobin	C18H16F3NO4	368.1110
Piperonyl butoxide	C19H30O5	339.2171
Pirimiphos ethyl	C13H24N3O3PS	334.1354
Primisulfuron-methyl	C15H12F4N4O7S	469.0441
Prochloraz	C15H16Cl3N3O2	376.0386
Procymidone	C13H11Cl2NO2	284.0245
Profenofos	C11H15BrClO3PS	372.9430
Prometon	C10H19N5O	226.1668
Prometryn	C10H19N5S	242.1439
Propachlor	C11H14ClNO	212.0842
Propaquizafop	C22H22N3O5Cl	444.1326
Propargite	C19H26O4S	351.1630
Propethamphos	C10H20NO4PS	282.0929
Propoxur	C11H15NO3	210.1130
Propyzamide	C12H11NOCl2	256.0296
Prosulfocarb	C14H21NOS	252.1422
Prosulfuron	C15H16N5O4SF3	420.0953
Prothioconazole	C14H15Cl2N3OS	344.0391
Prothiophos	C11H15Cl2O2PS2	344.9706
Pymetrozine	C10H11N5O	218.1042

Table SM4 (cont.). Pesticides not included in the reference standard mix (ESI positive).

Compound	Elemental composition	$[M+H]^+$
Pyraclostrobin	C19H18ClN3O4	388.1064
Pyrazophos	C14H20N3O5PS	374.0940
Pyridaben	C19H25ClN2OS	365.1454
Pyridate	C19H23ClN2O2S	379.1247
Pyrimethanil	C12H13N3	200.1188
Quinmerac	C11H8ClNO2	222.0322
Quinoclamine	C10H6ClNO2	208.0165
Quinoxyfen	C15H8FNOCl2	308.0045
Quizalofop-methyl	C18H15ClN2O4	359.0799
Reserpine	C33H40N2O9	609.2812
Resmethrin	C22H26O3	339.1960
Rimsulfuron	C14H17N5O7S2	432.0648
Rotenone	C23H22O6	395.1495
Sethoxydim	C17H29NO3S	328.1946
Silvex	C9H7Cl3O3	268.9539
Simetryn	C8H15N5S	214.1126
Spinosyn A	C41H65NO10	732.4687
Spinosyn D	C42H67NO10	746.4843
Strychnine	C21H22N2O2	335.1760
Sulfallate	C8H14ClNS2	224.0334
Sulfosulfuron	C19H21N3O7S2	468.0899
Sulfotep	C8H20O5P2S2	323.0306
Sulprofos	C12H19O2PS3	323.0363
Tebuthiuron	C9H16N4OS	229.1123
Teflubenzuron	C14H6Cl2F4N2O2	380.9821
Tepraloxydim	C17H24ClNO4	342.1472
Terbufos sulfone	C9H21O4PS3	321.0418
Terbufos sulfoxide	C9H21O3PS3	305.0469
Tetrachlorvinphos	C10H9Cl4O4P	364.9071
Thifensulfuron-methyl	C12H13N5O6S2	388.0385
Thiofanox	C9H18N2O2S	219.1167
Thiofanox (Na)	C9H17N2O2SNa	241.0987
Thiofanox-sulfone	C9H18N2O4S	251.1066
Thiofanox-sulfone(NH4)	C9H21N3O4S	268.1331

Table SM4 (cont.). Pesticides not included in the reference standard mix (ESI positive).

Compound	Elemental composition	$[M+H]^+$
Thiofanox-sulfoxide	C9H18N2O3S	235.1116
Thiofanox-sulfoxide (NH4)	C9H21N3O3S	252.1382
Thiophanate-ethyl	C14H18N4O4S2	371.0848
Thiram	C6H12N2S4	240.9962
Triasulfuron	C14H16ClN5O5S	402.0639
Triazophos	C12H16N3O3PS	314.0728
Tribenuron-methyl	C15H17N5O6S	396.0978
Tricyclazole	C9H7N3S	190.0439
Trietazine	C9H16ClN5	230.1172
Trifloxistrobin	C20H19F3N2O4	409.1375
Triflumuron	C15H10ClF3N2O3	359.0410
Trifluralin	C13H16F3N3O4	336.1171
Trisulfuron-methyl	C17H19F3N6O6S	493.1117
Trisulfuron-methyl (Na)	C17H18F3N6O6SNa	515.0937
Triticonazole	C17H20N3OCl	318.1373
Vamidothion	C8H18NO4PS2	288.0493
Zoxamide	C14H16Cl3NO2	336.0325

Table SM5. Pesticides included in the reference standard mix (ESI negative).

Compound	Elemental composition	$[M+H]^+$
Bentazone	C10H12N2O3S	239.0490
Bentazone F1	C7H6N2O3S	197.0021
Bromacil	C9H13BrN2O2	259.0082
Bromacil F1	C5H5N2O2Br	202.9456
Bromoxynil	C7H3Br2NO	273.8503
Chlorsulfuron	C12H12N5O4SCl	356.0221
Chlorsulfuron F1	C5H8N4O	139.0620
Dalapon	C3H4Cl2O2	140.9510
Dalapon F1	C3H3O2Cl	104.9743
Dalapon F2	C2H4Cl2	96.9612

Table SM5 (cont.). Pesticides included in the reference standard mix (ESI negative).

Compound	Elemental composition	$[M+H]^+$
Diuron	C9H10N2OCl2	231.0092
Diuron F1	C7H3NOCl2	185.9513
Fipronil	C12H4Cl2F6N4OS	434.9309
Fipronil F2	C11H2N4F3Cl2	315.9530
Fluazinam	C13H4Cl2F6N4O4	462.9436
Fluazinam F1	C13HN3O2F6Cl2	413.9272
Fludioxonil	C12H6F2N2O2	247.0319
Fludioxonil F2	C10H6N2O	169.0402
Flufenoxuron	C21H11ClF6N2O3	487.0284
Flufenoxuron F1	C21H10N2O3ClF5	467.0222
Flufenoxuron F2	C21H9N2O3ClF4	447.0160
Flufenoxuron F3	C7H5NOF2	156.0261
Fluoroxypyr	C7H5N2O3FCl2	252.9583
Fluoroxypyr F1	C7H4N2O3Cl2	232.9521
Fluoroxypyr F2	C5H3N2OFCl2	194.9528
Hexaflumuron	C16H8Cl2F6N2O3	458.9738
Hexaflumuron F1	C16H7N2O3F5Cl2	438.9676
Hexaflumuron F2	C8H5NOF4Cl2	275.9606
Ioxynil	C7H3I2NO	369.8226
Ioxynil F2	C6H3NI	214.9232
Ioxynil F3	C7H3NOI	242.9181
MCPA	C9H9ClO3	199.0162
MCPA F1	C7H5OCl	138.9951
Propanil	C9H9Cl2NO	215.9983
Propanil F1	C6H5NCl2	159.9721
Teflubenzuron	C14H6Cl2F4N2O2	378.9664
Teflubenzuron F1	C14H5N2O2F3Cl2	358.9602
Teflubenzuron F3	C7H2N2F2Cl2	220.9485
Teflubenzuron F4	C6H3NF2Cl2	195.9532
Terbacil	C9H13ClN2O2	215.0587
Terbacil F1	C5H5N2O2Cl	158.9961
Trinexapac acid	C11H12O5	223.0606
Trinexapac acid F1	C11H10O4	205.0501
Trinexapac acid F2	C10H12O3	179.0708

Table SM6. Pesticides not included in the reference standard mix (ESI negative).

Compound	Elemental composition	$[M+H]^+$
2,4-D	C8H6Cl2O3	218.9616
2,4-DB	C10H10Cl2O3	246.9929
3-phenoxybenzoic acid	C13H10O3	213.0552
6-chloronicotinic acid	C6H4ClNO2	155.9852
Absidic acid	C15H20O4	263.1283
Acetamiprid	C10H11N4Cl	221.0594
Acifluorfen	C14H7ClF3NO5	359.9887
Acrinathrin	C26H21F6NO5	540.1246
Bixafen	C18H12Cl2F3N3O	412.0231
Bromoxynil	C7H3Br2NO	273.8503
Chloroxuron	C15H15N2O2Cl	289.0744
Clothianidin	C6H8ClN5O2S	248.0009
Cyanazine	C9H13ClN6	239.0812
Cymoxanil	C7H10N4O3	197.0675
Cyproconazole	C15H18ClN3O	290.1060
Dicamba	C8H6Cl2O3	218.9616
Dichlorprop	C9H8Cl2O3	232.9772
Diflubenzuron	C14H9ClF2N2O2	309.0242
Diflufenican	C19H11F5N2O2	393.0662
DMSA	C8H12N2O2S	199.0541
DMST	C9H14N2O2S	213.0698
Fenazaquin	C20H22N2O	305.1654
Fenpropathrin	C22H23NO3	348.1600
Fludiozonil	C12H6F2N2O2	247.0319
Haloxyfop	C15H11ClF3NO4	360.0250
Imidacloprid	C9H10ClN5O2	254.0445
Iodosulfuron-methyl	C14H13IN5O6S	504.9553
Ioxynil	C7H3I2NO	369.8226
Lenacil	C13H18N2O2	233.1290
Malathion dicarboxylic acid	C6H11O6PS2	272.9656
Malathion monocarboxylic acid	C8H15O6PS2	300.9969
Mecoprop	C10H11ClO3	213.0318
Mestrione	C14H13NO7S	338.0334
Neburon	C12H16Cl2N2O	273.0561

Table SM6 (cont.). Pesticides not included in the reference standard mix (ESI negative).

Compound	Elemental composition	$[M+H]^+$
Norflurazone	C12H9F3N3OCl	302.0308
Pentachlorophenol	C6HCl5O	262.8392
Primisulfuron methyl	C15H12F4N4O7S	467.0285
Prothioconazole	C14H15Cl2N3OS	342.0235
Rimsulfuron	C14H17N5O7S2	430.0491
tau-Fluvalinate	C26H22ClF3N2O3	501.1193
Tebufenozide	C22H28N2O2	351.2073
Thiophanate-methyl	C12H14N4O4S2	341.0378
Toxaphene	C10H10Cl8	408.8212
Triflumuron	C15H10ClF3N2O3	357.0254

Table SM7. Results obtained in the qualitative validation. Recovery experiments were performed by triplicate at each fortification level.

Sample volume:	**100 mL**		**250 mL**		**500 mL**	
Compound	**0.01 µg/L**	**0.1 µg/L**	**0.01 µg/L**	**0.1 µg/L**	**0.01 µg/L**	**0.1 µg/L**
Atrazine	3/3	3/3	3/3	3/3	3/3	3/3
Atrazine-desethyl (DEA)	3/3	3/3	3/3	3/3	3/3	3/3
Atrazine-desisopropyl (DIA)	0/3	2/3	2/3	3/3	3/3	3/3
Bromacil	0/3	0/3	0/3	1/3	3/3	3/3
Carbendazim	0/3	3/3	2/3	3/3	3/3	3/3
Chlorpyrifos	0/3	0/3	0/3	0/3	0/3	0/3
Diuron	0/3	1/3	0/3	3/3	3/3	3/3
Imazalil	0/3	0/3	0/3	3/3	3/3	3/3
Linuron	0/3	0/3	0/3	3/3	3/3	3/3
Metolachlor	0/3	2/3	1/3	3/3	3/3	3/3
Prometryn	2/3	3/3	3/3	3/3	3/3	3/3
Simazine	2/3	3/3	3/3	3/3	3/3	3/3
Terbumeton	0/3	3/3	3/3	3/3	3/3	3/3
Terbumeton-desethyl (TED)	3/3	3/3	3/3	3/3	3/3	3/3
Terbuthylazine	2/3	3/3	3/3	3/3	3/3	3/3
Terbuthylazine-desethyl (TD)	3/3	3/3	3/3	3/3	3/3	3/3
Terbutryn	0/3	3/3	1/3	3/3	3/3	3/3
Thiabendazole	0/3	3/3	1/3	3/3	3/3	3/3
3,4-dichloroaniline (3,4- DCA)	0/3	0/3	0/3	2/3	3/3	3/3
3,5,6- trichloro-2-pyridinol (TCP)	0/3	0/3	0/3	2/3	3/3	3/3

x/3 (x indicates the number of samples out of the 3 analysed, in which the compound was identified

Table SM8. Regulation and occurrence of pesticides detected in the screening performed in this work[a].

Pesticide / TP	Authorized	Period of control[b]					
		Surface water (SW)			Groundwater (GW)		
		from	to	Samples analyzed	from	to	Samples analyzed
Acetamiprid	Yes		N.A.			N.A.	
Atrazine	No	June 1999	December 2015	2606	June 2002	June 2014	650
Atrazine-desethyl	*	May 2004	December 2015	1690	October 2011	June 2014	94
Atrazine-desisopropyl	*	May 2004	December 2015	1690	October 2011	June 2014	92
Azoxystrobin	Yes		N.A.			N.A.	
Bentazone	Yes		N.A.			N.A.	
Bromacil	No	March 2013	December 2015	898	November 2008	June 2015	134
Carbendazim	No		N.A.			N.A.	
Diazinon	No	January 2010	December 2015	N.A.	April 1997	June 2014	N.A.
Diuron	Yes	May 2004	December 2015	1889	June 2002	June 2014	692
Imazalil	Yes	May 2004	December 2015	1442	October 2011	December 2011	N.A.
Imidacloprid	Yes		N.A.			N.A.	
Isoproturon	No	May 2004	December 2015	N.A.	June 2002	June 2014	N.A.
Linuron	No	March 2013	December 2015	898	May 2013	June 2014	N.A.
MCPA	Yes		N.A.			N.A.	
Metalaxyl	Yes	March 2013	December 2015	N.A.	October 2011	June 2014	N.A.
Metolachlor	No	June 1999	October 2015	1560	June 2002	June 2014	625
Metolachlor oxanilic acid	*		N.A.			N.A.	
Nicosulfuron	Yes		N.A.			N.A.	
Paclobutrazol	Yes		N.A.			N.A.	
Propamocarb	Yes		N.A.			N.A.	
Propiconazole	Yes		N.A.			N.A.	
Simazine	No	June 1999	December 2015	2606	June 2002	June 2014	669
Tebuconazole	Yes		N.A.			N.A.	
Terbacil	No		N.A.			N.A.	
Terbumeton	No	March 2013	December 2015	898	May 2013	June 2014	82
Terbumeton-desethyl	*	March 2013	December 2015	898	May 2013	June 2014	82
Terbuthylazine	Yes	June 1999	December 2015	2248	June 2002	June 2014	669
Terbuthylazine-2-hydroxy	*		N.A.			N.A.	
Terbuthylazine-desethyl	*	March 2013	December 2015	898	May 2013	June 2014	82
Terbutryn	No	May 2004	December 2015	827	March 2010	November 2012	N.A.
Tetraconazole	Yes		N.A.			N.A.	
Tiabendazole	Yes	March 2013	December 2015	898	May 2013	June 2014	82

[a] Data supplied by JRB Authority **(**Confederación Hidrográfica del Júcar O.A, 2018)
[b] Time during which samples were collected to be analyzed before this investigation
*TP: Transformation Product
N.A.: Data not available

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2.1.3. Artículo científico II

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Research Paper

Ecological risk assessment of pesticides in the Mijares River (eastern Spain) impacted by citrus production using wide-scope screening and target quantitative analysis

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ABSTRACT

The widespread use of pesticides, especially in agricultural areas, makes necessary to control their presence in surrounding surface waters. The current study was designed to investigate the occurrence and ecological risks of pesticides and their transformation products in a Mediterranean river basin impacted by citrus agricultural production. Nineteen sites were monitored in three campaigns distributed over three different seasons. After a qualitative screening, 24 compounds was selected for subsequent quantitative analysis. As expected, the lower section of the river was most contaminated, with total concentration >5 μg/L in two sites near to the discharge area of wastewater treatment plants. The highest concentrations were found in September, after agricultural applications and when the river flow is reduced. Ecological risks were calculated using two mixture toxicity approaches (Toxic Unit and multi-substance Potentially Affected Fraction), which revealed high acute and chronic risks of imidacloprid to invertebrates, moderate-to-high risks of diuron, simazine and 2,4-D for primary producers, and moderate-to-high risks of thiabendazole for invertebrates and fish. This study shows that intensive agricultural production and the discharge of wastewater effluents containing pesticide residues from post-harvest citrus processing plants are threatening freshwater biodiversity. Further actions are recommended to control pesticide use and to reduce emissions.

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Highlights

- Pesticides were monitored in the Mijares River (Spain) in different seasons.
- A screening method was applied to select compounds for quantitative analysis.
- Quantitative analyses detected high concentrations of thiabendazole and imazalil.
- Wastewater effluents contributed to increase pesticide concentrations in the river.
- High risks were calculated for imidacloprid, diuron and thiabendazole.

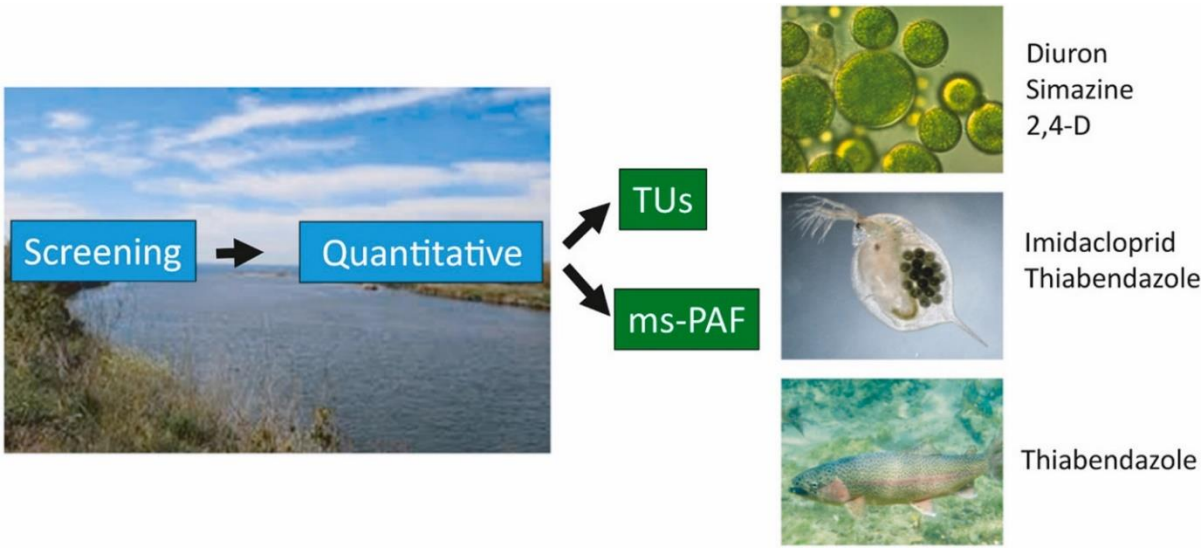
Abstract

The widespread use of pesticides, especially in agricultural areas, makes necessary to control their presence in surrounding surface waters. The current study was designed to investigate the occurrence and ecological risks of pesticides and their transformation products in a Mediterranean river basin impacted by citrus agricultural production. Nineteen sites were monitored in three campaigns distributed over three different seasons. After a qualitative screening, 24 compounds were selected for subsequent quantitative analysis. As expected, the lower section of the river was most contaminated, with total concentration >5 µg/L in two sites near to the discharge area of wastewater treatment plants. The highest concentrations were found in September, after agricultural applications and when the river flow is reduced. Ecological risks were calculated using two mixture toxicity approaches (Toxic Unit and multi-substance Potentially Affected Fraction), which revealed high acute and chronic risks of imidacloprid to invertebrates, moderate-to-high risks of diuron, simazine and 2,4-D for primary producers, and moderate-to-high risks of thiabendazole for invertebrates and fish. This study shows that intensive agricultural production and the discharge of wastewater effluents containing pesticide residues from post-harvest citrus processing plants are threatening freshwater biodiversity. Further actions are recommended to control pesticide use and to reduce emissions.

Keywords

Pesticides; surface water; chromatography coupled to mass spectrometry, ecological risk assessment, mixture toxicity.

Graphical abstract



1. Introducción

Pesticides are considered as a key tool to increase agricultural crop yields while reducing man power (Cooper and Dobson, 2007). Agricultural pesticides are prone to enter surface water ecosystems, and have been considered as one of the main causes of the freshwater biodiversity decline (Beketov et al., 2013). Spain is the second largest consumer of pesticides within the European Union (Eurostat, 2019). In 2018, the total amount of active substances commercialized was 73,286 Ton, which represents an increase of almost 2% as compared to the previous year (Ministerio de Agricultura Pesca y Alimentación, 2017). At present, more than 2000 compounds are authorized, containing several hundreds of active ingredients. The largest group of active substances commercialized for agricultural use corresponds to fungicides and bactericides (52%), followed by herbicides (23%), molluscicides and other plant protection products (16%), and insecticides (9%).

Surface water runoff, spray-drift and leaching are considered as the main entry routes for pesticides into surface water ecosystems. An alternative, less-researched pathway is wastewater treatment plants (WWTPs), which can discharge pesticide mixtures coming from washing equipment used in agriculture, fruit and vegetable post-harvest processing, and/or urban and domestic applications (Aguirre-Martínez and Martín-Díaz, 2020). In general, pesticides are poorly eliminated by conventional wastewater treatments (Gago-Ferrero et al., 2020). Possible explanations for the poor or even negative removal rates are deconjugation of metabolites and/or transformation products (TPs) of the pesticides, and desorption from particulate matter during wastewater treatment (Köck-Schulmeyer et al., 2013).

The European Union established a list of 45 priority substances that have been identified amongst those that pose a significant risk to the aquatic environment (Directive, 2013/39/EU). The fact that about 50% of these substances are pesticides indicates concerns about their potential negative side effects in freshwater ecosystems. However, many of these pesticides are obsolete compounds or have been recently banned. In 2018, the European Commission updated the Watch List of the Water Framework Directive (European Comission, 2018/840, 2018), including seven pesticides, five of them neonicotinoids. In the time of writing this paper, the Watch List was updated again, removing all the previous pesticides except metaflumizone, and adding 10 azole compounds, famoxadone and dimoxystrobin (European Comission,

2020/1161, 2020). In Spain and other southern European countries, the efforts to monitor the exposure to and risks of pesticides other than those included as part of the Water Framework Directive (WFD) are commonly insufficient. As discussed by several authors, post-registration monitoring is needed to assess the occurrence of pesticide mixtures and their retrospective risks for freshwater biodiversity, as well as to identify potential misuse practices and flaws of the prospective risk assessment framework (Rico et al., 2021, Vijver et al., 2017).

Comprehensive data on pesticide occurrence in water are crucial for a good characterization of the water quality. However, the large number of compounds that could be potentially present in water makes this task challenging. Most studies on pesticide risk assessment are based on concentration data obtained by target quantitative methods applied for a limited list of compounds. In the last years, gas chromatography-mass spectrometry (GC-MS) methods have moved to methods based on liquid chromatography-mass spectrometry (LC-MS), due to the change in the use of pesticides, from non-polar and volatile compounds to more polar and less volatile compounds, which are commonly less toxic and persistent in the environment. Nowadays, analytical techniques based LC coupled to tandem mass spectrometry (MS/MS) are widely applied for the determination of target pesticides and their TPs in water, at very low concentration levels (ng/L) (Marín et al., 2009, Sancho et al., 2004, Wille et al., 2012). As an integral complement to target MS/MS analysis, more limited in the scope, screening methods based on high resolution mass spectrometry (HRMS) allow the detection of a wide list of compounds and help to prioritize those that are more frequently found in environmental samples (Della-Flora et al., 2019, Hernández et al., 2015, Masiá et al., 2013, Pitarch et al., 2016). The application of HRMS-based screening methods is of great help to focus the subsequent quantitative analysis to those compounds that have been previously identified in water by wide-scope screening.

To date, only few studies have investigated the potential toxicological effects of pesticide mixtures at river catchment scale (Arenas-Sánchez et al., 2019, Le et al., 2017). One of the most commonly used mixture toxicity methods is the Toxic Unit (TU) approach, which relies on the summation of the individual toxic pressures exerted by each compound to a given standard test species assuming concentration addition (Liess and Ohe, 2005, Sprague, 1971). The major benefit of this approach is that it requires a limited number of toxicity data for each

compound. On the other hand, several studies demonstrate that the standard test species used for the calculation of TUs are not necessarily within the most sensitive taxa for some pesticide classes, potentially rendering the method estimations under protective for some species (Brock et al., 2016, Rico and Van den Brink, 2015). Another mixture toxicity method that is increasingly used is the multi-substance Potentially Affected Fraction (ms-PAF) approach (de Zwart and Posthuma, 2005, Posthuma et al., 2019) calculated on the basis of Species Sensitivity Distributions (SSDs) (Posthuma et al., 2002). One of its major strengths is that it uses as many toxicity data as possible to represent the sensitivity range of species assemblages in freshwater ecosystems, and provides probability estimates as regards to the number of species that will be affected given one or several pesticide concentrations. Its major limitation is that it requires a larger number of toxicity values to represent the sensitivity of different species of the ecosystem and to achieve robust risk estimates (Maltby et al., 2005, Wheeler et al., 2002). For some pesticides, the number of toxicity data available is often too limited, which forces researchers to exclude some compounds from the ms-PAF analysis (e.g. Rämö et al., 2018), thus hampering the risk characterization for all compounds contained in the pesticide mixture.

The objectives of this study were to investigate the occurrence of pesticides and their TPs into a Mediterranean river (Mijares River) by applying complementary analytical techniques and to assess their ecological risks. The Mijares River is directly impacted by WWTPs and, in some areas, it is expected to receive high loads of pesticides used in citrus production and post-harvest processing. A total of 57 surface water samples were collected in three different sampling campaigns over one year. After a preliminary screening to identify the most relevant pesticides, the samples were quantitatively analyzed by LC-MS/MS for the determination of 24 selected analytes. The results of the quantitative analysis were used to assess the risks of each pesticide individually and in mixtures for aquatic ecosystems making use of the TU and the ms-PAF approaches. This study highlights single compounds and pesticide mixtures that are posing an ecotoxicological risk to Mediterranean freshwater ecosystems in areas with predominant citrus production, and discusses the pros and cons of the implementation of each mixture toxicity techniques.

2. Experimental

2.1. Pesticide standards and reagents

A total of 19 pesticides and 5 TPs were selected for the quantitative analysis. More details regarding the standards and reagents used for their chemical analyses can be found in the **Supplementary Material (SM)**.

2.2. Study area

The study was carried out along the Mijares River, which is situated in the East coast of Spain (see **Fig. 1**). It is a relative small river (156 km) that originates at a height of 1600 m in the Sierra de Gúdar, in the municipality of El Castellar, Teruel province (TE), and discharges into the Mediterranean Sea between Almassora and Borriana, Castellón province (CS). Its basin covers 5466 km^2, which represents 13% of the total demarcation of the Júcar Water Basin Authority. The area is composed of featured reservoirs such as the one in Toranes (TE) and two others in Arenós and Sitjar (CS). The river regime is characterized by a period of moderate-to-high flow in February and June in the low course of the river, which is surpassed in October (the season with the highest water flow), and noticeable descents in January and especially in August.

The use of the river water for irrigation is highly important in this area. A total of 43,530 ha benefit from its water (94% corresponds to CS and the remaining 6% to TE). Most of the irrigated area (77%) is located in the lower section of the river (Plana Baixa region), where citrus fruits are the predominant crop with a percentage close to 87% of the irrigated area. In the rest of the sections of the catchment, especially the upper one, more than 80% of land cover is occupied by forested areas and a large part of the existing cultivation areas are abandoned (González, 2017).

Currently, in the upper part, a fertilizer factory and a fish farm are located in the neighboring municipality of Sarrión (TE). Moreover, there are four WWTPs which discharge their treated effluents to the river. They are located in four municipalities, two small ones, Montanejos and Toga, and two of bigger size, Vila-real and Almassora, which are considered as important sources of contamination in the surrounding environment (Fonseca et al., 2020). In addition,

in the middle section of the river, there is a Solid Waste Treatment Plant (SWTP) in Onda (CS).

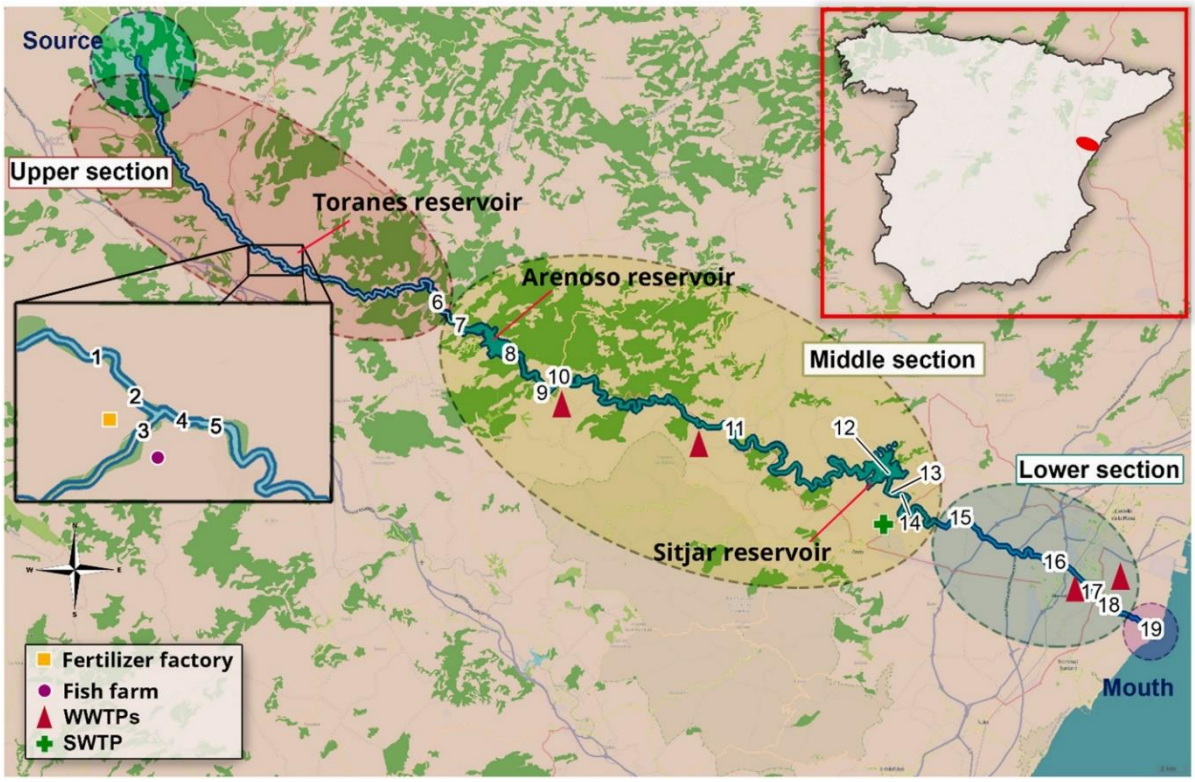


Fig. 1. Sampling sites (1−19) in the Mijares River. The bottom left inset shows the location of the Mijares River basin within Spain. The location of the fertilizer factory, fish farm, WWTPs and SWTP in the surrounding of the river are also displayed.

2.3. Sample collection

The Mijares River was sampled in 19 different sites (see **Fig. 1**), selected according to their characteristics and/or accessibility (see **Table S1 in SM**). Sites 1–6 were selected in the upper section, among which stand out site 2 (next to the fertilizer factory), sites 3 and 4 (close to the fish farm) and site 5 (Toranes reservoir). In the middle part, eight locations were studied corresponding to Arenós reservoir (sites 7–8), Montanejos WWTP (sites 9–10), Toga WWTP (site 11), Sitjar reservoir (site 12) and two sampling sites (13-14) (13- located downstream of the SWTP. In the lower section, four sampling locations (sites 15–18) were selected. Sites 17 and 18 are close to the discharge point of the WWTPs of Vila-real and Almassora, respectively. The last sampling site 19 is located in the estuary of the river.

In order to evaluate potential seasonal variation in the Mijares River, three sampling campaigns were conducted along different seasons, summer (June 2018), autumn (September 2018) and winter (February 2019). In every campaign, 19 surface water samples were collected, one from each sampling site, in polyethylene bottles, transported in refrigerated isothermal containers and stored in the dark at −20 °C until their analysis. A total of 57 river water samples were collected.

2.4. Instrumentation

2.4.1. QTOF MS

A hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (Xevo G2 QTOF) was interfaced to a Waters Acquity ultra-high performance liquid chromatography (UHPLC) system using an orthogonal Z-spray electrospray ionization (ESI) interface or to an Agilent 7890 A GC system using an atmospheric pressure chemical ionization source (APCI), all within a single instrument. For further details see the SM.

2.4.2. LC-MS/MS (QqQ)

Waters Acquity UPLC™, equipped with a quaternary pump system, interfaced to a Xevo TQ-S™ triple quadrupole (QqQ) mass spectrometer was used. See the SM for further details.

2.5. Sample treatment in screening analysis

Sample extraction and pre-concentration was made by solid-phase extraction (SPE). The procedure applied was based on the method previously developed by our research group (Pitarch et al., 2016). Briefly, a volume of 100 mL of centrifuged water sample was passed through an Oasis HLB cartridge (150 mg, Waters) and then, the analytes were eluted with 5 mL of methanol. The extract was divided into 2 aliquots (2.5 mL each), which were evaporated to dryness and reconstituted with 50 µL of hexane (for GC analysis) and 100 µL of methanol:water (10:90, v/v) (for LC analysis). Finally, 1 µL of the hexane extract and 20 µL of the methanol-water extract were injected into GC-QTOF MS and LC-QTOF MS, respectively.

2.6. Screening by QTOF MS

The presence of pesticides in water samples was firstly investigated by complementary wide-scope screening using both GC- and LC-QTOF MS (**Table 1**). The purpose was to identify the main pesticides and TPs present in surface water from the river in order to design the subsequent quantitative analysis and to apply it to those compounds that are actually present in water.

Table 1. Pesticides identified in the Mijares River after complementary screening based on GC-QTOF MS and LC-QTOF MS.

Acetamiprid (LC)	**Imidacloprid** (LC)
Atrazine (GC)	MCPA (LC)
Atrazine desethyl (DEA) (GC)	**Metalaxyl** (GC, LC)
Azoxystrobin (GC, LC)	**Metholachlor** (GC)
Carbaryl (GC)	2-Phenylphenol (GC)
Carbendazim (LC)	Propiconazole (GC, LC)
Chlorpyrifos ethyl (GC)	Pyrimethanil* (GC, LC)
Chlorpyrifos methyl (GC)	**Simazine** (GC, LC)
Diazinon (GC)	**Terbumeton** (LC)
Diflufenican (GC)	**Tebuconazole** (GC)
Dimethoate (GC, LC)	***Terbumeton desethyl*** (GC, LC)
Diuron (LC)	**Terbuthylazine** (GC, LC)
Fipronil (GC)	***Terbuthylazine 2-OH*** (LC)
Fluapyram* (GC)	***Terbuthylazine desethyl*** (GC, LC)
Imazalil (GC, LC)	**Terbutryn** (GC, LC)
Isoproturon (LC)	**Thiabendazole** (GC, LC)

TPs are shown in italic
In bold, pesticides included in the subsequent target quantitative analysis by LC-MS/MS
* Suspect compound, tentative identification

Pesticides and TPs investigated were included in a customized home-made database (around 550 compounds in LC and 425 in GC) (Fonseca et al., 2019, Pitarch et al., 2016). Detection and identification was based on mass accuracy, isotopic pattern (typically due to the presence of bromine or chlorine atoms), retention time (Rt) deviation and presence of fragment ions. For more details about the strategy employed, see (Hernández et al., 2014, Ibáñez et al., 2017) and the SM.

2.7. Sample treatment in quantitative analysis

Quantitative determination of pesticides was performed without any pre-treatment of the sample, except centrifugation, i.e. using direct injection of the water sample (Boix et al., 2015, Botero-Coy et al., 2018, Fonseca et al., 2020). Briefly, 2 mL of water was centrifuged at 12,000 rpm for 10 min. Subsequently, in an injection vial, 50 μL of an isotopic labeled internal standard (ILIS) solution of 1 ng/mL was added to 950 μL of the centrifuged water sample. Finally, 100 μL were injected into the LC-MS/MS system.

2.8. Quantitative analysis by LC-MS/MS

For the quantitative analysis of samples by LC-MS/MS, 24 target pesticides and TPs from different physicochemical families were selected. The experimental conditions are shown in **Table S2**.

Between samples, quality control (QC) were included. The QCs consisted on 3 surface water samples, selected among those analysed in this study, each one fortified at three concentration levels: 0.01, 0.1 and 1 μg/L. QCs recoveries were accepted between 60% and 140% (SANTE, 2019), giving in this way validity to the results obtained.

As samples were analysed by direct injection without any pre-concentration step, the lowest calibration level (LCL) was considered as the limit of quantification in water analysis (1 ng/L for all compounds). A compound was considered as "detected" when its concentration was below 1 ng/L and at least one q/Q ratio was accomplished. For the constructions of graphs, risk assessment evaluation, and for discussion of results obtained, the cut-off value used for detected positives was half the LCL (0.5 ng/L for all compounds).

2.9. Ecological risk assessment

Aquatic toxicity data for the pesticides that were detected in this study were retrieved from the US EPA ECOTOX database (ECOTOX, 2020) and the Pesticide Properties Database (PPDB, 2020). The toxicity data selection criteria (i.e., selected endpoints, exposure duration) for primary producers, invertebrates and vertebrates were based on those described by Rico et al. (2019b), and are described in the SM.

Risks for freshwater ecosystems were calculated following the TU approach and the msPAF approach for primary producers, invertebrates (acute and chronic) and vertebrates (acute and chronic), separately. TUs were calculated by dividing the measured concentration in each sampling site by the toxicity value of standard test species representing the different taxonomic groups and exposure durations. TUs for primary producers were calculated with the EC50–72/96 h for the green algae *Raphidocelis subcapitata*. TUs for acute and chronic exposure to aquatic invertebrates were calculated with EC50–48 h and NOEC-21d for the microcrustacean *Daphnia magna*, and TUs for acute and chronic exposure to vertebrates were calculated with LC50–96 h and NOEC-28d values for the fish *Oncorhynchus mykiss*, respectively (**Table 2**). When chronic toxicity data for *D. magna* or *O. mykiss* were not available, it was estimated by dividing the acute toxicity value by an extrapolation factor of 10. The TUs for the evaluated pesticide TPs were calculated based on the available toxicity data for the parent compounds. Finally, the mixture toxicity assessment following the TU approach was performed by summing the individual TUs for each compound measured in the sample assuming additivity of the measured substances. Aquatic risks were classified as low or insignificant when the sum of TUs was lower than 0.1, moderate when the sum of TUs was between 0.1 and 1, and high when the sum of TUs was above 1.

Aquatic risks following the msPAF approach were also calculated separately for each taxonomic group and exposure duration. First, an SSD was calculated for each pesticide that was detected at least once in the samples. The SSD parameters μ (median of log-transformed available toxicity values) and σ (standard deviation of log-transformed toxicity values or slope) were calculated assuming a log-normal distribution on the basis of the available toxicity data when there were at least three available data points. In such way, SSDs parameters could be calculated for 42 pesticide-taxonomic group and exposure duration combinations, out of the 75 needed (**Table 3**). Some extrapolation rules were applied to increase the number of compounds that could be evaluated following this approach. For example, acute μ were calculated based on the chronic ones (when these were available) by summing 1 to the chronic μ value and maintaining the same σ, while chronic μ were extrapolated by subtracting 1 to the

Table 2. Toxicity data (in µg/L) for standard test species used for the calculation of the Toxic Units. The selected standard test species are *Raphidocelis subcapitata* (primary producers), *Daphnia magna* (invertebrates) and *Oncorhynchus mykiss* (vertebrates). Data are only provided for the pesticides detected at least once in this study. Transformation products are not included (the Toxic Units for these were calculated using toxicity data for the parent compounds).

		Primary producers	**Invertebrates**		**Vertebrates**	
		Chronic	**Acute**	**Chronic**	**Acute**	**Chronic**
Pesticides	**Type** [a]	**EC50-72/96h**	**EC50-48h**	**NOEC-21d**	**LC50-96h**	**NOEC-28d**
2,4-D	H	100,000	92,926	46,200	358,000	35,800 [b]
Atrazine	H	16.0	27,100	1100	7300	730 [b]
Diuron	H	2.5	5700	96.0	7500	410
Imazalil	F	870	3500	350	1700	43.0
Imidacloprid	I	100,000	22,700	1300	229,100	9020
Linuron	H	10.0	310	130	7100	100
Metalaxyl	F	400	156,000	1000	68,100	6810
Metolachlor	H	30.0	23,500	1200	3900	390 [b]
Prometryn	H	11.7	12,660	1000	4570	457 [b]
Simazine	H	100	10,000	2500	45,300	4530 [b]
Tebuconazole	F	1240	4500	50.0	4400	10.0
Terbumeton	H	9.0	40,000	4000	14,000	1400
Terbuthylazine	H	2.0	21,200	19.0	3950	90.0
Terbutryn	H	2.4	2600	260	1500	150
Thiabendazole	F	9000	810	42.0	1970	21.0

[a] H: herbicide; F: fungicide; I: insecticide.
[b] Chronic toxicity value not available for *O. mykiss*. It has been calculated by dividing the acute toxicity value by 10.

Table 3. Number of available toxicity data and SSD parameters (μ = median of log-transformed toxicity data, σ = standard deviation of log-transformed toxicity data, n = number of species) for the pesticides detected in at least one of the monitored sampling sites (excluding the transformation products). For transformation products, we used the SSD parameters for the corresponding parent compounds. The hyphen (-) indicates that there was not enough data to estimate the SSD parameters.

Pesticides	Type[a]	TMoA[b]	Primary producers			Invertebrates						Vertebrates					
			Chronic			Acute			Chronic			Acute			Chronic		
			n	μ	σ	n	μ	σ	n	μ	σ	n	μ	σ	n	μ	σ
2,4-D	H	AM	8	3.33	1.59	16	5.03	0.70	1	-	-	32	5.5	1.0	1	[c]	[c]
Atrazine	H	PSII	41	1.34	0.96	40	3.91	1.01	28	1.59	0.80	34	4.2	0.5	18	1.56	1.09
Diuron	H	PSII	12	0.30	1.24	14	3.99	0.69	2	[c]	[c]	15	3.9	0.5	4	2.10	0.89
Imazalil	F	SB	1	-	-	1	-	-	1	-	-	3	3.4	0.3	1	[c]	[c]
Imidacloprid	I	ACh	2	-	-	38	1.54	1.31	11	0.31	1.14	5	3.9	1.4	1	[c]	[c]
Linuron	H	PSII	5	0.60	1.04	1	[d]	[d]	12	1.91	0.65	6	3.7	0.3	2	[c]	[c]
Metalaxyl	F	NA	1	-	-	2	-	-	1	-	-	3	3.9	0.6	1	[c]	[c]
Metolachlor	H	FA	11	1.67	1.05	2	-	-	1	-	-	8	4.0	0.4	1	[c]	[c]
Prometryn	H	PSII	3	0.70	0.28	3	4.08	0.41	2	[d]	[d]	7	3.7	0.3	3	1.90	1.34
Simazine	H	PSII	7	0.30	1.34	20	4.43	0.80	1	[d]	[d]	18	4.0	0.5	3	0.48	0.07
Tebuconazole	F	SB	1	-	-	8	3.59	0.71	6	1.42	0.51	7	3.9	1.2	4	1.90	1.09
Terbumeton	H	PSII	1	-	-	1	-	-	1	-	-	3	4.1	0.2	1	3.1	0.2
Terbuthylazine	H	PSII	3	2.27	0.68	1	-	-	1	-	-	5	3.7	0.3	1	2.7	0.3
Terbutryn	H	PSII	2	-	-	2	-	-	1	-	-	7	4.2	0.3	1	[c]	[c]
Thiabendazole	F	PR	1	-	-	2	-	-	1	-	-	4	3.9	0.9	1	[c]	[c]

[a] H: herbicide; F: fungicide; I: insecticide.

[b] AM: auxin mimics; PSII: inhibition of photosynthesis at PS II; SB: Sterol biosynthesis in membranes; ACh: acetylcholine inhibitor; NA: nucleic acids metabolism; FA: inhibition of fatty acid synthesis; PR: cytoskeleton and motor protein. For the $msPAFT_{MoA}$ and $msPAF_{Total}$ calculations, compounds classified as PSII and SB were further separated when the μ differed by more than 10% from the others.

[c] For PAF calculations, the chronic μ was calculated based on the acute one -1, and the σ was kept the same as the acute.

[d] For PAF calculations, the acute μ was calculated based on the chronic one +1, and the σ was kept the same as the chronic.

acute μ value and maintaining the same σ. The summation or subtraction of 1 to the chronic and acute μ correspond to an extrapolation factor of 10 between acute and chronic toxicity data, and allowed the evaluation of 9 additional cases (i.e., pesticide-taxonomic group and exposure duration combinations; **Table 3**). As for TPs, the same SSD parameters as for the parent compounds were used due to the lack of toxicity data to construct TP-specific SSDs.

The monitored pesticides were classified into seven Toxicological Modes of Action (TMoAs) following the classifications described by the Insecticide (IRAC, 2020), Fungicide (FRAC, 2020) and Herbicide (HRAC, 2020) Resistance Action Committees (**Table 3**). After this, the σ of the SSDs of the pesticides belonging to the same TMoA were compared, and when differences were larger than 10% they were assigned to a different TMoA. The toxic pressure of the compounds within each TMoA and their mixtures was calculated for each sample following the methods described by (de Zwart and Posthuma, 2005, Fonseca et al., 2020). First, the Hazard Unit (HU) was calculated for each compound in each sampling site by dividing the logarithm of the measured concentration by the μ of the corresponding SSD. Then, the msPAF corresponding to each TMoA ($msPAFT_{MoA}$) in each sample was calculated assuming concentration addition and using the Microsoft Excel© function (Eq. 1).

$$\mathrm{msPAF_{TMoA}} = NORM.DIST\,(HU_{TMoA}, 0, \sigma_{TMoA}, \quad (1)$$

Where HU_{TMoA} is the sum of the HUs within each TMoA, and σ_{TMoA} is the average σ for all compounds within the same TMoA. Subsequently, the total toxicity of the sample ($msPAF_{Total}$) was calculated using the response addition model (Eq. 2).

$$\mathrm{msPAF_{Total}} = \prod_{i=1}^{n}\left(1 - \mathrm{msPAF_{TMoA,1}}\right) \quad (2)$$

The $msPAF_{Total}$ for each sample was represented together with the relative contribution of each pesticide to the total toxic pressure. The PAF and the $msPAF_{Total}$ represent the fraction of aquatic species within each taxonomic group that will be affected by the exposure to an individual compound or the pesticide mixture, respectively. Usually, the Hazardous

Concentration for 5% of species (HC5), which is expected to protect the 95% of species, is taken as concentration threshold for ecological risk assessment (Posthuma et al., 2002). However, other authors have used the HC1 or the lower confidence limit of the HC5 as suitable threshold value (Maltby et al., 2009). In our study, ecological risks resulting from PAFs or msPAFs above 5% were classified as high, while values between 1% and 5% were classified as moderate, and values below 1% were classified as low or insignificant.

3. Results and discussion

The present study was designed to collect only water samples, focusing the analyses in the pesticides dissolved in water. This means that no sediment and suspended particulate matter (SPM) samples were collected. Although sediment and SPM analysis have been reported to be essential to better understand the type of pollution encountered (contemporary or historical pesticides input) (Barbieri et al., 2020, Zhang et al., 2020), the fact that most pesticides applied at present in the studied area are medium-high polar (i.e. better fitting with LC-MS analysis) implies that sorption on solid samples should be less important with respect to classical/old pesticides, which were mostly of low polarity (i.e. better fitting with GC-MS analysis). This may be certainly seen as a study limitation. However, data presented in this work on pesticides dissolved in the aqueous phase allow to have a good picture on the current situation of the study area. The strategy applied in this work can be considered as an starting point for future monitoring programmes focused on those pesticides actually used and posing the largest ecological risks.

3.1. Preliminary QTOF screening

After a general SPE pre-concentration step, the samples were screened for a list of more than 800 compounds (550 compounds in LC and 425 in GC), using the combined approach described in **Section 2.6**. The list contained a total of 337 compounds for which the reference standards were available. For the remaining compounds, only the exact mass and fragments reported in the literature were available. **Table 1** shows the pesticides and TPs identified in the screening. Most of compounds were identified/confirmed by agreement of Rt and experimental fragments with their reference standards. Only two fungicides, fluapyram and pyrimethanil, were tentatively identified (their reference standards were not available at our laboratory)

based on the information obtained by QTOF MS (i.e. accurate mass of the protonated molecule and of fragment ions), which was compared with online database, such as MassBank or MetLin, and/or with reported bibliography (Valera-Tarifa et al., 2020).

A total of 32 compounds (28 pesticides and 4 TPs) were identified using both QTOF MS methodologies (**Table 1**). As an example, **Figs. S1 and S2** illustrate the identification of thiabendazole (by LC-QTOF MS) and terbutryn (by GC-QTOF MS). Among parent compounds, there were 9 insecticides, 9 fungicides and 10 herbicides, the three groups of compounds commonly present in surface water (de Souza et al., 2020). Most pesticides found in the Mijares River have been reported in different studies of Mediterranean river catchments in Spain (Ccanccapa et al., 2016, Fonseca et al., 2019, Hernández et al., 2008, Marín et al., 2006, Masiá et al., 2013, Moreno-González et al., 2013, Rico et al., 2019a, Rousis et al., 2017). As regards to TPs, four compounds were herbicide triazines' TPs: atrazine desethyl (DEA), terbuthylazine 2-OH and –desethyl, and terbumeton desethyl, which are frequently detected in surface water and even groundwater (Ccanccapa et al., 2016, Marín et al., 2006, Masiá et al., 2013). From the results obtained after the wide-scope screening methodology, a list of pesticides was elaborated to carry out the next stage of target quantitative analysis. A total of 24 compounds (pesticides and TPs) were selected for the monitoring in the three subsequent campaigns. We prioritized the application of a LC-MS/MS method against a GC-MS/MS method, as most of the compounds found in the samples were LC-MS amenable. A few more compounds were added to the list of target analytes (atrazine-desisopropil, 2,4-D, linuron, prometryn, propamocarb, pyridaphention), based on our previous experience on surface water analysis, and on availability of reference standards in our laboratory. Several of the compounds selected are widely used in citrus production and processing (e.g. imazalil, metalaxyl, tebuconazole, thiabendazole).

3.2. Target analysis by LC-MS/MS (QqQ)

A total of 57 surface water samples were analysed by LC-MS/MS (QqQ) for the quantification of 24 target pesticides and TPs. QCs were analysed together (see **Table S3**). The results of the analysed samples are shown in **Tables S4–S6**, which correspond to the 1st (June 2018, summer), 2nd (September 2018, autumn) and 3rd (February 2019, winter) campaigns, respectively.

Table 4 shows the frequency of detection (% positive samples) for the compounds under study. The majority of compounds selected (19 out of 24) were found at least once in the analysed samples. The fungicide thiabendazole (26%) and the insecticide imidacloprid (23%) were the most commonly detected, with concentrations above the cut-off value of 0.5 ng/L. Four out of five pesticides (diuron, atrazine, terbutryn and simazine) included in the list of priority substances in the field of water policy were detected, but in all cases at concentrations levels below the maximum allowable concentration for surface waters (Directive, 2013/39/EU). Four out of the five investigated TPs were found, being the TPs of terbumeton (-desethyl) and therbuthylazine (-desethyl and –OH) those with the highest detection frequencies (9–10%). The maximum concentration level for TPs corresponded to terbuthylazine-OH (0.066 μg/L) in the mouth of the river (sample 19) during the first campaign (June 2018). The use of three triazine herbicides detected (atrazine, terbutryn and terbumeton) is prohibited in the countries of the European Union, so finding residues of these compounds or their TPs confirms their persistence in the environment, and hence the need to phase them out of the markets.

Table 4. Results obtained from quantitative analysis by LC-MS/MS (QqQ) of water samples collected in the three campaigns. All percentages were calculated from a total number of 57 samples. The lowest calibration level (LCL) was used as limit of quantification (1 ng/L for all pesticides). The half of LCL value was taken as the cut-off reference for detection frequency.

Family	Compounds	Positive samples(%)	Maximum level found (μg/L)
Fungicides			
Anilide	Metalaxyl	5	d
Benzimidazole	Thiabendazole	26	34.5
Carbamate	Propamocarb	0	-
Conazole	Imazalil	14	4.9
	Tebuconazole	2	d
Herbicides			
Chloroacetanilide	Metolachlor	2	d
Methylthiotriazine	Prometryn	4	d
Phenoxyacetic	2,4-D	5	0.61
Phenylurea	Diuron[a]	19	0.050
	Linuron	7	d

Table 4 (cont). Results obtained from quantitative analysis by LC-MS/MS (QqQ) of water samples collected in the three campaigns. All percentages were calculated from a total number of 57 samples. The lowest calibration level (LCL) was used as limit of quantification (1 ng/L for all pesticides). The half of LCL value was taken as the cut-off reference for detection frequency.

Family	Compounds	Positive samples (%)	Maximum level found (µg/L)
Triazine	Atrazine[a]	2	d
	Atrazine-desethyl	2	0.047
	Atrazine-desisopropil	0	-
	Terbumeton	7	d
	Terbumeton-desethyl	10	d
	Terbuthylazine	4	d
	Terbuthylazine-desethyl	9	0.025
	Terbuthylazine-OH	10	0.066
	Terbutryn[a]	5	0.035
	Simazine[a]	10	0.011
Insecticides			
Carbamate	Carbaryl	0	-
Organophophorus	Chlorpyrifos[a]	0	-
Organothiophosphate	Pyridaphention	0	-
Neonicotinoid	Imidacloprid[b]	23	0.27

[a] Compounds included in the List of Priority substances in the field of water policy (Directive 2013/39/EU). The maximum allowable concentration in inland surface waters: atrazine 2 µg/L, chlorpyrifos 0.1 µg/L, diuron 1.8 µg/L, simazine 4 µg/L, terbutryn 0.34 µg/L.
[b] Compound included in the Watch List (EC 2018/840). The maximum acceptable method detection limit for imidacloprid is 8.3 ng/L.

Only four pesticides exceeded in some occasions the value of 0.1 µg/L, namely 2,4-D, imidacloprid, thiabendazole and imazalil. The latter fungicides were detected at the highest concentrations, being thiabendazole the one that reached the highest concentration reported in this study (34.5 µg/L, sampling site 18, 2nd campaign). Both, thiabendazole and imazalil, are widely used in the post-harvest processing of citrus fruits in the Mediterranean area (Sanitat Vegetal. Generalitat Valenciana., 2020). Discharges of effluents from WWTPs that treat wastewater from agro-food industries can be an important route of water contamination in the investigated zone. In fact, previous studies have shown the presence of thiabendazole in effluents from these industries (Sánchez Pérez et al., 2014). These fungicides have been

frequently detected in surface water samples collected in this area (Ccanccapa et al., 2016, Fonseca et al., 2019, Hernández et al., 2015, Rousis et al., 2017), in untreated wastewaters (Marín et al., 2009) and occasionally in ground water (Hernández et al., 2008). Furthermore, the neonicotinoid insecticide imidacloprid has been recently prohibited for outdoor agricultural applications in the European Union (European Comission 2018/783, 2018, European Comission 2018/783, 2018) and is currently included in the Watch List of European Commission (European Comission 2018/840, 2018, European Comission 2018/840, 2018). The presence of imidacloprid in the surface water under study could be explained by its high persistence in sediments and soils (Bonmatin et al., 2015), and because its application as pet pest control and consequent environmental discharge by WTTP effluents (Sadaria et al., 2017).

The total accumulated concentrations of all pesticides in each sampling site are shown in **Fig. 2**. As expected, the upper section of the river was the less contaminated one and none of the sampling sites showed pesticide residue concentrations above the limit of quantification. The incidence of agriculture on water pollution in this area is presumably low, as more than 80% of land cover is occupied by forested areas and large part of the existing cultivation areas are abandoned (González, 2017). As expected, the fertilizer factory near to site 2 did not have any pesticide contribution to the river. The nearest sampling sites to the fish farm (sites 3 and 4) did not appear to influence the river pesticide exposure either.

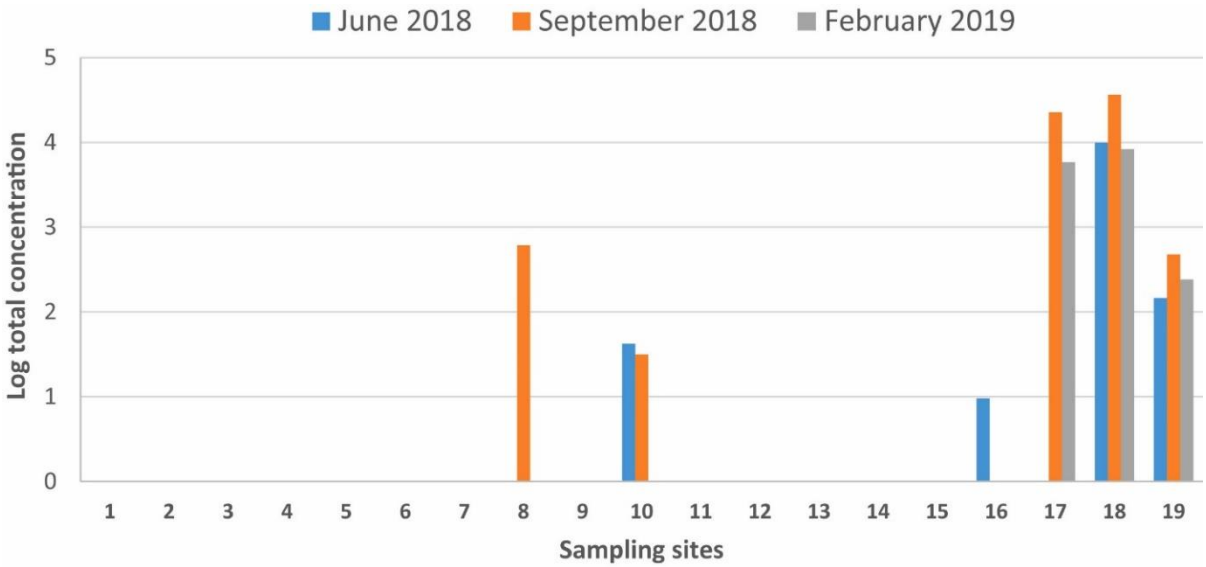


Fig. 2. Log total pesticide concentrations in the Mijares River in every sampling campaign (1st: June 2018; 2nd: September 2018, 3rd: February 2019).

With regards to the middle section of Mijares River, only two sites produced positive detections above the limit of quantification (sites 8 and 10) and the rest of sampling sites, did not show any remarkable positives. The concentration sum of pesticides downstream the Arenós reservoir (site 8) was above 600 ng/L, mainly due to the contribution of the phenoxyacetic herbicide 2,4-D, found in the second campaign at a relatively high concentration (611 ng/L) probably due to spray drift during it application in the late summer season. This herbicide is normally used to control dicotyledons, especially in wheat and barley crops. In the locations sampled downstream of WWTPs, Montanejos (site 10) showed some contamination by pesticides (total concentration 42 and 32 ng/L, in the first and the second campaign respectively). Although surface runoff from agricultural areas is expected to be one of the main sources of pesticide pollution together with spray drift, the contribution of WWTPs cannot be neglected (Fairbairn et al., 2016, Gago-Ferrero et al., 2020, Köck-Schulmeyer et al., 2013, López-Pacheco et al., 2019). However, the sampling site 11 (downstream WWTP Toga) did not appear to be contaminated, probably due to the small size of this village (only 100 inhabitants) and the limited agricultural production. In addition, the water samples collected downstream of the treatment plant of solid waste in Onda (sites 13 and 14) did not present any positives results, indicating that this treatment plant cannot be considered a pollution source for pesticides. This is in agreement with our previous study (Pitarch et al., 2016), which shows

that the major contamination source four groundwater in the surroundings of that SWTP corresponded to pesticides used in intensive citrus agriculture.

The lower section of the Mijares River was the most contaminated one, especially near to the river mouth. The sites with the highest pollution (>5000 ng/L) were located downstream of the WWTPs of Vila-real (site 17) and Almassora (site 18). Besides, these two sampling sites were among those with the highest number of positives (up to 10 pesticides were identified at site 18 in the 1st and 2nd campaigns), the next sampling site (19, Gola Almassora), the mouth of Mijares River, still presented an important pollution level. In this site, however, the total sum of concentrations was lower than 500 ng/L, indicating some natural pesticide attenuation.

Finally, it can be observed from **Fig. 2**, that the highest total concentration of pesticides in all the sampling sites was found in the second campaign (September 2018). This is in line with the pesticide application schemes, as many pesticides are reported to be applied until the end of summer (Planas et al., 2006), buy may also be explained by a lower flow and dilution capacity of the river during that period. In relation to pesticide groups, it is worth noting the high concentration of fungicides with respect to that of herbicides and insecticides (**Fig. 3**). Total fungicide concentrations as high as 59 µg/L were found in September. From the remaining groups, herbicides showed the second largest concentrations, and the only insecticide found did not exceed 0.5 µg/L.

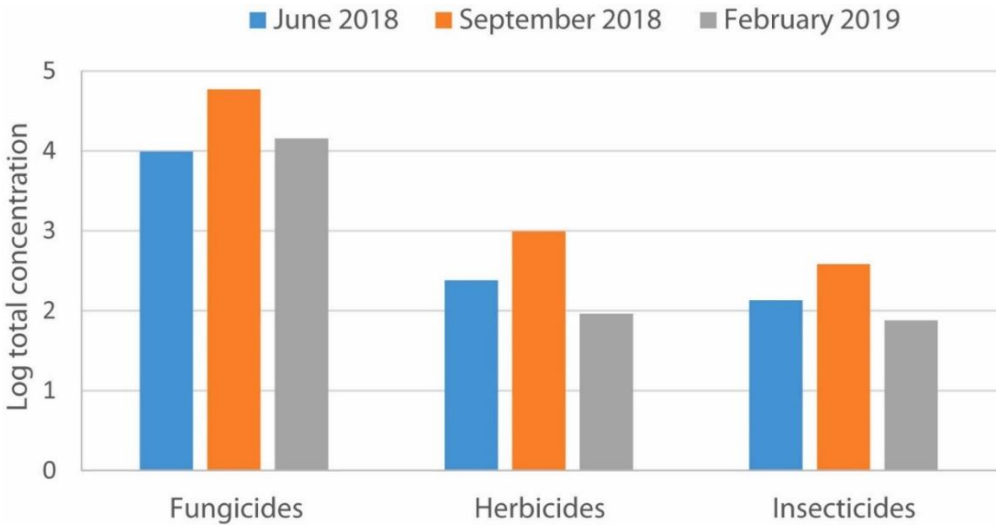


Fig. 3. Log total concentration of pesticide groups in the Mijares River in every sampling campaign (1st: June 2018; 2nd: September 2018, 3rd: February 2019).

An important finding of the present study was the high pesticide levels found in the surface water downstream of WWTP, which could be associated to the discharges of effluents from agro-food industries. Our results demonstrate that WWTP effluents can be considered important sources of pesticides into the aquatic environment, which is in agreement with previous findings (Gago-Ferrero et al., 2017, Golovko et al., 2021). Therefore, advanced technologies for removal of contaminants from wastewater are necessary to protect freshwater ecosystems (Prada-Vásquez et al., 2020).

3.3. Aquatic risk assessmen

The results of the ecological risk assessment following the TU approach show that mixture toxicity risks for primary producers were low or insignificant, with the sum of TUs being below 0.1 in all cases (**Fig. 4, Table S7**).

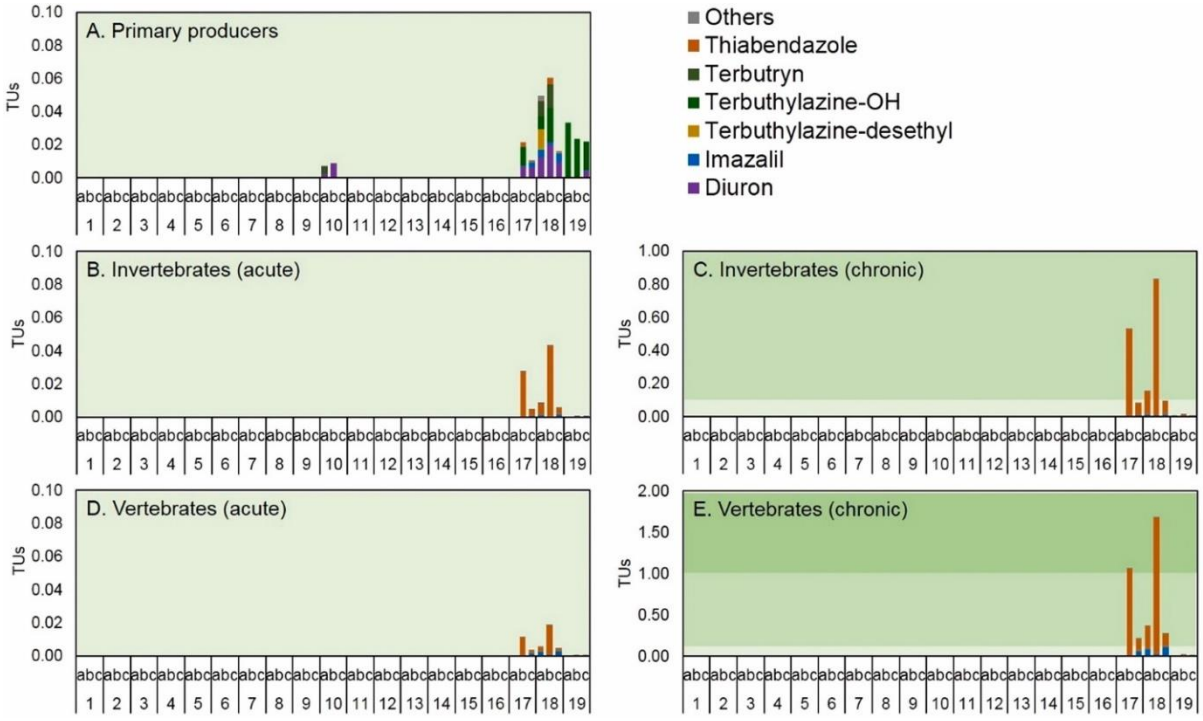


Fig. 4. Calculated sum of TUs and relative contribution of each pesticide to the total mixture toxicity. Only pesticides with TUs above 0.01 in at least one sampling site are shown. The shaded area in light, medium and intense green indicate low (sum of TUs<0.1), moderate ($0.1 \leq$ sum of TUs ≤ 1), and high (sum of TUs > 1) ecological risks, respectively. a, b, c refer to the samples taken in the first, second and third sampling campaigns (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

However, following the msPAF approach, moderate-to-high risks were calculated for sampling sites 8 (September), 10 (September), 17 (September and February) and 18 (in all sampling seasons). Toxicity was dominated by the herbicide diuron in the majority of samples, except for sampling site 18 in June, which was dominated by a combination of diuron and simazine, and sampling site 8 in September, which was dominated by 2,4-D (**Fig. 5, Table S8**).

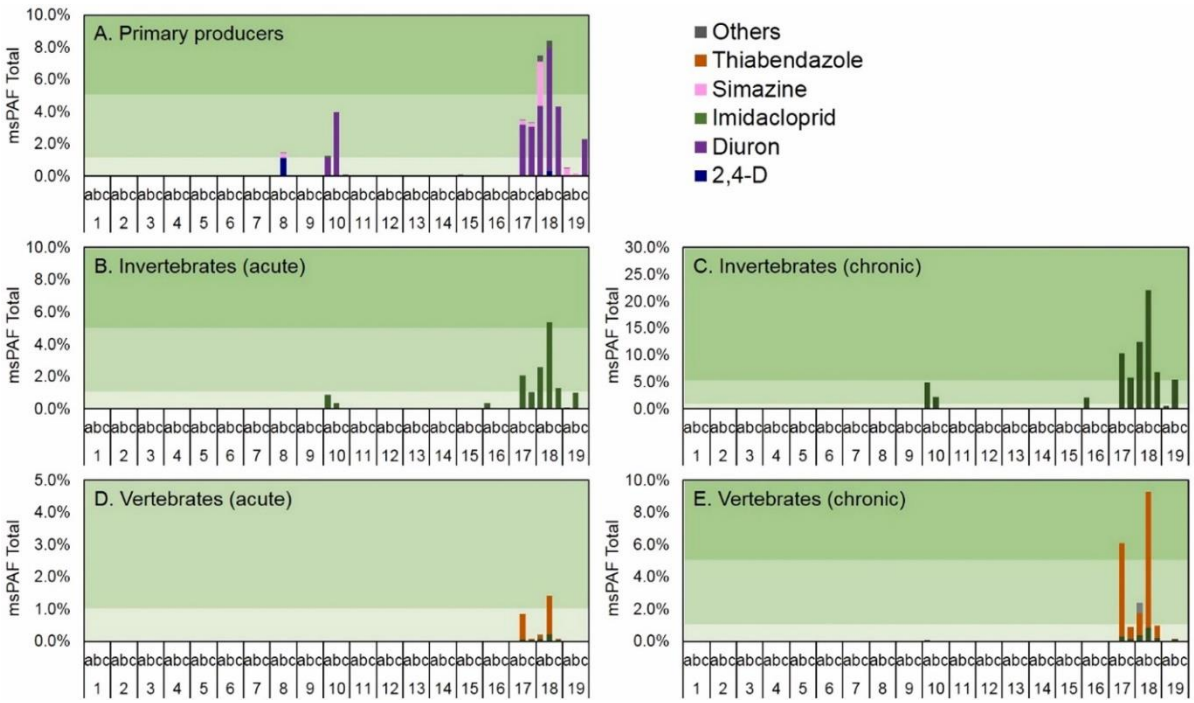


Fig. 5. Calculated msPAFTotal for each sample and relative contribution of each pesticide to the mixture toxicity. Only pesticides with a maximum calculated PAF of 1% in at least one sampling site are included. The shaded area in light, medium and intense green indicate low (msPAFTotal < 1%), moderate (1% ≤ msPAFTotal ≤ 5%), and high (msPAFTotal > 5%) ecological risks, respectively. a, b, c refer to the samples taken in the first, second and third sampling campaigns (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Diuron has been identified as one of the main pesticides causing risks for primary producers in Mediterranean watersheds (Arenas-Sánchez et al., 2019, Kuzmanović et al., 2015). It is particularly toxic to macrophytes such as *Myriophyllum spicatum* and *Apium nodiflorum* (Lambert et al., 2006), which explains the differences encountered between the results of the TU evaluation (based on the surrogate algae species *R. subcapitata*) and the ms-PAF evaluation (which also includes macrophytes). Similarly, the toxicity values

for *Myriophyllum aquaticum* NOEC-7d (lenght) and *Myriophyllum sibiricum* NOEC-14d (root growth) for 2,4-D have been found to be about 4–5 orders of magnitude lower than the calculated NOEC-96 h (growth rate) for *R. subcapitata* (**Table 2**) (Ebke et al., 2013, Roshon, 1997). The results of the acute risk assessment for aquatic invertebrates based on the TU approach showed low or insignificant risks, while moderate risks were calculated for chronic exposure in sampling sites 17 (in September) and 18 (in June and September), with a maximum sum of TUs of 0.83. In all cases, the toxic pressure was dominated by the fungicide thiabendazole (**Fig. 4, Table S9**). On the other hand, the results of the ms-PAF approach for acute exposure showed moderate risks for aquatic invertebrates in sites 17 (in September) and 18 (June and February), and high risks in site 18 in September. Chronic risks calculated with the ms-PAF approach were found to be moderate in sampling site 10 (in June and September) and high in sampling sites 17 (in September and February), 18 (all sampling seasons) and 19 (in September). The maximum calculated $msPAF_{Total}$ was 22% in sampling site 18 in September. According to the ms-PAF approach, toxicity was dominated by the insecticide imidacloprid in all cases (**Fig. 5, Table S10)**, which was not identified by the TU analysis. Imidacloprid, as well as other neonicotinoid insecticides, has been found to be orders of magnitude more toxic to aquatic insects as compared to *D. magna* (Morrissey et al., 2015, Roessink et al., 2013, Van den Brink et al., 2016), which explains the different results offered by the two approaches for this substance; and usually show larger than usual differences between acute and chronic toxicity values due to their particular toxic mode of action (Sánchez-Bayo and Tennekes, 2020). In a mesocosm study performed under Mediterranean conditions, Rico et al. (2018) found a significant decline of the aquatic insects *Cloeon dipterum* and chironomids, and the zooplankton Cyclopoida, after the application of one single dose of 0.20 µg/L of imidacloprid, which is very close to the highest concentration measured in our study (0.27 µg/L, **Table 4**), indicating a very likely abundance reduction of these taxonomic groups. It should be noted that risks of thiabendazole to invertebrates could not be evaluated following the ms-PAF approach due to lack of sufficient toxicity data (**Table 3**). However, previous studies have pointed towards a high toxicity of benzimidazole fungicides to aquatic invertebrates (Zubrod et al., 2019), and studies performed with another benzimidazole compound (carbendazim) highlighted flatworms, oligochaetes,

amphipods and cladocerans as particularly sensitive to prolonged exposure regimes (van Wijngaarden et al., 1998) such as the one found in our study.

Regarding the TU approach, acute toxicity to vertebrates was found to be insignificant. However, chronic mixture toxicity was found to be moderate in three samples (17 in February, and 18 in June and February), and high in sampling sites 17 and 18 during September, with a maximum sum of TUs of 1.7 (sampling site 18 in September). In these cases, toxicity was clearly dominated by thiabendazole, although the fungicide imazalil also had a small contribution (**Fig. 4, Table S11**). The analysis performed with the ms-PAF approach showed similar results, with thiabendazole triggering moderate-to-high risks, with a maximum $msPAF_{Total}$ of 9% in sampling site 18 during the sampling performed in September (**Fig. 5, Table S12**). It should be taken into account, however, that the chronic SSD for thiabendazole was extrapolated from the acute one (**Table 3**), which makes the ms-PAF assessment less accurate. However, the thiabendazole's NOEC-21d for *O. mykiss* (21 µg/L, **Table 2**) was well below the concentrations measured in this study (34 µg/L, **Table 4**), suggesting that thiabendazole may be posing a potential risk for fish populations. Thus, further experiments should be dedicated to assess the long-term effects of this fungicide to invertebrate and fish populations in the Mijares River.

Our study shows that, in the majority of cases, moderate-to-high risks for the different taxonomic groups were triggered by only one compound or two. This is in line with other mixture toxicity assessments performed in surface water ecosystems, which indicate that only a very limited number of compounds are responsible for biodiversity impacts in small-to-medium sized water basins (Arenas-Sánchez et al., 2019, Gustavsson et al., 2017, Munz et al., 2017, Verro et al., 2009). Our study also demonstrates that given the current data availability, risk characterization studies performed using solely the TU approach may disregard substances for which the standard test species is not sufficiently sensitive and/or for which there is wide variation in sensitivity among the species of the same taxonomic group (Morrissey et al., 2015). On the other hand, the implementation of the ms-PAF approach is limited by the number of toxicity data available, particularly for chronic risk assessments, and cannot be applied for all substances. Therefore, the combination of both methods seems a reasonable choice to identify compounds and mixtures that are posing an unacceptable risk, at least until further

experimental (or extrapolated) toxicity data become available to expand the application of the ms-PAF approach and its statistical power.

4. Conclusions

A comprehensive investigation was performed on the occurrence and risks of pesticides along a Mediterranean river impacted by citrus production and WWTPs in Spain. The upper section of the river presented low contamination, explained by the little agricultural activity, while the lower section, where citrus fruits are predominantly cultivated, was the most contaminated one, especially near the river mouth. The impact of wastewater effluents was evidenced by a notable increase of pesticide concentrations and by the higher number of compounds detected in the water samples collected downstream of WWTP discharges, especially in the lower section of the river. The highest total concentration of pesticides corresponded to the second campaign performed in September 2018, as most pesticide applications are made until late summer and dilution rates are lower during that period. The ecological risk assessment performed showed that imidacloprid poses high acute and chronic risks to aquatic invertebrates. Furthermore, moderate-to-high risks were calculated for primary producers due to diuron, and mixtures containing diuron, simazine and 2,4-D; and moderate-to-high risks were calculated for invertebrate and vertebrate species due to chronic exposure to the fungicide thiabendazole. This study demonstrates that, while intensive agricultural production is the main source of pesticide contamination in the Mijares River, freshwater biodiversity is primarily threatened in areas near to WWTPs and downstream of post-harvest citrus processing plants. Further actions are needed to control pesticide use and environmental emissions in agricultural areas of eastern Spain dominated by citrus production.

CRediT authorship contribution statement

Lubertus Bijlsma: Resources, Investigation, Formal analysis, Visualization, Writing - original draft. **Elena Pitarch:** Supervision, Visualization, Funding acquisition, Writing - original draft. **Félix Hernández:** Supervision, Visualization, Funding acquisition, Writing - review & editing. **Eddie Fonseca:** Formal analysis, Resources, Writing - review & editing. **José M. Marín:** Formal analysis, Resources, Writing - review & editing. **María Ibáñez:** Investigation, Formal analysis, Writing - review & editing. **Tania**

Portolés: Investigation, Formal analysis, Writing - review & editing. **Andreu Rico:** Resources, Investigation, Formal analysis, Writing - original draft.

Declaration of Competing Interest

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

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SUPPLEMENTARY MATERIAL

Ecological risk assessment of pesticides in the Mijares river (eastern Spain) impacted by citrus production using wide-scope screening and target quantitative analysis

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2. EXPERIMENTAL

2.1. Pesticide standards and reagents

Reference standards of pesticides, transformation products and isotopically labelled reference standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany), Fluka (Buchs, Switzerland), or Sigma-Aldrich (Madrid, Spain). Stock standard solutions (around 500 mg/L) were prepared in acetone and were stored at -20°C. Twenty-two mixtures of pesticide standards (individual concentration of each pesticide around 50 mg/L) were prepared by dilution of stock individual solutions in acetone. Working standard solutions containing all pesticides were prepared by dilution of mixtures with acetone (for sample fortification in GC), hexane (GC injection), methanol (for sample fortification in LC) and water (instrument injection in LC).

Acetonitrile and methanol (HPLC grade) were purchased from Scharlab (Barcelona, Spain). HPLC-grade water (resistivity of 18 MΩ cm) was obtained by purifying demineralised water (Millipore Ltd., Bedford, MA, USA). Formic acid (HCOOH, content > 98%) and ammonium acetate (NH_4Ac, reagent grade) were supplied by Scharlab.

Cartridges used for solid phase extraction were 150 mg Oasis HLB (Waters, Milfors, MA, USA).

2.3. Sample collection

Table S1. Description and location of the sampling sites along the Mijares River.

Sampling site	Source	Location	Province	Observations	UTM X	UTM Y	UTM Zone
1	Upper	Azud de Babor, Mora de Rubielos	Teruel	-	689781	4448005	30T
2	Upper	La Escaleruela, Sarrión	Teruel	Fertilizer factory	691742	4446731	30T
3	Upper	La Escaleruela, Sarrión	Teruel	Fish farm	691816	4446682	30T
4	Upper	La Escaleruela, Sarrión	Teruel	Fish farm (downstream)	691833	4446727	30T
5	Upper	Toranes, Albentosa	Teruel	Reservoir	692172	4446656	30T
6	Upper	Pozo de las Palomas (Los Cantos)Puebla de Arenoso	Castellón	-	703509	4443872	30T
7	Middle	Arenós, Puebla de Arenoso	Castellón	Upstream Reservoir	705419	4442293	30T
8	Middle	Arenós, Puebla de Arenoso	Castellón	Downstream Reservoir	708738	4440125	30T
9	Middle	Montanejos	Castellón	Upstream WWTP	712148	4438332	30T
10	Middle	Montanejos	Castellón	Effluent WWTP	712150	4438356	30T
11	Middle	Toga	Castellón	Effluent WWTP	725126	4435377	30T
12	Middle	Sitjar, Onda	Castellón	Reservoir	736137	4432693	30S
13	Middle	Onda	Castellón	Downstream SWTP, Power-plant	736525	4431287	30S
14	Middle	Onda	Castellón	Downstream SWTP, Gauging station	736713	4431282	30S
15	Lower	Onda	Castellón	-	740931	4429492	30S
16	Lower	Vila-real	Castellón	Santa Quiteria	748201	4426699	30S
17	Lower	Vila-real	Castellón	Effluent WWTP	751127	4424425	30S
18	Lower	Almassora	Castellón	Effluent WWTP	751477	4424111	30S
19	Mouth	Almassora	Castellón	Gola	755367	4421890	30S

UTM: Universal Transverse Mercator (hemisphere Northern)

2.4. Instrumentation

2.4.1 QTOF MS

LC-QTOF MS

A Waters Acquity UPLC system (Waters, Milford, MA, USA) was interfaced to a hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (XEVO G2 QTOF, Waters Micromass, Manchester, UK), using an orthogonal Z-spray-ESI interface, operating in both positive and negative ionisation modes. The chromatographic separation was performed using a Cortecs C_{18} analytical column (2.1 i.d. × 100 mm length, 2.7 µm particle size) from Waters, at a flow rate of 300 µL/min. The mobile phases used were (A) H_2O with 0.01% HCOOH and (B) MeOH with 0.01% HCOOH. The initial percentage of B was 10%, which was linearly increased to 90% in 14 min, followed by a 2 min isocratic period and, then, returned to initial conditions during 2 min. Nitrogen was used as drying and nebulizing gas. The gas flow was set at 1000 L/h. TOF-MS resolution was approximately 20,000 at full width half maximum (FWHM) at *m/z* 556. MS data were acquired over an *m/z* range of 50–1000. Capillary voltages of 0.7 and 3.0 kV were used in positive and negative ionisation modes, respectively. A cone voltage of 20 V was selected for both ionisation modes. Collision gas was argon 99.995% (Praxair, Valencia, Spain). The desolvation temperature was set to 600 °C and the source temperature to 130 °C. The column temperature was set to 40 °C.

For MS^E experiments, two acquisition functions with different collision energies were created. The low energy function (LE), selecting a collision energy of 4 eV, and the high energy (HE) function, with a collision energy ramp ranging from 15 to 40 eV, in order to obtain a greater range of fragment ions. The LE and HE functions settings were for both a scan time of 0.4 s.

Calibrations were automatically conducted from *m/z* 50 to 1000 with a 1:1 mixture of 0.05M NaOH:5% HCOOH, twenty five-fold diluted with ACN:H_2O (80:20). For automated accurate mass measurement, a solution of leucine encephalin (10 µg/mL) in ACN:H_2O (50:50) with 0.1% HCOOH was used as lock mass and pumped at a flow rate of 20 µL/min. The (de)protonated molecule of leucine encephalin (*m/z* 556.2771 in positive mode, *m/z* 554.2615 in negative mode) was used for recalibrating the mass axis and ensuring a robust accurate mass

measurement at any time. Mass data were acquired with MassLynx v 4.1 (Waters) and processed by ChromaLynx application manager (within MassLynx v 4.1).

GC-QTOF MS

An Agilent 7890A GC system (Palo Alto, CA, USA) equipped with an Agilent 7683 autosampler was coupled to the Xevo G2 QTOF, operating in APCI mode. The GC separation was performed using a fused silica DB-5MS capillary column with a length of 30 m x 0.25 mm i.d. and a film thickness of 0.25 µm (J&W Scientific, Folson, CA, USA). The oven temperature was programmed as follows: 90 °C (1 min); 5 °C/min to 300 °C (2 min). Pulsed splitless (50 psi) injections of 1 µL of sample extracts were carried out with an injector temperature of 280 °C and with a splitless time of 1 min. Helium 99.999 % (Praxair, Valencia, Spain) was used as carrier gas at a constant flow of 2 mL/min. The interface and source temperatures were set to 310 °C and 150 °C, respectively. The desolvation gas (N_2) was set at 300 L/h flow and the cone gas at 16 L/h. The voltage of the sampling cone was set at 20 V, the voltage of the extraction cone was 4 V, and the APCI corona pin was fixed at a current 1.7 µA. The ionization process occurred within an enclosed ion volume, which enabled control over the protonation/charge transfer processes. TOF MS resolution was approximately 20,000 (FWHM) at *m/z* 614. A scan time of 0.4 s was selected. MS data were acquired over an *m/z* range of 50-650. Heptacose was used for the daily mass calibration. Continuous internal calibration was performed using a background ion coming from the GC-column bleed as lock mass ($[M-H]^+$ of octamethylcyclotetrasiloxane, *m/z* 297.0830). Two injections were performed for sample: the first one promoting the formation of the molecular ion, and the second one, promoting the formation of the protonated molecule.

2.4.2. LC-MS/MS (QqQ)

A triple quadrupole mass spectrometer was interfaced to a Waters ACQUITY ultra performance liquid chromatography (UPLCTM) system (Waters Corp., Milford, MA, USA), equipped with a quaternary pump system. Chromatographic separation was carried out using an ACQUITY UPLC BEH C18 column (100 x 2.1 mm i.d., particle size 1.7 µm) (Waters). An optimized gradient was applied at a constant flow rate of 0.4 mL/min using methanol LC-MS (solvent A) and water LC-MS (solvent B), both 0.01% HCOOH and 1 mM ammonium acetate. The gradient elution was: 0 min, 5% A; 0-7 min linear from 5 to 90% A; 7-8 min, 90% A; 8-

8.1 min linear from 90 to 5 % A, return to initial conditions; 8.1–10 min 5% A, equilibration of the column. The injection volume was 100 µL.

A Xevo TQ-S^{TM} triple quadrupole mass spectrometer (Waters Micromass, Manchester, UK), equipped with ESI source was used. Determination of analytes was performed using ESI source in both positive and negative ion modes. Drying gas as well as nebulising gas was nitrogen (Praxair, Valencia, Spain). The cone gas flow rate was optimized at 250 L/h and the desolvation gas flow was set to 1200 L/h. The desolvation temperature was 650 °C. For operation in MS/MS mode, collision gas was Argon 99.995% (Praxair, Valencia, Spain) with a flow of 0.15 mL/min in the collision cell. Electrospray needle capillary voltage was fixed at 3.5 kV and 2 kV in positive and negative ionisation modes, respectively. The source temperature was set to 150 °C. The column temperature was maintained at 40 °C.

MassLynx software v 4.1 (Waters Corporation) was used to acquire data. TargetLynx application manager was used to quantify the concentration levels of the target analytes.

2.6. Screening by QTOF MS

When the reference standard was available, a compound was classified as "detected" when the molecular ion (i.e. in GC analysis), the (de)protonated molecule (in both GC and LC analysis) or a relevant adduct was observed (together with its characteristic isotopic pattern, if exits) at the expected Rt (±0.1 min deviation respect to a reference standard) with accurate mass (mass error ≤ 5 ppm). Then, the detected compound was considered as "identified/confirmed" when the molecular ion or (de)protonated molecule and at least one fragment ion were observed, both with mass errors below 5 ppm at the appropriate Rt. On the other hand, a compound (for which reference standard was not available at our laboratory) was considered as "tentatively identified" when one or more fragment ions were compatible with the chemical structure of the candidate or in agreement with previously reported data in the literature.

2.8. Quantitative analysis by LC-MS/MS

In order to allow the simultaneous quantification and reliable identification of the positive findings, two selected reaction monitoring (SRM) transitions (Q for quantification and q for

confirmation) were acquired for every compound (see **Table S2**). In addition, 8 isotopically labelled internal standard (ILIS) were used for matrix effects correction.

Quantification of pesticides was made using the Q transition and external calibration with standards in solvent, using relative areas with ILIS for several compounds. At least seven-points calibration curves (between 0.001-10 μg/L) were injected at the beginning and the end of each sequence. Linearity was assumed when regression coefficient was >0.99 with residuals lower than 30%. As samples were analysed by direct injection without any pre-concentration step, the lowest calibration level (LCL) was considered as the limit of quantification in water analysis (1 ng/L for all compounds).

The reliable identification of pesticides found in the samples was based on the ion ratio (peak area) calculation between both transitions (q/Q). The finding was considered as positive when the ion-ratio and the Rt of the compound in sample were within the tolerance ranges (± 30% for ion ratio, ± 0.1 min for Rt) in comparison with the reference standards injected in the calibration.

Table S2. LC-MS/MS conditions for pesticide quantification. Quantification (Q) and confirmation (q) transitions. Collision energy (CE).

Compounds	ESI	Cone (V)	Transition (Q)	CE (eV)	Transition (q)	CE (eV)
Atrazine	+	50	216.1 > 174.1	20	216.1 > 96.2	25
Atrazine-desethyl (DEA)	+	10	188.3 > 146.3	15	188.3 > 104.2	25
Atrazine-desisopropyl (DIA)	+	10	174.3 > 132.2	15	174.3 > 96.3	15
Carbaryl	+	30	202.0 > 145.1	20	202.0 > 127.2	30
Chlorpyrifos	+	10	350.0 > 97.0	30	350.0 > 198.0	20
2,4-D	-	10	219.0 > 125.0	25	219.0 > 161.0	15
Diuron	+	30	233.1 > 72.2	20	233.1 > 160.1	20
Imazalil	+	30	298.1 > 159.0	20	298.1 > 256.0	20
Imidacloprid	+	20	256.1 > 209.2	10	256.1 > 175.1	10
Linuron	+	10	249.0 > 160.0	15	249.0 > 182.0	15
Metalaxyl	+	35	280.1 > 220.1	15	280.1 > 160.1	25
Metolachlor	+	50	284.2 > 252.0	15	284.2 > 176.1	25

Table S2 (cont.). LC-MS/MS conditions for pesticide quantification. Quantification (Q) and confirmation (q) transitions. Collision energy (CE).

Compounds	ESI	Cone (V)	Transition (Q)	CE (eV)	Transition (q)	CE (eV)
Prometryn	+	10	242.0 > 158.0	20	242.0 > 200.0	15
Propamocarb	+	10	189.0 > 102.0	15	189.0 > 144.0	10
Pyridaphention	+	20	340.9 > 189.2	20	340.9 > 205.2	20
Simazine	+	10	202.3 > 132.1	15	202.3 > 124.2	15
Tebuconazole	+	10	308.0 > 70.0	15	308.0 > 125.0	30
Terbumeton	+	50	226.0 > 170.2	20	226.0 > 113.9	15
Terbumeton-desethyl	+	50	198.3 > 142.2	15	198.3 > 86.1	20
Terbuthylazine	+	10	230.3 > 174.2	15	230.3 > 104.2	25
Terbuthylazine-desethyl	+	20	202.2 > 145.9	25	202.2 > 79.0	25
Terbuthylazine-OH	+	30	212.2 > 156.1	15	212.2 > 86.1	20
Terbutryn	+	30	242.1 > 91.2	30	242.1 > 186.2	30
Thiabendazole	+	20	202.1 > 131.2	40	202.1 > 175.1	40
ILIS						
Atrazine-d_5	+	40	221.1 > 179.2	20		
Chlorpyrifos methyl-d_6	+	10	328.0 > 130.5	20		
Diuron-d_6	+	30	239.1 > 78.2	20		
Imazalil-d_5	+	30	302.0 > 159.0	20		
Metolachlor-d_6	+	25	290.2 > 258.2	15		
Simazine-d_5	+	30	207.2 > 137.0	20		
Terbuthylazine-d_5	+	10	235.2 > 179.2	15		
Thiabendazole-d_6	+	20	208.1 > 137.2	40		

2.9. Ecological risk assessment

The toxicity data selection criteria for primary producers, invertebrates and vertebrates were:

For primary producers, NOEC (No Observed Effect Concentration) values based on growth rate or yield obtained after an exposure period of 3-5 days and 7-28 days, for algae and macrophytes, respectively, were selected.

Acute toxicity data for invertebrates consisted of EC50s (mortality or immobilization) calculated after an exposure period of 2-4 days, while chronic toxicity data for invertebrates consisted of NOECs (growth rate, feeding inhibition, reproduction, mortality) calculated after and exposure period of 21 days.

Acute toxicity data for aquatic vertebrates (i.e., fish and amphibians) consisted of LC50s calculated after an exposure period of 2-4 days. Chronic toxicity data for vertebrates consisted of NOEC values (growth rate, development, behaviour, mortality) calculated after and exposure period of 21 days.

In the case that more than one toxicity value was available for a given taxon and pesticide combination, the geometric mean was calculated for the same endpoint and exposure duration, and the lowest value was conservatively selected.

3. RESULTS AND DISCUSSION

Table S3. Mean recoveries and RSD (in brackets) obtained for QCs after application of the LC-MS/MS procedure, corresponding to the three sampling campaigns. A total of 9 replicates (n=3 at each concentration level) were analyzed. The ILIS used for each compound is also shown.

Compounds	**ILIS**	**QC**					
		0.01 µg/L		**0.1 µg/L**		**1 µg/L**	
Atrazine	Atrazine-d_5	101	(4)	99	(3)	101	(3)
Atrazine-desethyl (DEA)	Atrazine-d_5	107	(13)	96	(16)	96	(14)
Atrazine-desisopropyl (DIA)	Atrazine-d_5	-		115	(18)	111	(20)
Carbaryl	-	82	(23)	67	(18)	64	(14)
Chlorpyrifos	-	158	(38)	80	(23)	71	(21)
2,4-D	-	-		96	(20)	94	(18)
Diuron	Diuron-d_6	116	(17)	102	(3)	106	(7)
Imazalil	Atrazine-d_5	148	(24)	105	(4)	107	(4)
Imidacloprid	Thiabendazole-d_6	116	(30)	113	(28)	111	(29)
Linuron	Diuron-d_6	104	(13)	109	(7)	120	(9)
Metalaxyl	-	81	(9)	80	(9)	84	(17)
Metolachlor	Metolachlor-d_6	84	(6)	86	(8)	92	(10)
Prometryn	Terbuthylazine-d_5	102	(8)	99	(9)	94	(5)

Table S3 (cont). Mean recoveries and RSD (in brackets) obtained for QCs after application of the LC-MS/MS procedure, corresponding to the three sampling campaigns. A total of 9 replicates (n=3 at each concentration level) were analyzed. The ILIS used for each compound is also shown.

Compounds	**ILIS**	**QC**					
		0.01 µg/L		**0.1 µg/L**		**1 µg/L**	
Propamocarb	-	111	(21)	97	(7)	100	(23)
Pyridaphention	-	85	(30)	83	(22)	81	(21)
Simazine	Simazine-d_5	120	(20)	109	(4)	110	(5)
Tebuconazole	Metolachlor-d_6	127	(19)	120	(14)	119	(12)
Terbumeton	Terbuthylazine-d_5	112	(9)	98	(13)	92	(6)
Terbumeton-desethyl	Simazine-d_5	104	(6)	97	(9)	98	(10)
Terbuthylazine	Terbuthylazine-d_5	109	(13)	103	(9)	105	(11)
Terbuthylazine-desethyl	Atrazine-d_5	97	(12)	103	(12)	107	(9)
Terbuthylazine-OH	Terbuthylazine-d_5	123	(21)	109	(12)	99	(5)
Terbutryn	Terbuthylazine-d_5	83	(14)	88	(10)	89	(14)
Thiabendazole	Thiabendazole-d_6	121	(22)	106	(7)	103	(6)

- Not available due to the lack of sensitivity

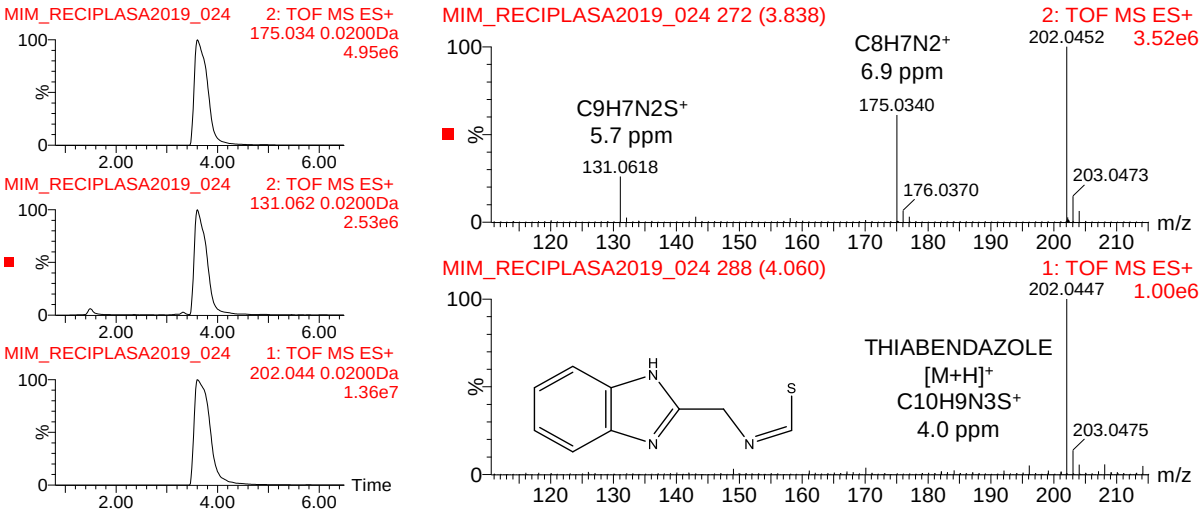

Figure S1. Detection and identification of fungicide thiabendazole in a surface water sample by LC-QTOF MS. Left: nw-XICs at 0.02 Da mass window for $[M+H]^+$ in LE function and main fragments in HE function. Right: LE (bottom) and HE (top) TOF mass spectra for the chromatographic peak at 3.9 min.

The LE spectrum of the chromatographic peak at 3.9 min showed an abundant signal at *m/z* 202.0447 (**Figure S1**) at the expected retention time of thiabendazole (C10H9N3S+, 4 ppm mass error). The HE spectrum also showed its characteristic main fragment ions at *m/z* 175.0340 (C8H7N2+, 6.9 ppm) and 131.0618 (C9H7N2S+, 5.7 ppm), confirming the identity of the pesticide.

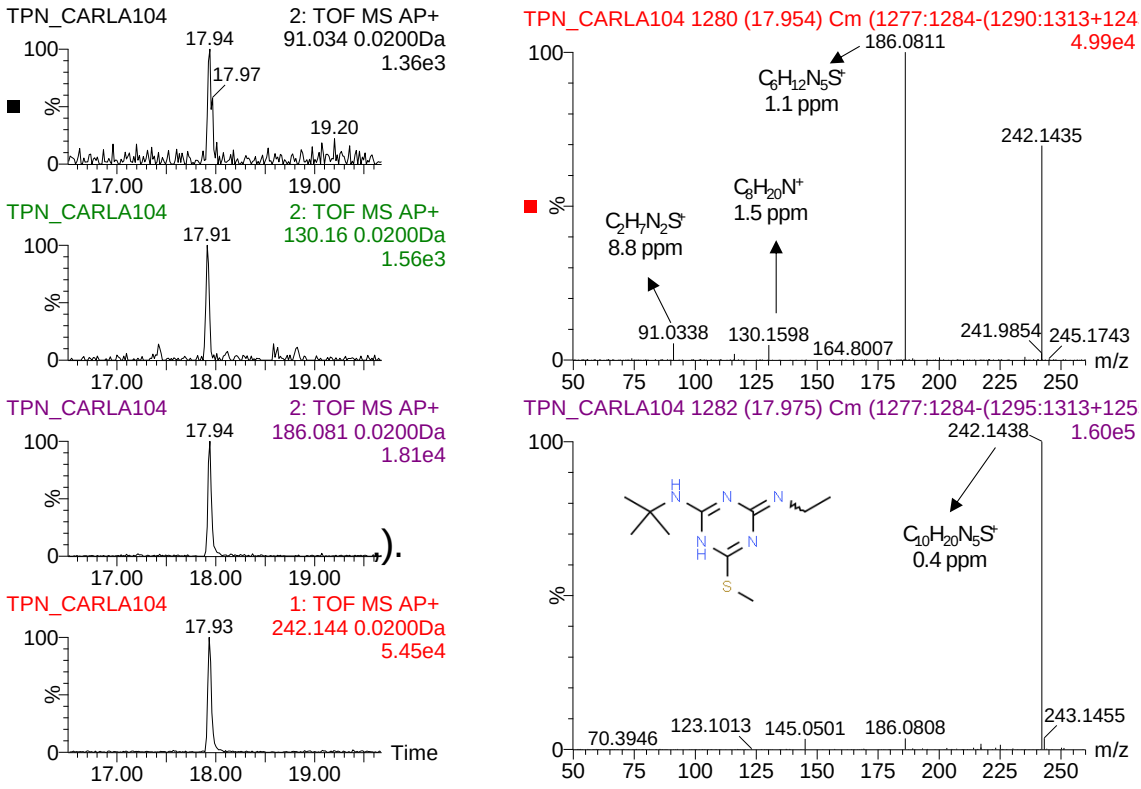

Figure S2. Detection and identification of terbutryn in a surface water sample by GC-QTOF MS. Left: nw-XICs at 0.02 Da mass window for [M+H]+ in LE function and main fragment in HE function. Right: LE (bottom) and HE (top) TOF mass spectra for the chromatographic peak at 17.93 min.

The LE spectrum of the chromatographic peak at 17.93 min showed an abundant signal at *m/z* 242.1439 (**Figure S2**) which could fit with the herbicide terbutryn (C10H20N5S+, 0.4 ppm mass error). The HE spectrum showed the characteristic main fragment ions of this compound at *m/z* values 186.0811 (C6H12N5S+, 1.1 ppm), 130.1598 (C8H20N+, 1.5 ppm) and 91.0338 (C2H7N2S+, 8.8 ppm). Moreover, the retention time deviation with respect to the reference standard was lower than 0.1 min.

Table S4. Concentration levels (ng/L) of pesticides in samples from the **first campaign** after applying LC-MS/MS (QqQ)

Compounds	Samples																		
	1a	**2a**	**3a**	**4a**	**5a**	**6a**	**7a**	**8a**	**9a**	**10a**	**11a**	**12a**	**13a**	**14a**	**15a**	**16a**	**17a**	**18a**	**19a**
Atrazine	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Atrazine-desethyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	47	-
Atrazine-desisopropil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carbaryl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chlorpyrifos	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2,4-D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diuron	-	-	-	-	-	-	-	-	-	5.3	-	-	-	-	d	-	-	31	-
Imazalil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3921	28
Imidacloprid	-	-	-	-	-	-	-	-	-	26	-	-	-	-	-	9.6	-	97	2.2
Linuron	-	-	-	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Metalaxyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Metolachlor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prometryn	-	-	-	-	-	d	-	-	-	-	-	-	-	-	-	-	d	-	-
Propamocarb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pyridaphention	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Simazine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11	2.3
Tebuconazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6.2	-
Terbumeton	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terbumeton-desethyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.3
Terbuthylazine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terbuthylazine-desethyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25	-
Terbuthylazine-OH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15	66
Terbutryn	-	-	-	-	-	-	-	-	-	11	-	-	-	-	-	-	-	22	-
Thiabendazole	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	-	-	5823	47

d. detected, concentration below to the limit value used for the quantification (LCL) and at least one q/Q ratio accomplished

Table S5. Concentration levels (ng/L) of pesticides in samples from the **second campaign** after applying LC-MS/MS (QqQ)

Compounds	Samples																		
	1b	2b	3b	4b	5b	6b	7b	8b	9b	10b	11b	12b	13b	14b	15b	16b	17b	18b	19b
Atrazine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Atrazine-desethyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Atrazine-desisopropil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carbaryl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chlorpyrifos	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2,4-D	-	-	-	-	-	-	-	611	-	-	-	-	-	-	-	-	-	136	-
Diuron	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	17	50	d
Imazalil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	395	1353	22
Imidacloprid	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	74	268	30
Linuron	-	-	-	-	-	-	-	d	-	-	-	-	-	-	-	-	d	-	-
Metalaxyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	d
Metolachlor	-	-	-	-	-	-	-	d	-	-	-	-	-	-	-	-	-	-	-
Prometryn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Propamocarb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pyridaphention	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Simazine	-	-	-	-	-	-	-	d	-	-	-	-	-	-	-	-	d	-	d
Tebuconazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terbumeton	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terbumeton-desethyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	d
Terbuthylazine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	-	-
Terbuthylazine-desethyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	-
Terbuthylazine-OH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23	40	46
Terbutryn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	35	-
Thiabendazole	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	22065	34547	377

d. detected, concentration below to the limit value used for the quantification (LCL) and at least one q/Q ratio accomplished

Table S6. Concentration levels (ng/L) of pesticides in samples from the **third campaign** after applying LC-MS/MS (QqQ)

Compounds	Samples																		
	1c	2c	3c	4c	5c	6c	7c	8c	9c	10c	11c	12c	13c	14c	15c	16c	17c	18c	19c
Atrazine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Atrazine-desethyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Atrazine-desisopropil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carbaryl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chlorpyrifos	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2,4-D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	-	-
Diuron	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	16	23	11
Imazalil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2647	4940	67
Imidacloprid	-	-	-	-	-	-	-	-	-	d	-	-	-	-	d	-	33	41	d
Linuron	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	-	-
Metalaxyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d
Metolachlor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prometryn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Propamocarb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pyridaphention	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Simazine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	-	-
Tebuconazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terbumeton	-	-	-	-	d	d	-	d	d	-	-	-	-	-	-	-	-	-	-
Terbumeton-desethyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	d	d
Terbuthylazine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d
Terbuthylazine-desethyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	d	d
Terbuthylazine-OH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	34
Terbutryn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thiabendazole	d	d	d	-	-	-	-	d	d	-	-	-	-	-	-	-	3123	3307	127

d. detected, concentration below to the limit value used for the quantification (LCL) and at least one q/Q ratio accomplished

Table S7. TUs for primary producers in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples														
	1a	1b	1c	2a	2b	2c	3a	3b	3c	4a	4b	4c	5a	5b	5c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S7 (cont.). TUs for primary producers in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples														
	6a	6b	6c	7a	7b	7c	8a	8b	8c	9a	9b	9c	10a	10b	10c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S7 (cont.). TUs for primary producers in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples														
	11a	11b	11c	12a	12b	12c	13a	13b	13c	14a	14b	14c	15a	15b	15c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S7 (cont.). TUs for primary producers in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples											
	16a	**16b**	**16c**	**17a**	**17b**	**17c**	**18a**	**18b**	**18c**	**19a**	**19b**	**19c**
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	**0.012**	**0.020**	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	**0.013**	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	**0.012**	<0.01	<0.01	**0.020**	<0.01	**0.033**	**0.023**	**0.017**
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	**0.015**	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S8. PAFs for primary producers in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples														
	1a	1b	1c	2a	2b	2c	3a	3b	3c	4a	4b	4c	5a	5b	5c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Linuron	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbumeton-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S8 (cont.). PAFs for primary producers in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples														
	6a	**6b**	**6c**	**7a**	**7b**	**7c**	**8a**	**8b**	**8c**	**9a**	**9b**	**9c**	**10a**	**10b**	**10c**
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	**0.013**	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	**0.019**	**0.055**	<0.01
Imazalil	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Imidacloprid	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbumeton	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbumeton-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table S8 (cont.). PAFs for primary producers in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples														
	11a	**11b**	**11c**	**12a**	**12b**	**12c**	**13a**	**13b**	**13c**	**14a**	**14b**	**14c**	**15a**	**15b**	**15c**
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Imidacloprid	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbumeton	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbumeton-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table S8 (cont.). PAFs for primary producers in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples											
	16a	**16b**	**16c**	**17a**	**17b**	**17c**	**18a**	**18b**	**18c**	**19a**	**19b**	**19c**
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	**0.047**	**0.045**	**0.072**	**0.098**	**0.059**	<0.01	<0.01	**0.034**
Imazalil	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Imidacloprid	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	**0.046**	<0.01	<0.01	**0.014**	<0.01	<0.01
Tebuconazole	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbumeton	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbumeton-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table S9. TUs for aquatic invertebrates acute in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples														
	1a	1b	1c	2a	2b	2c	3a	3b	3c	4a	4b	4c	5a	5b	5c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S9 (cont.). TUs for aquatic invertebrates acute in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples														
	6a	**6b**	**6c**	**7a**	**7b**	**7c**	**8a**	**8b**	**8c**	**9a**	**9b**	**9c**	**10a**	**10b**	**10c**
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S9 (cont.). TUs for aquatic invertebrates acute in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples														
	11a	11b	11c	12a	12b	12c	13a	13b	13c	14a	14b	14c	15a	15b	15c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S9 (cont.). TUs for aquatic invertebrates acute in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples											
	16a	**16b**	**16c**	**17a**	**17b**	**17c**	**18a**	**18b**	**18c**	**19a**	**19b**	**19c**
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	**0.027**	<0.01	<0.01	**0.043**	<0.01	<0.01	<0.01	<0.01

Table S9 (cont.). TUs for aquatic invertebrates chronic in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples														
	1a	1b	1c	2a	2b	2c	3a	3b	3c	4a	4b	4c	5a	5b	5c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S9 (cont.). TUs for aquatic invertebrates chronic in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples														
	6a	**6b**	**6c**	**7a**	**7b**	**7c**	**8a**	**8b**	**8c**	**9a**	**9b**	**9c**	**10a**	**10b**	**10c**
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S9 (cont.). TUs for aquatic invertebrates chronic in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples														
	11a	11b	11c	12a	12b	12c	13a	13b	13c	14a	14b	14c	15a	15b	15c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S9 (cont.). TUs for aquatic invertebrates chronic in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples											
	16a	**16b**	**16c**	**17a**	**17b**	**17c**	**18a**	**18b**	**18c**	**19a**	**19b**	**19c**
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	**0.011**	<0.01	**0.014**	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	**0.53**	**0.074**	**0.14**	**0.82**	**0.079**	<0.01	<0.01	<0.01

Table S10. PAFs for aquatic invertebrates acute in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples														
	1a	1b	1c	2a	2b	2c	3a	3b	3c	4a	4b	4c	5a	5b	5c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Metolachlor	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbumeton-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine-OH	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbutryn	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Thiabendazole	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table S10 (cont.). PAFs for aquatic invertebrates acute in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples														
	6a	6b	6c	7a	7b	7c	8a	8b	8c	9a	9b	9c	10a	10b	10c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Metolachlor	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbumeton-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine-OH	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbutryn	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Thiabendazole	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table S10 (cont.). PAFs for aquatic invertebrates acute in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples														
	11a	**11b**	**11c**	**12a**	**12b**	**12c**	**13a**	**13b**	**13c**	**14a**	**14b**	**14c**	**15a**	**15b**	**15c**
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Metolachlor	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbumeton-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine-OH	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbutryn	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Thiabendazole	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table S10 (cont.). PAFs for aquatic invertebrates acute in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples											
	16a	**16b**	**16c**	**17a**	**17b**	**17c**	**18a**	**18b**	**18c**	**19a**	**19b**	**19c**
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Imidacloprid	<0.01	<0.01	<0.01	<0.01	**0.021**	**0.011**	**0.026**	**0.054**	**0.013**	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Metolachlor	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbumeton-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine-OH	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbutryn	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Thiabendazole	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table S10 (cont.). PAFs for aquatic invertebrates chronic in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples														
	1a	1b	1c	2a	2b	2c	3a	3b	3c	4a	4b	4c	5a	5b	5c
2,4-D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Metolachlor	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbumeton-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine-OH	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbutryn	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Thiabendazole	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table S10 (cont.). PAFs for aquatic invertebrates chronic in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples														
	6a	**6b**	**6c**	**7a**	**7b**	**7c**	**8a**	**8b**	**8c**	**9a**	**9b**	**9c**	**10a**	**10b**	**10c**
2,4-D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	**0.049**	**0.022**	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Metolachlor	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbumeton-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine-OH	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbutryn	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Thiabendazole	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table S10 (cont.). PAFs for aquatic invertebrates chronic in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples														
	11a	**11b**	**11c**	**12a**	**12b**	**12c**	**13a**	**13b**	**13c**	**14a**	**14b**	**14c**	**15a**	**15b**	**15c**
2,4-D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Metolachlor	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbumeton-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine-OH	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbutryn	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Thiabendazole	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table S10 (cont.). PAFs for aquatic invertebrates chronic in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples											
	16a	**16b**	**16c**	**17a**	**17b**	**17c**	**18a**	**18b**	**18c**	**19a**	**19b**	**19c**
2,4-D	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Imidacloprid	**0.021**	<0.01	<0.01	<0.01	**0.10**	**0.058**	**0.12**	**0.22**	**0.069**	<0.01	**0.054**	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Metolachlor	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbumeton-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine-desethyl	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbuthylazine-OH	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Terbutryn	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Thiabendazole	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table S11. TUs for aquatic vertebrates acute in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples														
	1a	1b	1c	2a	2b	2c	3a	3b	3c	4a	4b	4c	5a	5b	5c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S11 (cont.). TUs for aquatic vertebrates acute in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples														
	6a	6b	6c	7a	7b	7c	8a	8b	8c	9a	9b	9c	10a	10b	10c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S11 (cont.). TUs for aquatic vertebrates acute in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples														
	11a	11b	11c	12a	12b	12c	13a	13b	13c	14a	14b	14c	15a	15b	15c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S11 (cont.). TUs for aquatic vertebrates acute in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples											
	16a	**16b**	**16c**	**17a**	**17b**	**17c**	**18a**	**18b**	**18c**	**19a**	**19b**	**19c**
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	**0.011**	<0.01	<0.01	**0.018**	<0.01	<0.01	<0.01	<0.01

Table S11 (cont.). TUs for aquatic vertebrate chronic in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples														
	1a	1b	1c	2a	2b	2c	3a	3b	3c	4a	4b	4c	5a	5b	5c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S11 (cont.). TUs for aquatic vertebrate chronic in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples														
	6a	6b	6c	7a	7b	7c	8a	8b	8c	9a	9b	9c	10a	10b	10c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S11 (cont.). TUs for aquatic vertebrate chronic in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples														
	11a	**11b**	**11c**	**12a**	**12b**	**12c**	**13a**	**13b**	**13c**	**14a**	**14b**	**14c**	**15a**	**15b**	**15c**
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S11 (cont.). TUs for aquatic vertebrates acute in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

Compounds	Samples											
	16a	**16b**	**16c**	**17a**	**17b**	**17c**	**18a**	**18b**	**18c**	**19a**	**19b**	**19c**
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	**0.062**	**0.091**	**0.031**	**0.11**	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	**1.1**	**0.15**	**0.28**	**1.6**	**0.16**	<0.01	**0.018**	<0.01

Table S12. PAFs for aquatic vertebrates acute in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples														
	1a	1b	1c	2a	2b	2c	3a	3b	3c	4a	4b	4c	5a	5b	5c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S12 (cont.). PAFs for aquatic vertebrates acute in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples														
	6a	6b	6c	7a	7b	7c	8a	8b	8c	9a	9b	9c	10a	10b	10c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S12 (cont.). PAFs for aquatic vertebrates acute in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples														
	11a	11b	11c	12a	12b	12c	13a	13b	13c	14a	14b	14c	15a	15b	15c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S12 (cont.). PAFs for aquatic vertebrates acute in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples											
	16a	16b	16c	17a	17b	17c	18a	18b	18c	19a	19b	19c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S12 (cont.). PAFs for aquatic vertebrates chronic in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples														
	1a	1b	1c	2a	2b	2c	3a	3b	3c	4a	4b	4c	5a	5b	5c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S12 (cont.). PAFs for aquatic vertebrates chronic in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples														
	6a	6b	6c	7a	7b	7c	8a	8b	8c	9a	9b	9c	10a	10b	10c
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S12 (cont.). PAFs for aquatic vertebrates chronic in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples														
	11a	**11b**	**11c**	**12a**	**12b**	**12c**	**13a**	**13b**	**13c**	**14a**	**14b**	**14c**	**15a**	**15b**	**15c**
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table S12 (cont.). PAFs for aquatic vertebrates chronic in the different sampling sites. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st: June 2018; 2nd: September 2018; 3rd: February 2019). NA: not available, there was not sufficient data to construct an SSD.

Compounds	Samples											
	16a	**16b**	**16c**	**17a**	**17b**	**17c**	**18a**	**18b**	**18c**	**19a**	**19b**	**19c**
2,4-D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Atrazine-desisopropil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Carbaryl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Chlorpyrifos	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Diuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imazalil	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imidacloprid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metalaxyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Metolachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Prometryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Propamocarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pyridaphention	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Simazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Tebuconazole	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbumeton-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-desethyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbuthylazine-OH	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Terbutryn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Thiabendazole	<0.01	<0.01	<0.01	<0.01	**0.042**	<0.01	<0.01	**0.065**	<0.01	<0.01	<0.01	<0.01

2.1.4. Discusión de resultados

Determinación de plaguicidas en aguas mediante UHPLC-HRMS

La ciencia enfrenta actualmente el reto de investigar los múltiples contaminantes que se encuentran en los diferentes compartimentos ambientales. El medio acuático no escapa a este problema. Los analizadores híbridos de cuadrupolo-tiempo de vuelo (QTOF) acoplados a cromatógrafos de líquidos o de gases son una herramienta analítica que permite detectar una gran cantidad de compuestos con propiedades fisicoquímicas muy diferentes y obtener, por tanto, una visión más realista de la carga de contaminantes en las muestras acuáticas. Si el objetivo es cuantificar los plaguicidas, se hace un análisis UHPLC-MS/MS con analizador de triple cuadrupolo.

En la presente tesis se llevó a cabo una investigación de barrido de plaguicidas y TPs mediante UHPLC-QTOF MS en muestras de aguas continentales tomadas en diferentes puntos de dos áreas situadas en el este mediterráneo de España para monitorizar los compuestos presentes más relevantes. El **artículo científico I** muestra los resultados del estudio realizado en aguas subterráneas y superficiales de la cuenca del Júcar, mientras que el **artículo científico II** se centra en el estudio de muestras de aguas superficiales tomadas a lo largo del río Mijares.

A continuación, se discuten los aspectos más relevantes de los dos artículos incluidos en el capítulo 2.1 de la presente tesis doctoral.

En el **artículo científico I**, se procedió, primeramente, a hacer una validación cualitativa de plaguicidas y TPs en aguas ambientales, aplicando un tratamiento previo mediante SPE y una posterior detección por UHPLC-QTOF MS. Se estudiaron 20 compuestos a dos niveles de concentración (0.01 y 0.1 μg/L) en una muestra de agua por triplicado. Los resultados fueron satisfactorios para un volumen de 500 mL a las dos concentraciones permitiendo alcanzar un elevado factor de preconcentración (1000). Sólo el plaguicida organofosforado clorpirifos no pudo identificarse, probablemente por falta de sensibilidad del instrumento, o bien porque se alcanzó el volumen de ruptura, pues ni siquiera a 100 mL se pudo detectar (ver **Tabla SM7** en el material suplementario). Sin embargo, teniendo en cuenta la importancia de este analito en el medio ambiente, se incluyó en el estudio posterior mediante UHPLC-MS/MS QqQ (ver **artículo científico II)**.

Una vez concluida la validación, se procedió a hacer un análisis de 8 muestras reales de agua superficial (SW) y 11 de agua subterránea (GW) tomadas en la cuenca del Júcar (ver **Figura 1** en el **artículo científico I**), siguiendo las metodologías de cribado de compuestos diana (*target screening*) y de identificación tentativa (*suspect screening*). En la primera, se seleccionan los analitos de interés con base en información cromatográfica y espectral; en la segunda, se trabaja sólo con información molecular. Para la primera se empleó una base de datos elaborada por nuestro grupo de investigación, la cual incluye más de 500 compuestos (plaguicidas y TPs) con su respectiva información cromatográfica y espectral, y para la segunda se trabajó únicamente con información molecular, pues no se disponía de los correspondientes patrones de referencia. Para identificar los compuestos, se siguieron las recomendaciones de la guía SANTE/11813/2017 (ver sección 2.7 del **artículo científico I**). En total, se identificaron 33 compuestos (27 plaguicidas y 6 TPs) por cribado de compuestos diana y 2 compuestos (1 plaguicida y 1 TP) por identificación tentativa.

La **Figura 2.2** muestra la frecuencia de detección de los plaguicidas encontrados en las 11 muestras de aguas subterráneas investigadas.

En todas las muestras de agua subterránea se encontraron los herbicidas triazínicos atrazina y simazina, junto con los TPs DEA y TED. En un 90% de las muestras (10 de 11) se observó terbutilazina y en un 72%, DIA. Además, en un 63 % de las muestras (7 de 11) se detectaron terbumetón y bromacil. Otros TPs encontrados fueron el T2H (54%) y el MOA (36%), este último detectado tentativamente pues no se disponía del estándar de referencia. Plaguicidas como el imazalil, la bentazona y el neonicotinoide imidacloprid (este último se encuentra en la lista de observación de sustancias a efectos de seguimiento a nivel europeo [43], fueron los menos detectados.

Si bien se trataba de una estimación semicuantitativa algunas muestras subterráneas presentaron bromacil (un herbicida) y TED a concentraciones superiores a 0.1 μg/L [39]. Pese a que el bromacil fue retirado en 2003, en el período 2013-2015 se detectó en varias muestras, aunque en estudios posteriores se observó una reducción gradual en su concentración en la cuenca del Júcar. En todo caso, en la campaña de 2017, fue el único compuesto que presentó una concentración superior a 0.1 μg/L en el 27% de las muestras analizadas por UHPLC-MS/MS QqQ. La presencia de bromacil en el agua subterránea indica que este plaguicida

podría haber experimentado el fenómeno de percolación, pues en 2017 no se detectó en aguas superficiales [113].

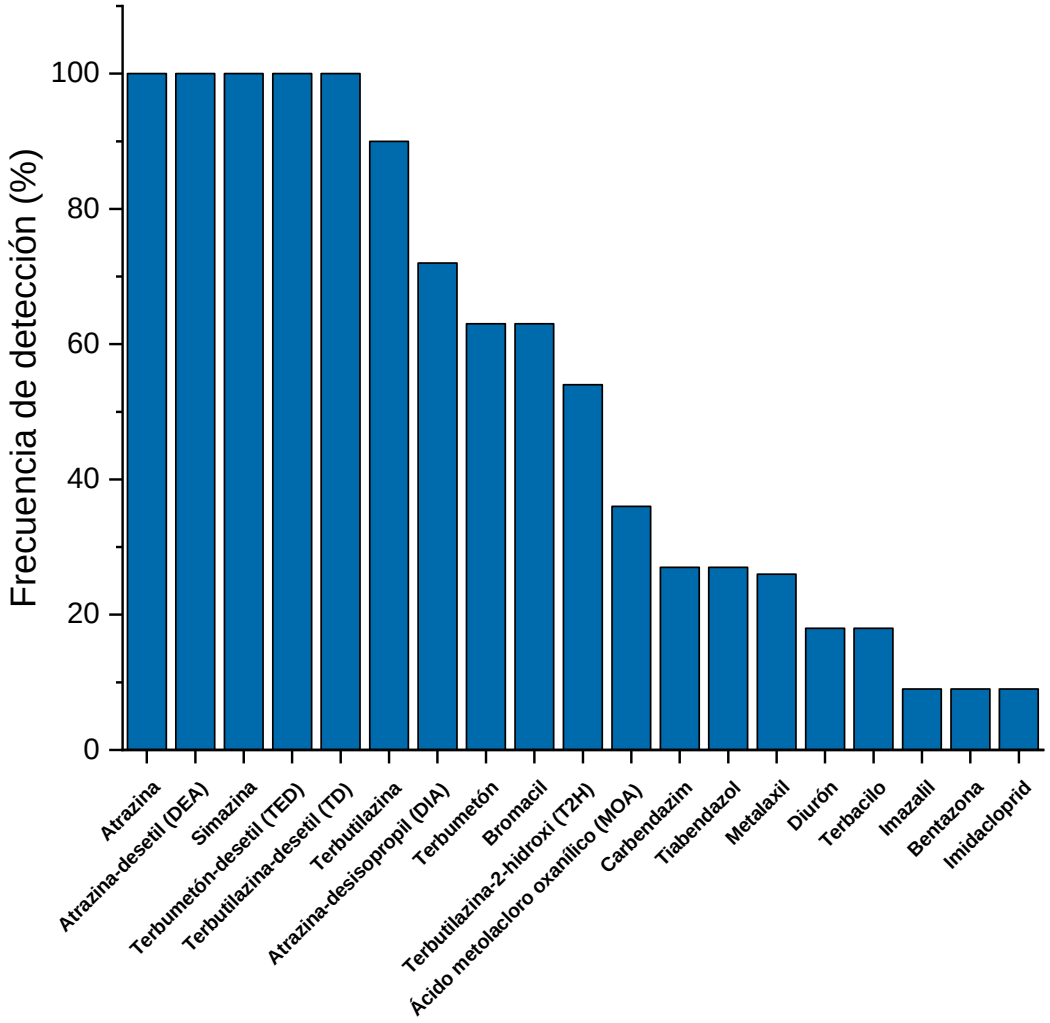

Figura 2.2. Plaguicidas y TPs detectados en muestras de agua subterránea en el cribado por UHPLC-QTOF MS. Muestras recolectadas entre marzo y abril de 2017. Cuenca del Júcar.

La **Figura 2.3** muestra el número de analitos presentes en las aguas subterráneas, donde GW32 es el punto de muestreo con mayor número de plaguicidas (16), seguido de los puntos GW43 y GW51 (14 analitos). La menor cantidad de analitos (7) se observó en los puntos GW41 y GW44.

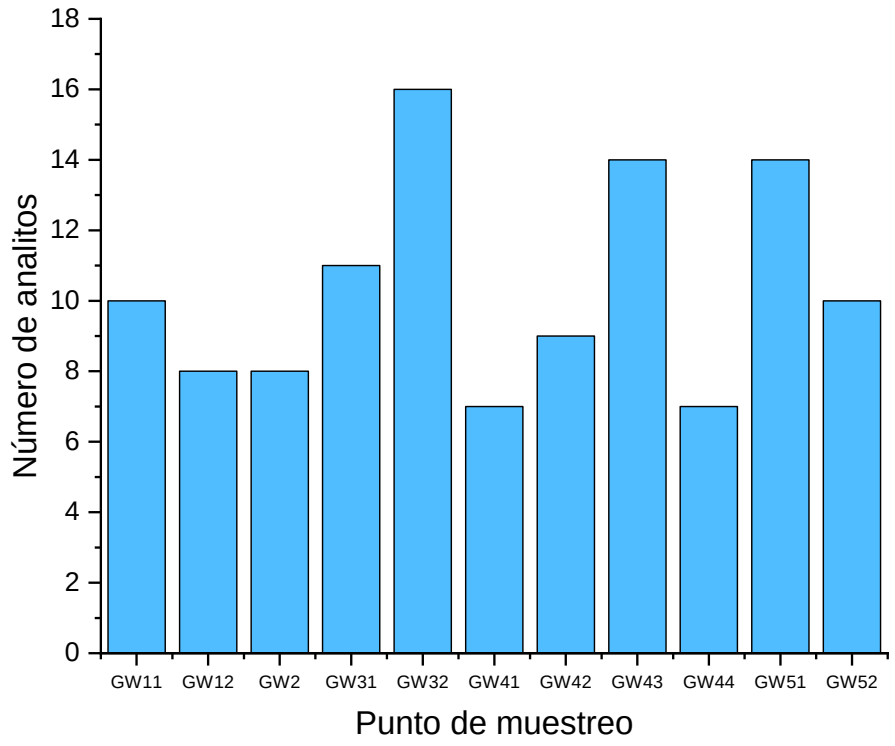

Figura 2.3. Número total de plaguicidas y TPs encontrados en cada punto de muestreo de agua subterránea en la cuenca del Júcar por UHPLC-QTOF MS. Muestras recolectadas entre marzo y abril de 2017.

La **Figura 2.4** muestra la frecuencia de detección de plaguicidas en las 11 muestras de aguas superficiales tomadas en la cuenca del Júcar. Del total de analitos detectados en las aguas superficiales, 11 estaban incluidos en la validación cualitativa previa. Todos los compuestos encontrados fueron confirmados con su respectivo estándar de referencia, excepto el MOA y el nicosulfurón.

Dos fungicidas, carbendazim y tiabendazol, se detectaron en todas las muestras de aguas superficiales. El tiabendazol se utiliza ampliamente en el tratamiento poscosecha de cítricos, mientras que el carbendazim, prohibido desde el 2014, se utilizaba como fungicida [114] y es un TP del metil-tiofanato y del benomilo [115], que se aplican en el tratamiento poscosecha de cítricos y en la producción de uva. Después de los fungicidas, los compuestos más detectados, presentes en un 62% de las muestras, fueron el imazalil, la terbutrina y el T2H. También se detectaron dos insecticidas que están en la lista de observación [43], el imidacloprid (12%) y el acetamiprid (25%). Algunos de los menos detectados (12%) fueron el ácido 2-metil-4-

clorofenoxiacético (MCPA), el paclobutrazol, un regulador de crecimiento de las plantas, y el herbicida linurón. Los únicos dos compuestos que excedieron el nivel de 0.1 μg/L fueron el imazalil, en dos de las muestras analizadas, y el tiabendazol, en una de las muestras.

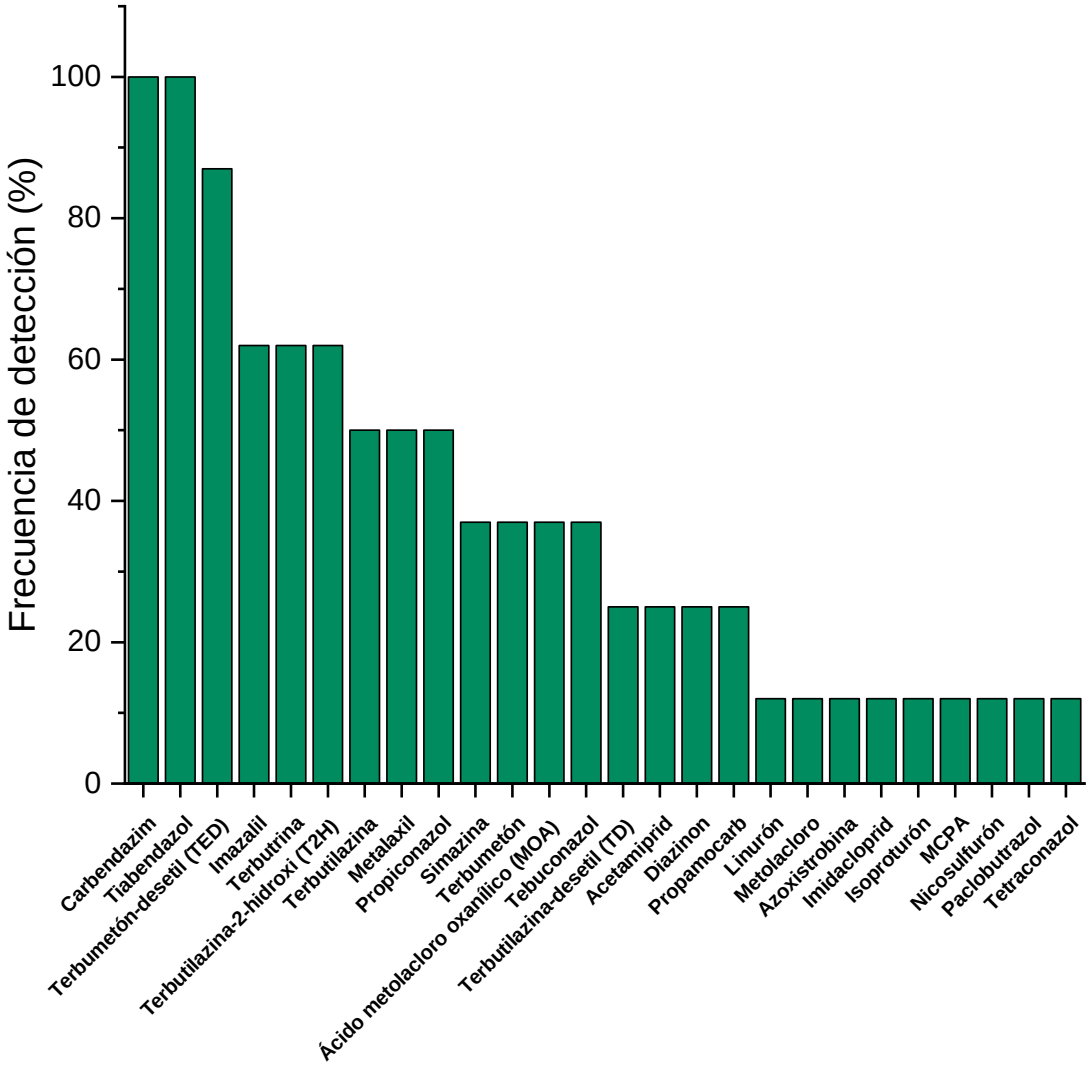

Figura 2.4. Plaguicidas y TPs detectados en muestras de agua superficial en el cribado por UHPLC-QTOF MS. Muestras recolectadas en mayo de 2017. Cuenca del Júcar.

Con respecto al número total de analitos presentes en aguas superficiales (ver **Figura 2.5**), destaca el punto SW8, donde se detectaron 18 compuestos, 17 plaguicidas confirmados con su respectivo estándar de referencia y el producto de transformación MOA, que fue identificado tentativamente. La elevada presencia de compuestos en este sitio puede deberse a la cercanía de una EDAR.

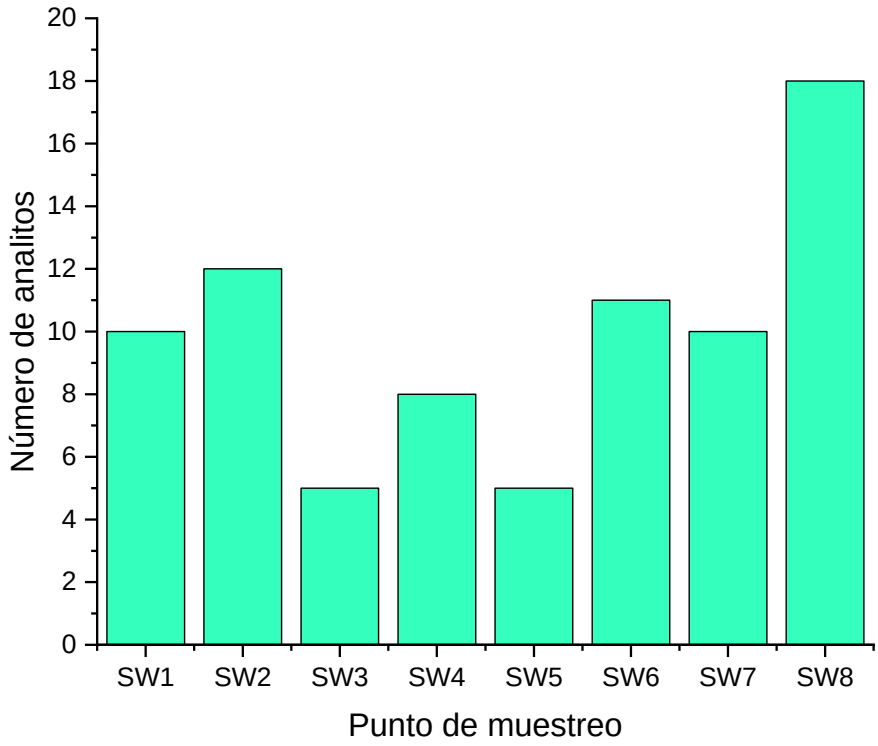

Figura 2.5. Número total de plaguicidas y TPs encontrados en cada punto de muestreo de agua superficial por UHPLC-QTOF MS. Muestras recolectadas en mayo de 2017. Cuenca del Júcar.

Tras la investigación en la cuenca del Júcar, se estudió la presencia de plaguicidas y TPs en el río Mijares (**artículo científico II)**. La aplicación de las técnicas complementarias GC-QTOF MS y UHPLC-QTOF MS forman parte de la primera parte del artículo científico II, ya que se utilizaron en una fase preliminar para determinar los plaguicidas más relevantes que pudieran encontrarse en el río Mijares. Al igual que en el estudio de la cuenca del Júcar, en el estudio del Mijares se trabajó con bases de datos creadas en nuestro centro de investigación, las cuales contemplan cerca de 550 compuestos para LC y 425 para GC. Una vez realizado el tratamiento de las muestras mediante SPE, se usaron factores de preconcentración de 500 y 1000 veces para los extractos a medir en LC y en GC, respectivamente. El análisis por GC-QTOF MS mostró la presencia de 24 compuestos (21 plaguicidas y 3 TPs). La comercialización de dos de los plaguicidas encontrados (clorpirifos y clorpirifos metil) ha quedado prohibida por la Autoridad Europea de Seguridad Alimentaria (EFSA), a partir de junio de 2020, por sus efectos perjudiciales para la salud humana [116]. Por su parte, el análisis por LC-QTOF MS mostró la presencia de 20 compuestos, 17 plaguicidas y 3 TPs, terbumetón desetil (TED), terbutilazina

desetil (TD) y terbutilazina-2-OH (T2H). También se detectaron dos insecticidas neocotinoides, el acetamiprid y el imidacloprid que aparecen incluidos en la lista de observación europea [43]. El primero representa un riesgo tóxico bajo para las abejas y otros polinizadores y el segundo, un riesgo alto [117].

Determinación de plaguicidas en aguas mediante UHPLC-MS/MS QqQ

Si bien el propósito de la toma de muestras en la cuenca del Júcar (**artículo científico I**) era hacer una estimación semicuantitativa de plaguicidas mediante UHPLC-QTOF MS, el objetivo del muestreo en el Mijares (**artículo científico II**) era obtener una cuantificación fiable mediante UHPLC-MS/MS QqQ, para luego llevar a cabo una evaluación de los riesgos ecológicos de los compuestos identificados.

Seguidamente, se discuten los resultados más relevantes del **artículo científico II** incluido en el capítulo 2.1 de esta tesis doctoral. Una vez concluido el análisis preliminar de amplio alcance mediante HRMS, se consideró una lista de 19 plaguicidas y 5 TPs para ser investigados cuantitativamente mediante UHPLC-MS/MS QqQ, sin ninguna etapa de preparación de muestra (inyección directa).

Primeramente, se optimizaron las condiciones de masas de los 24 compuestos y 8 ILIS seleccionados. Se obtuvieron los espectros de masa en modo barrido de iones completo aplicando diferentes voltajes de cono, mediante ionización por *electrospray* en polaridad positiva para todos los compuestos excepto el 2,4-D. Luego se seleccionó la molécula protonada, $[M+H]^+$, para cada uno de los analitos, y en el caso del 2,4-D, la molécula desprotonada. Una vez conocida la ionización de la molécula, se hizo un escaneo de iones producto a diferentes energías de colisión para obtener información estructural. A partir de los iones más abundantes se seleccionaron dos transiciones, una para la cuantificación (Q) y otra para la identificación (q) de cada uno de los compuestos. Además, se utilizaron 8 estándares internos marcados isotópicamente (ILIS) para corregir los efectos de la matriz. Para la identificación, se calcularon las relaciones (q/Q) de las muestras y se compararon con el valor teórico promedio obtenido a partir de las disoluciones preparadas para las curvas de calibración. Para confirmar los analitos como positivos, se estableció una tolerancia máxima del 30% y un tiempo de ± 0.1 min con respecto al estándar de referencia.

Para asegurar una cuantificación precisa de los resultados, se prepararon QC a partir de muestras reales fortificadas a tres niveles de concentración (n=9): 0.01, 0.1 y 1 µg/L. Los datos obtenidos señalan que 91, 97 y 91% de los compuestos a 0.01, 0.1 y 1 µg/L, respectivamente, tienen buenos porcentajes de recuperación (en el ámbito de aceptación de 60-140%), lo cual es satisfactorio según las guías internacionales [85]. Los únicos dos analitos que superaron el 140% de recuperación fueron el clorpirifos (158%) y el imazalil (148%), ambos a un nivel de fortificación de 0.01 µg/L. La atrazina desisopropil (DIA) y el ácido 2,4-diclorofenoxiacético (2,4-D) fueron los únicos dos plaguicidas que no se detectaron al nivel más bajo de fortificación. Los porcentajes de compuestos para diferentes ámbitos de recuperación promedio de los fármacos estudiados se presentan en la **Figura 2.6**.

Los datos señalan una desviación estándar relativa (RSD) menor al 20% en un 64, 88 y 83% de los compuestos, a 0.01, 0.1 y 1 µg/L, respectivamente. En el ámbito de 20% < RSD < 30%, los resultados fueron de un 32, 13 y 17%, mientras que sólo en un 5%, atribuido al clorpirifos a 0.01 µg/L, se observó una RSD mayor al 30%. Como era de esperar, la mayor variabilidad en los resultados la presentaron algunos analitos a bajas concentraciones (0.01 µg/L). En la **Figura 2.7,** se muestran los resultados de la RSD promedio obtenida en los plaguicidas y TPs estudiados.

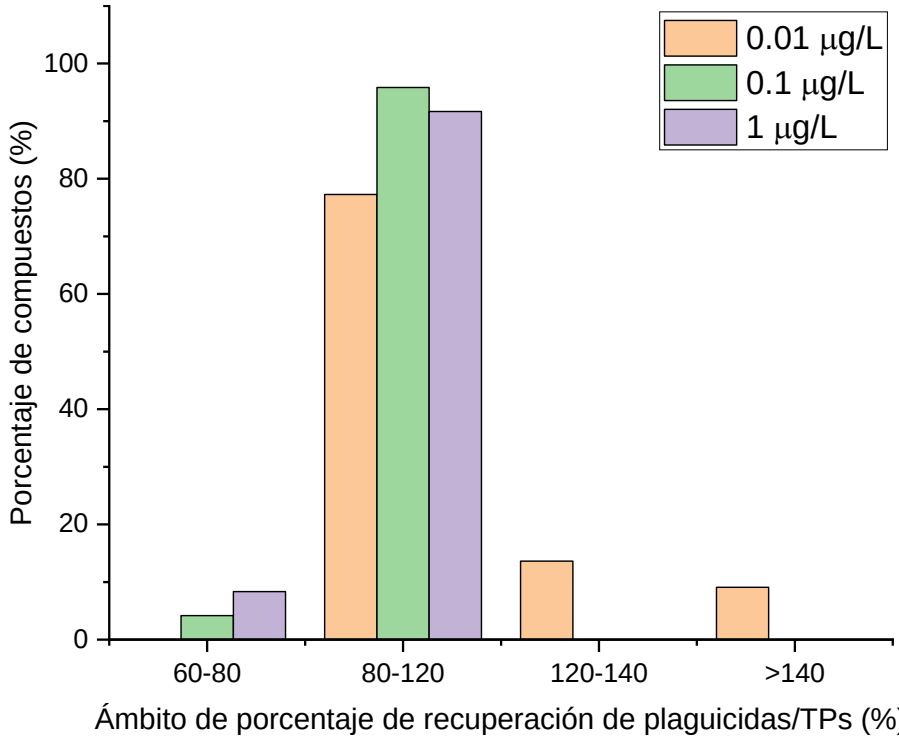

Figura 2.6. Porcentajes de recuperación promedio de QC de plaguicidas fortificados a tres niveles de concentración en las aguas superficiales del río Mijares.

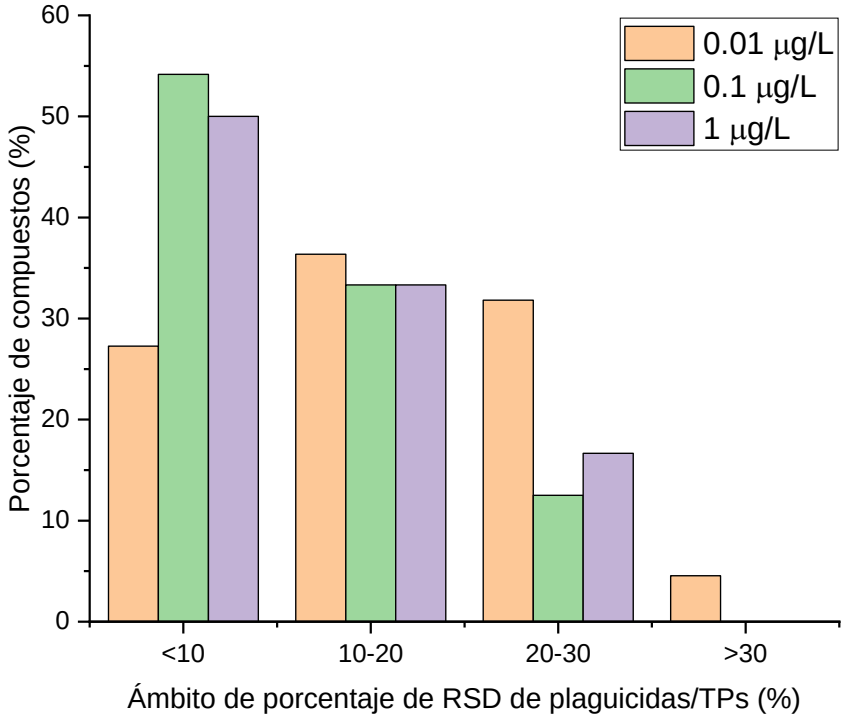

Figura 2.7. RSD promedio de QC de plaguicidas fortificados a tres niveles de concentración en las aguas superficiales del río Mijares.

Análisis de muestras reales de agua superficial

Se tomaron muestras del río Mijares en 19 sitios diferentes y durante tres campañas, para un total de 57 muestras de aguas superficiales. El muestreo se llevó a cabo en tres épocas diferentes, junio de 2018 (verano), septiembre de 2018 (otoño) y febrero de 2019 (invierno). Ver la **Figura 1** del **artículo científico II** para apreciar la distribución de los puntos de muestreo.

Las muestras se analizaron mediante la metodología cuantitativa UHPLC-MS/MS QqQ. Los puntos de muestreo 17, 18 y 19 (ver **Figuras 2.8–2.10)** destacaron por presentar la mayor detección de plaguicidas en las tres campañas de muestreo, probablemente debido a la presencia de una EDAR en las cercanías de los puntos 17 y 18, mientras que el 19 se ubica en la desembocadura del río. Es común detectar plaguicidas en las EDAR, porque las industrias de envasado o procesamiento de alimentos son fuentes puntuales de contaminación por plaguicidas. En efecto, a menudo estas industrias descargan sus aguas residuales en las EDAR municipales sin haber aplicado ningún tratamiento previo [118]. Ver el material suplementario del **artículo científico II** para obtener más detalles sobre los compuestos encontrados y su concentración, así como la ubicación de los puntos de muestreo.

En la primera campaña de muestreo se detectaron 14 analitos (10 plaguicidas y 4 TPs) a una concentración superior al límite de cuantificación. Los plaguicidas que presentaron la concentración más elevada fueron el imazalil y el tiabendazol en el punto de muestreo 18, con valores de 3.9 µg/L y 5.8 µg/L, respectivamente, mientras que el TP con la concentración más elevada fue la T2OH, con 0.066 µg/L. Estos tres compuestos se detectaron en los puntos de muestreo 18 y 19, y la concentración máxima para la T2OH se obtuvo en el punto de muestreo 19 (ver **Figura 2.8**).

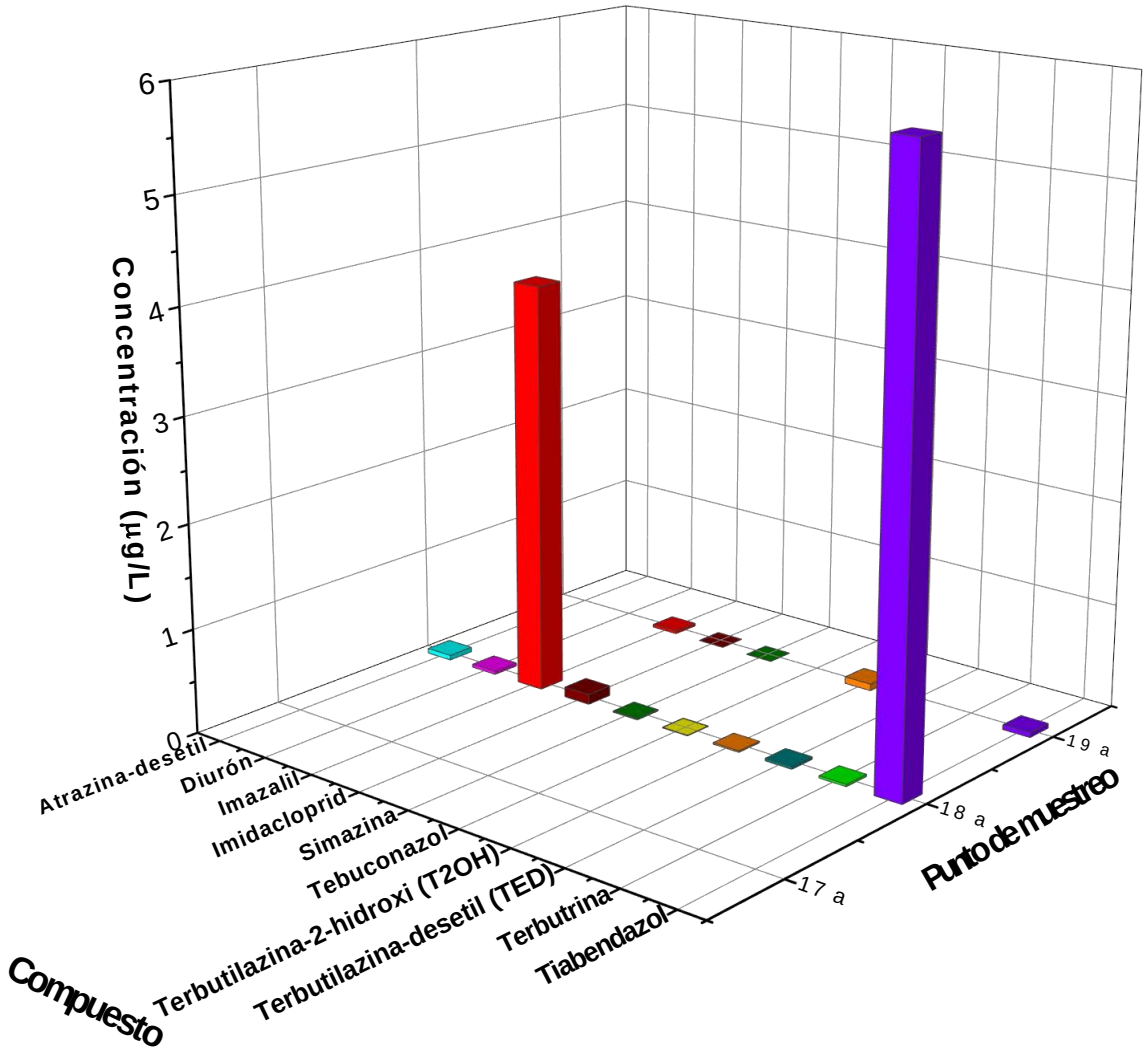

Figura 2.8. Concentración (µg/L) de plaguicidas/TPs detectados en muestras de aguas superficiales durante la primera campaña de muestreo (junio de 2018, verano). Río Mijares.

En la segunda campaña de muestreo se detectaron 14 analitos (11 plaguicidas y 3 TPs). Nuevamente, los plaguicidas que presentaron la concentración más elevada fueron imazalil y tiabendazol, con valores de 1.3 µg/L y 34.5 µg/L, respectivamente, mientras que el TP terbutilazina-2-hidroxi presentó una concentración máxima de 0.046 µg/L. La concentración máxima de los dos primeros se detectó en el sitio de muestreo 18, mientras que la terbutilazina-2-hidroxi se detectó en la desembocadura del río (sitio de muestreo 19) y a una concentración muy similar a la del sitio 18 (0.040 µg/L). En el sitio 17 se detectó tiabendazol a una concentración de 22 µg/L (ver **Figura 2.9**).

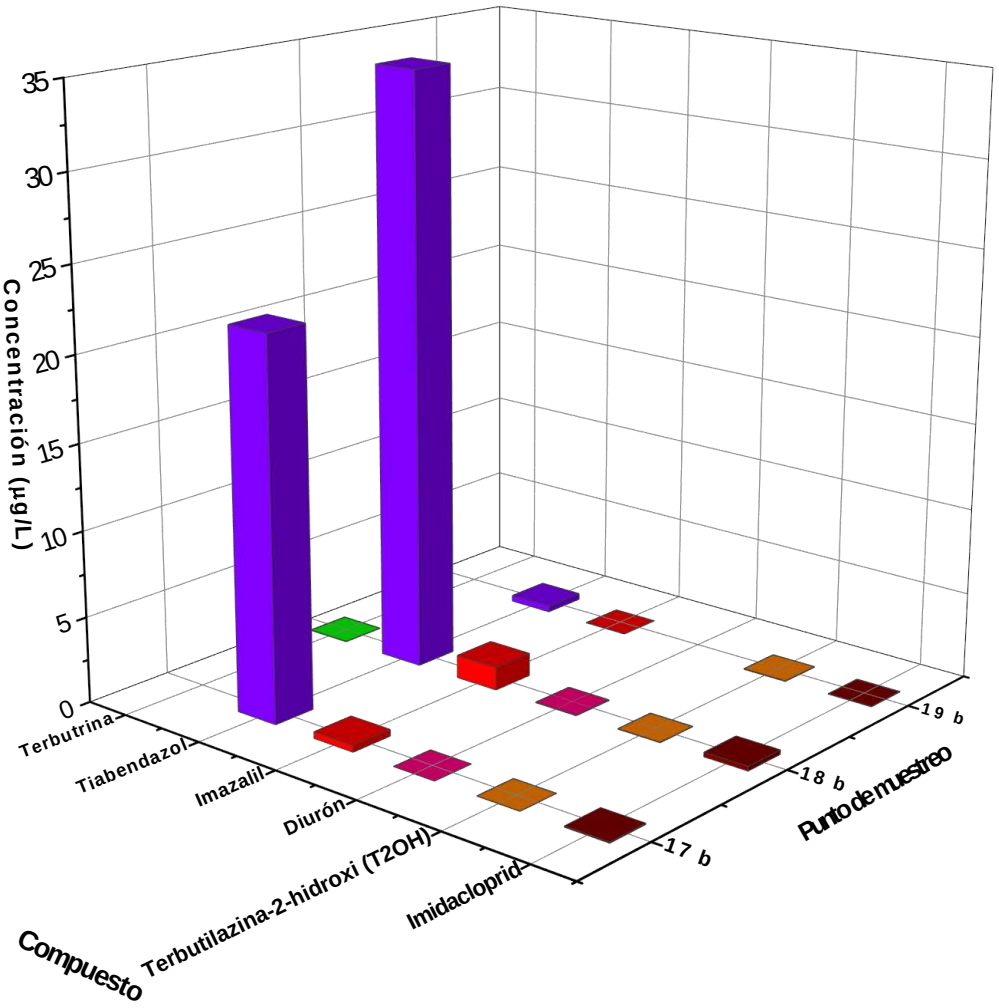

Figura 2.9. Concentración (µg/L) de plaguicidas/TPs detectados en muestras de aguas superficiales durante la segunda campaña de muestreo (septiembre de 2018, otoño). Río Mijares.

Finalmente, en la tercera campaña de muestreo se detectaron 13 analitos (10 plaguicidas y 3 TPs). Al igual que en las dos primeras campañas, el imazalil y el tiabendazol presentaron la concentración más elevada, 4.9 µg/L y 3.3 µg/L, respectivamente (ver **Figura 2.10**). Pese a que en esta campaña solo se cuantificó el TP T2OH, se consideraron como detectados (menor al límite de cuantificación) los TPs terbumetón-desetil y la terbutilazina-desetil.

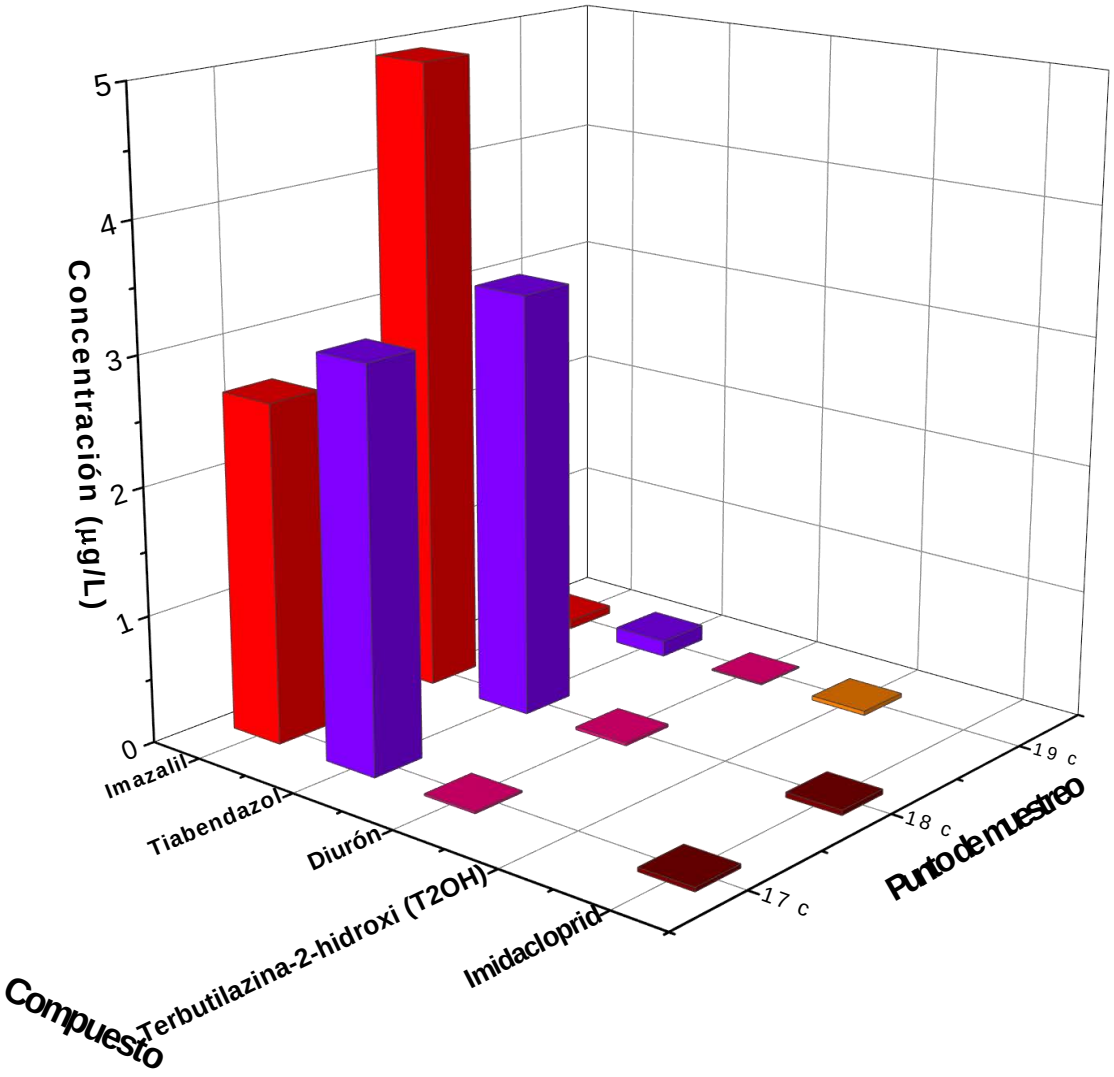

Figura 2.10. Concentración (µg/L) de plaguicidas/TPs detectados en muestras de aguas superficiales durante la tercera campaña de muestreo (febrero de 2019, invierno). Río Mijares.

Cuatro de los plaguicidas (atrazina, diurón, simazina y terbutrina) considerados entre las sustancias prioritarias en el ámbito de la política de aguas de la Unión Europea [41] se detectaron en las muestras analizadas pero en todos los casos los niveles de concentración estuvieron por debajo del máximo permitido en aguas superficiales. Por otra parte, el imidacloprid, incluido en la lista de observación de sustancias a efectos de seguimiento a nivel de la Unión en el ámbito de la política de aguas [43], se detectó en las tres campañas pero sólo en la segunda superó el valor de 0.1 µg/L, al alcanzar los 0.26 µg/L (punto de muestreo 18). En España, el uso de este plaguicida únicamente está autorizado en invernaderos para matar la mosca blanca y

pulgones en tomate, berenjena, calabacín, judía verde, melón, pepino y sandía, entre otros. En la misma campaña de muestreo se encontró 2,4-D a una concentración de 0.61 µg/L (punto de muestreo 8). El 2,4-D es un herbicida que se usa para combatir las malas hierbas dicotiledóneas en los cultivos de trigo y cebada. Estos dos plaguicidas, junto con los fungicidas imazalil y tiabendazol, fueron los que presentaron las concentraciones más altas en las tres campañas. Tanto el imazalil como el tiabendazol se detectaron en los puntos de muestreo 17, 18 y 19, en la segunda y tercera campaña, mientras que en la primera campaña solo se detectaron en los puntos 18 y 19. Estos dos fungicidas suelen aplicarse para combatir enfermedades de poscosecha en frutos cítricos.

Evaluación de riesgos ecológicos

A partir de los niveles de concentración obtenidos para los plaguicidas y TPs en las muestras de agua superficial, se llevó a cabo un estudio de evaluación del riesgo ecológico de los compuestos presentes en el río Mijares. Para ello, se emplearon dos aproximaciones, la de unidades de toxicidad (TU) y la de ms-PAF.

Los resultados de la evaluación del riesgo ecológico muestran que el imidacloprid presenta riesgos agudos y crónicos altos para los invertebrados. El $msPAF_{Total}$ máximo calculado fue de 22% en el sitio de muestreo 18 en el mes de septiembre. También se observan riesgos moderados para los productores primarios, por presencia de diurón y simazina, así como riesgos moderados y crónicos para especies acuáticas de invertebrados ($TU_{máx}$ = 0.83) y vertebrados ($TU_{máx}$ = 1.7), por presencia de tiabendazol.

2.2. INVESTIGACIÓN DE FÁRMACOS Y METABOLITOS EN AGUAS SUPERFICIALES DEL RÍO MIJARES

2.2.1. Introducción

2.2.2. Artículo científico III

Occurrence and ecological risks of pharmaceuticals in Mediterranean river in Eastern Spain

Environmental International, 144 (2020) 106004

2.2.3. Discusión de resultados

2.2.1. Introducción

Una de las principales vías de contaminación del medio acuático son las estaciones depuradoras de aguas residuales (EDAR), por la cantidad de sustancias químicas, muchas veces tóxicas, que suelen acarrear sus descargas. Es más, dependiendo de los tratamientos que se apliquen en las EDAR, los xenobióticos, entre ellos los fármacos, pueden llegar a las aguas receptoras prácticamente inalterados.

El nivel de concentración de fármacos en el agua (de unos pocos ng/L) afecta no solo la calidad del agua sino a las especies que habitan en ella. De hecho, se ha observado que los fármacos pueden causar alteraciones morfológicas, metabólicas y sexuales en las especies acuáticas, generar resistencia antibiótica en microorganismos patógenos acuáticos, e interrumpir el proceso de biodegradación en los efluentes de las EDAR [26].

Los compuestos farmacológicos se pueden clasificar según su aplicación, tal y como se muestra en la **Tabla 2.1** (únicamente se hace referencia a los grupos terapéuticos estudiados en esta tesis), en donde se incluyen ejemplos de fármacos ampliamente utilizados en España con algún efecto adverso.

De los grupos terapéuticos mencionados, los antibióticos merecen especial atención, no sólo porque se usan ampliamente en los seres humanos y en los sectores veterinario y agrícola, sino por los efectos tóxicos que pueden presentar, tanto en los microorganismos como en los animales superiores. Por ejemplo, se ha observado resistencia bacteriana y microbiana, alteraciones en la expresión génica, actividades anormales de proteínas y enzimas, y malformaciones en ratas, peces y ranas. La resistencia bacteriana a los antibióticos es uno de los desafíos más importantes de la medicina contemporánea, ya que puede entorpecer el tratamiento de infecciones en humanos, provocar una amenaza pandémica, incrementar los costes de salud, prolongar las estancias hospitalarias y elevar la mortalidad [119,120].

Los fármacos se excretan del cuerpo principalmente por vía renal o hepatobiliar, ya sea como productos inalterados o como productos biotransformados (metabolitos), cuya estructura se ha modificado para aumentar la hidrosolubilidad y facilitar su eliminación. Una vez fuera del organismo humano, sea por factores bióticos o abióticos, o por una combinación de ambos, estos compuestos pueden sufrir diferentes reacciones y generar TPs. Los TPs pueden ser más

estables y más tóxicos que el compuesto original, y pueden afectar de varias formas a los organismos acuáticos [121]. Por esta razón, es importante incluirlos en las metodologías analíticas y en las evaluaciones del riesgo para el medio acuático.

Tabla 2.1. Grupos terapéuticos de productos farmacéuticos y efectos adversos en el medioambiente.

Grupo terapéutico	Ejemplo	Efectos adversos
Analgésicos Para controlar dolores y fiebre	Paracetamol	El pez bagre negro (*Rhamdia quelen*) mostró reducción en los niveles de hemoglobina, hematocrito y testosterona, hepatotoxicidad, e interrupción en el eje hipotalámico-pituitario-gonadal [122].
Agentes antihelmínticos Para tratar parásitos	Levamisol	Estudios en el salmón del Atlántico (*Salmo salar* L.) demostraron leves patologías en las branquias[123].
Antibióticos Para infecciones bacterianas	Azitromicina	Inhibe el crecimiento del alga *Pseudokirchneriella subcapitata* [124].
Antidepresivos Para trastornos depresivos y de ansiedad, dolor crónico neuropático y deshabituación tabáquica	Venlafaxina	Afecta el desarrollo del helecho *Polystichum setiferum*. Al parecer ocasiona toxicidad letal aguda y toxicidad subletal crónica en el desarrollo mayor de esta planta [125].
Antiepilépticos Para convulsiones o crisis epilépticas	Gabapentina	Afecta varias funciones vitales del pez cebra (*Danio rerio*); entre otras, provoca neurotoxicidad y toxicidad [126].
Antihipertensivos Para hipertensión arterial y prevención de enfermedades cardíacas, renales y cerebrovasculares	Enalapril	Pruebas en embriones de pez cebra mostraron toxicidad independientemente de su concentración [127].

Tabla 2.1 (cont.). Grupos terapéuticos de productos farmacéuticos y efectos adversos en el medioambiente.

Grupo terapéutico	Ejemplo	Efectos adversos
Antiulcerosos Para enfermedades acidopépticas y trastornos digestivos	Omeprazol	Puede afectar la bomba de protones de organismos como las microalgas *Tetraselmis suecica*, perturbar el pH de la homeostasis y provocar una muerte celular rápida en células expuestas a concentraciones de 2.8 ng/célula [128].
Ansiolíticos Acción depresora del sistema nervioso central	Lorazepam	Pruebas de transición luz/oscuridad mostraron que disminuye significativamente la actividad hipotalámica del pez cebra (*Danio rerio*)[129].
Agentes beta-bloqueantes Buscan, entre otros, reducir la presión arterial y aliviar las migrañas	Metoprolol	Produce cambios fisiológicos en el mejillón cebra de agua dulce, *Dreissena polymorpha*; también se puede bioacumular a concentraciones bajas y ambientalmente relevantes [130].
Hipolipemiantes Para reducir niveles de lípidos en sangre y disminuir accidentes cardiovasculares	Atorvastatin	Citotoxidad en truchas arcoíris (*Oncorhynchus mykiss*) en respuesta a su exposición a atorvastatina[131].
Antiinflamatorios no esteroideos Para tratar dolor, inflamación y fiebre	Naproxeno	Toxicidad en algas (*Pseudokirchneriella subcapitata*), rotíferos (*Brachionus calyciflorus*) y crustáceos (*Thamnocephalus platyurus* y *Ceriodaphnia dubia*) en respuesta a pruebas con naproxeno [132].

En 2018, la Comisión Europea 2018/840/UE estableció una lista de observación de sustancias a las que debe darse seguimiento en el medio acuático de la Unión Europea para decidir su consideración como sustancia prioritaria en los controles del agua. Cinco antibióticos (la penicilina amoxicilina, la fluoroquinolona ciprofloxacino), y los macrólidos, azitromicina, claritromicina y eritromicina) fueron incluidos en la lista. Sin embargo, en 2020, la lista se actualizó: se retiraron los tres macrólidos, se mantuvieron la amoxicilina y el ciprofloxacino, se incorporaron tres compuestos azólicos (clotrimazol, fluconazol, miconazol) así como el antidepresivo venlafaxina y su metabolito O-desmetilvenlafaxina [44].

Dados los efectos adversos que ocasionan los fármacos en el medio ambiente, es preciso disponer de datos fiables que adviertan sobre su nivel de concentración, su destino y su comportamiento. De ahí la importancia de aplicar o, si es el caso, desarrollar, métodos analíticos rápidos, sensibles, selectivos y robustos, que permitan verificar su presencia en las matrices ambientales [133]. Una metodología basada en UHPLC-MS/MS con analizador QqQ es una buena opción para cuantificar fármacos target a concentraciones muy bajas (del orden de unos pocos ng/L), de ahí que se utilizara en la investigación que dio pie al **artículo científico III**. Por otra parte, el uso de la espectrometría de masas de alta resolución (HRMS) abre la posibilidad de identificar una cantidad muy amplia de fármacos y complementar la lista de analitos que se pueden determinar mediante metodologías target.

Las metodologías analíticas permiten cuantificar la presencia de fármacos en distintas matrices ambientales (en agua, por ejemplo), lo que a su vez permite determinar su riesgo. Los compuestos se pueden evaluar de forma individual o como mezclas de compuestos. Para evaluar la probabilidad de que un fármaco, a una determinada concentración, produzca un efecto adverso en un organismo acuático, se suele utilizar el concepto de distribución de sensibilidad de especies (SSD). Esta herramienta ecotoxicológica resulta útil para establecer criterios de calidad ambiental y de evaluación de riesgos ecológicos[134]. La variación en la sensibilidad se puede describir mediante una distribución estadística. Así, la representación gráfica de la SSD (**Figura 2.11**) se obtiene contrastando las medidas de efecto (eje de las abscisas), es decir, la concentración más alta a la que no se produce un efecto significativo sobre los individuos expuestos (NOEC), la concentración que mata al 50% de los organismos

(LC50) y la concentración que tiene un efecto sobre el 50% de los organismos (EC50), con la fracción potencialmente afectada (PAF) (eje de las ordenadas).

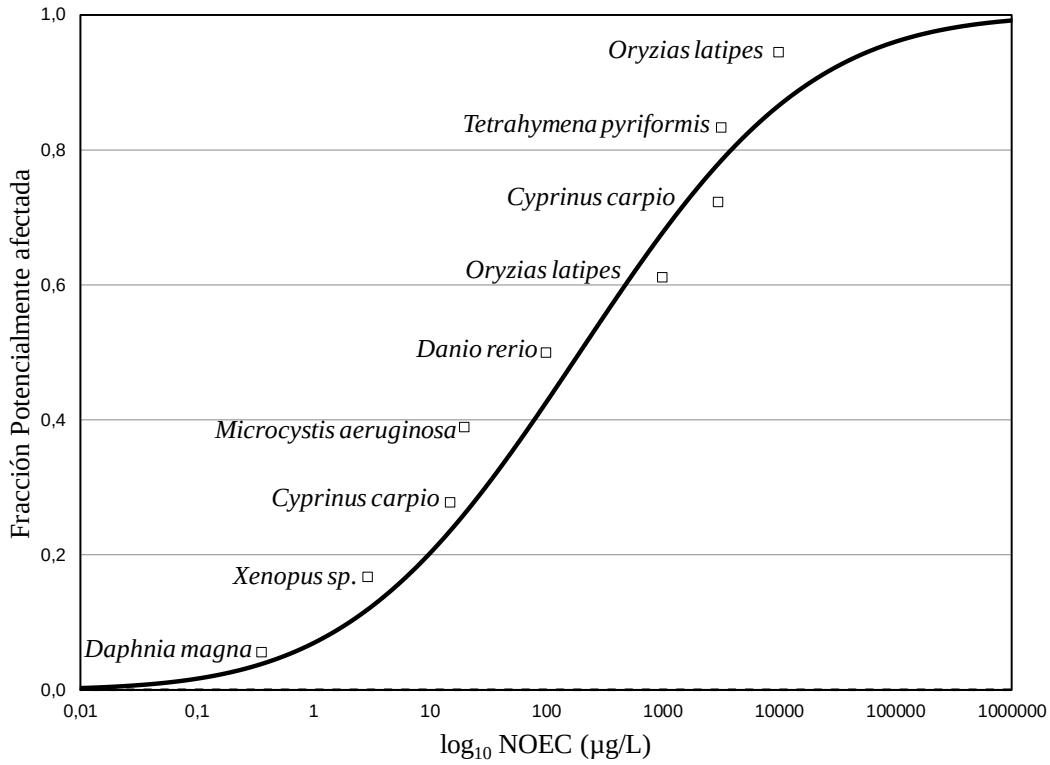

Figura 2.11. Distribución de sensibilidad de especies.

La curva de distribución de sensibilidad de especies permite predecir el efecto de una mezcla de compuestos sobre un conjunto de especies, pues se puede calcular la fracción de esas especies que podría resultar afectada por una sustancia a una concentración determinada (msPAF). Para calcular la msPAF se utiliza un modelo estadístico de adición para cada grupo terapéutico ($msPAF_{TC}$). Luego, a partir de la contribución relativa de cada $msPAF_{TC,}$ se puede obtener la contribución relativa de cada grupo terapéutico a la toxicidad total, usando el modelo de acción independiente o adición de efectos para los diferentes grupos terapéuticos ($msPAF_{Total}$).

En el caso de los antibióticos, a partir de la concentración a la cual se espera que no ocurran efectos adversos en un organismo específico (PNEC), se puede establecer el riesgo de promover resistencia bacteriana con ese fármaco. Para ello, se calcula el cociente de riesgo

(RQ), dividiendo la concentración de antibiótico por el PNEC de resistencia. Un RQ mayor a 1 indica que se corre el riesgo de desarrollar resistencia a antibióticos, ya que la concentración ambiental supera la PNEC.

2.2.2. Artículo científico III

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Occurrence and ecological risks of pharmaceuticals in a Mediterranean river in Eastern Spain

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ABSTRACT

Pharmaceuticals are biologically active molecules that may exert toxic effects to a wide range of aquatic organisms. They are considered contaminants of emerging concern due to their common presence in wastewaters and in the receiving surface waters, and the lack of specific regulations to monitor their environmental occurrence and risks. In this work, the environmental exposure and risks of pharmaceuticals have been studied in the Mijares River, Eastern Mediterranean coast (Spain). A total of 57 surface water samples from 19 sampling points were collected in three monitoring campaigns between June 2018 and February 2019. A list of 40 compounds was investigated using a quantitative target UHPLC-MS/MS method. In order to complement the data obtained, a wide-scope screening of pharmaceuticals and metabolites was also performed by UHPLC-HRMS. The ecological risks posed by the pharmaceutical mixtures were evaluated using species sensitivity distributions built with chronic toxicity data for aquatic organisms. In this study, up to 69 pharmaceuticals and 9 metabolites were identified, out of which 35 compounds were assessed using the quantitative method. The highest concentrations in water corresponded to acetaminophen, gabapentin, venlafaxine, valsartan, ciprofloxacin and diclofenac. The compounds that were found to exert the highest toxic pressure on the aquatic ecosystems were principally analgesic/anti-inflammatory drugs and antibiotics. These were: phenazone > azithromycin > diclofenac, and to a lower extent norfloxacin > ciprofloxacin > clarithromycin. The monitored pharmaceutical mixtures are expected to exert severe ecological risks in areas downstream of WWTP discharges, with the percentage of aquatic species affected ranging between 65% and 82% in 3 out of the 19 evaluated sites. In addition, five antibiotics were found to exceed antibiotic resistance thresholds, thus potentially contributing to resistance gene enrichment in environmental bacteria. This work illustrates the wide use and impact of pharmaceuticals in the area under study, and the vulnerability of surface waters if only conventional wastewater treatments are applied. Several compounds included in this study should be incorporated in future water monitoring programs to help in the development of future regulations, due to their potential risk to the aquatic environment.

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Highlights

- Seasonal variation of pharmaceuticals present in a Spanish river was monitored.
- Use of different MS instruments permitted comprehensive monitoring.
- Four out of five antibiotics included in the EU Watch List were found.
- Ecological risks were mainly driven by phenazone > azithromycin > diclofenac.
- Severe ecological risks observed in 3 out of 19 sampling sites downstream of WWTPs.

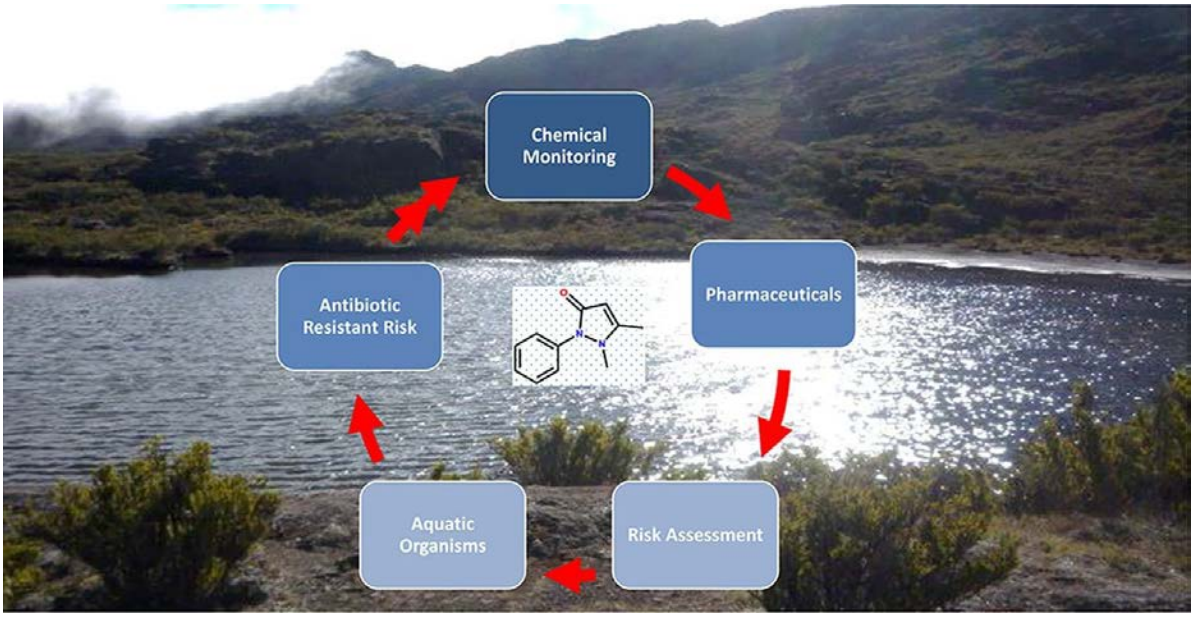
Abstract

Pharmaceuticals are biologically active molecules that may exert toxic effects to a wide range of aquatic organisms. They are considered contaminants of emerging concern due to their common presence in wastewaters and in the receiving surface waters, and the lack of specific regulations to monitor their environmental occurrence and risks. In this work, the environmental exposure and risks of pharmaceuticals have been studied in the Mijares River, Eastern Mediterranean coast (Spain). A total of 57 surface water samples from 19 sampling points were collected in three monitoring campaigns between June 2018 and February 2019. A list of 40 compounds was investigated using a quantitative target UHPLC-MS/MS method. In order to complement the data obtained, a wide-scope screening of pharmaceuticals and metabolites was also performed by UHPLC-HRMS. The ecological risks posed by the pharmaceutical mixtures were evaluated using species sensitivity distributions built with chronic toxicity data for aquatic organisms. In this study, up to 69 pharmaceuticals and 9 metabolites were identified, out of which 35 compounds were assessed using the quantitative method. The highest concentrations in water corresponded to acetaminophen, gabapentin, venlafaxine, valsartan, ciprofloxacin and diclofenac. The compounds that were found to exert the highest toxic pressure on the aquatic ecosystems were principally analgesic/anti-inflammatory drugs and antibiotics. These were: phenazone > azithromycin > diclofenac, and to a lower extent norfloxacin > ciprofloxacin > clarithromycin. The monitored pharmaceutical mixtures are expected to exert severe ecological risks in areas downstream of WWTP discharges, with the percentage of aquatic species affected ranging between 65% and 82% in 3 out of the 19 evaluated sites. In addition, five antibiotics were found to exceed antibiotic resistance thresholds, thus potentially contributing to resistance gene enrichment in environmental bacteria. This work illustrates the wide use and impact of pharmaceuticals in the area under study, and the vulnerability of surface waters if only conventional wastewater treatments are applied. Several compounds included in this study should be incorporated in future water monitoring programs to help in the development of future regulations, due to their potential risk to the aquatic environment.

Keywords

Pharmaceuticals and metabolites; surface water; screening; liquid chromatography mass spectrometry; environmental impact; ecological risk assessment.

Graphical abstract



1. Introduction

The prevention of water bodies deterioration is an urgent issue nowadays. Among other matters, it is necessary to accurately monitor the presence of a wide variety of organic contaminants in order to preserve the ecological status of aquatic ecosystems. In this context, pharmaceuticals are of current concern due to their widespread use and frequent detection in the water cycle. Pharmaceuticals can reach water bodies from different sources, such as a human consumption (Botero-Coy et al., 2018, García-Galán et al., 2016), landfill leachates (Lu et al., 2016, Masoner et al., 2014), use of wastewater treatment plants (WWTPs) sludge as fertilizers (Behera et al., 2011), effluents from hospitals (Della-Flora et al., 2019, Verlicchi et al., 2012) or improper disposal of unused or expired medicines (Bashaar et al., 2017, Tong et al., 2011). Due to the poor removal efficiency of most conventional WWTPs (Al-Odaini et al., 2010, Behera et al., 2011), it is not surprising that pharmaceuticals are found in treated effluents and reach receiving surface waters (Botero-Coy et al., 2018, Collado et al., 2014, Gao et al., 2012, Gracia-Lor et al., 2012, Hernández et al., 2019a, Ibáñez et al., 2017, Paíga et al., 2017, Picó et al., 2020, Rico et al., 2016) and even drinking water sources (Boleda et al., 2014, Bruce et al., 2010, Praveena et al., 2019).

Pharmaceuticals are biologically active molecules designed to target a varied range of human receptors and that display different toxicological modes of action, depending on the biological endpoint that is evaluated. A recent review on the environmental exposure and toxicity data for 22 pharmaceuticals shows that hormones, antiepileptics, anti-inflammatories and antibiotics are generally the TCs posing the highest ecotoxicological risks (Pereira et al., 2020). However, consumption patterns and removal efficiencies vary across different river basins, which result in diverse complex mixtures, that need to be evaluated case-by-case (Altenburger et al., 2015).

A significant amount of research has been carried out on the occurrence of pharmaceuticals in surface waters, but only data from parent compounds are normally reported (Boix et al., 2015, Ferrer et al., 2010, Grabic et al., 2012, Gracia-Lor et al., 2011, Huntscha et al., 2012, Ibáñez et al., 2009). However, there are more and more data available evidencing that the unaltered compounds are just the “top of the iceberg”, because they usually represent a small part of the total amount of the compounds excreted in urine (Hernández et al., 2019a).

In the last few years, several papers have reported the occurrence of many metabolites in surface and wastewaters (Boix et al., 2016, Della-Flora et al., 2019, Gracia-Lor et al., 2014, Ibáñez et al., 2017, Langford and Thomas, 2011, Rúa-Gómez and Püttmann, 2012). Apart from analytical drawbacks, such as the lack of reference standards and the absence of priority compounds lists, the evaluation of the toxicity of metabolites and transformation products (TPs) involves considerable effort (Lindholm-Lehto et al., 2016). However, it is of importance as they can be as persistent and/or toxic as the parent compound and can have negative effects on different aquatic organisms (Rivera-Jaimes et al., 2018). For this reason, they should be gradually included in analytical methods and in aquatic risk assessments (Hernández et al., 2019a, Santana-Viera et al., 2016).

Until recently, environmental regulations barely included maximum allowable concentration levels for pharmaceuticals in surface waters. The European Commission (European Comission, 2018) establishes a Watch List of substances that must be followed up as part of public policies. The objective of that list is to collect data from the Member States about the concentration levels of the included pharmaceuticals in the water bodies and to decide, in a later stage, whether they can be considered as priority substances in the regular monitoring of water quality. Five antibiotics (i.e. the fluoroquinolone ciprofloxacin, the penicillin amoxicillin and the macrolides azithromycin, clarithromycin, erythromycin) have already been included in the current Watch List. Recent studies indicate that the aquatic risk of pharmaceuticals, such as carbamazepine and ciprofloxacin, has increased from 10 to 20 times in the last 20 years due to the demographic concentration in urban areas and the low dilution capacity of surface waters in (semi-)arid areas (Oldenkamp et al., 2019). The presence of antibiotics in the environment is of special concern, as it can lead to the development of bacterial resistance genes, a fact that has already been observed even in pristine areas such as the Antarctic (Hernández et al., 2019b) and which may represent a serious problem in fighting some diseases (Mokh et al., 2017). Recent investigations show that urban WWTPs constitute hotspots for antibiotic emissions, contributing to the enrichment of resistance genes in surface water ecosystems (Buelow et al., 2020). In this regard, threshold concentrations for antibiotic resistance have been proposed for a wide range of antibiotics to aid the assessment of their respective resistance development risks (Bengtsson-Palme and Larsson, 2016, Rico et al., 2017), and to prioritize compounds and management practices that should be implemented at a watershed scale.

One of the main reasons for the increase of data on the presence of pharmaceuticals in water is the relevant role of modern environmental analytical chemistry (Hernández et al., 2019a). Most data reported nowadays are based on target quantitative methods commonly using ultra high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS), which offers excellent sensitivity, selectivity and robustness (Beccaria and Cabooter, 2020, Campos-Mañas et al., 2017, García-Galán et al., 2016, van Nuijs et al., 2010). However, the application of target methodologies may provide incomplete results as other compounds present in the sample could remain ignored in the analysis. Then, a screening based on high resolution MS (HRMS) becomes necessary in order to identify as many contaminants as possible, even when reference standards are not available at the laboratory (Aceña et al., 2015, Boix et al., 2016, Hernández et al., 2015a, Hernández et al., 2015b).

The aim of this study was to assess the occurrence and ecological risks of a wide variety of pharmaceuticals and metabolites in the Mijares River, located in Eastern Mediterranean Spain. A total of 57 surface water samples were collected in three different campaigns over one year. Samples were quantitatively analyzed by UHPLC-MS/MS for the determination of 40 target pharmaceuticals. Additionally, a screening by UHPLC-HRMS was performed in order to complement the quantitative results obtained. The results of the quantitative analysis were used to perform a probabilistic risk assessment for aquatic organisms, which helped to highlight individual compounds and pharmaceutical mixtures that are posing an ecotoxicological risk. Moreover, the monitored antibiotics were evaluated in regards to their resistance development risks. Overall, this study contributes to the identification of pharmaceutical compounds that need to be further monitored and that are candidates to be included in future updates of the Water Framework Directive and regional monitoring plans.

2. Material and methods

2.1. Chemicals and materials

See Supplementary Material (S.M.)

2.2. Description of the sampling sites and sample collection

The Mijares River originates in Aragón (at 1.600 m in Sierra de Gúdar, in the municipality El Castellar, province of Teruel) and ends in the Mediterranean Sea, Castellón, Eastern Spain (see **Fig. 1**). It is 156 km long with a 5.466 km^2 wide basin, which represents 13% of the total demarcation of the Jucar Hydrographic Confederation. The river is an important source of irrigation water in the lower basin, which is an important agricultural area with predominance of citrus crops (Garófano-Gómez et al., 2013).

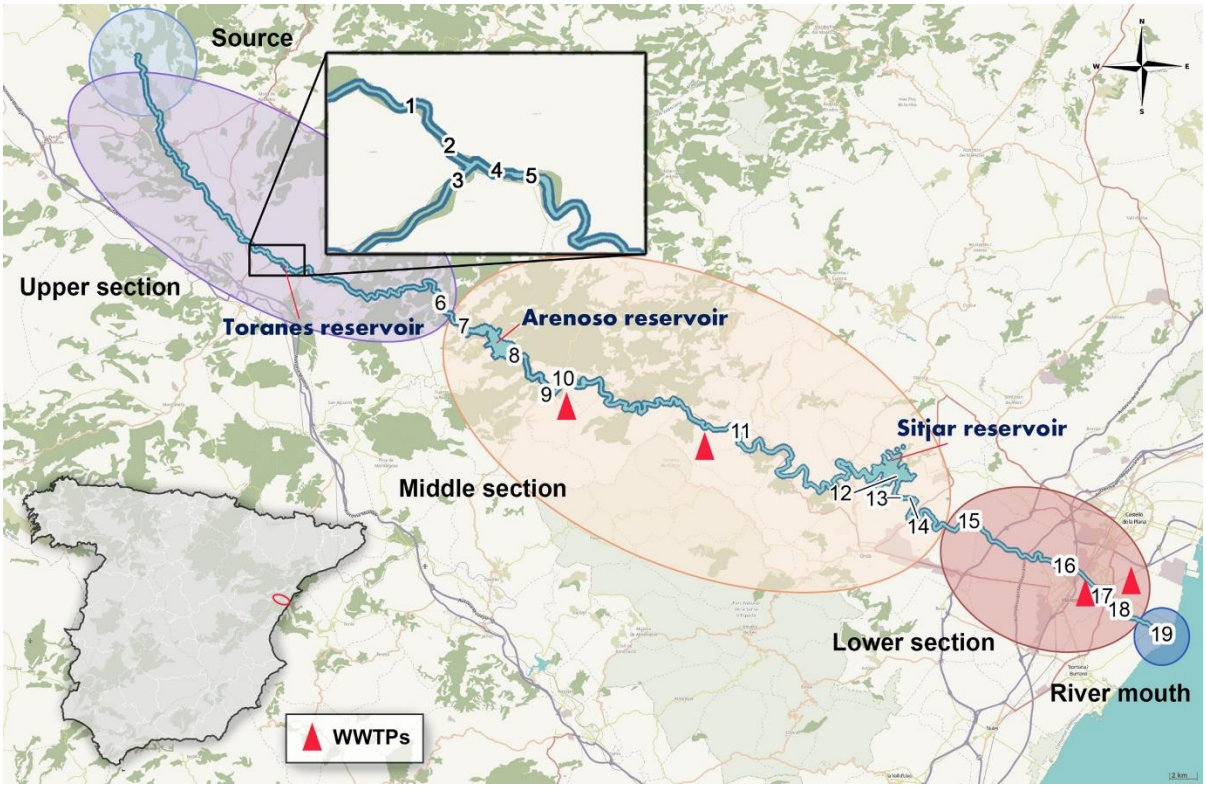


Fig. 1. Location of the sampling sites and WWTPs along the Mijares River.

Water samples were taken at 19 different points (see **Fig. 1**), covering almost all the Mijares River, from its source until its estuary: points 1–6 are sited in the upper section of the river, 7–14 in the middle section, 15–18 in the lower section, and point 19 in the river mouth. All sampling sites were selected based on different characteristics and/or accessibility (**Table S1**). In the municipality of Sarrión (Teruel), three sampling points were considered due to their proximity to a fertilizer factory (point 2) or to a fish farm (points 3 and 4). The potential contribution of small towns in terms of emerging contaminants might be attributed to four WWTPs discharging their effluents into the river. For this reason, several sampling sites were selected downstream of the WWTPs: points 9 and 10, near Montanejos (WWTP flow 627 m^3/day; population served 1.513 p.e); 11 near Toga (WWTP flow 21 m^3/day; population served 66 p.e); 17 near Vila-real (WWTP flow 3.666 m^3/day; population served 16.449 p.e); and 18 near Almassora (WWTP flow 7.386 m^3/day; population served 34.337 p.e) (**Table S2**) (EPSAR, 2020). Also, two sampling sites (13 and 14), located downstream of a solid waste treatment plant (SWTP) near Onda (Castellón), were included in this study. Waters from three reservoirs located in the Mijares River were also sampled: 5 (Toranes reservoir, Teruel), 7 and 8 (Arenós reservoir, Castellón) and 12 (Sitjar reservoir, Castellón).

Three sampling campaigns were conducted in order to monitor pharmaceuticals concentrations along different periods: June 2018 (1st campaign, summer), September 2018 (2nd campaign, autumn) and February 2019 (3rd campaign, winter). In every campaign, 19 surface water samples, one from each sampling point, were collected in polyethylene bottles, transported to the laboratory on the same day (within max. 6 h) in refrigerated isothermal containers, and stored at −20 °C until analysis.

2.3. Sample treatment

2.3.1 Quantitative analysis

The procedure applied for quantitative UHPLC-MS/MS analysis was based on methodology previously developed by our research group (Boix et al., 2015, Botero-Coy et al., 2018) using direct injection of the sample, without any pre-concentration. Briefly, 2 mL of water was centrifuged at 12.000 rpm for 10 min. Subsequently, 50 µL of isotopically labelled internal standard (ILIS) mix solution of 1 µg/L was added to 950 µL of the centrifuged water sample

(final ILIS concentration in the sample injected, 50 ng/L). Finally, 50 µL was injected into the UHPLC-MS/MS system.

2.3.2 Screening analysis

The UHPLC-HRMS screening required a previous generic sample extraction and pre-concentration. This was performed by solid-phase extraction (SPE), following the procedure described by Pitarch et al. (2016). **Figure S1** shows a flowchart of the extraction procedure. Briefly, 100 mL of water was passed through an Oasis® HLB (150 mg) cartridge. After elution, the extract was reconstituted with 100 µL of methanol:water (10:90, v/v) and 20 µL were injected into the UHPLC-QTOF MS.

2.4. Instrumentation

Quantitative analysis was performed using a Waters ACQUITY UPLC® (Waters Corp.) equipped with a binary pump system was interfaced to a Xevo TQ-S™ triple quadrupole (QqQ) mass spectrometer (Waters Corp.). For qualitative screening a Waters ACQUITY UPLC® (Waters Corp.) interfaced to a hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (XEVO G2 QTOF, Waters) was used. For more details related to the instrumentation used see S.M.

2.5. Quantitative LC-MS/MS analysis and quality assurance

In total, 40 pharmaceuticals (**Table 1**) from different therapeutical classes were selected for target quantitative analysis by LC-MS/MS (QqQ). The experimental conditions are shown in **Table S3**. At least, seven-point calibration curves (0.005–20 µg/L) were injected at the beginning and the end of each sequence. As the samples were analysed by direct injection, without any pre-concentration step, the lowest calibration level (LCL) was taken as the limit of quantification in samples (**Table S3**). A compound was considered as "detected" when its concentration was below LCL and at least one q/Q ratio was accomplished allowing in this way its reliable identification. For the constructions of graphs, risk assessment evaluation, and for discussion of results obtained, the cut-off value used for detected positives was half of their LCL.

Quality control (QC) samples, consisting on three surface waters each fortified at three concentration levels (0.01, 0.1 and 1 µg/L), were analysed together with the samples (see **Table S4**). QCs recoveries between 60 and 140% were considered satisfactory (SANTE, 2019). For many compounds, the corresponding ILIS was used for matrix effects correction, ensuring an accurate quantification (**Table S3**). The ratio between the qualitative and quantitative transitions (q/Q ratio) as well retention time deviation (±0.1 min) were used for the reliable identification of positive findings (SANTE, 2019).

Table 1. Target pharmaceuticals and results obtained by UHPLC-MS/MS (QqQ) quantitative analysis of water samples collected in the three campaigns. Percentages were calculated from a total number of 57 samples. Lowest calibration level (LCL), used as limit of quantification. The value of LCL/2 was taken as the cut-off reference for detection frequency.

Family	Compound	Positive samples (%)	Positive samples > 0.1 µg/L (%)	Maximum level found (µg/L)	LCL (ng/L)
Analgesics	Acetaminophen √	65	2	0.20	5
	Tramadol √	17	14	1.9	5
Anthelmintic agents	Levamisol	16	2	0.11	5
Antibiotics	Clindamycin	16	4	0.13	5
	Sulfadiazine	5	0	0.020	5
	Sulfamethoxazole √	19	9	0.20	5
	Tetracycline	9	0	0.011	5
	Trimetroprim	12	7	0.72	5
	Azithromycin* √	16	10	1.6	50
	Ciprofloxacin*[a]	33	5	1.1	50
	Clarithromycin* √	14	12	0.33	5
	Erythromycin*	17	2	0.12	5
	Furaltadone	0	0	-	5
	Lincomycin	9	0	0.011	5
	Metronidazole	10	2	0.11	5
	Nalidixic acid	2	2	d	5
	Norfloxacin[a]	25	5	0.94	50
	Oxolinic acid	19	0	d	5
	Roxithromycin	0	0	-	5

Table 1 (cont.). Target pharmaceuticals and results obtained by UHPLC-MS/MS (QqQ) quantitative analysis of water samples collected in the three campaigns. Percentages were calculated from a total number of 57 samples. Lowest calibration level (LCL), used as limit of quantification. The value of LCL/2 was taken as the cut-off reference for detection frequency.

Family	Compound	Positive samples (%)	Positive samples > 0.1 μg/L (%)	Maximum level found (μg/L)	LCL (ng/L)
Antidepressants	Venlafaxine √	40	14	0.80	5
Antiepileptics	Gabapentin √	42	16	1.9	5
	Carbamazepine √	19	0	0.026	5
	Primidone	26	17	1.0	5
Antihipertensives	Enalapril	0	0	-	5
	Irbesartan √	23	12	1.7	5
	Losartan √	19	12	0.68	5
	Valsartan √	39	16	1.6	5
Antiulcer drugs	Omeprazole sulfide-4-hydroxy √	19	7	0.15	5
	Pantoprazole	14	0	0.013	5
Benzodiazepines	Alprazolam	19	0	0.020	5
	Lorazepam √	16	0	0.094	10
Beta-blocker agents	Metoprolol	14	0	0.057	5
	Salbutamol	17	0	0.023	5
Hypolipidemic agents	Atorvastatin	12	2	0.21	5
	Bezafibrate[b]	9	0	d	1000
	Gemfibrozil[b]	0	0	-	1000
Nonsteroidal anti-inflammatory	Diclofenac √	33	16	0.94	5
	Ketoprofen[b] √	0	0	-	1000
	Naproxen[b] √	14	0	d	1000
	Phenazone	21	14	2.0	10

*Compounds included in the Watch List of the Commission Decision 2018/840.
√ Compounds also detected in the UHPLC-QTOF MS screening.
[a] Results in positive samples should be taken as guidance values since accurate quantification could not be made.
[b] Compounds with LCL higher than 0.1 μg/L, so positive samples > 0.1 μg/L is not applicable.
d, detected: concentration below LCL and at least one q/Q ratio was accomplished.

2.6. UHPLC-HRMS screening

A great number of organic micro-pollutants were investigated by screening based on UHPLC-QTOF MS. Accurate-mass data generated at low and high collision energy were processed by ChromaLynx™ Application Manager (within MassLynx) in combination with a home-made database, containing a large number of pharmaceuticals and their main metabolites. In total,

the presence of > 900 compounds were investigated (see **Table S5** in **S.M.**). This software applies a "post-target" processing method by monitoring exact masses of the suspect analytes and obtains the corresponding narrow-window Extracted Ion Chromatogram (nw-EICs).

The database included, at least, the name and elemental composition of the parent compounds (occasionally adducts). Information on retention time (Rt), main fragment ions and adducts was also added when reference standards were available, which greatly helped to facilitate and support the identification process.

When a chromatographic peak was observed at the corresponding exact mass but the reference standard was not available, the characteristic isotope pattern (if chlorine or bromine atoms were present) as well as fragment ions were evaluated and their compatibility with the chemical structure of the suspect compound was assessed. Tentative identification was reinforced by agreement with MS/MS product ions reported in literature or available databases (preferably in exact mass). For more information see (Hernández et al., 2015a, Hernández et al., 2015b).

2.7. Ecological risk assessment

The probability that exposure concentrations result in unacceptable effects for aquatic organisms was calculated based on the Species Sensitivity Distributions (SSD) approach (Posthuma et al. 2002). The Potentially Affected Fraction (PAF) was calculated for individual compounds, and the multi-substance Potentially Affection Fraction (msPAF), for contaminant mixtures, following the methods described by de Zwart and Posthuma (2005). Risks were calculated using the SSDs provided by Posthuma et al. (2019) for chronic exposure. In their study, the SSD parameters μ (median of the log-transformed toxicity values) and σ (standard deviation of log-transformed toxicity values or slope) were calculated using a log-normal distribution on the basis of chronic toxicity data (primarily No Observed Effect Concentrations, NOECs) for bacteria, algae, invertebrates and fish. Since for some compounds chronic toxicity data is very limited, acute-to-chronic extrapolation techniques and read-across (i.e., Quantitative-Structure Activity Relationships, QSARs) was often applied for their derivation. The robustness of the SSD parameters was evaluated on the basis of the methods described by Posthuma et al. (2019), which consider four quality aspects: (1) the availability of a sufficient number of data to calculate the SSD μ and σ, (2) the biodiversity coverage, (3) the

origin of the toxicity data (i.e., experimental, extrapolated or read-across), and (4) the type of extrapolation (in case the data was extrapolated). The SSD parameters of the compounds that were detected at least once in this study are provided in **Table S6** together with their quality scores, while a detailed description of the quality scores is provided in **Table S7**. When there was no chronic toxicity data for a specific compound, the μ was derived by subtracting 1 to the μ of the SSD built with acute toxicity data (i.e., assuming an acute-to-chronic extrapolation factor of 10 for the species assemblage), and using a σ of 0.7. A σ of 0.7 was also applied to the chronic SSDs that had a σ that was considered too large or too low according to the criteria established by Posthuma et al. (2019). The σ value of 0.7 is the average SSD slope for the 12,386 chemicals evaluated by Posthuma et al. (2019).

The monitored pharmaceuticals were classified into eleven Therapeutic Classes (TCs). Then, the toxic pressure of the compounds within each of the TCs and their mixtures was calculated for each sample. First, the Hazard Unit (HU) was calculated for each compound in each sampling site by dividing the logarithm of the measured concentration by the SSD μ. These HUs are used to adjust for differences in the potency of the evaluated compounds. Next, the concentration addition model was used to calculate the msPAF corresponding to each TC ($msPAF_{TC}$) in each sample using the Microsoft Excel © function (**Eq. (1)**).

$$msPAF_{TC} = NORM.DIST\,(HU_{TC}, 0, \sigma_{TC}, 1) \quad (1)$$

Where HU_{TC} is the sum of the HUs for each compound in the TC, and σ_{TC} is the average σ for all compounds in the TC.

After obtaining the $msPAF_{TC}$ for each TC, the total toxicity of the sample ($msPAF_{Total}$) was calculated using the response addition model (**Eq. (2)**).

$$msPAF_{Total} = 1 - \prod_{i=1}^{n}\left(1 - msPAF_{TC,i}\right) \quad (2)$$

Finally, the $msPAF_{Total}$ for each sample was represented with the relative contribution of each TC to the total toxic pressure. In our study, the PAF and the $msPAF_{Total}$ represent the fraction of species of the aquatic ecosystem that will be affected (i.e., the NOEC is exceeded) by the

chronic exposure to an individual compound or the pharmaceutical mixture, respectively. In this study, PAF and $msPAF_{Total}$ values between 5% and 25% were considered to result in moderate ecological risks, while values above 25% were considered to induce severe risks (see section 3.4 for rationale).

2.8. Antibiotic resistance risks

The risks of promoting antibiotic resistance in environmental bacteria were calculated using the resistance Predicted No Effect Concentrations (PNECs) proposed by Bengtsson-Palme and Larsson (2016) for all the evaluated antibiotics except furaltadone, oxolinic acid and sulfadiazine, for which resistance PNECs are not available. Risk Quotients (RQs) were calculated by diving the measured antibiotic concentrations by the resistance PNECs, so that a RQ quotient larger than one indicates a potential risk of antibiotic resistance development.

3. Results and discussion

3.1. Quantitative analysis by UHPLC-MS/MS (QqQ)

3.1.1. Quality control samples

Especial emphasis was made on QCs evaluation in order to support the reliability of quantitative data reported. **Table S4** shows the average results obtained for 9 QCs analysed (one QC per spiking level and per sampling campaign, this is, 3 replicates per each spiking level). It should be noted that QCs at lowest fortification level were only performed in the first campaign (n = 3). Recoveries were generally between 60 and 140% (SANTE, 2019), and mostly in the 80–120% range. The use of analyte-ILIS and the absence of complex sample treatment process surely facilitated obtaining satisfactory quality controls, with a few exceptions. The most relevant were for the antibiotics ciprofloxacin and norfloxacin, whose recovery values were slightly above 200% and poorly reproducible. The lack of sensitivity of our instrumentation in negative mode prevented the determination of the drugs measured under this mode (bezafibrate, gemfibrozil, ketoprofen and naproxen) at the low fortification levels tested and only QC recoveries at 1 µg/L could be calculated for these compounds. The antibiotics clarithromycin and roxithromycin showed unsatisfactory recoveries in some cases, especially at the highest level of fortification, probably because their analyte-ILIS was not

available and therefore matrix effects could not be corrected. Regarding data reported in this paper for water samples, the unsatisfactory QCs recoveries only affected to ciprofloxacin and norfloxacin, and therefore those values must be taken as semi-quantitative. The reason might be the low ILIS concentration used (50 ng/L). In fact, in subsequent works performed in our group we increased the amount of ILIS added to the samples obtaining a significant improvement in the results.

3.1.2. Analysis of surface samples

A total of 57 river water samples (19 per campaign) were analysed by LC-MS/MS (QqQ) for 40 target pharmaceuticals. The compounds were selected based on their frequent occurrence in effluent wastewater and surface water samples analysed in previous studies (Botero-Coy et al., 2018, Hernández et al., 2015a, Hernández et al., 2015b). The concentrations found in the samples analysed are included in **Tables S8, S9 and S10,** corresponding to the first (June 2018, summer), second (September 2018, autumn) and third (February 2019, winter) campaigns. **Table 1** shows the frequency of detection (% positive samples) of the pharmaceuticals investigated. As indicated in section 2.5, the cut-off value used for the compounds detected was half of their LCL.

Thirty-five out of the 40 compounds evaluated in this study were measured at least once in the samples. The analgesic acetaminophen was the most frequently detected (65% of samples above the cut-off value 2. 5 ng/L). The antiepileptic gabapentin (42% above 2.5 ng/L), the antidepressant venlafaxine (40% above 2.5 ng/L), the antihypertensive valsartan (39% above 2.5 ng/L), the antibiotic ciprofloxacin (33% above 25 ng/L) and the anti-inflammatory drug diclofenac (33% above 2.5 ng/L) were also frequently found. A notable amount of pharmaceuticals (66% of the compounds detected) exceeded, in at least one of the samples, the concentration level of 0.1 µg/L (value set by European Union countries). The compounds with the highest percentage of exceedances were primidone, gabapentin, valsartan and diclofenac. Seven drugs (tramadol, azithromycin, ciprofloxacin, gabapentin, irbesartan, valsartan and phenazone) slightly surpassed 1 µg/L, particularly in the sites 17 and 18, but never exceeded 2 µg/L. Some of the pharmaceuticals detected in the Mijares River are currently included in the Watch List of substances for European-wide monitoring in the field of water policy

(European Comission, 2018), such as the antibiotics ciprofloxacin, clarithromycin, erythromycin and azithromycin. As an example, **Figure S2** shows the positive findings of losartan (antihypertensive), diclofenac (NSAID) and erythromycin (antibiotic) in three surface water samples investigated.

The spatial distribution along the Mijares River, expressed as the sum of the average concentration of the 3 campaigns of each individual pharmaceutical, is shown in **Fig. 2**. As expected, the upper section was the less contaminated (<100 ng/L for total pharmaceuticals), even in the points near the fertilizer factory (site 2) and the fish farm (sites 3 and 4), which presented a similar pattern to the rest of upper sites demonstrating no relevant contribution of pharmaceutical residues into the Mijares River.

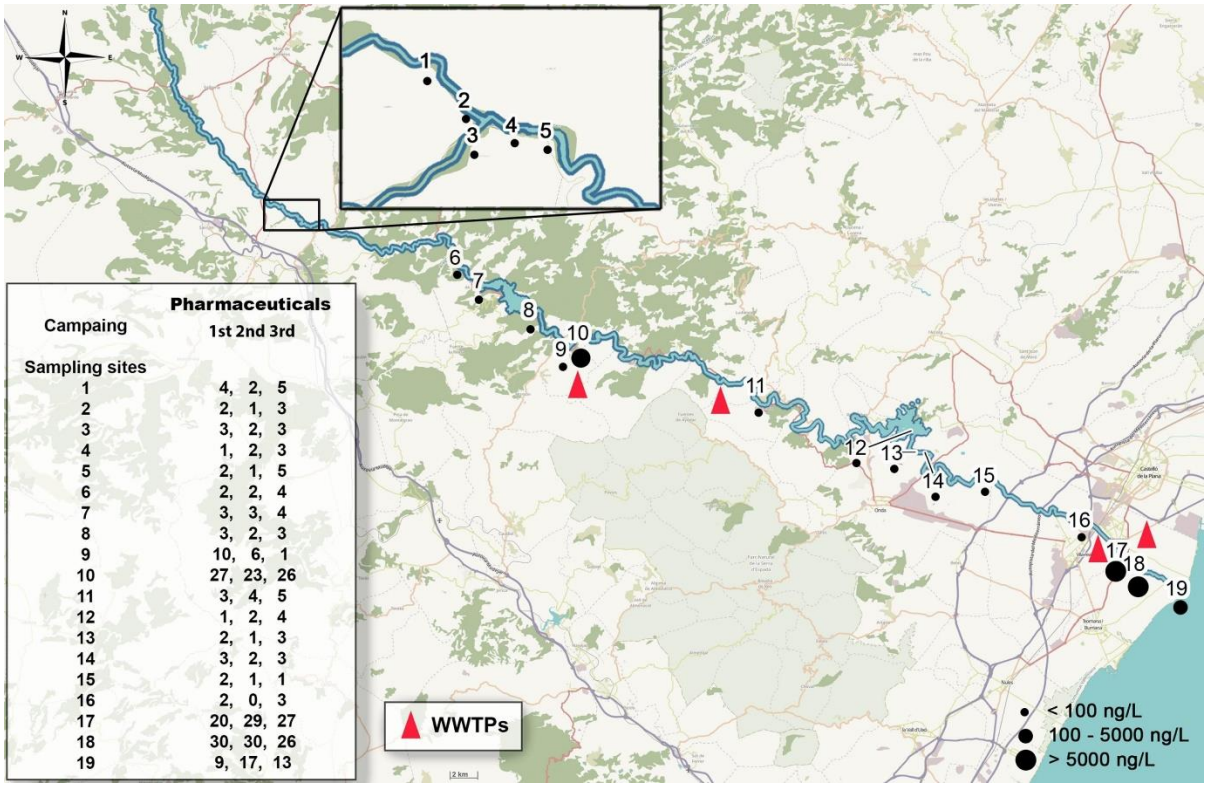


Fig. 2. Spatial distribution as total average concentration of pharmaceuticals in Mijares River. In the left side, the number of pharmaceuticals found in each sampling site per campaign is shown (1st: June 2018; 2nd: September 2018; 3rd: February 2019).

As regards to the middle section, most of the sampling points showed mean concentrations of pharmaceuticals lower than 100 ng/L (7: upstream Arenoso reservoir; 11: Toga; 12: Sitjar

reservoir; 12–13: Onda SWTP). It is worth noticing the sample collected downstream Montanejos WWTP (point 10), with a total concentration of pharmaceuticals above 5000 ng/L and high number of positives (up to 27 pharmaceuticals in the 1st campaign). On the contrary, the sampling site 11 (downstream WWTP Toga) did not appear to be very contaminated, which may be explained by the small size of this village with only 100 inhabitants. Moreover, in the sample collected downstream of the SWTP located in Onda (points 13 and 14) very few pharmaceuticals were found (<100 ng/L), indicating that no relevant pollution in terms of pharmaceuticals comes from this plant. This is in agreement with data reported on groundwater from that area, where pesticides were found as the most relevant contaminants due to the intensive agriculture in the surrounding area, focused on citrus crops (Pitarch et al., 2016).

As expected, the lower section of the river was the most contaminated, especially in the area nearest to the estuary. The most polluted sites (total concentration > 5000 ng/L) were located downstream of the two WWTPs, near Vila-real (point 17) and Almassora (point 18). Surface water collected in these two sampling sites presented the highest number of positives (between 20 and 30, depending on the campaign). The last sampling site, near the river mouth into the Mediterranean Sea (19, Gola Almassora), also presented a notable pharmaceuticals pollution, but with mean total concentrations below 5000 ng/L.

3.2. Seasonal variation

The total concentration for the different pharmaceutical families in each sampling campaign is shown in **Fig. 3**. Antihypertensive, anti-inflammatory agents and antibiotics presented the highest concentrations. No clear trends were observed as a function of the sampling season, although a slight increase in concentrations of antihypertensives, antidepressants, antibiotics and analgesics seemed to occur in winter (3rd sampling). This fact is not surprising in the case of antibiotics due to the increase of their consumption to treat respiratory infections in colder periods (Letsinger et al. 2019).

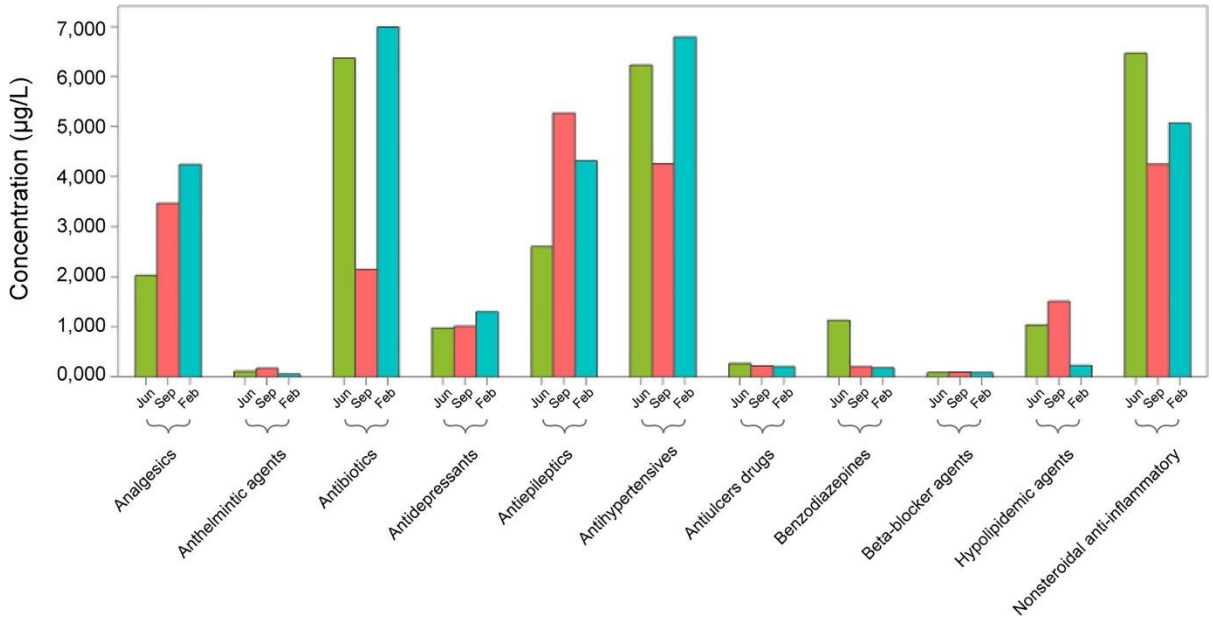


Fig. 3. Total pharmaceutical concentrations (µg/L) (grouped by families) in the Mijares River in every sampling campaign (1st campaign: June 2018; 2nd campaign: September 2018; 3rd campaign: February 2019). NSAIDs: Nonsteroidal anti-inflammatory drugs.

Due to the higher pollution observed in sampling sites 10, 17 and 18, specific data from these samples were evaluated to highlight possible seasonal trends. The antibiotics azithromycin, clarithromycin and trimethoprim were present at higher concentrations in winter at the three sampling sites. Other compounds were also found at higher concentrations in winter, at least in 2 out of the 3 sampling sites: the antibiotics clindamycin, erythromycin, sulfamethoxazole and metronidazole; the antihypertensives irbesartan, losartan and valsartan; the benzodiazepine alprazolam; the antiepileptic primidone; and the analgesic tramadol. The fact that pharmaceuticals presented higher concentrations in winter is in agreement with other river monitoring campaigns (Conley et al., 2008, Daneshvar et al., 2010, Lindholm-Lehto et al., 2016). Moreover, during cold periods, there is less degradation of the compounds in the WWTPs due to the low temperatures and irradiation, which result in higher analyte concentration levels in the effluent wastewater and, therefore, in the receiving surface water (Azzouz and Ballesteros, 2013, Golovko et al., 2014, Lindholm-Lehto et al., 2016).

3.3. Screening of pharmaceuticals and metabolites

A qualitative screening using UHPLC-QTOF MS was applied to samples collected in the second campaign to complement quantitative data and obtain information about other compounds that could be present in the samples. **Table S11** shows the detection frequency of pharmaceuticals. In total, 41 pharmaceuticals were detected, and up to 35 were confirmed with reference standards. Six more compounds were tentatively identified on the basis of the interpretation of accurate-mass data acquired, but could not be confirmed because the reference standard was not available at our laboratory.

Compounds with the highest detection frequency were acetaminophen and venlafaxine, identified in 4 out of the 19 samples. Six pharmaceuticals (azithromycin, carbamazepine, diclofenac, irbesartan, lidocaine and sulfamethoxazole) were found in 3 samples (16%). As expected, the upper section (points 1–6) presented the lowest number of findings, illustrating the little anthropogenic influence on this area. Regarding sites located downstream of the SWTP in Onda (13 and 14), no analytes were found indicating that no relevant pharmaceutical pollution comes from this plant, which is in agreement with quantitative results obtained in the three campaigns. As expected, the highest number of findings corresponded to water samples collected WWTP downstream, especially near Vila-real (point 17) and Almassora (point 18).

Fig. 4 shows a summary of the results obtained in the screening, grouped by pharmaceutical families. Antihypertensives and non-steroidal anti-inflammatory drugs (NSAIDs) were most frequently detected, each representing 20% of the findings, followed by antibiotics (12%). The remaining families were below 10%. Other compounds, mainly identified in points 17 and 18, were amisulpride (antipsychotic), cetirizine (antihistamine), dimetridazole (antiparasitic), iopromide (X-ray contrast agent), rimantadine (antiviral agent), each one with 2.2%, and lidocaine (anesthetic, 4.4%). Most of the compounds identified by HRMS screening have been often reported in surface water by the scientific literature (Gómez et al., 2010; Hernández et al., 2015b; Ibañez et al., 2009; López et al., 2014; Masiá et al., 2013).

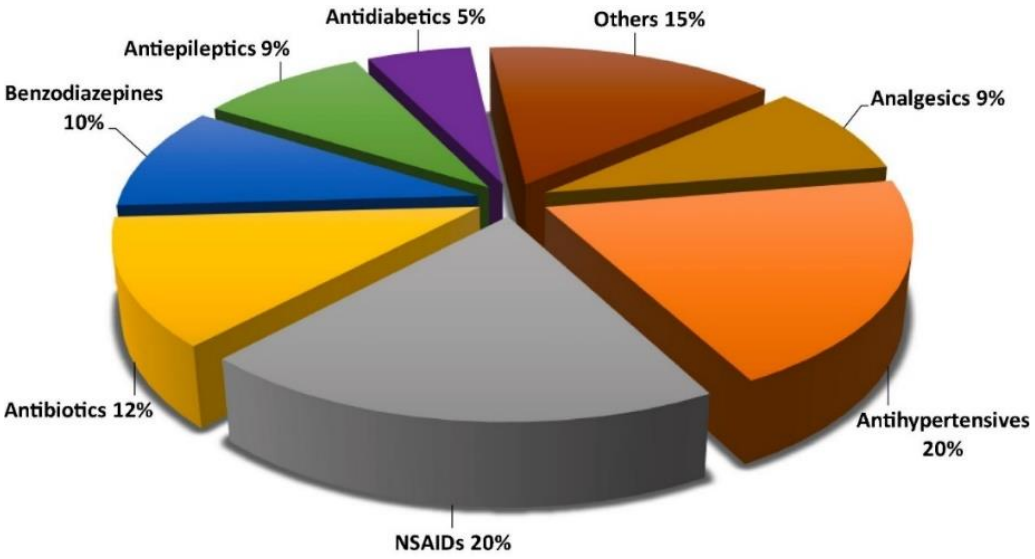


Fig. 4. Percentages of the different families of pharmaceuticals identified in the Mijares River by UHPLC-QTOF MS screening. NSAIDs: Nonsteroidal anti-inflammatory drugs. The "Others" category includes the following types of pharmaceuticals: anesthetics, antihistamines, antiparasitics, antipsychotics, antiviral and X-ray contrast agents.

From the 41 pharmaceuticals identified in the screening, 16 were already included in the target quantitative method applied in this work (marked with √ in **Table 1**). It must be taken into account that the quantitative UHPLC-MS/MS method offer much better sensitivity than the screening methodology, as it was optimized for a limited number of compounds and the TQS instrument has higher sensitivity than our QTOF instrument. It is therefore noteworthy that the detection frequency depends, not only on the concentration of the compound, but on the sensitivity of the method towards that particular compound. Hence, a lower detection frequency should not necessarily be associated to lower presence. The results from this screening will be useful to update the analytical methodology, by adding the compounds identified in the screening to the list of target analytes for quantitative UHPLC-MS/MS analysis.

The excellent potential of UHPLC-HRMS also allowed to investigate pharmaceutical metabolites with the aim to generate useful data for future monitoring, including relevant metabolites detected in surface water. The screening of metabolites was focused on the most contaminated samples (i.e. those collected in sampling sites 10, 17, 18 and 19) to facilitate

their detection and identification. **Table 2** shows the nine metabolites (tentatively) identified in surface water. 6 out of 9 metabolites could be confirmed with reference standards.

Table 2. Metabolites and/or transformation products of pharmaceuticals identified in surface water samples by UHPLC-QTOF MS.

Compounds	**Samples**			
	10b	**17b**	**18b**	**19b**
4-AA (4-Aminoantipyrine)	✓	–	–	–
4-AAA (4-Acetylaminoantipyrine)	✓	✓	✓	✓
4-FAA (4-Formylaminoantipyrine)	✓	✓	✓	✓
Carbamazepine-10,11-epoxide	–	✓	✓	–
Clopidogrel carboxylic acid	–	✓	✓	–
O-Desmethyl venlafaxine	–	t	t	–
4-OH Omeprazole sulphide	–	✓	✓	–
Losartan carboxylic acid	–	t	t	–
Nordiazepam (N-desmethyldiazepam)	–	t	t	–

✓: confirmed with reference standard, ((de)protonated molecule and at least one fragment ion were observed at the expected retention time).
t: tentative identification ((de)protonated molecule was observed and at least one ion fragment was justified).

4-acetylaminoantipyrine (4-AAA) and 4-formylaminoantipyrine (4-FAA), metabolites of the antipyretic drug dipyrone (metamizole), were identified in the 4 samples analysed. Furthermore, 4-OH omeprazole sulphide, carbamazepine-10,11-epoxide and clopidogrel carboxylic acid were also found in 2 out of the 4 samples, while 4-aminoantipyrine (4-AA) (another metabolite of dipyrone) was only identified in 1 of the surface water samples. These metabolites have also been found in surface water in previous studies performed by our group (Boix et al., 2016, Boix et al., 2014, Gracia-Lor et al., 2014).

Three metabolites could only be tentatively identified as the reference standards were not available at our laboratory. The potential of QTOF MS for investigation of metabolites is illustrated in **Figure S3,** which shows the tentative identification of nordiazepam (N-desmethyldiazepam) in a sample that also contained the parent compound diazepam (for more details, see **S.M.**)

3.4. Ecological risk assessment

The results of the ecological risk assessment performed with SSDs built with chronic NOECs show that the majority of the sampling sites are exposed to a low mixture toxic pressure ($msPAF_{Total}$ below 5%; **Fig. 5**). However the site 19 was considered to be moderately impacted, with msPAFs ranging between 5% and 25%; and sites 10, 17 and 18 were severely impacted, with calculated $msPAF_{Total}$ above 25%. Particularly, in sites 17 and 18 (in all sampling campaigns), and in 10 (in summer), the percentage of affected aquatic species ranged between 65% and 82%, indicating a very high ecotoxicological risk (**Fig. 5**).

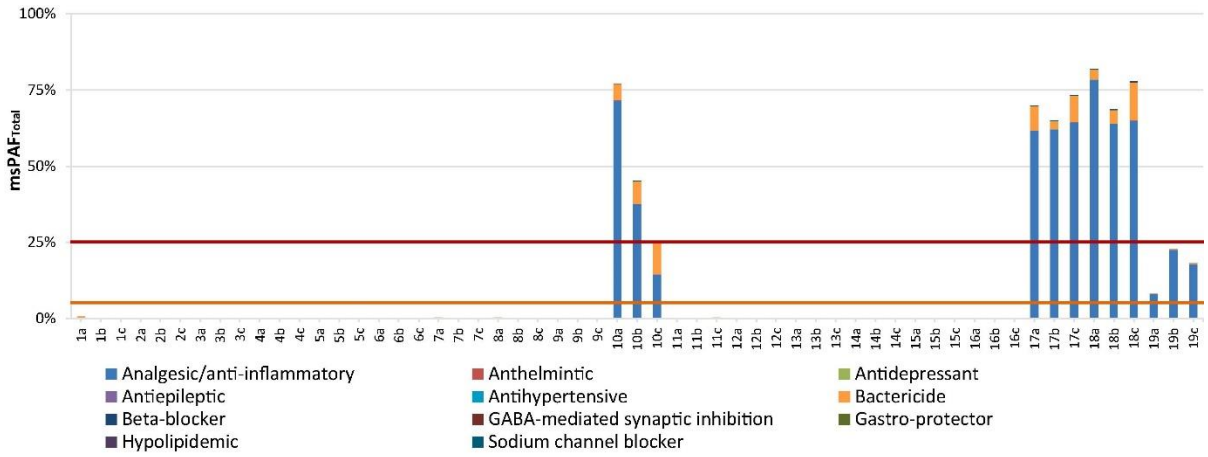


Fig. 5. Calculated total chronic toxicity ($msPAF_{Total}$) for each sample and relative contribution of each specific therapeutic class to the total toxic pressure. The orange line indicates an $msPAF_{Total}$ of 5%, and the red line an $msPAF_{Total}$ of 25%. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st campaign: June 2018; 2nd campaign: September 2018; 3rd campaign: February 2019). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In all cases, toxicity was dominated by the analgesic/anti-inflammatory TC ($msPAF_{TC}$ 15–81%). Within this TC, toxicity was clearly dominated by phenazone, although diclofenac also had an important contribution (**Tables S12-14**). The second TC with the highest calculated toxicity were the bactericides (antibiotics), with a $msPAF_{TC}$ ranging between 5% and 12% in sampling sites 10 (all sampling campaigns), 17 (summer and winter) and 18 (autumn and winter). Within this TC, toxicity was dominated by azithromycin in autumn and winter (in sites 10, 17 and 18). In summer, the toxicity of this TC was dominated by norfloxacin, although

other antibiotics such as ciprofloxacin and clarithromycin also contributed to the toxicity of the mixture. Regarding each of the monitored compounds in isolation, the highest ecological risks were established for phenazone > azithromycin > diclofenac, with individual PAFs above 10% in at least one sampling site; and to a lower extent norfloxacin, ciprofloxacin and clarithromycin, with individual PAFs above 1% in at least one sampling site (**Tables S12-S14)**.

The method based on SSDs, and the calculated msPAFs, is a more ecological relevant approach when compared to other methods (e.g. Toxic Unit) to assess the risk of chemical mixtures to aquatic ecosystems. This is basically because it integrates toxicity data for as many taxa as possible and accounts for their sensitivity differences on the basis of a statistical distribution. The capacity of the SSD approach to represent ecosystem effects has been evaluated on the basis of field monitoring studies and micro- and mesocosm experiments performed mainly with pesticides (e.g. Schäfer et al., 2013, Rico et al., 2018). Due to the absence of validation studies performed with pharmaceuticals, it is somewhat difficult to characterize the level of impact caused by each of the established risk categories. We expect that in the sites classified with severe risks (PAF or $msPAF_{Total}$ above 25%), the NOEC exceedances contributes to a loss of species that results in significant indirect ecological effects and in effects on important ecological functions. However, further investigations should be performed to quantify these effects and to validate the SSD method with pharmaceutical compounds.

One of the major drawbacks of the SSD approach for its implementation in pharmaceutical risk assessment is the limited amount of experimental chronic toxicity data available. In this way, chronic SSDs often need to be based on extrapolated or read-across toxicity data. For example, the μ of the chronic SSD for phenazone were based on the extrapolation of the μ for the acute one (2.5 μg/L), which was in turn constructed with a limited number of QSAR-based toxicity data (Posthuma et al. 2019; **Table S7**). Toxicity studies performed with other non-steroidal anti-inflammatory drugs, such as diclofenac, have shown cellular toxicity, genotoxicity, immunodepression, growth inhibition and estrogenic effects on fish at environmentally relevant concentrations (Hoeger et al., 2005, Hong et al., 2007, Xu et al., 2019). Therefore, experiments aimed at assessing the chronic toxicity of phenazone on fish are highly recommended. Regarding the other high priority compounds, the SSDs for azithromycin, ciprofloxacin and clarithromycin were based on a relatively large number of

toxicity data, but relied on acute-to-chronic toxicity data extrapolations, while the SSD for norfloxacin was based on available chronic toxicity data (**Table S7**). Previous studies show that these compounds are highly toxic to aquatic microorganisms, including cyanobacteria and some diatoms (Guo et al., 2015). Therefore, their ecotoxicological risks may be associated to the alteration of the structure of microbial communities and primary producers, most likely those associated to hard substrates, downstream of areas with significant WWTP influence (i.e., Montanejos, site 10, and in the mouth of the river, sites 17 and 18). Furthermore, several studies show that ecosystem functions mediated by these microorganisms (e.g. nitrification, denitrification, anaerobic ammonium oxidation) can be affected by prolonged exposure to concentrations similar to those that have been found in this study (Roose-Amsaleg and Laverman, 2016).

Although a large number of pharmaceuticals have been monitored in this study, the results of the aquatic risk assessment show that only a very limited number of compounds has a potential contribution to the total toxicity of the sample. This is in line with other studies evaluating the potential ecotoxicological of pharmaceutical mixtures, which demonstrate that usually a reduced number of compounds (≤ 5) significantly contribute to the total toxicity of the sample (Schäfer et al., 2013, Arenas-Sánchez et al., 2019). In our study, two TCs were the main responsible for the toxicity observed in the most polluted sites (i.e., analgesic/anti-inflammatory drugs and antibiotics). In principle, effects other than additive or antagonistic between these pharmaceutical groups are not expected on the impacted ecosystem, as they affect species in well separated trophic levels (i.e., cyanobacteria and fish). In addition, toxicity studies assessing the effects of non-steroidal anti-inflammatory drug mixtures on fish and other aquatic organisms (Cleuvers, 2004, Sehonova et al., 2017), or antibiotic mixtures on algae (González-Pleiter et al., 2013) generally demonstrate additivity, confirming that the concentration addition model used in this study for chemicals within the same TC is not expected to underestimate, neither overestimate, the calculated risks.

3.5. Antibiotic resistance risks

RQs exceeding the value of 1 were calculated in 3 out of the 19 evaluated sampling sites of the Mijares River (sites 10, 17 and 18). Resistance PNECs were exceeded by five antibiotics

(see **Fig. 6**), being ciprofloxacin the compound with the highest RQ (17.3), followed by azithromycin (6.5), norfloxacin (1.9), trimethoprim (1.5) and clarithromycin (1.3).

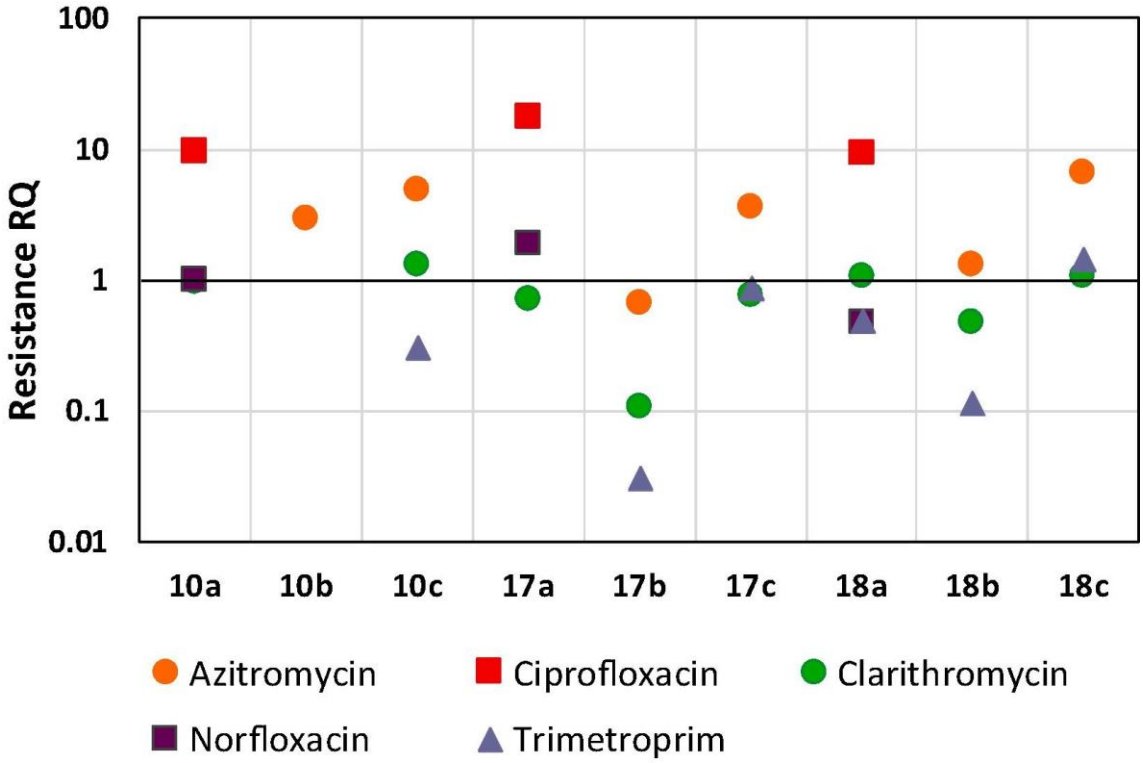


Fig. 6. Calculated RQs for the antibiotics that are expected to result in antibiotic resistance risks (RQ > 1) in at least one of the samples. Only the sites with RQs higher than one are represented. a, b, c refer to the samples taken in the first, second and third sampling campaigns, respectively (1st campaign: June 2018; 2nd campaign: September 2018; 3rd campaign: February 2019.

In some samples, exceedance of resistance thresholds occurred for more than one antibiotic (e.g. ciprofloxacin and norfloxacin; azithromycin and clarithromycin). Overall the antibiotics with the highest resistance development risk belong to the fluoroquinolone and the macrolide classes, which are classified as antibiotics of critical importance for human health (WHO, 2019). This study shows that WWTPs discharges into the Mijares River are contributing to environmental concentrations that may contribute to the enrichment of resistance genes in aquatic bacterial communities. However, the link between these indicators and the risks to the human population are not that straightforward. The assessment of the human transmission risks depends on the exposure levels (via bathing, irrigation, drinking), and require a

complementary *in-situ* evaluation of fecal contamination, resistant bacteria, genes and mobile genetic elements (Huijbers et al., 2019), which is out of the scope of this study. At this stage, however, this study evidences that antibiotics in the EU Watch List (and others co-occurring with them) should be evaluated, not only regarding their potential ecotoxicological side-effects, but also regarding their contribution to antibiotic resistance development in the environment.

4. Conclusions

A comprehensive investigation has been made on the occurrence and risks of pharmaceuticals in the Mijares River (Eastern Spain). Up to 35 pharmaceuticals were quantified in the water samples analyzed. The impact of wastewater effluents was evidenced by a notable increase of pharmaceutical concentrations as well as in the number of compounds detected in the samples collected downstream of WWTP discharges. The effect of the WWTP was observed even for small populations located along the river. The compounds most frequently found were acetaminophen, gabapentin, venlafaxine, valsartan, ciprofloxacin and diclofenac.

The complementary use of target quantitative methodology and qualitative wide-scope screening, allowed to have a more complete overview on the pharmaceuticals present in water. Accurate-mass data acquired by UHPLC-HRMS also allowed to investigate the presence of metabolites, leading to the identification of nine compounds, of which 4-acetylaminoantipyrine (4-AAA), 4-formylaminoantipyrine (4-FAA), 4-OH omeprazole sulphide, carbamazepine-10,11-epoxide and clopidogrel carboxylic acid were the most detected. Further studies on the occurrence and risks of these metabolites are recommended.

A probabilistic risk assessment for aquatic organisms has been performed, indicating moderate-to-severe ecological risks in four sampling points downstream of WWTP discharges. The toxicity of the pharmaceutical mixture was dominated by analgesic/anti-inflammatory drugs and antibiotics, and the compounds with the highest contribution to the toxicity were phenazone > azithromycin > diclofenac > norfloxacin, ciprofloxacin > clarithromycin. Out of these six compounds, only three are currently included in the EU Watch List. Out of the 13 antibiotic compounds evaluated in this study, 5 were found to exceed threshold concentrations for antibiotic resistance, particularly in the sampling sites downstream of WWTP discharges.

Therefore, this study supports the advancement of water sanitation methods to minimize ecological and antibiotic resistance risks in the Mijares River.

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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SUPPLEMENTARY MATERIAL

Occurrence and ecological risks of pharmaceuticals in a Mediterranean river in Eastern Spain

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2. EXPERIMENTAL

2.1. Chemicals and materials

Pharmaceutical reference standards were purchased from Sigma-Aldrich (St Louis, MO, USA), LGC Promochem (London, UK), Toronto Research Chemicals (Ontario, Canada), Across Organics (Geel, Belgium), Bayer Hispania (Barcelona, Spain), Fort Dodge Veterinaria (Gerona, Spain), Vetoquinol Industrial (Madrid, Spain) and Aventis Pharma (Madrid, Spain). Isotopically labelled internal standards (ILIS) were acquired from CDN Isotopes (Quebec, Canada), Toronto Research Chemicals, Cambridge Isotope Laboratories (Andover, MA, USA), Sigma-Aldrich (St Louis, MO, USA) and Cerilliant (Texas, USA). All reference standards presented purity higher than 93%. Standard stock solutions of each compound were prepared at 500 mg/L in methanol or acetonitrile and stored at -20 °C. Intermediate solutions (50 mg/L) were prepared by ten-fold dilution of the stock solution with methanol. Mixed working solutions containing all analytes were prepared from intermediate solutions by appropriate dilution with HPLC-grade water.

Acetonitrile and methanol (HPLC grade) were purchased from Scharlab (Barcelona, Spain). HPLC-grade water (resistivity of 18 MΩ cm) was obtained by purifying demineralised water (Millipore Ltd., Bedford, MA, USA). Formic acid (HCOOH, content > 98%) and ammonium acetate (NH_4Ac, reagent grade) were supplied by Scharlab.

Cartridges used for solid phase extraction were 150 mg Oasis HLB (Waters, Milford, MA, USA).

2.2. Description of the sampling sites and sample collection

Table S1. Description and location of the sampling sites along the Mijares River.

Sampling point	Source	Location	Province	Observations	UTM X	UTM Y	UTM Zone	Hemisphere
1	Upper	Azud de Babor, Mora de Rubielos	Teruel	-	689781	4448005	30T	Northern
2	Upper	La Escaleruela, Sarrión	Teruel	Fertilizer factory	691742	4446731	30T	Northern
3	Upper	La Escaleruela, Sarrión	Teruel	Fish farm	691816	4446682	30T	Northern
4	Upper	La Escaleruela, Sarrión	Teruel	Fish farm (downstream)	691833	4446727	30T	Northern
5	Upper	Toranes, Albentosa	Teruel	Reservoir	692172	4446656	30T	Northern
6	Upper	Pozo de las Palomas (Los Cantos) Puebla de Arenoso	Castellon	-	703509	4443872	30T	Northern
7	Middle	Arenós, Puebla de Arenoso	Castellon	Upstream Reservoir	705419	4442293	30T	Northern
8	Middle	Arenós, Puebla de Arenoso	Castellon	Downstream Reservoir	708738	4440125	30T	Northern
9	Middle	Montanejos	Castellon	Upstream WWTP	712148	4438332	30T	Northern
10	Middle	Montanejos	Castellon	Effluent WWTP	712150	4438356	30T	Northern
11	Middle	Toga	Castellon	Effluent WWTP	725126	4435377	30T	Northern
12	Middle	Sitjar, Onda	Castellon	Reservoir	736137	4432693	30S	Northern
13	Middle	Onda	Castellon	Downstream SWTP, Power-plant	736525	4431287	30S	Northern
14	Middle	Onda	Castellon	Downstream SWTP, Gauging station	736713	4431282	30S	Northern
15	Lower	Onda	Castellon	-	740931	4429492	30S	Northern
16	Lower	Vila-real	Castellon	Santa Quiteria	748201	4426699	30S	Northern
17	Lower	Vila-real	Castellon	Effluent WWTP	751127	4424425	30S	Northern
18	Lower	Almassora	Castellon	Effluent WWTP	751477	4424111	30S	Northern
19	Mouth	Almassora	Castellon	Gola	755367	4421890	30S	Northern

UTM: Universal Transverse Mercator

Table S2. Types of treatment in different conventional WWTPs (EPSAR, 2020).

	Wastewater Treatment Plant			
Treatment (water line)	Montanejos	Toga	Vila-real	Almassora
Preliminary				
Screening of coarse matter	✓	✓	✓	✓
Screening	✓	✓	✓	✓
Homogenization tank				✓
Grit removal		✓	✓	✓
Grease removal			✓	✓
Primary				
Physical-chemical				✓
Clarifying			✓	✓
Secondary				
Prolonged aeration	✓	✓		
Activated sludge			✓	✓
Nitrogen removal	✓	✓		✓
Phosphorous removal		✓		✓
Disinfection by chlorination				✓

2.3. Sample treatment

2.3.2. Screening analysis

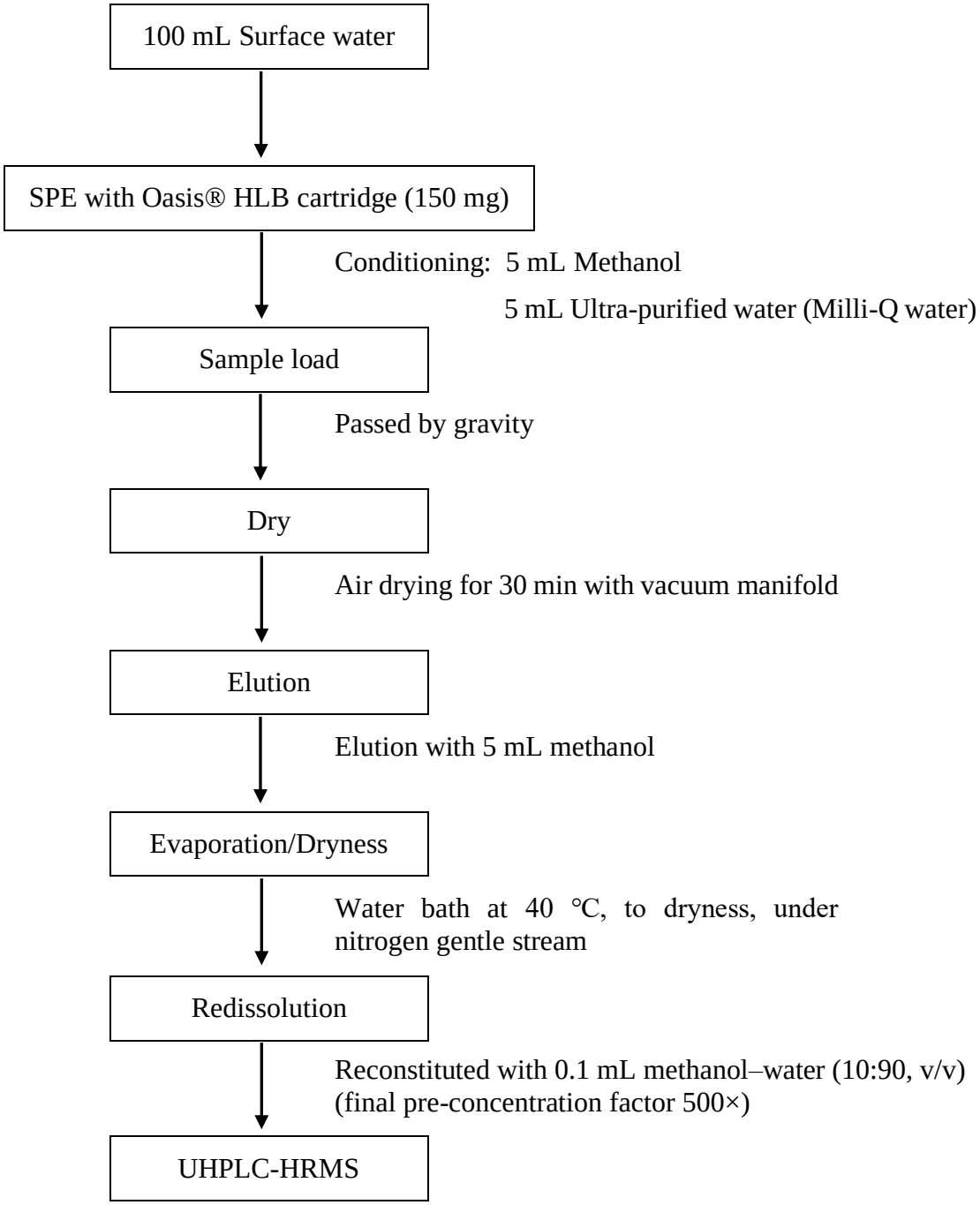

Figure S1. Procedure applied for sample preparation in HRMS screening analysis

2.4. Instrumentation

UHPLC-MS/MS (QqQ)

UHPLC analysis was carried out with a Waters ACQUITY ultra performance liquid chromatography (UPLC®) system (Waters, Milford, MA, USA), equipped with a binary solvent manager and a sample manager. The UHPLC separation was performed using a CORTECS C_{18} analytical column (100 x 2.1 mm i.d., particle size 2.7 µm) (Waters). An optimized gradient was applied at a constant flow rate of 0.4 mL/min using water LC-MS (solvent A) and methanol LC-MS (solvent B), both 0.01% HCOOH and 1 mM ammonium acetate.

The mobile phase gradient was: 0 min, 5% B; 0-7 min linearly increased from 5 to 95% B; 7-8 min, 95% B; 8-8.1 min, returning to initial conditions; maintained at 5% B until 9.5 min, for equilibration of the column. The sample manager temperature was 10 °C. The injection volume was 50 µL.

A Xevo TQ-S™ triple quadrupole mass spectrometer (Waters, Manchester, UK), equipped with T-Wave devices and an electrospray ionization (ESI) interface operated in positive and negative ion modes, was used. Drying gas as well as nebulising gas was nitrogen (Praxair, Valencia, Spain). The cone gas flow rate was optimized at 250 L/h and the desolvation gas flow was set to 1200 L/h. The desolvation temperature was 650 °C. For operation in MS/MS mode, collision gas was Argon 99.995% (Praxair, Valencia, Spain) with a flow of 0.15 mL/min in the collision cell. Electrospray needle capillary voltage was fixed at 3.5 kV and 2 kV in positive and negative ionisation modes, respectively. The source temperature was set to 150 °C. The column temperature was maintained at 40 °C.

MassLynx software v 4.1 (Waters Corporation) was used to acquire data. TargetLynx application manager was used to quantify the concentration levels of the target analytes.

UHPLC-HRMS

UHPLC-HRMS analysis was performed using a Waters ACQUITY UPLC® ultra-high performance liquid chromatography (UHPLC) system (Waters, Milford, MA, USA) interfaced to a hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (XEVO G2 QTOF, Waters Micromass, Manchester, UK), using an orthogonal Z-spray electrospray ionization (ESI) interface operated in both positive and negative ionisation modes.

The UHPLC separation was performed using an CORTECS C_{18} column (2.1 i.d. × 100 mm length, 2.7 µm particle size, Waters), at a flow rate of 300 µL/min. The mobile phases were (A) H_2O with 0.01% HCOOH and (B) MeOH with 0.01% HCOOH. The mobile phase gradient was: 10% B at 0 min; 90% B at 14 min, linearly increased; 90% B maintained for two minutes more; return to initial conditions at 16.1 min (10% B) and maintained until 18 min. The column oven was kept at 40 °C and the sample manager at 10 °C. The injection volume was 20 µL.

MS data were acquired over a *m/z* range of 50-1000. Nitrogen was used as drying and nebulizing gas. The gas flow was set at 1000 L/h. TOF-MS resolution was approximately 18000 at full width half maximum (FWHM) at *m/z* 556. Capillary voltages of 0.7 kV and 3.0 kV were used in positive and negative ionisation modes, respectively. A cone voltage of 20 V was selected for both ionisation modes. Collision gas was argon 99.995% (Praxair, Valencia, Spain). The desolvation temperature was 600 °C and the source temperature 130 °C.

For MS^E experiments, two acquisition functions with different collision energies were used: the low energy (LE), selecting a collision energy of 4 eV in order to obtain information about the protonated molecule and adducts (if present), and the high energy (HE) function, with a collision energy ramp ranging from 15 to 40 eV, in order to obtain a greater range of fragment ions. The LE and HE functions settings were for both a scan time of 0.3 s. Data were automatically processed by ChromaLynx XS software (MassLynx v 4.1, Waters).

2.5. Quantitative LC-MS/MS analysis and quality assurance

Table S3. UHPLC-MS/MS (QqQ) conditions used for pharmaceuticals.

Compounds	ESI	Cone (V)	Transition (Q)	CE (eV)	Transition (q)	CE (eV)	LCL (ng/L)
Acetaminophen	+	10	152.0 > 110.0	15	152.0 > 93.0	20	5
					152.0 > 65.0	25	
Alprazolam	+	10	309.0 > 281.0	25	309.0 > 205.0	25	5
					309.0 > 274.0	25	
Atorvastatin	+	10	559.0 > 440.0	20	559.0 > 466.0	15	5
					559.0 > 292.0	25	
Azithromycin	+	10	749.4 > 591.4	25	749.4 > 82.9	45	50
					749.4 > 116.1	45	
Bezafibrate	-	10	360.0 > 274.0	20	360.0 > 154.0	25	1000
					360.0 > 85.0	15	
Carbamazepine	+	10	237.0 > 194.0	20	237.0 > 179.0	25	5
					237.0 > 192.0	10	
Ciprofloxacin	+	10	332.0 > 231.0	25	332.0 > 288.0	15	50
					332.0 > 314.0	20	
Clarithromycin	+	10	590.0 > 158.0	20	590.0 > 116.0	25	5
					590.0 > 98.0	25	
Clindamycin	+	10	425.1 > 126.0	20	425.1 > 337.0	20	5
					425.1 > 389.0	15	
Diclofenac	+	10	296.2 > 214.2	30	296.2 > 250.0	10	5
					296.2 > 278.0	5	
Enalapril	+	10	377.0 > 234.0	15	377.0 > 117.0	25	5
					377.0 > 303.0	15	
Erythromycin	+	10	734.0 > 158.0	25	734.0 > 576.0	15	10
					734.0 > 558.0	15	
Furaltadone	+	10	325.0 > 100.0	20	325.0 > 252.0	15	5
					325.0 > 281.0	10	
Gabapentin	+	10	172.0 > 137.0	15	172.0 > 154.2	15	5
					172.0 > 95.0	20	
Gemfibrozil	-	10	249.0 > 113.0	10	249.0 > 121.0	20	1000
					249.0 > 127.0	10	
Irbesartan	+	10	429.0 > 207.0	25	429.0 > 195.0	20	5
					429.0 > 180.0	25	
Ketoprofen	-	10	253.0 > 79.0	10	253.0 > 92.0	20	1000
					253.0 > 209.0	10	
Levamisol	+	10	205.0 > 178.0	20	205.0 > 91.0	25	5
					205.0 > 123.0	25	
Lincomycin	+	10	407.0 > 126.0	20	407.0 > 359.0	15	5
					407.0 > 389.0	15	
Lorazepam	+	10	321.0 > 275.0	20	321.0 > 303.0	15	10
					321.0 > 229.0	25	
Losartan	+	10	423.1 > 207.1	15	423.1 > 377.1	15	5
					423.1 > 405.1	10	
Metropolol	+	10	268.2 > 116.0	15	268.2 > 74.0	20	5
					268.2 > 191.0	15	
Metronidazole	+	10	172.0 > 127.9	15	172.0 > 82.1	20	5
					172.0 > 55.9	20	
Nalidixic acid	+	10	233.0 > 187.0	25	233.0 > 215.0	10	5
					233.0 > 159.0	25	
Naproxen	-	10	229.0 > 170.0	20	229.0 > 185.0	10	1000
					185.0 > 169.0	20	
Norfloxacin	+	10	320.0 > 233.0	25	320.0 > 276.0	15	50
					320.0 > 302.0	20	

Table S3 (cont.). UHPLC-MS/MS (QqQ) conditions used for pharmaceuticals.

Compounds	ESI	Cone (V)	Transition (Q)	CE (eV)	Transition (q)	CE (eV)	LCL (ng/L)
Omeprazole sulfide, 4-hydroxy	+	10	316.0 > 168.0	20	316.0 > 149.0	20	5
					316.0 > 283.0	15	
Oxolinic acid	+	10	262.0 > 216.0	25	262.0 > 244.0	15	5
					262.0 > 158.0	25	
Pantoprazole	+	10	384.0 > 200.0	10	384.0 > 138.0	25	5
					384.0 > 153.0	15	
Phenazone	+	10	189.3 > 131.1	20	189.3 > 104.1	20	10
					189.3 > 58.1	20	
Primidone	+	10	219.2 > 162.0	10	219.2 > 91.0	20	5
					219.2 > 119.2	15	
Roxithromycin	+	10	679.0 > 158.0	25	679.0 > 116.0	25	5
					679.0 > 98.0	25	
Salbutamol	+	10	240.0 > 148.0	15	240.0 > 222.1	10	5
					240.0 > 166.1	10	
Sulfadiazine	+	10	251.0 > 156.0	15	251.0 > 92.0	25	5
					251.0 > 108.0	20	
Sulfamethoxazole	+	10	254.0 > 92.0	25	254.0 > 156.0	15	5
					254.0 > 108.0	20	
Tetracycline	+	10	445.0 > 154.0	25	445.0 > 410.0	15	5
					445.0 > 427.0	10	
Tramadol	+	10	264.0 > 58.0	10	264.0 > 121.0	25	5
					264.0 > 246.0	10	
Trimetroprim	+	10	291.0 > 123.0	25	291.0 > 230.0	20	5
					291.0 > 261.0	25	
Valsartan	+	10	436.0 > 207.0	25	436.0 > 235.0	15	5
					436.0 > 261.0	15	
Venlafaxine	+	10	278.0 > 58.0	15	278.0 > 260.0	10	5
					278.0 > 121.0	25	
ILIS							
Acetaminophen-d_4	+	10	156.0 > 114.0	10			
Atorvastatin-d_5	+	10	564.0 > 445.0	20			
Azithromycin-d_3	+	10	752.2 > 594.2	25			
Carbamazepine 10,11-epoxide-d_{10}	+	10	263.0 > 190.0	25			
Ciprofloxacin-d_8	+	10	340.1 > 322.1	20			
Cocaethylene-d_8	+	10	326.0 > 204.0	20			
Diclofenac-d_4	+	10	300.1 > 219.2	20			
Erythromycin-^{13}C-d_3	+	10	738.1 > 161.9	35			
Irbesartan-d_6	+	10	435.1 > 213.3	25			
Norfloxacin-d_5	+	10	325.0 > 238.0	20			
Omeprazole-d_3	+	10	349.0 > 198.0	10			
Simvastatin-d_6	+	10	425.2 > 199.1	10			
Sulfamethoxazole-$^{13}C_6$	+	10	260.0 > 162.0	15			
Valsartan-d_8	+	10	444.0 > 207.0	25			
Venlafaxin-d_6	+	10	284.3 > 64.1	25			

Quantitative (Q) and qualitative (q) transitions. Collision energy (CE). Lowest calibration level (LCL), used as the limit of quantification. Isotopically labelled internal standards (ILIS).

3. RESULTS AND DISCUSSION

Table S4. Mean recoveries and RSD (in brackets) for QCs (both in %) at the three concentration levels tested, after applying the UHPLC-MS/MS procedure for the determination of pharmaceuticals to water samples corresponding to the three sampling campaigns. A total of 9 replicates (n=3 at each level) has been considered, with the exception of 0.01 µg/L (a total of 3 replicates). The ILIS used for each compound is shown.

Compounds	**ILIS**	**QCs**		
		0.01 µg/L	**0.1 µ/L**	**1 µg/L**
Acetaminophen	Acetaminophen-d_4	116 (7)	87 (13)	86 (15)
Alprazolam	-	101 (18)	113 (16)	118 (26)
Atorvastatin	Atorvastatin-d_5	118 (18)	111 (6)	108 (12)
Azithromycin	Azitromycin-d_3	-	43 (27)	87 (34)
Bezafibrate	-	-	-	68 (33)
Carbamazepine	Carbamazepine	139 (19)	105 (16)	[a]
Ciprofloxacin	Ciprofloxacin-d_8	*	*	*
Clarithromycin	-	99 (6)	129 (35)	*
Clindamycin	-	136 (17)	112 (26)	110 (25)
Diclofenac	Diclofenac-d_4	115 (7)	106 (6)	98 (3)
Enalapril	-	119 (13)	111 (7)	109 (11)
Erythromycin	Erythromycin-$^{13}Cd_3$	105 (12)	99 (38)	111 (17)
Furaltadone	-	148 (7)	140 (6)	134 (18)
Gabapentin	-	[b]	112 (10)	105 (4)
Gemfibrozil	-	-	-	49 (43)
Irbesartan	Irbesartan-d_6	127 (3)	110 (21)	120 (15)
Ketoprofen	-	-	-	84 (30)
Levamisol	Cocaethylene-d_8	125 (11)	99 (18)	117 (10)
Lincomycin	-	132 (1)	132 (19)	132 (16)
Lorazepam	-	105 (3)	98 (12)	104 (17)
Losartan	-	135 (25)	119 (15)	116 (14)
Metropolol	-	123 (1)	117 (6)	124 (6)
Metronidazole	-	121 (6)	118 (8)	122 (11)
Nalidixic acid	-	125 (2)	90 (12)	89 (9)
Naproxen	-	-	-	70 (26)
Norfloxacin	Norfloxacin-d_5	*	*	*
Omeprazole sulfide, 4-hydroxy	Omeprazole-d_3	123 (3)	93 (13)	84 (9)
Oxolinic acid	-	103 (38)	93 (11)	92 (6)
Pantoprazole	-	125 (6)	116 (12)	115 (16)

Table S4 (cont.). Mean recoveries and RSD (in brackets) for QCs (both in %) at the three concentration levels tested, after applying the UHPLC-MS/MS procedure for the determination of pharmaceuticals to water samples corresponding to the three sampling campaigns. A total of 9 replicates (n=3 at each level) has been considered, with the exception of 0.01 µg/L (a total of 3 replicates). The ILIS used for each compound is shown.

Compounds	**ILIS**	**QCs**		
		0.01 µg/L	**0.1 µ/L**	**1 µg/L**
Phenazone	-	74 (2)	76 (16)	81 (21)
Primidone	-	104 (16)	101 (10)	102 (10)
Roxithromycin	-	107 (24)	*	*
Salbutamol	-	126 (7)	160 (29)	155 (25)
Sulfadiazine	Sulfamethoxazole-$^{13}C_6$	120 (13)	104 (29)	105 (13)
Sulfamethoxazole	Sulfamethoxazole-$^{13}C_6$	97 (15)	103 (8)	105 (16)
Tetracycline	-	52 (26)	60 (28)	55 (19)
Tramadol	-	149 (2)	117 (8)	125 (9)
Trimetroprim	-	139 (1)	132 (14)	136 (11)
Valsartan	Valsartan-d_8	140 (60)	100 (25)	91 (18)
Venlafaxine	Venlafaxin-d_6	132 (5)	100 (15)	117 (7)

[a] Not calculated due to the lack of linearity at high concentration levels
[b] Not calculated due to the presence of the compound in the "blank" sample used for QCs preparation at concentration higher than 0.01 µg/L
- Not available due to the lack of sensitivity
* Poor reproducible recovery values, usually higher than 200%

Table S5. Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	Elemental composition
4-Hydroxy-N-desmethyl Tamoxifen	C25H27NO2
Δ1,4-Androstadiene-3,17-dione	C19H24O2
1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one	C14H10NOCl2
1,4-Androstadiene-3,17-dione	C19H24O2
1,4-Dihydroxy Alprazolam	C17H13ClN4O2
10,11-Dihydro-10,11-Dihydroxy Carbamazepine	C15H14N2O3
10,11-Dihydroxy Carbamazepine	C15H12N2O3
10-Hydroxy Amitriptyline	C20H23NO
10-Hydroxy Carbazepine	C15H14N2O2
10-Hydroxy Imipramine	C19H24N2O
10-Hydroxy Warfarin	C19H16O5
14-Hydroxy Clarithromycin	C38H69NO14
16 β-hydroxy Stanozolol	C21H32N2O2
1-Hydroxy Alprazolam	C17H13ClN4O
2,3-Dihydroxy Carbamazepine	C15H12N2O3
2-Amino Flubendazole	C14H10FN3O
2-Hydroxy Atorvastatin	C33H35FN2O6
2-Hydroxy Carbamazepine	C15H12N2O2
2-Hydroxy Desipramine	C18H22N2O
2-Hydroxyclomipramine	C19H23ClN2O
2-Hydroxydesmethylimipramine	C18H22N2O
2-Hydroxydesmethyltrimipramine	C19H24N2O
2-Hydroxyimipramine	C19H24N2O
2-Hydroxymethyl Olanzapine	C17H20N4OS
2-Hydroxytrimipramine	C20H26N2O
3-(Hydroxy-carboxymethyl)hydratopic acid	C11O5H12
3-(Keto-carboxymethyl)hydratopic acid	C11O5H10
3-(N-Acetyl-L-cystein-S-yl) Acetaminophen	C13H16N2O5S
3,5-dichlorosalicylic acid	C7H4Cl2O3
3-[(4-Carboxy-4-methylpentyl)oxy]-4-	C15H20O5
3',4'-Dihydroxy Flurbiprofen	C15H13FO4
3''-Hydroxy Pravastatin	C23H36O8
3'-Desmethoxy Aliskiren	C29H51N3O6
3'-Desmethoxy Aliskiren 3'-Carboxylic Acid	C29H49N3O7
3'-Mercaptoacetaminophen Disulfide	C16H16N2O4S2
3''-Hydroxy Simvastatin	C25H38O6

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	**Elemental composition**
3-amino-2-oxazolidona	C3H6N2O2
3-amino-5-morfolinometill-2- oxazolidinona	C8H15N3O3
3-Carboxy Mefenamic Acid	C15H13NO4
3-Cysteinyl Acetaminophen	C11H14N2O4S
3-Hydroxy Acetaminophen	C8H9NO3
3-Hydroxy Bromazepam	C14H10BrN3O2
3-Hydroxy Carbamazepine	C15H12N2O2
3-Hydroxy Desloratadine	C19H19ClN2O
3-hydroxy Diclofenac	C14H11Cl2NO3
3-Hydroxy Lidocaine	C14H22N2O2
3-Hydroxy Omeprazole	C17H19N3O4S
3-Hydroxy Phenytoin	C15H12N2O3
3-hydroxy Stanozolol	C21H32N2O2
3'-Hydroxy Trimethoprim	C14H18N4O4
3-Hydroxy Valproic acid	C8H16O3
3-Hydroxymethyl Meclofenamic Acid	C14H11Cl2NO3
3-Hydroxymethyl Mefenamic Acid	C15H15NO3
3-Methoxy Acetaminophen	C9H11NO3
3-Oxo Valproic acid	C8H14O3
3α-Hydroxy Pravastatin	C23H35O7
4'-Hydroxy Flurbiprofen	C15H13FO3
4'-Hydroxy Nimesulide	C13H12N2O6S
4'-Hydroxy Tamoxifen	C26H29NO2
4-Acetylamino Antipyrine	C13H15N3O2
4-Amino Antipyrine	C11H13N3O
4-Aminosalicylic acid	C7H7NO3
4-Chlorobenzoic acid	C7H5ClO2
4-Desmethoxy Omeprazole	C16H17N3O2S
4-Dimethylamino Antipyrine	C13H17N3O
4-Formylamino Antipyrine	C12H13N3O2
4-Hydroxy Alprazolam	C17H13ClN4O
4-Hydroxy Atorvastatin	C33H35FN2O6
4-Hydroxy Diclofenac	C14H11Cl2NO3
4-Hydroxy Levamisole	C11H12N2OS
4-Hydroxy Mepivacaine	C15H22N2O2
4-Hydroxy Midazolam	C18H13ClFN3O

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	Elemental composition
4-Hydroxy Nitrofurantoin	C8H6N4O6
4-Hydroxy Omeprazole sulfide	C16H17N3O2S
4-Hydroxy Propranolol	C16H21NO3
4-Hydroxy Stanozolol	C21H32N2O2
4-Hydroxy Tamoxifen	C26H29NO2
4'-Hydroxy Trimethoprim	C14H18N4O4
4-Hydroxy Valproic acid	C8H16O3
4-Hydroxy Valsartan	C24H29N5O4
4-Hydroxy-N-desmethyl Tamoxifen	C25H27NO2
4-Methylamino Antipyrine/Ampyrone	C12H15N3O
4-Nitro Sulfamethoxazole	C10H9N3O5S
4-Oxo Norfloxacin	C16H16FN3O4
4-Oxo Valproic acid	C8H14O3
5-(4-hydroxy-2,5-dimethylphenoxy)-2,2-dimethyl-Pentanoic acid	C15H22O4
5-[2-(hydroxymethyl)-5-methylphenoxy]-2,2-dimethyl-Pentanoic acid	C15H22O4
5'-Oxo Amisulpride	C17H25N3O5S
5-Aminosalicylic Acid	C7H7NO3
5-Hydroxy Desloratadine	C19H19ClN2O
5-Hydroxy Diclofenac	C14H11Cl2NO3
5-Hydroxy Omeprazole	C17H19N3O4S
5-Hydroxy Propafenone	C21H27NO4
5-Hydroxy Propranolol	C16H21NO3
5-Hydroxy Valproic acid	C8H16O3
5-Hydroxymethyl Cimetidine	C10H16N6OS
5-Methoxysalicylic acid	C8H8O4
5-O-Desmethyl Omeprazole	C16H17N3O3S
5-O-Desmethyl Omeprazole Sulfide	C16H17N3O2S
5-Oxopyrrolidinyl Sulpiride	C15H21N3O5S
6'-Hydroxymethyl Simvastatin	C25H38O6
6-Methyl Salicylic acid	C8H8O3
7-Amino Clonazepam	C15H12ClN3O
7-Amino Flunitrazepam	C16H14FN3O
7-Amino Nitrazepam	C15H13N3O
7-Hydroxy Methotrexate	C20H22N8O6
7-Hydroxy N-Desalkyl Quetiapine	C17H17N3OS
7-Hydroxy Propranolol	C16H21NO3

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	**Elemental composition**
7-Hydroxy Quetiapine	C21H25N3O3S
7-Hydroxy Risperidone	C23H27FN4O3
8-Hydroxy Clomipramine	C19H23ClN2O
8-Hydroxy Mirtazapine	C17H19N3O
9-Hydroxy Risperidone	C23H27FN4O3
Abacavir	C14H18N6O
Abamectin B1	C48H72O14
Aceclofenac	C16H13Cl2NO4
Acenocoumarol	C19H15NO6
Acepromazine	C19H22N2OS
Acetaminophen/paracetamol	C8H9NO2
Acetazolamida	C4H6N4O3S2
Acetobutolol	C18H28N2O4
Acetopromazine	C19H22N2OS
Acetylsalicylic acid	C9H8O4
Aciclovir	C8H11N5O3
Albendazole	C12H15N3O2S
Albendazole sulfone	C12H15N3O4S
Albendazole sulfoxide	C12H15N3O3S
Albendazole-2-aminosulfone	C10H13N3O2S
Albuterol	C13H21NO3
Aldosterone	C21H28O5
Alendronate	C3H5N3O2
Alendronic acid	C4H13NO7P2
Aliskiren	C30H53N3O6
Alprazolam	C17H13ClN4
Alprazolam 5-Oxide	C17H13ClN4O
Altrenogest	C21H26O2
Amantadine	C10H17N
Amidotrizoic acid	C11H9I3N2O4
Amikacin	C22H43N5O13
Amiloride	C6H8ClN7O
Aminomebendazole	C14H11N3O
Aminopyridine	C5H6N2
Aminosidine	C23H45N5O14
Amiodarona	C25H29I2NO3

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	**Elemental composition**
Amisulpride	C17H27N3O4S
Amisulpride N-oxide	C17H27N3O5S
Amitriptyline	C20H23N
Amlodipine	C20H25N2O5Cl
Amoxicillin	C16H19N3O5S
Amperozide	C23H29F2N3O
Ampicillin	C16H19N3O4S
Amprolium	C14H18N4
Androstanolone	C19H30O2
Androstenediol	C19H30O2
Androstenedione	C19H26O2
Androsterone	C19H30O2
Ansamycin	C46H62N4O11
Antipyrine/Phenazone	C11H12N2O
Apramycin	C21H41N5O11
Arbidol	C22H28BrClN2O4S
Arprinocid	C12H9ClFN5
Astemizol	C28H31FN4O
Atazanavir	C38H52N6O7
Atenolol	C14H22N2O3
Atenolol acid	C14H21NO4
Atomoxetine	C17H21NO
Atorvastatin	C33H35FN2O5
Atropine	C17H23NO3
Avoparcine	C89H102ClN9O36
Azaperol	C19H24FN3O
Azaperone	C19H22FN3O
Azathioprine	C9H7N7O2S
Azepromazine	C19H22N2OS
Azithromycin	C38H72N2O12
Aztreonam	C13H17N5O8S2
Bacitrazine A	C66H103N17O16S
Bamethan	C12H19NO2
Bamethane	C12H19NO2
Baquiloprim	C17H20N6
Barbital	C8H12N2O3

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	**Elemental composition**
Bendiocarb	C11H13NO4
Bendroflumethiazide	C15H14F3N3O4S2
Benzylhydrochlorothiazide	C14H14ClN3O4S2
Betamethasone/Dexamethasone	C22H29FO5
Betaxolol	C18H29NO3
Bezafibrate	C19H20ClNO4
Bicalutamide	C18H14F4N2O4S
Bifenthrin	C23H22ClF3O2
Bisacodilo	C22H19NO4
Bisoprolol	C18H31NO4
Bolandiol	C18H28O2
Bolasterone	C21H32O2
Boldenone	C19H26O6
Boldione	C19H24O2
Bromazepam	C14H10BrN3O
Brombuterol	C12H18Br2N2O
Broom-clenbuterol	C12H18N2OClBr
Broom-clenproperol	C11H16N2OBr
Broom-mapenterol	C14H20N2OF3Br
Budesonide	C25H34O6
Bufencarb	C26H38N2O4
Buphenine, Nylidrin	C19H25NO2
Bupivacaine	C18H28N2O
Buprenorphine	C29H41NO4
Busulfan	C6H14O6S2
Cambendazole	C14H14N4O2S
Candesartan	C24H20N6O3
Capecitabine	C15H22FN3O6
Captopril	C9H15NO3S
Carazolol	C18H22N2O2
Carbadox	C11H10N4O4
Carbamazepine	C15H12N2O
Carbamazepine 10,11-Epoxide	C15H12N2O2
Carbamazepine-o-quinone	C15H10N2O3
Carbenicillin	C17H18N2O6S
Carbimazole	C7H10N2O2S

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	Elemental composition
Carboxyibuprofen	C13H16O4
Carbuterol	C13H21N3O3
Carnidazole	C8H12N4O3S
Carozolole	C18H23N2O2
Carprofen	C15H12ClNO2
Carvedilol	C24H26N2O4
Cefaclor	C15H14N3O4SCl
Cefadroxil	C16H17N3O5S
Cefalexin	C16H17N3O4S
Cefalonium	C20H18N4O5S2
Cefalotin	C16H16N2O6S2
Cefazoline	C14H14N8O4S3
Cefepime	C19H24N6O5S2
Cefoperazone	C25H27N9O8S2
Cefotaxim	C16H17N5O7S2
Cefquinome	C23H24N6O5S2
Ceftiofur	C19H17N5O7S3
Ceftizoxime	C13H13N5O5S2
Ceftriaxone	C18H18N8O7S3
Cefuroxime	C16H16N4O8S
Celiprolol	C20H33N3O4
Cephacetrile	C13H13N3O6S
Cephadroxile	C16H17N3O5S
Cephalexin	C16H17N3O4S
Cephapirin	C17H17N3O6S2
Cephazolin	C14H14N8O4S3
Cephradine	C16H19N3O4S
Cetirizine	C21H25ClN2O3
Chloramphenicol	C11H12N2O5Cl2
Chlorbrombuterol	C12H18BrClN2O
Chlordiazepoxide	C16H14ClN3O
Chlormadinon	C21H27ClO3
Chlorprenaline	C11H16ClNO
Chlorpromazine	C17H19ClN2S
Chlorprotixene	C18H18ClNS
Chlortestosterone	C19H27ClO2

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	**Elemental composition**
Chlortetracyclin	C22H23ClN2O8
Chrysoine	C12H10N2O5S
Cimaterol	C12H17N3O
Cimbuterol	C13H19N3O
Cimetidine	C10H16N6S
Cimetidine sulfoxide	C10H16N6O2S
Cinoxacin	C12H10N2O5
Ciprofloxacin	C17H18N3O3F
Ciprofloxacin N-Oxide	C17H18FN3O4
Ciprofloxacin Piperazinyl-N4-sulfate	C17H18FN3O6S
Ciproheptadina	C21H21N
cis-3-Hydroxy Glyburide	C23H28ClN3O6S
cis-4-Hydroxy Tamoxifen Discontinued	C26H29NO2
Citalopram	C20H21FN2O
Citalopram alcohol	C19H18FNO2
Citalopram aldehyde	C18H14FNO2
Citalopram propionic acid	C18H14FNO3
Citalopram-N-Oxide	C20H21FN2O2
Clarythromycin	C38H69NO13
Clavulanic acid	C8H9NO5
Clemidazole penicillin	C35H38ClN5O4S
Clenbuterol	C12H18Cl2N2O
Clencyclohexerol	C14H20Cl2N2O2
Clenisopenterol	C13H20N2OCl2
Clenproperol	C11H16Cl2N2O
Climbazole	C15H17ClN2O2
Clinafloxacine	C17H17ClFN3O3
Clindamycin	C18H33ClN2O5S
Clindamycin Sulfoxide	C18H33ClN2O6S
Clobazam	C16H13ClN2O2
Clofibrate	C12H15ClO3
Clofibric acid	C10H11ClO3
Clomipramine	C19H23ClN2
Clonazepam	C15H10N3ClO3
Clopidogrel	C16H16NO2SCl
Clopidogrel carboxylic acid	C15H14ClNO2S

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	**Elemental composition**
Clostebol	C19H27ClO2
Cloxacillin	C19H18ClN3O5S
Clozapine	C18H19ClN4
Clozapine-N-oxide	C18H19ClN4O
Codeine	C18H21NO3
Colistin A	C53H100N16O13
Colistin B	C52H98N16O13
Corticosterone	C21H30O4
Cortisol/Hydrocortisone	C21H30O5
Cortisone	C21H28O5
Crotamiton	C13H17NO
Cyclobenzaprine	C20H21N
Cyclophosphamide	C7H15Cl2N2O2P
Cyfluthrin	C22H18Cl2FNO3
Cylathrin	C22H18Cl2FNO3
Cytarabine	C9H13N3O5
D,L N,O-Didesmethyl Venlafaxine	C15H23NO2
D,L N-Desmethyl Venlafaxine	C16H25NO2
D,L-N,N-Didesmethyl-O-desmethyl Venlafaxine	C14H21NO2
D,L-O-Desmethyl Venlafaxine	C16H25NO2
Danofloxacin	C19H20FN3O3
Dapsone	C12H12N2O2S
Deacetyl Diltiazem N-Oxide	C20H24N2O4S
Deacetyl-O-Demethyl Diltiazem	C19H22N2O3S
Decoquinate	C24H35NO5
Dehydro Nifedipine	C17H16N2O6
Dehydro Warfarin	C19H14O4
Delmadinone	C21H25ClO3
Deltamethrin	C22H19Br2NO3
Demecocycline	C21H21ClN2O8
Demethyl Clomipramine	C18H21ClN2
Demethyl Imipramine	C18H22N2
Desacetyl Diltiazem	C20H24N2O3S
Desalkyl Verapamil D617	C17H26N2O2
Desethylene Ciprofloxacin	C15H16FN3O3
Desmethyl Amisulpride	C16H25N3O4S

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	**Elemental composition**
Desmethyl Diltiazem	C21H24N2O4S
Desmethyl Ofloxacin	C17H18FN3O4
Desmethyl Ranitidine	C12H20N4O3S
Desmethyl Diazepam	C15H11ClN2O
Desmethyl Doxepine	C18H19NO
Desmethylene Paroxetine	C18H20FNO3
Desmethyl Sertraline	C16H15Cl2N
Desmethyl Trimipramine	C19H24N2
Desoximetasone	C22H29FO4
Desoxycortisone	C21H30O4
Dexamethasone	C22H29FO5
Dianabol	C20H28O2
Diatrizoate	C11H8I3N2NaO4
Diaveridine	C13H16N4O2
Diazepam	C16H13ClN2O
Diclazuril	C17H9Cl3N4O2
Diclofenac	C14H11Cl2NO2
Dicloxacillin	C19H17Cl2N3O5S
Didesmethyl Citalopram	C18H17FN2O
Didesmethyl Imipramine	C17H20N2
Dienestrol	C18H18O2
Diethylstilbestrol	C18H20O2
Digoxin	C41H64O14
Dihydro Fenofibrate	C20H23ClO4
Dihydro Ketoprofen	C16H16O3
Dihydro Androsterone	C19H32O2
Dihydro Streptomycin	C21H41N7O12
Dihydroxy Carbazepine	C15H14N2O3
Diltiazem	C22H26N2O4S
Diltiazem N-Oxide	C22H26N2O5S
Dimethylamino Phenazone	C13H17N3O
Dimethylaniline	C8H11N
Dimetisterone	C23H32O2
Dimetridazole	C5H7N3O2
Dinitolmide	C8H7N3O5
Dioxacarb	C11H13NO4

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	Elemental composition
Diphenhydramine	C17H21NO
Diphenhydramine N-Oxide	C17H21NO2
Disopyramide	C21H29N3O
Dobutamine	C18H23NO3
Docetaxel	C43H53NO14
Donepezil	C24H29NO3
Doramectine	C50H74O14
Doxapram	C24H30N2O2
Doxazosine	C23H25N5O5
Doxepin	C19H21NO
Doxifluridine	C9H11FN2O5
Doxorubicin	C27H29NO11
Doxycycline	C22H24N2O8
Doxytetracycline	C22H26N2O9
Drostanolone	C20H32O2
Duloxetine	C18H19NOS
Emamectin B1a	C49H75NO13
Emamectin B1b	C48H73NO13
Emamectine	C49H75NO13
Embutramide	C17H27NO3
Enalapril	C20H28N2O5
Enalaprilat	C18H24N2O5
Enoxacin	C15H17FN4O3
Enoxaparin	C26H40N2O36S5
Enrofloxacin	C19H22FN3O3
Eosine	C20H8Br4O5
Epicillin	C16H21N3O4S
Epinandrolone	C18H26O2
Epirubicin	C27H29NO11
Epitrenbolone	C18H22O2
Eprinomectin	C50H75NO14
Eprosartan	C23H24N2O4S
Equilenin	C18H18O2
Ergoline	C14H16N2
Erythromycin A	C37H67NO13
Erythromycin B	C37H67NO12

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	**Elemental composition**
Erythromycin C	C36H65NO13
Erythrosine	C20H8I4O5
Escitalopram	C20H21FN2O
Esomeprazole	C17H19N3O3S
Espectinomicin	C14H24N2O7
Estradiol	C18H24O2
Estriol	C18H24O3
Estrone	C18H22O2
Etilefrin	C10H15NO2
Etofibrate	C18H18ClNO5
Etoposide	C29H32O13
Ezetimibe	C24H21F2NO3
Famotidine	C8H15N7O2S3
Febantel	C20H22N4O6S
Fenacetine	C10H13NO2
Fenbendazole	C15H13N3O2S
Fenirofibrate	C17H17ClO4
Fenobarbital	C12H12N2O3
Fenofibrate	C20H21ClO4
Fenofibric acid	C17H15ClO4
Fenoprofen	C15H14O3
Fenoterol	C17H21NO4
Fenpropathrin	C22H23NO3
Fentanyl	C22H28N2O
Fenvalerate	C25H22ClNO3
Fleroxacin	C17H18F3N3O3
Florfenicol	C12H14Cl2FNO4S
Florfenicol amine	C10H14FNO3S
Flubendazol	C16H12FN3O3
Fluconazole	C13H12N6OF2
Flucythrinate	C26H23F2NO4
Flufenamic acid	C14H10F3NO2
Flugestone	C21H29FO4
Flumequine	C14H12NO3F
Flumethasone	C22H28F2O5
Flunixin	C14H11F3N2O2

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	**Elemental composition**
Fluometuron	C10H11F3N2O
Fluorometholone	C21H29FO5
Fluorouracil	C4H3FN2O2
Fluoxetine	C17H18F3NO
Fluoxymesterone	C20H29FO3
Flurazepam	C21H23ClFN3O
Fluvalinate	C26H22ClF3N2O3
Folic acid	C19H19N7O6
Formoterol	C19H24N2O4
Formyl Ciprofloxacin	C18H18FN3O4
Framycetin	C23H46N6O13
Furaltadone	C13H16N4O6
Furazolidone	C8H7N3O5
Furosemide	C12H11ClN2O5S
Gabapentin	C9H17NO2
Gatifloxacin	C19H22FN3O4
Gemcitabine	C9H11F2N3O4
Gemfibrozil	C15H22O3
Gemifloxacin	C18H20FN5O4
Gentamicin C1	C21H43N5O7
Gentamicin C2	C20H41N5O7
Gentisic acid	C7H6O4
Glibenclamida	C23H28N3ClO5S
Grepafloxacin	C19H22FN3O3
Griseofulvin	C17H17O6
Halofuginone	C16H17BrClN3O3
Haloperidol	C21H23ClFNO2
Haloperidol n-oxide	C21H23ClFNO3
Hexestrol	C18H22O2
Hydralazine	C8H8N4
Hydrochlorothiazide	C7H8ClN3O4S2
Hydrocodone	C18H21NO3
Hydrocortisone	C21H30O5
Hydroxy Atorvastatin	C33H35FN2O6
Hydroxy Clenhepterol	C15H24N2O2Cl2
Hydroxy Dihydro Nifedipine Lactone	C16H12N2O6

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	**Elemental composition**
Hydroxy Diazepam	C16H13ClN2O2
Hydroxy Dimetridazole	C5H7N3O3
Hydroxy Ibuprofen	C13H18O3
Hydroxy Ipronidazole	C7H11N3O3
Hydroxy Mebendazole	C16H15N3O3
Hydroxy Methylclenbuterol	C12H18Cl2N2O2
Hydroxy Metronidazole	C6H9N3O4
Hydroxy Metronidazole	C6H9N3O4
Hydroxy Norpseudoephedrine	C9H13NO2
Hydroxy Pentobarbital	C11H18N2O4
Hydroxy Phenobarbital	C12H12N2O4
Hydroxy Phenylbutazone	C19H20N2O3
Hydroxy Warfarin	C19H16O5
Ibandronic acid	C9H23NO7P2
Ibuprofen	C13H18O2
Ibur	C14H18N4O4
Ifosfamide	C7H15Cl2N2O2P
Imidazol	C3H4N2
Imidocarb	C19H20N6O
Imipenem	C12N3O4S
Imipramine N-Oxide	C19H24N2O
Indomethacin	C19H16ClNO4
Iopamidol	C17H22I3N3O8
Iopromide	C18H24I3N3O8
Ipronidazole	C7H11N3O2
Irbesartan	C25H28N6O
Irinotecan	C33H38N4O6
Isoniazid	C6H7N3O
Isoprenaline	C11H17NO3
Isoprocarb	C11H15NO2
Isoproterenol	C11H17NO3
Isoxsuprine	C18H23NO3
Ivermectin	C48H74O14
Josamycin	C42H69NO15
Kanamycin	C18H36N4O11
Ketoprofen	C16H14O3

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	Elemental composition
Ketotriclabendazole	C14H9Cl3N2OS
Kitasamycin	C35H59NO13
Labetalol	C19H24N2O3
Lacosamide	C13H18N2O3
Lamotrigine	C9H7Cl2N5
Lansoprazole	C16H14F3N3O2S
Lasalocid	C34H54O8
Latanoprost	C26H40O5
Leukomycin	C11H12Cl2N2O5
Levamisole	C11H12N2S
Levetiracetam	C8H14N2O2
Levofloxacin	C18H20FN3O4
Levomepromazine/Methotrimeprazine	C19H24N2OS
Levothyroxine	C15H11NI4O4
Lidocaine	C14H22N2O
Lincomycin	C18H34N2O6S
Linestrenol	C20H28O
Lisuride	C20H26N4O
Lomefloxacin	C17H19F2N3O3
Loratadine	C22H23N2O2Cl
Lorazepam	C15H10Cl2N2O2
Losartan	C22H23N6ClO
Losartan Carboxaldehyde	C22H21ClN6O
Losartan Carboxylic Acid	C22H21ClN6O2
Mabuterol	C13H18ClF3N2O
Mahexerol	C15H22N2OF3Cl
Mapenterol	C14H20ClF3N2O
Marbofloxacin	C17H19N4O4F
Mebendazole	C16H13N3O3
Meclofenamic acid	C14H11Cl2NO2
Medazepam	C16H15ClN2
Mefenamic acid	C15H15NO2
Meloxicam	C14H13N3O4S2
Memantine	C12H21N
Meprobamate	C9H18N2O4
Meropenem	C17H25N3O5S

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	**Elemental composition**
Mestanolone	C20H32O2
Mestilbol	C19H22O2
Mestranol	C21H26O2
Metaproterenol	C11H17NO3
Metenolone	C20H30O2
Metformin	C4H11N5
Methacycline	C22H22N2O8
Methandienone	C20H28O2
Methandriol	C20H32O2
Methandrostenol	C20H28O2
Methapyrilene	C14H19N3S
Methiciclin	C17H20N2O6S
Methimazole	C4H6N2S
Metoclopramide	C14H22N3ClO2
Metoprolol	C15H25NO3
Metoprolol acid	C14H21NO4
Metronidazole	C6H9N3O3
Mevastatin	C23H34O5
Midazolam	C18H13N3ClF
Midecamycin	C41H67NO15
Minocycline	C23H27N3O7
Mirtazapine	C17H19N3
Misoprostol	C22H38O5
Mitomycine	C15H18N4O5
Mizolastine	C24H25FN6O
Monensin	C36H62O11
Monoacetyldapsone	C14H14N2O3S
Mono-oxidation atazanavir	C38H52N6O8
Montelukast	C35H36O3SNCl
Moxidectin	C37H53NO8
Moxifloxacin	C21H24N3O4F
Moxifloxacin N-Sulfate	C21H24FN32O7S
N-(2,6-Dichlorophenyl) Anthranilic Acid	C13H9Cl2NO2
N-(2-Hydroxyethyl) Lorazepam	C17H14Cl2N2O3
N,N,O-Tridesmethyl Diltiazem	C19H20N2O4S
N,N-Didesmethyl N-tert-Butoxycarbonyl	C25H30N2O6S

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	Elemental composition
N,O-Didesmethyl Verapamil	C25H34N2O4
N-1-(Hydroxyethyl) Flurazepam	C17H14ClFN2O2
N-Acetyl Benzo Quinoneimine	C8H7NO2
N-Acetyl Sulfadiazine	C12H12N4O3S
N-Acetyl Sulfamethazine	C14H16N4O3S
N-Acetyl Sulfamethoxazole	C12H13N3O4S
N-Acetyl-4-Aminosalicylic acid	C9H9NO4
N-Acetyl-5-Aminosalicylic acid	C9H9NO4
Nadolol	C17H27NO4
Nafcilline	C21H22N2O5S
Nalidixic acid	C12H12N2O3
Nandrolone	C18H26O2
Naproxen	C14H14O3
Narasin	C43H72O11
N-Demethyl Erythromycin A	C36H65NO13
N-Demethyl Olanzapine	C16H18N4S
N-Demethyl Roxithromycin	C40H74N2O15
N-desalkyl Quetiapine sulfoxide	C17H17N3SO
N-Deschlorobenzoyl Indomethacin	C12H13NO3
N-Desmethyl Clarithromycin	C37H67NO13
N-Desmethyl-4'-hydroxy Tamoxifen	C25H27NO2
N-Desmethyl Clindamycin	C17H31ClN2O5S
N-Desmethyl Clindamycin sulfoxide	C17H31ClN2O6S
N-Desmethyl Clozapine	C17H17ClN4
N-Desmethyl Mirtazapine	C16H17N3
N-Desmethyl Tramadol	C15H23NO2
N-Despropyl Propafenone	C18H21NO3
Nemadectin	C36H52O8
Neomycin	C23H46N6O13
Neomycin A	C12H26N4O6
Neomycin C	C23H46N6O13
N-Hydroxy Norfloxacin	C16H18FN3O4
N-Hydroxy Sulfamethoxazole	C10H11N3O4S
N-Hydroxy Pentobarbital	C11H18N2O4
Nicarbazin	C19H18N6O6
Nicotine	C10H14N2

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	**Elemental composition**
Nifedipine	C17H18N2O6
Niflumic acid	C13H9F3N2O2
Nifursol	C12H7N5O9
Nigericin	C40H68O11
Nimesulide	C13H12N2O5S
Nimorazol	C9H14N4O3
Nitrazepam	C15H11N3O3
Nitrofurantoin	C8H6N4O5
Nitrofurazone	C6H6N4O4
Nitroglicerine	C3H5N3O9
Nitromide	C7H5N3O5
N-Methyl Amisulpride	C18H29N3O4S
N-Methyl Mapenterol	C15H22N2OF3Cl
Norandrostenediona	C18H24O2
Norcitalopram	C19H19FN2O
Norclobazam	C15H11ClN2O2
Nordiazepam	C15H11ClN2O
Norethandrolone	C20H30O2
Norethindrone	C20H26O2
Norfloxacin	C16H18FN3O3
Norfloxacin N-oxide	C16H17FN3O4
Norfluoxetine	C16H16F3NO
Norflurazepam	C15H10ClFN2O
Norgestrel/Levonorgestrel	C21H28O2
Norlidocaine	C12H18N2O
Norpseudoephedrine	C9H13NO
Norquetiapine	C17H17N3S
Norsertraline	C16H15Cl2N
Norverapamil	C26H36N2O4
Norvinisterone	C20H28O2
Noscapine	C22H23NO7
Novobiocin	C31H36N2O11
O-Dealkyl Cetirizine	C19H23ClN2O
O-Demethyl Metoprolol	C14H23NO3
O-Desacetyl-N-desmethyl Diltiazem	C19H22N2O3S
O-Desalkyl Quetiapine	C19H21N3OS

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	Elemental composition
O-Desmethyl Indomethacin	C18H14ClNO4
O-Desmethyl Naproxen	C13H12O3
O-Desmethyl-N-deschlorobenzoyl Indomethacin	C11H11NO3
O-Desmethyl Nortramadol	C14H21NO2
O-Desmethyl Tramadol	C15H23NO2
O-Desmethyl Verapamil	C26H36N2O4
Ofloxacin	C18H20FN3O4
Ofloxacin N-Oxide	C18H20FN305
Olanzapine	C17H20N4S
Olanzapine 2-Carboxaldehyde	C17H18N4OS
Olaquindox	C12H13N3O4
Oleandomycin	C35H61NO12
Omeprazole	C17H19N3O3S
Omeprazole Acid	C17H17N3O5S
Omeprazole Sulfide	C17H19N3O2S
Omeprazole Sulfone	C17H19N3O4S
Omeprazole Sulfone N-Oxide	C17H19N3O5S
Orciprenaline	C11H17NO3
Ornidazole	C7H10ClN3O3
Ortho-Hydroxy Hippuric	C9H9NO4
Oseltamivir	C16H28N2O4
Oseltamivir Carboxylate	C14H24N2O4
Oxacillin	C19H19N3O5S
Oxamyl	C7H13N3O3S
Oxandrolone	C19H30O3
Oxazepam	C15H11N2O2Cl
Oxcarbazepine	C15H12N2O2
Oxfendazole	C15H13N3O3S
Oxfendazole sulfone	C15H13N3O4S
Oxibendazole	C12H15N3O3
Oxo Ciprofloxacin	C17H16FN3O4
Oxo Clopidogrel	C16H16ClNO3S
Oxolinic acid	C13H11NO5
Oxprenolol	C15H23NO3
Oxyclozanide	C13H6Cl5NO3
Oxymetholone	C21H32O3

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	**Elemental composition**
Oxyphenbutazone	C19H20N2O3
Oxytetracycline	C22H24N2O9
Paclitaxel	C47H51NO14
Pancuronium	C35H60N2O4
Pantoprazol	C16H15F2N3O4S
Pantoprazole Sulfide	C16H15F2N3O3S
Pantoprazole Sulfone	C16H15F2N3O5S
Paromomycin	C23H45N5O14
Paroxetine	C19H20FNO3
Pavabid	C20H22ClNO4
Pefloxacin	C17H20FN3O3
Penconazole	C13H15Cl2N3
Penethamate	C22H31N3O4S
Penethicillin	C17H20N2O5S
Penicillin	C9H12N2O4S
Penicillin G	C16H18N2O4S
Penicillin M	C14H21N3O6S
Penicillin V	C16H18N2O5S
Pentagestrone	C26H38O3
Pentoxifylline	C13H18N4O3
Permethrin	C21H20Cl2O3
Phenacetin	C10H13NO2
Phenothrin	C23H26O3
Phenoxymethyl Penicillin	C16H18N2O5S
Phenylbutazone	C19H20N2O2
Phenylthiouracil	C10H8N2OS
Phenytoin	C15H12N2O2
Phloxine B	C20H4Br4Cl4O5
Phosphestrol	C18H22O8P2
Phtalylsulphatiazol	C17H13N3O5S2
Pilocarpine	C11H16N2O2
Pindolol	C14H20N2O2
Pipedimic acid	C14H17N5O3
Piperacillin	C23H27N5O7S
Pirantel	C11H14N2S
Pirbuterol	C12H20N2O3

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	Elemental composition
Piridoxin	C8H11NO3HC1
Pirlimycin	C17H31ClN2O5S
Piromidic acid	C14H16N4O3
Piroxicam	C15H13N3O4
Pitosin	C43H66N12O12S2
Pravastatin	C23H36O7
Prazepam	C19H17ClN2O
Prednisolone	C21H28O5
Prednisone	C21H26O5
Pregabalin	C8H17NO2
Prilocaine	C13H20N2O
Primidone	C12H14N2O2
Procaine	C13H20N2O2
Procaterol	C16H22N2O3
Progesterone	C21H30O2
Promazine	C17H20N2S
Promecarb	C12H17NO2
Promethazine	C17H20N2S
Propafenona	C21H27NO3
Propenidazol	C11H13N3O5
Propicilline	C18H22N2O5S
Propiomazine	C20H24N2OS
Propranolol	C16H21NO2
Propylthiouracil	C7H10N2OS
Propyphenazone	C14H18N2O
Prothipendyl	C16H19N3S
Quetiapine	C21H25N3O2S
Quetiapine sulfone	C21H25N3O4S
Quetiapine sulfoxide	C21H25N3O3S
Quinapril	C25H30N2O5
Quinbolone	C24H32O2
Quinestrol	C25H32O2
Ractopamine	C18H23NO3
Raloxifene	C28H27NO4S
Ramifenazone	C14H19N3O
Ramipril	C23H32N2O5

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	**Elemental composition**
Ranitidine	C13H22N4O3S
Ranitidine N-Oxide	C13H22N4O4S
Ranitidine S-Oxide	C13H22N4O4S
Reproterol	C18H23N5O5
Ribavirin	C8H12N4O5
Rifampicin	C43H58N4O12
Rifamycin	C37H47NO12
Rifaximine	C43H51N3O11
Rimantadine	C12H21N
Risedronic acid	C7H11NO7P2
Risperidone	C23H27FN4O2
Ritodrine	C17H21NO3
Rivastigmine	C14H22N2O2
Robenidine	C15H13Cl2N5
Rolitetracycline	C27H33N3O8
Ronidazole	C6H8N4O4
Rosoxacin	C17H14N2O3
Roxythromycin	C41H76N2O15
Rufloxacin	C17H18FN3O3S
Salbutamol	C13H21NO3
Salicilic acid	C7H6O3
Salinomycin	C42H70O11
Salmeterol	C25H37NO4
Sarafloxacin	C20H17F2N3O3
S-Desethyl S-Methyl Amisulpride	C16H25N3O4S
Secnidazol	C7H11N3O3
Selamectin	C43H63NO11
SEM	C11H15NO2
Semduramycin	C45H75O16
Sertraline	C17H17NCl2
Sertraline Carbamic acid	C18H17Cl2NO2
Sildenafil	C22H30N6O4S
Simvastatin	C25H38O5
Simvastatin Hydroxy Acid	C25H40O6
Sitagliptin	C16H15F6N5O
S-Methyl-3-thioacetaminophen	C9H11NO2S

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	Elemental composition
Sotalol	C12H20N2O3S
Sparfloxacin	C19H22F2N4O3
Spiramycin I	C43H74N2O14
Spiramycin II	C45H76N2O15
Spiramycin III	C46H78N2O15
Stanolone	C19H30O2
Stanozolol	C21H32N2O
Stilbestrol	C18H20O2
Streptomycin	C21H39N7O12
Succinilsulfatiazol	C13H15N3O6S2
Sulfabenzamide	C13H12N2O3S
Sulfacetamide	C8H10N2O3S
Sulfachlorpyrazine	C10H9ClN4O2S
Sulfaclozine	C10H9ClN4O2S
Sulfadiazine	C10H10N4O2S
Sulfadimethoxine/sulfadimetacine	C12H14N4O4S
Sulfadimidine/sulfametazine	C12H14N4O2S
Sulfadoxine	C12H14N4O4S
Sulfaethoxypyridazine	C12H14N4O3S
Sulfafenazol	C15H14N4O2S
Sulfaguanidine	C7H10N4O2S
Sulfalen	C11H12N4O3S
Sulfamerazine	C11H12N4O2S
Sulfameter/sulfamethoxydiazine	C11H12N4O3S
Sulfamethazine	C12H14N4O2S
Sulfamethiazol	C9H10N4O2S2
Sulfamethoxazol	C10H11N3O3S
Sulfamethoxypyridazine	C11H12N4O3S
Sulfamonomethoxine	C11H12N4O3S
Sulfamoxole	C11H13N3O3S
Sulfanilamide	C6H8N2O2S
Sulfanitran	C14H13N3O5S
Sulfaperine	C11H12N4O2S
Sulfapirazol	C16H16N4O2S
Sulfapyridine	C11H11N3O2S
Sulfaquinoxaline	C14H12N4O2S

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	**Elemental composition**
Sulfasalazine	C18H14N4O5S
Sulfathiazole	C9H9N3O2S2
Sulfatolamide	C14H19N5O4S3
Sulfatroxazole	C11H13N3O3S
Sulfisomidine	C12H14N4O2S
Sulfisoxazole	C11H13N3O3S
Sulfo Ciprofloxacin	C17H18FN3O6S
Sulpiride	C15H23N3O4S
Suxibuzone	C24H26N2O6
Tadalafil	C22H19N3O4
Talidomida	C13H10N2O4
Tamoxifen	C26H29NO
Tamoxifen N-Oxide	C26H29NO2
Tamsulosin	C20H28N2O5S
Tapazole	C4H6N2S
Tartrazine	C16H12N4O9S2
Tau-fluvalinate	C26H22ClF3N2O3
Tefluthrin	C17H14ClF7O2
Tegafur	C8H9FN2O3
Telmisartan	C33H30N4O2
Telobuterol	C12H18ClNO
Temafloxacin	C21H18F3N3O3
Terbutaline	C12H19NO3
Testosterone	C19H28O2
Tetracycline	C22H24N2O8
Tetrazepam	C16H17ClN2O
Theophylline	C7H8N4O2
Thiamazole	C4H6N2S
Thiamphenicol	C12H15NO5SCl2
Thioridazine 2-Sulfone	C21H26N2O2S2
Thioridazine 5-Sulfoxide	C21H26N2OS2
Thiouracil	C4H4N2OS
Thiurea	CH4N2S
Tiagabine	C20H25NO2S2
Tiamulin	C28H47NO4S
Tilmicosin	C46H80N2O13

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	Elemental composition
Timolol	C13H24N4O3S
Tinidazole	C8H13N3O4S
Tobramycin	C18H37N5O9
Tolbutamida	C12H18N2O3S
Tolfenamic acid	C14H12ClNO2
Toltrazuril	C18H14F3N3O4S
Toltrazurilsulfon	C18H14F3N3O6S
Topiramate	C12H21NO8S
Torasemide	C16H20N4O3S
Tramadol	C16H25NO2
Trazodone	C19H22N5ClO
Trenbolone	C18H22O2
Triamcinolone	C21H27FO6
Triamcinolone acetonide	C24H31FO6
Triazolam	C17H12Cl2N4
Triclabendazole	C14H9Cl3N2OS
Triclabendazole sulfoxide	C14H9Cl3N2O2S
Triflupromazine/Fluopromazine	C18H19F3N2S
Trifuraline	C13H16F3N3O4
Trimethoprim	C14H18N4O3
Trimipramine	C20H26N2
Trimipramine N-Oxide	C20H26N2O
Trovafloxacin	C20H15F3N4O3
Tryptamin	C10H12N2
Tulobuterol	C12H18ClNO
Tylosin A	C46H77NO17
Tylosin B	C39H65NO14
Tylosin C	C45H75NO17
Tylosin D	C46H79NO17
Uranine	C20H12O5
Valnemulin	C31H52N2O5S
Valsartan	C24H29N5O3
Vedaprofen	C19H22O2
Venlafaxine	C17H27NO2
Verapamil	C27H38N2O4
Vildagliptin	C17H25N3O2

Table S5 (cont.). Compounds (name and elemental composition) included in the database. Metabolites are indicated in italics.

Compound	**Elemental composition**
Vinorelbina	C45H54N4O8
Virginiamycin-M1	C28H35N3O7
Virginiamycin-S1	C43H49N7O10
Warfarin	C19H16O4
Xylazine	C12H16N2S
Zafirlukast	C31H33N3O6S
Zanamivir	C12H19N4O7
Zilpaterol	C14H19N3O2
Zopiclone	C17H17ClN6O3
α,4-Diydroxy Midazolam	C18H13ClFN3O2
α-Hydroxy Metoprolol	C15H25NO4
α-Hydroxy Midazolam	C18H13ClFN3O
α-Zearalanol	C18H26O5
α-Zearalanone	C18H24O5
β-Boldenone	C19H26O6
β-Trenbolone	C18H22O2
β-Zearalanol	C18H26O5

Table S6. Pharmaceuticals detected in this study, together with their therapeutic class, their species sensitivity distributions (SSD) parameters and the quality score associated to them.

Pharmaceutical	Therapeutic class	μ	σ	Quality score
Acetaminophen	Analgesic/anti-inflammatory	2.11	1.11	1211
Alprazolam	GABA-mediated synaptic inhibition	2.23	0.70	0
Atorvastatin	Hypolipidemic	1.61	0.70	1311*
Azitromycin	Bactericide	1.40	1.29	1124
Bezafibrate	Hypolipidemic	3.64	1.04	1124
Carbamazepine	Sodium channel blocker	2.38	1.25	1111
Ciprofloxacin	Bactericide	2.86	1.29	1124
Clarithromycin	Bactericide	2.45	1.29	1124
Clindamycin	Bactericide	3.59	0.77	0
Diclofenac	Analgesic/anti-inflammatory	1.93	1.50	1111
Erythromycin	Bactericide	2.15	0.70	1111*
Gabapentin	Sodium channel blocker	3.57	1.11	0
Irbesartan	Antihipertensive	3.80	0.52	1323
Levamisol	Anthelmintic	3.06	0.61	0
Lincomycin	Bactericide	2.69	1.36	1224
Lorazepam	GABA-mediated synaptic inhibition	2.21	0.63	0
Losartan	Antihypertensive	5.20	0.45	1325
Metoprolol	Beta-blocker	3.83	1.04	1111
Metronidazole	Bactericide	4.99	0.70	1123
Nalidixic acid	Bactericide	2.76	0.70	0
Naproxen	Analgesic/anti-inflammatory	3.33	1.12	1224
Norfloxacin	Bactericide	1.49	0.79	1211
Omeprazole sulfide, 4-hydroxy	Gastro-protector	2.82	0.68	0
Oxolinic acid	Bactericide	4.20	1.29	1323
Pantoprazole	Gastro-protector	1.34	0.70	0
Phenazone	Analgesic/anti-inflammatory	-0.60	0.70	0
Primidone	Antiepileptic	3.19	1.15	0
Salbutamol	Beta-blocker	2.40	0.96	0
Sulfadiazine	Bactericide	3.28	1.29	1124
Sulfamethoxazole	Bactericide	2.36	1.29	1123
Tetracycline	Bactericide	2.42	1.29	1111
Tramadol	Analgesic/anti-inflammatory	2.62	0.65	0
Trimetroprim	Bactericide	4.11	1.29	1111
Valsartan	Antihypertensive	4.15	0.55	1324
Venlafaxine	Antidepressant	2.32	0.79	1325

* The μ was considered too large or too low, and was replaced by 0.7.

Table S7. Description of the species sensitivity distributions (SSD) parameter quality scores (adapted from Posthuma et al. 2019).

Digit	Quality aspect	Modality	Meaning
1	Data availability	0	No sufficient data to calculate an SSD, the μ was estimated from the acute SSD μ-1, and a σ of 0.7.
		1	Sufficient data to calculate an SSD (μ and σ were calculated)
2	Biodiversity coverage	1	Number of taxa > 10.
		2	Number of taxa > 5.
		3	Number of taxa > 2.
3	Data origin	1	Experimental chronic NOECs available.
		2	Chronic NOECs were extrapolated.
4	Extrapolation type	1	Not extrapolated.
		2	Chronic EC50 divided by 3.
		3	Acute NOEC divided by 3.
		4	Acute EC50 divided by 10.
		5	All available toxicity data extrapolated following the above rules.

Table S8. Concentrations (ng/L) of pharmaceuticals in water samples from the **first campaign**.

Compounds	Samples																		
	1a	**2a**	**3a**	**4a**	**5a**	**6a**	**7a**	**8a**	**9a**	**10a**	**11a**	**12a**	**13a**	**14a**	**15a**	**16a**	**17a**	**18a**	**19a**
Acetaminophen	-	-	-	-	-	48	30	44	-	11	-	-	-	12	-	-	15	d	-
Alprazolam	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	7	12	-
Atorvastatin	-	-	-	-	-	-	-	-	-	13	-	-	-	-	-	-	-	9	-
Azitromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bezafibrate	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	-	d	-
Carbamazepine	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	16	23	18
Ciprofloxacin	d	d	d	d	d	d	d	d	d	616	d	d	d	d	d	d	1105	587	d
Clarithromycin	-	-	-	-	-	-	-	-	-	241	-	-	-	-	-	-	175	262	-
Clindamycin	-	-	-	-	-	-	-	-	-	171	-	-	-	-	-	-	58	54	-
Diclofenac	-	-	-	-	-	-	-	-	9	571	-	-	-	-	-	-	147	870	6
Enalapril	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Erythromycin	-	-	-	-	-	-	-	-	-	76	-	-	-	-	-	-	24	79	-
Furaltadone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gabapentin	-	-	-	-	-	-	-	-	d	1128	-	-	-	-	-	-	869	d	-
Gemfibrozil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Irbesartan	-	-	-	-	-	-	-	-	15	637	-	-	-	-	-	-	-	1076	10
Ketoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Levamisol	-	-	-	-	-	-	-	-	-	87	-	-	-	-	-	-	32	40	-
Lincomycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	-
Lorazepam	-	-	-	-	-	-	-	-	-	94	-	-	-	-	-	-	39	79	-
Losartan	-	-	-	-	-	-	-	-	11	638	-	-	-	-	-	-	65	268	-
Metoprolol	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	29	57	-
Metronidazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	106	-
Nalidixic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Naproxen	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	-	d	-
Norfloxacin	60	-	d	-	-	-	d	d	d	494	d	-	d	d	d	d	936	231	d
Omeprazole sulfide, 4-hydroxy	-	-	-	-	-	-	-	-	d	116	-	-	-	-	-	-	-	149	d
Oxolinic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pantoprazole	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	-	8	-
Phenazone	-	-	-	-	-	-	-	-	-	1273	-	-	-	-	-	-	700	1984	9
Primidone	-	-	-	-	-	-	-	-	20	1013	-	-	-	-	-	-	246	264	46
Roxithromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salbutamol	-	-	-	-	-	-	-	-	-	13	-	-	-	-	-	-	6	23	-
Sulfadiazine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sulfamethoxazole	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	24	39	-
Tetracycline	11	7	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tramadol	-	-	-	-	-	-	-	-	-	644	-	-	-	-	-	-	-	1224	-
Trimetroprim	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	242	-
Valsartan	d	-	d	-	-	-	-	-	20	1623	d	-	-	-	-	-	412	976	10
Venlafaxine	-	-	-	-	-	-	-	-	d	139	-	-	-	-	-	-	335	805	-

d, detected: concentration below the limit of quantification, Underlined: guidance value

Table S9. Concentrations (ng/L) of pharmaceuticals in water samples from the **second campaign**.

Compounds	Samples																		
	1b	**2b**	**3b**	**4b**	**5b**	**6b**	**7b**	**8b**	**9b**	**10b**	**11b**	**12b**	**13b**	**14b**	**15b**	**16b**	**17b**	**18b**	**19b**
Acetaminophen	-	24	9	6	10	95	90	27	8	21	28	-	18	10	-	-	197	22	-
Alprazolam	-	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	9	6	d
Atorvastatin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	19	-
Azitromycin	-	-	-	-	-	-	-	-	-	737	-	-	-	-	-	-	166	325	d
Bezafibrate	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	d	d	-
Carbamazepine	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	9	13	13
Ciprofloxacin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clarithromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	27	116	-
Clindamycin	-	-	-	-	-	-	-	-	-	30	-	-	-	-	-	-	38	53	-
Diclofenac	d	-	-	-	-	-	d	-	d	263	d	d	-	d	8	-	424	578	8
Enalapril	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Erythromycin	-	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	43	47	d
Furaltadone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gabapentin	d	-	-	-	-	-	-	d	-	403	-	-	-	-	-	-	1924	1818	114
Gemfibrozil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Irbesartan	-	-	-	-	-	-	-	-	d	839	-	-	-	-	-	-	870	1147	29
Ketoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Levamisol	-	-	-	-	-	-	-	-	-	108	-	-	-	-	-	-	47	32	-
Lincomycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	d	6
Lorazepam	-	-	-	-	-	-	-	-	-	80	-	-	-	-	-	-	55	55	-
Losartan	-	-	-	-	-	-	-	-	-	80	-	-	-	-	-	-	119	169	d
Metoprolol	-	-	-	-	-	-	-	-	-	16	-	-	-	-	-	-	20	44	-
Metronidazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	45	-
Nalidixic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Naproxen	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	d	d	-
Norfloxacin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Omeprazole sulfide, 4-hydroxy	-	-	-	-	-	-	-	-	-	75	-	-	-	-	-	-	40	111	d
Oxolinic acid	-	-	d	d	-	-	-	-	-	-	d	d	-	-	-	-	-	-	-
Pantoprazole	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	d	d	-
Phenazone	-	-	-	-	-	-	-	-	-	140	-	-	-	-	-	-	576	701	43
Primidone	-	-	-	-	-	-	-	-	d	408	-	-	-	-	-	-	228	212	118
Roxithromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salbutamol	-	-	-	-	-	-	d	-	-	d	-	-	-	-	-	-	11	11	-
Sulfadiazine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	20
Sulfamethoxazole	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	163	174	24
Tetracycline	-	-	-	-	-	d	-	-	d	-	-	-	-	-	-	-	-	-	-
Tramadol	-	-	-	-	-	-	-	-	-	772	-	-	-	-	-	-	933	1119	85
Trimetroprim	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15	57	-
Valsartan	-	-	-	-	-	-	-	-	-	148	-	-	-	-	-	-	323	523	12
Venlafaxine	-	-	-	-	-	-	-	-	d	200	-	-	-	-	-	-	344	468	8

d. detected, concentration below the limit of quantification

Table S10. Concentrations (ng/L) of pharmaceuticals in water samples from the **third campaign**.

Compounds	Samples																		
	1c	**2c**	**3c**	**4c**	**5c**	**6c**	**7c**	**8c**	**9c**	**10c**	**11c**	**12c**	**13c**	**14c**	**15c**	**16c**	**17c**	**18c**	**19c**
Acetaminophen	27	7	30	11	17	5	10	7	-	d	d	22	-	d	d	13	11	-	5
Alprazolam	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	20	20	d
Atorvastatin	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	23	212	-
Azitromycin	-	-	-	-	-	-	-	-	-	1218	d	-	-	-	-	-	887	1617	d
Bezafibrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carbamazepine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	19	26	11
Ciprofloxacin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clarithromycin	-	-	-	-	-	-	-	-	-	325	-	-	-	-	-	-	187	262	-
Clindamycin	-	-	-	-	-	-	-	-	-	59	-	-	-	-	-	-	90	132	-
Diclofenac	-	-	-	-	-	-	-	-	-	324	-	-	-	-	-	-	245	939	-
Enalapril	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Erythromycin	-	-	-	-	-	-	-	-	-	18	-	-	-	-	-	-	84	122	-
Furaltadone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gabapentin	20	-	13	14	12	20	15	9	-	866	-	6	d	d	-	d	1144	983	-
Gemfibrozil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Irbesartan	-	-	-	-	-	-	-	-	-	66	-	-	-	-	-	-	1414	1684	9
Ketoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Levamisol	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	21	36	-
Lincomycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11
Lorazepam	-	-	-	-	-	-	-	-	-	21	-	-	-	-	-	-	57	65	-
Losartan	-	-	-	-	-	-	-	-	-	142	-	-	-	-	-	-	461	676	-
Metoprolol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24	32	-
Metronidazole	-	-	-	-	-	-	-	-	-	25	-	-	-	-	-	-	18	82	-
Nalidixic acid	-	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	-	-
Naproxen	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	d	d	-
Norfloxacin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Omeprazole sulfide, 4-hydroxy	-	-	-	-	-	-	-	-	-	30	-	-	-	-	-	-	17	150	-
Oxolinic acid	d	d	-	d	d	-	d	-	d	d	-	-	-	-	-	-	-	-	-
Pantoprazole	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-	-	d	13	-
Phenazone	-	-	-	-	-	-	-	-	-	22	-	-	-	-	-	-	880	1134	29
Primidone	d	-	-	-	-	-	-	-	-	459	-	-	-	-	-	-	306	303	90
Roxithromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salbutamol	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	16	19	-
Sulfadiazine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15
Sulfamethoxazole	-	-	-	-	-	-	-	-	-	101	-	-	-	-	-	-	126	199	22
Tetracycline	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tramadol	-	-	-	-	-	-	-	-	-	377	-	-	-	-	-	-	1668	1949	73
Trimetroprim	-	-	-	-	-	-	-	-	-	152	-	-	-	-	-	-	439	723	d
Valsartan	-	-	d	-	d	d	d	-	-	222	d	d	d	-	-	-	694	1397	-
Venlafaxine	d	d	-	-	d	d	-	d	-	68	d	d	d	d	-	d	520	691	d

d. detected, concentration below the limit of quantification

Table S11. Results obtained in SW samples by UHPLC-HRMS screening. SW samples collected in September 2018.

Compounds	Samples																			Detection frequency (%)
	1b	2b	3b	4b	5b	6b	7b	8b	9b	10b	11b	12b	13b	14b	15b	16b	17b	18b	19b	
Acetaminophen	-	-	-	-	-	d	d	d	-	-	d	-	-	-	-	-	-	-	-	21
Aliskiren	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Amisulpride	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Antypirine (Phenazone)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Azythromycin	-	-	-	-	-	-	-	-	-	✓	-	-	-	-	-	-	✓	✓	-	16
Bisoprolol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	t	t	-	11
Carbamazepine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	✓	16
Cetirizine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Clarithromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	d	d	-	11
Codeine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Diazepam	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Diclofenac	-	-	-	-	-	-	-	-	-	✓	-	-	-	-	-	-	✓	✓	-	16
Dimetridazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Eprosartan	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Flufenamic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	t	t	-	11
Gabapentin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Iopromide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Irbesartan	-	-	-	-	-	-	-	-	-	✓	-	-	-	-	-	-	✓	✓	-	16
Ketoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Lamotriginine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Levofloxacin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Lidocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	d	16
Lorazepam	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Losartan	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Meclofenamic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Naproxen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	t	t	-	11
Niflumic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Nordiazepam	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	-	-	5
Ofloxacin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Oxazepam	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Oxcarbazepine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Rimantadine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Sitagliptin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Sulfamethoxazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	✓	16
Temazepam	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	t	t	-	11
Telmisartan	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Tolfenamic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	t	t	-	11
Tramadol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	t	t	-	11
Valsartan	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11
Venlafaxine	-	-	-	-	-	-	-	-	-	✓	-	-	-	-	-	-	✓	✓	✓	21
Vildagliptin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	✓	-	11

✓: confirmed with reference standard, ((de)protonated molecule and at least one fragment ion were observed at the expected retention time). t: tentative identification without reference standard, ((de)protonated molecule was observed and at least one ion fragment was justified). d: detected (only (de)protonated molecule was observed at the expected retention time). Pharmaceuticals included in the quantitative methodology by UHPLC-MS/MS are shown in bold

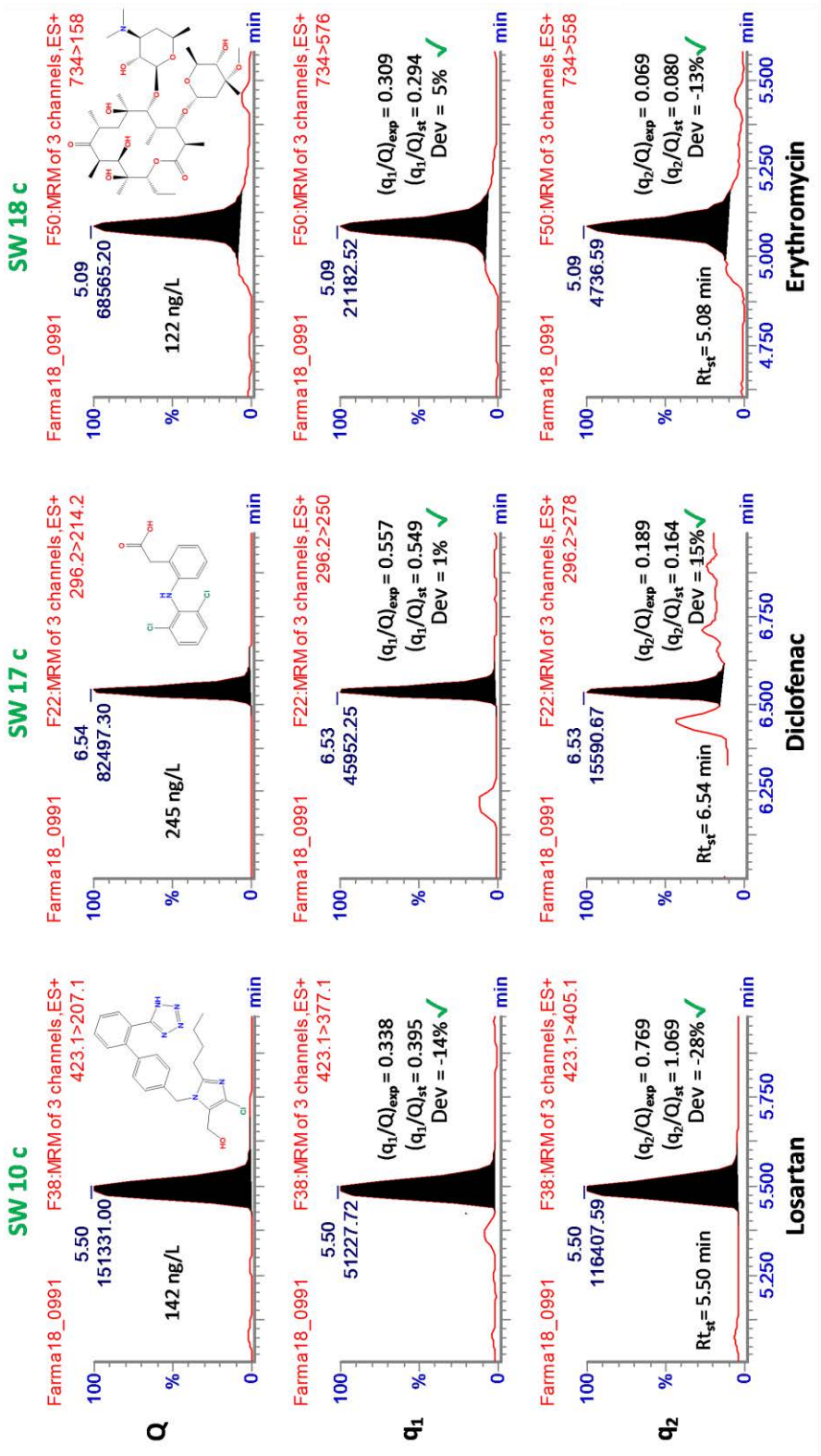

Figure S2. UHPLC-MS/MS chromatograms of positive findings in three surface water samples.

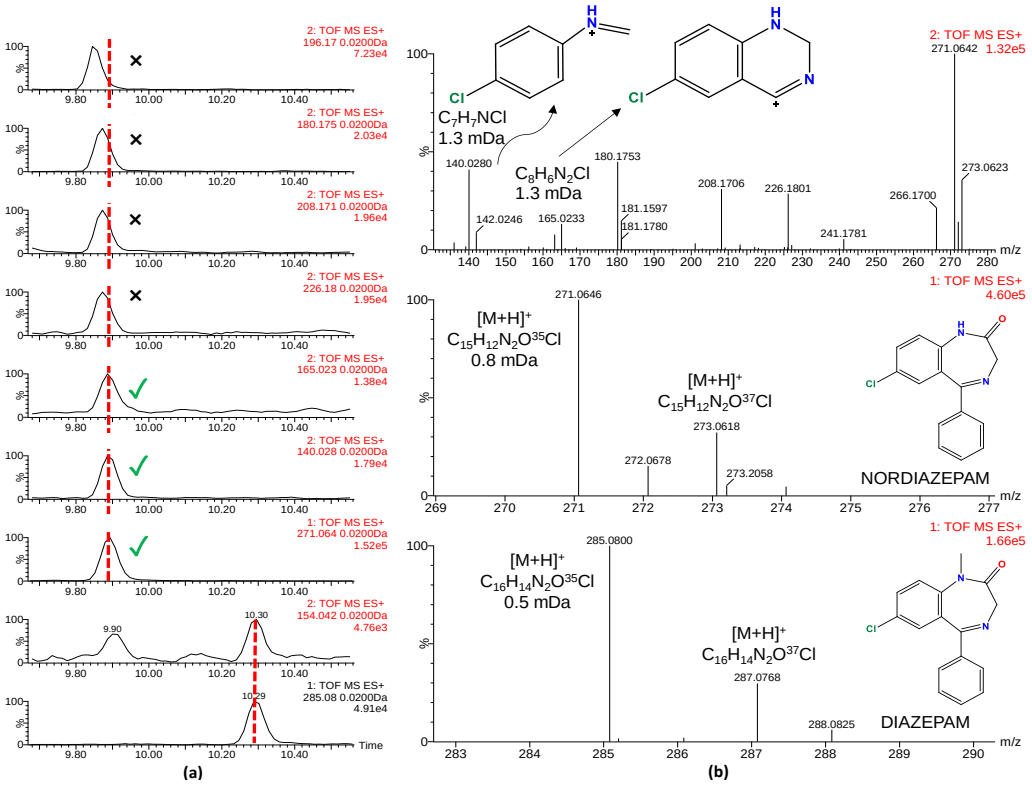

Figure S3. Detection and tentative identification of nordiazepam in a surface water sample that also contained diazepam. (a) nw-XICs at 0.02 Da mass window for $[M+H]^+$ in LE function and main fragments in LE/HE functions. (b) LE (top), LE (middle) TOF mass spectra for the chromatographic peak at 9.89 min (nordiazepam), and (b) LE (bottom) spectrum of diazepam (peak at 10.29 min).

The LE spectrum of the chromatographic peak at 9.89 min showed an abundant signal at *m/z* 271.0646 (**Figure S3**b, middle) and the characteristic isotope profile of a chlorine atom. It might correspond to the metabolite nordiazepam ($C_{15}H_{12}N_2OCl^+$), with a mass error of 0.8 mDa in relation to its theoretical exact mass. The HE spectrum (**Figure S3**b, top) showed two fragment ions at *m/z* 140.0280 ($C_7H_7NCl^+$, corresponding to the loss of C_6H_5CN) and 165.0233 ($C_8H_6N_2Cl^+$, due to the C_6H_6 loss), both with mass errors lower than 2 mDa. These data, and the justification of the fragments, strongly supported the tentative identification of the compound as nordiazepam. Interestingly, the extracted ion chromatogram (XIC) at *m/z* 285.0800 (**Figure S3**a, bottom), corresponding to the protonated molecule of the parent compound, also showed a peak at 10.29 min (**Figure S3**a), which retention time and LE (**Figure S3**b, bottom) and HE spectra (data not shown) perfectly fitted with the reference standard.

Table S12. Individual compound PAF calculated in the first sampling campaign (summer).

Compounds	Samples																		
	1a	**2a**	**3a**	**4a**	**5a**	**6a**	**7a**	**8a**	**9a**	**10a**	**11a**	**12a**	**13a**	**14a**	**15a**	**16a**	**17a**	**18a**	**19a**
Acetaminophen						0.10		<0.1		<0.1				<0.1			<0.1	<0.1	
Alprazolam										<0.1							<0.1	<0.1	
Atorvastatin										<0.1								<0.1	
Azitromycin																			
Bezafibrate										<0.1								<0.1	
Carbamazepine										<0.1							<0.1	<0.1	<0.1
Ciprofloxacin	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.43	0.82	<0.1
Clarithromycin										0.86							0.64	0.93	
Clindamycin										<0.1							<0.1	<0.1	
Diclofenac									0.41	7.42							3.31	9.28	0.27
Enalapril																			
Erythromycin										<0.1							<0.1	<0.1	
Furaltadone																			
Gabapentin									<0.1	<0.1							<0.1	<0.1	
Gemfibrozil																			
Irbesartan									<0.1	<0.1								<0.1	<0.1
Ketoprofen																			
Levamisol										<0.1							<0.1	<0.1	
Lincomycin																		<0.1	
Lorazepam										<0.1							<0.1	<0.1	
Losartan									<0.1	<0.1							<0.1	<0.1	
Metoprolol										<0.1							<0.1	<0.1	
Metronidazole																		<0.1	
Nalidixic acid																			
Naproxen										<0.1								<0.1	
Norfloxacin	<0.1		<0.1				<0.1	<0.1	<0.1	1.21	<0.1		<0.1	<0.1	<0.1	<0.1	2.84	0.38	<0.1
Omeprazole sulfide, 4-hydroxy									<0.1	<0.1								<0.1	<0.1
Oxolinic acid																			
Pantoprazole										<0.1								<0.1	
Phenazone										84.34							73.80	90.04	2.08
Primidone									<0.1	0.28							<0.1	<0.1	<0.1
Roxithromycin																			
Salbutamol										<0.1							<0.1	<0.1	
Simvastatin																			
Sulfadiazine																			
Sulfamethoxazole										<0.1							0.10	0.17	
Tetracycline	<0.1	<0.1			<0.1														
Tramadol										<0.1								<0.1	
Trimetroprim																		<0.1	
Valsartan	<0.1		<0.1						<0.1	<0.1	<0.1						<0.1	<0.1	<0.1
Venlafaxine									<0.1	<0.1							<0.1	<0.1	

Table S13. Individual compound PAF calculated in the second sampling campaign (autumn).

Compounds	Samples																		
	1b	**2b**	**3b**	**4b**	**5b**	**6b**	**7b**	**8b**	**9b**	**10b**	**11b**	**12b**	**13b**	**14b**	**15b**	**16b**	**17b**	**18b**	**19b**
Acetaminophen		<0.1	<0.1	<0.1	<0.1	0.24	0.22	<0.1	<0.1	<0.1	<0.1		<0.1	<0.1			0.56	<0.1	
Alprazolam										<0.1							<0.1	<0.1	<0.1
Atorvastatin																	<0.1	<0.1	
Azitromycin										11.69							4.52	7.12	0.99
Bezafibrate										<0.1							<0.1	<0.1	
Carbamazepine										<0.1							<0.1	<0.1	<0.1
Ciprofloxacin																			
Clarithromycin																	<0.1	0.43	
Clindamycin										<0.1							<0.1	<0.1	
Diclofenac	0.13						0.13		0.13	4.76	0.13	0.13		0.13	0.37		6.29	7.47	0.37
Enalapril																			
Erythromycin											<0.1						<0.1	<0.1	<0.1
Furaltadone																			
Gabapentin	<0.1							<0.1		<0.1							0.15	0.14	<0.1
Gemfibrozil																			
Irbesartan									<0.1	<0.1							<0.1	<0.1	<0.1
Ketoprofen																			
Levamisol										<0.1							<0.1	<0.1	
Lincomycin																	<0.1	<0.1	<0.1
Lorazepam										<0.1							<0.1	<0.1	
Losartan										<0.1							<0.1	<0.1	<0.1
Metoprolol										<0.1							<0.1	<0.1	
Metronidazole																	<0.1	<0.1	
Nalidixic acid																			
Naproxen										<0.1							<0.1	<0.1	
Norfloxacin																			
Omeprazole sulfide, 4-hydroxy										<0.1							<0.1	<0.1	<0.1
Oxolinic acid			<0.1	<0.1							<0.1	<0.1							
Pantoprazole										<0.1							<0.1	<0.1	
Phenazone										35.90							69.72	73.83	13.71
Primidone									<0.1	<0.1							<0.1	<0.1	<0.1
Roxithromycin																			
Salbutamol							<0.1			<0.1							<0.1	<0.1	
Simvastatin																			
Sulfadiazine																		<0.1	<0.1
Sulfamethoxazole										<0.1							0.73	0.78	0.10
Tetracycline						<0.1			<0.1										
Tramadol										<0.1							<0.1	<0.1	<0.1
Trimetroprim																	<0.1	<0.1	
Valsartan										<0.1							<0.1	<0.1	<0.1
Venlafaxine									<0.1	<0.1							<0.1	<0.1	<0.1

Table S14. Individual compound PAF calculated in the third sampling campaign (winter).

Compounds	Samples																		
	1c	**2c**	**3c**	**4c**	**5c**	**6c**	**7c**	**8c**	**9c**	**10c**	**11c**	**12c**	**13c**	**14c**	**15c**	**16c**	**17c**	**18c**	**19c**
Acetaminophen	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1		<0.1	<0.1	<0.1		<0.1	<0.1	<0.1	<0.1		<0.1
Alprazolam										<0.1							<0.1	<0.1	<0.1
Atorvastatin										<0.1							<0.1	<0.1	
Azitromycin										15.35	0.99						12.96	17.72	0.99
Bezafibrate																			
Carbamazepine																	<0.1	<0.1	<0.1
Ciprofloxacin																			
Clarithromycin										1.13							0.68	0.93	
Clindamycin										<0.1							<0.1	<0.1	
Diclofenac										5.38							4.56	9.65	
Enalapril																			
Erythromycin										<0.1							<0.1	<0.1	
Furaltadone																			
Gabapentin	<0.1		<0.1	<0.1	<0.1	<0.1	<0.1	<0.1		<0.1		<0.1	<0.1	<0.1		<0.1	<0.1	<0.1	
Gemfibrozil																			
Irbesartan										<0.1							<0.1	<0.1	<0.1
Ketoprofen																			
Levamisol										<0.1							<0.1	<0.1	
Lincomycin																			<0.1
Lorazepam										<0.1							<0.1	<0.1	
Losartan										<0.1							<0.1	<0.1	
Metoprolol																	<0.1	<0.1	
Metronidazole										<0.1							<0.1	<0.1	
Nalidixic acid											<0.1								
Naproxen										<0.1							<0.1	<0.1	
Norfloxacin																			
Omeprazole sulfide, 4-hydroxy										<0.1							<0.1	<0.1	
Oxolinic acid	<0.1	<0.1		<0.1	<0.1		<0.1		<0.1	<0.1									
Pantoprazole										<0.1							<0.1	<0.1	
Phenazone										6.56							78.21	82.55	9.04
Primidone	<0.1									0.11							<0.1	<0.1	<0.1
Roxithromycin																			
Salbutamol										<0.1							<0.1	<0.1	
Simvastatin																			
Sulfadiazine																			<0.1
Sulfamethoxazole										0.46							0.57	0.88	<0.1
Tetracycline																			
Tramadol										<0.1							<0.1	<0.1	<0.1
Trimetroprim										<0.1							<0.1	<0.1	<0.1
Valsartan			<0.1		<0.1	<0.1	<0.1			<0.1	<0.1	<0.1	<0.1				<0.1	<0.1	
Venlafaxine	<0.1	<0.1			<0.1	<0.1		<0.1		<0.1	<0.1	<0.1	<0.1	<0.1		<0.1	<0.1	<0.1	<0.1

References

Posthuma, L., van Gils, J., Zijp, M.C., van de Meent, D., de Zwartd, D., 2019. Species Sensitivity Distributions for Use in Environmental Protection, Assessment, and Management of Aquatic Ecosystems for 12 386 Chemicals. Environ. Toxicol. Chem. 38, 703–711. https://doi.org/10.1002/etc.4373.

EPSAR, 2020. Entitat de Sanejament d'aigües. Generalitat Valenciana [WWW Document]. URL http://www.epsar.gva.es/instalaciones/buscador-edar.aspx (accessed 06.08.20).

2.2.3. Discusión de resultados

En el **artículo científico III** se presentan los resultados de la cuantificación de 40 fármacos investigados durante tres campañas de muestreo en las aguas del río Mijares. Para complementar los resultados del estudio cuantitativo se llevó a cabo un screening de amplio alcance de fármacos y metabolitos. También, se evaluó el riesgo ecológico de las mezclas farmacéuticas encontradas en aguas superficiales, utilizando distribuciones de sensibilidad de especies elaboradas con datos de toxicidad crónica para organismos acuáticos.

Determinación de fármacos en aguas mediante UHPLC-MS/MS QqQ

La presencia de fármacos en el medio ambiente preocupa tanto a la comunidad en general como a la comunidad científica en particular. Una de las principales vías de contaminación del medio ambiente son los residuos farmacológicos que, tras ser excretados, pasan a las EDAR y de ahí a las aguas receptoras. Para detectar estos compuestos es preciso recurrir a técnicas sumamente sensibles, dados los bajos niveles de concentración a los que suelen encontrarse. En este artículo, se buscó determinar la presencia de fármacos en el río Mijares utilizando la metodología UHPLC-MS/MS con analizador QqQ.

Como primer paso, se optimizaron las condiciones de masas de los 40 fármacos objeto de estudio, así como de los 14 ILIS seleccionados. Los espectros de masas se obtuvieron en modo full scan a diferentes voltajes de cono y aplicando ionización por electrospray en polaridad positiva y negativa. Una vez obtenido el espectro de masas, se seleccionó, para cada uno de los compuestos, ya sea la molécula protonada, $[M+H]^+$ o la molécula desprotonada, $[M-H]^-$. Luego se llevó a cabo el experimento en modo escaneo de iones producto, a diferentes energías de colisión. Tras seleccionar tres transiciones para aumentar la confiabilidad en la etapa de confirmación, se efectuó el experimento en modo SRM con cada uno de los compuestos. Sólo 4 de los 40 fármacos estudiados, se determinaron en modo de ionización negativa.

A modo de ejemplo, la **Figura 2.12** muestra los espectros en modo full scan para la fenazona a diferentes energías de colisión.

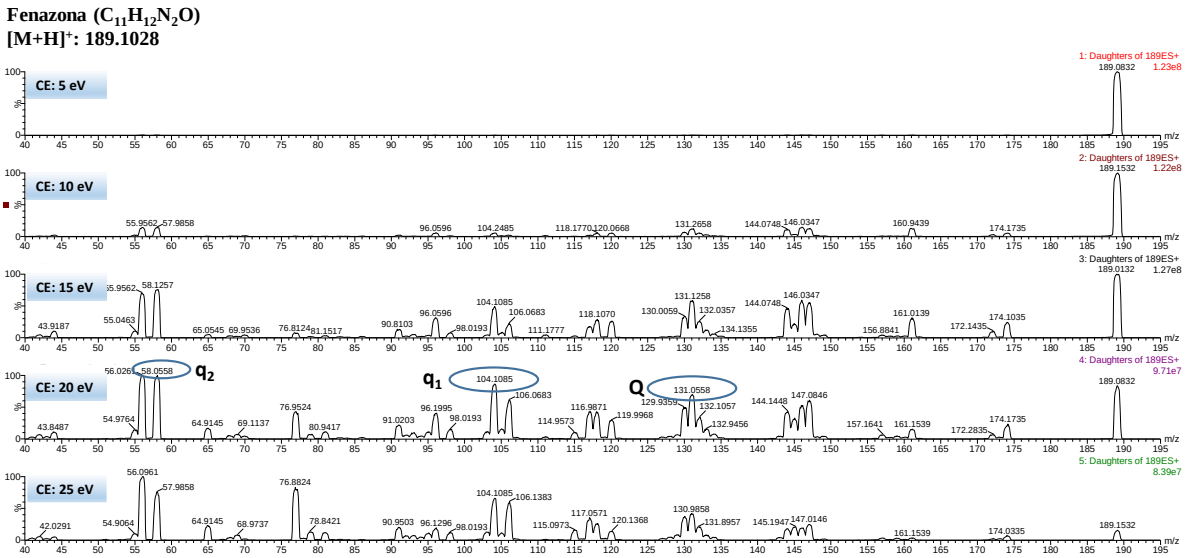

Figura 2.12. Espectros en modo full scan correspondientes a la optimización de la energía de colisión (CE) para la fenazona.

La metodología UHPLC-MS/MS QqQ no incluyó ningún paso de preparación de muestra, salvo la centrifugación previa a la inyección directa. Este modo de inyección es posible merced a las prestaciones en sensibilidad y selectividad de los instrumentos actuales [135]. Al eliminar la preconcentración se reducen las posibilidades de contaminación, el volumen de muestra y el uso de disolvente, al tiempo que se incrementa el rendimiento de preparación de muestras [136].

Con el objetivo de aumentar la confianza en los resultados obtenidos del análisis mediante UHPLC-MS/MS QqQ, se prepararon QC a a tres niveles de concentración (0.01, 0.1 y 1 µg/L) a partir de muestras reales. Los porcentajes de compuestos para diferentes ámbitos de recuperación promedio de los fármacos estudiados se presentan en la **Figura 2.13**. Los datos señalan que un 91, 97 y 91% de los compuestos a 0.01, 0.1 y 1 µg/L, respectivamente, presentaron porcentajes de recuperación en el rango 60-140%, lo cual cumple con los requisitos establecidos en el apartado C43 de la guía SANTE para recuperaciones individuales.

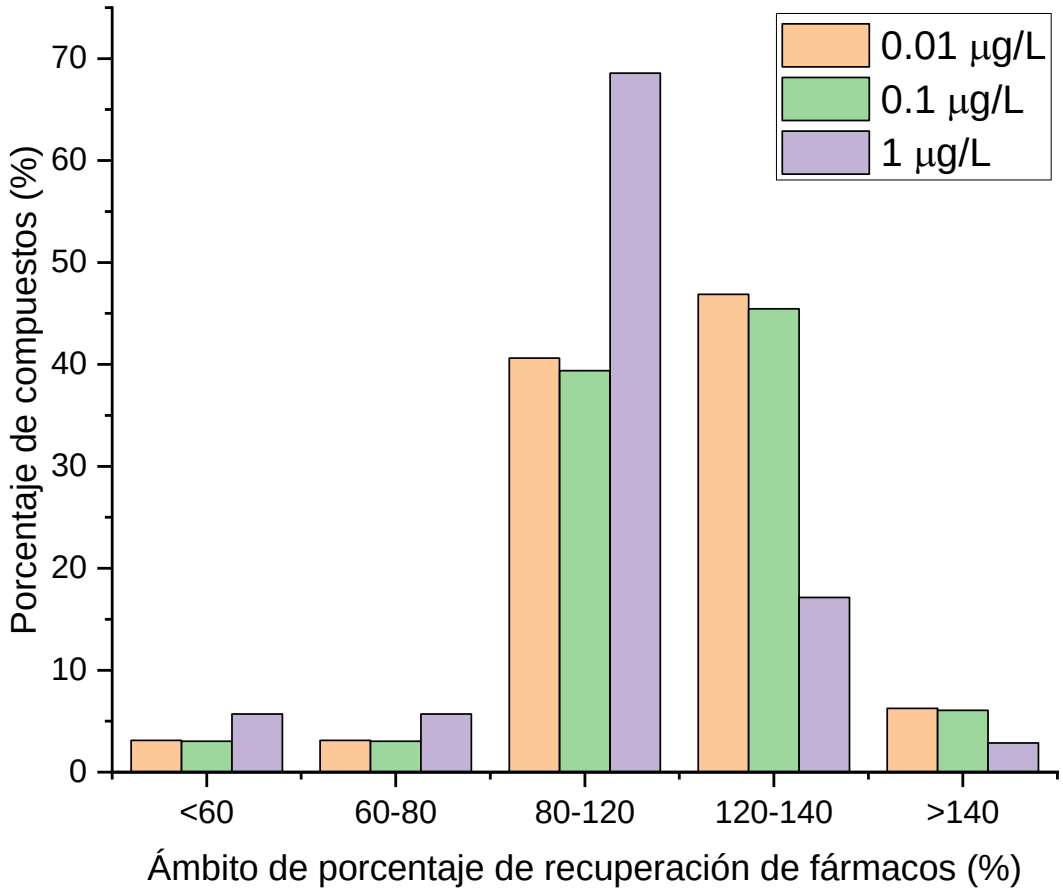

Figura 2.13. Porcentajes de recuperación promedio de los fármacos estudiados a tres niveles de concentración diferentes [0.01 µg/L (n=3), 0.1 µg/L (n=9), 1 µg/L (n=9)] en aguas superficiales.

A partir de los resultados de los QC, se obtuvieron los valores de RSD (ver **Tabla 2.14**), los cuales fueron inferiores al 20% en un 84, 73 y 74% de los compuestos a 0.01, 0.1 y 1 µg/L, respectivamente. Este es un resultado aceptable para las concentraciones a las que se está trabajando. En el ámbito de 20% < RSD < 30%, los resultados fueron de un 9, 21 y 7%, mientras que para una RSD superior al 30% fueron inferiores al 9%.

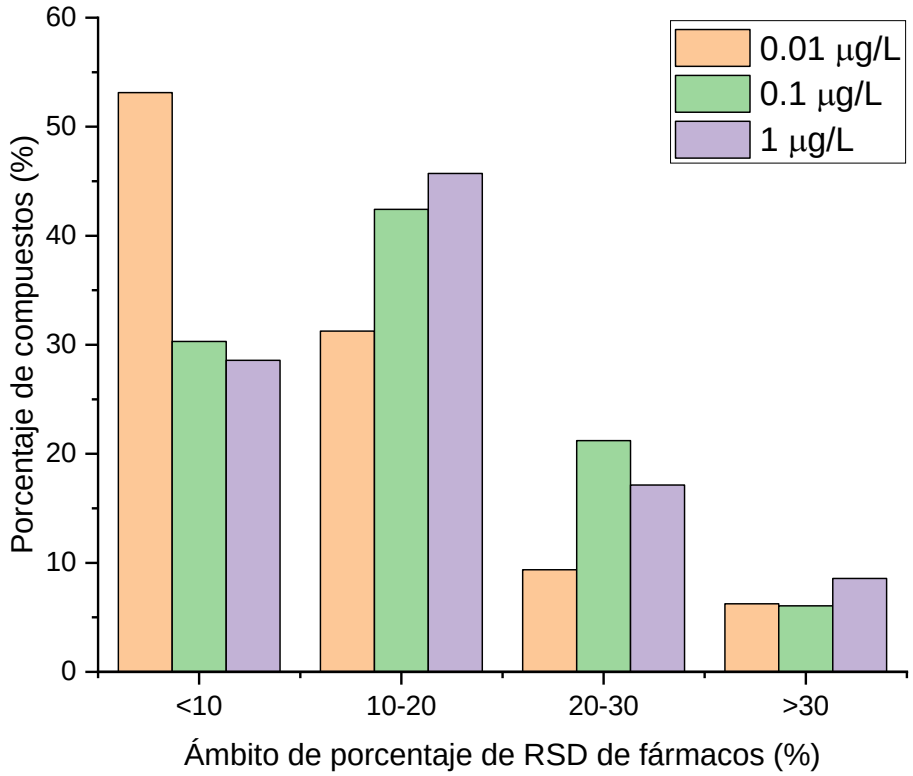

Figura 2.14. Coeficiente de variación promedio de los fármacos estudiados a tres niveles de concentración diferentes [0.01 µg/L (n=3), 0.1 µg/L (n=9), 1 µg/L (n=9)] en aguas superficiales.

Análisis de muestras de agua superficial del río Mijares

Se tomaron 57 muestras de agua superficial en 19 puntos del río Mijares durante tres campañas (junio 2018, septiembre 2018 y febrero 2019). El análisis de las muestras mediante el método cuantitativo UHPLC-MS/MS QqQ permitió detectar 35 de los 40 compuestos que se estaban investigando. En general, la detección fue baja en los puntos de muestreo ubicados en la parte superior y media del río, excepto en el punto 10, ubicado aguas abajo de una EDAR. Por el contrario, en la parte inferior del río, más densamente poblada, la cantidad de fármacos detectada también fue mayor, especialmente cerca de los municipios de Vila-real y Almassora, aguas abajo de dos EDAR (ver **Figura 2**, **artículo científico III**). Los fármacos detectados a mayores concentraciones fueron: acetaminofén, gabapentina, venlafaxina, valsartán, ciprofloxacino y diclofenaco. También se detectaron cuatro de los cinco antibióticos incluidos

en la lista de observación de la Unión Europea 2018/840 (ciprofloxacino, azitromicina, claritromicina y eritromicina).

En la primera campaña de muestreo, se encontraron 31 fármacos, los cuales se citan seguidamente en orden de frecuencia de detección, junto al grupo terapéutico al que pertenecen y el ámbito de concentración, superior al nivel de calibración más bajo: ciprofloxacino (100%, antibiótico, 0.58-1.1 µg/L), norfloxacino (74%, antibiótico, 0.060-0.94 µg/L), valsatán (42%, antihipertensivo, 0.010-1.6 µg/L), acetaminofén (37%, analgésico, 0.011-0.048 µg/L), primidona (26%, antiepiléptico, 0.020-1.0 µg/L) y diclofenaco (26%, antiinflamatorio no esteroideo, 0.006-0.87 µg/L) (ver **Figura 2.15**). En las **Figuras 2.15-2.17** se presentan solamente los resultados obtenidos en los puntos de muestreo 10, 17 y 18, los más contaminados. Para los demás puntos de muestreo, ver el material suplementario del **artículo científico III**.

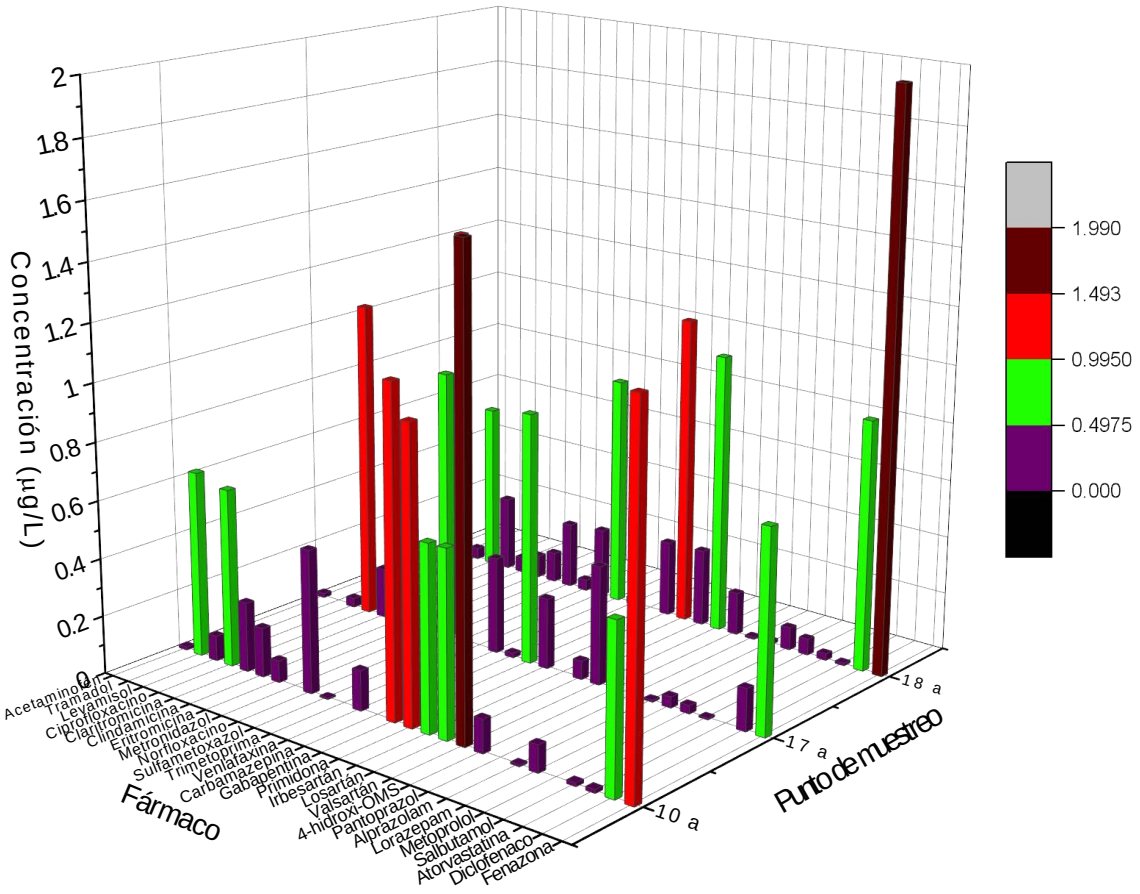

Figura 2.15. Concentración (µg/L) de fármacos detectados en muestras de aguas del río Mijares durante la primera campaña de muestreo (junio de 2018, verano).

En la segunda campaña de muestreo se detectaron 32 fármacos (ver **Figura 2.16**). Seguidamente se citan siguiendo el orden fijado para el primer muestreo; acetaminofén (79%, analgésico, 0.006-0.19 µg/L), diclofenaco (58%, antiinflamatorio no esteroideo, 0.008-0.57 µg/L), gabapentina (32%, antiepiléptico, 0.11-1.9 µg/L), irbesartán (26%, antihipertensivo, 0.029-1.1 µg/L), primidona (26%, antiepiléptico, 0.11-0.40 µg/L) y venlafaxina (26%, antidepresivo, 0.008-0.46 µg/L).

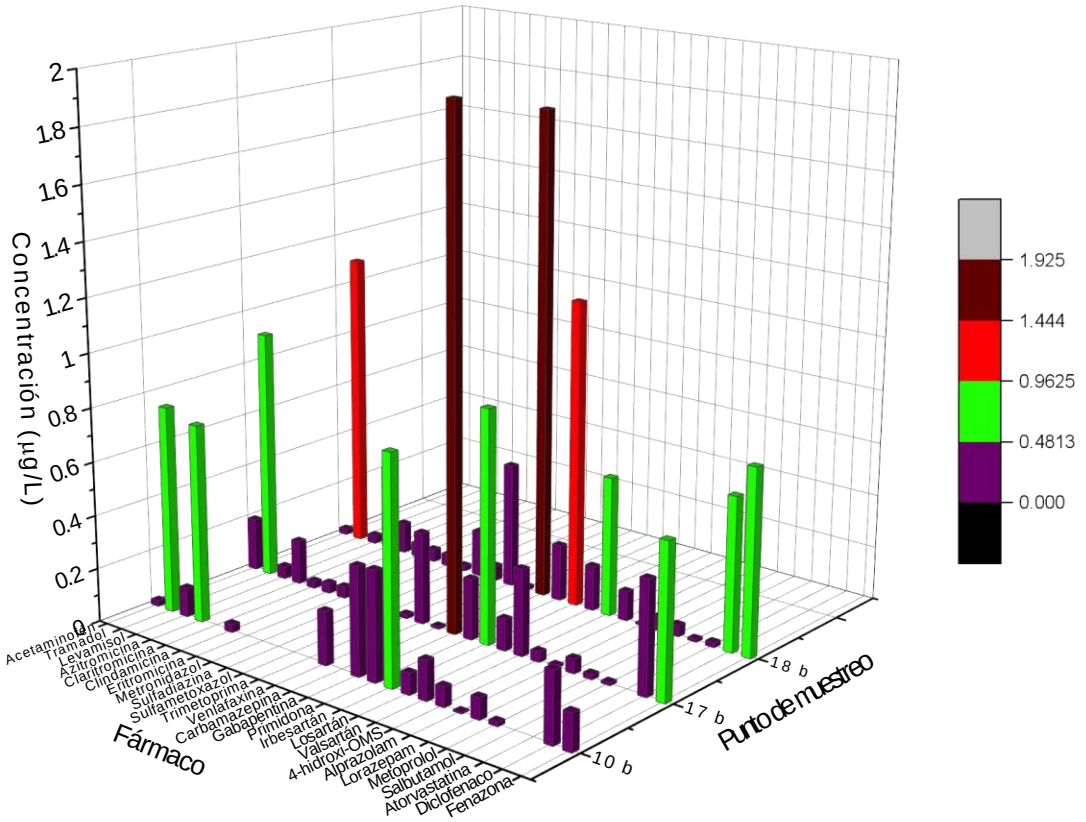

Figura 2.16. Concentración (µg/L) de fármacos encontrados en muestras de aguas del río Mijares durante la segunda campaña de muestreo (setiembre de 2018, otoño).

Finalmente, en la tercera y última campaña de muestreo, se detectaron 31 fármacos (ver **Figura 2.17**), que también se citan según el orden establecido: acetaminofén (84%, analgésico, 0.005-0.30 µg/L), gabapentina (74% antiepiléptico, 0.060-1.1 µg/L), venlafaxina (74%, antidepresivo, 0.068-0.69 µg/L), valsartán (53%, antihipertensivo, 0.017-1.3 µg/L), azitromicina (26%, antibiótico, 0.88-1.6 µg/L), y primidona (26%, antiepiléptico, 0.090-0.45 µg/L).

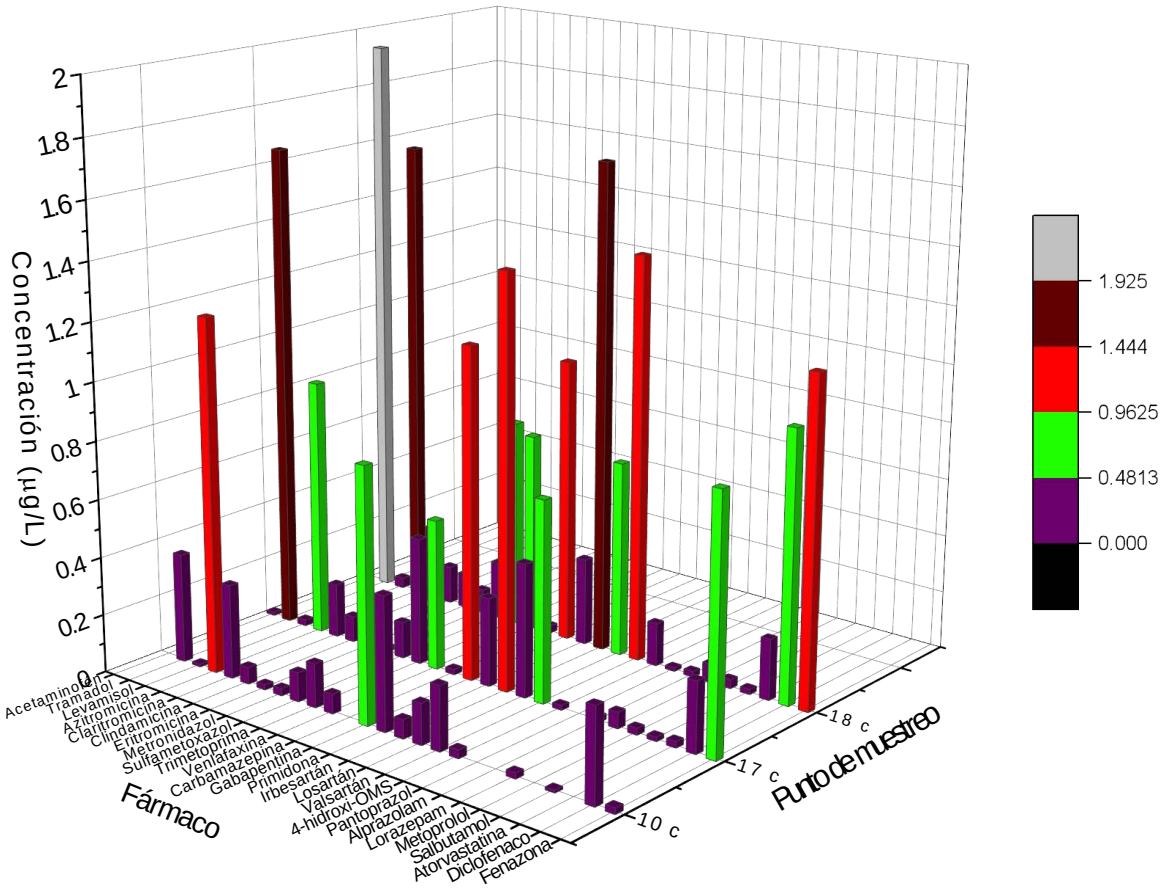

Figura 2.17. Concentración (µg/L) de fármacos encontrados en muestras de aguas del río Mijares durante la tercera campaña de muestreo (febrero de 2019, invierno).

En general, no se observaron tendencias claras en función de la temporada de muestreo. En la tercera campaña, realizada en invierno, sí se notó un ligero aumento en la concentración de antihipertensivos, antidepresivos, antibióticos y analgésicos. En el caso de los antibióticos, el aumento no es de extrañar, pues su consumo tiende a aumentar en esta época del año, sobre todo para tratar infecciones respiratorias. Con respecto a los puntos donde se encontró la mayor cantidad y la mayor concentración de analitos (10, 17 y 18), la azitromicina, la claritromicina y la trimetoprima presentaron los niveles de concentración más altos durante el invierno.

Determinación de fármacos en aguas mediante UHPLC-HRMS

Para complementar los resultados obtenidos con el análisis cuantitativo, las muestras de agua de la segunda campaña se sometieron a un análisis cualitativo por UHPLC-HRMS. El resultado del cribado reveló la presencia de una gran cantidad de fármacos (**Figura 2.18)** y metabolitos.

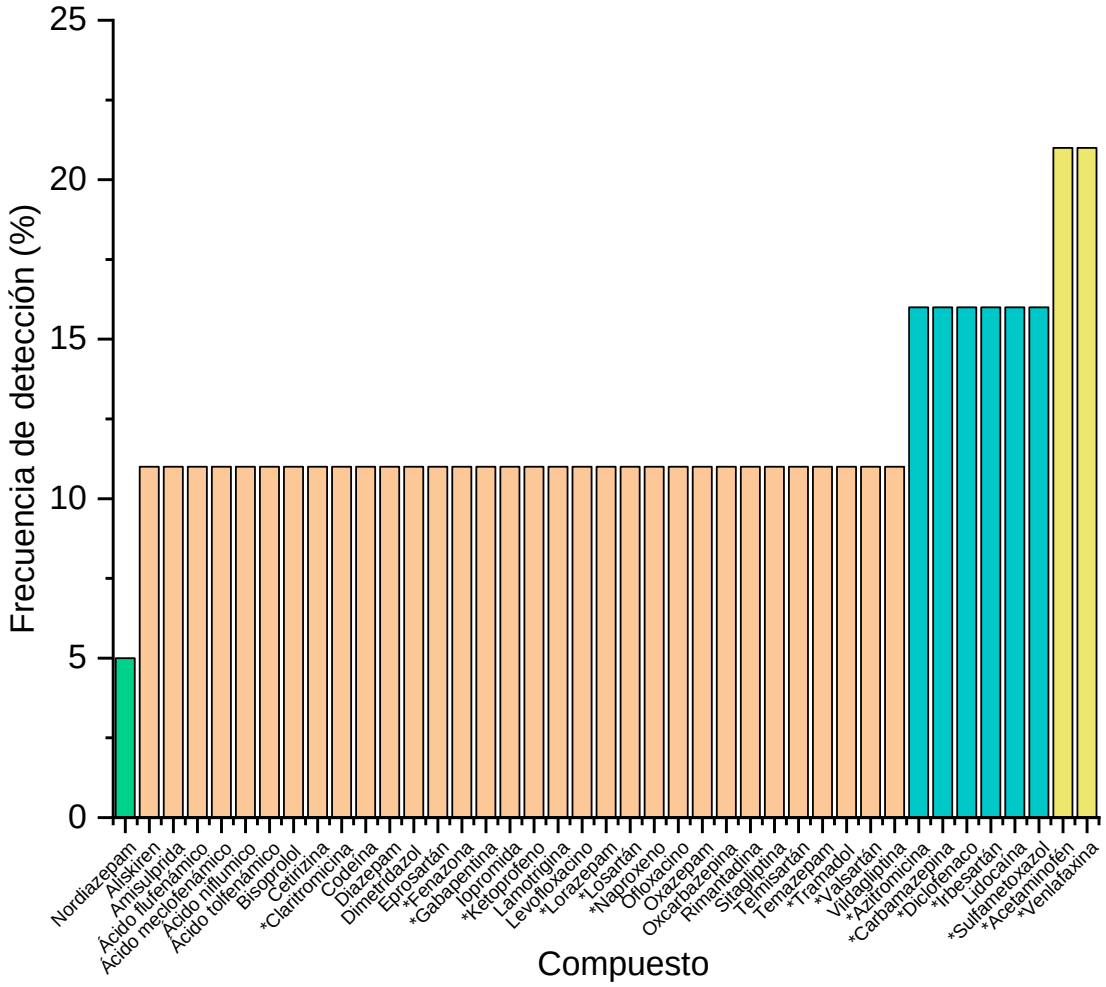

Figura 2.18. Fármacos detectados en muestras de agua superficial en el cribado por UHPLC-QTOF MS. Muestras recolectadas en setiembre de 2018.

Se detectaron 41 fármacos, 35 de los cuales se confirmaron con estándares de referencia y el resto de manera tentativa, observando la molécula protonada y al menos uno de los iones fragmento debidamente justificado a partir de la interpretación de los datos de masa exacta. De los 41 compuestos detectados, 16 se incluyeron en el análisis por UHPLC-MS/MS QqQ (ver compuestos con asterisco en la **Figura 2.18**). Los fármacos con mayor frecuencia de detección

fueron el acetaminofén y la venlafaxina, ambos con un 21% y observados en 4 de los 19 puntos de muestreo. El segundo lugar lo ocuparon 6 compuestos (azitromicina, carbamazepina, diclofenaco, irbesartán, lidocaína y sulfametoxazol), todos con un 16% de frecuencia de detección. Los grupos farmacéuticos más detectados fueron los antihipertensivos y los antiinflamatorios no esteroideos, con un 20% cada uno, seguidos de los antibióticos, con un 12%.

Con respecto a los metabolitos, el cribado por UHPLC-HRMS permitió detectar 9 compuestos, 6 de los cuales se confirmaron con estándares de referencia (**Figura 2.19)**. Los analitos más detectados fueron: 4-acetilaminoantipirina (4-AAA) y 4-formilaminoantipirina (4-FAA). Uno de los metabolitos detectados, el O-desmetilvenlafaxina, aparece incluido en la última lista de observación de sustancias a nivel de la Unión [44].

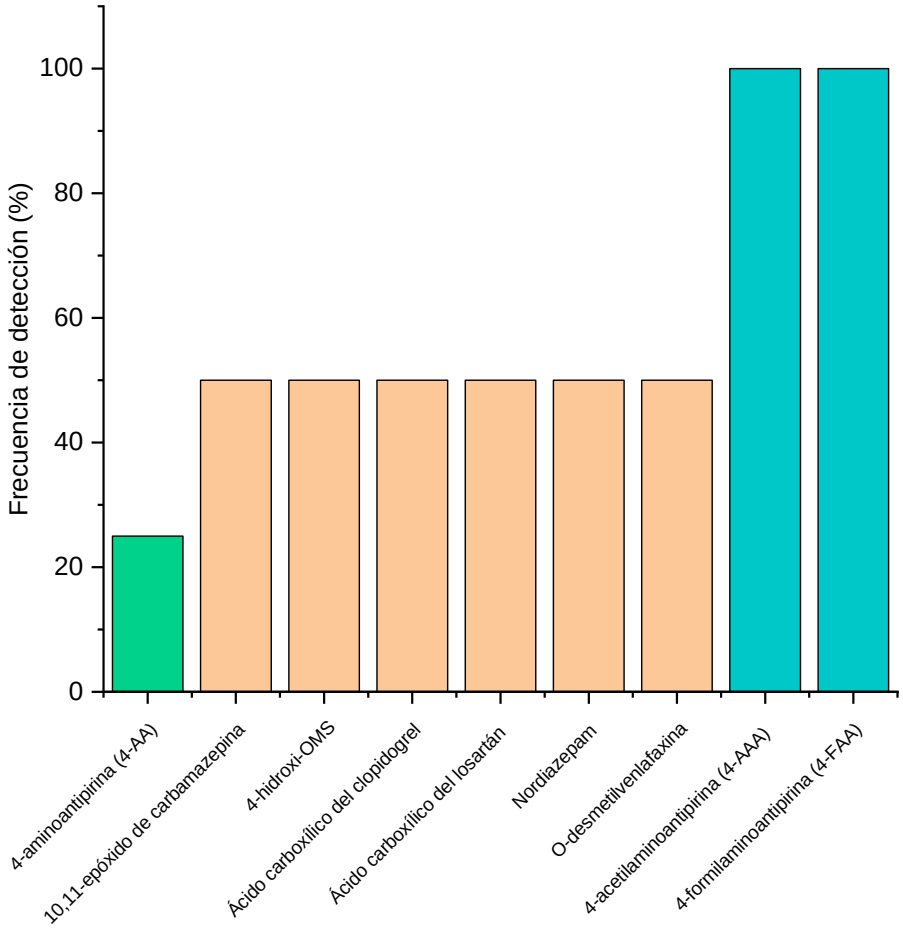

Figura 2.19. Metabolitos detectados en muestras de agua superficial en el cribado por UHPLC-QTOF MS. Muestras recolectadas en septiembre de 2018.

Evaluación de riesgos ecológicos

El riesgo ecológico en el río Mijares se evaluó a partir de los niveles de concentración de los fármacos detectados en las muestras de agua superficial.

Los productos farmacéuticos que mayor toxicidad aportaron a los ecosistemas acuáticos fueron la fenazona > la azitromicina > el diclofenaco, todos con valores individuales de PAF superiores al 10% (ver **Tabla 2.2**). El segundo lugar lo ocuparon el norfloxacino > ciprofloxacino > claritromicina. Los riesgos asociados fueron bajos, moderados y graves, respectivamente, de acuerdo con los valores de msPAF. No obstante, en todas las campañas de muestreo, el porcentaje de especies acuáticas afectadas varió entre un 65% y un 82% en los puntos 17 y 18, y 10 en el verano, lo que apunta a un riesgo ecotoxicológico muy alto.

Tabla 2.2. Fármacos y grupos terapéuticos que más aportan a la toxicidad de los ecosistemas acuáticos (río Mijares).

Producto farmacéutico	**Aporte a la toxicidad**	**PAF[a] (%)**	**msPAF (%)**	**Clasificación del riesgo[b]**
Fármacos	fenazona > azitromicina > diclofenaco	> 10	< 5	Bajo (en mayoría de los puntos)
	norfloxacino > ciprofloxacino > claritromicina	> 1	5 - 25	Moderado (19)
			> 25	Grave (10, 17, 18)
Grupo terapéutico	analgésicos / antiinflamatorios	na[c]	15 - 81	Todos los puntos
	antibióticos	na[c]	5 - 12	(10 todas las campañas) (17 verano e invierno) (18 otoño e invierno)

[a] Porcentaje obtenido en al menos un punto de muestreo
[b] Entre paréntesis los puntos de muestreo
[c] na = no se aplica

En todos los casos, la toxicidad estuvo dominada por los analgésicos/antiinflamatorios, que arrojaron $msPAF_{TC}$ de entre un 15% y un 81%, y cuyos principales contribuyentes tóxicos fueron la fenazona y el diclofenaco. El segundo lugar lo ocuparon los antibióticos. En este grupo terapéutico, la mayor contribución tóxica la aportó la azitromicina en los puntos de muestreo 10, 17 y 18, en otoño e invierno. En verano, el norfloxacino tuvo una contribución determinante, seguida del ciprofloxacino y la claritromicina.

Resistencia a antibióticos

El ingreso continuo de antibióticos al medioambiente, a través de los efluentes de las EDAR, supone un riesgo de selección de bacterias resistentes. Las EDAR contienen bacterias ambientales, humanas y de animales, lo que las convierte en un sitio ideal para la selección, y posterior diseminación, al medio ambiente de bacterias y genes resistentes [137].

En efecto, la presencia constante de antibióticos en aguas superficiales puede crear resistencia bacteriana [138] e incluso "superbacterias": cepas resistentes a múltiples fármacos[139]. Con esto se genera un problema de salud pública realmente serio, pues los tratamientos se vuelven más largos y complejos y las enfermedades más difíciles de diagnosticar.

Es importante, entonces, que los gobiernos y los responsables de la salud pública evalúen la presencia de antibióticos en el medioambiente, no solo por sus posibles efectos ecotoxicológicos, sino por su contribución al desarrollo de la resistencia antibiótica.

En el río Mijares, cinco antibióticos excedieron los umbrales de resistencia; es decir, se podría estar enriqueciendo el gen de resistencia de las bacterias ambientales a los antibióticos. En los puntos de muestreo 10, 17 y 18, que tienen en sus cercanías una EDAR, el cociente de riesgo (RQ) excedió el valor de 1. En orden de mayor a menor, los antibióticos que excedieron el PNEC de resistencia fueron el ciprofloxacino (RQ 17.3), la azitromicina (RQ 6.5), el norfloxacino (RQ 1.9), la trimetoprima (RQ 1.5) y la claritromicina (RQ 1.3). En general, los antibióticos con mayor riesgo de desarrollar resistencia pertenecen a las familias de las fluoroquinolonas y los macrólidos, todos ellos antibióticos de importancia crítica para la salud humana.

2.3. INVESTIGACIÓN DE FÁRMACOS EN LAS AGUAS RESIDUALES DE UNA EDAR CON TRATAMIENTO CONVENCIONAL

2.3.1. Introducción

2.3.2. Artículo científico IV

Investigation of pharmaceuticals in a conventional wastewater treatment plant:

Removal efficiency, seasonal variation and impact of a nearby hospital

2.3.3. Discusión de resultados

2.3.1. Introducción

En las últimas décadas, la esperanza de vida se ha incrementado en por lo menos 25 años, sobre todo en los países que van a la cabeza de la innovación farmacéutica [140]. En el caso de España, la esperanza de vida llegó a 83.4 años en 2017, un aumento de 4.1 años respecto al año 2000. Este aumento, superior a la media de la Unión Europea (EU) (3.6 años) se debió, en gran medida, a una menor tasa de mortalidad por padecimientos cardiovasculares, principalmente cardiopatías isquémicas y enfermedades cerebrovasculares. Lamentablemente, la tasa de obesidad sigue en aumento, y otras enfermedades, como el cáncer de pulmón, el cáncer colorrectal y la enfermedad pulmonar obstructiva crónica siguen ocupando un lugar destacado. En el caso de la enfermedad de Alzheimer, la situación no es tan alentadora, pues la tasa de mortalidad por esta causa ha aumentado sustancialmente [141].

El consumo de medicamentos por zona geográfica se puede estimar a partir del valor de DHD (dosis diarias definidas (DDD) por cada 1000 habitantes por día) que reportan los países. Así, en la **Figura 2.20**, elaborada por el Centro Europeo para el Control y Prevención de Enfermedades (ECDC), se muestra el consumo de antibióticos de uso sistémico en la UE durante 2018 [142]. Las cifras confirman que España, junto con Francia, Polonia y Rumania, figura entre los países que más antibióticos consumen en la UE.

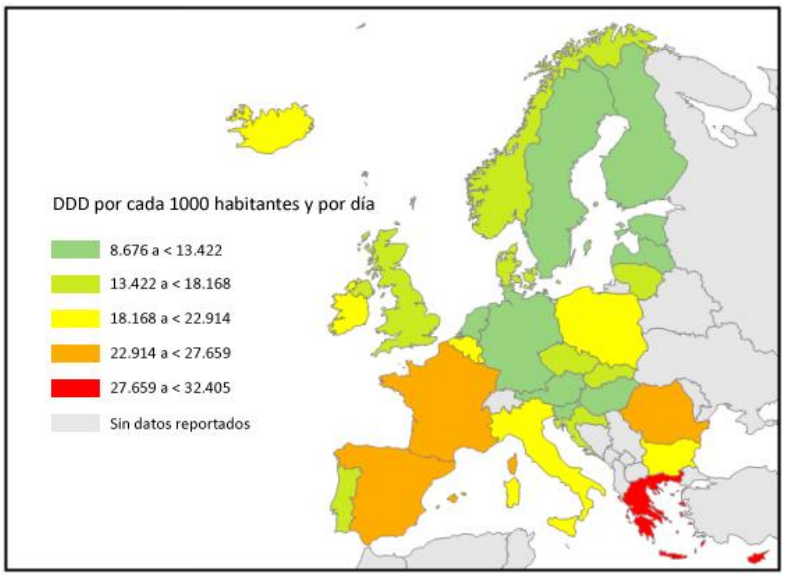

Figura 2.20. Consumo de antibióticos de acción sistémica en la UE en 2018. Fuente: Adaptado ECDC, 2020 [142].

En España, entre 1997 y 2015, el rango de valores de DHD para antibióticos de uso sistémico osciló entre 14.9 y 17.7 (**Figura 2.21**). Ese valor subió a 25.6 en 2016 [25], entre otros, porque en los informes del Plan Nacional frente a la Resistencia a los Antibióticos (PRAN) se comenzaron a incluir los antibióticos dispensados con receta privada, además de los antibióticos dispensados a nivel hospitalario [143].

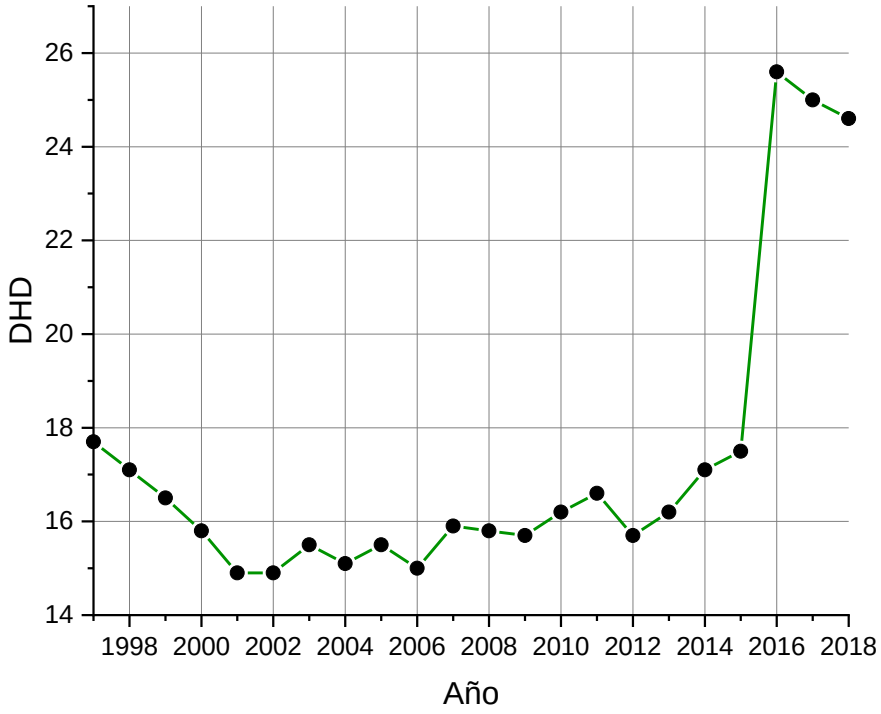

Figura 2.21. Consumo de antibióticos en España. Fuente: Elaborada con base en AEMPS, 2019.

En todo caso, el crecimiento constante que ha experimentado España en los últimos años en relación con el gasto en medicamentos y atención médica refleja la importancia que ha adquirido la industria farmacéutica en este país. España es actualmente uno de los principales mercados farmacéuticos europeos y su potencial para fabricar medicamentos, invertir en infraestructura relacionada, diversificar e incursionar en otros mercados es considerable. Las perspectivas de este sector industrial para el 2025 parecen ser muy alentadoras [144].

Esta demanda, cada vez mayor, de fármacos ha conducido, sobre todo a partir de la década de los noventa, a que se preste más atención a la posible presencia de estas sustancias en el medioambiente.

Los efluentes de las aguas residuales son una de las principales vías de entrada de fármacos al medioambiente. La finalidad de las estaciones depuradoras es colectar las aguas residuales y reducir, o si es posible eliminar, las sustancias contaminantes que se hayan añadido al agua durante su uso. Para ello, se aplican métodos físicos, físicoquímicos, biológicos y de otro tipo. De esa forma, los efluentes tratados se pueden descargar directamente a las aguas receptoras (aguas superficiales, aguas costeras, embalses, entre otras). A continuación, se describen brevemente las diferentes etapas que tienen lugar en una EDAR [145].

La *etapa de pretratamiento* es el primer paso y consiste en separar los materiales de mayor tamaño (‘pozo de gruesos’) y eliminar los residuos sólidos que se encuentran flotando en el agua residual que entra a la EDAR. Siguen, luego, las operaciones de desbaste, tamizado, desarenado y desengrasado. En el desbaste, una serie de rejas permite separar físicamente el material grueso de menor tamaño y el material no soluble en agua. El tamizado —proceso de filtración que utiliza un soporte delgado con ranuras de paso— ayuda a reducir el contenido de sólidos en suspensión. Los materiales con un tamaño superior a los 0.2 mm se eliminan en la etapa de desarenado. La estructura hidráulica del desarenador permite eliminar gravas, partículas minerales, granos y semillas, entre otros. La última etapa del pretratamiento es el desengrasado, donde se eliminan los sólidos y los líquidos que no se mezclan con el agua y que son menos densos que ella. Esta etapa se puede llevar a cabo de forma simultánea con el desarenado en sistemas llamados desarenadores-desengrasadores aireados.

El *tratamiento primario* es la siguiente etapa y consiste en la decantación primaria y la aplicación de tratamientos fisicoquímicos. La decantación primaria trabaja por gravedad y tiene como finalidad separar los sólidos sedimentables que podrían suponer una demanda importante de oxígeno en los tratamientos posteriores (contaminación biodegradable). En los tratamientos fisicoquímicos se agregan reactivos químicos para disminuir la presencia de sólidos en suspensión. Los sólidos coloidales se eliminan incrementando su tamaño y su densidad mediante procesos de coagulación-floculación.

En el *tratamiento secundario o biológico* se elimina la materia orgánica por medio de un reactor biológico. En esta etapa se incorporan microorganismos que actúan sobre una parte de la materia orgánica para oxidarla y producir agua, dióxido de carbono, amoníaco, subproductos y energía. La energía que se libera permite que una fracción de la materia orgánica se convierta, por síntesis, en tejido celular nuevo y se produzca la floculación. Una vez consumido el contenido orgánico, comienza la respiración endógena: el nuevo tejido celular se autoconsume (autooxidación) para obtener la energía necesaria para poder mantenerse. Para apoyar las reacciones de oxidación, síntesis y respiración endógena generalmente se introduce oxígeno en el reactor biológico (se incorpora aire). La floculación hace que se formen agregados de mayor densidad que el líquido circundante (lodos o fangos). En una etapa posterior, una parte de estos lodos se separa por sedimentación, mientras que la otra se recircula en el reactor biológico para mantener una concentración determinada de microorganismos vivos. Esta fase se denomina proceso de lodos activos o lodos activados.

Muchas plantas convencionales descargan sus efluentes en las aguas receptoras tras haber finalizado los tratamientos primario y secundario. El problema es que su capacidad para eliminar los fármacos y otros xenobióticos es limitada: normalmente los lodos activados no adsorben estos productos eficazmente y los microorganismos que se utilizan en el tratamiento secundario no los digieren por completo. Además, los xenobióticos inhiben la actividad microbiana, lo que dificulta aún más la eliminación de los fármacos [119].

La aplicación de un *tratamiento terciario* permite mejorar la calidad del vertido final. Algunas estaciones depuradoras aplican la filtración y/o la desinfección, aunque parece que la adsorción de carbón activado y la ozonización dan mejores resultados [146]. En el caso de la ozonización, se generan TPs con posibles efectos biológicos, pero estas sustancias se podrían tratar con filtros de arena, si bien los costes de operación podrían verse incrementados [147].

La eliminación de los fármacos depende de las propiedades fisicoquímicas de las sustancias presentes en la EDAR y de las condiciones de los tratamientos, es decir, del tiempo de retención del lodo, del tiempo de retención hidráulica y de la temperatura [148]. En general, los fármacos de carácter básico muestran coeficientes de reparto más altos que los fármacos neutros y ácidos. Los fármacos con un log $K_{ow} > 5$ y masas moleculares altas quedan fácilmente retenidos

en los lodos y se eliminan de las fases acuosas, mientras que los de valores bajos (log K_{ow} < 2.5) tienden a permanecer en la fase acuosa [119].

La eficacia de eliminación (RE) de los fármacos en una EDAR se puede estimar a partir de la carga diaria (g/día) de analitos en la fase acuosa del influente (IWW) y el efluente (EWW), teniendo en cuenta la concentración de los compuestos y el caudal diario de las aguas residuales [149]. También se puede estimar a partir de los datos de concentración de los analitos encontrados en las aguas del IWW y el EWW, sin tomar en cuenta los valores de caudal [150]. Sin embargo, es preferible estimar la RE a partir de la carga diaria del IWW y el EWW, pues la irregularidad del caudal durante las semanas de muestreo a la entrada y salida de la EDAR, así como la cantidad de lluvia, afectan directamente los datos de concentración [149].

Muchos estudios han comprobado la presencia de fármacos en compartimentos ambientales, como masas de aguas, sedimento, suelo y biota. Dada la variedad de propiedades fisicoquímicas que presentan estos compuestos y sus TPs, y, en muchos casos, dadas las dificultades que encuentran las EDAR para eliminarlos, es importante continuar investigando su presencia en estas instalaciones. De esta forma se podrán mejorar procesos, aplicar tratamientos novedosos y conocer el nivel de concentración y el tipo de contaminantes que continuamente son incorporados a las aguas receptoras. Igualmente importante resulta evaluar su repercusión ambiental.

2.3.2. Artículo científico IV

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Investigation of pharmaceuticals in a conventional wastewater treatment plant: Removal efficiency, seasonal variation and impact of a nearby hospital

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ABSTRACT

Discharges from the wastewater treatment plants (WWTPs) are among the main sources of contamination to receiving surface water, therefore the quality of treated wastewater needs to be properly monitored. However, not only the effluents of larger WWTPs employing advanced treatment processes have been considered, but also those from more conventional WWTPs. In this study, the occurrence and behavior of pharmaceuticals have been investigated in a conventional WWTP which receives wastewater from an urban area and a near-by hospital. 24-h composite samples were collected during one week before (influent wastewater, IWW) and after (effluent wastewater, EWW) treatment along three monitoring campaigns distributed over one year. Moreover, seven daily IWW samples discharged from a hospital were also collected. A preliminary wide-scope screening using liquid chromatography (LC) coupled to high resolution mass spectrometry allowed to identify a wide number of pharmaceuticals in the samples. Based on the screening findings, a list of 40 compounds was established for subsequent target quantitative analyses by LC-tandem mass spectrometry. Up to 75% of the compounds investigated were present in all wastewater samples. Analyte concentrations in hospital discharge samples were significantly higher, evidencing an important contribution in terms of pharmaceuticals content. Antibiotics showed the highest concentrations during the winter season, which could be related to the increase in the prescription of these compounds to treat respiratory infections. Data from this work show that the biological treatment applied was able to eliminate nearly half of the compounds under study, although still 12 pharmaceuticals were not or poorly removed.

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Highlights

- 13 out of 16 antibiotics analyzed were found in both raw and treated wastewater.
- Antibiotic concentrations were notably higher in winter.
- Most of pharmaceuticals reached the WWTP mainly through the hospital discharge.
- Removal efficiencies of WWTP were estimated by comparing daily loads in IWW and EWW.
- Around 50% of pharmaceuticals were totally or partially removed from the WWTP.

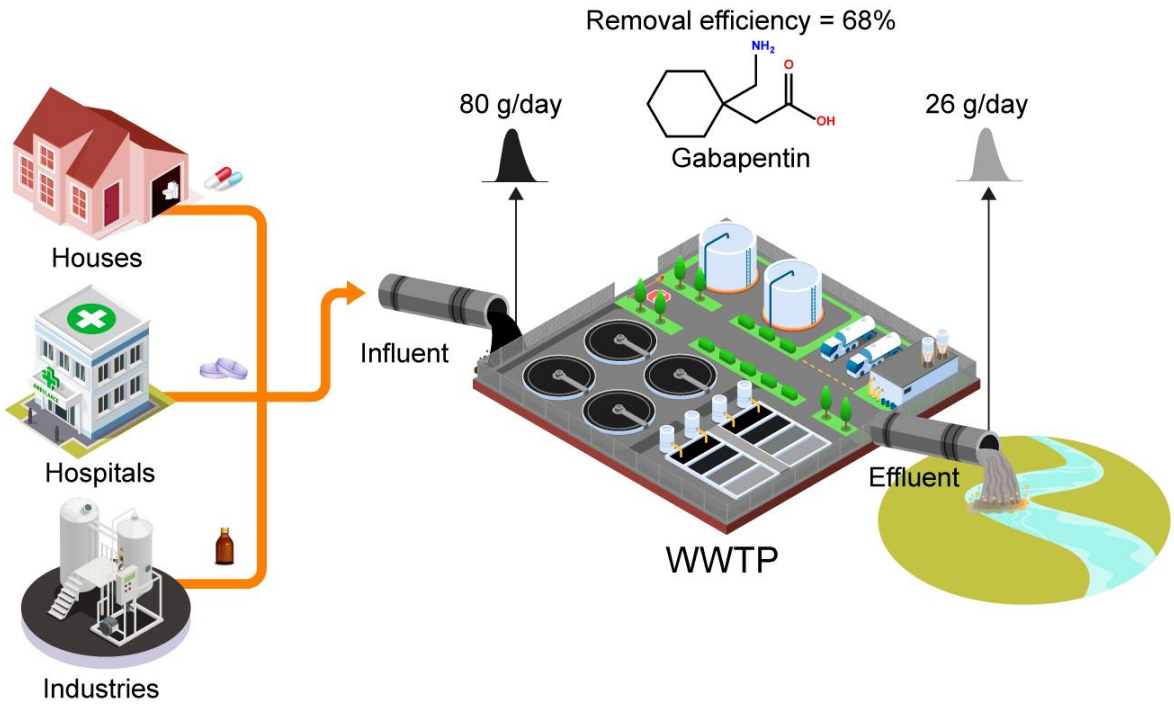
Abstract

Discharges from the wastewater treatment plants (WWTPs) are among the main sources of contamination to receiving surface water, therefore the quality of treated wastewater needs to be properly monitored. However, not only the effluents of larger WWTPs employing advanced treatment processes have been considered, but also those from more conventional WWTPs. In this study, the occurrence and behavior of pharmaceuticals have been investigated in a conventional WWTP which receives wastewater from an urban area and a near-by hospital. 24-h composite samples were collected during one week before (influent wastewater, IWW) and after (effluent wastewater, EWW) treatment along three monitoring campaigns distributed over one year. Moreover, seven daily IWW samples discharged from a hospital were also collected. A preliminary wide-scope screening using liquid chromatography (LC) coupled to high resolution mass spectrometry allowed to identify a wide number of pharmaceuticals in the samples. Based on the screening findings, a list of 40 compounds was established for subsequent target quantitative analyses by LC-tandem mass spectrometry. Up to 75% of the compounds investigated were present in all wastewater samples. Analyte concentrations in hospital discharge samples were significantly higher, evidencing an important contribution in terms of pharmaceuticals content. Antibiotics showed the highest concentrations during the winter season, which could be related to the increase in the prescription of these compounds to treat respiratory infections. Data from this work show that the biological treatment applied was able to eliminate nearly half of the compounds under study, although still 12 pharmaceuticals were not or poorly removed.

Keywords

Pharmaceuticals; antibiotics, wastewater treatment; hospital discharge; WWTP removal efficiency.

Graphical abstract



1. Introduction

The investigation on the occurrence of contaminants of emerging concern (CECs), specifically pharmaceuticals, in the aquatic environment has gained much interest due to their widely use and frequent detection in the water cycle at concentrations even higher than classical persistent and/or priority substances [1], [14], [4]. CECs are normally not included in the routine analysis due to the lack on regulation and high analytical cost, but their presence may have a negative impact on the environment and shows on human public health [2], [20], [21], [26]. Environmental regulations have barely included the control of pharmaceuticals in water bodies. However, due to the growing concern about this subject, policy makers have become aware of this potential environmental and public health problem. Hence, the European Commission updated the Watch List of the Water Framework Directive [13] to obtain more EU-wide monitoring data, with the final goal to better regulate priority pollutants in the aquatic environment [18]. Five antibiotics have been already included in the Watch List i.e. the penicillin amoxicillin, the fluoroquinolone ciprofloxacin and three macrolides erythromycin, clarithromycin and azithromycin. Yet in the near future, the requirements of water quality will be probably modified and become stricter, especially in relation to pharmaceutical discharges from the wastewater treatment plants (WWTPs), since the quality of wastewater effluent is of great relevance as it is one of the main sources of contamination to receiving surface water [17].

Conventional treatments applied by WWTPs do not commonly remove these compounds efficiently, and they can thus end-up in effluent wastewater (EWW) at relatively high concentrations, frequently exceeding 1 μg/L [22], [3], [34]. Consequently, it is not surprising that pharmaceuticals are found in receiving surface water [11], [15], [50] and even in drinking water [10], [32], [40]. A number of papers have highlighted the need for improving the treatment applied in the WWTPs, employing additional tertiary treatment processes [46]. Although additional advanced oxidation processes (AOPs) are recommended to improve the elimination of pollutants, they will imply additional costs that may be difficult to bear for relatively small WWTPs.

The efficiency of treatment and thus the extent of pharmaceutical removal by a WWTP can be restricted depending on the compounds concentration, chemical structure, solubility, charge and the existence of viable bacteria in the WWTP with degradative capabilities [12]. Previous studies have demonstrated that the removal efficiency (RE) for pharmaceuticals can vary among different and even in the same treatment processes [31], [36], [47]. Therefore, regular monitoring campaigns are required to obtain information about the actual functioning of the WWTP and to evaluate the potential impact of treated water on the aquatic environment. Detection, reliable identification and accurate quantification of CECs is a challenge in modern analytical chemistry. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is the most widely applied technique for the determination of pharmaceuticals in wastewater, focusing on a limited list of target compounds [16], [31], [45]. However, the use of pharmaceuticals between regions varies spatially and temporally due to different regulations, prescription practices, etc., so the application of target methods may not be sufficient as many compounds other than analytes remain ignored in the analysis. Therefore, wide-scope screening methodologies making use of high-resolution mass spectrometry (HRMS) become necessary in order to detect and identify a high number of contaminants, allowing to select the most relevant compounds for subsequent quantitative target analysis [19], [24], [25], [51].

The objectives of this work were: (1) Investigate the contribution of a continuous discharge from a hospital located in the nearby area to a small WWTP in the north of Spain; (2) Estimate the removal efficiency of the WWTP for a selected group of pharmaceuticals after application of a conventional treatment; (3) Evaluate the seasonal variation of pharmaceuticals detected in the WWTP. For this purpose, a preliminary screening by LC coupled to quadruple time of flight (QTOF) MS was carried out in order to detect and identify the most abundant pharmaceuticals in wastewater. Then, a list of 40 target pharmaceuticals was established for subsequent quantitative analysis based on LC-MS/MS with triple quadrupole (QqQ). A total of 42 samples, 21 IWW (influent wastewater) and 21 EWW (effluent wastewater), from the WWTP were quantitatively analyzed in three sampling campaigns distributed over a year. Additionally, 7 wastewater samples from the hospital were also analyzed during the first monitoring. The comparison of daily loads (g/day) in influent and effluent water allowed the estimation of RE for the selected pharmaceuticals.

2. Materials and methods

2.1. Pharmaceutical standards and reagents

40 pharmaceuticals (**Table 1**) from different groups and physicochemical characteristics were selected for target quantitative analysis. More details are included in the Supplementary Material (SM).

Table 1. LC-MS/MS conditions (cone value 10V) for pharmaceuticals selected.

Family	Compounds	Transition (Q)	CE (eV)	Transition (q)	CE (eV)	LCL* (ng/L)
Analgesic	Acetaminophen	152.0 > 110.0	15	152.0 > 93.0	20	5
				152.0 > 65.0	25	
	Acetaminophen-d_4	*156.0 > 114.0*	*10*	-	-	-
Benzodiazepine	Alprazolam	309.0 > 281.0	25	309.0 > 205.0	25	5
				309.0 > 274.0	25	
Hypolipidemic agent	Atorvastatin	559.0 > 440.0	20	559.0 > 466.0	15	5
				559.0 > 292.0	25	
	Atorvastatin-d_5	*564.0 > 445.0*	*20*	-	-	-
Antibiotic	Azithromycin[a]	749.4 > 591.4	25	749.4 > 82.9	45	50
				749.4 > 116.1	45	
	Azithromycin-d_3	*752.2 > 594.2*	*25*	-	-	-
Hypolipidemic agent	Bezafibrate (-)	360.0 > 274.0	20	360.0 > 154.0	25	1000
				360.0 > 85.0	15	
Antiepileptic	Carbamazepine	237.0 > 194.0	20	237.0 > 179.0	25	5
				237.0 > 192.0	10	
	Carbamazepine 10,11-epoxide-d_{10}	*263.0 > 190.0*	*25*	-	-	-
Antibiotic	Ciprofloxacin[a]	332.0 > 231.0	25	332.0 > 288.0	15	50
				332.0 > 314.0	20	
	Ciprofloxacin-d_8	*340.1 > 322.1*	*20*	-	-	-
Antibiotic	Clarithromycin[a]	590.0 > 158.0	20	590.0 > 116.0	25	5
				590.0 > 98.0	25	
Antibiotic	Clindamycin	425.1 > 126.0	20	425.1 > 337.0	20	5
				425.1 > 389.0	15	
Nonsteroidal anti-inflammatory	Diclofenac	296.2 > 214.2	30	296.2 > 250.0	10	5
				296.2 > 278.0	5	
	Diclofenac-d_4	*300.1 > 219.2*	*20*	-	-	-
Antihypertensive	Enalapril	377.0 > 234.0	15	377.0 > 117.0	25	5
				377.0 > 303.0	15	
Antibiotic	Erythromycin[a]	734.0 > 158.0	25	734.0 > 576.0	15	10
				734.0 > 558.0	15	
	Erythromycin-^{13}C-d_3	*738.1 > 161.9*	*35*	-	-	-
Antibiotic	Furaltadone	325.0 > 100.0	20	325.0 > 252.0	15	5
				325.0 > 281.0	10	

Table 1. (cont.). LC-MS/MS conditions (cone value 10V) for pharmaceuticals selected.

Family	Compounds	Transition (Q)	CE (eV)	Transition (q)	CE (eV)	LCL* (ng/L)
Antiepileptic	Gabapentin	172.0 > 137.0	15	172.0 > 154.2	15	5
				172.0 > 95.0	20	
Hypolipidemic agent	Gemfibrozil (-)	249.0 > 113.0	10	249.0 > 121.0	20	1000
				249.0 > 127.0	10	
Antihypertensive	Irbesartan	429.0 > 207.0	25	429.0 > 195.0	20	5
				429.0 > 180.0	25	
	Irbesartan-d_6	*435.1 > 213.3*	*25*	-	-	-
Nonsteroidal anti-inflammatory	Ketoprofen (-)	253.0 > 79.0	10	253.0 > 92.0	20	1000
				253.0 > 209.0	10	
				253.0 > 209.0	10	
Anthelmintic agent	Levamisole	205.0 > 178.0	20	205.0 > 91.0	25	5
				205.0 > 123.0	25	
	Cocaethylene-d_8	*326.0 > 204.0*	*20*	-	-	-
Antibiotic	Lincomycin	407.0 > 126.0	20	407.0 > 359.0	15	5
				407.0 > 389.0	15	
Benzodiazepine	Lorazepam	321.0 > 275.0	20	321.0 > 303.0	15	10
				321.0 > 229.0	25	
Antihypertensive	Losartan	423.1 > 207.1	15	423.1 > 377.1	15	5
				423.1 > 405.1	10	
Beta-blocker agent	Metropolol	268.2 > 116.0	15	268.2 > 74.0	20	5
				268.2 > 191.0	15	
Antibiotic	Metronidazole	172.0 > 127.9	15	172.0 > 82.1	20	5
				172.0 > 55.9	20	
Antibiotic	Nalidixic acid	233.0 > 187.0	25	233.0 > 215.0	10	5
				233.0 > 159.0	25	
Nonsteroidal anti-inflammatory	Naproxen (-)	229.0 > 170.0	20	229.0 > 185.0	12	1000
				185.0 > 169.0	20	
Antibiotic	Norfloxacin	320.0 > 233.0	25	320.0 > 276.0	15	50
				320.0 > 302.0	20	
	Norfloxacin-d_5	*325.0 > 238.0*	*20*	-	-	-
Antiulcer drug	Omeprazole sulfide, 4-hydroxy[a]	316.0 > 168.0	20	316.0 > 149.0	20	5
				316.0 > 283.0	15	
	Omeprazole-d_3	*349.0 > 198.0*	*10*	-	-	-
Antibiotic	Oxolinic acid	262.0 > 216.0	25	262.0 > 244.0	15	5
				262.0 > 158.0	25	
Antiulcer drug	Pantoprazole	384.0 > 200.0	10	384.0 > 138.0	25	5
				384.0 > 153.0	15	
Nonsteroidal anti-inflammatory	Phenazone	189.3 > 131.1	20	189.3 > 104.1	20	10
				189.3 > 58.1	20	
Antiepileptic	Primidone	219.2 > 162.0	10	219.2 > 91.0	20	5
				219.2 > 119.2	15	

Table 1. (cont.). LC-MS/MS conditions (cone value 10V) for pharmaceuticals selected.

Family	Compounds	Transition (Q)	CE (eV)	Transition (q)	CE (eV)	LCL* (ng/L)
Antibiotic	Roxithromycin	679.0 > 158.0	25	679.0 > 116.0	25	5
				679.0 > 98.0	25	
Beta-blocker agent	Salbutamol	240.0 > 148.0	15	240.0 > 222.1	10	5
				240.0 > 166.1	10	
Antibiotic	Sulfadiazine	251.0 > 156.0	15	251.0 > 92.0	25	5
				251.0 > 108.0	20	
Antibiotic	Sulfamethoxazole	254.0 > 92.0	25	254.0 > 156.0	15	5
				254.0 > 108.0	20	
	Sulfamethoxazole-$^{13}C_6$	*260.0 > 162.0*	*15*	-	-	-
Antibiotic	Tetracycline	445.0 > 154.0	25	445.0 > 410.0	15	5
				445.0 > 427.0	10	
Analgesic	Tramadol	264.0 > 58.0	10	264.0 > 121.0	25	5
				264.0 > 246.0	10	
Antibiotic	Trimetroprim	291.0 > 123.0	25	291.0 > 230.0	20	5
				291.0 > 261.0	25	
Antihypertensive	Valsartan	436.0 > 207.0	25	436.0 > 235.0	15	5
				436.0 > 261.0	15	
	Valsartan-d_8	*444.0 > 207.0*	*25*	-	-	-
Antidepressant	Venlafaxine	278.0 > 58.0	15	278.0 > 260.0	10	5
				278.0 > 121.0	25	
	Venlafaxin-d_6	*284.3 > 64.1*	*25*	-	-	-

Notes: All compounds were measured in positive mode, with the exception of 4 compounds that were measured in negative mode (marked as (–)). Quantification (Q) and confirmation (q) transitions. Collision energy (CE). Lowest calibration level (LCL, *x5 for raw and x2 for treated samples), estimated as the limit of quantification. In italic, ILIS used for quantification of their corresponded analyte.

[a] Compounds included in the Watch List of the Commission Decision 2018/840 [13].

2.2. Description of the wastewater treatment plant

The WWTP from Ricao, located in Asturias (Northern Spain), treats urban wastewater of different municipalities belonging to the public sanitation system of the Güeña, Sella and Piloña rivers. The WWTP also receives different authorized industrial discharges, mainly related to the chemical, pharmaceutical, food and services sectors, so the characteristics of their discharges are usually heterogenous.

The WWTP Ricao is designed to treat discharges from an equivalent population of 54,000 inhabitants. Its maximum pre-treatment flow rate is 41,208 m^3/day and a maximum of 20,640 m^3/day when an A20 type biological process with anaerobic, anoxic chambers and aerated carousel channels is applied. The biological process is designed for organic matter, nitrogen

and phosphorus removal. This treatment is a conventional treatment of active sludge, which incorporates at the reactor inlet an anaerobic zone that receives the influent residual water and the recirculated sludge, producing the fermentation reaction and phosphate elimination. The biological reactor has a capacity of 16,076 m^3 and the biologically treated effluent ends in two circular decanters (28 meters in diameter and 3,50 m of useful height). The treated water from the WWTP is discharged to the Sella River.

The quality parameters of the effluent of the WWTP must be in accordance with the discharge authorization nº V/33/01838 of 21 April 2015 (see **Table S1** in SM), which mainly includes the parameters defined in the Water Framework [18], such as biochemical or chemical oxygen demand (BOD5 or COD), organic matter, suspended solids and nutrients (nitrogen and phosphorus).

2.3. Sample collection

A preliminary sampling and HRMS screening was carried out before performing the three campaigns of quantitative analysis. To this aim, a 24-h composite IWW and a 24-h composite EWW sample from the WWTP Ricao were collected in June 2018. In addition, a 24-h composite sample from a continuous discharge of a hospital located in the surrounding area was also collected. 24-h composite wastewater samples were collected using a time-proportional sampling mode (100 mL, every 15 min). All these samples were screened by LC-QTOF MS. For quantitative LC-MS/MS analyses, IWW and EWW samples (24-h composite) were collected over seven consecutive days along three campaigns: 1st (September 2018), 2nd (January 2019) and 3rd (April 2019). Additionally, in the 1st campaign, seven 24-h composite samples reaching the WWTP from the hospital were also collected. **Table S2** in SM shows sampling dates and the corresponding wastewater flow rates.

All samples were collected in high-density polyethylene bottles, stored at <−20 °C, and transported to the laboratory after the last sample of the week was collected. Upon reception in the laboratory, samples were stored in the dark at -20 °C until analysis (i.e. within 2 weeks).

2.4. Sample treatment

A generic solid-phase extraction (SPE) procedure based on Gracia-Lor et al. [21] was applied for the screening analysis. In order to reduce matrix complexity, IWW and hospital discharge samples were previously diluted x4 with Milli-Q water.

The procedure for quantitative determination of pharmaceuticals was based on those previously developed by our research group [7], [8], employing direct injection of the (diluted) samples. In this work, a simple dilution x5 (IWW and hospital discharge) or x2 (EWW) with Milli-Q water was made in order to reduce matrix complexity.

More details are included in SM and Section 2.4.

2.5. Instrumentation

Qualitative screening was performed using a Waters Acquity UPLC (Waters Corp.) interfaced to a hybrid quadrupole-TOF mass spectrometer (Xevo G2 QTOF, Waters Corp.), using a Z-spray electrospray (ESI) was used. Two acquisition functions with different collision energies were used for MS^E experiments: the low energy (LE), selecting a collision energy of 4 eV in order to obtain information about the protonated molecule and adducts (if present), and the high energy (HE) function, with a collision energy ramp ranging from 15 to 40 eV, in order to obtain a greater range of fragment ions. The LE and HE functions settings were for both a scan time of 0.3 s

Quantitative analyses were performed using a Waters Acquity H-Class UPLC (Waters Corp.), equipped with a binary pump system, was interfaced to a triple quadrupole (Xevo TQ-STM, Waters Corp.) mass spectrometer (Waters Corp.) with an ESI source. See SM for more details.

2.6. Analysis

2.6.1. Qualitative screening: QTOF data processing

Accurate-mass data provided by QTOF, generated at low and high collision energy (MS^E mode) during the same run, were processed using ChromaLynx XS software (within MassLynx) in combination with a homemade database containing around 1.000 pharmaceuticals and 250 metabolites [24], [28]. The data were automatically processed and the chromatograms obtained (Extracted Ion Chromatogram, EIC) with a narrow mass window

(nw-EIC) of 20 mDa for each *m/z* ion selected. The different approaches of data processing are described in SM.

2.6.2. Target quantitative analysis

On the basis of screening results, 40 pharmaceuticals were selected in order to perform quantitative target analysis by LC-MS/MS. The experimental conditions are shown in **Table 1**. In order to facilitate accurate quantification, up to fourteen isotopically labeled internal standard (ILIS) were used for matrix effects correction. All compounds, including ILIS, were measured in positive ionization mode, with only 4 exceptions as shown in **Table 1**.

Quality controls (QC) consisted of two samples of different type, each fortified at two levels: 0.5 and 5 μg/L (IWW), and 0.2 and 2 μg/L (EWW). QCs recoveries between 60% and 140% were considered satisfactory [43].

For fourteen pharmaceuticals (see **Table 1**), quantification was performed using internal standard method with their corresponding ILIS. In the case of levamisole, cocaethylene-d8 was used as ILIS based on our previous experience [7]. The rest of compounds were quantified by external standards with calibration curves prepared in solvent. The limit of quantification was estimated from the lowest calibration level (LCL) taking into account the sample dilution: LCLx5 (for IWW and hospital discharge) and LCLx2 (for EWW). Positive samples will be considered as "detected" when the concentration was below LCL and at least one q/Q ratio was accomplished. For the constructions of graphs, the detected positives were given the value of half of their LCL.

3. Results and discussion

3.1. Preliminary QTOF screening

With the objective to identify a large number of pharmaceuticals in wastewaters, three different sample types (hospital discharge, IWW and EWW) were subjected to wide-scope screening by LC-QTOF MS after SPE pre-concentration to enable the detection of analytes at the low concentrations normally present.

A large number of pharmaceuticals and relevant metabolites from different therapeutic groups were investigated. Several compounds could be confirmed by comparison of retention time and experimental fragments because the reference standard was available at the laboratory. However, other compounds could only be tentatively identified (suspect screening) due to the lack of analytical standard (**Table 2)**.

Table 2. Pharmaceuticals identified in wastewater samples from the WWTP after UHPLC-QTOF MS screening.

IWW	**EWW**	**Hospital discharge**	
4-FAA	*4-FAA*	*4-AA*	Levofloxacin[a]
4-AAA	*4-AAA*	*4-FAA*	Lidocaine[a]
Acetaminophen	Amperozide[a]	*4-MAA*	**Losartan**
Amperozide[a]	**Carbamazepine**	*4-AAA*	*Meclofenamic acid*[a]
Diclofenac	*Clopidogrel carboxylic acid*	**Acetaminophen**	**Metronidazole**
Fenofibric acid	**Diclofenac**	*Acethyl-sulfamethoxazole*[a]	**Naproxen**
Gabapentin	**Gabapentin**	Amoxicilline[a]	Nemantine[a]
Gemfibrozil	**Irbesartan**	Amperozide[a]	*o-Desmethyl venlafaxine*
Irbesartan	Lamotrigine[a]	Atenolol[a]	Ofloxacin[a]
Ketoprofen	*Meclofenamic acid*	**Atorvastatin**	***Omeprazole sulfide 4-OH***
Naproxen	Narasin[a]	**Ciprofloxacin**	Oxcarbazepine[a]
Narasin[a]	*o-Desmethyl venlafaxine*	*Clopidogrel carboxylic acid*	Pregabalin[a]
o-Desmethyl venlafaxine	Oxcarbazepine[a]	**Diclofenac**	Propanolol[a]
Oxcarbazepine[a]		Eprosartan[a]	Quetiapine[a]
Venlafaxine		Esomeprazole[a]	Rimantadine[a]
		Fenofibric acid	**Sulfamethoxazole**
		Gabapentin	Sulfapyridine[a]
		Gemfibrozil	**Trimethoprim**
		Irbesartan	**Valsartan**
		Ketoprofen	**Venlafaxine**

Notes: 4-AA: 4-aminoantipyrine; 4-AAA: 4-acethylaminoantipyrine; 4-FAA: 4-formylaminoantipyrine; 4-MAA: 4-methylaminoantipyrine. Metabolites are shown in italic. In bold, pharmaceuticals included in the subsequent quantitative analysis by UHPLC-MS/MS.
[a] Suspect compound, tentative identification.

In such cases, the presence of the protonated molecule and fragment ions was evaluated in the low energy (LE) and high energy (HE) functions, respectively, as well as the characteristic isotope pattern when Cl or Br were present. Tentative identification was based on the information obtained by LC-QTOF MS (i.e. accurate mass of the protonated molecule and

fragment ions), which was compared with online databases, such as MassBank or MetLin, or previously reported fragments in the literature. In total, 40 pharmaceuticals and/or their metabolites were identified in the three samples studied. 17 out of 40 compounds could be confirmed with their corresponding reference standard, while the remaining were tentatively identified on the basis of the accurate mass information provided by QTOF MS. The compounds confirmed with standards corresponded to pharmaceuticals and/or metabolites commonly found in wastewaters [11], [27], [28], [38], [41], [7], [8]. As expected, the greatest number of pharmaceuticals was found in the hospital wastewater, while the EWW presented the lowest number of positives.

As an example, **Fig. 1** shows a finding of the analgesic acetaminophen in hospital wastewater (chromatographic peak at 2.00 min). It can be observed the presence of the protonated molecule and several fragment ions, all with mass errors <5 ppm, in the HE spectrum (**Fig. 1**a, top) and the LE spectrum (**Fig. 1**a, bottom) of this peak. The nw-XICs for five *m/z* fragment ions are also depicted and were perfectly aligned, demonstrated that they all come from the same compound **(Fig. 1**b).

Fig. 2 illustrates the process followed for the tentative identification, taking as example an angiotensin II receptor antagonist found in the hospital discharge water sample. The LE spectrum in ESI positive of the chromatographic peak detected at 7.41 min, showed an abundant signal at *m/z* 425.1542 (**Fig. 2**a, bottom). This would correspond to the protonated molecule of eprosartan ($C_{23}H_{25}N_2O_4S^+$, with a mass error of 1.6 ppm in relation with its theoretical exact mass). The HE spectrum showed four fragment ions at *m/z* 295.1447 ($C_{18}H_{19}N_2O_2^+$, 0 ppm), 273.1059 ($C_{15}H_{17}N_2OS^+$, -1.1 ppm), 207.1131 ($C_{11}H_{15}N_2O_2^+$, -1.4 ppm) and 135.0445 ($C_8H_7O_2^+$, -0.7 ppm) (**Fig. 2**a, top). As it can be seen, the structure of these fragment ions was justified on the basis of their measured accurate masses, and all were compatible with the structure of the candidate compound. In addition, the four fragment ions were in accordance with the scientific literature (MassBank [33]). All these data strongly support the tentative identification of the compound as eprosartan.

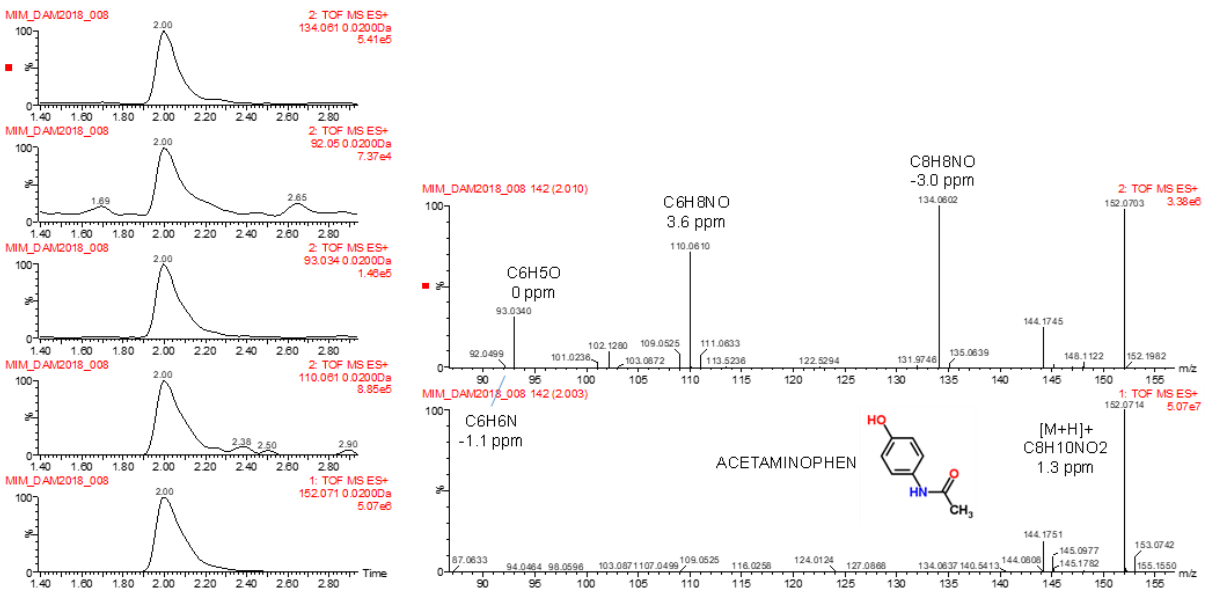


Fig. 1. Detection and identification of acetaminophen in the analysis by LC-QTOF MS of the sample corresponded to the hospital discharge. (a) LE (bottom) and HE (top) mass spectra of the chromatographic peak at retention time 2.00 min. (b) XICs with 0.02 Da mass window for the protonated molecule in LE and different ions observed in HE.

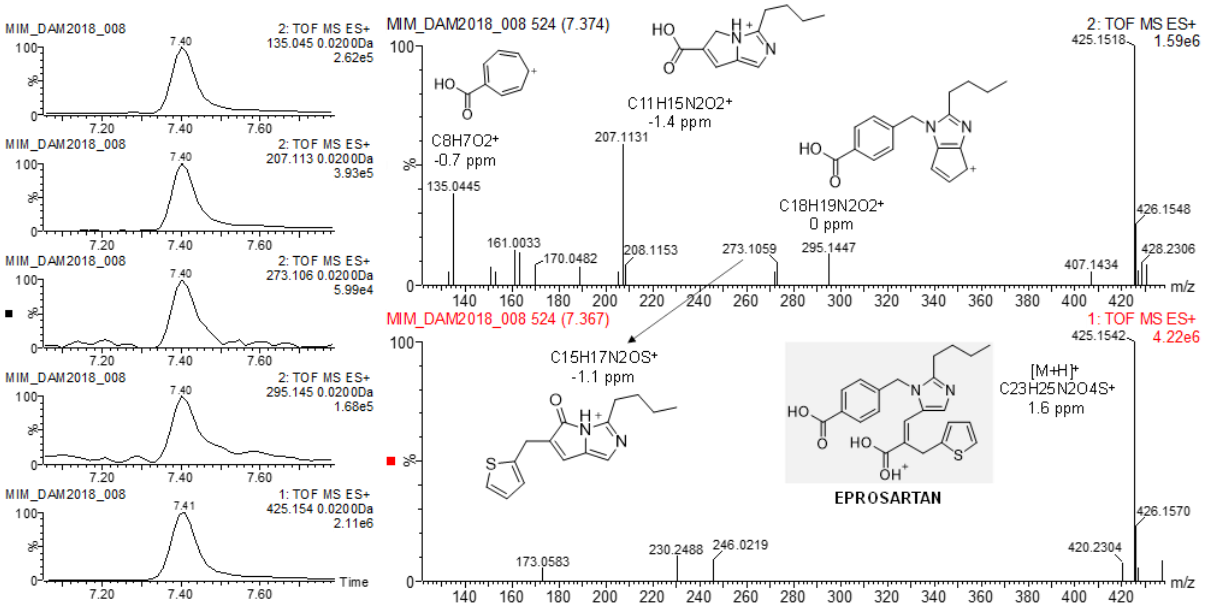


Fig. 2. Detection and tentative identification of eprosartan in the analysis by LC-QTOF MS of the sample corresponded to the hospital discharge. (a) LE (bottom) and HE (top) mass spectra of the chromatographic peak at retention time 7.41 min. (b) XICs with 0.02Da mass window for the protonated molecule in LE and different ions observed in HE.

From the results of the wide-scope screening, a list of pharmaceuticals was established to perform target quantitative analysis in the next monitoring campaigns. In total, 40 compounds were selected including the 17 pharmaceuticals that were identified and confirmed by QTOF MS. Those compounds tentatively identified that could not be confirmed due to the absence of reference standards in our laboratory, remained as priority compounds for subsequent works in the area, because of the working calendar did not allow us to wait the acquisition of such new standards. The rest of the pharmaceuticals until completing the list of target compounds were added based on our previous experience on wastewater analysis from different WWTPs, and on their occurrence in such type of samples (their reference standards were also available in our laboratory).

3.2. Quantitative analysis by LC-MS/MS

3.2.1. Quality control analysis

The analytical methodology applied for the quantitative determination of the 40 pharmaceuticals has been previously developed and validated in our laboratory [7], [8], where particular attention was paid to the evaluation of the matrix effects. Due to the high complexity and variability of the sample matrices studied in the present work, special emphasis was made on the analysis of representative quality control sample in order to support the reliability of quantitative data reported (see Section 2.6.2). **Table 3** summarizes the average QCs recoveries for IWW and EWW, which were in general, satisfactory with values between 60% and 140% [43] (See **Tables S3-S5** in SM for detailed information).

Table 3. Average recoveries (%) of QCs analyzed in the three sampling campaigns for wastewaters (IWW and EWW) from the WWTP.

		IWW		**EWW**	
Compounds	**ILIS**	**0.5 µg/L**	**5 µg/L**	**0.2 µg/L**	**2 µg/L**
Acetaminophen	Acetaminophen-d_4	92	100	83	100
Alprazolam	-	87	107	84	94
Atorvastatin	Atorvastatin-d_5	106	112	90	88
Azitromycin	Azitromycin-d_3	***34***[a]	***48***[a]	***30***[a]	65[a]
Bezafibrate	-	102[b]	78[a]	136[b]	82[a]
Carbamazepine	Carbamazepine 10,11-epoxide-d_{10}	113	[c]	109	[c]
Clarithromycin	-	127[a]	***161***[a]	122	***145***[b]
Clindamycin	-	105	119	117	121

Table 3 (cont.). Average recoveries (%) of QCs analyzed in the three sampling campaigns for wastewaters (IWW and EWW) from the WWTP.

Compounds	ILIS	IWW		EWW	
		0.5 µg/L	**5 µg/L**	**0.2 µg/L**	**2 µg/L**
Diclofenac	Diclofenac-d_4	94	109	102	110
Enalapril	-	83	87	85	87
Erythromycin	Erythromycin-$^{13}Cd_3$	83	94	82	107[a]
Furaltadone	-	107	106	106	105
Gabapentin	-	114	115	133	113
Gemfibrozil	-	-	112[b]	-	101[b]
Irbesartan	Irbesartan-d_6	85	121	117	107
Ketoprofen	-	-	109[b]	-	84[b]
Levamisole	Cocaethylene-d_8	95	127	107	136
Lincomycin	-	120	124	110	101
Lorazepam	-	105	80	77	94
Losartan	-	88	90	89	91
Metoprolol	-	102[b]	119[b]	104[b]	127[b]
Metronidazole	-	98	102	100	106
Nalidixic acid	-	84	84	81	78
Naproxen	-	-	67[a]	-	90
Omeprazole sulfide, 4-OH	Omeprazole-d_3	100	87	97	89
Oxolinic acid	-	74	70	79	70
Pantoprazole	-	104	99	103	82
Phenazone	-	101	113	87	98
Primidone	-	98	99	91	97
Roxithromycin	-	114[a]	127[b]	139	***145***[b]
Salbutamol	-	102	118	131	126
Sulfadiazine	Sulfamethoxazole-$^{13}C_6$	95	100	80	90
Sulfamethoxazole	Sulfamethoxazole-$^{13}C_6$	107	109	103	109
Tetracycline	-	80	81	89	69
Tramadol	-	104[b]	112[b]	107[b]	116[b]
Trimetroprim	-	123[b]	***149***[b]	125[b]	***156***[b]
Valsartan	Valsartan-d_8	78	91	90	91
Venlafaxine	Venlafaxin-d_6	86	111	84	110

Notes: In bold and italic, recoveries out of accepted range (60-140 %) are shown. – Value not available due to the lack of sensitivity, which prevents reaching the lowest concentrations tested.
[a] Average of two available values.
[b] Only one available value.
[c] Not calculated due to lack of linearity at high concentration levels.

The complexity of the matrix samples analysed in combination with the low analyte concentrations make this type of analysis complicated, being quite difficult finding a

compromise to get fully satisfactory data for all compounds. Thus, some exceptions, among the 40 pharmaceuticals investigated, were observed. The most remarkable were the antibiotics ciprofloxacin and norfloxacin, for which poor reproducibility and average recoveries out of the established range 60-140% were obtained. Three more compounds, all analysed in negative ESI (gemfibrozil, ketoprofen and naproxen), could not be properly evaluated due to the lack of sensitivity in negative ionization mode at the fortified levels tested. The antibiotics clarithromycin, roxithromycin and trimetroprim presented average recoveries near the acceptable range, but slightly greater than 140%, especially at the high fortification levels. A possible explanation could be that these antibiotics are more prone to matrix enhancement resulting in apparent higher recoveries, which could not be corrected due to the lack of analyte ILIS. For the antibiotic azithromycin, the average recovery was also near the acceptable range, but slightly below 50%.

Regarding the impact of the above mentioned exceptions in data reported, it was limited to only those cases where positive detections were found. The most noticeable corresponded to the antibiotics ciprofloxacin and norfloxacin, which could not be quantified with the required accuracy, despite being found in all samples at relatively high concentrations. For these two compounds, guidance data are presented, which should be considered as approximate concentration range.

3.2.2. Occurrence of pharmaceuticals in wastewaters

A total of 21 IWW 24-h composite samples were collected from the WWTP along the three sampling campaigns (see Section 2). During the same period, 21 EWW 24-h composite samples were also collected in order to evaluate the removal efficiency of the WWTP. **Table 4** summarizes the average weekly concentrations of pharmaceuticals in IWW and EWW samples in the three sampling campaigns. For more details see **Tables S6-S11** in SM. As it can be seen, 34 out of 40 pharmaceuticals were found, illustrating the wide presence of these emerging contaminants in wastewater, even after the treatment applied in the WWTP based on a combined biological process (anaerobic-anoxic-aerobic). Among them, the four antibiotics included in the European Watch List [13] – the fluoroquinolone ciprofloxacin and three macrolides, erythromycin, clarithromycin and azithromycin – were also found. Only six compounds from the target list were not detected in any of the samples analyzed: three

antibiotics (furaltadone, lincomycin and roxithromycin), two hypolipidemic agents (bezafibrate and gemfibrozil) and one anti-inflammatory (ketoprofen).

Table 4. Average weekly concentrations (ng/L) of pharmaceuticals in influent and effluent wastewater samples from the WWTP in the three sampling campaigns.

	IWW				EWW			
Compounds	**1st**	**2nd**	**3rd**	**Average**	**1st**	**2nd**	**3rd**	**Average**
Acetaminophen	6490	4564	5030	**5361**	-	d	-	**d**
Alprazolam	-	d	-	**d**	d	d	d	**d**
Atorvastatin	87	d	88	**88[a]**	d	-	-	**d**
Azitromycin	186	328	-	**257[a]**	-	d	-	**d**
Bezafibrate	-	-	-	**-**	-	-	-	**-**
Carbamazepine	d	-	-	**d**	d	-	d	**d**
Ciprofloxacin	149,700	8191	1270	**53,054**	3640	2242	700	**2194**
Clarithromycin	97	192	-	**145[a]**	48	107	37	**64**
Clindamycin	-	-	-	**-**	d	-	d	**d**
Diclofenac	232	56	223	**170**	143	26	126	**98**
Enalapril	50	-	29	**40[a]**	-	-	-	**-**
Erythromycin	25	64	d	**45[a]**	28	d	13	**21[a]**
Furaltadone	-	-	-	**-**	-	-	-	**-**
Gabapentin	4013	1836	3775	**3208**	1555	528	1125	**1069**
Gemfibrozil	-	-	-	**-**	-	-	-	**-**
Irbesartan	223	63	181	**156**	175	57	159	**130**
Ketoprofen	-	-	-	**-**	-	-	-	**-**
Levamisole	29	-	-	**29[b]**	28	d	13	**21[a]**
Lincomycin	-	-	-	**-**	-	-	-	**-**
Lorazepam	34	-	25	**30[a]**	44	d	21	**33[a]**
Losartan	168	27	67	**87**	12	10	15	**12**
Metoprolol	d	d	d	**d**	d	d	d	**d**
Metronidazole	d	37	54	**46[a]**	d	d	d	**d**
Nalidixic acid	-	-	-	**-**	d	-	-	**d**
Naproxen	2365	-	-	**2365[b]**	-	-	-	**-**
Norfloxacin	880	10,386	530	**3932**	800	2455	350	**1202**
Omeprazole sulfide. 4-OH	66	d	50	**58[a]**	38	d	54	**46[a]**
Oxolinic acid	-	d	-	**d**	-	-	15	**15[b]**
Pantoprazole	-	d	d	**d**	19	d	d	**19[a]**
Phenazone	32	42	-	**37[a]**	d	-	-	**d**
Primidone	76	-	50	**63[a]**	72	11	40	**41**
Roxithromycin	-	-	-	**-**	-	-	-	**-**
Salbutamol	d	d	d	**d**	d	-	d	**d**
Sulfadiazine	-	-	d	**d**	-	-	d	**d**
Sulfamethoxazole	74	d	34	**54[a]**	33	13	14	**20**
Tetracycline	44	103	55	**67**	19	-	16	**18**
Tramadol	625	119	471	**405**	594	112	398	**368**
Trimetroprim	137	96	231	**155**	15	21	37	**24**
Valsartan	507	136	446	**363**	26	31	37	**31**
Venlafaxine	162	43	123	**109**	172	35	119	**109**

Notes: d: detected, not quantified. Concentration below LCL and at least one q/Q ratio was accomplished. Underlined: estimated concentration.

[a] Average data from two samplings.

[b] Data from only one sampling.

In IWW samples, the highest concentrations corresponded to the analgesic acetaminophen (5.4 μg/L), the anti-inflammatory naproxen (2.4 μg/L) and the antiepileptic gabapentin (3.2 μg/L). The majority of the pharmaceuticals showed markedly lower average concentrations in treated waters, which indicates that most of them were eliminated/retained in the WWTP, at least partially. Thus, in EWW most concentrations did not exceeded the average weekly value of 0.1 μg/L, with a few exceptions such as gabapentin (1.1 μg/L), irbesartan (0.13 μg/L) and tramadol (0.37 μg/L). Several compounds, such as clindamycin, levamisole, lorazepam, oxolinic acid, pantoprazole, tramadol and venlafaxine, were found at similar concentration levels in IWW and EWW, or even at higher concentrations in EWW, which suggests the non-removal of these compounds using the primary treatment applied in the WWTP. Pharmaceutical elimination in WWTPs is probably a complex process as many plants are equipped with the main objective of removing biodegradable carbon, nitrogen and phosphorus compounds and microbiological organisms [37] and not equipped to remove complex contaminants. The finding of higher concentrations in the treated water has been reported several times in the scientific literature [22], [29], [8]. The low removal efficiency of the WWTP for these compounds together with the possible release of conjugates (usually glucuronides and sulphates) during the treatment of wastewater might be possible causes of the increase in concentrations [30], [48]. In addition, matrix effects (commonly ionization suppression) are much higher in IWW than in EWW which may hamper the detection/quantification of some compounds in IWW, particularly when they are present at very low concentrations [5].

Special attention should be paid to the presence of antibiotics in wastewater, especially EWW, due to their potential hazardous to the aquatic environment. Recent investigations show that WWTPs constitute hotspots for antibiotic emissions, contributing to the enrichment of resistance genes in surface water ecosystems [9]. In Spain, several macrolide antibiotics were determined [23], being azithromycin the compound detected at the highest concentration level, both in IWW and EWW. Moreover, Rodriguez-Mozaz et al. performed a comprehensive monitoring of antibiotics in wastewater samples of WWTPs from 7 European countries, where Spain presented the highest concentrations for azithromycin, ciprofloxacin, clindamycin, clarithromycin, metronidazole and sulfamethoxazole [42]. The results obtained in these works were in agreement with the present study, where thirteen of the 16 antibiotics investigated were

detected in both IWW and EWW and of which azithromycin, ciprofloxacin, trimethoprim, clarithromycin and norfloxacin showed in general the highest concentrations.

The comparison of average concentrations for the several pharmaceutical families studied allows to obtain interesting conclusions (see **Fig. 3**). The season with lowest total concentrations for nearly all families of compounds was winter (green bars, 2nd campaign, January 2019), in both IWW and EWW, but there was an evident exception with the group of antibiotics, which concentrations in wastewater were notably higher in winter. A fact that is not surprising due to the expected increase in the prescription of antibiotics to fight respiratory infections typically during winter. It is also illustrative, by comparing the top and bottom graphics, the notable decrease in concentrations for all families in the EWW (bottom). This evidences a certain removal efficiency in the WWTP as will be discussed in **Section 3.2.4**.

Although the results obtained in this study correspond to the dissolved phase of wastewater samples, in every campaign a preliminary analysis of a sludge sample was also performed. Compared to the wastewater analyzed at the same period, much less pharmaceuticals could be quantified in the particulate material, surely due to their absence or their very low concentrations. This could be explained by the medium-high polarity of the compounds under study, making them more soluble in the aqueous phase and being hardly adsorbed on the sludge. This suggests that analysis of the particulate phase should not significantly modify the results presented in this work.

3.2.3. Contribution of the hospital discharge

In the first campaign (September 2018), in addition to the seven IWW samples from the WWTP, another seven 24-h composite samples were collected from a continuous discharge of a hospital in the nearby area. The results of quantitative analysis by LC-MS/MS for IWW and hospital discharge samples are included in **Tables S6** and **S12**, respectively, in SM.

In the hospital samples, 28 out of the 40 pharmaceuticals investigated were detected. In general, pharmaceutical concentrations were similar along the sampling week. Some exceptions were erythromycin, losartan, pantoprazole, phenazone, sulfamethoxazole, trimethoprim and valsartan, which presented greater variations (RSD above 50%). The highest concentrations in hospital samples corresponded to the widely consumed analgesic

acetaminophen (159 μg/L), the antiepileptic gabapentin (23 μg/L) and the anti-inflammatory naproxen (2.9 μg/L).

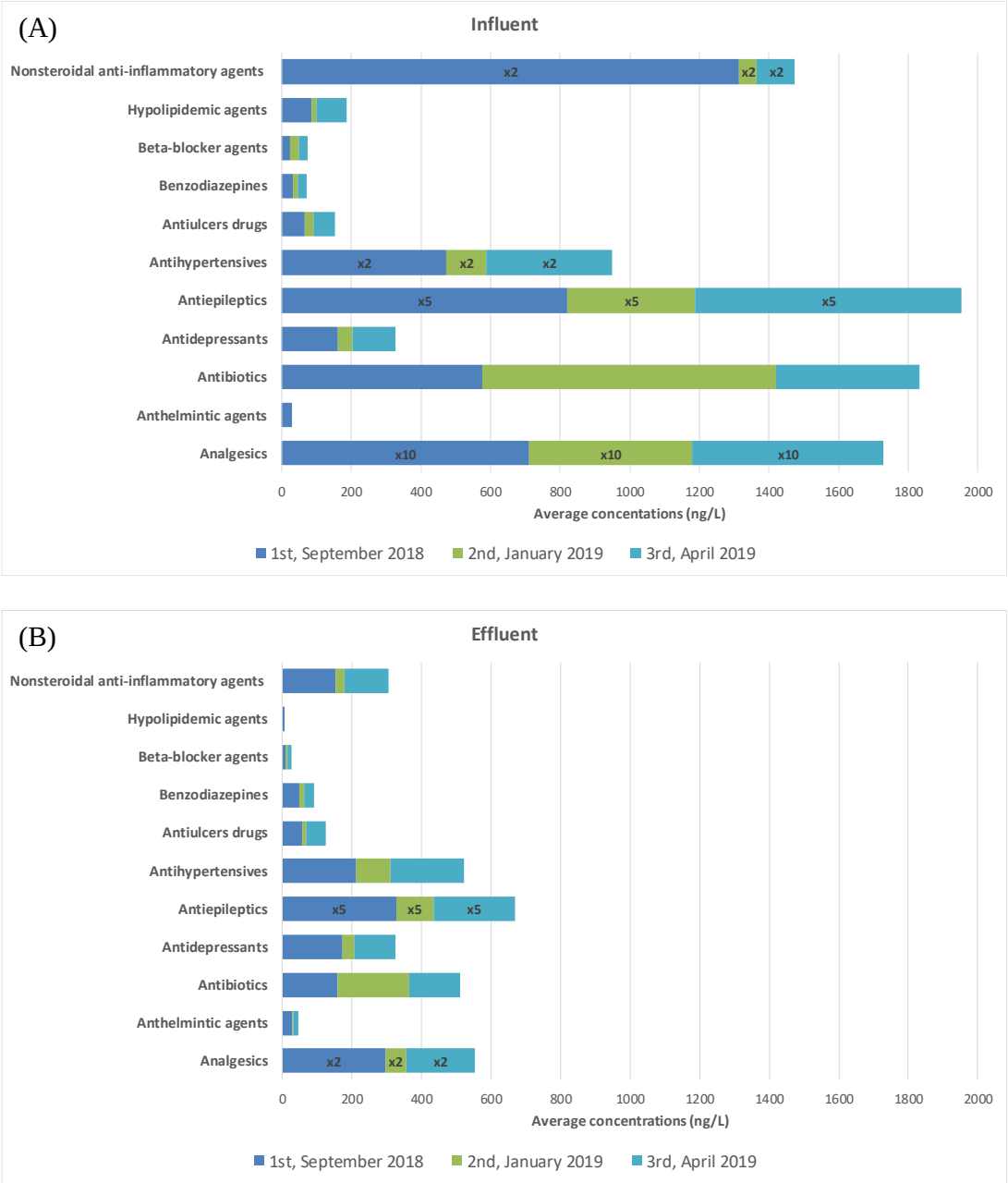


Fig. 3. Average concentrations of different pharmaceutical families in the influent (A) and the effluent (B) of the WWTP Ricao along three sampling campaigns. To build the graphs, data reported as "d" (detected) have been assigned a value equal to half of the LCL. Ciprofloxacin and norfloxacin have not been included in the total of antibiotics as their concentrations were indicative. The annotation (x2, x5 and x10) into the bars indicates that concentration level is 2, 5 or 10 times higher than the level presented in the graphic.

Similarly, 28 out the 40 compounds were also detected in IWW collected during the same days, of which 24 coincided with those found in hospital water. In general, the concentration levels in IWW were rather consistent throughout the whole week, with the exception of phenazone, which presented greater variation (RSD greater than 50%). Similarly to the hospital discharge, the highest concentrations corresponded to the analgesic acetaminophen (8.7 μg/L), the antiepileptic gabapentin (4.7 μg/L) and the anti-inflammatory naproxen (2.4 μg/L), whose concentrations were significantly lower than in the hospital wastewater, and in agreement with data reported in the literature (e.g. [16], [35], [44]).

Fig. 4 shows the average weekly concentrations of pharmaceuticals in the hospital discharge and in IWW during the first campaign. In order to represent the compounds detected (but not quantified), a concentration value equal to half of their LCL was estimated. In general, concentrations in the hospital samples were clearly higher than in IWW of the WWTP, except for five compounds – clarithromycin, irbesartan, levamisole, primidone and tetracycline – which showed mean concentrations slightly higher in the IWW. The results suggest that a large part of the pharmaceuticals studied reached the WWTP mainly through the discharge from the hospital. This was expected, and it is in agreement with Bellver-Domingo et al. [4], who reported hospitals as one of the main facilities that discharge anti-inflammatories into Valencian urban wastewater.

3.2.4. Estimation of removal efficiencies of pharmaceuticals in the WWTP

The efficiency of pharmaceuticals removal in a WWTP can be estimated from the compound concentrations and/or from pharmaceutical daily loads in IWW and in EWW. Most estimations are based on analyte concentrations [21], [39], [49], [6], [8], however in this study we have used daily loads (g/day), which were calculated taking into account the concentrations in wastewater and the daily flows of IWW and EWW. Although the use of concentrations is a useful approach, the estimation based on total loads seems more realistic as it takes into account the total amount of pharmaceuticals entering into the WWTP and the total loads in the treated water, and therefore it takes into account the influence of the amount of water in each case (see **Table S2** in SM). Thus, we compared the daily loads at the entrance and the exit of the next day, assuming a residence time at the WWTP of 24 h. From the seven daily loads, we are able to calculate the average daily loads for the whole week (g/day), which were finally used for

RE estimation. Data on daily and average weekly loads are shown in **Tables S13-S18** and **S19**, respectively, in SM, for the three sampling campaigns. From these data, the daily and average RE (%) were calculated for each campaign, as shown in **Tables S20-S22** of SM.

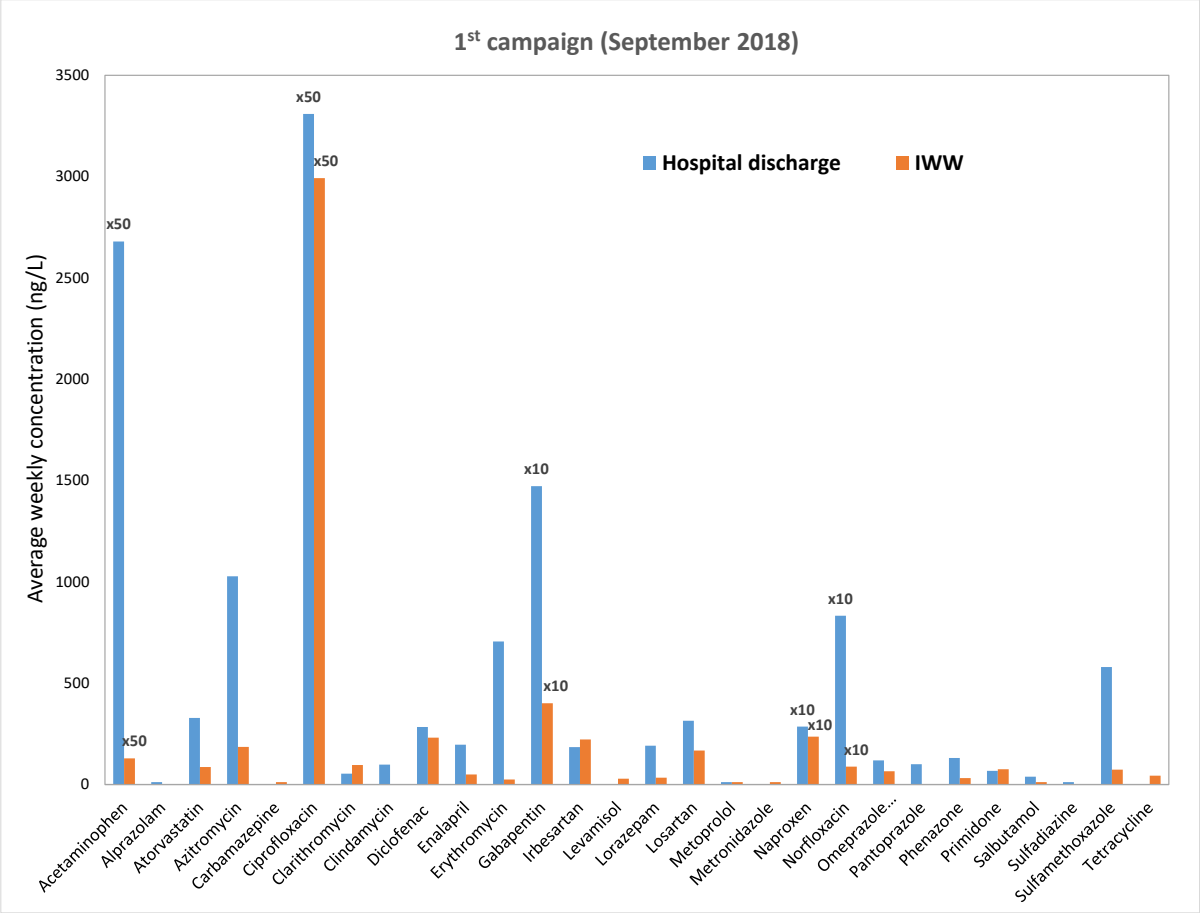


Fig. 4. Concentrations of pharmaceuticals (ng/L) calculated as the average of the seven days from the September campaign in hospital discharge and in IWW from the WWTP. To build the graph, data reported as "d" (detected) have been assigned a value equal to half of the LCL. Ciprofloxacin and norfloxacin data are indicative. The annotation (x10 and x50) on the bars indicates that concentration level is 10 or 50 times higher than the level presented in the graphic.

Fig. 5 shows the average RE of pharmaceuticals in each monitoring campaign (the two antibiotics with estimated concentrations, ciprofloxacin and norfloxacin, are not included). Different behaviours were observed, with a first group including 34% of the compounds which were removed almost completely, with average RE above 75% (acetaminophen, atorvastatin,

azithromycin, enalapril, losartan, metronidazole, naproxen, salbutamol, tetracycline, trimetroprim and valsartan). A second group included pharmaceuticals for which the elimination was not total, but greater than 50% (diclofenac, gabapentin and phenazone). Another six compounds presented slightly variable RE along the three campaigns, with a tendency to poor removal (RE ≤ 40%) (irbesartan, levamisole, lorazepam, primidone, tramadol and venlafaxine). A fourth group corresponded to 18% of compounds detected which did not seem to be eliminated, with RE near 0% or even negative RE (alprazolam, clindamycin, metoprolol, nalidixic acid, pantoprazole and sulfadiazine). The remaining analytes showed highly variable elimination data along the three sampling campaigns, with no clear tendency (carbamazepine, clarithromycin, erythromycin, omeprazole sulphide 4-OH, oxolonic acid and sulfamethoxazole).

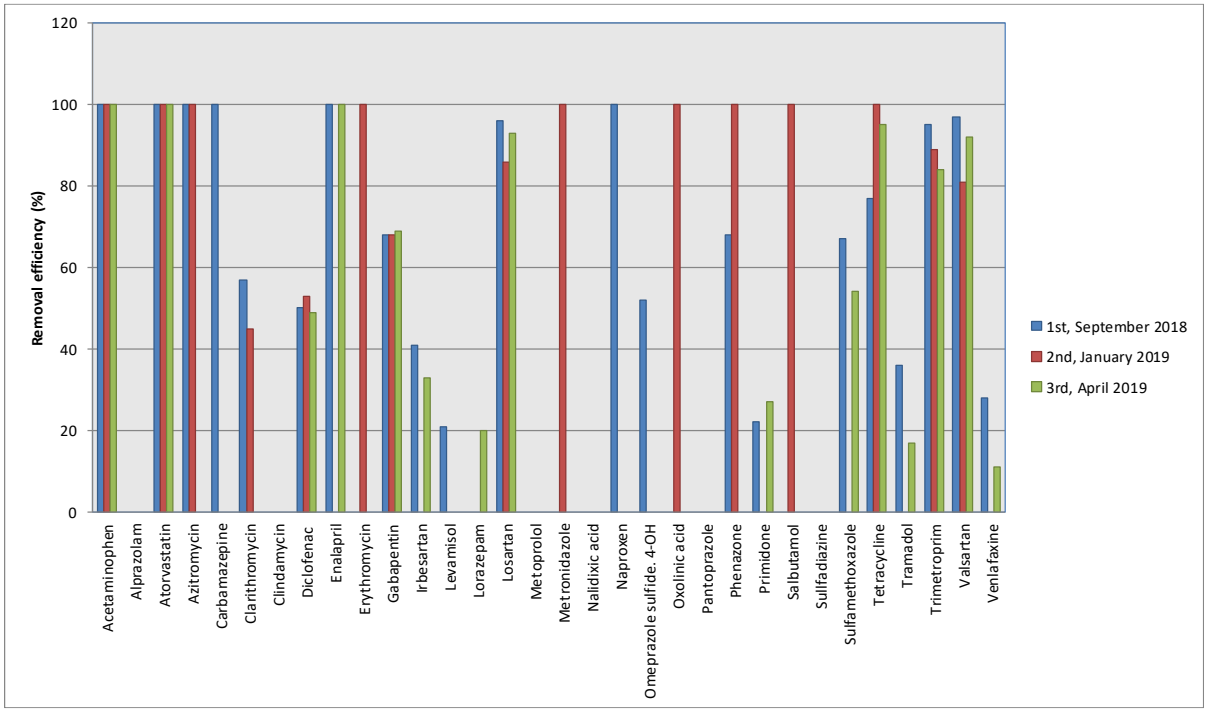


Fig. 5. Average removal efficiency (%) for pharmaceuticals in the WWTP estimated for the three monitoring campaigns (the absence of a bar indicates RE is near or below 0%).

In summary, 14 out of 32 pharmaceuticals detected, which account for 44% of the compounds, were removed (more than 50%) in the WWTP using a conventional treatment based on a combined biological process (anaerobic-anoxic-aerobic). The fact that RE were calculated

based on three weekly sampling campaigns in different periods of the year (i.e. different climatic conditions) makes data reported more robust, especially for those pharmaceuticals that showed consistent behavior. The results obtained are mostly in agreement with those reported elsewhere [8], [21], [22], [29], [31], [40], [45].

It is important to remark the potential impact on the aquatic environment of emerging contaminants present in treated wastewater. Although around half of the pharmaceuticals investigated in this work were partially or totally removed in the WWTP, the use of a secondary and an optional tertiary treatment process seems necessary in order to improve the removal of these compounds and to protect the environment, although those additional treatments are always associated with a higher cost [37]. Yet in the near future, the requirements of water quality will be surely modified and become stricter, especially in relation to pharmaceutical discharges from WWTPs, since the quality of wastewater effluent is of great relevance as it is one of the main sources of contamination to receiving surface water [17]. Frequent monitoring campaigns are needed to determine the quality of treated water in terms of emerging contaminants, but risk assessment studies also are required to establish the potential harmful effects on these compounds on the aquatic environment. Conducting monitoring campaigns making use of advanced analytical techniques will be necessary to update European regulations particularly in relation to the quality of wastewater effluents.

4. Conclusions

The occurrence of pharmaceuticals in wastewater from a conventional WWTP has been investigated, as well as their possible elimination as a result of the treatment applied. IWW and EWW samples from the WWTP were collected in three seasonal campaigns, as well as raw wastewater samples from a hospital discharge nearby the plant to evaluate the impact in terms of pharmaceuticals content. Due to the high number of pharmaceuticals that may be present in this type of samples, a preliminary wide-scope screening using LC-HRMS with QTOF MS was applied to identify the most relevant/abundant compounds in the samples. Based on data from the screening, 40 compounds were selected for subsequent target quantitative analysis by LC-MS/MS with QqQ.

Most of pharmaceuticals detected in IWW from the WWTP were identified in hospital discharge samples at concentrations significantly higher, which seems to indicate that a large part of pharmaceuticals reach the WWTP mainly through the discharge from the hospital.

The removal efficiency of pharmaceuticals was estimated from daily loads in the IWW and in EWW, which were calculated for the three one-week campaigns. From the 32 compounds detected in the water samples, the wide majority presented lower concentrations in treated water compared to raw wastewater. Thus, around 50% of the compounds were totally (>80%) or partially (RE > 50%) removed using the conventional biological treatment, but still a large number of compounds could not be efficiently eliminated. Most of concentrations in EWW did not exceed the average weekly value of 0.1 μg/L, with a few exceptions such as gabapentin (1.1 μg/L), irbesartan (0.13 μg/L) and tramadol (0.37 μg/L). Other compounds, such as clindamycin, levamisole, lorazepam, oxolinic acid, pantoprazole and venlafaxine, were found at similar concentrations in IWW and EWW, which suggests the non-removal of these compounds in the WWTP. The fact that some pharmaceuticals still remain in the treated wastewater may suppose a risk for the aquatic environment. Therefore, additional treatments are required to improve the removal of these emerging contaminants, as well as conducting periodically ambitious monitoring campaigns to evaluate the performance of the WWTP and the potential impact of treated water on the aquatic environment.

Finally, the study of seasonal variation demonstrated that concentration levels of antibiotics were notably higher in winter due to typical infections of that period of the year.

CRediT authorship contribution statement

Lubertus Bijlsma: Resources, Investigation, Formal analysis, Visualization, Writing - original draft. **Elena Pitarch**: Supervision, Visualization, Funding acquisition, Writing - original draft. **Eddie Fonseca**: Formal analysis, Resources, Writing - review & editing. **María Ibáñez**: Investigation, Formal analysis, Writing - review & editing. **Ana María Botero**: Formal analysis, Resources, Writing - review & editing. **Javier Claros**: Resources, Writing - review & editing. **Laura Pastor**: Resources, Writing - review & editing. **Félix Hernández**: Supervision, Visualization, Funding acquisition, Writing - review & editing.

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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SUPPLEMENTARY MATERIAL

Investigation of pharmaceuticals in a conventional wastewater treatment plant: removal efficiency, seasonal variation and impact of a nearby hospital

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2. MATERIALS AND METHODS

2.1. Pharmaceutical standards and reagents

Pharmaceutical reference standards were purchased from Sigma-Aldrich (St Louis, MO, USA), LGC Promochem (London, UK), Toronto Research Chemicals (Ontario, Canada), Across Organics (Geel, Belgium), Bayer Hispania (Barcelona, Spain), Fort Dodge Veterinaria (Gerona, Spain), Vetoquinol Industrial (Madrid, Spain) and Aventis Pharma (Madrid, Spain) and the isotope labelled internal standards (ILIS) were form CDN Isotopes (Quebec, Canada); Toronto Research Chemicals, Cambridge Isotope Laboratories (Andover, MA, USA) Sigma-Aldrich (St Louis, MO, USA) and Cerilliant (Texas, USA). All reference standards presented purity higher than 93%. Standard stock solutions of each compound were prepared at 500 mg/L in methanol or acetonitrile and were stored at -20°C. Intermediate solutions (50 mg/L) were prepared by dilution of the stock solution ten-fold with methanol. Mixed working solutions containing all analytes were prepared from intermediate solutions by appropriate dilution with water, and were used for preparation of the aqueous calibration standards and for spiking samples in the study.

HPLC-grade methanol (MeOH), HPLC-grade acetonitrile (ACN), formic acid (HCOOH, content > 98%) and ammonium acetate (NH_4AC, reagent grade), were purchased from Scharlab (Barcelona, Spain). HPLC grade water was obtained from distilled water passed through a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Description of the wastewater treatment plant

Table S1. Water quality parameters of effluent wastewater (EWW) from the wastewater treatment plant (WWTP) Ricao according to the discharge authorization nº V/33/01838 of 21 April 2015.

Parameter	Discharge limit
pH	Between 6-9
Suspended solids	< 15 mg/L
BOD_5	< 12 mg O_2/L
COD	< 60 mg O_2/L
Total ammonium	< 3 mg NH_4/L
Nitrates	< 35 mg NO_3/L
Kjeldahl nitrogen*	< 5 mg N/L
Total nitrogen	< 15 mg N/L
Total phosphorus	< 2 mg P/L

* The sum of ammonia-nitrogen plus organically bound nitrogen but does not include nitrate-nitrogen or nitrite-nitrogen

2.3. Sample collection

Table S2. Sampling campaigns of wastewater samples from the WWTP Ricao.

Date	Day	Input flow (m^3/day)		
		IWW-hospital	**IWW**	**EWW**
1st Campaign				
24/09/2018	Monday	na	22.120	16.980
25/09/2018	Tuesday	na	20.828	16.830
26/09/2018	Wednesday	na	17.184	18.110
27/09/2018	Thursday	na	17.992	16.680
28/09/2018	Friday	na	22.208	16.590
29/09/2018	Saturday	na	20.796	15.860
30/09/2018	Sunday	na	18.996	15.680
2nd Campaign				
28/01/2019	Monday	-	23.880	22.190
29/01/2019	Tuesday	-	20.140	19.810
30/01/2019	Wednesday	-	19.588	19.970
31/01/2019	Thursday	-	26.176	22.540
01/02/2019	Friday	-	21.984	21.100
02/02/2019	Saturday	-	30.480	22.550
03/02/2019	Sunday	-	23.480	21.486
3rd Campaign				
22/04/2019	Monday	-	20.412	19.840
23/04/2019	Tuesday	-	23.680	22.740
24/04/2019	Wednesday	-	23.040	22.630
25/04/2019	Thursday	-	18.752	18.824
26/04/2019	Friday	-	21.144	21.450
27/04/2019	Saturday	-	19.648	19.990
28/04/2019	Sunday	-	19.520	19.880

na, data not available

2.4. Analytical procedure

A generic solid-phase extraction (SPE) procedure was applied to broaden the applicability of the HRMS screening to a large number of pharmaceutical compounds. In order to reduce matrix complexity, influent wastewater (IWW) and hospital discharge samples were previously diluted x4 with Milli-Q water (a 25 mL-aliquot of raw water was taken and then 75 mL of Milli-Q water was added). Briefly, 100 mL of the diluted sample were passed by gravity through Oasis HLB (200 mg, Waters) cartridges, previously conditioned with 5 mL of methanol and 5 mL of HPLC-grade water. After drying under vacuum for 20 min, the analytes were eluted with 10 mL of methanol. The extract was evaporated under a gentle nitrogen stream at 35 °C down to dryness and reconstituted with 500 µL of methanol:water (10:90, v/v) (pre-concentration factor x 50). Finally, 20 µL of the extracts were injected into the LC-QTOF MS.

For a quantitative determination, a volume of 1 mL of wastewater (IWW and hospital discharge dilution x5; EWW dilution x2) was centrifuged at 12.000 rpm for 10 min. Then, 200 µL-aliquot of the centrifuged sample was taken (IWW and hospital wastewater) and 750 µL Milli-Q water and 50 µL of mix isotope labelled internal standard (ILIS) solution (1 µg/L) were added. For EWW, a 500 µL-aliquot of the centrifuged sample was taken and then 450 µL Milli-Q water and 50 µL of mix ILIS solution (1 µg/L) were added. Finally, 50 µL of the diluted samples were injected into the liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

2.5. Instrumentation

LC-HRMS analysis was performed using a Waters ACQUITY UPLC ultra-high performance liquid chromatography (UHPLC) system (Waters, Milford, MA, USA) interfaced to a hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (Xevo G2 QTOF, Waters Micromass, Manchester, UK), using an orthogonal Z-spray electrospray ionization (ESI) interface operating in both positive and negative ionisation modes.

The UHPLC separation was performed using a CORTECS C_{18} column (2.1 i.d. × 100 mm length, 2.7 µm particle size, Waters), at a flow rate of 300 µL/min. The mobile phases were (A) H_2O with 0.01% HCOOH and (B) MeOH with 0.01% HCOOH. The mobile phase gradient

was: 10% B at 0 min, 90% B at 14 min linearly increased, 90% B at 16 min, and finally 10% B at 18 min in order to return to initial conditions. The injection volume was 20 µL.

MS data were acquired over a *m/z* range of 50-1000. Nitrogen was used as drying and nebulizing gas. The gas flow was set at 1000 L/h. TOF-MS resolution was approximately 18000 at full width half maximum (FWHM) at *m/z* 556. Capillary voltages of 0.7 kV and 3.0 kV were used in positive and negative ionisation modes, respectively. A cone voltage of 20 V was selected for both ionisation modes. Collision gas was argon 99.995% (Praxair, Valencia, Spain). The desolvation temperature was 600 °C and the source temperature 130 °C. Data were automatically processed by ChromaLynx XS software (MassLynx v 4.1, Waters).

A triple quadrupole (QqQ) mass spectrometer, used for quantitative analyses, was interfaced to a Waters ACQUITY ultra performance liquid chromatography (UPLC^{TM}) system (Waters Corp., Milford, MA, USA), equipped with a binary pump system. A CORTECS C_{18} column (100 x 2.1 mm i.d., particle size 2.7 µm) (Waters) was used. An optimized gradient was applied at a constant flow rate of 0.4 mL/min using methanol LC-MS (solvent A) and water LC-MS (solvent B), both 0.01% HCOOH and 1 mM ammonium acetate. The gradient elution was: 0 min, 5% A; 0-7 min linear from 5 to 95% A; 7-8 min, 95% A; 8-8.1 min linear from 95 to 5 % A, return to initial conditions; 8.1–9.5 min 5% A, equilibration of the column. The injection volume was 50 µL.

A Xevo TQ-S^{TM} triple quadrupole mass spectrometer (Waters Micromass, Manchester, UK), equipped with ESI source was used. Determination of analytes was performed using ESI source in both positive and negative ion modes. Drying gas as well as nebulising gas was nitrogen (Praxair, Valencia, Spain). The cone gas flow rate was optimized at 250 L/h and the desolvation gas flow was set to 1200 L/h. The desolvation temperature was 650 °C. For operation in MS/MS mode, collision gas was Argon 99.995 % (Praxair, Valencia, Spain) with a flow of 0.15 mL/min in the collision cell. Electrospray needle capillary voltage was fixed at 3.5 kV and 2 kV in positive and negative ionisation modes, respectively. The source temperature was set to 150 °C. The cone value was 10V. The column temperature was maintained at 40 °C.

MassLynx software v 4.1 (Waters Corporation) was used to acquire data. TargetLynx application manager was used to quantify the concentration levels of the target analytes.

2.6. Analysis

2.6.1. *Qualitative screening: QTOF data processing*

Different approaches for QTOF data processing were used depending on the availability of references standards. Briefly, information about elemental composition, retention time, main fragment ions and adduct formation was included in the target list when the standard was available, facilitating and enhancing the reliability of the identification. On the contrary, when the standard was not available (suspect screening), the only information was the elemental composition of the parent compound (occasionally adducts). In such cases, the presence of the protonated molecule and fragment ions was evaluated in the low energy (LE) and high energy (HE) functions, respectively, as well as the characteristic isotope pattern when Cl or Br were present. Making use of the experimental accurate-mass data, the compatibility of fragment ions with the chemical structure of the suspect compound was evaluated, and finally a tentative identification was feasible. The tentative identification was supported by MS/MS product ions reported in the literature or databases for the suspect compound (either in exact or nominal mass), when available.

2.6.2. *Target* quantitative analysis

Three SRM transitions were acquired for every compound allowing the quantification and the reliable identification of positive findings. Quantification was made using the quantification transition (Q) and external calibration with standards in solvent. When the analyte-ILIS was available, relative areas were used for quantification. At least seven-points calibration curves (5-20.000 ng/L) were injected at the beginning and the end of each sequence. Linearity was assumed when regression coefficient was >0.99 with residuals lower than 30%.

Quality controls (QC) were processed in the same manner as the samples and were injected in every batch of samples analysed.

The identification of compounds in the samples was based on the ion ratios (peak area) between the confirmation (q1 and q2) and quantification (Q) transitions. The finding was considered as confirmed when at least one ion-ratio (q1/Q and/or q2/Q) and the retention time of the compound in the sample were within the tolerance ranges (± 30% for ion ratios, ± 0.1 min for retention time) in comparison with the reference standards injected in the calibration. In case

of reasonable doubts (e.g. deviations slightly above than tolerance limits), the parameters used for identification were tested in the QCs injected in the same sequence of analysis as the questionable samples.

3. RESULTS AND DISCUSSION

Table S3. Mean recoveries (n=2, %) for QCs at two concentration levels after the application of LC-MS/MS procedure to wastewater samples corresponded to the **1st campaign**. The ILIS used for each compound are shown.

Compounds	ILIS	Hospital		IWW		EWW	
		0.5 µg/L	**5 µg/L**	**0.5 µg/L**	**5 µg/L**	**0.2 µg/L**	**2 µg/L**
Acetaminophen	Acetaminophen-d_4	[a]	[a]	132	125	97	100
Alprazolam	-	82	87	110	106	101	125
Atorvastatin	Atorvastatin-d_5	103	104	107	103	106	106
Azitromycin	Azitromycin-d_3	81	60	***27***	***47***	***15***	69
Bezafibrate	-	-	***49***	-	63	-	81
Carbamazepine	Carbamazepine 10,11-epoxide-d_{10}	89	[b]	91	[b]	74	[b]
Ciprofloxacin	Ciprofloxacin-d_8	*	*	*	*	*	*
Clarithromycin	-	118	*	139	*	124	*
Clindamycin	-	135	130	77	81	108	121
Diclofenac	Diclofenac-d_4	116	108	98	96	96	94
Enalapril	-	111	104	102	107	104	106
Erythromycin	Erythromycin-$^{13}Cd_3$	95	108	78	103	90	108
Furaltadone	-	84	100	82	74	115	95
Gabapentin	-	101	122	105	109	***149***	117
Gemfibrozil	-	-	-	-	-	-	-
Irbesartan	Irbesartan-d_6	100	108	92	100	150	106
Ketoprofen	-	-	-	-	-	-	-
Levamisole	Cocaethylene-d_8	106	125	99	121	138	176
Lincomycin	-	116	89	97	110	124	108
Lorazepam	-	112	98	111	93	77	100
Losartan	-	115	93	106	103	105	104
Metoprolol	-	103	110	110	121	112	126
Metronidazole	-	72	86	98	99	103	116
Nalidixic acid	-	81	95	90	94	97	96
Naproxen	-	-	-	-	62	-	78
Norfloxacin	Norfloxacin-d_5	*	*	*	*	*	*
Omeprazole sulfide, 4-OH	Omeprazole-d_3	73	***57***	96	80	67	64
Oxolinic acid	-	99	83	94	79	107	85
Pantoprazole	-	129	124	119	121	118	69
Phenazone	-	138	148	114	134	81	96
Primidone	-	87	88	102	105	94	106
Roxithromycin	-	*	*	135	*	***154***	*
Salbutamol	-	100	96	111	104	140	139
Sulfadiazine	Sulfamethoxazole-$^{13}C_6$	88	96	110	120	73	87
Sulfamethoxazole	Sulfamethoxazole-$^{13}C_6$	117	110	114	116	109	111
Tetracycline	-	86	57	95	79	83	37
Tramadol	-	84	93	104	103	108	115
Trimetroprim	-	84	98	116	124	118	132
Valsartan	Valsartan-d_8	87	126	68	70	86	103
Venlafaxine	Venlafaxin-d_6	96	105	96	114	107	118

In bold and italic, recoveries outside the accepted range (60-140%)

[a] Presence of analyte in the blank sample at high concentration level, which prevents recovery calculation

[b] Value not calculated due to lack of linearity at high concentration level

- Value not available due to lack of sensitivity, which prevents reaching the low concentration levels tested

** Poorly reproducible recovery values, in general greater than 200%*

Table S4. Mean recoveries (n=2, %) for QCs at two concentration levels after the application of LC-MS/MS procedure to wastewater samples corresponded to the **2nd campaign**. The ILIS used for each compound are shown.

Compounds	ILIS	IWW		EWW	
		0.5 µg/L	**5 µg/L**	**0.2 µg/L**	**2 µg/L**
Acetaminophen	Acetaminophen-d_4	72	92	93	139
Alprazolam	-	78	140	79	81
Atorvastatin	Atorvastatin-d_5	95	112	60	114
Azitromycin	Azitromycin-d_3	***40***	***49***	***45***	60
Bezafibrate	-	-	-	-	-
Carbamazepine	Carbamazepine 10,11-epoxide-d_{10}	137	[a]	133	[a]
Ciprofloxacin	Ciprofloxacin-d_8	*	*	*	*
Clarithromycin	-	*	***160***	140	***145***
Clindamycin	-	140	***148***	138	133
Diclofenac	Diclofenac-d_4	104	131	96	140
Enalapril	-	84	88	86	93
Erythromycin	Erythromycin-$^{13}Cd_3$	60	71	87	-
Furaltadone	-	135	140	131	140
Gabapentin	-	105	103	120	108
Gemfibrozil	-	-	-	-	-
Irbesartan	Irbesartan-d_6	95	***153***	91	124
Ketoprofen	-	-	109	-	84
Levamisole	Cocaethylene-d_8	74	84	65	78
Lincomycin	-	139	140	97	93
Lorazepam	-	126	75	79	107
Losartan	-	95	104	95	101
Metoprolol	-	***222***	***200***	***234***	***227***
Metronidazole	-	106	112	105	110
Nalidixic acid	-	89	77	85	77
Naproxen	-	-	-	-	121
Norfloxacin	Norfloxacin-d_5	*	*	*	*
Omeprazole sulfide, 4-OH	Omeprazole-d_3	84	79	96	94
Oxolinic acid	-	75	70	77	66
Pantoprazole	-	103	96	103	101
Phenazone	-	104	110	102	110
Primidone	-	106	105	99	103
Roxithromycin	-	*	*	***142***	***145***
Salbutamol	-	82	137	***148***	140
Sulfadiazine	Sulfamethoxazole-$^{13}C_6$	105	108	107	119
Sulfamethoxazole	Sulfamethoxazole-$^{13}C_6$	100	106	98	108
Tetracycline	-	85	105	127	119
Tramadol	-	***240***	***281***	***241***	***294***
Trimetroprim	-	***242***	***274***	***249***	***264***
Valsartan	Valsartan-d_8	99	107	91	102
Venlafaxine	Venlafaxin-d_6	81	117	62	104

In bold and italic, recoveries outside the accepted range (60-140%)

[a] Value not calculated due to lack of linearity at high concentration level

- Value not available due to lack of sensitivity, which prevents reaching the low concentration levels tested

** Poorly reproducible recovery values, in general greater than 200%*

Table S5. Mean recoveries (n=2, %) for QCs at two concentration levels after the application of LC-MS/MS procedure to wastewater samples corresponded to the **3rd campaign**. The ILIS used for each compound are shown.

Compounds	ILIS	IWW		EWW	
		0.5 µg/L	**5 µg/L**	**0.2 µg/L**	**2 µg/L**
Acetaminophen	Acetaminophen-d_4	72	82	60	61
Alprazolam	-	73	75	73	76
Atorvastatin	Atorvastatin-d_5	116	122	105	***45***
Azitromycin	Azitromycin-d_3	[b]	[b]	[b]	[b]
Bezafibrate	-	102	92	136	82
Carbamazepine	Carbamazepine 10,11-epoxide-d_{10}	111	[a]	121	[a]
Ciprofloxacin	Ciprofloxacin-d_8	*	*	*	*
Clarithromycin	-	114	***162***	101	*
Clindamycin	-	98	128	106	108
Diclofenac	Diclofenac-d_4	79	99	113	97
Enalapril	-	64	66	65	62
Erythromycin	Erythromycin-$^{13}Cd_3$	110	107	70	105
Furaltadone	-	104	104	73	81
Gabapentin	-	133	134	129	114
Gemfibrozil	-	-	112	-	101
Irbesartan	Irbesartan-d_6	67	111	110	91
Ketoprofen	-	-	-	-	-
Levamisole	Cocaethylene-d_8	113	***177***	117	***155***
Lincomycin	-	125	123	109	103
Lorazepam	-	77	71	74	74
Losartan	-	63	62	67	67
Metoprolol	-	93	116	95	127
Metronidazole	-	91	96	91	93
Nalidixic acid	-	72	82	60	61
Naproxen	-	-	72	-	70
Norfloxacin	Norfloxacin-d_5	*	*	*	*
Omeprazole sulfide, 4-OH	Omeprazole-d_3	121	103	128	110
Oxolinic acid	-	***54***	60	***53***	60
Pantoprazole	-	90	80	88	75
Phenazone	-	86	94	79	89
Primidone	-	85	88	80	82
Roxithromycin	-	92	127	120	*
Salbutamol	-	112	112	104	100
Sulfadiazine	Sulfamethoxazole-$^{13}C_6$	71	73	61	65
Sulfamethoxazole	Sulfamethoxazole-$^{13}C_6$	106	105	101	108
Tetracycline	-	60	60	***58***	***50***
Tramadol	-	104	121	106	116
Trimetroprim	-	130	***174***	132	***180***
Valsartan	Valsartan-d_8	67	96	93	68
Venlafaxine	Venlafaxin-d_6	81	103	82	107

In bold and italic, recoveries outside the accepted range (60-140%)

[a] Value not calculated due to lack of linearity at high concentration levels

[b] Value not calculated due to the absence of the chromatographic peak

- Value not available due to lack of sensitivity, which prevents reaching the low concentration levels tested

** Poorly reproducible recovery values, in general greater than 200%*

Table S6. Concentrations (ng/L) of pharmaceuticals in 24-h IWW samples collected during the **first campaign** (September 2018). Average concentration for the seven days.

Compounds	**IWW1_1**	**IWW2_1**	**IWW3_1**	**IWW4_1**	**IWW5_1**	**IWW6_1**	**IWW7_1**	**Average**
Acetaminophen	5185	8713	7336	6649	7171	7733	2643	**6490**
Alprazolam	-	-	-	-	-	-	-	**-**
Atorvastatin	86	90	72	81	84	113	80	**87**
Azitromycin	195	177	195	168	175	207	184	**186**
Bezafibrate	-	-	-	-	-	-	-	**-**
Carbamazepine	d	d	d	d	d	d	d	**d**
Ciprofloxacin	99440	460160	88450	48500	-	-	51780	**149700**
Clarithromycin	117	96	127	76	70	101	95	**97**
Clindamycin	-	-	-	-	-	-	-	**-**
Diclofenac	407	247	199	197	165	252	157	**232**
Enalapril	45	66	51	49	49	58	34	**50**
Erythromycin	d	35	30	32	d	33	d	**25***
Furaltadone	-	-	-	-	-	-	-	**-**
Gabapentin	3561	3875	4311	3641	4087	4678	3942	**4013**
Gemfibrozil	-	-	-	-	-	-	-	**-**
Irbesartan	215	240	189	248	224	264	185	**223**
Ketoprofen	-	-	-	-	-	-	-	**-**
Levamisole	26	d	43	29	27	32	27	**29***
Lincomycin	-	-	-	-	-	-	-	**-**
Lorazepam	34	29	41	35	-	-	-	**34**
Losartan	156	181	156	178	165	221	120	**168**
Metoprolol	d	d	d	d	d	d	d	**d**
Metronidazole	d	d	d	d	d	d	d	**d**
Nalidixic acid	-	-	-	-	-	-	-	**-**
Naproxen	2357	2366	2373	2370	-	2369	2358	**2365**
Norfloxacin	456	1270	1970	1030	550	178	731	**880**
Omeprazole sulfide, 4-OH	64	65	65	52	66	90	63	**66**
Oxolinic acid	-	-	-	-	-	-	-	**-**
Pantoprazole	-	-	-	-	-	-	-	**-**
Phenazone	93	-	d	d	-	d	56	**32***
Primidone	80	71	69	84	63	82	88	**76**
Roxithromycin	-	-	-	-	-	-	-	**-**
Salbutamol	-	d	d	d	d	d	d	**d**
Sulfadiazine	-	-	-	-	-	-	-	**-**
Sulfamethoxazole	70	129	70	56	57	101	36	**74**
Tetracycline	49	53	-	-	-	-	-	**44**
Tramadol	574	570	649	593	660	730	603	**625**
Trimetroprim	180	199	126	109	93	146	109	**137**
Valsartan	457	552	390	602	444	651	456	**507**
Venlafaxine	146	151	154	153	180	191	161	**162**

d: detected, *not quantified. Concentration below the limit value of quantification*
Underlined: guidance concentration data
**For the calculation of average concentration only the quantified values were considered*

Table S7. Concentrations (ng/L) of pharmaceuticals in 24-h EWW samples collected during the **first campaign** (September 2018). Average concentration for the seven days.

Compounds	EWW1_1	EWW2_1	EWW3_1	EWW4_1	EWW5_1	EWW6_1	EWW7_1	Average
Acetaminophen	-	-	-	-	-	-	-	-
Alprazolam	d	d	d	d	d	6	d	**d**
Atorvastatin	-	d	-	-	d	-	-	**d**
Azitromycin	-	-	-	-	-	-	-	-
Bezafibrate	-	-	-	-	-	-	-	-
Carbamazepine	-	-	-	-	d	d	-	**d**
Ciprofloxacin	1160	-	610	1560	11080	3770	-	**3640**
Clarithromycin	53	44	57	34	58	47	43	**48**
Clindamycin	d	d	10	d	d	d	10	**d**
Diclofenac	182	151	141	148	126	135	119	**143**
Enalapril	-	-	-	-	-	-	-	-
Erythromycin	36	24	26	41	23	23	22	**28**
Furaltadone	-	-	-	-	-	-	-	-
Gabapentin	1669	1638	1599	1550	1391	1535	1506	**1555**
Gemfibrozil	-	-	-	-	-	-	-	-
Irbesartan	150	134	167	194	191	192	195	**175**
Ketoprofen	-	-	-	-	-	-	-	-
Levamisole	37	30	27	28	25	24	27	**28**
Lincomycin	-	-	-	-	-	-	-	-
Lorazepam	44	44	41	31	57	41	47	**44**
Losartan	12	13	10	d	11	15	d	**12***
Metoprolol	d	d	d	-	10	14	d	**d**
Metronidazole	d	d	-	d	-	d	-	**d**
Nalidixic acid	d	d	d	d	-	-	d	**d**
Naproxen	-	-	-	-	-	-	-	-
Norfloxacin	1120	710	135	2080	530	515	515	**800**
Omeprazole sulfide, 4-OH	39	40	39	37	34	36	40	**38**
Oxolinic acid	-	-	-	-	-	-	-	-
Pantoprazole	21	19	19	19	17	18	23	**19**
Phenazone	d	d	-	d	d	d	d	**d**
Primidone	66	59	69	74	77	74	84	**72**
Roxithromycin	-	-	-	-	-	-	-	-
Salbutamol	-	d	d	d	d	d	d	**d**
Sulfadiazine	-	-	-	-	-	-	-	-
Sulfamethoxazole	44	46	36	31	19	18	38	**33**
Tetracycline	-	30	18	-	-	-	d	**19***
Tramadol	550	529	503	530	867	910	271	**594**
Trimetroprim	12	15	10	-	24	-	d	**15***
Valsartan	-	31	-	-	35	19	18	**26**
Venlafaxine	184	180	162	175	155	160	191	**172**

d: detected, not quantified. Concentration below the limit value of quantification
Underlined: guidance concentration data
**For the calculation of average concentration only the quantified values were considered*

Table S8. Concentrations (ng/L) of pharmaceuticals in 24-h IWW samples collected during the **second campaign** (January 2019) from the WWTP Ricao. Average concentration for the seven days.

Compounds	**IWW1_2**	**IWW2_2**	**IWW3_2**	**IWW4_2**	**IWW5_2**	**IWW6_2**	**IWW7_2**	**Average**
Acetaminophen	6060	4240	9475	6495	4020	960	695	**4564**
Alprazolam	-	d	d	-	-	d	-	**d**
Atorvastatin	d	d	d	d	d	d	d	**d**
Azitromycin	295	355	255	380	435	d	250	**328***
Bezafibrate	-	-	-	-	-	-	-	**-**
Carbamazepine	-	-	-	-	-	-	-	**-**
Ciprofloxacin	10570	11360	8795	9285	8920	5125	3280	**8191**
Clarithromycin	195	230	300	140	210	130	140	**192**
Clindamycin	-	-	-	-	-	-	-	**-**
Diclofenac	75	70	65	40	30	d	d	**56***
Enalapril	-	-	-	-	-	-	-	**-**
Erythromycin	d	60	25	d	70	d	100	**64***
Furaltadone	-	-	-	-	-	-	-	**-**
Gabapentin	1210	2155	1985	2065	1740	860	2840	**1836**
Gemfibrozil	-	-	-	-	-	-	-	**-**
Irbesartan	45	90	60	75	45	d	65	**63***
Ketoprofen	-	-	-	-	-	-	-	**-**
Levamisole	-	-	-	-	-	-	-	**-**
Lincomycin	-	-	-	-	-	-	-	**-**
Lorazepam	-	-	-	-	-	-	-	**-**
Losartan	25	31	30	27	25	d	d	**27***
Metoprolol	d	d	d	d	-	-	-	**d**
Metronidazole	d	33	41	d	25	d	51	**37***
Nalidixic acid	-	-	-	-	-	-	-	**-**
Naproxen	-	-	-	-	-	-	-	**-**
Norfloxacin	20155	18545	10690	8985	6850	3695	3785	**10386**
Omeprazole sulfide, 4-OH	d	d	d	d	d	d	d	**d**
Oxolinic acid	d	d	d	d	-	d	-	**d**
Pantoprazole	d	d	d	d	d	d	d	**d**
Phenazone	d	45	40	d	d	-	d	**42***
Primidone	-	-	-	-	-	-	-	**-**
Roxithromycin	-	-	-	-	-	-	-	**-**
Salbutamol	-	d	d	d	-	d	-	**d**
Sulfadiazine	-	-	-	-	-	-	-	**-**
Sulfamethoxazole	d	d	d	d	d	-	-	**d**
Tetracycline	150	130	30	-	d	-	-	**103***
Tramadol	135	198	133	133	85	35	113	**119**
Trimetroprim	115	128	190	95	85	30	33	**96**
Valsartan	135	270	165	160	105	d	90	**136***
Venlafaxine	35	60	35	d	d	d	d	**43***

d: detected, *not quantified. Concentration below the limit value of quantification*
Underlined: guidance concentration data
**For the calculation of average concentration only the quantified values were considered*

Table S9. Concentrations (ng/L) of pharmaceuticals in 24-h EWW samples collected during the **second campaign** (January 2019) from the WWTP Ricao. Average concentration for the seven days.

Compounds	EWW1_2	EWW2_2	EWW3_2	EWW4_2	EWW5_2	EWW6_2	EWW7_2	Average
Acetaminophen	-	d	-	d	-	-	d	**d**
Alprazolam	d	-	d	d	d	d	-	**d**
Atorvastatin	-	-	-	-	-	-	-	**-**
Azitromycin	d	d	d	d	d	d	d	**d**
Bezafibrate	-	-	-	-	-	-	-	**-**
Carbamazepine	-	-	-	-	-	-	-	**-**
Ciprofloxacin	2164	2596	2634	3072	1918	1726	1584	**2242**
Clarithromycin	60	79	154	94	146	124	92	**107**
Clindamycin	-	-	-	-	-	-	-	**-**
Diclofenac	34	17	36	38	28	12	16	**26**
Enalapril	-	-	-	-	-	-	-	**-**
Erythromycin	d	d	d	d	d	d	d	**d**
Furaltadone	-	-	-	-	-	-	-	**-**
Gabapentin	416	480	614	724	536	372	556	**528**
Gemfibrozil	-	-	-	-	-	-	-	**-**
Irbesartan	64	36	80	72	60	42	48	**57**
Ketoprofen	-	-	-	-	-	-	-	**-**
Levamisole	d	d	d	d	d	d	d	**d**
Lincomycin	-	-	-	-	-	-	-	**-**
Lorazepam	d	-	d	d	d	d	d	**d**
Losartan	-	d	10	10	d	-	d	**10***
Metoprolol	d	-	d	d	d	-	-	**d**
Metronidazole	-	-	d	d	d	-	-	**d**
Nalidixic acid	-	-	-	-	-	-	-	**-**
Naproxen	-	-	-	-	-	-	-	**-**
Norfloxacin	2764	3010	4612	2624	1822	1030	1322	**2455**
Omeprazole sulfide, 4-OH	d	d	d	d	d	d	d	**d**
Oxolinic acid	-	-	-	-	-	-	-	**-**
Pantoprazole	d	-	d	d	d	d	d	**d**
Phenazone	-	-	-	-	-	-	-	**-**
Primidone	d	10	12	11	10	d	d	**11***
Roxithromycin	-	-	-	-	-	-	-	**-**
Salbutamol	-	-	-	-	-	-	-	**-**
Sulfadiazine	-	-	-	-	-	-	-	**-**
Sulfamethoxazole	12	d	11	18	11	d	d	**13***
Tetracycline	-	-	-	-	-	-	-	**-**
Tramadol	151	80	149	148	107	59	89	**112**
Trimetroprim	17	22	30	25	12	d	d	**21***
Valsartan	17	34	52	34	30	12	36	**31**
Venlafaxine	36	26	44	56	38	20	26	**35**

d: detected, not quantified. Concentration *below the limit value of quantification*
Underlined: guidance concentration data
**For the calculation of average concentration only the quantified values were considered*

Table S10. Concentrations (ng/L) of pharmaceuticals in 24-h IWW samples collected during the **third campaign** (April 2019) from the WWTP Ricao. Average concentration for the seven days.

Compounds	IWW1_3	IWW2_3	IWW3_3	IWW4_3	IWW5_3	IWW6_3	IWW7_3	Average
Acetaminophen	9341	27	101	9414	7627	7170	1531	**5030**
Alprazolam	-	-	-	-	-	-	-	-
Atorvastatin	77	96	65	114	98	98	68	**88**
Azitromycin	-	-	-	-	-	-	-	-
Bezafibrate	-	-	-	-	-	-	-	-
Carbamazepine	-	-	-	-	-	-	-	-
Ciprofloxacin	1700	1200	900	1400	1100	1600	990	**1270**
Clarithromycin	-	-	-	-	-	-	-	-
Clindamycin	-	-	-	-	-	-	-	-
Diclofenac	184	293	134	331	207	270	142	**223**
Enalapril	17	39	33	31	32	27	28	**29**
Erythromycin	-	d	d	d	d	d	d	**d**
Furaltadone	-	-	-	-	-	-	-	-
Gabapentin	2572	3332	3791	5103	4042	3608	3978	**3775**
Gemfibrozil	-	-	-	-	-	-	-	-
Irbesartan	124	163	162	185	217	241	173	**181**
Ketoprofen	-	-	-	-	-	-	-	-
Levamisole	-	-	-	-	-	-	-	-
Lincomycin	-	-	-	-	-	-	-	-
Lorazepam	25	25	25	25	27	25	25	**25**
Losartan	51	77	83	73	56	63	66	**67**
Metoprolol	d	-	d	35	d	d	-	**d**
Metronidazole	d	d	d	d	72	36	d	**54***
Nalidixic acid	-	-	-	-	-	-	-	-
Naproxen	-	-	-	-	-	-	-	-
Norfloxacin	990	400	850	430	460	270	300	**530**
Omeprazole sulfide, 4-OH	51	52	39	59	53	55	42	**50**
Oxolinic acid	-	-	-	-	-	-	-	-
Pantoprazole	d	d	d	d	d	d	d	**d**
Phenazone	-	-	-	-	-	-	-	-
Primidone	42	48	55	57	44	60	46	**50**
Roxithromycin	-	-	-	-	-	-	-	-
Salbutamol	d	d	d	d	d	d	d	**d**
Sulfadiazine	d	d	d	d	d	d	d	**d**
Sulfamethoxazole	25	25	25	30	54	44	d	**34***
Tetracycline	103	-	-	33	30	-	-	**55**
Tramadol	403	417	477	477	494	475	556	**471**
Trimetroprim	137	196	266	270	317	305	129	**231**
Valsartan	319	644	419	583	442	400	313	**446**
Venlafaxine	76	124	114	154	138	131	125	**123**

d: detected, not quantified. Concentration below *the limit value of quantification*
Underlined: guidance concentration data
**For the calculation of average concentration only the quantified values were considered*

Table S11. Concentrations (ng/L) of pharmaceuticals in 24-h EWW samples collected during the **third campaign** (April 2019) from the WWTP Ricao. Average concentration for the seven days.

Compounds	EWW1_3	EWW2_3	EWW3_3	EWW4_3	EWW5_3	EWW6_3	EWW7_3	Average
Acetaminophen	-	-	-	-	-	-	-	-
Alprazolam	d	-	d	-	d	d	d	**d**
Atorvastatin	-	-	-	-	-	-	-	-
Azitromycin	-	-	-	-	-	-	-	-
Bezafibrate	-	-	-	-	-	-	-	-
Carbamazepine	-	d	d	d	d	-	d	**d**
Ciprofloxacin	1300	900	750	720	430	470	370	**700**
Clarithromycin	58	50	29	32	-	23	31	**37**
Clindamycin	-	d	d	-	d	-	d	**d**
Diclofenac	192	113	97	80	109	129	159	**126**
Enalapril	-	-	-	-	-	-	-	-
Erythromycin	14	17	11	15	10	10	15	**13**
Furaltadone	-	-	-	-	-	-	-	-
Gabapentin	1112	1101	1135	1086	1229	1132	1082	**1125**
Gemfibrozil	-	-	-	-	-	-	-	-
Irbesartan	154	143	251	136	90	161	175	**159**
Ketoprofen	-	-	-	-	-	-	-	-
Levamisole	11	10	10	10	11	14	22	**13**
Lincomycin	-	-	-	-	-	-	-	-
Lorazepam	25	20	23	22	21	17	19	**21**
Losartan	18	18	16	13	15	11	13	**15**
Metoprolol	-	d	d	d	d	d	d	**d**
Metronidazole	d	d	d	d	d	d	-	**d**
Nalidixic acid	-	-	-	-	-	-	-	-
Naproxen	-	-	-	-	-	-	-	-
Norfloxacin	560	640	430	240	140	250	170	**350**
Omeprazole sulfide, 4-OH	53	50	52	51	56	60	53	**54**
Oxolinic acid	18	12	d	d	d	d	-	**15***
Pantoprazole	d	d	d	d	d	d	d	**d**
Phenazone	-	-	-	-	-	-	-	-
Primidone	45	44	41	40	37	38	38	**40**
Roxithromycin	-	-	-	-	-	-	-	-
Salbutamol	d	d	d	d	d	d	d	**d**
Sulfadiazine	d	d	d	d	d	d	d	**d**
Sulfamethoxazole	11	12	10	15	17	17	17	**14**
Tetracycline	18	13	18	-	-	-	-	**16**
Tramadol	405	389	365	375	400	419	433	**398**
Trimetroprim	36	40	27	29	41	51	35	**37**
Valsartan	37	44	45	28	36	38	30	**37**
Venlafaxine	118	115	116	115	121	123	125	**119**

d: detected, not quantified. Concentration below the limit *value of quantification*
Underlined: guidance concentration data
**For the calculation of average concentration only the quantified values were considered*

Table S12. Concentrations (ng/L) of pharmaceuticals in 24-h samples corresponded to the hospital discharge collected during the **first campaign** (September 2018). Average concentration for the seven days.

Compounds	**Hospital1_1**	**Hospital2_1**	**Hospital3_1**	**Hospital4_1**	**Hospital5_1**	**Hospital6_1**	**Hospital7_1**	**Average**
Acetaminophen	144160	145630	117290	144600	115150	158840	112340	**134000**
Alprazolam	d	d	d	9	-	-	-	**d**
Atorvastatin	294	277	745	206	221	266	294	**329**
Azitromycin	1140	1387	998	1134	1349	1059	130	**1028**
Bezafibrate	-	-	-	-	-	-	-	**-**
Carbamazepine	-	-	-	-	-	-	-	**-**
Ciprofloxacin	1180	4197	139000	197000	23200	113000	680000	**165500**
Clarithromycin	73	-	-	-	35	-	-	**54**
Clindamycin	86	167	88	83	-	71	-	**99**
Diclofenac	385	237	189	221	236	331	392	**284**
Enalapril	253	212	59	233	189	171	263	**197**
Erythromycin	3132	296	303	525	61	436	190	**706**
Furaltadone	-	-	-	-	-	-	-	**-**
Gabapentin	13995	18114	2311	13377	22947	18943	13439	**14732**
Gemfibrozil	-	-	-	-	-	-	-	**-**
Irbesartan	240	292	163	114	117	185	184	**185**
Ketoprofen	-	-	-	-	-	-	-	**-**
Levamisole	-	-	-	-	-	-	-	**-**
Lincomycin	-	-	-	-	-	-	-	**-**
Lorazepam	235	199	96	189	-	264	169	**192**
Losartan	105	451	96	181	146	778	450	**315**
Metoprolol	d	d	25	34	-	d	d	**d**
Metronidazole	-	-	-	-	-	-	-	**-**
Nalidixic acid	-	-	-	-	-	-	-	**-**
Naproxen	2868	2857	2859	2881	-	2853	-	**2863**
Norfloxacin	2150	9600	9200	14800	7260	10650	4700	**8340**
Omeprazole sulfide, 4-OH	159	118	80	105	117	127	127	**119**
Oxolinic acid	-	-	-	-	-	-	-	**-**
Pantoprazole	101	48	25	85	204	71	174	**101**
Phenazone	40	-	367	-	72	-	47	**131**
Primidone	-	-	-	-	68	-	-	**68**
Roxithromycin	-	-	-	-	-	-	-	**-**
Salbutamol	58	43	22	33	35	42	42	**39**
Sulfadiazine	d	d	4	d	d	d	d	**d**
Sulfamethoxazole	-	-	-	d	-	144	1016	**580***
Tetracycline	-	-	-	-	-	-	-	**-**
Tramadol	1528	1388	1787	1490	1564	1740	1061	**1509**
Trimetroprim	31	d	16	29	40	348	1299	**293***
Valsartan	3404	3152	1949	5925	2737	63	2924	**2879**
Venlafaxine	1119	865	764	917	1161	1477	946	**1035**

d: detected, not quantified. Concentration below the limit value of quantification
Underlined: guidance concentration data
**For the calculation of average concentration only the quantified values were considered*

Table S13. Daily loads (g/day) of pharmaceuticals in 24-h IWW samples collected during the **first campaign** (September 2018) from the WWTP Ricao.

Compounds	**IWW1_1**	**IWW2_1**	**IWW3_1**	**IWW4_1**	**IWW5_1**	**IWW6_1**	**IWW7_1**
Acetaminophen	115	181	126	120	159	161	50.2
Alprazolam	-	-	-	-	-	-	-
Atorvastatin	1.9	1.9	1.2	1.5	1.9	2.3	1.5
Azitromycin	4.3	3.7	3.4	3.0	3.9	4.3	3.5
Bezafibrate	-	-	-	-	-	-	-
Carbamazepine	d	d	d	d	d	d	d
Ciprofloxacin	2200	9500	1520	870	-	-	980
Clarithromycin	2.6	2.0	2.2	1.4	1.5	2.1	1.8
Clindamycin	-	-	-	-	-	-	-
Diclofenac	9.0	5.1	3.4	3.5	3.7	5.2	3.0
Enalapril	1.0	1.4	0.88	0.87	1.1	1.2	0.64
Erythromycin	d	0.33	0.27	0.29	d	0.33	d
Furaltadone	-	-	-	-	-	-	-
Gabapentin	78.8	80.7	74.1	65.5	90.8	97.3	74.9
Gemfibrozil	-	-	-	-	-	-	-
Irbesartan	4.8	5.0	3.2	4.5	5.0	5.5	3.5
Ketoprofen	-	-	-	-	-	-	-
Levamisole	0.58	d	0.73	0.52	0.60	0.66	0.50
Lincomycin	-	-	-	-	-	-	-
Lorazepam	0.75	0.59	0.70	0.63	-	-	0.75
Losartan	3.4	3.8	2.7	3.2	3.7	4.6	2.3
Metoprolol	d	d	d	d	d	d	d
Metronidazole	d	d	d	d	d	d	d
Nalidixic acid	-	-	-	-	-	-	-
Naproxen	52.1	49.3	40.8	42.6	-	49.3	44.8
Norfloxacin	10	26	34	18	12	4	14
Omeprazole sulfide. 4-OH	1.4	1.4	1.1	0.93	1.5	1.9	1.2
Oxolinic acid	-	-	-	-	-	-	-
Pantoprazole	-	-	-	-	-	-	-
Phenazone	2.0	-	d	d	-	d	1.1
Primidone	1.8	1.5	1.2	1.5	1.4	1.7	1.7
Roxithromycin	-	-	-	-	-	-	-
Salbutamol	-	d	d	d	d	d	d
Sulfadiazine	-	-	-	-	-	-	-
Sulfamethoxazole	1.5	2.7	1.2	1.0	1.3	2.1	0.67
Tetracycline	1.1	1.1	-	-	-	-	-
Tramadol	12.7	11.9	11.1	10.7	14.6	15.2	11.5
Trimetroprim	4.0	4.1	2.2	2.0	2.1	3.0	2.1
Valsartan	7.8	9.3	7.1	10.0	7.4	10.3	7.2
Venlafaxine	3.2	3.1	2.6	2.8	4.0	4.0	3.1

d: detected, not quantified. Concentration *below the limit value of quantification*
Underlined: guidance concentration data

Table S14. Daily loads (g/day) of pharmaceuticals in 24-h EWW samples collected during the **first campaign** (September 2018) from the WWTP Ricao.

Compounds	**EWW1_1**	**EWW2_1**	**EWW3_1**	**EWW4_1**	**EWW5_1**	**EWW6_1**	**EWW7_1**
Acetaminophen	-	-	-	-	-	-	-
Alprazolam	d	d	d	d	d	6	d
Atorvastatin	-	d	-	-	d	-	-
Azitromycin	-	-	-	-	-	-	-
Bezafibrate	-	-	-	-	-	-	-
Carbamazepine	-	-	-	-	d	d	-
Ciprofloxacin	20	-	11	26	184	60	-
Clarithromycin	0.90	0.74	1.0	0.57	1.0	0.75	0.67
Clindamycin	d	d	0.19	d	d	d	0.16
Diclofenac	3.1	2.5	2.6	2.5	2.1	2.1	1.9
Enalapril	-	-	-	-	-	-	-
Erythromycin	0.61	0.40	0.47	0.68	0.38	0.36	0.34
Furaltadone	-	-	-	-	-	-	-
Gabapentin	28.3	27.6	29.0	25.9	23.1	24.3	23.6
Gemfibrozil	-	-	-	-	-	-	-
Irbesartan	2.5	2.3	3.0	3.2	3.2	3.0	3.1
Ketoprofen	-	-	-	-	-	-	-
Levamisole	0.63	0.50	0.49	0.47	0.41	0.38	0.42
Lincomycin	-	-	-	-	-	-	-
Lorazepam	0.75	0.74	0.74	0.52	0.95	0.65	0.74
Losartan	0.20	0.22	0.18	d	0.18	0.24	d
Metoprolol	d	d	d	-	0.17	0.22	d
Metronidazole	d	d	-	d	-	d	-
Nalidixic acid	d	d	d	d	-	-	d
Naproxen	-	-	-	-	-	-	-
Norfloxacin	19	12	2	35	9	8	8
Omeprazole sulfide. 4-OH	0.66	0.67	0.71	0.62	0.56	0.57	0.63
Oxolinic acid	-	-	-	-	-	-	-
Pantoprazole	0.36	0.32	0.34	0.32	0.28	0.29	0.36
Phenazone	d	d	-	d	d	d	d
Primidone	1.1	1.0	1.2	1.2	1.3	1.2	1.3
Roxithromycin	-	-	-	-	-	-	-
Salbutamol	-	d	d	d	d	d	d
Sulfadiazine	-	-	-	-	-	-	-
Sulfamethoxazole	0.77	0.65	0.52	0.32	0.29	0.60	0.77
Tetracycline	-	0.50	0.33	-	-	-	d
Tramadol	9.3	8.9	9.1	8.8	14.4	14.4	4.2
Trimetroprim	0.20	0.25	0.18	-	0.4	-	d
Valsartan	-	0.52	-	-	0.58	0.30	0.28
Venlafaxine	3.1	3.0	2.9	2.9	2.6	2.5	3.0

d: detected, not quantified. Concentration *below the limit value of quantification*
Underlined: guidance concentration data

Table S15. Daily loads (g/day) of pharmaceuticals in 24-h IWW samples collected during the **second campaign** (January 2019) from the WWTP Ricao.

Compounds	**IWW1_2**	**IWW2_2**	**IWW3_2**	**IWW4_2**	**IWW5_2**	**IWW6_2**	**IWW7_2**
Acetaminophen	145	85	186	170	88	29	16
Alprazolam	-	d	d	-	-	d	-
Atorvastatin	d	d	d	d	d	d	d
Azitromycin	7.0	7.1	5.0	9.9	9.6	d	5.9
Bezafibrate	-	-	-	-	-	-	-
Carbamazepine	-	-	-	-	-	-	-
Ciprofloxacin	252	229	172	243	196	156	77
Clarithromycin	4.7	4.6	5.9	3.7	4.6	4.0	3.3
Clindamycin	-	-	-	-	-	-	-
Diclofenac	1.8	1.4	1.3	1.0	0.66	d	d
Enalapril	-	-	-	-	-	-	-
Erythromycin	d	1.2	0.49	d	1.5	d	2.3
Furaltadone	-	-	-	-	-	-	-
Gabapentin	29	43	39	54	38	26	67
Gemfibrozil	-	-	-	-	-	-	-
Irbesartan	1.1	1.8	1.2	2.0	1.0	d	1.5
Ketoprofen	-	-	-	-	-	-	-
Levamisole	-	-	-	-	-	-	-
Lincomycin	-	-	-	-	-	-	-
Lorazepam	-	-	-	-	-	-	-
Losartan	0.60	0.61	0.58	0.69	0.55	d	d
Metoprolol	d	d	d	d	-	-	-
Metronidazole	d	0.66	0.79	d	0.55	d	1.2
Nalidixic acid	-	-	-	-	-	-	-
Naproxen	-	-	-	-	-	-	-
Norfloxacin	481	374	209	235	151	113	89
Omeprazole sulfide. 4-OH	d	d	d	d	d	d	d
Oxolinic acid	d	d	d	d	-	d	-
Pantoprazole	d	d	d	d	d	d	d
Phenazone	d	0.91	0.78	d	d	-	d
Primidone	-	-	-	-	-	-	-
Roxithromycin	-	-	-	-	-	-	-
Salbutamol	-	d	d	d	-	d	-
Sulfadiazine	-	-	-	-	-	-	-
Sulfamethoxazole	d	d	d	d	d	-	-
Tetracycline	3.4	2.6	0.59	-	d	-	-
Tramadol	3.2	4.0	2.6	3.5	1.9	1.1	2.6
Trimetroprim	2.7	2.6	3.7	2.5	1.9	0.91	0.76
Valsartan	3.0	5.3	3.3	3.6	2.2	d	1.9
Venlafaxine	0.84	1.2	0.69	d	d	d	d

d: detected, not quantified. Concentration below the limit value of quantification
Underlined: guidance concentration *data*

Table S16. Daily loads (g/day) of pharmaceuticals in 24-h EWW samples collected during the **second campaign** (January 2019) from the WWTP Ricao.

Compounds	**EWW1_2**	**EWW2_2**	**EWW3_2**	**EWW4_2**	**EWW5_2**	**EWW6_2**	**EWW7_2**
Acetaminophen	-	d	-	d	-	-	d
Alprazolam	d	-	d	d	d	d	-
Atorvastatin	-	-	-	-	-	-	-
Azitromycin	d	d	d	d	d	d	d
Bezafibrate	-	-	-	-	-	-	-
Carbamazepine	-	-	-	-	-	-	-
Ciprofloxacin	48	51	53	69	40	39	34
Clarithromycin	1.3	1.6	3.1	2.1	3.1	2.8	2.0
Clindamycin	-	-	-	-	-	-	-
Diclofenac	0.75	0.34	0.72	0.86	0.59	0.27	0.34
Enalapril	-	-	-	-	-	-	-
Erythromycin	d	d	d	d	d	d	d
Furaltadone	-	-	-	-	-	-	-
Gabapentin	9.2	9.5	12	16	11	8.4	12
Gemfibrozil	-	-	-	-	-	-	-
Irbesartan	1.4	0.71	1.6	1.6	1.3	0.95	1.0
Ketoprofen	-	-	-	-	-	-	-
Levamisole	d	d	d	d	d	d	d
Lincomycin	-	-	-	-	-	-	-
Lorazepam	0.75	-	0.73	0.75	0.81	0.78	0.76
Losartan	-	d	0.20	0.23	d	-	d
Metoprolol	d	-	d	d	d	-	-
Metronidazole	-	-	d	d	d	-	-
Nalidixic acid	-	-	-	-	-	-	-
Naproxen	-	-	-	-	-	-	-
Norfloxacin	61	60	92	59	38	23	28
Omeprazole sulfide. 4-OH	d	d	d	d	d	d	d
Oxolinic acid	-	-	-	-	-	-	-
Pantoprazole	d	-	d	d	d	d	d
Phenazone	-	-	-	-	-	-	-
Primidone	d	0.20	0.24	0.25	0.21	d	d
Roxithromycin	-	-	-	-	-	-	-
Salbutamol	-	-	-	-	-	-	-
Sulfadiazine	-	-	-	-	-	-	-
Sulfamethoxazole	0.27	d	0.22	0.41	0.24	d	d
Tetracycline	-	-	-	-	-	-	-
Tramadol	3.4	1.6	3.0	3.3	2.3	1.3	1.9
Trimetroprim	0.38	0.44	0.60	0.56	0.25	d	d
Valsartan	-	0.67	1.0	0.77	0.63	0.27	0.77
Venlafaxine	0.80	0.52	0.88	1.3	0.80	0.45	0.56

d: detected, not quantified. Concentration *below the limit value of quantification*
Underlined: guidance concentration *data*

Table S17. Daily loads (g/day) of pharmaceuticals in 24-h IWW samples collected during the **third campaign** (April 2019) from the WWTP Ricao.

Compounds	**IWW1_3**	**IWW2_3**	**IWW3_3**	**IWW4_3**	**IWW5_3**	**IWW6_3**	**IWW7_3**
Acetaminophen	191	0.6	2.3	177	161	141	30
Alprazolam	-	-	-	-	-	-	-
Atorvastatin	1.6	2.3	1.5	2.1	2.1	1.9	1.3
Azitromycin	-	-	-	-	-	-	-
Bezafibrate	-	-	-	-	-	-	-
Carbamazepine	-	-	-	-	-	-	-
Ciprofloxacin	34	28	21	26	24	31	19
Clarithromycin	-	-	-	-	-	-	-
Clindamycin	-	-	-	-	-	-	-
Diclofenac	3.8	6.9	3.1	6.2	4.4	5.3	2.8
Enalapril	0.35	0.92	0.75	0.58	0.68	0.52	0.55
Erythromycin	-	d	d	d	d	d	d
Furaltadone	-	-	-	-	-	-	-
Gabapentin	52	79	87	96	85	71	78
Gemfibrozil	-	-	-	-	-	-	-
Irbesartan	2.5	3.9	3.7	3.5	4.6	4.7	3.4
Ketoprofen	-	-	-	-	-	-	-
Levamisole	-	-	-	-	-	-	-
Lincomycin	-	-	-	-	-	-	-
Lorazepam	0.51	0.59	0.58	0.47	0.57	0.49	0.49
Losartan	1.0	1.8	1.9	1.4	1.2	1.2	1.3
Metoprolol	d	-	d	0.6	d	d	-
Metronidazole	d	d	d	d	1.5	0.71	d
Nalidixic acid	-	-	-	-	-	-	-
Naproxen	-	-	-	-	-	-	-
Norfloxacin	20	9.3	19	8.1	9.7	5.4	5.6
Omeprazole sulfide. 4-OH	1.0	1.2	0.89	1.1	1.1	1.1	0.81
Oxolinic acid	-	-	-	-	-	-	-
Pantoprazole	d	d	d	d	d	d	d
Phenazone	-	-	-	-	-	-	-
Primidone	0.85	1.1	1.3	1.1	0.92	1.2	0.89
Roxithromycin	-	-	-	-	-	-	-
Salbutamol	d	d	d	d	d	d	d
Sulfadiazine	d	d	d	d	d	d	d
Sulfamethoxazole	0.50	0.59	0.58	0.55	1.1	0.86	d
Tetracycline	2.1	-	-	0.61	0.63	-	-
Tramadol	8.2	9.9	11	8.9	10	9.3	11
Trimetroprim	2.8	4.6	6.1	5.1	6.7	6.0	2.5
Valsartan	6.3	15	9.5	11	9.5	8.0	6.2
Venlafaxine	1.6	2.9	2.6	2.9	2.9	2.6	2.4

d: detected, not quantified. Concentration *below the limit value of quantification*
Underlined: guidance concentration *data*

Table S18. Daily loads (g/day) of pharmaceuticals in 24-h EWW samples collected during the **third campaign** (April 2019) from the WWTP Ricao.

Compounds	**EWW1_3**	**EWW2_3**	**EWW3_3**	**EWW4_3**	**EWW5_3**	**EWW6_3**	**EWW7_3**
Acetaminophen	-	-	-	-	-	-	-
Alprazolam	d	-	d	-	d	d	d
Atorvastatin	-	-	-	-	-	-	-
Azitromycin	-	-	-	-	-	-	-
Bezafibrate	-	-	-	-	-	-	-
Carbamazepine	-	d	d	d	d	-	d
Ciprofloxacin	26	21	17	14	9.2	9.4	7.3
Clarithromycin	1.2	1.1	0.66	0.60	-	0.46	0.62
Clindamycin	-	d	d	-	d	-	d
Diclofenac	3.8	2.6	2.2	1.5	2.3	2.6	3.2
Enalapril	-	-	-	-	-	-	-
Erythromycin	0.28	0.39	0.25	0.28	0.21	0.20	0.30
Furaltadone	-	-	-	-	-	-	-
Gabapentin	22	25	26	20	26	23	22
Gemfibrozil	-	-	-	-	-	-	-
Irbesartan	3.1	3.3	5.7	2.6	1.9	3.2	3.5
Ketoprofen	-	-	-	-	-	-	-
Levamisole	0.22	0.23	0.23	0.19	0.24	0.28	0.44
Lincomycin	-	-	-	-	-	-	-
Lorazepam	0.50	0.45	0.52	0.41	0.45	0.34	0.38
Losartan	0.36	0.41	0.36	0.24	0.32	0.22	0.26
Metoprolol	-	d	d	d	d	d	d
Metronidazole	d	d	d	d	d	d	-
Nalidixic acid	-	-	-	-	-	-	-
Naproxen	-	-	-	-	-	-	-
Norfloxacin	11	15	10	4.6	2.9	4.8	3.4
Omeprazole sulfide. 4-OH	1.1	1.1	1.2	1.0	1.2	1.2	1.1
Oxolinic acid	0.36	0.27	d	d	d	d	-
Pantoprazole	d	d	d	d	d	d	d
Phenazone	-	-	-	-	-	-	-
Primidone	0.89	1.0	0.93	0.75	0.79	0.76	0.76
Roxithromycin	-	-	-	-	-	-	-
Salbutamol	d	d	d	d	d	d	d
Sulfadiazine	d	d	d	d	d	d	d
Sulfamethoxazole	0.22	0.27	0.23	0.28	0.36	0.34	0.34
Tetracycline	0.36	0.30	0.41	-	-	-	-
Tramadol	8.0	8.8	8.3	7.1	8.6	8.4	8.6
Trimetroprim	0.71	0.91	0.61	0.55	0.88	1.0	0.70
Valsartan	0.73	1.0	1.0	0.53	0.77	0.76	0.60
Venlafaxine	2.3	2.6	2.6	2.2	2.6	2.5	2.5

d: detected, not quantified. Concentration *below the limit value of quantification*
Underlined: guidance concentration data

Table S19. Average daily loads (g/day) of pharmaceuticals in IWW and EWW samples collected during the three sampling campaigns from the WWTP Ricao.

Compuestos	IWW				EWW			
	1st	2nd	3rd	Average	1st	2nd	3rd	Average
Acetaminophen	130	103	100	**111**	-	d	-	**d**
Alprazolam	-	d	-	**d**	d	d	d	**d**
Atorvastatin	1.7	d	1.8	**1.8**[a]	d	-	-	**d**
Azitromycin	3.7	7.4	-	**5.6**[a]	-	d	-	**d**
Bezafibrate	-	-	-	**-**	-	-	-	**-**
Carbamazepine	d	-	-	**d**	d	-	d	**d**
Ciprofloxacin	3014	189	26	**1076**	60	48	15	**41**
Clarithromycin	1.9	4.4	-	**3.2**[a]	0.80	2.3	0.77	**1.3**
Clindamycin	-	-	-	**-**	0.18	-	d	**0.18**[a]
Diclofenac	4.7	1.2	4.6	**3.5**	2.4	0.55	2.6	**2**
Enalapril	1.0	-	0.62	**0.82**[a]	-	-	-	**-**
Erythromycin	0.31	1.4	d	**0.84**[a]	0.46	d	0.27	**0.37**[a]
Furaltadone	-	-	-	**-**	-	-	-	**-**
Gabapentin	80	42	78	**67**	26	11	23	**20**
Gemfibrozil	-	-	-	**-**	-	-	-	**-**
Irbesartan	4.5	1.4	3.8	**3.2**	2.9	1.2	3.3	**2.5**
Ketoprofen	-	-	-	**-**	-	-	-	**-**
Levamisole	0.60	-	-	**0.60**[b]	0.47	d	0.26	**0.37**[a]
Lincomycin	-	-	-	**-**	-	-	-	**-**
Lorazepam	0.68	-	0.53	**0.61**[a]	0.73	0.76	0.44	**0.58**
Losartan	3.4	0.61	1.4	**1.8**	0.20	0.22	0.31	**0.24**
Metoprolol	d	d	d	**d**	0.20	d	d	**0.20**
Metronidazole	d	0.80	1,1	**1.0**[a]	d	d	d	**d**
Nalidixic acid	-	-	-	**-**	d	-	-	**d**
Naproxen	46	-	-	**46**[b]	-	-	-	**-**
Norfloxacin	17	236	11	**88**	13	52	7.4	**24**
Omeprazole sulfide. 4-OH	1.3	d	1.0	**1.2**[a]	0.63	d	1.1	**0.88**[a]
Oxolinic acid	-	d	-	**d**	-	-	0.32	**0.32**[b]
Pantoprazole	-	d	d	**d**	0.32	d	d	**0.32**[b]
Phenazone	1.6	0.85	-	**1.2**[a]	d	-	-	**d**
Primidone	1.5	-	1.1	**1.3**[a]	1.2	0.23	0.84	**0.75**
Roxithromycin	-	-	-	**-**	-	-	-	**-**
Salbutamol	d	d	d	**d**	d	-	d	**d**
Sulfadiazine	-	-	d	**d**	-	-	d	**d**
Sulfamethoxazole	1.5	d	0.7	**1.1**[a]	0.56	0.29	0.29	**0.38**
Tetracycline	1.1	2.2	1.1	**1.5**	0.42	-	0.36	**0.39**[a]
Tramadol	13	2.7	9.76	**8.3**	9.9	2.4	8.3	**6.8**
Trimetroprim	2.8	2.2	4.8	**3.3**	0.26	0.45	0.77	**0.49**
Valsartan	8.4	3.2	9.4	**7.0**	0.42	0.69	0.77	**0.63**
Venlafaxine	3.3	0.9	2.6	**2.2**	2.9	0.76	2.5	**2.0**

d: detected, not quantified. Concentration *below the limit value of quantification*
Underlined: guidance concentration data
[a] *Average of two samplings*
[b] *Value of only one sampling*

Table S20. Removal efficiency (%) of pharmaceuticals investigated in the WWTP Ricao during the **first sampling campaign** (September 2018).

Compounds	**1**	**2**	**3**	**4**	**5**	**6**	**Average**
Acetaminophen	100	100	100	100	100	100	**100**
Alprazolam	0	0	0	0	0	0	**0**
Atorvastatin	100	100	100	100	100	100	**100**
Azitromycin	100	100	100	100	100	100	**100**
Bezafibrate	-	-	-	-	-	-	-
Carbamazepine	100	100	100	100	100	100	**100**
Ciprofloxacin	n.c.	-	n.c.	n.c.	n.c.	n.c.	n.c.
Clarithromycin	71	48	74	29	52	68	**57**
Clindamycin	0	<0	0	0	0	<0	**0**
Diclofenac	72	50	28	41	42	64	**50**
Enalapril	100	100	100	100	100	100	**100**
Erythromycin	<0	<0	<0	<0	<0	<0	**<0**
Furaltadone	-	-	-	-	-	-	-
Gabapentin	65	64	65	65	73	76	**68**
Gemfibrozil	-	-	-	-	-	-	-
Irbesartan	53	39	0[(a)]	29	39	44	**41**
Ketoprofen	-	-	-	-	-	-	-
Levamisole	11	<0	36	21	37	35	**21**
Lincomycin	-	-	-	-	-	-	-
Lorazepam	2[(a)]	<0	26[(a)]	<0	<0	<0	**<0**
Losartan	94	95	100	94	93	100	**96**
Metoprolol	-	-	100[(a)]	<0[(a)]	-	-	**-**
Metronidazole	-	-	-	<0[(a)]	-	<0[(a)]	**-**
Nalidixic acid	-	-	<0[(a)]	-	-	<0[(a)]	**-**
Naproxen	100	100	100	100	-	100	**100**
Norfloxacin	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
Omeprazole sulfide. 4-OH	52	48	44	39	61	66	**52**
Oxolinic acid	-	-	-	-	-	-	-
Pantoprazole	<0	<0	<0	<0	<0	<0	**<0**
Phenazone	95	-	22[(a)]	72	55	49	**68**
Primidone	44	15	<0[(a)]	15	15	22	**22**
Roxithromycin	-	-	-	-	-	-	-
Salbutamol	<0[(a)]	-	-	-	-	-	**-**
Simvastatin	-	-	-	-	-	-	-
Sulfadiazine	-	-	-	-	-	-	-
Sulfamethoxazole	50	76	57	68	77	71	**67**
Tetracycline	53	70	56	100	100	83	**77**
Tramadol	30	23	21	<0[(a)]	1[(a)]	72	**36**
Trimetroprim	94	96	100	80	100	100	**95**
Valsartan	93	100	100	94	96	97	**97**
Venlafaxine	66	7	<0[(a)]	7	37	24	**28**

n.c., it could not be calculated by having only indicative *concentration data*

[(a)] Anomalous data (outlier), not considered *for obtaining the average*

** Removal efficiency has been* considered *as 0% since the data is highly variable, but with a tendency to non-elimination*

Table S21. Removal efficiency (%) of pharmaceuticals investigated in the WWTP Ricao during the **second sampling campaign** (January 2019).

Compounds	1	2	3	4	5	6	Average
Acetaminophen	100	100	100	100	100	100	**100**
Alprazolam	-	-	-	0	0	100[(a)]	**-**
Atorvastatin	100	100	100	100	100	100	**100**
Azitromycin	100	100	100	100	100	[(a)]	**100**
Bezafibrate	-	-	-	-	-	-	-
Carbamazepine	-	-	-	-	-	-	**-**
Ciprofloxacin	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	**n.c.**
Clarithromycin	66	34	64	16	39	50	**45**
Clindamycin	-	-	-	-	-	-	**-**
Diclofenac	81	49	33	44	59	<0[(a)]	**53**
Enalapril	-	-	-	-	-	-	**-**
Erythromycin	-	100	100	-	100	-	**100**
Furaltadone	-	-	-	-	-	-	-
Gabapentin	67	72	58	79	78	54	**68**
Gemfibrozil	-	-	-	-	-	-	-
Irbesartan	34	12	<0	36	4	<0	**0***
Ketoprofen	-	-	-	-	-	-	-
Levamisole	0	0	0	0	0	0	**0**
Lincomycin	-	-	-	-	-	-	-
Lorazepam	[(a)]	<0	<0	<0	<0	<0	**<0**
Losartan	100	67	61	100	100	[(a)]	**86**
Metoprolol	100[(a)]	-	-	-	-	-	**-**
Metronidazole	100	100	100	-[(a)]	100	100	**100**
Nalidixic acid	-	-	-	-	-	-	**-**
Naproxen	-	-	-	-	-	-	**-**
Norfloxacin	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	**n.c.**
Omeprazole sulfide. 4-OH	-	-	-	-	-	-	**-**
Oxolinic acid	100	100	100	100	-[(a)]	100	**100**
Pantoprazole	100 [(a)]	-	-	-	-	-	**-**
Phenazone	100	100	100	100	100	-[(a)]	**100**
Primidone	<0	<0	<0	<0	0	0	**<0**
Roxithromycin	-	-	-	-	-	-	-
Salbutamol	-[(a)]	100	100	100	-[(a)]	100	**100**
Simvastatin	-	-	-	-	-	-	-
Sulfadiazine	-	-	-	-	-	-	-
Sulfamethoxazole	-	<0	<0	<0	-	0	**<0**
Tetracycline	100	100	100	-[(a)]	100	-[(a)]	**100**
Tramadol	51	25	<0	35	29	<0	**0***
Trimetroprim	84	77	85	90	100	100	**89**
Valsartan	78	81	77	82	88	<0[(a)]	**81**
Venlafaxine	38	27	<0	<0	<0	<0	**0***

n.c., it could not be calculated by having only indicative concentration data

(a) Anomalous data (outlier), not considered for obtaining the average

* Removal efficiency has been considered as 0% since *the data is highly variable, but with a tendency to non-elimination*

Table S22. Removal efficiency (%) of pharmaceuticals investigated in the WWTP Ricao during the **third sampling campaign** (April 2019).

Compounds	**1**	**2**	**3**	**4**	**5**	**6**	**Average**
Acetaminophen	100	100	100	100	100	100	**100**
Alprazolam	-[(a)]	0	-[(a)]	0	0	0	**0**
Atorvastatin	100	100	100	100	100	100	**100**
Azitromycin	-	-	-	-	-	-	-
Bezafibrate	-	-	-	-	-	-	-
Carbamazepine	0	0	0	0	-[(a)]	0	**0**
Ciprofloxacin	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	**n.c.**
Clarithromycin	<0	<0	<0	-[(a)]	<0	<0	**<0**
Clindamycin	0	0	-[(a)]	0	-[(a)]	0	**0**
Diclofenac	32	68	51	62	41	40	**49**
Enalapril	100	100	100	100	100	100	**100**
Erythromycin	<0	<0	<0	<0	<0	<0	**<0**
Furaltadone	-	-	-	-	-	-	-
Gabapentin	52	67	77	72	74	70	**69**
Gemfibrozil	-	-	-	-	-	-	-
Irbesartan	-29[(a)]	-47[(a)]	31	44	30	26	**33**
Ketoprofen	-	-	-	-	-	-	-
Levamisole	<0	<0	<0	<0	<0	<0	**<0**
Lincomycin	-	-	-	-	-	-	-
Lorazepam	11	12	28	4	40	23	**20**
Losartan	100	80	87	100	100	-[(a)]	**93**
Metoprolol	-	0[(a)]	-	100[(a)]	-	-	-
Metronidazole	-	-	-	-	100 [(a)]	100 [(a)]	-
Nalidixic acid	-	-	-	-	-	-	-
Naproxen	-	-	-	-	-	-	-
Norfloxacin	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	**n.c.**
Omeprazole sulfide. 4-OH	-10	4	-8	-9	-8	2	**0***
Oxolinic acid	<0[(a)]	0	0	0	0	-[(a)]	**0**
Pantoprazole	-	-	-	-	-	-	-
Phenazone	-	-	-	-	-	-	-
Primidone	-18[(a)]	18	41	26	17	35	**27**
Roxithromycin	-	-	-	-	-	-	-
Salbutamol	-	-	-	-	-	-	-
Simvastatin	-	-	-	-	-	-	-
Sulfadiazine	-	-	-	-	-	-	-
Sulfamethoxazole	45	62	51	34	70	61	**54**
Tetracycline	86	<0[(a)]	-[(a)]	100	100	-[(a)]	**95**
Tramadol	-8[(a)]	16	36	4	20	8	**17**
Trimetroprim	67	87	91	83	85	88	**84**
Valsartan	84	93	94	93	92	93	**92**
Venlafaxine	-69[(a)]	11	18	10	15	3	**11**

n.c., it could not be calculated by having only indicative concentration data

(a) Anomalous data (outlier), not considered for obtaining the average

* Removal efficiency has been considered as 0% since the data is highly variable, but with a tendency to non-elimination

2.3.3. Discusión de resultados

El **artículo científico IV** contiene los resultados de un estudio que buscaba determinar la presencia de fármacos en las aguas residuales de una EDAR convencional con tratamiento biológico. Para ello, se seleccionaron 40 fármacos meta a partir de un cribado preliminar por UHPLC-QTOF MS. En la **Tabla 2.3** se indican las DHD y los porcentajes de excreción de los 40 fármacos estudiados.

Tabla 2.3. Consumo de fármacos en España y porcentajes de excreción.

Grupo terapéutico[a]	**Fármaco**	**Consumo (DHD)**[b]	**% Excreción**[c]
Analgésicos (2019)	Acetaminofén	27.3	>5 forma inalterada[d]
	Tramadol	2.77	30 renal, 60 fecal[e]
Agente antihelmíntico	Levamisol	n.d.	n.d.
Antibióticos (2018)	Azitromicina	1.50	5 forma inalterada[d]
	Ciprofloxacino	0.875	44.7 renal, 25 fecal[d]
	Claritromicina	0.553	20-30 forma inalterada renal, 70-80 fecal[d]
	Clindamicina	0.0907	n.d.
	Eritromicina	0.04307	12-15 forma inalterada renal[d]
	Furaltadona	n.d.	n.d.
	Lincomicina	0.000967	1-31 renal, >40 fecal[d]
	Metronidazol	n.d.	60-80 renal, 6-15 fecal[d]
	Ácido nalidíxico	n.d.	n.d.
	Norfloxacino	0.133	33-48 renal[d]
	Ácido oxolínico	n.d.	n.d.
	Roxitromicina	0.00315	10 renal, 65 fecal[d]
	Sulfadiazina	0.00137	6 -40 mg/100 ml renal[d]
	Sulfametoxazol+ Trimetoprima	0.349	sulfametoxazol 15-30 trimetoprima 50 renal[d]
	Tetraciclina	$8.44x10^{-6}$	n.d.
	Trimetoprima	0.00167	n.d.
Antidepresivos (2013)	Venlafaxina	8.63	87[e]
Antiepilépticos (2016)	Carbamazepina	0.92	72 renal, 28 fecal[d]
	Gabapentina	2.00	40[e]
	Primidona	0.10	40[d]

Tabla 2.3 (cont.). Consumo de fármacos en España y porcentajes de excreción.

Grupo terapéutico[a]	**Fármaco**	**Consumo (DHD)**[b]	**% Excreción**[c]
Antihipertensivos (2019)	Enalapril	42.97	40 enalaprilato, 20 inalterada renal[d]
	Irbesartán	4.36	20 renal, 80 fecal[e]
	Losartán	12.83	35-43 renal, 58-50 fecal[d]
	Valsartán	12.22	13 renal, 83 fecal[e]
Antiulcerosos[f] (2012)	4-hidroxi-sulfuro-omeprazol[g]	n.d.	80 renal, 20 fecal[d] (para el Omeprazol)
	Pantoprazol	12.04	80 renal, 20 fecal[d]
Ansiolíticos (2019)	Alprazolam	15.41	n.d.
	Lorazepam	22.25	77 renal[d]
Agentes betabloqueantes (2019)	Metoprolol	0.429	95 renal (>5 forma inalterada)[d]
	Salbutamol	n.d.	n.d.
Hipolipemiantes (2019)	Atorvastatina	60.24	<2 renal, >90 fecal[e]
	Bezafibrato	0.0795	95 renal, 3 fecal[d]
	Gemfibrozilo	1.22	70 renal, 6 fecal[d]
Antiinflamatorios no (2016)	Diclofenaco	3.15	60 renal, 40 fecal[d]
	Ketoprofeno	0.03	75-90 renal, 1-8 fecal[d]
	Naproxeno	8.56	95 renal, <3 fecal[d]
	Fenazona	n.d.	95 renal[d]

[a] Entre paréntesis se indica el año en que se reporta el valor de la DHD.
[b] AEMPS[24].
[c] Los datos corresponden a una administración por vía oral, excepto para la sulfadiazina y la fenazona, de uso cutáneo, y la eritromicina, que se aplica por vía intravenosa.
[d] CIMA[151].
[e] Thomas L. Lemke et al., 2013[152]
[f] En el caso del omeprazol, DHD = 104.02
[g] Metabolito del omeprazol.
n.d.: datos no disponibles.

Las muestras de aguas residuales de la EDAR se recogieron durante tres campañas de muestreo realizadas entre septiembre de 2018 y abril de 2019. De manera complementaria, en la primera campaña se muestreó la descarga de un hospital que lleva sus efluentes a la estación

depuradora. Las muestras, previamente centrifugadas y diluidas, se analizaron por inyección directa (DI) con posterior determinación mediante UHPLC-MS/MS con analizador QqQ.

A continuación, se presentan los resultados de las tres campañas de muestreo, el impacto de la descarga hospitalaria, la eficiencia de eliminación de la estación depuradora y la variación estacional de los 40 fármacos seleccionados.

Análisis de las muestras de agua tomadas en las tres campañas de muestreo

Las 42 muestras (21 IWW y 21 EWW) de agua residual que se tomaron entre septiembre de 2018 y abril de 2019 se analizaron mediante UHPLC-MS/MS (QqQ). En todas las secuencias de análisis se introdujeron QC a dos niveles de concentración, tanto para las muestras del IWW como del EWW. En general, salvo algunas excepciones, los resultados fueron satisfactorios. Ahora bien, 34 de los 40 fármacos seleccionados se detectaron en un rango relativamente amplio de concentraciones. Los únicos que no se detectaron fueron: furaltadona, lincomicina y roxitromicina (antibióticos); bezafibrate y gemfibrozil (hipolipemiantes), y ketoprofeno (antiinflamtorio).

En el primer muestreo, se detectaron 23 fármacos (22 en IWW y 17 en EWW) (ver **Figura 2.22**). En el influente, la mayor concentración la presentaron analgésicos, antibióticos, antiepilépticos, antihipertensivos y antiinflamatorios no esteroideos; a saber, acetaminofén, 2.6-8.7 µg/L; azitromicina, 0.17-0.21 µg/L; gabapentina, 3.6-4.7 µg/L; valsatán, 0.39-0.65 µg/L y naproxeno, 2.35-2.37 µg/L. Como era de esperar, los niveles de concentración en las aguas del efluente fueron menores: tramadol, 0.27-0.91 µg/L; venlafaxina, 0.15-0.19 µg/L; gabapentina, 1.3-1.6 µg/L; irbesartán, 0.13-0.19 µg/L; y diclofenaco, 0.11-0.18 µg/L.

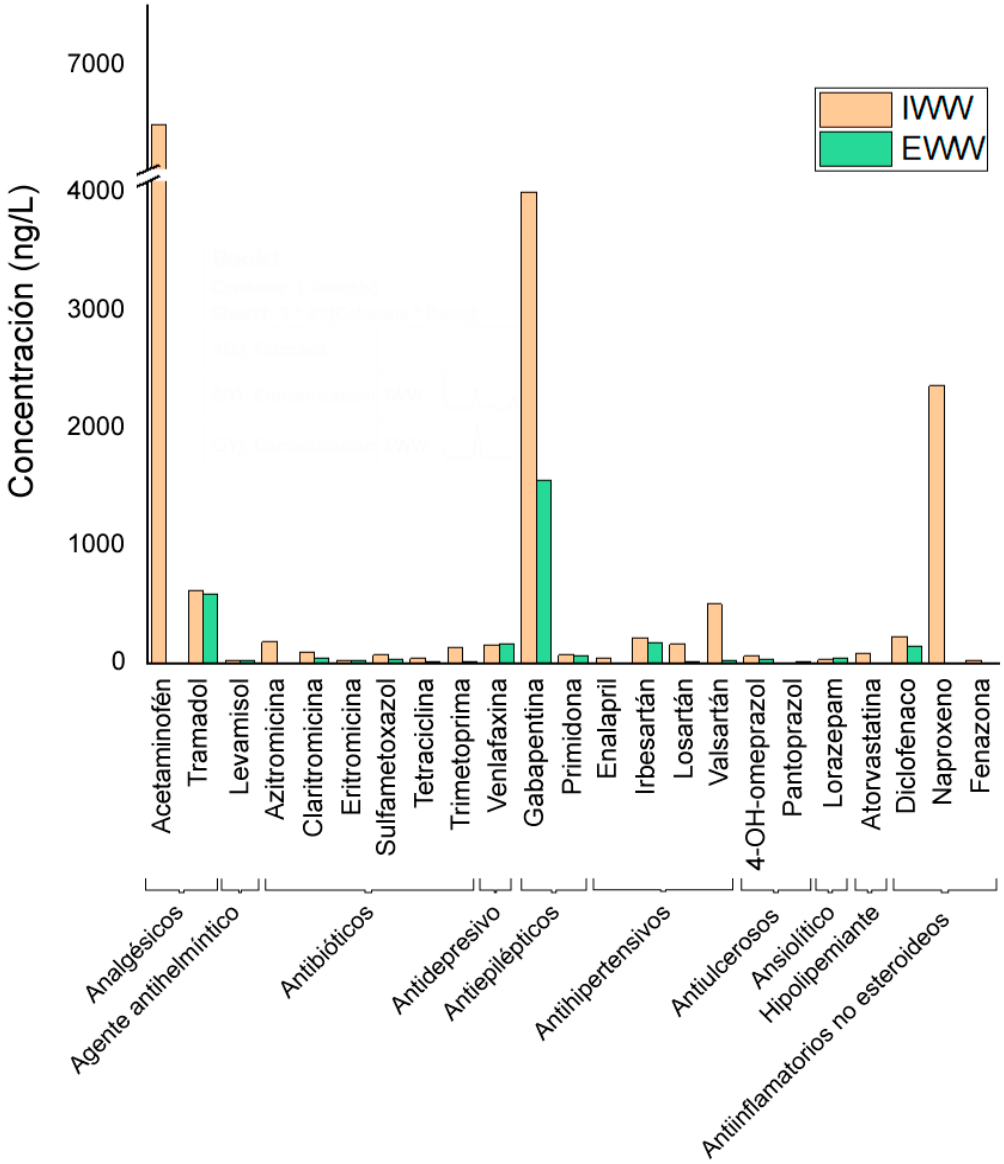


Figura 2.22. Concentración (µg/L) promedio de fármacos presentes en el influente y el efluente de la EDAR (septiembre de 2018).

En el segundo muestreo se encontraron 17 fármacos (15 en IWW y 11 en EWW) (ver **Figura 2.23**). También en este caso las mayores concentraciones se observaron en analgésicos, antibióticos, antiepilépticos, antihipertensivos y antiinflamatorios no esteroideos: acetaminofén, 0.69-9.4 µg/L; azitromicina, 0.25-0.43 µg/L; gabapentina, 0.86-2.8 µg/L; valsartán, 0.090-0.27 µg/L y diclofenaco, 0.030-0.075 µg/L. En el efluente, la mayor concentración se observó en analgésicos, antibióticos, antidepresivos, antiepilépticos y

antihipertensivos: tramadol, 0.059-0.15 µg/L; claritromicina, 0.060-0.15 µg/L; venlafaxina, 0.020-0.056 µg/L; gabapentina, 0.37-0.72 µg/L; irbesartán, 0.036-0.080 µg/L.

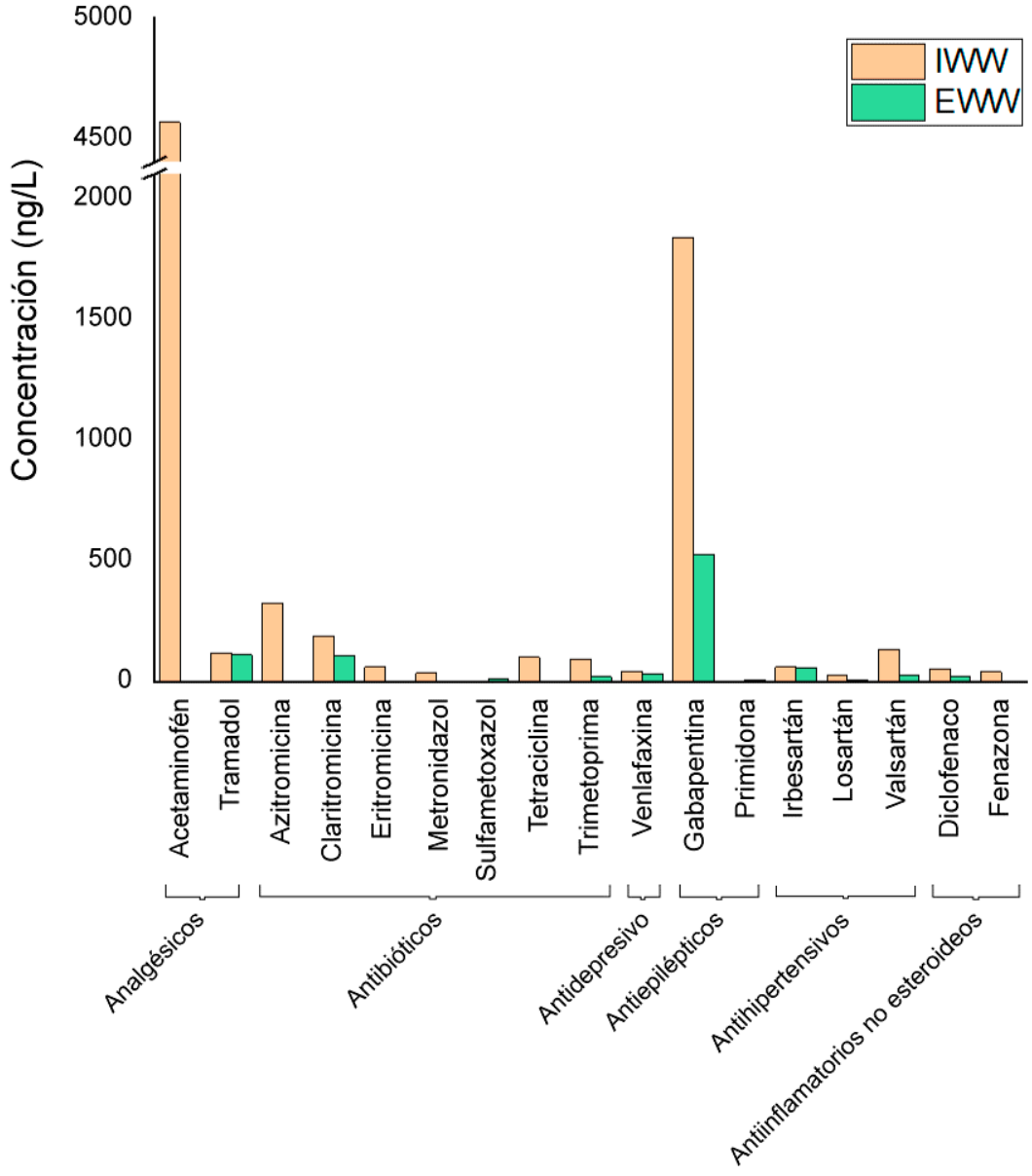


Figura 2.23. Concentración (µg/L) promedio de fármacos presentes en el influente y el efluente de la EDAR (enero de 2019).

En el tercer muestreo (abril de 2019) se encontraron 21 fármacos (17 en IWW y 17 en EWW) (ver **Figura 2.24)**. En el influente, las mayores concentraciones se observaron en analgésicos, antibióticos, antiepilépticos, antihipertensivos y antiinflamatorios no esteroideos; a saber:

acetaminofén, 0.027-9.4 µg/L; trimetoprima, 0.12-0.31 µg/L; gabapentina, 2.5-5.1 µg/L; valsartán, 0.31-0.64 µg/L y diclofenaco, 0.13-0.31 µg/L. Esos mismos grupos terapéuticos aportaron las mayores concentraciones en el efluente: tramadol, 0.36-0.41 µg/L; claritromicina, 0.023-0.058 µg/L; gabapentina, 1.0-1.2 µg/L; irbesartán, 0.090-0.25 µg/L y diclofenaco, 0.080-0.19 µg/L.

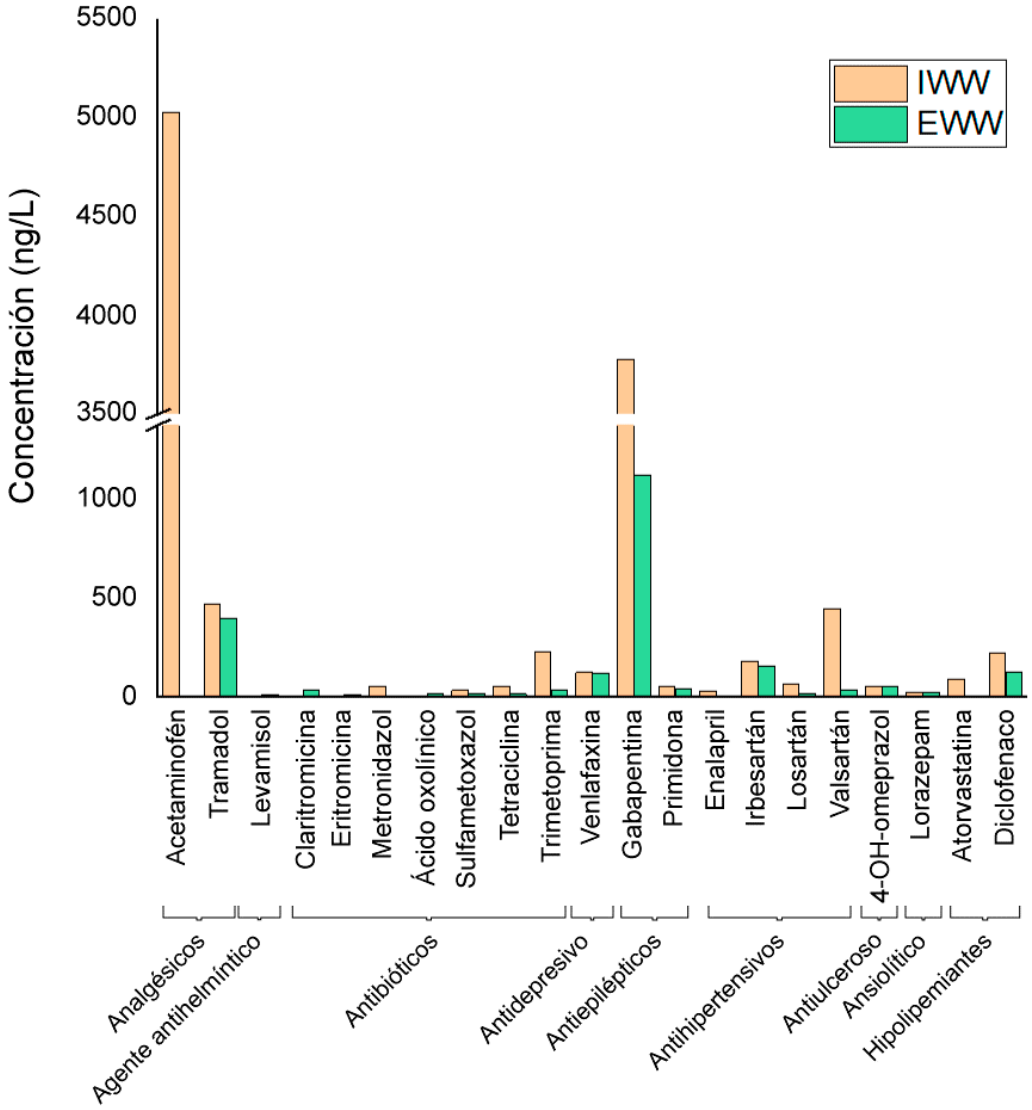


Figura 2.24. Concentración (µg/L) promedio de fármacos presentes en el influente y el efluente de la EDAR (abril de 2019).

Varios de los fármacos que se detectaron a bajas concentraciones en las aguas residuales (claritromicina, carbamazepina, metoprolol y ketoprofeno) se consumen relativamente poco

en España (ver **Tabla 2.3**), pero tienen un porcentaje de excreción alto (>70 %). Otros, como el acetaminofén, presentan un consumo alto pero un porcentaje de excreción bajo en forma inalterada (< 30 %). Finalmente, fue notable la presencia de fármacos como gabapentina, irbesartán, losartán y valsartán, posiblemente por presentar un consumo alto y un porcentaje de excreción intermedio (30-70%).

Impacto de la descarga del hospital

El impacto de la descarga hospitalaria a la EDAR se evaluó únicamente en la primera campaña de muestreo. Un análisis de siete muestras de aguas de IWW y siete muestras de aguas de descarga hospitalaria reveló la presencia de 28 de los 40 fármacos investigados en ambos tipos de aguas, con una coincidencia de 24 compuestos y una concentración superior en las muestras hospitalarias, excepto en el caso de la claritromicina, el irbesartán, el levamisol, la primidona y la tetraciclina. En la **Figura 3** del **artículo científico IV** se muestra la concentración (ng/L) promedio semanal de los fármacos detectados en las aguas hospitalarias y en las aguas del influente de la estación depuradora.

Los fármacos que presentaron la mayor concentración en ambos tipos de aguas fueron el acetaminofén, la gabapentina y el naproxeno, con valores de 159, 23 y 2.9 µg/L, respectivamente, en las aguas hospitalarias, y de 4.7, 8.7 y 2.4 µg/L, respectivamente, en el IWW. Si bien en las aguas hospitalarias no se detectaron carbamazepina, levamisol, metronidazol o tetraciclina, estos fármacos sí aparecieron en las muestras del IWW, lo que sugiere que podrían estar ingresando a la planta de una fuente distinta a la hospitalaria. Por su parte, los fármacos alprazolam, clindamicina, pantroprazol y sulfadiazina se detectaron únicamente en las aguas hospitalarias, lo que sugiere que, o bien se degradaron en el trayecto hacia la planta, o bien sufrieron una dilución significativa, de manera que no fue posible detectarlos. Esta última suposición es razonable porque, para estas cuatro moléculas, la concentración promedio más alta durante la semana fue de 0.1 µg/L.

Eficiencia de eliminación de fármacos en la EDAR

La eficiencia de eliminación (RE) de los fármacos detectados en la EDAR se estimó a partir de los resultados obtenidos durante las tres campañas de muestreo de aguas de IWW y EWW. Así, se observaron varios comportamientos de eliminación:

- Los siguientes compuestos se eliminaron casi por completo (RE >75%): acetaminofén, atorvastatina, azitromicina, enalapril, losartán, metronidazol, naproxeno, salbutamol, tetraciclina, trimetroprima y valsartán.
- Los siguientes compuestos se eliminaron de forma parcial (50% < RE <75%): diclofenaco, gabapentina y fenazona.
- Los siguientes compuestos presentaron valores de eliminación ligeramente variables durante las tres semanas de muestreo y su eliminación se consideró deficiente (RE ≤ 40%): irbesartán, levamisol, lorazepam, primidona, tramadol y venlafaxina.
- Los siguientes compuestos presentaron un valor RE cercano a 0 o inferior a 0: alprazolam, clindamicina, metoprolol, ácido nalidíxico, pantoprazol y sulfadiazina.
- Los siguientes compuestos presentaron valores de eliminación muy variables durante los tres muestreos: carbamazepina, claritromicina, eritromicina, 4-hidroxi-sulfuro-omeprazol, ácido oxolínico y sulfametoxazol. Este comportamiento puede deberse a las propiedades fisicoquímicas de los analitos, a factores bióticos y abióticos en la estación depuradora o a características propias del influente. La planta recibe descargas industriales del sector químico, farmacéutico, alimentario y de servicios, por lo que el influente se convierte en una matriz muy heterogénea y compleja de analizar.

Variación estacional

Los valores de concentración obtenidos durante las tres campañas de muestreo no permiten establecer tendencias estacionales por compuesto a lo largo del año de control. Sin embargo, al comparar la concentración total promedio de los diferentes grupos terapéuticos, se encontró que los valores más bajos se presentaron durante el invierno, excepto en el caso de los antibióticos, tanto en el IWW como en el EWW (**Figura 2, artículo científico IV**). Esta situación podría deberse a que los antibióticos se prescriben con mucha mayor frecuencia

durante el primer trimestre del año para combatir infecciones bacterianas en la población española (**Figura 2.25)**.

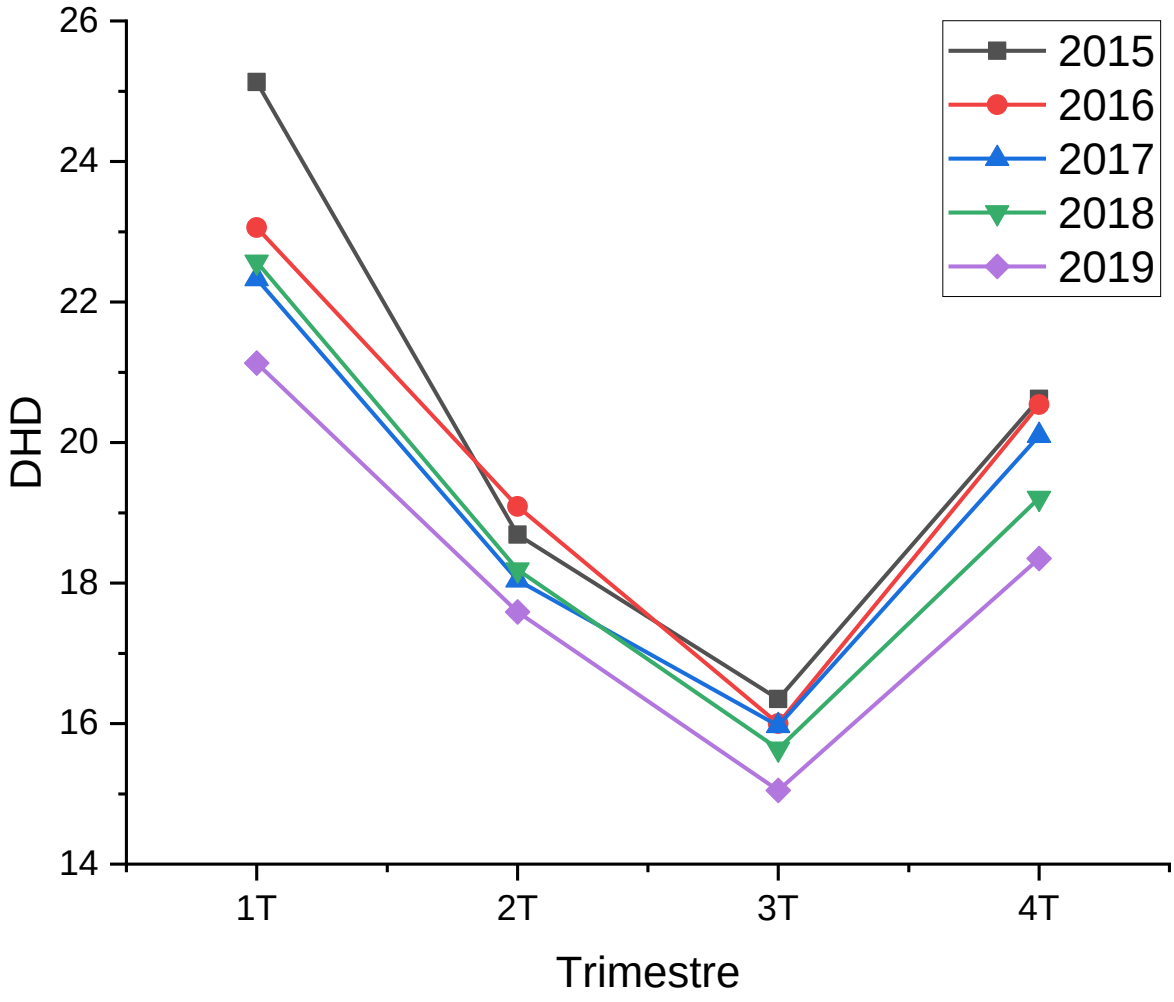
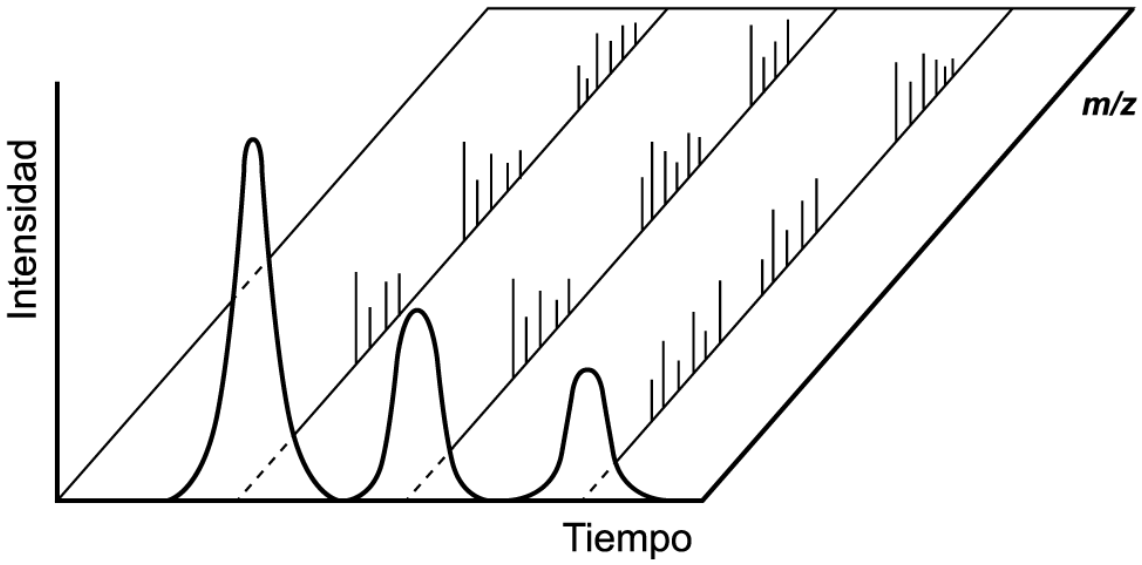


Figura 2.25. Consumo trimestral de antibióticos de uso sistémico en España durante el período 2015-2019. Fuente: Elaborada con base en PRAN, 2018.

CAPÍTULO 3

CONCLUSIONES Y TRABAJO FUTURO



3.1. Conclusiones

La conclusión general que se extrae de los trabajos realizados en la presente tesis doctoral es que se ha podido evaluar el potencial de las técnicas cromatográficas de líquidos y de gases acopladas a la espectrometría de masas de alta resolución y baja resolución, que, de forma complementaria, permiten tener una herramienta analítica muy útil y versátil para detectar, identificar y cuantificar un gran número de compuestos y productos de degradación con propiedades fisicoquímicas muy diferentes en muestras de agua medioambientales y residuales.

De los trabajos presentados en esta tesis doctoral se pueden derivar las siguientes conclusiones específicas:

1) El screening mediante LC-QTOF MS y GC-QTOF MS brindó la posibilidad de detectar una gran cantidad de compuestos sin tener que hacer una preselección de contaminantes o una optimización individualizada de parámetros como la selección de transiciones y energías de colisión, pues se adquiere un espectro completo de masas, con alta resolución y medidas de masa exacta y con buena sensibilidad.

2) El acoplamiento UHPLC-MS/MS con analizador QqQ permitió la confirmación y cuantificación de plaguicidas, fármacos y TPs en muestras de aguas naturales (superficiales y subterráneas), así como aguas residuales provenientes del influente y efluente de una EDAR.

3) La inyección directa de muestras de aguas superficiales, sin ningún tratamiento previo, y de aguas residuales con una dilución previa, y su posterior medición por UHPLC-MS/MS QqQ, permitió una cuantificación rápida y eficaz de los compuestos estudiados.

4) El uso de estándares internos marcados isotópicamente (ILIS) corrigió de manera aceptable los valores del control de calidad (QC) para los analitos en que fueron utilizados, lo cual permitió una cuantificación más fiable y precisa de los resultados.

5) Del análisis mediante UHPLC-QTOF MS de las aguas de la cuenca hidrográfica del Júcar, se encontró una notable cantidad de plaguicidas y TPs, de amplio uso para riego y principal

fuente de suministro de agua potable. Este hecho demuestra la vulnerabilidad del área y la necesidad de aplicar metodologías de análisis cuantitativos basadas en listas actualizadas de compuestos diana para vigilar de cerca la calidad del agua superficial y subterránea.

6) Del análisis cuantitativo mediante UHPLC-MS/MS QqQ de las aguas superficiales del río Mijares, se detectaron plaguicidas, algunos de los cuales superaron en alguna ocasión el valor 0.1 µg/L (2,4-D, imazalil, imidacloprid y tiabendazol). Se observó, que la parte baja del río Mijares, donde predominan los cultivos de cítricos, presentó el mayor nivel de contaminación por plaguicidas, especialmente en el área más cercana a la desembocadura. El impacto de los efluentes de las EDAR cercanas a los puntos de muestreo de esta zona, se hizo evidente por el número de compuestos detectados en las muestras recolectadas aguas abajo de las descargas de las estaciones.

7) Tras la evaluación de los riesgos acuáticos de los plaguicidas detectados en el río Mijares, se concluyó que siguiendo el enfoque TU los riesgos de toxicidad fueron bajos para los productores primarios, y moderados para especies de invertebrados (exposición crónica) y vertebrados (toxicidad aguda) debido a la exposición al fungicida tiabendazol. Por otro lado, mediante el enfoque ms-PAF, se obtuvieron riesgos moderados para los productores primarios a partir de mezclas de herbicidas diurón, simazina y 2,4-D, y riesgos moderados a altos para especies de invertebrados y vertebrados debido a la exposición del tiabendazol. Además, el imidacloprid presenta altos riesgos agudos y crónicos para los invertebrados acuáticos.

8) El análisis cuantitativo mediante UHPLC-MS/MS QqQ de las aguas superficiales del río Mijares permitió detectar un número considerable de fármacos, especialmente en las muestras tomadas aguas debajo de los vertidos de las EDAR. Los compuestos que más frecuentemente detectados fueron acetaminofén, gabapentina y venlafaxina y los que presentaron de mayores concentraciones fenazona, tramadol y gabapentina. No se encontraron tendencias claras en función de la temporada de muestreo, excepto en la tercera campaña (invierno), donde hubo un leve aumento en la concentración de

antibióticos, posiblemente porque en esta época se incrementa su uso para tratar infecciones respiratorias.

9) En la evaluación del riesgo ecológico de los fármacos detectados en el río Mijares, los compuestos que ejercieron mayor toxicidad sobre los ecosistemas acuáticos fueron principalmente los analgésicos/antiinflamatorios y los antibióticos. Además, cinco de los antibióticos excedieron los umbrales de resistencia (ciprofloxacino, azitromicina, norfloxacino, trimetoprima, y claritromicina) en las aguas abajo de las EDAR. Estos resultados sugieren que las descargas de las EDAR en el río Mijares están contribuyendo a crear concentraciones de antibióticos que podrían contribuir al enriquecimiento de genes de resistencia en comunidades bacterianas acuáticas.

10) El uso complementario de la metodología cuantitativa y el cribado cualitativo permitió tener una visión más completa de los fármacos y metabolitos presentes en el agua. De los resultados obtenidos mediante UHPLC-HRMS se llegó a la identificación de nueve metabolitos, de los cuales 4-acetilaminoantipirina (4-AAA), 4-formilaminoantipirina (4-FAA), 4-OH omeprazol sulfuro, carbamazepina-10,11-epóxido y ácido clopidogrel carboxílico fueron los más detectados. Estos hallazgos indican que sería recomendable realizar más estudios sobre la aparición y los riesgos de estos metabolitos en las aguas.

11) Está claro que los efluentes de las estaciones de tratamiento de aguas residuales y las descargas hospitalarias son una fuente importante de contaminación del medio ambiente acuático. Debido a la elevada cantidad de fármacos que pueden estar presentes en este tipo de muestras, fue necesario la aplicación preliminar de un cribado de amplio alcance por UHPLC-QTOF MS, que arrojó un número significativo de detecciones que fueron la base para el análisis cuantitativo mediante UHPLC-MS/MS QqQ.

12) La estimación de la eficiencia de eliminación mediante el tratamiento biológico convencional mostró comportamientos diferentes entre los fármacos detectados. Alrededor de un 50% de los compuestos se eliminaron totalmente mediante el tratamiento biológico convencional, y una gran cantidad de compuestos no se pudo eliminar de manera eficiente. La presencia de algunos de los fármacos estudiados en las aguas residuales

tratadas puede suponer un riesgo para el medio ambiente acuático. Por lo tanto, probablemente deberían implementarse tratamientos adicionales en la EDAR para mejorar la eliminación de estos contaminantes emergentes, así como realizar mediciones periódicas para la evaluación de la estación depuradora y el potencial impacto de los efluentes en el medio acuático.

3.1. Conclusions

The general conclusion drawn from the work undertaken during this doctoral thesis is that it has been possible to evaluate the potential of liquid and gas chromatographic techniques coupled to high resolution and low resolution mass spectrometry, which, in a complementary way, allow us to have a very useful and versatile analytical tool to detect, identify and quantify a large number of compounds and degradation products with very different physicochemical properties in environmental and wastewater samples.

The following specific conclusions can be derived from the work presented in this doctoral thesis:

1) Screening by LC-QTOF MS and GC-QTOF MS provided the possibility of detecting a large number of compounds without having to make a preselection of contaminants or an individualized optimization of parameters such as the selection of transitions and collision energies, as a complete mass spectrum was obtained, with high resolution and accurate mass measurements and with good sensitivity.

2) The UHPLC-MS/MS coupling with a QqQ analyzer allowed the confirmation and quantification of pesticides, pharmaceuticals and TPs in natural water samples (surface water and groundwater) as well as wastewater from WWTP influents and effluents.

3) Direct injection of surface water samples, without any prior treatment, and wastewater samples with prior dilution, and their subsequent measurement by UHPLC-MS/MS QqQ, allowed rapid and efficient quantification of the compounds studied.

4) The use of isotopically labeled internal standards (ILIS) corrected in an acceptable manner the quality control (QC) values for the analytes in which they were used, which allowed a more reliable and precise quantification of the results.

5) The analysis by UHPLC-QTOF MS of the waters of the Júcar River basin found a notable amount of pesticides and TPs, widely used for irrigation and the main source of drinking water supply. This fact demonstrates the vulnerability of the area and the need to apply

quantitative analysis methodologies based on updated lists of target compounds to closely monitor surface and groundwater quality.

6) The quantitative analysis by UHPLC-MS/MS QqQ of the surface water of the Mijares River, revealed pesticides, some of which exceeded 0.1 µg/L at some point (2,4-D, imazalil, imidacloprid and thiabendazole). It was observed that the lower part of the Mijares River, where citrus crops predominate, had the highest level of pesticide contamination, especially in the area closest to the mouth of the river. The impact of the effluents from the WWTPs near the sampling points in this area was evident from the number of compounds detected in the samples collected downstream of the stations' discharges.

7) After the aquatic risk assessment of the pesticides detected in the Mijares River, it was concluded that, following the TU approach, the toxicity risks were low for primary producers, and moderate for invertebrate species (chronic exposure) and vertebrates (acute toxicity) due to exposure to the fungicide thiabendazole. On the other hand, using the ms-PAF approach, moderate risks were obtained for primary producers from mixtures of diuron, simazine and 2,4-D herbicides, and moderate to high risks for invertebrate and vertebrate species due to thiabendazole exposure. In addition, imidacloprid presents high acute and chronic risks to aquatic invertebrates.

8) Quantitative analysis by UHPLC-MS/MS QqQ of the surface waters of the Mijares River allowed the detection of a considerable number of drugs, especially in the samples taken downstream of the WWTP discharges. The most frequently detected compounds were acetaminophen, gabapentin and venlafaxine and those with the highest concentrations were phenazone, tramadol and gabapentin. No clear trends were found according to the sampling season, except in the third campaign (winter), where there was a slight increase in the concentration of antibiotics, possibly because their use to treat respiratory infections increases at that time of year.

9) In the ecological risk assessment of the drugs detected in the Mijares River, the compounds that exerted the greatest toxicity on aquatic ecosystems were mainly analgesics/anti-

inflammatory drugs and antibiotics. In addition, five of the antibiotics exceeded resistance thresholds (ciprofloxacin, azithromycin, norfloxacin, trimethoprim, and clarithromycin) in the waters downstream of the WWTPs. These results suggest that WWTP discharges into the Mijares River are contributing to antibiotic concentrations that could contribute to the enrichment of resistance genes in aquatic bacterial communities.

10) The complementary use of quantitative methodology and qualitative screening provided a more complete picture of the drugs and metabolites present in the water. From the results obtained by UHPLC-HRMS, nine metabolites were identified, of which 4-acetylaminoantipyrine (4-AAA), 4-formylaminoantipyrine (4-FAA), 4-OH omeprazole sulfide, carbamazepine-10,11-epoxide and clopidogrel carboxylic acid were the most detected. These findings indicate that further studies on the occurrence and risks of these metabolites in waters would be advisable.

11) It is clear that effluents from sewage treatment plants and hospital discharges are a major source of contamination of the aquatic environment. Due to the high amount of pharmaceuticals that may be present in this type of samples, preliminary application of a wide-range screening by UHPLC-QTOF MS was necessary, which yielded a significant number of detections that were the basis for quantitative analysis by UHPLC-MS/MS QqQ.

12) The estimation of the elimination efficiency by conventional biological treatment showed different behaviors among the detected drugs. About 50% of the compounds were completely removed by conventional biological treatment, and a large number of compounds could not be removed efficiently. The presence of some of the studied drugs in treated wastewater may pose a risk to the aquatic environment. Therefore, additional treatments should probably be implemented in the WWTPs to improve the removal of these emerging pollutants, as well as periodic measurements for the evaluation of the WWTPs and the potential impact of the effluents on the aquatic environment.

3.2. Sugerencias para trabajo futuro

Los resultados obtenidos en esta tesis doctoral permiten sugerir algunas líneas de trabajo para futuras investigaciones que pueden resultar de interés:

- Desarrollar, optimizar y validar una metodología para la cuantificación de contaminantes por GC-MS/MS con analizador de triple cuadrupolo (QqQ) para complementar los resultados obtenidos mediante el cribado por GC-QTOF MS.

- Ampliar la capacidad analítica de la metodología cuantitativa de LC-MS/MS QqQ para fármacos y especialmente para productos de degradación encontrados en el cribado realizado por LC-QTOF-MS en esta tesis doctoral, en otros resultados disponibles en la literatura científica y para los nuevos compuestos incluidos en la nueva lista de observación de sustancias.

- Aumentar la cantidad de plaguicidas y productos de transformación de la validación cualitativa realizada por LC-QTOF MS, para identificar, mediante la detección de amplio alcance, un mayor número de contaminantes relevantes e incluirlos en las metodologías cuantitativas por LC-MS/MS QqQ.

- Explorar el potencial del acoplamiento de la cromatografía de líquidos y la cromatografía de gases con la espectrometría de masas de movilidad iónica (IMS), LC-IMS QTOF MS y GC-APCI-IMS QTOF MS, respectivamente, para la detección e identificación de fármacos y productos de degradación presentes en las aguas residuales y de descarga hospitalaria.

3.2. Suggestions for future work

The results obtained in this doctoral thesis allow to suggest some lines of work for future research that may be of interest:

- Develop, optimize and validate a methodology for the quantification of contaminants by GC-MS/MS with a triple quadrupole analyzer (QqQ) to complement the results obtained by GC-QTOF MS screening.

- Extend the analytical capability of the quantitative LC-MS/MS QqQ methodology for pharmaceuticals, degradation products found in the screening performed by LC-QTOF-MS in this PhD thesis and in other results available in the scientific literature, and for new compounds included in the watch list of substances.

- Increase the number of pesticides and transformation products from the qualitative validation performed by LC-QTOF MS, in order to identify, by wide-range screening, a larger number of relevant contaminants and include them in quantitative methodologies by LC-MS/MS QqQ.

- Explore the potential of coupling liquid chromatography and gas chromatography with ion mobility mass spectrometry (IMS), LC-IMS QTOF MS and GC-APCI-IMS QTOF MS, respectively, for the detection and identification of pharmaceuticals and degradation products present in wastewater and hospital discharge water.

CAPÍTULO 4

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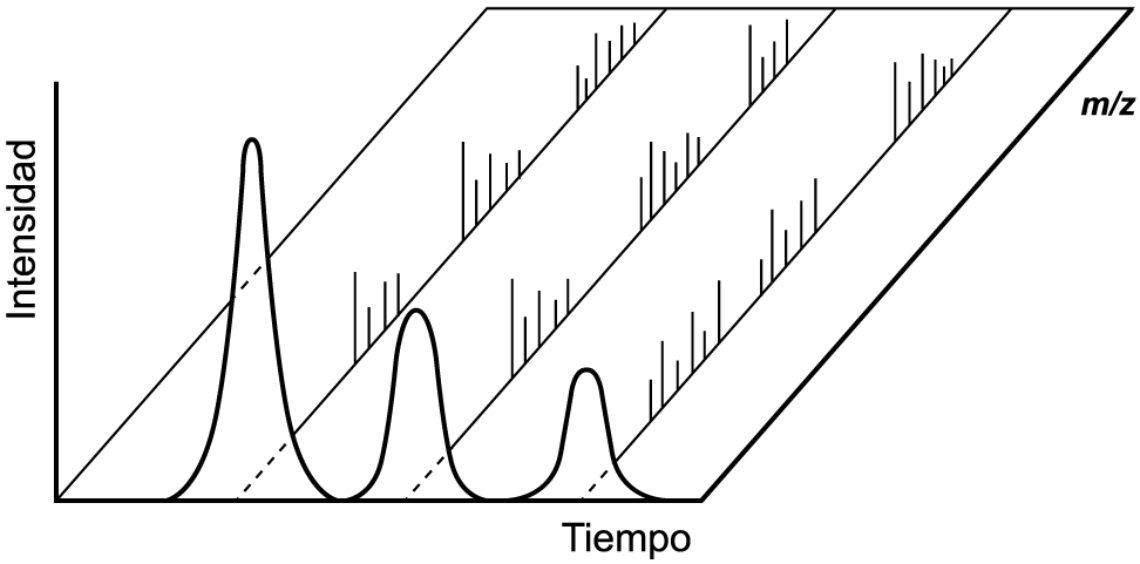

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ANEXO

Tablas y aceptación de coautores

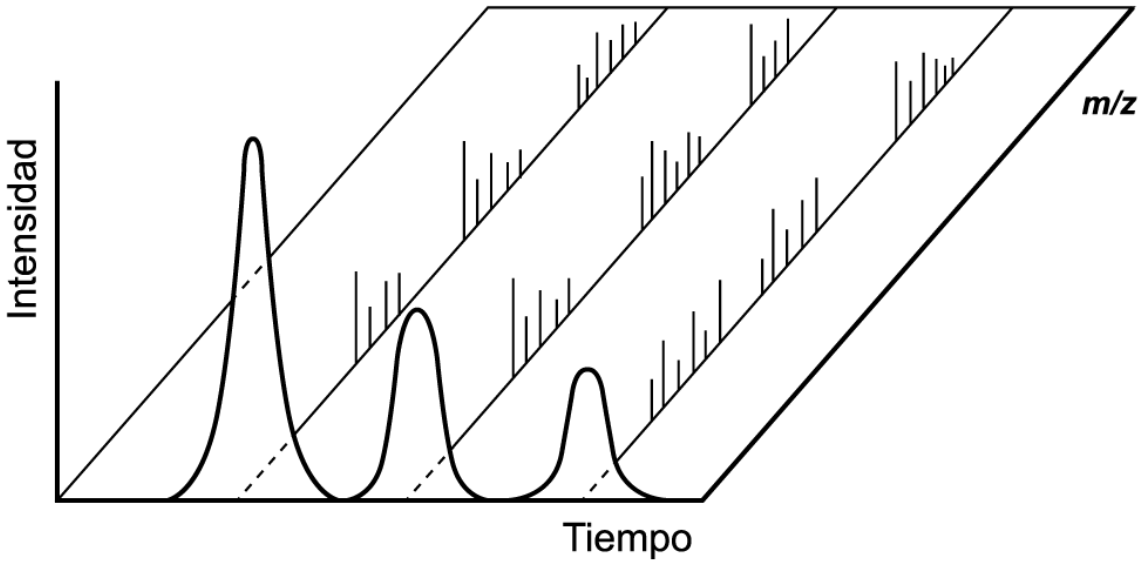


Tabla A1. Propiedades de plaguicidas y productos de transformación estudiados y detectados en la tesis doctoral.

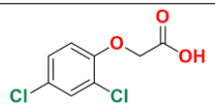
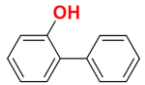
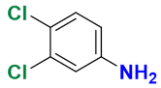
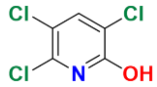
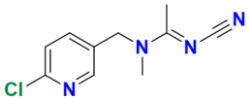
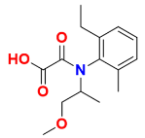
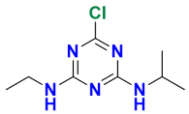
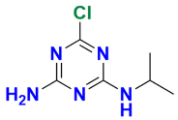
Plaguicida /Número CAS/ Acción biocida[1]	Fórmula química/ Masa exacta (Da)[2]	Estructura química[3]	Solub. en agua[a] (mg/L a 20 °C)[1]	p*Ka* (25 °C)[1]	Coeficiente de adsorción[b], Koc[1]	Vida media[c] (d)[1]	log Kow (pH 7, 20 °C)[1]
2,4-D 94-75-7 Herbicida	$C_8H_6Cl_2O_3$ 219.9694		24300	3.4	39.3	4.4	-0.82
2-Fenilfenol 90-43-7 Fungicida	$C_{12}H_{10}O$ 170.0732		560	9.4	n.d	4	3.18
3,4-Dicloroanilina 95-76-1 TP	$C_6H_5Cl_2N$ 160.9799		580	2.97	195[4]	n.d	2.69
3,5,6-tricloro-2-piridinol (TCP) 6515-38-4 TP	$C_5H_2Cl_3NO$ 196.9202		80.9	n.d	149	226	3.21
Acetamiprid 135410-20-7 Insecticida	$C_{10}H_{11}ClN_4$ 222.0672		2950	0.7	200	1.6	0.8
Ácido metolacloro oxanílico (MOA) 152019-73-3 TP	$C_{15}H_{21}NO_4$ 279.1471		360000	n.d	17.0	325	n.d
Atrazina 1912-24-9 Herbicida	$C_8H_{14}ClN_5$ 215.0938		35	1.7	100	75	2.7
Atrazina-desetil (DEA) 6190-65-4 TP	$C_6H_{10}ClN_5$ 187.0625		2700	n.d	110	45	1.51

Tabla A1 (cont.). Propiedades de plaguicidas y productos de transformación estudiados y detectados en la tesis doctoral.

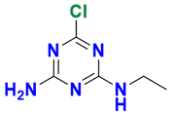
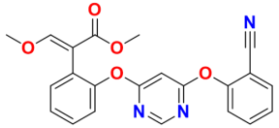
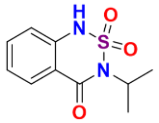
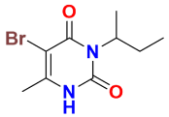
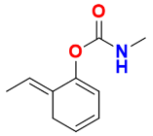
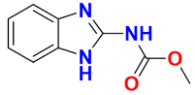
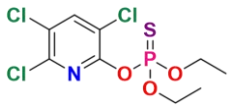
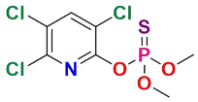
Plaguicida /Número CAS/ Acción biocida[1]	Fórmula química/ Masa exacta (Da)[2]	Estructura química[3]	Solub. en agua[a] (mg/L a 20 °C)[1]	p*Ka* (25 °C)[1]	Coeficiente de adsorción[b], Koc[1]	Vida media[c] (d)[1]	log Kow (pH 7, 20 °C)[1]
Atrazina-desisopropil (DIA) 1007-28-9 TP	$C_5H_8ClN_5$ 173.0468		980	n.d	130	n.d	1.15
Azoxistrobina 131860-33-8 Fungicida	$C_{22}H_{17}N_3O_5$ 403.1168		6.7	n.d	589	78	2.5
Bentazona 25057-89-0 Herbicida	$C_{10}H_{12}N_2O_3S$ 240.0569		7112	3.51	55.3	20	-0.46
Bromacil 314-40-9 Herbicida	$C_9H_{13}BrN_2O_2$ 260.0160		815	9.27	32	60	1.88
Carbaril 63-25-2 Insecticida	$C_{12}H_{11}NO_2$ 201.0790		9.1	10.4	300	16	2.36
Carbendazim 10605-21-7 Fungicida	$C_9H_9N_3O_2$ 191.0695		8.0	4.2	225[5]	40	1.48
Clorpirifos (clorpirifos etil) 2921-88-2 Insecticida	$C_9H_{11}Cl_3NO_3PS$ 348.9263		1.05	n.d	5509	386	4.7
Clorpirifos metil 5598-13-0 Insecticida	$C_7H_7Cl_3NO_3PS$ 320.8950		2.74	n.d	4645	12	4.00

Tabla A1 (cont.). Propiedades de plaguicidas y productos de transformación estudiados y detectados en la tesis doctoral.

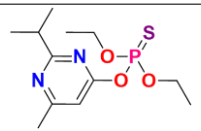
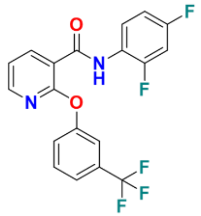
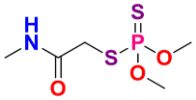
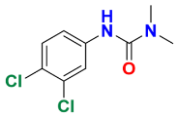
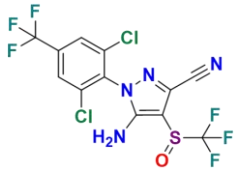
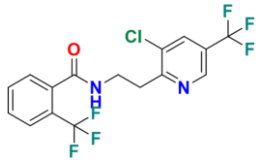
Plaguicida /Número CAS/ Acción biocida[1]	Fórmula química/ Masa exacta (Da)[2]	Estructura química[3]	Solub. en agua[a] (mg/L a 20 °C)[1]	p*Ka* (25 °C)[1]	Coeficiente de adsorción[b], Koc[1]	Vida media[c] (d)[1]	log Kow (pH 7, 20 °C)[1]
Diazinon 333-41-5 Insecticida	$C_{12}H_{21}N_2O_3PS$ 304.1010		60	2.6	609	9.1	3.69
Diflufenican 83164-33-4 Herbicida	$C_{19}H_{11}F_5N_2O_2$ 394.0741		0.05	n.d	5504	94.5	4.2
Dimetoato 60-51-5 Insecticida	$C_5H_{12}NO_3PS_2$ 228.9996		25900	n.d	9[5]	2.5	0.75
Diuron 330-54-1 Herbicida	$C_9H_{10}Cl_2N_2O$ 232.0170		35.6	n.d	680	146.6	2.87
Fipronil 120068-37-3 Insecticida	$C_{12}H_4Cl_2F_6N_4OS$ 435.9387		3.78	n.d	n.d	142	3.75
Fluopiram 658066-35-4 Fungicida	$C_{16}H_{11}ClF_6N_2O$ 396.0464		16.0	n.d	n.d	309	3.3

Tabla A1 (cont.). Propiedades de plaguicidas y productos de transformación estudiados y detectados en la tesis doctoral.

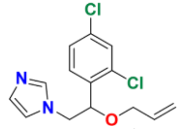
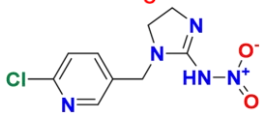
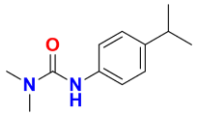
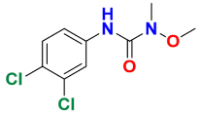
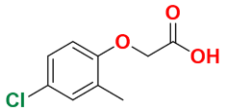
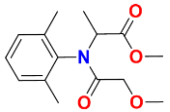
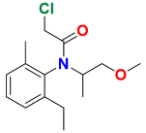
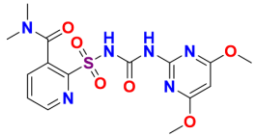
Plaguicida /Número CAS/ Acción biocida[1]	Fórmula química/ Masa exacta (Da)[2]	Estructura química[3]	Solub. en agua[a] (mg/L a 20 °C)[1]	p*Ka* (25 °C)[1]	Coeficiente de adsorción[b], Koc[1]	Vida media[c] (d)[1]	log Kow (pH 7, 20 °C)[1]
Imazalil 35554-44-0 Fungicida	$C_{14}H_{14}Cl_2N_2O$ 296.0483		184	6.49	4166[5]	76.3	2.56
Imidacloprid 138261-41-3 Insecticida	$C_9H_{10}ClN_5O_2$ 255.0523		610	n.d	n.d	191	0.57
Isoproturon 34123-59-6 Herbicida	$C_{12}H_{18}N_2O$ 206.1419		70.2	n.d	n.d	12	2.5
Linuron 330-55-2 Herbicida	$C_9H_{10}Cl_2N_2O_2$ 248.0119		63.8	n.d	842.8	57.6	3
MCPA (ácido 2-metil-4-clorofenoxiacético) 94-74-6 Herbicida	$C_9H_9ClO_3$ 200.0240		29390	3.73	10^5	24	-0.81
Metalaxil 57837-19-1 Fungicida	$C_{15}H_{21}NO_4$ 279.1471		8400	0	162	36	1.75
Metolaclor 51218-45-2 Herbicida	$C_{15}H_{22}ClNO_2$ 283.1339		530	n.d	120	90	3.4
Nicosulfuron 111991-09-4 Herbicida	$C_{15}H_{18}N_6O_6S$ 410.1009		7500	4.78	30	26	0.61

Tabla A1 (cont.). Propiedades de plaguicidas y productos de transformación estudiados y detectados en la tesis doctoral.

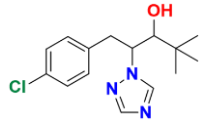
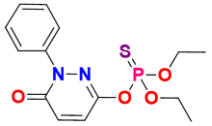
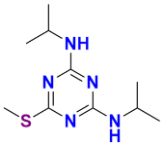
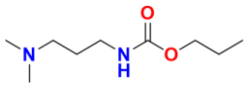
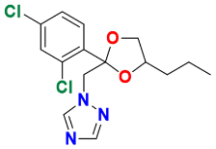
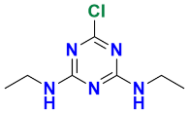
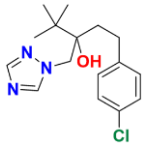
Plaguicida /Número CAS/ Acción biocida[1]	Fórmula química/ Masa exacta (Da)[2]	Estructura química[3]	Solub. en agua[a] (mg/L a 20 °C)[1]	p*Ka* (25 °C)[1]	Coeficiente de adsorción[b], Koc[1]	Vida media[c] (d)[1]	log Kow (pH 7, 20 °C)[1]
Paclobutrazol 76738-62-0 Fungicida	$C_{15}H_{20}ClN_3O$ 293.1295		22.9	n.d	400	112	3.11
Piridafentión 119-12-0 Insecticida	$C_{14}H_{17}N_2O_4PS$ 340.0647		100	n.d	7211	18	3.2
Prometrina 7287-19-6 Herbicida	$C_{10}H_{19}N_5S$ 241.1361		33	4.1	400	41	3.34
Propamocarb 24579-73-5 Fungicida	$C_9H_{20}N_2O_2$ 188.1525		900000	9.5	n.d	14	0.84
Propiconazol 60207-90-1 Fungicida	$C_{15}H_{17}Cl_2N_3O_2$ 341.0698		150	1.09	1086	71.8	3.72
Simazina 122-34-9 Herbicida	$C_7H_{12}ClN_5$ 201.0781		5	1.62	130	60	2.3
Tebuconazol 107534-96-3 Fungicida	$C_{16}H_{22}ClN_3O$ 307.1451		36	5.0	n.d	63	3.7

Tabla A1 (cont.). Propiedades de plaguicidas y productos de transformación estudiados y detectados en la tesis doctoral.

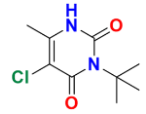
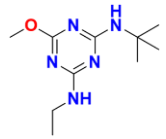
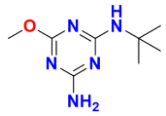
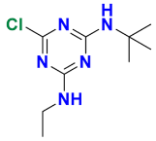
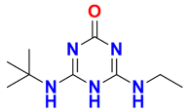
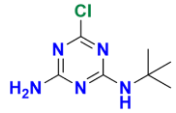
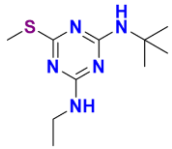
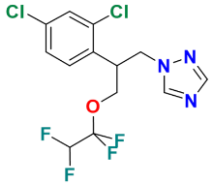
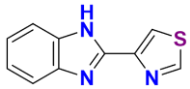
Plaguicida /Número CAS/ Acción biocida[1]	Fórmula química/ Masa exacta (Da)[2]	Estructura química[3]	Solub. en agua[a] (mg/L a 20 °C)[1]	p*Ka* (25 °C)[1]	Coeficiente de adsorción[b], Koc[1]	Vida media[c] (d)[1]	log Kow (pH 7, 20 °C)[1]
Terbacil 5902-51-2 Herbicida	$C_9H_{13}ClN_2O_2$ 216.0666		710	9.5	55	115	1.89
Terbumetón 33693-04-8 Herbicida	$C_{10}H_{19}N_5O$ 225.1590		130	4.68[6]	295	300	3.04
Terbumetón-desetil (TED) 30125-64-5 TP	$C_8H_{15}N_5O$ 197.1277		n.d	4.39[6]	n.d	n.d	1.93[6]
Terbutilazina 5915-41-3 Herbicida	$C_9H_{16}ClN_5$ 229.1094		6.6	1.9	n.d	72	3.4
Terbutilazina-2-hidroxi (T2OH) 66753-07-9 TP	$C_9H_{17}N_5O$ 211.1433		7.19	n.d	n.d	n.d	559
Terbutilazina-desetil (TD) 30125-63-4 TP	$C_7H_{12}ClN_5$ 201.0781		327.1	n.d	n.d	54	2.3
Terbutrina 886-50-0 Herbicida	$C_{10}H_{19}N_5S$ 241.1361		25	4.3	2432	74	3.66

Tabla A1 (cont.). Propiedades de plaguicidas y productos de transformación estudiados y detectados en la tesis doctoral.

Plaguicida /Número CAS/ Acción biocida[1]	**Fórmula química/ Masa exacta (Da)**[2]	**Estructura química**[3]	**Solub. en agua**[a] **(mg/L a 20 °C)**[1]	**p*Ka* (25 °C)**[1]	**Coeficiente de adsorción**[b]**, Koc**[1]	**Vida media**[c] **(d)**[1]	**log Kow (pH 7, 20 °C)**[1]
Tetraconazol 112281-77-3 Fungicida	$C_{13}H_{11}Cl_2F_4N_3O$ 371.0215		156.6	0.65	n.d	61	3.56
Tiabendazol 148-79-8 Fungicida	$C_{10}H_7N_3S$ 201.0361		30	4.73	3983	500	2.39

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6. Conrad A, Dedourge O, Cherrier R, Couderchet M, Biagianti S. Leaching of terbumeton and terbumeton-desethyl from mini-columns packed with soil aggregates in laboratory conditions. *Chemosphere*. 2006;65(9):1600-1609. doi:10.1016/j.chemosphere.2006.03.046

[a] Solubilidad en agua

[b] Coeficiente de adsorción de carbono orgánico o suelo/agua (L/kg)

[c] Vida media de degradación aeróbica en suelo

Tabla A2. Propiedades de fármacos y metabolitos estudiados y detectados en la tesis doctoral.

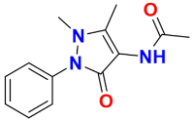
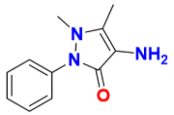
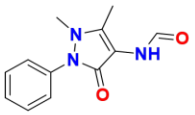
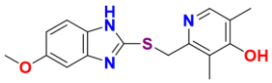
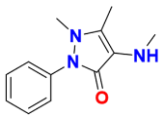
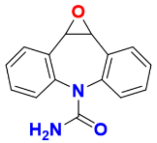
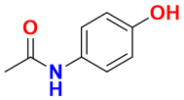
Fármaco /Número CAS/ Grupo terapéutico[1]	Fórmula química/ Masa exacta (Da)[1]	Estructura química[2]	Solub. en agua (mol/L)[3,4]	p*Ka*[3–5]	Coeficiente de adsorción, Koc[4]	Vida media (d)[4]	log Kow[1,3]
4-acetilaminoantipirina (4-AAA) 83-15-8 Metabolito	$C_{13}H_{15}N_3O_2$ 245.1164		1.76	0.180	162	4.65	0.659
4-aminoantipirina (4-AA) 83-07-8 Metabolito	$C_{11}H_{13}N_3O$ 203.1059		0.0943	9.5	47.6	3.55	0.588
4-formilaminoantipirina (4-FAA) 1672-58-8 Metabolito	$C_{12}H_{13}N_3O_2$ 231.1008		1.78	nd	173	5.15	0.423
4-hidroxi-sulfuro-omeprazol 103876-98-8 Metabolito	$C_{16}H_{17}N_3O_2S$ 315.1041		2.43	nd	1940	4.76	1.981
4-metilaminoantipirina (4-MAA) 519-98-2 Metabolito	$C_{12}H_{15}N_3O$ 217.1215		2.02	nd	215	4.89	1.051
10,11-epóxido de carbamazepina 36507-30-9 Metabolito	$C_{15}H_{12}N_2O_2$ 252.0899		3.5	n.d	883	6.65	0.068
Acetaminofén 103-90-2 Analgésico	$C_8H_9NO_2$ 151.0633		0.0943	9.50	47.6	3.55	0.314

Tabla A2 (cont.). Propiedades de fármacos y metabolitos estudiados y detectados en la tesis doctoral.

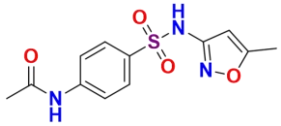
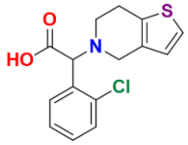
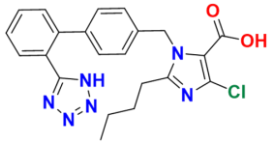
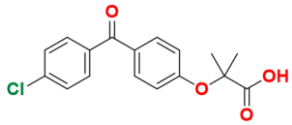
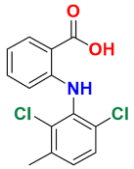
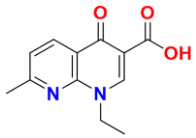
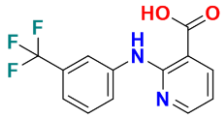
Fármaco /Número CAS/ Grupo terapéutico[1]	Fórmula química/ Masa exacta (Da)[1]	Estructura química[2]	Solub. en agua (mol/L)[3,4]	p*Ka*[3–5]	Coeficiente de adsorción, Koc[4]	Vida media (d)[4]	log Kow[1,3]
Acetilsulfametoxazol 21312-10-7 Metabolito	$C_{12}H_{13}N_3O_4S$ 295.0627		1.16	n.d	238	4.65	0.326
Ácido carboxílico del clopidogrel 90055-55-3 Metabolito	$C_{15}H_{14}ClNO_2S$ 307.0434		n.d	n.d	n.d	n.d	1.13
Ácido carboxílico del losartan 124750-92-1 Metabolito	$C_{22}H_{21}ClN_6O_2$ 436.1415		1.06	n.d	2660	6.63	5.07
Ácido fenofíbrico 42017-89-0 Hipolipemiante	$C_{17}H_{15}ClO_4$ 318.0659		0.0000202	3.88 -2.1	1760	3.55	2.525
Ácido meclofenámico 644-62-2 AINE	$C_{14}H_{11}Cl_2NO_2$ 295.0167		1.16	3.79 -3.6	2680	4.43	5.2
Ácido nalidíxico 389-08-2 Antibiótico	$C_{12}H_{12}N_2O_3$ 232.0848		0.000392	8.6	208	3.35	0.997
Ácido niflúmico 4394-00-7 AINE	$C_{13}H_9F_3N_2O_2$ 282.0616		0.0000572	1.88 5.51	465	3.54	1.615

Tabla A2 (cont.). Propiedades de fármacos y metabolitos estudiados y detectados en la tesis doctoral.

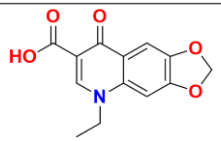
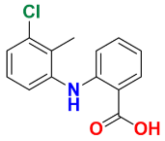
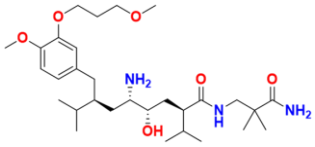
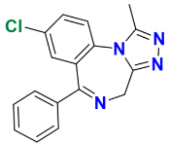
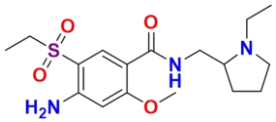
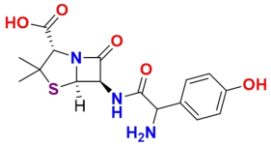
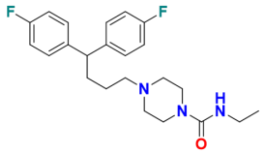
Fármaco /Número CAS/ Grupo terapéutico[1]	Fórmula química/ Masa exacta (Da)[1]	Estructura química[2]	Solub. en agua (mol/L)[3,4]	p*Ka*[3–5]	Coeficiente de adsorción, Koc[4]	Vida media (d)[4]	log Kow[1,3]
Ácido oxolínico 14698-29-4 Antibiótico	$C_{13}H_{11}NO_5$ 261.0637		0.0000123	5.58 -4.3	811	3.35	0.94
Ácido tolfenámico 13710-19-5 AINE	$C_{14}H_{12}ClNO_2$ 261.0557		0.00000295	5.11	1760	4.63	2.403
Aliskiren 173334-57-1 Antihipertensivo	$C_{30}H_{53}N_3O_6$ 551.3934		5.1	9.49	72800	3.36	2.94
Alprazolam 28981-97-7 Ansiolítico	$C_{17}H_{13}ClN_4$ 308.0829		0.000251	18.3 5.08	597	3.73	2.1
Amisulprida 71675-85-9 Antipsicótico	$C_{17}H_{27}N_3O_4S$ 369.1722		2.57	9.37	1280	3.35	1.398
Amoxicilina 26787-78-0 Antibiótico	$C_{16}H_{19}N_3O_5S$ 365.1045		0.00933	3.23 7.43	88.8	35.6	-3.064
Amperrozida 75558-90-6 Antipsicótico	$C_{23}H_{29}F_2N_3O$ 401.2279		2.02	15.61 7.56	1170	4.49	4.12

Tabla A2 (cont.). Propiedades de fármacos y metabolitos estudiados y detectados en la tesis doctoral.

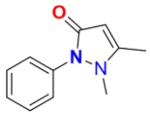
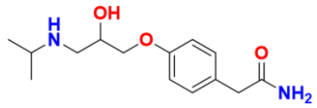
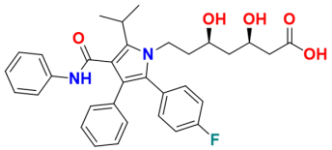
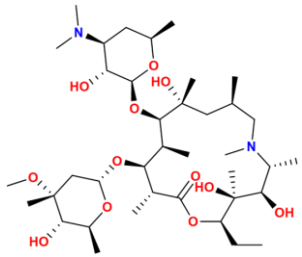
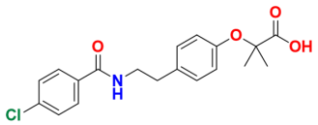
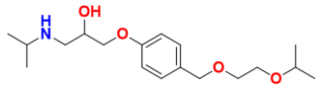
Fármaco /Número CAS/ Grupo terapéutico[1]	Fórmula química/ Masa exacta (Da)[1]	Estructura química[2]	Solub. en agua (mol/L)[3,4]	p*Ka*[3–5]	Coeficiente de adsorción, Koc[4]	Vida media (d)[4]	log Kow[1,3]
Antipirina (Fenazona) 60-80-0 AINE	$C_{11}H_{12}N_2O$ 188.0950		1.58	1.45	112	3.36	0.651
Atenolol 29122-68-7 Agente betabloqueante	$C_{14}H_{22}N_2O_3$ 266.1630		0.053	9.54	302	3.53	-0.465
Atorvastatina 134523-00-5 Hipolipemiante	$C_{33}H_{35}FN_2O_5$ 558.2530		1.15	4.46	242000	85.8	3.442
Azitromicina 83905-01-5 Antibiótico	$C_{38}H_{72}N_2O_{12}$ 748.5085		2.60	8.5	43400	15.2	1.888
Bezafibrato 41859-67-0 Hipolipemiante	$C_{19}H_{20}ClNO_4$ 361.1081		0.92	3.83 -0.84	13900	3.53	1.591
Bisoprolol 66722-44-9 Agente betabloqueante	$C_{18}H_{31}NO_4$ 325.2253		2.74	9.5	3900	4.29	1.237

Tabla A2 (cont.). Propiedades de fármacos y metabolitos estudiados y detectados en la tesis doctoral.

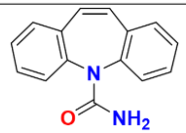
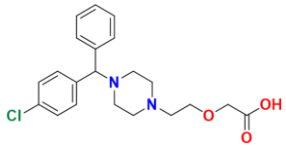
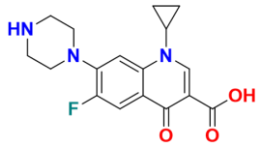
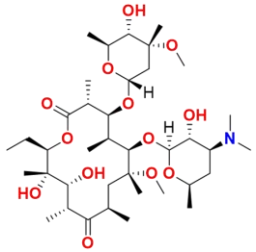
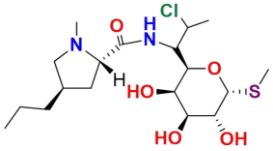
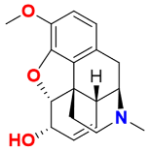
Fármaco /Número CAS/ Grupo terapéutico[1]	Fórmula química/ Masa exacta (Da)[1]	Estructura química[2]	Solub. en agua (mol/L)[3,4]	p*Ka*[3–5]	Coeficiente de adsorción, Koc[4]	Vida media (d)[4]	log Kow[1,3]
Carbamazepina 298-46-4 Antiepiléptico	$C_{15}H_{12}N_2O$ 236.0950		0.000491	15.96 -3.8	544	6.54	0.792
Cetirizina 83881-51-0 Antialérgico	$C_{21}H_{25}ClN_2O_3$ 388.1554		1.27	1.52 2.92 8.27	1110	4.52	0.891
Ciprofloxacino 85721-33-1 Antibiótico	$C_{17}H_{18}FN_3O_3$ 331.1332		0.0333	6.09	305	3.35	1.809
Claritromicina 81103-11-9 Antibiótico	$C_{38}H_{69}NO_{13}$ 747.4769		3.41	8.99	45300	15.2	1.66
Clindamicina 18323-44-9 Antibiótico	$C_{18}H_{33}ClN_2O_5S$ 424.1799		0.0000724	7.6	167	37	2.001
Codeína 76-57-3 Analgésico	$C_{18}H_{21}NO_3$ 299.1521		0.0302	8.2	196000	143	1.14

Tabla A2 (cont.). Propiedades de fármacos y metabolitos estudiados y detectados en la tesis doctoral.

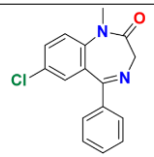
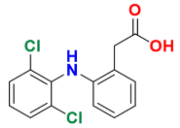
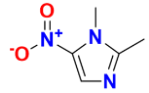
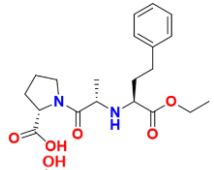
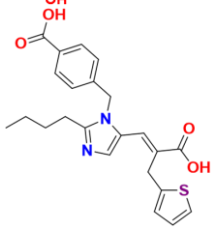
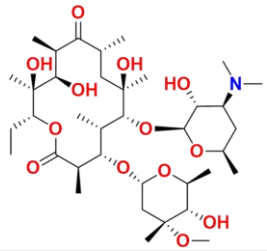
Fármaco /Número CAS/ Grupo terapéutico[1]	Fórmula química/ Masa exacta (Da)[1]	Estructura química[2]	Solub. en agua (mol/L)[3,4]	p*Ka*[3–5]	Coeficiente de adsorción, Koc[4]	Vida media (d)[4]	log Kow[1,3]
Diazepam 439-14-5 Ansiolítico	$C_{16}H_{13}ClN_2O$ 284.0716		0.000175	3.4	773	3.35	2.91
Diclofenaco 15307-86-5 AINE	$C_{14}H_{11}Cl_2NO_2$ 295.0167		0.00000641	4.15	5740	4.68	2.219
Dimetridazol 551-92-8 Antiparasitario	$C_5H_7N_3O_2$ 141.0538		0.13	n.d	43.3	4.47	0.566
Enalapril 75847-73-3 Antihipertensivo	$C_{20}H_{28}N_2O_5$ 376.1998		0.0436	3.67 5.2	180	3.35	2.125
Eprosartán 133040-01-4 Antihipertensivo	$C_{23}H_{24}N_2O_4S$ 424.1457		1.17	3.63 6.93	49800	14.1	4.96
Eritromicina 114-07-8 Antibiótico	$C_{37}H_{67}NO_{13}$ 733.4612		0.000355	8.88	44300	15.5	1.141

Tabla A2 (cont.). Propiedades de fármacos y metabolitos estudiados y detectados en la tesis doctoral.

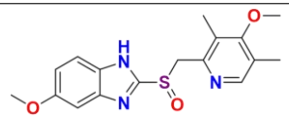
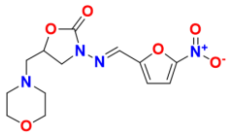
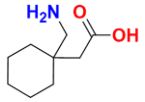
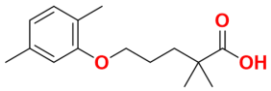
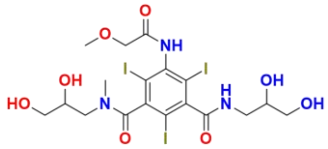
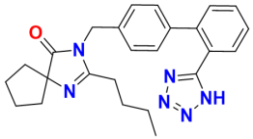
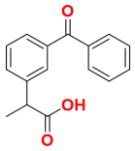
Fármaco /Número CAS/ Grupo terapéutico[1]	Fórmula química/ Masa exacta (Da)[1]	Estructura química[2]	Solub. en agua (mol/L)[3,4]	p*Ka*[3–5]	Coeficiente de adsorción, Koc[4]	Vida media (d)[4]	log Kow[1,3]
Esomeprazol 119141-88-7 Antiulceroso	$C_{17}H_{19}N_3O_3S$ 345.1147		2.41	9.68 4.77	931	3.36	0.032
Furaltadona 139-91-3 Antibiótico	$C_{13}H_{16}N_4O_6$ 324.1070		0.0023	n.d	264	5.68	0.386
Gabapentina 60142-96-3 Antiepiléptico	$C_9H_{17}NO_2$ 171.1259		1.93	3.7	127	3.72	1.391
Gemfibrozilo 25812-30-0 Hipolipemiante	$C_{15}H_{22}O_3$ 250.1569		1.04	4.42 -4.8	216	3.53	2.59
Iopromida 73334-07-3 Agente de diagnóstico	$C_{18}H_{24}I_3N_3O_8$ 790.8697		1.46	11.09 -1.7	48.7	34.9	-1.028
Irbesartán 138402-11-6 Antihipertensivo	$C_{25}H_{28}N_6O$ 428.2325		1.25	7.4 4.12	21500	34.8	4.564
Ketoprofeno 22071-15-4 AINE	$C_{16}H_{14}O_3$ 254.0943		0.000388	4.2	696	4.64	1.651
Lamotrigina 84057-84-1 Antiepiléptico	$C_9H_7Cl_2N_5$ 255.0079		2.13	5.7	179	6.23	2.805

Tabla A2 (cont.). Propiedades de fármacos y metabolitos estudiados y detectados en la tesis doctoral.

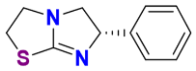
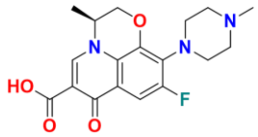
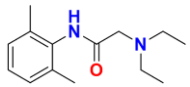
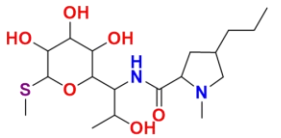
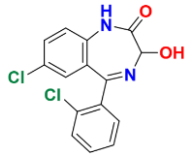
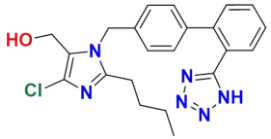
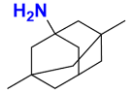
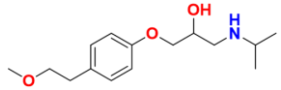
Fármaco /Número CAS/ Grupo terapéutico[1]	Fórmula química/ Masa exacta (Da)[1]	Estructura química[2]	Solub. en agua (mol/L)[3,4]	p*Ka*[3–5]	Coeficiente de adsorción, Koc[4]	Vida media (d)[4]	log Kow[1,3]
Levamisol 14769-73-4 Agente antihelmíntico	$C_{11}H_{12}N_2S$ 204.0721		0.0055	6.98	248	3.45	1.851
Levofloxacino 100986-85-4 Antibiótico	$C_{18}H_{20}FN_3O_4$ 361.1438		1.59	6.25	482	3.36	1.995
Lidocaína 137-58-6 Anestésico	$C_{14}H_{22}N_2O$ 234.1732		0.0176	7.72	331	4.86	2.35
Lincomicina 154-21-2 Antibiótico	$C_{18}H_{34}N_2O_6S$ 406.2138		0.00229	12.37 7.97	60.3	36.8	0.69
Lorazepam 846-49-1 Ansiolítico	$C_{15}H_{10}Cl_2N_2O_2$ 320.0119		0.00025	13	1540	4.28	2.4
Losartán 114798-26-4 Antihipertensivo	$C_{22}H_{23}ClN_6O$ 422.1622		1.19	5.5	13500	14.1	4.641
Memantina 19982-08-2 Antiparkinson	$C_{12}H_{21}N$ 179.1674		2.75	10.27	4910	802	3.524
Metoprolol 37350-58-6 Agente betabloqueante	$C_{15}H_{25}NO_3$ 267.1834		0.0632	9.7	1280	4.47	0.702

Tabla A2 (cont.). Propiedades de fármacos y metabolitos estudiados y detectados en la tesis doctoral.

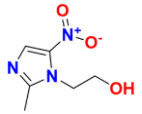
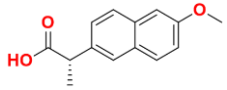
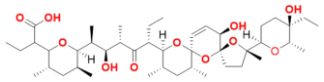
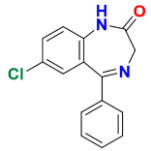
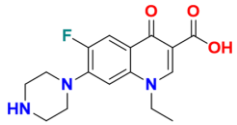
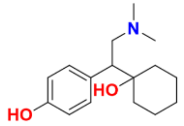
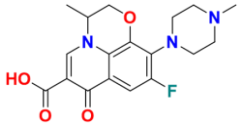
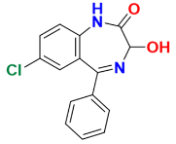
Fármaco /Número CAS/ Grupo terapéutico[1]	Fórmula química/ Masa exacta (Da)[1]	Estructura química[2]	Solub. en agua (mol/L)[3,4]	p*Ka*[3–5]	Coeficiente de adsorción, Koc[4]	Vida media (d)[4]	log Kow[1,3]
Metronidazol 443-48-1 Antibiótico	$C_6H_9N_3O_3$ 171.0644		0.0573	2.57 15.42	57.3	4.46	-0.143
Naproxeno 22204-53-1 AINE	$C_{14}H_{14}O_3$ 230.0943		0.0000604	4.2	555	3.53	1.794
Narasina 55134-13-9 Antibiótico	$C_{43}H_{72}O_{11}$ 764.5075		0.000132	4.5 -3	136000	103	6.2
Nordiazepam (N-desmetildiazepam) 1088-11-5 Metabolito	$C_{15}H_{11}ClN_2O$ 270.0560		1.76	12.3 2.85	1640	3.54	2.9
Norfloxacino 70458-96-7 Antibiótico	$C_{16}H_{18}FN_3O_3$ 319.1332		0.279	5.77 8.68	305	3.35	1.689
O-desmetilvenlafaxina 93413-62-8 Metabolito	$C_{16}H_{25}NO_2$ 263.1885		3.19	n.d	277	4.71	1.582
Ofloxacino 82419-36-1 Antibiótico	$C_{18}H_20FN_3O_4$ 361.1438		0.0776	5.45 6.2	482	3.36	1.995
Oxazepam 604-75-1 Ansiolítico	$C_{15}H_{11}ClN_2O_2$ 286.0509		0.000112	10.61 -1.5	359	4.27	2.2

Tabla A2 (cont.). Propiedades de fármacos y metabolitos estudiados y detectados en la tesis doctoral.

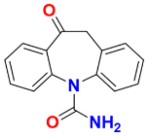
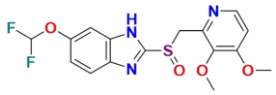
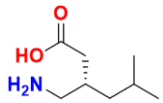
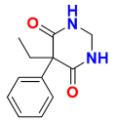
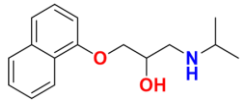
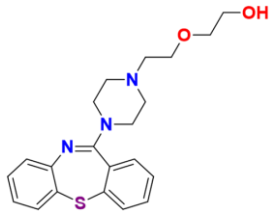
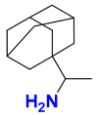
Fármaco /Número CAS/ Grupo terapéutico[1]	**Fórmula química/ Masa exacta (Da)**[1]	**Estructura química**[2]	**Solub. en agua (mol/L)**[3,4]	**p*Ka***[3–5]	**Coeficiente de adsorción, Koc**[4]	**Vida media (d)**[4]	**log Kow**[1,3]
Oxcarbazepina 28721-07-5 Antiepiléptico	$C_{15}H_{12}N_2O_2$ 252.0899		1.75	13.73	833	4.88	0.023
Pantoprazol 102625-70-7 Antiulceroso	$C_{16}H_{15}F_2N_3O_4S$ 383.0751		1.60	3.92 8.19	390	4.29	0.624
Pregabalina 148553-50-8 Antiepiléptico	$C_8H_{17}NO_2$ 159.1259		2.14	4.8 10.23	75.7	3.54	-0.475
Primidona 125-33-7 Antiepiléptico	$C_{12}H_{14}N_2O_2$ 218.1055		0.00229	11.5 -6.2	78.6	3.34	1.378
Propanolol 525-66-6 Agente betabloqueante	$C_{16}H_{21}NO_2$ 259.1572		0.00106	9.46	251	3.35	2.023
Quetiapina 111974-69-7 Antipsicótico	$C_{21}H_{25}N_3O_2S$ 383.1667		0.00396	10.14	3300	585	3.578
Rimantadina 13392-28-4 Antiviral	$C_{12}H_{21}N$ 179.1674		0.00396	10.14	3300	585	3.578

Tabla A2 (cont.). Propiedades de fármacos y metabolitos estudiados y detectados en la tesis doctoral.

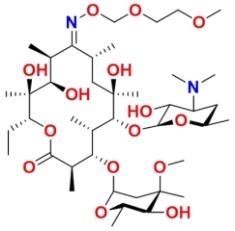
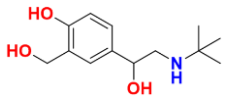
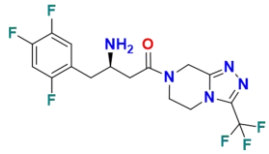
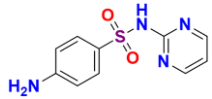
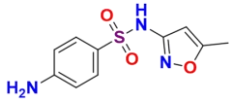
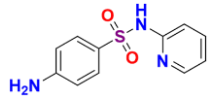
Fármaco /Número CAS/ Grupo terapéutico[1]	Fórmula química/ Masa exacta (Da)[1]	Estructura química[2]	Solub. en agua (mol/L)[3,4]	p*Ka*[3–5]	Coeficiente de adsorción, Koc[4]	Vida media (d)[4]	log Kow[1,3]
Roxitromicina 80214-83-1 Antibiótico	$C_{41}H_{76}N_2O_{15}$ 836.5246		2.30	12.45 9.08	31300	13.6	3.24
Salbutamol 18559-94-9 Agente betabloqueante	$C_{13}H_{21}NO_3$ 239.1521		0.0600	10.30	107	4.28	0.469
Sitagliptina 486460-32-6 Antihiperglucemiante	$C_{16}H_{15}F_6N_5O$ 407.1181		2.05	8.78	303	3.55	1.684
Sulfadiazina 68-35-9 Antibiótico	$C_{10}H_{10}N_4O_2S$ 250.0524		0.000340	6.42	115	3.36	-1.465
Sulfametoxazol 723-46-6 Antibiótico	$C_{10}H_{11}N_3O_3S$ 253.0521		0.00212	6.16 1.97	91.6	3.35	0.255
Sulfapiridina 144-83-2 Antibiótico	$C_{11}H_{11}N_3O_2S$ 249.0572		0.00130	8.51	149	3.36	-0.608

Tabla A2 (cont.). Propiedades de fármacos y metabolitos estudiados y detectados en la tesis doctoral.

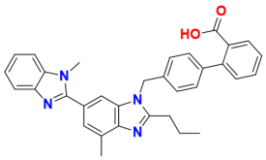
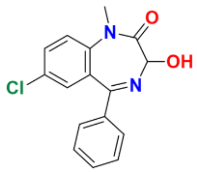
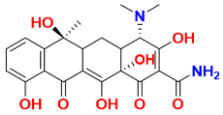
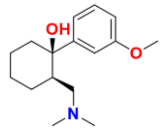
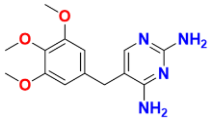
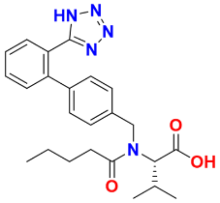
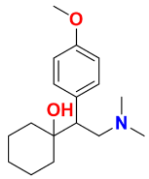
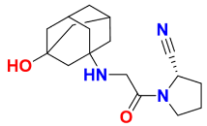
Fármaco /Número CAS/ Grupo terapéutico[1]	Fórmula química/ Masa exacta (Da)[1]	Estructura química[2]	Solub. en agua (mol/L)[3,4]	p*Ka*[3–5]	Coeficiente de adsorción, Koc[4]	Vida media (d)[4]	log Kow[1,3]
Telmisartán 144701-48-4 Antihipertensivo	$C_{33}H_{30}N_4O_2$ 514.2369		1.55	3.65 6.13	129000	37	5.046
Temazepam 846-50-4 Ansiolítico	$C_{16}H_{13}ClN_2O_2$ 300.0666		1.75	10.68 -1.4	1130	2.95	2.19
Tetraciclina 60-54-8 Antibiótico	$C_{22}H_{24}N_2O_8$ 444.1533		0.00140	3.3	558	148	-0.789
Tramadol 27203-92-5 Analgésico	$C_{16}H_{25}NO_2$ 263.1885		3.60	9.41	567	3.36	2.6
Trimetoprima 738-70-5 Antibiótico	$C_{14}H_{18}N_4O_3$ 290.1379		0.00134	7.12	115	4.24	2.398

Tabla A2 (cont.). Propiedades de fármacos y metabolitos estudiados y detectados en la tesis doctoral.

Fármaco /Número CAS/ Grupo terapéutico[1]	Fórmula química/ Masa exacta (Da)[1]	Estructura química[2]	Solub. en agua (mol/L)[3,4]	p*Ka*[3–5]	Coeficiente de adsorción, Koc[4]	Vida media (d)[4]	log Kow[1,3]
Valsartán 137862-53-4 Antihipertensivo	$C_{24}H_{29}N_5O_3$ 435.2270		1.02 n.d	4.73 n.d	589 n.d	7.37 n.d	4.409 n.d
Venlafaxina 93413-69-5 Antidepresivo	$C_{17}H_{27}NO_2$ 277.2042		2.60	14.42 8.91	653	3.36	1.903
Vildagliptina 274901-16-5 Antihiperglucemiante	$C_{17}H_{25}N_3O_2$ 303.1947		5.2	14.71 9.03	919	230	0.786

1. Royal Society of Chemistry. ChemSpider. Home Page. http://www.chemspider.com/Chemical-Structure. Accessed December 23, 2020.
2. PerkinElmer Informatics. ChemDraw® Proffesional 17.1 software. 2017:Waltham, MA.
3. National Center for Biotechnology Information. PubChem Database. https://pubchem.ncbi.nlm.nih.gov/compound. Accessed December 24, 2020.
4. EPA. CompTox Chemicals Dashboard. US Environmental Protection Agency's Chemical Safety for Sustainability Research Program. https://comptox.epa.gov/dashboard. Accessed December 22, 2020.
5. DrugBank. Detailed Drug and Drug Target Information. https://go.drugbank.com/. Accessed December 22, 2020.

n.d: dato no disponible

Tabla A3. Normas de calidad ambiental (NCA) para los plaguicidas estudiados en la presente tesis doctoral incluidos en la lista de sustancias prioritarias.

N°	Nombre de la sustancia	**NCA-MA** Aguas superficiales continentales (µg/L)	**NCA-MA** Otras aguas superficiales (µg/L)	**NCA-CMA** Aguas superficiales continentales (µg/L)	**NCA-CMA** Otras aguas superficiales (µg/L)
(1)	*Alacloro*	0.3	0.3	0.7	0.7
(2)	Antraceno	0.1	0.1	0.1	0.1
(3)	*Atrazina*	0.6	0.6	2.0	2.0
(4)	Benceno	10	8	50	50
(5)	Difeniléteres bromados			0.14	0.14
(6)	Cadmio y sus compuestos (en función de las clases de dureza del agua)	≤ 0.08 (Clase 1) 0.08 (Clase 2) 0.09 (Clase 3) 0.15 (Clase 4) 0.25 (Clase 5)	0.2	≤ 0.45 (Clase 1) 0.45 (Clase 2) 0.6 (Clase 3) 0.9 (Clase 4) 1.5 (Clase 5)	≤ 0.45 (Clase 1) 0.45 (Clase 2) 0.6 (Clase 3) 0.9 (Clase 4) 1.5 (Clase 5)
(6bis)	Tetracloruro de carbono	12	12	No aplicable	No aplicable
(7)	Cloroalcanos C_{10-13}	0.4	0.4	1.4	1.4
(8)	*Clorfenvinfós*	0.1	0.1	0.3	0.3
(9)	*Clorpirifos (Clorpirifos-etilo)*	0.03	0.03	0.1	0.1
(9bis)	Plaguicidas de tipo ciclodieno: *Aldrina* *Dieldrina* *Endrina* *Isodrina*	Σ = 0.01	Σ = 0.005	No aplicable	No aplicable
(9ter)	*DDT* total	0.025	0.025	No aplicable	No aplicable
	p,p'-DDT	0.01	0.01	No aplicable	No aplicable
(10)	1,2-Dicloroetano	10	10	No aplicable	No aplicable
(11)	Diclorometano	20	20	No aplicable	No aplicable
(12)	Ftalato de di(2-etilhexilo) (DEHP)	1.3	1.3	No aplicable	No aplicable
(13)	*Diurón*	0.2	0.2	1.8	1.8
(14)	*Endosulfán*	0.005	0.0005	0.01	0.004
(15)	Fluoranteno	0.0063	0.0063	0.12	0.12

Tabla A3 (cont.). Normas de calidad ambiental (NCA) para las sustancias prioritarias y algunos otros contaminantes.

N°	Nombre de la sustancia	**NCA-MA** Aguas superficiales continentales (µg/L)	**NCA-MA** Otras aguas superficiales (µg/L)	**NCA-CMA** Aguas superficiales continentales (µg/L)	**NCA-CMA** Otras aguas superficiales (µg/L)
(16)	*Hexaclorobenceno*			0.05	0.05
(17)	Hexaclorobutadieno			0.6	0.6
(18)	*Hexaclorociclohexano*	0.02	0.002	0.04	0.02
(19)	*Isoproturón*	0.3	0.3	1.0	1.0
(20)	Plomo y sus compuestos	1.2	1.3	14	14
(21)	Mercurio y sus compuestos			0.07	0.07
(22)	*Naftaleno*	2	2	130	130
(23)	Níquel y sus compuestos	4	8.6	34	34
(24)	Nonilfenoles (4-Nonilfenol)	0.3	0.3	2.0	2.0
(25)	Octilfenoles ((4-(1,1′,3,3′-tetrametilbutil)- fenol))	0.1	0.01	No aplicable	No aplicable
(26)	Pentaclorobenceno	0.007	0.0007	No aplicable	No aplicable
(27)	*Pentaclorofenol*	0.4	0.4	1	1
(28)	Hidrocarburos aromáticos policíclicos (HAP)	No aplicable	No aplicable	No aplicable	No aplicable
	Benzo(a)pireno	1.7×10^{-4}	1.7×10^{-4}	0.27	0.027
	Benzo(b) fluoranteno	Ver nota 1	Ver nota 1	0.017	0.017
	Benzo(k) fluoranteno	Ver nota 1	Ver nota 1	0.017	0.017
	Benzo(g,h,i)perileno	Ver nota 1	Ver nota 1	8.2×10^{-3}	8.2×10^{-3}
	Indeno(1,2,3- cd)pireno	No aplicable	No aplicable	No aplicable	No aplicable
(29)	*Simazina*	1	1	4	4
(29bis)	Tetracloroetileno	10	10	No aplicable	No aplicable
(29ter)	Tricloroetileno	10	10	No aplicable	No aplicable
(30)	Compuestos de tributilestaño (Catión de tributilestaño)	0.0002	0.0002	0.0015	0.0015
(31)	Triclorobencenos	0.4	0.4	No aplicable	No aplicable

Tabla A3 (cont.). Normas de calidad ambiental (NCA) para las sustancias prioritarias y algunos otros contaminantes.

N°	Nombre de la sustancia	**NCA-MA** Aguas superficiales continentales (µg/L)	**NCA-MA** Otras aguas superficiales (µg/L)	**NCA-CMA** Aguas superficiales continentales (µg/L)	**NCA-CMA** Otras aguas superficiales (µg/L)
(32)	Triclorometano	2.5	2.5	No aplicable	No aplicable
(33)	*Trifluralina*	0.03	0.03	No aplicable	No aplicable
(34)	*Dicofol*	1.3×10^{-3}	3.2×10^{-5}	No aplicable	No aplicable
(35)	Ácido perfluorooctano-sulfónico y sus derivados (PFOS)	6.5×10^{-4}	1.3×10^{-4}	36	7.2
(36)	*Quinoxifeno*	0.15	0.015	2.7	0.54
(37	Dioxinas y compuestos similares			No aplicable	No aplicable
(38)	*Aclonifeno*	0.12	0.012	0.12	0.012
(39)	*Bifenox*	0.012	0.0012	0.04	0.004
(40)	*Cibutrina*	0.0025	0.0025	0.016	0.016
(41)	*Cipermetrina*	8×10^{-5}	8×10^{-6}	6×10^{-4}	6×10^{-5}
(42)	*Diclorvós*	6×10^{-4}	6×10^{-5}	7×10^{-4}	7×10^{-5}
(43)	Hexabromociclododecano (HBCDD)	0.0016	0.0008	0.5	0.05
(44)	*Heptacloro y epóxido de heptacloro*	2×10^{-7}	1×10^{-8}	3×10^{-4}	3×10^{-5}
(45)	*Terbutrina*	0.065	0.0065	0.34	0.034

MA: media anual.
CMA: concentración máxima admisible
Nota 1: Por lo que respecta al grupo de sustancias prioritarias de hidrocarburos aromáticos policíclicos (HAP) (n° 28), las NCA de la biota y las correspondientes NCA-MA en el agua se refieren a la concentración de benzo(a)pireno, en cuya toxicidad se basan. El benzo(a)pireno puede considerarse como un marcador de los otros HAP, ya que solo tal sustancia debe ser objeto de seguimiento a efectos de comparación con las NCA de la biota o las correspondientes NCA-MA en el agua.
En cursiva las sustancias clasificadas como plaguicidas (“IUPAC, Pesticide properties database,” 2007)

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Escola de Doctorat - ED

Elena Pitarch Arquimbau, como coautor/ coautora doy mi **autorización** a **Eddie Alexander Fonseca Rubí** para la presentación de las siguientes publicaciones como parte de su tesis doctoral.

Relación de publicaciones:

Ecological risk assessment of pesticides in the Mijares River (eastern Spain) impacted by citrus production using wide-scope screening and target quantitative analysis. Journal of Hazardous Materials, 412 (2021) 125277. https://doi.org/10.1016/j.jhazmat.2021.125277

Occurrence and ecological risks of pharmaceuticals in a Mediterranean river in Eastern Spain. Environment International, 144 (2020) 106004. https://doi.org/10.1016/j.envint.2020.106004

Investigation of pharmaceuticals in a conventional wastewater treatment plant: removal efficiency, seasonal variation and impact of a nearby hospital. Journal of Environmental Chemical Engineering (Manuscrito aceptado)

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MARIA ELENA PITARCH ARQUIMBAU

Castelló, 19 de abril de 2021

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"(...)

"Aquellas tesis doctorales que opten por la incorporación de artículos (compendio de publicaciones) deben de ajustarse, en la medida de lo posible, a la siguiente estructura: -Introducción/objetivos - Un capítulo por artículo incorporado - Discusión general de los resultados - Conclusiones. -Aceptación de los coautores de que el doctorando presente el trabajo como tesis y renuncia expresa de estos a presentarlo como parte de otra tesis doctoral."

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Relación de publicaciones:

Investigation of pesticides and their transformation products in the Júcar River Hydrographical Basin (Spain) by wide-scope high-resolution mass spectrometry screening. Environmental Research, 177 (2019) 108570. https://doi.org/10.1016/j.envres.2019.108570

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Lugar, fecha y firma

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Ecological risk assessment of pesticides in the Mijares River (eastern Spain) impacted by citrus production using wide-scope screening and target quantitative analysis. Journal of Hazardous Materials, 412 (2021) 125277. https://doi.org/10.1016/j.jhazmat.2021.125277

Occurrence and ecological risks of pharmaceuticals in a Mediterranean river in Eastern Spain. Environment International, 144 (2020) 106004. https://doi.org/10.1016/j.envint.2020.106004

Investigation of pharmaceuticals in a conventional wastewater treatment plant: removal efficiency, seasonal variation and impact of a nearby hospital. Journal of Environmental Chemical Engineering (Manuscrito aceptado)

Asimismo, **renuncio** a poder utilizar estas publicaciones como parte de otra tesis doctoral.

Y para que conste firmo el presente documento,

Lugar, fecha y firma

Castellon, 22 april 2021

Todo ello, atendiendo al artículo 23 de la Normativa de los Estudios de Doctorado, regulados por el RD 99/2011, en la Universitat Jaume I (Aprobada por el Consejo de Gobierno núm. 19 de 26 de Enero de 2012, modificada por el Consejo de Gobierno núm. 29 de 27 de Noviembre de 2012 y con posterior modificación por el Consejo de Gobierno núm. 37 de 25 de Julio de 2013):

"(...)

"Aquellas tesis doctorales que opten por la incorporación de artículos (compendio de publicaciones) deben de ajustarse, en la medida de lo posible, a la siguiente estructura: -Introducción/objetivos - Un capítulo por artículo incorporado - Discusión general de los resultados - Conclusiones. -Aceptación de los coautores de que el doctorando presente el trabajo como tesis y renuncia expresa de estos a presentarlo como parte de otra tesis doctoral."

UNIVERSITAT JAUME I
Escola de Doctorat · ED

Ignacio Morell Evangelista, como coautor/ coautora doy mi **autorización** a **Eddie Alexander Fonseca Rubí** para la presentación de las siguientes publicaciones como parte de su tesis doctoral.

Relación de publicaciones:

Investigation of pesticides and their transformation products in the Júcar River Hydrographical Basin (Spain) by wide-scope high-resolution mass spectrometry screening. Environmental Research, 177 (2019) 108570. https://doi.org/10.1016/j.envres.2019.108570

Asimismo, **renuncio** a poder utilizar estas publicaciones como parte de otra tesis doctoral.

Y para que conste firmo el presente documento,

Lugar, fecha y firma

Castelló, 19 abril 2021

Todo ello, atendiendo al artículo 23 de la Normativa de los Estudios de Doctorado, regulados por el RD 99/2011, en la Universitat Jaume I (Aprobada por el Consejo de Gobierno núm. 19 de 26 de Enero de 2012, modificada por el Consejo de Gobierno núm. 29 de 27 de Noviembre de 2012 y con posterior modificación por el Consejo de Gobierno núm. 37 de 25 de Julio de 2013):

"(...)

"Aquellas tesis doctorales que opten por la incorporación de artículos (compendio de publicaciones) deben de ajustarse, en la medida de lo posible, a la siguiente estructura: -Introducción/objetivos - Un capítulo por artículo incorporado - Discusión general de los resultados - Conclusiones. -Aceptación de los coautores de que el doctorando presente el trabajo como tesis y renuncia expresa de estos a presentarlo como parte de otra tesis doctoral."

UNIVERSITAT JAUME I
Escola de Doctorat · ED

Tania Portolés Nicolau, como coautor/ coautora doy mi **autorización** a **Eddie Alexander Fonseca Rubí** para la presentación de las siguientes publicaciones como parte de su tesis doctoral.

Relación de publicaciones:

Ecological risk assessment of pesticides in the Mijares River (eastern Spain) impacted by citrus production using wide-scope screening and target quantitative analysis. Journal of Hazardous Materials, 412 (2021) 125277. https://doi.org/10.1016/j.jhazmat.2021.125277

Asimismo, **renuncio** a poder utilizar estas publicaciones como parte de otra tesis doctoral.

Y para que conste firmo el presente documento,

TANIA| PORTOLES| NICOLAU

Firmado digitalmente por TANIA|PORTOLES|NICOLAU
Fecha: 2021.04.19 15:25:50 +02'00'

Lugar, fecha y firma

Todo ello, atendiendo al artículo 23 de la Normativa de los Estudios de Doctorado, regulados por el RD 99/2011, en la Universitat Jaume I (Aprobada por el Consejo de Gobierno núm. 19 de 26 de Enero de 2012, modificada por el Consejo de Gobierno núm. 29 de 27 de Noviembre de 2012 y con posterior modificación por el Consejo de Gobierno núm. 37 de 25 de Julio de 2013):

"(...)

"Aquellas tesis doctorales que opten por la incorporación de artículos (compendio de publicaciones) deben de ajustarse, en la medida de lo posible, a la siguiente estructura: -Introducción/objetivos - Un capítulo por artículo incorporado - Discusión general de los resultados - Conclusiones. -Aceptación de los coautores de que el doctorando presente el trabajo como tesis y renuncia expresa de estos a presentarlo como parte de otra tesis doctoral."

UNIVERSITAT JAUME I
Escola de Doctorat · ED

José Manuel Marín Ramos, como coautor/ coautora doy mi **autorización** a **Eddie Alexander Fonseca Rubí** para la presentación de las siguientes publicaciones como parte de su tesis doctoral.

Relación de publicaciones:

Ecological risk assessment of pesticides in the Mijares River (eastern Spain) impacted by citrus production using wide-scope screening and target quantitative analysis. Journal of Hazardous Materials, 412 (2021) 125277. https://doi.org/10.1016/j.jhazmat.2021.125277

Asimismo, **renuncio** a poder utilizar estas publicaciones como parte de otra tesis doctoral.

Y para que conste firmo el presente documento,

Lugar, fecha y firma

Castelló de la Plana, 19 Abril 2021

Todo ello, atendiendo al artículo 23 de la Normativa de los Estudios de Doctorado, regulados por el RD 99/2011, en la Universitat Jaume I (Aprobada por el Consejo de Gobierno núm. 19 de 26 de Enero de 2012, modificada por el Consejo de Gobierno núm. 29 de 27 de Noviembre de 2012 y con posterior modificación por el Consejo de Gobierno núm. 37 de 25 de Julio de 2013):

"(...)

"Aquellas tesis doctorales que opten por la incorporación de artículos (compendio de publicaciones) deben de ajustarse, en la medida de lo posible, a la siguiente estructura: -Introducción/objetivos - Un capítulo por artículo incorporado - Discusión general de los resultados - Conclusiones. -Aceptación de los coautores de que el doctorando presente el trabajo como tesis y renuncia expresa de estos a presentarlo como parte de otra tesis doctoral."

UNIVERSITAT JAUME I
Escola de Doctorat · ED

Ana María Botero Coy, como coautor/ coautora doy mi **autorización** a **Eddie Alexander Fonseca Rubí** para la presentación de las siguientes publicaciones como parte de su tesis doctoral.

Relación de publicaciones:

Investigation of pharmaceuticals in a conventional wastewater treatment plant: removal efficiency, seasonal variation and impact of a nearby hospital. Journal of Environmental Chemical Engineering (Manuscrito aceptado)

Asimismo, **renuncio** a poder utilizar estas publicaciones como parte de otra tesis doctoral.

Y para que conste firmo el presente documento,

Ana María Botero Coy
Firmado digitalmente por Ana María Botero Coy
Fecha: 2021.04.20 13:19:46 +02'00'
Lugar, fecha y firma

Todo ello, atendiendo al artículo 23 de la Normativa de los Estudios de Doctorado, regulados por el RD 99/2011, en la Universitat Jaume I (Aprobada por el Consejo de Gobierno núm. 19 de 26 de Enero de 2012, modificada por el Consejo de Gobierno núm. 29 de 27 de Noviembre de 2012 y con posterior modificación por el Consejo de Gobierno núm. 37 de 25 de Julio de 2013):

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"Aquellas tesis doctorales que opten por la incorporación de artículos (compendio de publicaciones) deben de ajustarse, en la medida de lo posible, a la siguiente estructura: -Introducción/objetivos - Un capítulo por artículo incorporado - Discusión general de los resultados - Conclusiones. -Aceptación de los coautores de que el doctorando presente el trabajo como tesis y renuncia expresa de estos a presentarlo como parte de otra tesis doctoral."

UNIVERSITAT JAUME I
Escola de Doctorat · ED

Arianna Renau Pruñonosa, como coautor/ coautora doy mi **autorización** a **Eddie Alexander Fonseca Rubí** para la presentación de las siguientes publicaciones como parte de su tesis doctoral.

Relación de publicaciones:

Investigation of pesticides and their transformation products in the Júcar River Hydrographical Basin (Spain) by wide-scope high-resolution mass spectrometry screening. Environmental Research, 177 (2019) 108570. https://doi.org/10.1016/j.envres.2019.108570

Asimismo, **renuncio** a poder utilizar estas publicaciones como parte de otra tesis doctoral.

Y para que conste firmo el presente documento,

Castellón de la Plana, 20 de Abril de 2021

Lugar, fecha y firma

ARIANNA| RENAU| PRUÑONOSA

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Fecha: 2021.04.20 21:54:03 +02'00'

Todo ello, atendiendo al articulo 23 de la Normativa de los Estudios de Doctorado, regulados por el RD 99/2011, en la Universitat Jaume I (Aprobada por el Consejo de Gobierno núm. 19 de 26 de Enero de 2012, modificada por el Consejo de Gobierno núm. 29 de 27 de Noviembre de 2012 y con posterior modificación por el Consejo de Gobierno núm. 37 de 25 de Julio de 2013):

"(...)

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UNIVERSITAT JAUME I
Escola de Doctorat · ED

Emma Gracia Lor, como coautor/ coautora doy mi **autorización** a **Eddie Alexander Fonseca Rubí** para la presentación de las siguientes publicaciones como parte de su tesis doctoral.

Relación de publicaciones:

Investigation of pesticides and their transformation products in the Júcar River Hydrographical Basin (Spain) by wide-scope high-resolution mass spectrometry screening. Environmental Research, 177 (2019) 108570. https://doi.org/10.1016/j.envres.2019.108570

Asimismo, **renuncio** a poder utilizar estas publicaciones como parte de otra tesis doctoral.

Y para que conste firmo el presente documento,

GRACIA LOR EMMA - 18444705Q

Firmado digitalmente por GRACIA LOR EMMA - 18444705Q
Nombre de reconocimiento (DN): c=ES, serialNumber=IDCES-18444705Q, givenName=EMMA, sn=GRACIA LOR, cn=GRACIA LOR EMMA - 18444705Q
Fecha: 2021.04.19 16:42:29 +02'00'

Madrid, 19 de abril de 2021

Todo ello, atendiendo al artículo 23 de la Normativa de los Estudios de Doctorado, regulados por el RD 99/2011, en la Universitat Jaume I (Aprobada por el Consejo de Gobierno núm. 19 de 26 de Enero de 2012, modificada por el Consejo de Gobierno núm. 29 de 27 de Noviembre de 2012 y con posterior modificación por el Consejo de Gobierno núm. 37 de 25 de Julio de 2013):

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"Aquellas tesis doctorales que opten por la incorporación de artículos (compendio de publicaciones) deben de ajustarse, en la medida de lo posible, a la siguiente estructura: -Introducción/objetivos - Un capítulo por artículo incorporado - Discusión general de los resultados - Conclusiones. -Aceptación de los coautores de que el doctorando presente el trabajo como tesis y renuncia expresa de estos a presentarlo como parte de otra tesis doctoral."

UNIVERSITAT JAUME I
Escola de Doctorat · ED

Teodoro Estrela Monreal, como coautor/ coautora doy mi **autorización** a **Eddie Alexander Fonseca Rubí** para la presentación de las siguientes publicaciones como parte de su tesis doctoral.

Relación de publicaciones:

Investigation of pesticides and their transformation products in the Júcar River Hydrographical Basin (Spain) by wide-scope high-resolution mass spectrometry screening. Environmental Research, 177 (2019) 108570. https://doi.org/10.1016/j.envres.2019.108570

Asimismo, **renuncio** a poder utilizar estas publicaciones como parte de otra tesis doctoral.

Y para que conste firmo el presente documento,

Madrid, 20 de abril de 2021

Todo ello, atendiendo al artículo 23 de la Normativa de los Estudios de Doctorado, regulados por el RD 99/2011, en la Universitat Jaume I (Aprobada por el Consejo de Gobierno núm. 19 de 26 de Enero de 2012, modificada por el Consejo de Gobierno núm. 29 de 27 de Noviembre de 2012 y con posterior modificación por el Consejo de Gobierno núm. 37 de 25 de Julio de 2013):

"(...)

"Aquellas tesis doctorales que opten por la incorporación de artículos (compendio de publicaciones) deben de ajustarse, en la medida de lo posible, a la siguiente estructura: -Introducción/objetivos - Un capítulo por artículo incorporado - Discusión general de los resultados - Conclusiones. -Aceptación de los coautores de que el doctorando presente el trabajo como tesis y renuncia expreso de estos a presentarlo como parte de otra tesis doctoral."

UNIVERSITAT JAUME I
Escola de Doctorat · ED

Andreu Rico Artero, como coautor/ coautora doy mi **autorización** a **Eddie Alexander Fonseca Rubi** para la presentación de las siguientes publicaciones como parte de su tesis doctoral.

Relación de publicaciones:

Ecological risk assessment of pesticides in the Mijares River (eastern Spain) impacted by citrus production using wide-scope screening and target quantitative analysis. Journal of Hazardous Materials, 412 (2021) 125277. https://doi.org/10.1016/j.jhazmat.2021.125277

Occurrence and ecological risks of pharmaceuticals in a Mediterranean river in Eastern Spain. Environment International, 144 (2020) 106004. https://doi.org/10.1016/j.envint.2020.106004

Asimismo, **renuncio** a poder utilizar estas publicaciones como parte de otra tesis doctoral.

Y para que conste firmo el presente documento,

Firmado por ANDREU RICO ARTERO - NIF:29202419D el día 22/04/2021 con un certificado emitido por ACCVCA-120

Valencia, a 22 de Abril del 2021

Todo ello, atendiendo al artículo 23 de la Normativa de los Estudios de Doctorado, regulados por el RD 99/2011, en la Universitat Jaume I (Aprobada por el Consejo de Gobierno núm. 19 de 26 de Enero de 2012, modificada por el Consejo de Gobierno núm. 29 de 27 de Noviembre de 2012 y con posterior modificación por el Consejo de Gobierno núm. 37 de 25 de Julio de 2013):

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UNIVERSITAT JAUME I
Escola de Doctorat · ED

Miguel Ángel Pérez Martín, como coautor/ coautora doy mi **autorización** a **Eddie Alexander Fonseca Rubí** para la presentación de las siguientes publicaciones como parte de su tesis doctoral.

Relación de publicaciones:

Investigation of pesticides and their transformation products in the Júcar River Hydrographical Basin (Spain) by wide-scope high-resolution mass spectrometry screening. Environmental Research, 177 (2019) 108570. https://doi.org/10.1016/j.envres.2019.108570

Asimismo, **renuncio** a poder utilizar estas publicaciones como parte de otra tesis doctoral.

Y para que conste firmo el presente documento,

PEREZ MARTIN, MIGUEL ANGEL (AUTENTICACIÓN)
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Lugar, fecha y firma

Valencia, 19 de abril de 2021

Todo ello, atendiendo al articulo 23 de la Normativa de los Estudios de Doctorado, regulados por el RD 99/2011, en la Universitat Jaume I (Aprobada por el Consejo de Gobierno núm. 19 de 26 de Enero de 2012, modificada por el Consejo de Gobierno núm. 29 de 27 de Noviembre de 2012 y con posterior modificación por el Consejo de Gobierno núm. 37 de 25 de Julio de 2013):

"(...)

"Aquellas tesis doctorales que opten por la incorporación de artículos (compendio de publicaciones) deben de ajustarse, en la medida de lo posible, a la siguiente estructura: -Introducción/objetivos - Un capítulo por artículo incorporado - Discusión general de los resultados - Conclusiones. -Aceptación de los coautores de que el doctorando presente el trabajo como tesis y renuncia expresa de estos a presentarlo como parte de otra tesis doctoral."

UNIVERSITAT JAUME I
Escola de Doctorat · ED

Sara María Jiménez Argudo, como coautor/ coautora doy mi **autorización** a **Eddie Alexander Fonseca Rubí** para la presentación de las siguientes publicaciones como parte de su tesis doctoral.

Relación de publicaciones:

Investigation of pesticides and their transformation products in the Júcar River Hydrographical Basin (Spain) by wide-scope high-resolution mass spectrometry screening. Environmental Research, 177 (2019) 108570. https://doi.org/10.1016/j.envres.2019.108570

Asimismo, **renuncio** a poder utilizar estas publicaciones como parte de otra tesis doctoral.

Y para que conste firmo el presente documento,

Lugar, fecha y firma

Todo ello, atendiendo al artículo 23 de la Normativa de los Estudios de Doctorado, regulados por el RD 99/2011, en la Universitat Jaume I (Aprobada por el Consejo de Gobierno núm. 19 de 26 de Enero de 2012, modificada por el Consejo de Gobierno núm. 29 de 27 de Noviembre de 2012 y con posterior modificación por el Consejo de Gobierno núm. 37 de 25 de Julio de 2013):

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"Aquellas tesis doctorales que opten por la incorporación de artículos (compendio de publicaciones) deben de ajustarse, en la medida de lo posible, a la siguiente estructura: -Introducción/objetivos - Un capítulo por artículo incorporado - Discusión general de los resultados - Conclusiones. -Aceptación de los coautores de que el doctorando presente el trabajo como tesis y renuncia expresa de estos a presentarlo como parte de otra tesis doctoral."

Firmado por JIMENEZ ARGUDO SARA MARIA - DNI 04603334E el día 19/04/2021 con un certificado emitido por AC Administración Pública

UNIVERSITAT JAUME I
Escola de Doctorat · ED

Laura Pastor Alcañiz, como coautor/ coautora doy mi **autorización** a **Eddie Alexander Fonseca Rubi** para la presentación de las siguientes publicaciones como parte de su tesis doctoral.

Relación de publicaciones:

Investigation of pharmaceuticals in a conventional wastewater treatment plant: removal efficiency, seasonal variation and impact of a nearby hospital. Journal of Environmental Chemical Engineering (Manuscrito aceptado)

Asimismo, **renuncio** a poder utilizar estas publicaciones como parte de otra tesis doctoral.

Y para que conste firmo el presente documento,

LAURA PASTOR ALCAÑIZ - NIF:48437060A

Valencia, 19 de abril de 2021

Lugar, fecha y firma

Todo ello, atendiendo al artículo 23 de la Normativa de los Estudios de Doctorado, regulados por el RD 99/2011, en la Universitat Jaume I (Aprobada por el Consejo de Gobierno núm. 19 de 26 de Enero de 2012, modificada por el Consejo de Gobierno núm. 29 de 27 de Noviembre de 2012 y con posterior modificación por el Consejo de Gobierno núm. 37 de 25 de Julio de 2013):

"(...)

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UNIVERSITAT JAUME I
Escola de Doctorat · ED

Javier Claros Bedoya, como coautor/ coautora doy mi **autorización** a **Eddie Alexander Fonseca Rubí** para la presentación de las siguientes publicaciones como parte de su tesis doctoral.

Relación de publicaciones:

Investigation of pharmaceuticals in a conventional wastewater treatment plant: removal efficiency, seasonal variation and impact of a nearby hospital. Journal of Environmental Chemical Engineering (Manuscrito aceptado)

Asimismo, **renuncio** a poder utilizar estas publicaciones como parte de otra tesis doctoral.

Y para que conste firmo el presente documento.

Lugar, fecha y firma

Javier Claros

Valencia, 19-4-2021

Todo ello, atendiendo al artículo 23 de la Normativa de los Estudios de Doctorado, regulados por el RD 99/2011, en la Universitat Jaume I (Aprobada por el Consejo de Gobierno núm. 19 de 26 de Enero de 2012, modificada por el Consejo de Gobierno núm. 29 de 27 de Noviembre de 2012 y con posterior modificación por el Consejo de Gobierno núm. 37 de 25 de Julio de 2013):

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UNIVERSITAT JAUME I
Escola de Doctorat · ED

Francisco González Breijo, como coautor/ coautora doy mi **autorización** a **Eddie Alexander Fonseca Rubí** para la presentación de las siguientes publicaciones como parte de su tesis doctoral.

Relación de publicaciones:

Investigation of pesticides and their transformation products in the Júcar River Hydrographical Basin (Spain) by wide-scope high-resolution mass spectrometry screening. Environmental Research, 177 (2019) 108570. https://doi.org/10.1016/j.envres.2019.108570

Asimismo, **renuncio** a poder utilizar estas publicaciones como parte de otra tesis doctoral.

Y para que conste firmo el presente documento,

en Pinar del Río, Cuba. El día 19 de abril de 2021.

Lugar, fecha y firma

Todo ello, atendiendo al artículo 23 de la Normativa de los Estudios de Doctorado, regulados por el RD 99/2011, en la Universitat Jaume I (Aprobada por el Consejo de Gobierno núm. 19 de 26 de Enero de 2012, modificada por el Consejo de Gobierno núm. 29 de 27 de Noviembre de 2012 y con posterior modificación por el Consejo de Gobierno núm. 37 de 25 de Julio de 2013):

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UNIVERSITAT JAUME·I