Universidad de la República

Facultad de Ciencias

Programa de Posgrado en Ciencias Ambientales

Dinámica y efecto de plaguicidas en matrices ambientales: aproximaciones de campo y experimentales

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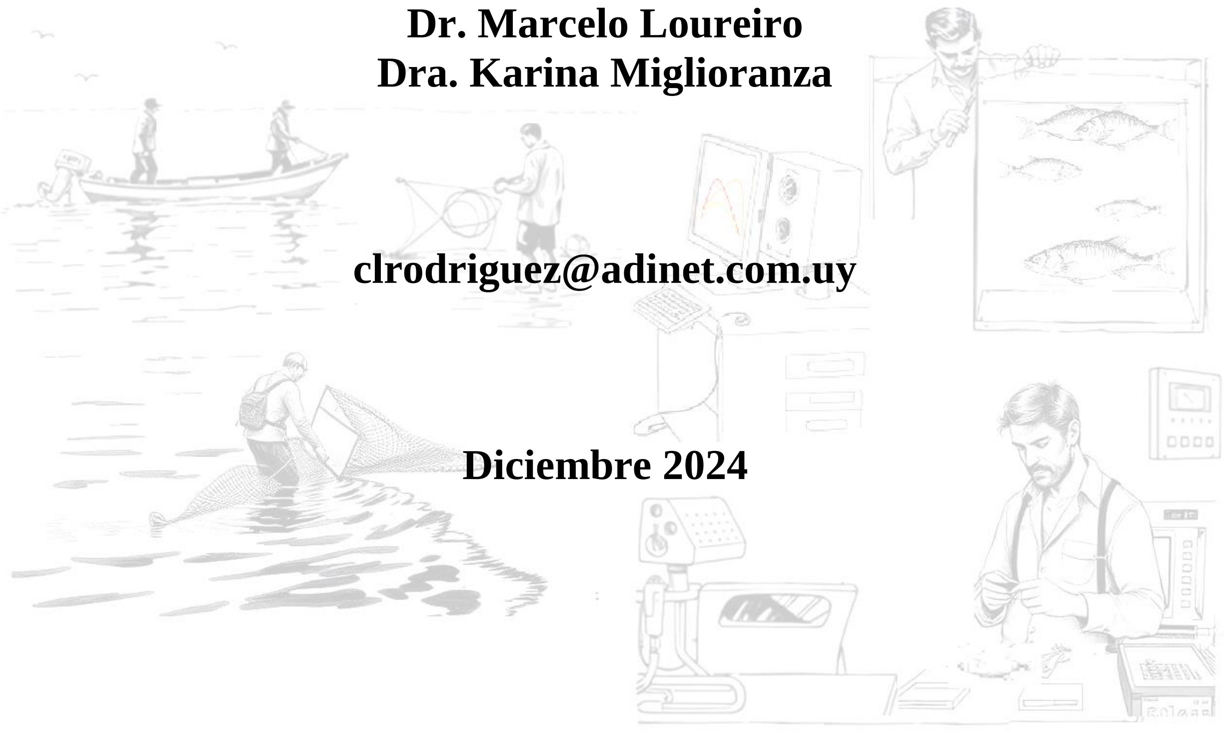
Diciembre 2024



Agradecimientos

Quiero expresar mi agradecimiento a todas las personas que han sido parte fundamental en la realización de esta tesis.

En primer lugar, agradezco a mis tutores Dr. Franco Teixeira de Mello y Dr. Andrés Pérez Parada por darme la oportunidad de desarrollar mis estudios bajo su supervisión. Ambos, con dedicación y paciencia me guiaron a lo largo de todo el doctorado.

A mis compañeros de laboratorio. Gracias Canario, Ale, Martín, Guille y Franco por el apoyo en los muestreos de campo y por las largas horas de laboratorio, abriendo pescado hasta la madrugada. Sin ese esfuerzo este trabajo no habría sido posible. Agradezco también a Margenny, Maite Burwood, Claudia Baladán, Samanta Stebniki, Carmen Casás y Clementina Calvo por la colaboración en los muestreos.

Al equipo de los laboratorios de Facultad de Química y GACT-Paysandú por el procesamiento analítico de las muestras, especialmente a Horacio Heinzen, Silvina Niell y Andrés. Al equipo del laboratorio de DINACEA, en especial a Natalia Barboza y Alejandro Mangarelli.

A la Dra. Andrea Hued por recibirme en su laboratorio y compartir generosamente su tiempo y conocimientos conmigo. A Alejo y Marina por su dedicación y ayuda con los bioensayos y mediciones enzimáticas. A todos los compañeros del IDEA – UNC por hacer que mi estancia en Córdoba fuera excelente.

Un agradecimiento a la Unidad Coordinadora del Proyecto GCP/URU/031/GFF, a la Comisión Académica de Postgrado (CAP) y al Sistema Nacional de Investigadores (SNI).

A los diferentes organismos desde los que se recibieron aportes para la realización de este trabajo: Municipio de Atlántida, Comisión de Cuenca de la Laguna del Cisne y OSE.

Muchas gracias a mi familia. A María Ángel por el apoyo incondicional, a Santiago y Emiliano por alegrarme la vida. A mi madre y hermanos por la motivación para continuar en este camino.

RESUMEN

Dinámica y efecto de plaguicidas en matrices ambientales: aproximaciones de campo y experimentales

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La presencia de plaguicidas en los sistemas acuáticos representa una de las amenazas ambientales más importantes a escala global. Desde 2020, América Latina se ha convertido en la región donde se aplican la mayor cantidad de plaguicidas a nivel global. En particular, en Uruguay la intensificación agrícola asociada a los cultivos de secano y el uso masivo de plaguicidas han llevado a un deterioro significativo en la calidad de los cuerpos de agua. En este sentido, el desarrollo de planes de monitoreo integrales que involucren el análisis en varias matrices ambientales y la realización de análisis de riesgo representan una herramienta fundamental para una correcta evaluación y gestión de plaguicidas. Los objetivos de la presente tesis fueron monitorear la presencia y destino de múltiples plaguicidas en agua superficial y peces en la Laguna del Cisne (LC), utilizada para el suministro de agua potable. Determinar el posible impacto ambiental de estos contaminantes en el sistema mediante diferentes evaluaciones de riesgo ecotoxicológico (ERE) y realizar bioensayos de exposición en una especie de pez nativa para evaluar los efectos del compuesto más representativo del sistema. La estrategia aplicada permitió generar el más completo set de datos de ocurrencia de plaguicidas en un sistema acuático de Uruguay. Mediante muestreos mensuales (Abril 2018- Marzo 2019) se logró determinar la presencia de 25 plaguicidas en agua superficial. La distribución temporal fue relacionada al uso estacional de plaguicidas en los cultivos de la cuenca, con máximos de ocurrencia en primavera-verano. Los plaguicidas más frecuentes del sistema fueron el insecticida clorantraniliprole (CHL) y los herbicidas glifosato (su metabolito AMPA) y metolacloro. Se observó una contaminación por organoclorados, principalmente p,p′-DDT, con concentraciones en algunos casos superiores en dos órdenes de magnitud a los valores guía internacional para la protección de la biota acuática. Se encontraron seis compuestos actualmente prohibidos por la legislación nacional, incluida la atrazina, que presentó una alta frecuencia de ocurrencia. La ERE se realizó en función de las concentraciones en agua a través de aproximaciones determinísticas (RQ) y probabilísticas (PRA). Ambos enfoques evidenciaron un muy alto riesgo ecotoxicológico de plaguicidas organoclorados (DDT y sus metabolitos DDD y DDE), organofosforados (etión y clorpirifós) y piretroides (especialmente bifentrina, permetrina y cipermetrina) en este sistema. En el análisis PRA, la concentración perjudicial para el 5% de las especies (HC5) fue mayor para la especie más sensible en exposición a largo plazo. Contrariamente, en exposiciones agudas el riesgo fue mayor para todas las especies. En tejido de peces se encontraron ocho compuestos diferentes, con concentraciones desde 1000 a 453000 $ng \cdot kg^{-1}$, siendo propiconazole, chlorpyrifos, y p,p′-DDE los compuestos más frecuentes. Se encontró que la bioacumulación está directamente relacionada con el contenido lipídico y la talla de los individuos. Se observó una complementariedad entre ambas matrices en el tipo de plaguicidas detectados, relacionado con las propiedades fisicoquímicas de estos. Para conocer los efectos subletales del compuesto más frecuente y con un alto riego ecotoxicológico en el sistema (CHL), se realizaron bioensayos de toxicidad aguda (96hs) en madrecita (*Cnesterodon decemmaculatus*) a concentraciones de 1/10 (1.5 mg/L) y 1/100 (0.15 mg/L) de la LC50. Se evidenció que la exposición a

CHL impacta negativamente en la salud de los peces, con posibles consecuencias a nivel individual y ecológico. La actividad locomotora (distancia recorrida, velocidad media y máxima y tiempo de inmovilidad) disminuyó significativamente en los peces expuestos a ambas concentraciones, en comparación con el control. La actividad de la acetilcolinesterasa (AChE) no evidenció efectos colinérgicos, posiblemente debido al modo de acción de CHL. Los músculos y el cerebro fueron los órganos más afectados por el estrés oxidativo. La actividad de la catalasa (CAT) mostró una disminución significativa en el cerebro y músculo, mientras que en las branquias fue mayor a bajas concentraciones. La actividad de la Glutatión S-transferasa (GST) mostró un efecto estimulador en el hígado a la concentración más alta, y no se registraron cambios significativos en las transaminasas y el índice somático. La exposición parece inducir varios mecanismos de defensa en la especie, incluyendo fenómenos de hormesis para mantener la homeostasis en los organismos. En el caso de los insecticidas dirigidos a los músculos, como las diamidas, los parámetros locomotores pueden ser uno de los biomarcadores más eficaces para evaluar el impacto de la exposición. Este estudio representa el primero sobre los efectos de la exposición a CHL en esta especie, considerada un bioindicador regional. La información generada en esta tesis constituye un insumo fundamental para fortalecer los programas de monitoreo y gestión de plaguicidas a nivel nacional e internacional. En este sentido, contribuye al diseño de planes de monitoreo y al desarrollo de metodologías que permiten implementar diferentes alternativas de ERE para la formulación de normativas más eficaces. En el contexto del sistema de LC, los resultados obtenidos proporcionan una descripción detallada de la línea base, la cual debería servir como modelo para ajustar medidas de gestión ambiental.

PALABRAS CLAVE: Plaguicidas, Monitoreo ambiental, Laguna del Cisne, Evaluación de riesgo ecotoxicológico, Bioensayos de toxicidad

ABSTRACT

Dynamics and Effects of Pesticides in Environmental Matrices: Field and Experimental Approaches

MSc. César Rodríguez Bolaña

The presence of pesticides in aquatic systems represents one of the most significant environmental threats globally. Since 2020, Latin America has become the region with the highest application of pesticides worldwide. In particular, in Uruguay, the intensification of agriculture associated with rainfed crops and the massive use of pesticides have led to a significant deterioration in the quality of water bodies. In this context, the development of comprehensive monitoring plans that involve multi-matrix environmental analysis and risk assessments is a fundamental tool for proper pesticide evaluation and management. The objectives of this thesis were to monitor the presence and fate of multiple pesticides in surface water and fish in Laguna del Cisne (LC), a reservoir used for drinking water supply. Additionally, the study aimed to determine the potential environmental impact of these contaminants on the system through different ecotoxicological risk assessments (ERA) and to conduct exposure bioassays on a native fish species to evaluate the effects of the most representative compound in the system. The applied strategy generated the most comprehensive dataset on pesticide occurrence in an aquatic system in Uruguay. Through monthly sampling (April 2018-March 2019), 25 pesticides were detected in surface water. The temporal distribution was linked to the seasonal use of pesticides in the watershed, with peak occurrences in spring-summer. The most frequent pesticides in the system were the insecticide chlorantraniliprole (CHL) and the herbicides glyphosate (and its metabolite AMPA) and metolachlor. Contamination by organochlorines, mainly p,p′-DDT, was observed, with concentrations in some cases exceeding international guideline values for the protection of aquatic biota by two orders of magnitude. Six compounds currently banned by national legislation, including atrazine, which showed a high frequency of occurrence, were detected. The ERA was performed based on water concentrations through deterministic (RQ) and probabilistic (PRA) approaches. Both approaches revealed a very high ecotoxicological risk from organochlorine pesticides (DDT and its metabolites DDD and DDE), organophosphates (ethion and chlorpyrifos), and pyrethroids (especially bifenthrin, permethrin, and cypermethrin) in this system. In the PRA, the hazardous concentration for 5% of species (HC5) was higher for the most sensitive species during long-term exposure. Conversely, in acute exposures, the risk was higher for all species. In fish tissue, eight different compounds were found, with concentrations ranging from 1,000 to 453,000 ng•kg−1, with propiconazole, chlorpyrifos, and p,p′-DDE being the most frequent. Bioaccumulation was found to be directly related to lipid content and the size of the individuals. A complementarity was observed between both matrices in the types of pesticides detected, related to their physicochemical properties. To determine the sublethal effects of the most frequent compound with high ecotoxicological risk in the system (CHL), acute toxicity bioassays (96h) were conducted on Cnesterodon decemmaculatus at concentrations of 1/10 (1.5 mg/L) and 1/100 (0.15 mg/L) of the LC50. The exposure to CHL was found to negatively impact fish health, with potential consequences at both individual and ecological levels. Locomotor activity (distance traveled, mean and maximum speed, and immobility time) significantly decreased in fish exposed to both concentrations compared to the control. Acetylcholinesterase (AChE) activity did not show cholinergic effects, possibly due to CHL's mode of action. Muscles and the brain were the organs most affected by oxidative stress. Catalase (CAT) activity showed a significant decrease in the brain and muscle, while in the gills, it was higher at low concentrations. Glutathione S-transferase (GST) activity showed a stimulatory effect in the liver at the highest concentration, and no significant changes were observed in transaminases and the somatic index. The exposure appeared to induce several defense mechanisms in the species, including hormesis phenomena, to maintain homeostasis in the organisms. In the case of insecticides targeting muscles, such as diamides, locomotor parameters may be one of the most effective biomarkers for assessing the impact of exposure. This study is the first to analyze the effects of CHL exposure on this species, considered a regional bioindicator. The information generated in this thesis is a critical resource for strengthening pesticide monitoring and management programs at both national and international levels. In this regard, it contributes to the design of monitoring plans and the development of methodologies that allow the implementation of various ERA alternatives for more effective regulatory frameworks. In the context of the LC system, the results provide a detailed baseline that should serve as a model for adjusting environmental management measures.

Keywords: Pesticides, Environmental monitoring, Laguna del Cisne, Ecotoxicological risk assessment, Toxicity bioassays

ÍNDICE

Acrónimos y siglas

AChE: Acetilcolinesterasa

ALP: Fosfatasa alcalina

ALT: Alanina aminotransferasa

AST: Aminotransferasa

CAT: Catalasa

CHL: Clorantraniliprol

DINACEA: Dirección Nacional de Calidad y Evaluación Ambiental

DGSA: Dirección General de Servicios Agrícolas

EPA: Agencia de Protección Ambiental de los Estados Unidos de América (por sus siglas en inglés)

ERE: Evaluación de Riesgo Ecotoxicológicoo

GST: Glutatión S-transferasa

IBR: Respuesta de los biomarcadores integrada (Integrated biomarker response, por sus siglas en inglés)

LC: Laguna del Cisne

MA: Ministerio de Ambiente

MGAP: Ministerio de Ganadería, Agricultura y Pesca

OAN: Observatorio Ambiental Nacional

OMS: Organización Mundial de la Salud

POP´s: Plaguicidas Orgánicos Persistentes

PRA: Evaluación probabilística de riesgo (Probabilistic Risk Assessment, por sus siglas en inglés).

RQ: Evaluación determinística de riesgo (Risk Quotient, por sus siglas en inglés).

1. Introducción

La agricultura moderna es altamente dependiente de la utilización de paquetes tecnológicos, entre ellos los plaguicidas, que son empleados para proteger y aumentar el rendimiento de los cultivos (Sharma et al., 2019; Khan et al., 2023; Thakur & Sharma, 2024). Estos compuestos son en su mayoría sustancias químicas destinadas a prevenir, destruir o controlar cualquier tipo de plaga, considerándose como plaga cualquier organismo que cause perjuicio o que interfiera de cualquier forma en la producción, almacenamiento, transporte y comercialización de productos agrícolas (World Health Organization, 2009). Suelen clasificarse en función de los tipos de plaga que combaten, siendo los más importantes los herbicidas (empleados para controlar plantas), fungicidas (para controlar hongos) e insecticidas (para controlar insectos); así como en base a su mecanismo de acción, persistencia ambiental, toxicidad entre otras características (Kaur et al., 2019) (Ver Anexo 1).

Desde 2020, América Latina se ha convertido en la región donde se aplican la mayor cantidad de plaguicidas a nivel global, siendo los países sudamericanos responsables de casi el 90% del uso en esta región (FAOStat, 2024). Las diversas prácticas agrícolas desarrolladas en Sudamérica determinan que los tipos de compuestos varíen entre países, e incluso entre áreas de un mismo país. En la cuenca del Río de la Plata, dedicada en su mayoría a la producción de cereales (por ejemplo, maíz) y oleaginosas (Williams & Thompson, 2022) existe un dominio en el uso de herbicidas sobre los demás tipos de compuestos, representando el 89,9%, 76,1% y 58,7% del total de plaguicidas utilizados en 2022 en Argentina, Brasil y Uruguay respectivamente (FAOStat, 2024). Por su parte, Argentina y Uruguay también presentan los mayores valores de aplicación *per capita* de Latinoamérica (FAOStat, 2024).

En Uruguay, el sector agrícola desempeña un papel significativo en la economía, representando una de las principales contribuciones al Producto Interno Bruto (PBI) nacional (Soutullo et al., 2020; García-Préchac et al., 2022). En este sentido, la intensificación de la agricultura experimentada en las últimas décadas, principalmente debido al desarrollo de los cultivos de secano, ha llevado al uso masivo de plaguicidas (Cespedes-Payret et al., 2009; Soutullo et al., 2020; Fernández-Nion & Díaz-Isasa, 2024). Para el período 2000-2020, se reportó un aumento en las cantidades de herbicidas aplicados por hectárea, pasando de 2,58 a 7,97 kg/ha (Palladino et al., 2023). Solamente en 2022, el país importó un total de 14,8 millones de kg de ingredientes activos de plaguicidas (DGSA, 2023).

La introducción de tales volúmenes de plaguicidas en el ambiente representa un riesgo para la salud humana y la de los ecosistemas, ya que estos pueden producir múltiples consecuencias negativas a nivel de los organismos, poblaciones y comunidades (Sharma et al., 2019; de Souza et al., 2020; Souza et al., 2022; Khan et al., 2023). Sumado a ello, la presencia de compuestos prohibidos para su uso actual, comúnmente llamados "legados o heredados", como el caso de plaguicidas organoclorados y algunos organofosforados y piretroides; también representan un riesgo para el ambiente dada su elevada persistencia ambiental y tendencia a bioacumularse en la biota (Pérez-Parada et al., 2018; Kumar et al., 2021).

1.1. Destino ambiental de los plaguicidas

El destino ambiental y el comportamiento de los plaguicidas (entre ellos el transporte, su transformación química y persistencia) están estrechamente relacionados con sus propiedades fisicoquímicas, como su solubilidad en agua (SW), el coeficiente de partición octanol-agua (Kow), la presión de vapor (Pv), la constante de ley de Henry (K), el coeficiente de absorción en carbono orgánico (Koc) y la constante de disociación (Ka), entre otros (Figura 1).

De esta manera, los compuestos con alta solubilidad en agua son más susceptibles de transportarse por escorrentía superficial o a través del suelo (lixiviación) alcanzando fácilmente los cuerpos de agua; mientras que los de baja solubilidad son más propensos a adsorberse al suelo y a sedimentos, aumentando el riesgo de acumulación (Triegel & Guo, 2018; Grondona et al., 2023). El coeficiente K_{ow} representa una medida de la lipofilia de los compuestos, de esta manera plaguicidas con alto K_{ow} tienden a bioacumularse, particularmente en tejidos grasos (Pérez-Parada et al., 2018; Ghosh & Sarower, 2024). Por su parte, Pv mide la tendencia de una sustancia a evaporarse, por lo que plaguicidas con alto Pv presentan una mayor tendencia a transportarse a través del aire, mientras que compuestos menos volátiles permanecen más tiempo en el suelo o en el agua (Aparicio et al., 2015; Kaur et al., 2019). Otras características importantes que influyen en el destino ambiental de los plaguicidas son el K_{oc} (capacidad para unirse a las partículas del suelo) y la persistencia, medida como DT_{50} (tiempo donde se degrada la mitad de la concentración). En este sentido, aquellos con alto Koc y persistencia (e.g.

DDT, dieldrín, clorpirifós, endosulfán) suelen representar un mayor riesgo ambiental debido a que tienden a adherirse fuertemente a las partículas del suelo y permanecer en él durante muchos años, aumentando su potenciales efectos de exposición, bioacumulación y toxicidad a largo plazo (Aparicio et al., 2015; Vryzas, 2018; Silva et al., 2019).

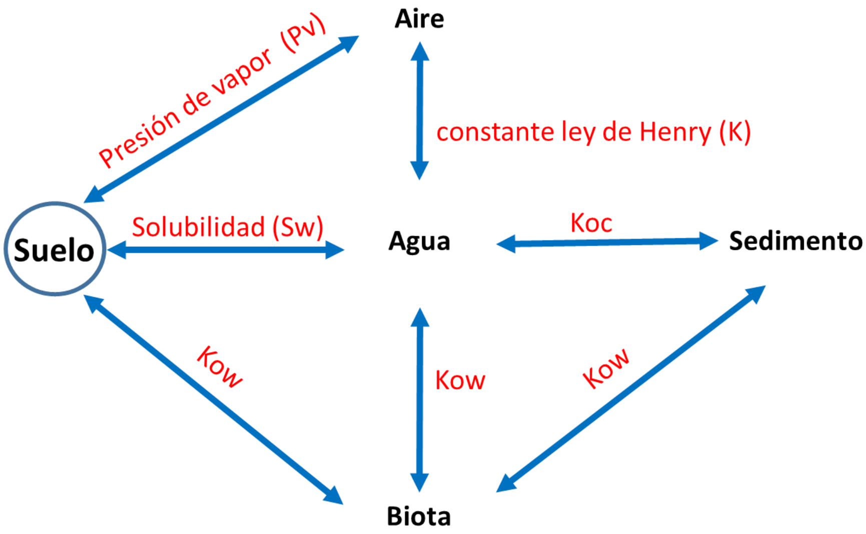

Figura 1. Principales propiedades fisicoquímicas de los plaguicidas y su influencia en el destino ambiental de los compuestos una vez aplicados al suelo (circulo azul).

Por lo tanto, una vez aplicadas en las áreas objetivo, las moléculas de los plaguicidas pueden ser transportadas (e.g. por volatilización, escorrentía), disipadas (e.g. por degradación microbiológica y fotolítica) o permanecer inalteradas a través de interacciones lábiles con el suelo o las plantas (Aparicio et al., 2015; Pérez-Parada et al., 2018; Silva et al., 2019).

La persistencia de estos compuestos en el suelo representa un sumidero, desde el cual los plaguicidas pueden ser transportados hacia las aguas continentales por escorrentía superficial o deposición atmosférica. Son particularmente propensos a este transporte los compuestos con alta solubilidad, baja adsorción al suelo y alta volatilidad (Lupi et al., 2016; Vryzas, 2018; Chow et al., 2020).

Es así como existe una mayor llegada de plaguicidas en los sistemas acuáticos con cuencas agrícolas en comparación con cuencas no agrícolas, observándose una relación directa entre la concentración y la ocurrencia de plaguicidas en las aguas superficiales y la cantidad y frecuencia de plaguicidas aplicados en la cuenca (Metcalfe et al., 2019; de Souza et al., 2020; Souza et al., 2022)

En el medio acuático, los plaguicidas pueden ser adsorbidos por las partículas orgánicas e inorgánicas suspendidas, las cuales luego pueden depositarse en los sedimentos (en general aquellos con baja solubilidad y alto Koc); o ser ingeridas por la biota (preferentemente alto Kow) pudiendo existir bioacumulación y biomagnificación (Vryzas, 2018; Pérez-Parada et al., 2018; Kumar et al., 2021).

Además, los plaguicidas que no se hidrolizan (no se descomponen por acción del agua) permanecen en esta matriz por más tiempo y pueden ingresar a la biota a través de los tejidos (Pizzochero et al., 2019). Por otra parte, ciertos plaguicidas sufren transformaciones fotoquímicas y químicas, como óxido-reducción o hidrólisis, que muchas veces generan metabolitos más tóxicos que sus precursores (Arnot & Mackay, 2018; Ghosh & Sarower, 2024).

Estas diferentes vías de transferencia, transformación y acumulación en diferentes matrices generan dinámicas espaciales y temporales complejas entre el sistema terrestre y el acuático que requiere especial atención a la hora de ser estudiada (Figura 2).

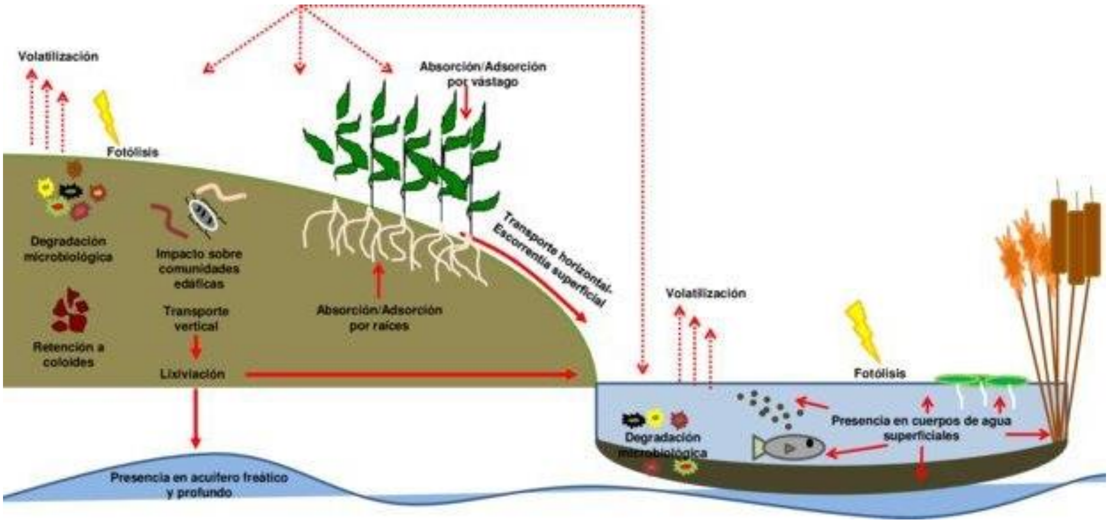

Figura 2. Esquema representando el transporte y destino ambiental de los plaguicidas en el medio ambiente. Tomado de Aparicio et al., 2015.

1.2. Plaguicidas en matrices bióticas y abióticas

El estudio de la compleja dinámica ambiental de los plaguicidas en los sistemas acuáticos exige un monitoreo exhaustivo de su presencia y concentración en las diferentes matrices ambientales como ser agua y biota (González et al., 2013; Carazo-Rojas et al., 2018; Montagner et al., 2022; Slaby et al., 2022).

El análisis de plaguicidas en agua permite evaluar la presencia y los aportes recientes de plaguicidas en el sistema, posibilitando detectar los usos estacionales de estos compuestos y evaluar sus efectos en la calidad del agua (Carazo-Rojas et al., 2018; Kapsi et al., 2019). En general esta matriz es en la que se registra la mayor cantidad de compuestos, siendo representativa de lo que ocurre en la cuenca (Metcalfe et al., 2019; de Souza et al., 2020; Montagner et al., 2022). Asimismo, la toma de muestras es muy accesible y se cuentan con metodologías de análisis altamente estandarizados. Otra ventaja es que muchas normativas y regulaciones ambientales así como datos de ecotoxicología se basan en los límites de concentración en agua (Chow et., 2020; Casallanovo et al., 2021).

Sin embargo, las muestras de agua presentan la desventaja de ser muy dependientes de las condiciones ambientales, por ejemplo, el viento o la turbulencia del agua pueden generar resuspensión de sedimentos, aumentando la turbidez del sistema, lo que podría mezclar los plaguicidas o adherirlos con mayor facilidad a la materia orgánica, estando más disponibles para la biota (Pérez-Parada et al., 2018). Además, como se mencionó anteriormente, las características fisicoquímicas de los compuestos determinan en gran medida que tipo de plaguicidas son susceptibles de encontrarse en agua. En este sentido, para plaguicidas lipofílicos (baja solubilidad y alto Kow) el análisis de esta matriz únicamente, podría no ser representativa de la carga de contaminantes en el sistema (Carazo-Rojas et al., 2018; Wang et al., 2019).

En este sentido, la capacidad de bioacumulación y biomagificación que presentan los peces los convierte en una matriz ideal para detectar contaminantes hidrofóbicos, así como evaluar su efecto potencial en los organismos y los patrones históricos de contaminación (Arnot & Gobas, 2006; Ernst et al., 2018; Perez-Parada et al., 2018; Pizzochero et al., 2019; Ghosh & Sarower, 2024).

Por lo tanto, el análisis complementario entre estas dos matrices permite capturar una mayor variedad de compuestos con diferentes características fisicoquímicas, conocer su destino ambiental en el sistema, evaluar la exposición de la biota acuática y detectar patrones temporales en relación a los usos del suelo. Este conocimiento resulta fundamental para una evaluación más realista de la calidad ambiental y para una correcta gestión de los sistemas acuáticos. En este sentido, una parte de este trabajo intenta capturar la dinámica temporal y espacial de múltiples plaguicidas en agua y peces en la Laguna del Cisne.

1.3.Bioacumulación de plaguicidas en peces

Las interacciones entre matrices abióticas y bióticas juegan un rol fundamental en la dinámica de los plaguicidas en un sistema acuático. Los organismos acuáticos pueden incorporar los plaguicidas y sus metabolitos en distintos tejidos, por la simple exposición en el medio (bioconcentración) o a través de la dieta (bioacumulación) (Arnot & Gobas, 2006; Pérez-Parada et al., 2018). Asimismo, puede ocurrir un proceso de biomagnificación, donde las concentraciones de los plaguicidas se incrementan a medida que aumenta la posición trófica (Arnot & Mackay, 2018) (Figura 3).

Las tasas de acumulación dependen de diversos factores, siendo la intensidad del uso de plaguicidas en la cuenca, la duración de la exposición y las características fisicoquímicas de los contaminantes, algunas de las variables más relevantes (Arnot & Gobas, 2006; Ernst et al., 2018).

Dentro de las propiedades de los compuestos, su volatilidad, persistencia en el medio y solubilidad o afinidad a los tejidos biológicos resultan las más importantes (Pérez-Parada et al., 2018). En este sentido, los plaguicidas que representan mayor preocupación desde el punto de vista de la salud y el medio ambiente, son aquellos compuestos persistentes y lipofílicos (Arnot & Gobas, 2006.; Pérez-Parada 2018). Los plaguicidas lipofílicos, con valores de log K_{ow}> 3 tienen mayor capacidad de interactuar con partículas y sedimentos suspendidos o acumularse en tejidos biológicos (van der Oost et al., 2003; Montagner et al., 2022). Asimismo, suelen presentar una mayor tendencia a la bioacumulación y biomagnificación sobretodo en especies o tejidos con alto contenido lipídico (Pérez-Parada et al., 2018). Asimismo, los plaguicidas polares, es decir aquellos que poseen mayor solubilidad están presentes principalmente en la fase acuosa.

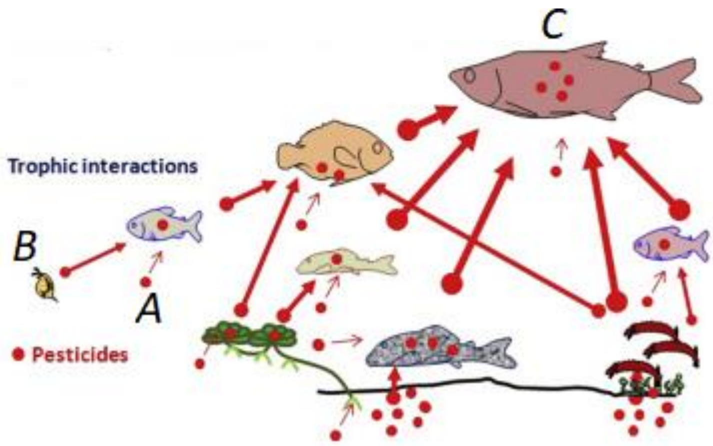

Figura 3. Vías de exposición de los peces a los contaminantes en el medio acuático y los diferentes procesos de absorción de estos compuestos: bioconcentración (A), bioacumulación (B) y biomagnificación (C). Tomado de Pérez-Parada et al., 2018.

En el caso de los peces, las características biológicas de las especies como el tipo de alimentación, tamaño, estado nutricional y reproductivo de los individuos pueden afectar la tasa de acumulación y biotransformación de plaguicidas en los organismos (van der Oost et al., 2003; Arnot & Gobas, 2006; Barni et al., 2014; Anzalone et al., 2024).
De esta manera, especies con alto contenido lipídico tienden a acumular más compuestos en sus tejidos (Enrst et al., 2018). La misma relación se observa con la talla, donde individuos más grandes tienden a acumular un mayor número de compuestos (Pérez-Parada et al., 2018). El régimen de alimentación también condiciona el grado de exposición, ya que especies filtradoras se encuentran más expuestas a compuestos presentes en el agua, mientras que las especies detritívoras a los particionados en el sedimento (Wang et al., 2019; Anzalone et al., 2024).

El análisis del proceso de bioacumulación de los plaguicidas en peces permite comprender cómo estos contaminantes se mueven entre los distintos compartimentos en los sistemas acuáticos y evaluar sus efectos potenciales sobre los organismos. En este sentido, la exposición a los plaguicidas y sus metabolitos, ya sea de forma crónica (prolongada y a bajas concentraciones) o aguda (puntual y en elevada concentración) puede estimarse mediante la cuantificación de sus residuos en distintos tejidos, como ser tejido muscular, adiposo o hepático (van der Oost et al., 2003; Ernst et al., 2018; Pérez-Parada et al., 2018).

1.4.Ecotoxicología

La ecotoxicología comprende el estudio del destino y los efectos causados por los contaminantes, naturales o sintéticos, sobre los individuos, poblaciones, comunidades y ecosistemas, con el objetivo de proveer herramientas de gestión que permitan prevenir, mitigar o remediar tales efectos (Connell et al., 2009; Carriquiriborde, 2021). Es una ciencia multidisciplinar que integra conocimientos de ecología, toxicología, fisiología, química analítica, biología molecular y estadística y que emplea métodos tanto de campo como de laboratorio (Ullah & Zorriehzahra 2015; Calisi, 2023).

1.4.1 Evaluación de Riesgo Ecotoxicológico

Desde la ecotoxicología para identificar, cuantificar y caracterizar el riego de un contaminante para el ambiente, se han desarrollado diversas herramientas de Evaluación de Riesgos Ecotoxicológicos (ERE) (Carriquiriborde, 2021; Maertens et al., 2022). Estas incorporan la probabilidad de un determinado contaminante genere efectos ecológicamente adversos y son utilizadas habitualmente por la academia y con fines reglamentarios en muchos países.

En ecosistemas acuáticos las ERE´s a menudo se basan en la utilización de aproximaciones determinísticas, como los cocientes de riesgo (RQ, Risk Quotient). Este abordaje implica la comparación de las concentraciones ambientales (exposición) predichas o estimadas en el ambiente; y sus efectos ecológicos (toxicidad) en especies centinela (RQ= exposición/toxicidad) (Carazo-Rojas et al., 2018; Kapsi et al., 2019; Iturburu et al., 2019; Mac Loughlin et al., 2022; Mentzel et al., 2022). Si bien este enfoque determinista es usado frecuentemente en gestión del riesgo, ha sido criticado por sus supuestos precautorios, que conducen a una sobreestimación de los potenciales efectos ecológicos de los contaminantes en el ambiente (Nagai, 2021; Maertens et al., 2022; Mentzel et al., 2022).

Por otro lado, la evaluación probabilística de riesgo (PRA, Probabilistic Risk Assessment) ofrece una alternativa más refinada que el método RQ y permite además realizar estimaciones cuantitativas del riesgo (Nagai, 2017; Maertens et al., 2022; Mentzel et al., 2022; McNamara et al., 2023). Este enfoque probabilístico considera la variabilidad en la sensibilidad de las distintas especies a un mismo estresor para construir una distribución de la sensibilidad de las especies (SSD) (Posthuma et al., 2002; Nagai, 2021; Bertrand & Iturburu, 2023).

El abordaje PRA es actualmente utilizado por agencias gubernamentales de Estados Unidos (USEPA, 2014), la Unión Europea ((EFSA (European Food Safety Authority), 2018); Australia y Canadá (Lee-Steere, 2009; Bhuller et al., 2021). En Latinoamérica, varios países (Brasil, Bolivia, Colombia, Ecuador, Perú y Venezuela) han incorporado un marco de ERE en sus legislaciones sobre uso de plaguicidas, aunque en todos los casos esta se basa en la evaluación determinística del riesgo (Carriquiriborde et al., 2014; Casallanovo et al., 2021). Estos marco regulatorios menos rigurosos ponen de manifiesto la necesidad de fortalecer las capacidades de monitoreo y regulatorias para proteger mejor la salud pública y el medio ambiente.

1.4.2 Bioensayos de toxicidad

Los bioensayos son herramientas ampliamente utilizadas en el campo de la ecotoxicología (Ullah & Zorriehzahra, 2015; Bertrand and Iturburu, 2023). Estas pruebas de toxicidad permiten realizar mediciones experimentales del efecto de agentes químicos o físicos en sistemas biológicos, estableciendo relaciones concentración-respuesta bajo condiciones controladas en campo o en laboratorio. En este sentido, han permitido evaluar el impacto de un contaminante sobre los organismos, comparar la sensibilidad de una o más especies a distintos tóxicos y establecer límites permitidos para los distintos contaminantes, entre otras ventajas (Castro et al., 2004; Xu et al., 2020).

Cuando se busca conocer el potencial tóxico de una sustancia sobre un organismo blanco, se espera que el efecto observable se logre a la concentración más baja posible Comúnmente se evalúa la concentración requerida para matar el 50% de los organismos, denominada concentración letal 50% (CL50) (Ullah & Zorriehzahra, 2015).). Este parámetro permite comparar diferentes sustancias y decidir cuál es más eficaz para alcanzar el efecto deseado. También las CL50 permiten comparar las mismas sustancias en diferentes organismos. Para realizar un bioensayo es importante identificar el tipo de sustancia a evaluar, en que organismo se probará y qué tipo de respuesta a ser evaluada (Zhang et al., 2023).

Los organismos acuáticos, en particular los peces, son ampliamente utilizados como bioindicadores debido a su sensibilidad a la exposición de plaguicidas y a la amplia gama de respuestas que permiten ser evaluadas (Castro et al., 2004; Bertrand & Iturburu, 2023). A su vez, los resultados que se obtienen tienen una importante significancia ecológica debido a su relevancia en el funcionamiento de los sistemas acuáticos (Slaninova et al., 2009; Pérez-Parada et al., 2018). Al tratarse de organismos vertebrados, son normalmente considerados como buenos representantes de organismos de mayor complejidad y por lo tanto los efectos tóxicos observados en ellos son más fáciles de comprender e interpretar (Ullah & Zorriehzahra, 2015).

En los últimos años, el uso de especies nativas en bioensayos de toxicidad se ha vuelto prominente en América del Sur, ya que permite evaluar la exposición y el efecto de los plaguicidas en los sistemas acuáticos, en función de las prácticas agrícolas y las condiciones ambientales regionales (Bertrand & Iturburu, 2023). Dentro de estas especies destaca el pez madrecita (*Cnesterodon decemmaculatus*), considerada un bioindicador acuático regional de calidad ambiental (Rautenberg et al., 2022).

Su amplia distribución y abundancia en Argentina, Brasil y Uruguay, su tolerancia a ambientes alterados, pequeño tamaño y facilidad de mantener en condiciones de laboratorio, la han convertido en un modelo ideal para evaluar el efecto de diversos tipos de herbicidas e insecticidas (Ossana et al., 2016; Bonifacio et al., 2017; Bernal-Rey et al., 2020; Bonifacio et al., 2020; Bertrand & Iturburu, 2023; Pautasso et al., 2023; Ruiz de Arcaute et al., 2023), siendo actualmente la especie predominante para la realización de bioensayos en la región (Bertrand & Iturburu, 2023).

Los ERE y los bioensayos de toxicidad son por tanto, herramientas complementarias en la evaluación de riesgo ecotoxicológico. Mientras que los primeros permiten una evaluación rápida y preliminar del riesgo potencial de los plaguicidas en ecosistemas acuáticos, los segundos proporcionan una validación y comprensión detallada de sus efectos biológicos en los organismos. En este sentido, para una evaluación más completa y precisa del riesgo ecotoxicológico, la aplicación de ambos métodos resulta fundamental.

1.4.3 Biomarcadores y estrés oxidativo en peces

Los bioensayos que incorporan biomarcadores de estrés oxidativo proporcionan una evaluación más completa del impacto de los contaminantes en los organismos. Muchos plaguicidas pueden inducir alteraciones por diversos mecanismos bioquímicos, afectando diferentes niveles de organización biológica, como molecular, celular, histológica, poblacional, comunitaria y ecosistémica (van der Oost et al. 2003; Sharma & Verma, 2019; Gonçalves et al., 2021; Calisi, 2023). La magnitud y el tipo de anomalías ocasionadas en los organismos expuestos a plaguicidas dependen de diversos factores, desde ambientales (dosis y duración de la exposición, tipo de estresor, temperatura, entre otros) hasta bióticos (especie, edad, dieta, estado fisiológico) (Slaninova et al., 2009). Estas alteraciones se reflejan y pueden ser estudiadas a través de diferentes biomarcadores, a partir de los cuales es posible cuantificar el grado de estrés, en función de la magnitud de los cambios en los marcadores seleccionados (Slaninova et al., 2009; Bonifacio et al., 2020; Gonçalves et al., 2021).

Los biomarcadores pueden ser de distinto tipo (exposición, sensibilidad y efecto) y de distinto carácter (químicos, moleculares, bioquímicos, morfológicos, genéticos). Los biomarcadores de exposición indican que el contaminante presente en el medio ingresó en el organismo. Por su parte, los biomarcadores de susceptibilidad evidencian la vulnerabilidad de los individuos a los efectos de un contaminante, en función de su capacidad para metabolizarlo Sin embargo, son los biomarcadores de efecto los que permiten determinar el efecto que estos contaminantes producen en el organismo, al proporcionar información sobre su grado de alteración (Slaninova et al., 2009).

Los biomarcadores pueden ser evaluados a distintos niveles de organización biológica, debido a que los efectos de un contaminante en niveles de organización superiores, siempre van precedidos por alteraciones en niveles anteriores (Figura 4). En este sentido, las primeras alteraciones observadas por la exposición a estos compuestos se producen a nivel molecular y los biomarcadores más estudiados a este nivel se corresponden con enzimas involucradas en la biotransformación y la detoxificación, como la catalasa (CAT), super-óxido dismutasa (SOD), glutatión-S-transferasas (GST´s) y glutatión peroxidasas (GPx) (van der Oost et al., 2003; Gonçalves et al., 2021).

El metabolismo celular está continuamente generando especies reactivas de oxígeno (ROS), también denominadas radicales libres, y los organismos responden a la presencia de ROS intensificando la actividad de estas enzimas (Rohani, 2023). Los plaguicidas pueden inducir estrés oxidativo, como resultado de un desequilibrio entre la producción de ROS y los mecanismos de defensa antioxidante de los peces, lo que puede causar daños o la muerte de las células o del organismo (Slaninova et al., 2009; Bonifacio et al., 2020; de Arcaute et al., 2023). Así, la evaluación de los niveles y actividades de estas enzimas, permiten conocer las respuestas adaptativas de los individuos ante la formación de ROS debida a la exposición de sustancias tóxicas para el organismo. Varios plaguicidas (y sus metabolitos) han demostrado ser promotores de la formación de ROS que contribuyen al daño oxidativo, entre ellos los organohalogenados, organofosforados, carbamatos, piretroides y triazinas (Slaninova et al., 2009).

Otras enzimas adecuadas para evaluar la respuesta a la contaminación por plaguicidas son las colinesterasas, utilizadas como biomarcadores de efectos neurotóxicos (Bernal-Rey et al., 2020; Gonçalves et al., 2022) y las transaminasas, utilizadas como referencia del daño hepático en peces (Bonifacio et al., 2020).

A medida que la exposición y el efecto continúan, diferentes biomarcadores pueden ser utilizados en los siguientes niveles de organización biológica (Figura 4). A nivel celular, la presencia y frecuencia de micronúcleos (MN) es comúnmente empleada para determinar el daño genético, así como anormalidades nucleares (NA) (Ossana et al., 2016; Pautasso et al., 2023; Rohani, 2023).

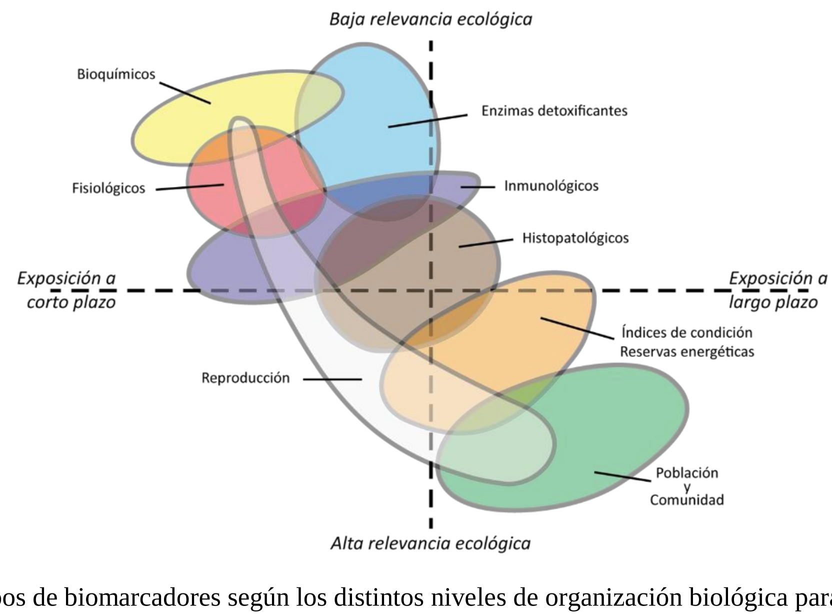

Figura 4. Tipos de biomarcadores según los distintos niveles de organización biológica para la evaluación de las respuestas de un sistema biológico a un xenobiótico, en función del tiempo de exposición y su relevancia ecológica en los sistemas biológicos. Modificado de Peakall & Shugart (2013).

Un siguiente nivel, son los marcadores histológicos y ultra estructurales donde generalmente se evalúan efectos subletales y crónicos de la exposición a xenobióticos, siendo las branquias y el hígado los más relevantes (Shah & Parveen 2022; Rohani, 2023). Otros marcadores utilizados, son aquellos de carácter morfológico, como el factor de condición e índices hepáticos (Bonifacio el al., 2017).

Finalmente, los parámetros de comportamiento proporcionan la ventaja de integrar las respuestas individuales y poblacionales a las respuestas bioquímicas. En particular, la actividad locomotora (velocidad, distancia recorrida, inmovilidad) representa una excelente herramienta para vincular los procesos fisiológicos y ecológicos de los organismos expuestos a un contaminante (Sharma et al., 2019).

En este sentido, el uso de múltiples biomarcadores a diferentes niveles de organización, brindan un análisis integral del efecto de los contaminantes en los organismos (van der Oost, 2003; Sharma et al., 2019; Calisi, 2023; Rohani, 2023).

1.5.Antecedentes en Uruguay

En las últimas décadas Uruguay ha experimentado un deterioro significativo en la calidad de sus cuerpos de agua. La presión antrópica, relacionada con la expansión agrícola y urbana; la forestación y las descargas de efluentes industriales y urbanos, ha provocado de forma cada vez más frecuente eventos de eutrofización (Alvareda et al., 2020; Goyenola et al., 2021; Dias Tadeu et al., 2023; Kruk et al., 2023; Teixeira de Mello et al., 2024) y contaminación por plaguicidas, tanto en aguas recreativas (Williman et al., 2017; Ridolfiet al., 2014; Nardo et al., 2015; Ernst et al., 2018; Griffero et al., 2019; Soutullo et al., 2020), como en fuentes de agua para suministro de agua potable (Ministerio de Ambiente 2021; Frontera et al., 2024).

Ante este escenario, existe una creciente preocupación por los efectos potencialmente adversos de los plaguicidas para la salud humana y los ecosistemas acuáticos, que han llevado a un impulso en la investigación nacional sobre esta problemática. Desde instituciones académicas y gubernamentales se vienen desarrollando metodologías analíticas de detección y planes de monitoreo ambiental que buscan evaluar la presencia de estos compuestos en ecosistemas acuáticos nacionales. Actualmente, la mayor cantidad de información generada sobre ocurrencia de plaguicidas en agua superficial es realizada a nivel gubernamental por el MA, cuyos planes de monitoreo se basan en la toma puntual de muestras de agua y con una frecuencia estacional o menor.

El fortalecimiento en la gestión de plaguicidas a nivel nacional presentó un hito en 2018, cuando se comenzó a ejecutar el proyecto GCP/URU/031/GFF. El mismo tenía como objetivo aportar a la construcción de capacidades para el monitoreo ambiental de plaguicidas, permitiendo ajustar y acordar interinstitucionalmente protocolos para mejorar las capacidades analíticas de plaguicidas a escala nacional. La metodología implementada en el mismo sirvió como insumo para el desarrollo de una parte de esta tesis.

Por otra parte, el análisis de la matriz peces es aún incipiente en el país, y las evaluaciones se han originado desde la academia, reportando una exposición subletal y continúa de las comunidades de peces a múltiples plaguicidas (Stabile, 2018; Ernst et al., 2018, Soutullo et al, 2020). Sin embargo, los posibles efectos de la exposición a plaguicidas en la biota acuática, en particular sobre los peces siguen sin estudiarse en nuestro país.

Tampoco se ha analizado la ocurrencia de plaguicidas en matriz agua y peces simultáneamente, mientras que existe un único antecedente de evaluación de riesgo ecotoxocológico por contaminación de contaminantes emergentes incluyendo algunos plaguicidas en función de sus concentraciones en agua (Griffero et al., 2019).

En Uruguay la reglamentación sobre plaguicidas data de 1977 (Decreto 149/977, https://www.impo.com.uy/bases/decretos/149-1977), lo que la convierte en una de las más antiguas de Latinoamérica (Carriquiriborde et al., 2014). La constante innovación en los plaguicidas comercializados, junto con un marco normativo laxo ha llevado a que se aprueben muchas sustancias que están prohibidas en países con legislaciones más rigurosas (Pesticide Action Network, 2022). Esto disminuye sustancialmente la eficacia de las actuales medidas de protección para el medio ambiente y la salud humana. En este sentido, la actual normativa sobre registro y autorización de plaguicidas (Decreto N° 317/007, https://www.impo.com.uy/bases/decretos/317-2007) contempla la caracterización del riesgo de estos compuestos únicamente durante el proceso de registro y renovación (luego de cuatro años), y se basa en información sobre las propiedades fisicoquímicas de las sustancias, y en sus potenciales efectos ecotoxicológicos y en la salud humana, con información aportada por las mismas empresas que buscan registrar o renovar los productos (Oficina de Programación y Política Agropecuaria, 2022). El rol gubernamental en este proceso ha consistido en confirmar las informaciones técnicas presentadas (Correa, 2020). En Uruguay, solo recientemente la DGSA está realizando ERE, pero únicamente para polinizadores, durante las etapas de registro de nuevos productos y renovaciones, aplicando el modelo BeeRex (basado en RQ) de la EPA (Oficina de Programación y Política Agropecuaria, 2022).

En consecuencia, aún existe un gran desconocimiento sobre el destino y los riesgos asociados con la exposición a plaguicidas en los sistemas acuáticos nacionales. En este sentido, la aplicación de herramientas de campo y experimentales, que contemplen múltiples matrices y diferentes metodologías de evaluación de impacto ecológico, representarían una herramienta fundamental para obtener una evaluación más completa de los efectos ambientales debido al uso de plaguicidas en estos ecosistemas. La Figura 5 ejemplifica el abordaje seguido en esta tesis, donde se ha buscado integrar los abordajes de campo y experimental.

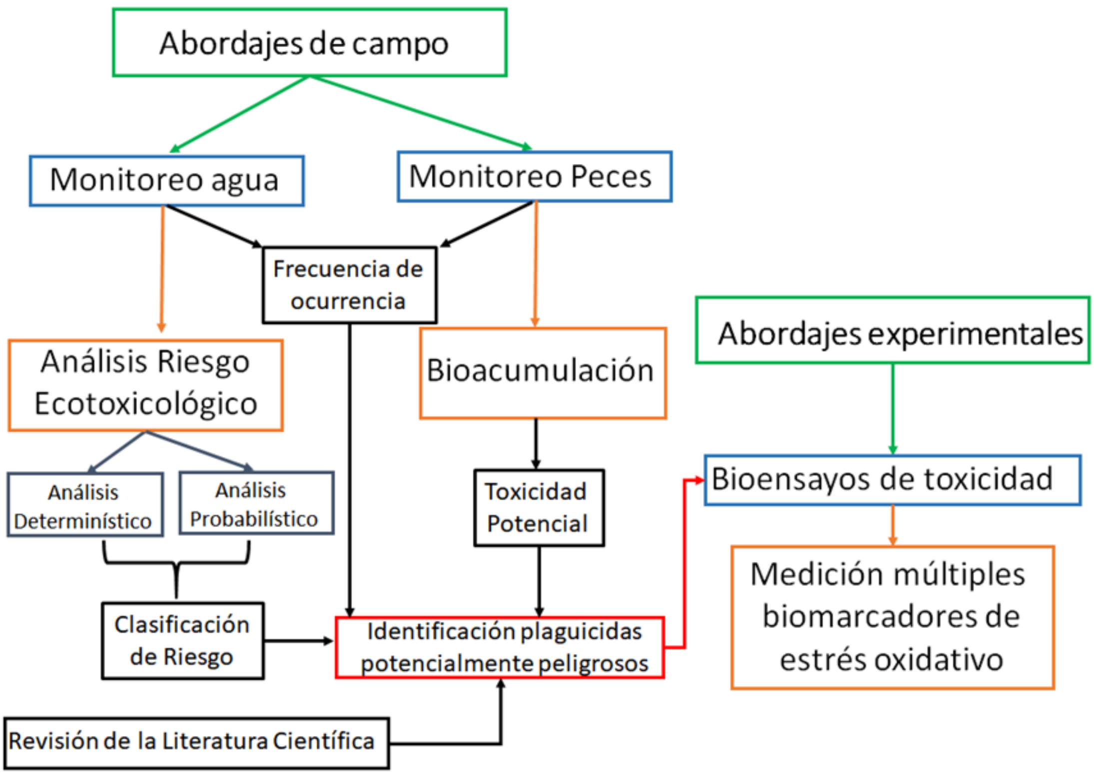

Figura 5. Abordajes de campo y experimentales para evaluar la dinámica e impactos ecológicos de los plaguicidas en los sistemas acuáticos. El uso complementario de ambos enfoques permite una evaluación más robusta de la calidad del agua, permitiendo llevar adelante una gestión más eficaz de los recursos hídricos.

Dentro de los abordajes de campo, el muestreo de alta frecuencia en agua superficial y en biota (biomonitoreo) permitiría identificar variaciones temporales de contaminación y patrones de bioacumulación en el sistema. Asimismo, la integración de esta información con datos de toxicidad posibilita la estimación del riesgo potencial que presentan, mediante la realización de análisis de riesgo ecotoxicológico. Por su parte, las aproximaciones experimentales complementan estos datos de campo mediante la evaluación de los efectos tóxicos directos en los organismos.

La información obtenida en esta tesis pretende impulsar planes de monitoreo efectivos, desarrollar estrategias de mitigación y ayudar a la formulación de normativas eficaces.

2. OBJETIVOS

2.1. Objetivo General:

Monitorear la presencia y destino de distintos plaguicidas en agua superficial y peces en una laguna utilizada para el suministro de agua potable; analizar la existencia de bioacumulación en peces y determinar el posible impacto ambiental de estos contaminantes en el sistema mediante evaluaciones de riesgo ecotoxicológico y bioensayos de exposición en la especie nativa *Cnesterodon decemmaculatus*

2.2 Objetivos específicos:

1. Analizar la distribución temporal de múltiples plaguicidas de uso actual y “heredados” en agua superficial y peces, y evaluar los factores involucrados en su dinámica ambiental.

2. Implementar una Evaluación de Riesgo Ecológico (ERE) mediante aproximaciones determinísticas (RQ) y probabilísticas (PRA), en función a las concentraciones ambientales encontradas en agua superficial.

3. Evaluar experimentalmente los efectos de un plaguicida de riesgo ecotoxicológico alto mediante la estimación de biomarcadores de efecto a diferentes niveles de organización biológica en una especie de pez nativo.

Organización de la tesis:

Los objetivos específicos se desarrollan a lo largo de los siguientes capítulos incluyendo diferentes enfoques metodológicos, tanto de campo como experimentales en tres capítulos. En el Capítulo 1 se evalúa la dinámica temporal y espacial de 88 plaguicidas en agua superficial y 38 en músculo del pez detritívoro sabalito (*Cyphocharax voga*), la especie más representativa del sistema LC. De esta manera se logra establecer la influencia de las propiedades fisicoquímicas de los plaguicidas encontrados, del uso del suelo (pasado y actual) y de las características biológicas de esta especie, en la ocurrencia mensual en agua y en los procesos de acumulación. Los resultados asociados a este capítulo se encuentran publicados en Science of the Total Environment (https://doi.org/10.1016/j.scitotenv.2023.162310).

En el Capítulo 2 se evalúa el riesgo ecológico para la biota acuática mediante la aplicación de ERE aplicando metodologías determinísticas y probabilísticas, para 28 plaguicidas encontrados en agua superficial. Esto permite identificar la temporalidad del riesgo a lo largo de un ciclo anual y los compuestos con mayor riesgo ecológico. Además, permite analizar las ventajas y desventajas de ambas metodologías en la evaluación de ERE como herramienta para la implementación de un marco regulatorio nacional en los ecosistemas acuáticos en Uruguay. Los resultados asociados a este capítulo se encuentran publicados en Science of the Total Environment (https://doi.org/10.1016/j.scitotenv.2023.168704).

En el capítulo 3 se realizan bioensayos de toxicidad en madrecita (Cnesterodon decemmaculatus), para evaluar los efectos subletales del Chlorantraniliprole (CHL) siendo este el compuesto más frecuente y de alto riego ecológico en el sistema. La estimación de diferentes biomarcadores enzimáticos: acetilcolinesterasa (AChE); Catalasa (CAT); Glutatión S-transferasa (GST) y transaminasas; morfológicos (índices corporales) y de comportamiento, permite establecer las posibles consecuencias de compuesto a nivel individual y ecológico. Los resultados asociados a este capítulo se encuentran actualmente en revisión en la revista Environmental Toxicology and Chemistry y se presenta en formato de manuscrito.

Finalmente, se presenta una síntesis de las contribuciones más importantes de la tesis, con una discusión general integradora de todos ellos y perspectivas importantes para el desarrollo de futuras investigaciones en la temática.

Capítulo 1:

Monitoreo multicompartimental de plaguicidas heredados y de uso actual en una laguna subtropical utilizada como fuente de agua potable (Laguna del Cisne, Uruguay)

El contenido de este capítulo se encuentra publicado en la revista Science of the Total Environment y se presenta en el formato original del artículo:

Rodríguez-Bolaña, C., Pérez-Parada, A., Tesitore, G., Goyenola, G., Kröger, A., Pacheco, M., Gérez, N., Berton, A., Zinola, G., Gil, G., Mangarelli, A., Pequeño, F., Besil, N., Niell, S., Heinzen, H., & Teixeira de Mello, F. (2023). Multicompartmental monitoring of legacy and currently used pesticides in a subtropical lake used as a drinking water source (Laguna del Cisne, Uruguay). *Science of the Total Environment*, 874, 162310. DOI: https://doi.org/10.1016/j.scitotenv.2023.162310

Science of the Total Environment 874 (2023) 162310

Contents lists available at ScienceDirect

Science of the Total Environment

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

Multicompartmental monitoring of legacy and currently used pesticides in a subtropical lake used as a drinking water source (Laguna del Cisne, Uruguay)

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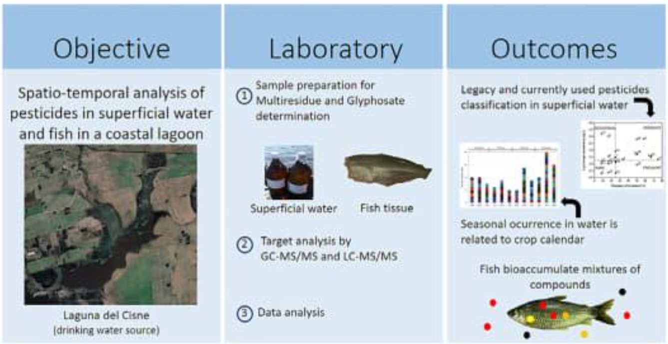
HIGHLIGHTS

- Levels of legacy and current use pesticides in superficial water and fish were determined.
- Seasonal and temporal variations were related to crop and livestock calendars.
- Organochlorine pesticides still represent a major concern to the environment.
- Multiple compounds were found co-occurring in fish muscle tissue.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Yolanda Picó

Keywords:
Rainfed agriculture
Pesticide residues
Multicompartmental monitoring
Freshwater
Drinking-water source

ABSTRACT

A pilot annual monitoring survey (April 2018–March 2019) was conducted to investigate the presence of pesticides in superficial water and fish in Laguna del Cisne, one of the most critical drinking water sources in Uruguay. A total of 25 pesticide residues were detected in superficial water (89.3 % of the samples). Pesticide's temporal distribution was associated with crops and livestock practices, with higher occurrences in spring and summer than in autumn and winter. The most frequent compounds in superficial water were the insecticide chlorantraniliprole, and the herbicides glyphosate (including its metabolite AMPA) and metolachlor. The levels of Organochlorine pesticide, p,p′-DDT, was in some cases two order of magnitude above the international water quality guidelines for Ambient Water Criteria. In fishes, eight different pesticides were detected, at concentrations from 1000 to 453,000 $ng \cdot kg^{-1}$. The most frequent pesticides found were propiconazole, chlorpyrifos, and p,p′-DDE. The widespread occurrence of pesticides in fish suggests potential exposure effects on fish populations and the aquatic ecosystem. The sampling approach of this work allowed monitoring the continuous concentrations of several pesticides in surface waters and fishes to establish the influence from past and current agriculture practices in Laguna del Cisne basin. For safety measures, continuous monitoring programs must be performed in this system to prevent toxicity impacts on aquatic organisms and human health.

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http://dx.doi.org/10.1016/j.scitotenv.2023.162310
Received 9 September 2022; Received in revised form 13 January 2023; Accepted 10 February 2023
Available online 23 February 2023

1. Introduction

Modern agriculture is highly dependent on pesticides, primarily to protect crops and enhance yields (Sharma et al., 2019; Sjerps et al., 2019; Rizzo et al., 2021; Syafrudin et al., 2021). Despite their recognized importance in the control of pests and diseases, their use has been implicated in the loss of biodiversity and impairment of ecosystem functions, including significant impacts on the aquatic ecosystem (Pérez-Parada et al., 2018; Sharma et al., 2019; de Souza et al., 2020).

Currently-used pesticides (CUPs) and those that have been discontinued or banned for agricultural use, usually so-called legacy pesticides (LPs), may persist and contaminate surface waters and accumulate in biota (Pérez-Parada et al., 2018; Bueno and Cunha, 2020; Kumar et al., 2021). Surface runoff after rain events is a widely accepted pathway for the mobility of most pesticides from agricultural soils and landfills to aquatic systems (Carazo-Rojas et al., 2018; Triegel and Guo, 2018; Andrade et al., 2021; Syafrudin et al., 2021). Lixiviation and atmospheric deposition also represent additional inputs, mainly to LPs (X. Huang et al., 2020; H. Huang et al., 2020; Jin et al., 2021; Riaz et al., 2021; Montagner et al., 2022).

The fate and behavior of pesticides in the aquatic systems are highly related to their physicochemical properties, such as aqueous solubility (S_W), octanol-water partition coefficient (K_{OW}), vapor pressure (Pv), Henry's law constant (HC), organic carbon constant (K_{OC}) and dissociation constant (Ka) (Shen and Wania, 2005; Triegel and Guo, 2018; Pérez-Parada et al., 2018). These characteristics determine a preferential partitioning between environmental compartments, low soluble in water compounds, like many LPs and their metabolites can accumulate in fishes and sediments (Pérez-Parada et al., 2018; Gong et al., 2020; Kumari, 2020) representing high acute toxicity for aquatic biota (Sharma et al., 2019; Mazzoni et al., 2020). In contrast, CUPs are hypothesized to be safer than LPs, because they are less persistent and bioaccumulative (Pérez-Parada et al., 2018; de Souza et al., 2022). However, several authors have largely proved their ecological risk and impact on non-target organisms (Zeng et al., 2018; Iturburu et al., 2019; Chen et al., 2021; de Souza et al., 2022).

At the global scale, only a few studies have included the simultaneous monitoring of pesticides in different matrices including water and biota of a wide range of CUPs and LPs (Abrantes et al., 2010; Masiá et al., 2013; Belenguer et al., 2014; Masiá et al., 2015; Carazo-Rojas et al., 2018; Slaby et al., 2022; Montagner et al., 2022). However, most of these studies do not analyze the spatiotemporal distribution performing an annual sample.

In Uruguay, the intensification of agriculture mainly related to rainfed crops and afforestation has led to massive use of pesticides (Rizzo et al., 2021; Scarlato et al., 2022). >25 million kg of active ingredients of pesticides were imported in 2020 (DGSA. Direccion de Servicios Agricolas, 2021). These represent a potential risk to human and ecosystem health due to environmental implications of pesticides such as degradation and desertification of soils (Rizzo et al., 2021; García-Préchac et al., 2022), biodiversity decrease and loss of ecosystem functions (Ernst et al., 2018; Soutullo et al., 2020), water pollution, and pesticide residue accumulation in food products (Mañay et al., 2004).

The available data on pesticide residue levels in superficial water in Uruguay mainly refers to the Río Uruguay and Río de la Plata (Mañay et al., 2004, Barra et al., 2006; Williman et al., 2017; Ridolfi et al., 2014; Nardo et al., 2015; Lupi et al., 2019; Girones et al., 2020; Soutullo et al., 2020). In fishes, the analysis of pesticide residues is incipient, with very few antecedents (Ernst et al., 2018; Soutullo et al., 2020) while none of these studies analyzed both compartments at the same time.

Atlantic coastal lagoons in Uruguay are sites with international importance for conservation (Rodriguez et al., 2017; Rodríguez-Gallego et al., 2017), and recent studies reported the presence of pesticides in the Protected Area of Laguna de Rocha (Nardo et al., 2015; Griffero et al., 2019) and Laguna de Castillos (Griffero et al., 2019). In particular, Laguna del Cisne (LC) is the largest lagoon in the department of Canelones (Southern end of the Atlantic coastal lagoons) and provides a drinking water source for approximately 100.000 surrounding inhabitants. More than a decade of increasing crop area has been establishing an environmental and health risk for the system (Goyenola et al., 2017; Gazzano et al., 2021). Due to serious social and ecological impacts attributed to the intensive use of pesticides in its watershed (Gazzano et al., 2021), since 2016, the local government has established the implementation of precautionary measures aiming at an agroecological transition of the basin (Gonzalez-Fernández and Orcasberrro, 2018).

This study presents results of one year (2018–2019) of pesticide residue surveillance in water, and biota from Laguna del Cisne with the aim to: (i) Analyze the Spatio-temporal distribution of CUPs and LPs pesticides in superficial water, and fishes, and evaluate the possible relationship with the annual crop calendar; (ii) Understand the relationships between the temporal distribution of pesticides in biotic and abiotic environmental matrices. For this purpose, we analyzed the monthly distribution of pesticides in a sedimentivore fish toothless characins (*Cyphocharax voga*, Characiformes), the most representative fish species of the studied system.

2. Materials and methods

2.1. Study area

The Laguna del Cisne (34°45′S; 55°49′W) is the largest lentic system in the department of Canelones, Uruguay. Is a small shallow system (total area = 127 ha, and mean depth = 2.0 m). Receives contributions from a basin of 50 km^2, with the streams Piedra del Toro and Cañada del Cisne as main tributaries (37.3 and 28.9 % of the total basin area respectively, sensu Goyenola et al., 2011) (Fig. 1). The eastern zone of the lagoon includes the "El Estero" wetland, with covers 24.8 % of the catchment and drains the highest fruit and vegetable production in the area.

A drinking water facility is found on the lagoon's shore (see Fig. 1). Although neighboring areas range from intensive crop to natural pasture, agriculture is the dominant land use. More than a decade of increasing crop area has established an environmental and health risk for the system (Goyenola et al., 2011; Scarlato et al., 2022). The system has been classified as a eutrophic-dystrophic shallow lake with high phosphorus levels (frequently exceeding 500 μg-P.L^{-1}), but low phytoplanktonic productivity (Goyenola et al., 2017).

2.2. Sample collection

Water and fish samples were collected monthly at different sites from LC since April 2018 to March 2019 (Fig. 1). Fish were collected using eight Nordic multi-mesh gillnets (Appelberg, 2000) placed in 4 areas from 1 to 1.5 m water deep. To evaluate possible relationships between pesticides concentrations in fish and water, we selected *Cyphocharax voga*. Its high frequency (present in all sampling campaigns), size (max length = 26.3 cm, enough to obtain muscle samples), trophic role (link between benthic and pelagic food webs), and high fat content in muscle makes this species a suitable model as potential bioindicator (Sagrario and Ferrero, 2013; Barni et al., 2016).

Individuals of *C. voga* captured monthly were measured for standard length (SL ±1.0 mm), total weight (TW ± 0.1 g), gonad weight (GW ± 0.01 g), and liver weight (LW ± 0.01 g). In the laboratory, 50–100 g of dorso-lateral muscle tissue was dissected after removing the scales. Each sample was generated from 3 to 5 individuals and was composed with fish of similar sizes. Between three (in June 2018) and six samples (March 2019) were obtained per month, completing a total of 51 samples of *C. voga* including 117 individuals. Dissected specimens were stored in aluminum foil and freeze-dried at −18 °C until analysis (Colazzo et al., 2019).

Water samples were collected in glass bottles (1 L) previously rinsed with hexane and acetone. Samples were taken directly from the bottles below the surface and stored at 4 °C during a maximum of seven days before analysis. In each sampling campaign, seven samples were taken, completing 84 water samples in the entire period.

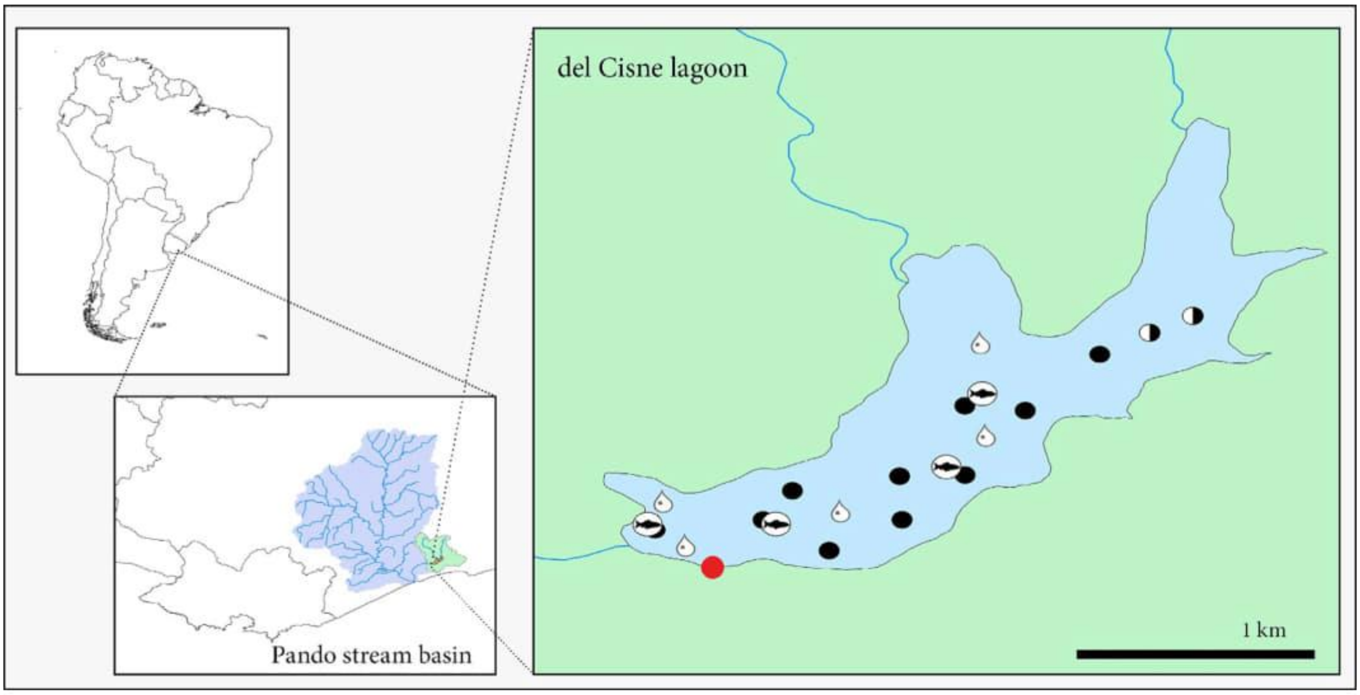

Fig. 1. Sampling sites in the Laguna del Cisne, basin, Uruguay. White drops: water sampling sites; Black/White dots: fishes samples sites; Red dot: drinking water facility. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.3. Chemicals and reagents

Analytical standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The purity of all the standards was >98 %. HPLC-grade acetonitrile (MeCN), dichloromethane (DCM), ethyl acetate (EtAc) and methanol (MeOH) were supplied by J.T. Baker (Darmstadt, Germany). Water used for LC-MS/MS analysis was obtained from a Direct-Q3 Ultrapure Water System from Millipore (Billerica, MA, USA). Formic acid (FA) NaCl and $Na_2B_4O_7{\cdot}10\ H_2O$ was from Sigma Aldrich (Steinheim, Germany).

Individual stock standard solutions of the target compounds were prepared in pure MeCN, MeOH, or EtAc and stored at −18 °C. Stock solutions were prepared from the standard substances at 2000 mg L^{-1} in a proper solvent. Working solutions were prepared by appropriate dilution of the stock solutions in MeCN for liquid chromatography (LC) and EtAc for gas chromatography (GC) amenable compounds. Bulk anhydrous $MgSO_4$ > 98 % purity, dispersive solid-phase extraction (d-SPE) grade C18 (octadecyl silica) and PSA (primary secondary amine) 40–60 μm were purchased from Scharlab (Barcelona, Spain). Triphenyl phosphate (TPP) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and used as the surrogate compound (SC). SC solutions were prepared in EtAc.

2.4. Apparatus

High-speed blender made of stainless steel Skymsen (SC, Brazil) was used to chop frozen fish. Automatic pipettes suitable for handling volumes of 1–10 μL, 100–1000 μL and 1–10 mL were from Socorex (Lausanne, Switzerland). An analytical scale capable of weighing 1 mg was from Shimadzu (Kyoto, Japan). A centrifuge Eppendorf (Hamburg, Germany) providing 3000g was used. Organic aliquots were evaporated using Biotage AB TurboVap® LV Workstation (Uppsala, Sweden).

2.5. Scope of pesticides

According to local records and field investigations, the pesticides were selected to represent the most relevant pesticides LPs (e.g. banned insecticides) and CUPs (DGSA. Direccion de Servicios Agricolas, 2018). The complete list of the chosen pesticides for each matrix and their physicochemical properties are presented in Supplementary Material (Table S1).

2.6. Glyphosate and AMPA analysis in water

Glyphosate and its metabolite aminophosphonic acid (AMPA) were determined in freshwater by the official laboratory at Ministry of Livestock, Agriculture and Fisheries of Uruguay (MGAP). This laboratory uses ISO 21458:2008 method in routine analysis. Briefly, the method includes 9-fluorenylmethoxycarbonyl chloride derivatization and determination of residues by liquid chromatography – fluorescence detection (LC-FLD). Achieved limits of detection (LOD) are 0.1 and 0.25 μg/L for Glyphosate and AMPA, respectively. Limits of quantitation (LOQs) are 0.2 and 0.5 μg/L for Glyphosate and AMPA.

2.7. Sample preparation for multiresidue determination of pesticides in water

Two different methodologies were used for the analysis of pesticide residues in water. The first one was based on a liquid-liquid extraction (LLE) with DCM based on EPA 508 method (EPA 508, 1995) with subsequent instrumental determination using GC–MS/MS (gas chromatography-tandem mass spectrometry).

Then, 0.5 L of raw water were placed in a glass decantation ball and extracted with 3 DCM portions of 200 mL. The organic phases are brought together and brought to almost dryness in mild stream of N_2 gas. Subsequently, they are taken up in 1 mL EtAc and analyzed by GC–MS/MS.

Additionally, direct sample introduction technique was used for LC-MS/MS (liquid chromatography – tandem mass spectrometry) monitoring of LC amenable pesticides (Pareja et al., 2011). The choice of this method was based on the analytical practicality of monitoring certain pesticides with higher solubility in water (Pareja et al., 2011). Here, 0.9 mL of water is added to 0.1 mL of acetonitrile and injected directly on the LC-MS/MS instrument (no concentration factor exists). The water samples are not filtered, so they include pesticides associated with organic particles. The quantification limits reported for this matrix are included in Table S2.

2.8. Sample preparation for multiresidue determination of pesticides in fish

Fish samples were analyzed accordingly to previous reports (Colazzo et al., 2019; Ernst et al., 2018; Pareja et al., 2021). Frozen fish homogenate (10 g) was weighed into a 50 mL polypropylene centrifuge tube. A 10 μL aliquot of TPP 10 μg mL^{-1} solution was added to each tube as SC and let it stand for 1 min. 10 mL of ACN has been added afterward. The tube was shaken vigorously by hand for 1 min. Then, 1.5 g of NaCl and 4.0 g of $MgSO_4$ were added and the resulting mixture was shaken vigorously by hand for 4 min. Each tube was centrifuged at 3500 rpm for 5 min. Clean-up was performed using d-SPE. A 7 mL aliquot of the organic layer was transferred into a 15 mL polypropylene tube containing 350 mg PSA, 180 mg C18, and 1000 mg $MgSO_4$. The tube was vortexed for 1 min and centrifuged at 3500 rpm for 5 min. A 1 mL aliquot of the extract was filtered through 0.45 um PVDF filter and transferred into a 2 mL screw cap auto sampler vial and directly injected in LC-MS/MS. On the other hand, a 4 mL aliquot of the extract was transferred into a conic glass test tube and driven to dryness under N_2 stream in the evaporation equipment. Finally, the extract was redissolved in 1 mL EtAc for GC–MS/MS analysis. The equivalent tissue concentration per sample extract was 1 g·mL^{-1}. The quantification limits reported for this matrix are included in Table S2.

2.9. LC-MS/MS

An AB Sciex™ API 4000 (Concord, Canada) Quadrupole-linear ion trap (QTrap®) was operated in triple quadrupole MS/MS mode coupled to Agilent 1200 LC system (Agilent Technologies, Palo Alto, USA). An Agilent Technologies Zorbax Eclipse XDB-C18 (150 mm × 4.6 mm, 5 μm) analytical column was used. Column oven temperature was set at 20 °C. The mobile phase consisted of (A): 0.1 % formic acid in water and (B) MeCN and the following elution program was used: It was run at 0.6 mL min^{-1} starting with 70 % component A at injection time and stable for 3 min, gradually changing to 0 % A (100 % B) over 22 min and stable for 5 min, then to 30 % A (70 % B) over 5 min. This eluent composition was kept for 5 min and kept there until 40 min after injection. Tandem MS detection was performed using the multiple reaction monitoring (MRM) mode. The optimal MRM conditions for each analyte were optimized using direct infusion in the ESI+ mode. Source temperature was 500 °C, the ionization voltage was 5000 V, curtain gas was nitrogen at 20 psi and the nebulizer gas was air at 50 psi. Scheduled multiple reaction monitoring was used with a setting of a 90 s detection window covering the expected retention time of each analyte and the target scan time was 2 s for all pesticides. Analyst 1.5 software from AB Sciex™ was used for instrument control and data processing. Optimized conditions and settings (selected transitions, collision energies, etc.) used in this study are listed in Table S3a.

2.10. GC–MS/MS

A Shimadzu TQ 8050 was used for analysis of GC amenable pesticides in all tested matrixes. The injection volume was 1 μL in splitless. Separation was conducted in RXi-5MS Sil capillary column (5 % diphenyl/ 95 % dimethyl polysiloxane, 30 m; 0.25 mm id; 0.25 μm film) from Restek (Bellefonte, PA, USA). The injector temperature was 280 °C. The carrier gas used was high purity Helium at a constant flow rate of 1 mL min^{-1}. The interface temperature was 300 °C and the ionization source temperature was230°C A detector voltage of 1.25 kV was used and Argon (200 kPa) was used as collision gas. In all experiments, the monitoring mode was operated in MRM, adjusting the CE voltages and using SmartPesticide Database (SPDB) and MRM Optimization Tool (Restek, Bellefonte, PA, USA). Optimized conditions and settings for the compounds used in this study are listed in Table S3b. GC–MS Solution version 4.11 SU2 with MS libraries was used for instrument control and data processing.

2.11. Analytical quality assurance and quality control (QA/QC)

Identification of the compounds was assessed through those requirements established at SANTE guidelines for different MS-based techniques (SANTE/11312/2021). LC-MS/MS and GC–MS/MS were operated under MRM mode. Identification was based on retention time matching (±0.1 min); two MRM transitions plus the ion ratios within ±30 % relative tolerance to those calibration standards from the same analytical sequence. The limits of quantification (LOQs) shown in Table S2were determined according to the criteria established at SANTE based on the lowest concentration with acceptable accuracy in recovery experiments. Quantification was done via matrix-matched calibration. LC-MS/MS and GC–MS/MS quantitation was performed through external calibration.

2.12. Information on agrochemicals

Information on logarithmic octanol-water partition coefficient (log Kow), soil degradation (DT_{50}, aerobic), and ecotoxicology for fish (Acute dose 96 h LC_{50} (mg L^{-1})) were obtained from website databases (IUPAC Footprint, 2017) (Table S1). Annual statistics of used agrochemicals were accessed via the websites of the Ministry of Agriculture (DGSA. Direccion de Servicios Agricolas, 2018).

2.13. Determination of the total lipid content

Lipid extraction was carried out using a modified Bligh & Dyer method (Ramalhosa et al., 2012; Ernst et al., 2018). Five grams of sample, 20 mL of MeOH, and 10 mL of DCM were added and vortexed for 5 min in a conical flask. The layers were separated by centrifugation for 5 min at 3000 rpm. The lower layer was transferred to a pear-shaped flask with a Pasteur pipette. Evaporation was done under a N_2 stream at 35 °C and the extraction was dried in an oven at 80 °C until constant weight.

The total lipid content was obtained through gravimetric control. All samples were analyzed twice to control the accuracy of the method. The results were expressed as a percentage of lipid weight over the total weight of the sample.

2.14. Land use

Laguna del Cisne includes agriculture, forest, animal breeding, wetlands, and urbanized zones as mainland uses (Gazzano et al., 2021). The principal crops are soybeans, corn, wheat, potato, and grapes. An increase in land under cultivation, including transgenic soybean, between 2001 and 2015, and its use as a source of drinking water determined the application of precautionary measures in 2016, aimed at an agro-ecological transition of the basin. It is currently the only aquatic system with such protection measures in Uruguay (Gazzano et al., 2021).

There is a significant lack of information on the productive activities carried out in the region, land cover of each type of crop, annual rate of changes in land use, farming methods, and percentage of smaller farms (Bálsamo, 2018; Gonzalez-Fernández and Orcasberrro, 2018).

Land-use activities are resulted from double cropping and crop–pasture rotations with different management techniques (Table 1). Soybean represents the largest summer crop under the continuous annual cropping under no-till (and maize to a lesser extent) (Cespedes-Payret et al., 2009; Rizzo et al., 2021). The sowing of soybean (*Glicine* max) and maize (*Zea mays*) occurred during September–December. Harvest is performed during April–May, and a winter crop of common wheat (*Triticum aestivum*) is sown in later May–August and harvested before the next summer crop (Table 1). Crop-pasture rotations are also observed in the area during autumn, when grassland is sown after summer crops harvest with two productions cycles (autumn and spring), potatoes appears as another important crop in the area. Autumn sowing generally takes place between December and later March, while spring sowing extends from July to December.

Long-term crops include exotic forest plantations (mainly *Eucalyptus* spp.), grapes, and a smaller scale citrus, apple, and pear. In this case, management strategies to ensure higher crop productivity involve continuous weed, insect, and fungus control (Cespedes-Payret et al., 2009; Scarlato et al., 2022). Sometimes, farmers use pesticides preventively, which can

Table 1
Sowing calendar of main Crops cultivated in Laguna del Cisne. Blue: planting season; Green: Growing period; Orange: harvest period.

	Autumn			Winter			Spring			Summer		
Land use	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Soybeans	Harvest						Planting		Growing period			
Maize	Harvest					Planting			Growing period			Harvest
Wheat			Planting			Growing period		Harvest				
Grassland	Planting		Growing period					Harvest (Forage)				Planting
Potato	Growing period		Harvest		Planting		Growing period		Harvest		Planting	

lead to inefficiencies and overuse (Gazzano et al., 2021; Scarlato et al., 2022).

Finally, there is a large vegetable cultivation under greenhouse conditions during winter. These cultivation includes: tomato, onion, pepper, carrot, strawberry. All of the above mentioned gives the region of Laguna del Cisne an important role in supplies the domestic market of different vegetable species (Gonzalez-Fernández and Orcasberrro, 2018).

2.15. Data analysis

We estimated the frequency of occurrence (%FO) for each pesticide as the number of times it was registered over the total number in each compartment. An Olmstead-Tukey diagram (Fisher, 1983) was constructed to classify pesticide residues in superficial water of average concentration per compound and frequency of occurrence (FO%). Substances found above the median values of average concentration and FO% were classified as "dominant"; those with an average concentration above the median but FO% lower than the median value as "occasional". Substances with high FO % but low average concentration were considered "frequent", and substances with low average concentration and low FO% were "rare" (Ernst et al., 2018).

For results below the LOQ, we assigned a value of zero. Shapiro-Wilks test was applied to evaluate normality while Levene's test was used to test homogeneity of variance. Spearman's correlation analysis was used for examining pesticide concentration in surface water between seasons, and differences were evaluated by one-way (ANOVA) with Dunnett's T3 post hoc test.

To determine the similarity in the presence and absence of pesticides in surface water between the lagoon sites according to their spatial proximity, a cluster analysis (multivariate classification analysis) was performed using the UPGMA algorithm and Jaccard's similarity index (Jaccard, 1908).

For fishes, the results of pesticides residues concentration in muscle tissues were expressed as ng·kg-1. A Kruskal Wallis test was performed to examine differences between physicochemical characteristics of each pesticide (log Kow and water solubility), and their occurrence in fishes and superficial water matrix.

For each individual of *C. voga*, three indices were calculated using the following formulas:

$$\text{Hepatosomatic index (HSI)} : (\text{HW}/\text{TW}) * 100;$$

$$\text{Gonadosomatic index (GSI)} : (\text{GW}/\text{TW}) * 100;$$

$$\text{Condition Factor (K)} : \left(\text{TW}/\text{SL}^{\text{b}}\right) * 100;$$

TW = total weight (g), EW = eviscerated weight (g), HW = liver weight (g), GW = gonad weight (g), SL = standard length (cm) and b as parameters from the weight–length relationship.

To analyze the possible relationships between pesticide concentration and biological variables we carried out Generalized Linear Models (GLM) from the negative binomial family. In this case we used pesticide concentrations as dependent variable and body weight, body length, K, HIS, GSI, and lipid content as independent variables. All variables were standardized previously in order to evaluate the size effect of each one over pesticide concentration. Model selection was carried out using a likelihood ratio test (LRT) seeking to obtain the simplest model possible. Statistical analyses were performed using the statistical software R (R Core Team, 2018).

To identify relationships among co-occurrence of pesticides in fishes and superficial water, we conducted a redundancy analysis (RDA; Legendre et al., 2011). For the response and explanatory variables, we Ln $(ax+1)$ transformed when 0 values were present (Jaeger, 2008).

3. Results and discussion

In this study, various pesticide residues were identified occurring in water and fish. The results obtained during the study are summarized in Table 2 and Table 4 for those compounds detected in water and *C. voga*, respectively. The scope of analysis for each matrix and their corresponding LOQ is found at Table S2. Several compounds were not analyzed simultaneously in all the matrices due to their particular scope selected in validation studies.

3.1. Pesticides occurrence in water

Our monitoring program detected 25 pesticides in surface waters (6 fungicides, 8 herbicides, and 11 insecticides), with a high FO(%) (89.3 % of the samples showed at least one compound). These findings would indicate that the 20 m of exclusion in the use of pesticides in the streams and 100 m of exclusion on the lagoon margin, would be an insufficient management measures, due to the high mobility of these compounds (Pérez-Parada et al., 2018; Bueno and Cunha, 2020). In these sense, the riparian zone is essential for mitigating pesticide run-off and depends on the characteristics of vegetation, soils, and hydrology (Triegel and Guo, 2018; Cole et al., 2020; Mary-Lauyé et al., 2023). In LC, the vegetation cover in riparian zones consists of natural grassland subject to grazing, which can cause soil erosion and changes in the types of vegetation (Cole et al., 2020). The scarcity of forests in buffer zones is common in Uruguay, due to the roles of multiple factors, including climate and livestock presence (Mary-Lauyé et al., 2023). In addition, the hydrological conditions of LC are highly dependent on precipitation events, due to the low altitudinal variations of the basin, during periods of intense rainfall, the total floodable area can reach 190 ha and cover all the riparian zone (Goyenola et al., 2011). This might cause an increase in the mobilization and transport of pesticides to the system during flooding events. For these reasons, an increase in the exclusion zone in streams and the lagoon would be necessary to reduce pesticide input to surface waters. There are several national environmental monitoring programs (e.g. US EPA, Canadian Council of Ministers of Environment, European Environment Agency) that analyze the occurrence of pesticides in aquatic systems (basically in surface water) over the long term. Many of these programs focus on organochlorine and organophosphorus compounds because of their environmental concerns. In Uruguay, the National Monitoring Program is carried out by the Uruguayan Ministry of Environment in several water bodies (DINAMA, 2020). Our results showed that 15 pesticides have the maximum priority level (3) and ten have level 2 (significant importance) (DINAMA, 2020). For currently used

Table 2
Average concentration (Meanc) (ng·L^{-1}) of pesticides present in water in the 7 site samples in Laguna del Cisne. Classification of pesticide residues in Occasional, Rare, Frequent and Dominant based on average concentration and frequency of occurrence using the whole samples following the Olmsted-Tukey diagram (Fig. 2). Superindex 1 Compound analyzed as from June 2018; 2 Compound analyzed as from August 2018.

N	Pesticide	C	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR
1	2.4-D	O	590	–	–	1500	–	–	–	–	–	–	–	–
2	Acetochlor[2]	R	–	–	–	–	–	–	–	15	10	16	–	–
3	alpha-BHC[2]	R	–	–	–	–	–	–	–	–	–	–	2	–
4	AMPA	D	847	713	890	580	750	810	600	NA	NA	NA	NA	NA
5	Atrazine	D	–	–	–	–	–	–	–	47	50	49	52	41
6	Azoxystrobin	F	18	–	–	–	–	–	–	31	–	31	5	–
7	Bifenthrin	O	–	–	–	–	78	–	–	8	–	9	–	–
8	Chlorantraniliprole	D	32	8	–	10	10	10	–	63	–	60	56	–
9	Chlorpyrifos	F	–	8	8	–	–	–	–	9	–	9	15	4
10	Cypermethrin	O	–	50	–	–	–	–	–	–	20	–	–	–
11	Cyproconazole[1]	R	–	–	–	–	–	–	–	36	–	–	5	7
12	Diazinon	R	–	–	–	–	–	–	–	–	–	–	1	3
13	Ethion	O	–	–	–	–	–	–	–	–	10	–	130	109
14	Glyphosate	D	620	360	–	850	–	228	1045	NA	NA	NA	NA	NA
15	Metalaxyl[1]	R	–	–	–	–	–	–	–	21	–	–	17	9
16	Metribuzin[1]	R	–	–	–	–	–	–	8	–	–	–	14	–
17	p,p′-DDD	D	15	–	15	25	–	–	–	43	9	60	6	2
18	p,p′-DDE	R	32	–	–	–	–	–	–	–	–	–	2	4
19	p,p′-DDT	D	60	21	–	–	–	–	–	34	230	–	9	14
20	Permethrin	R	–	21	–	–	12	–	–	–	–	–	–	–
21	Pyraclostrobin[1]	R	–	–	–	–	–	–	–	–	–	–	–	17
22	Simazine[1]	R	–	–	–	–	–	–	–	–	–	–	22	0
23	S-Metolachlor	D	–	–	–	–	145	20	10	52	9	61	18	5
24	Tebuconazole	R	–	–	–	–	–	–	–	–	–	–	2	3
25	Thiabendazole	O	–	–	–	–	1580	–	–	–	–	–	–	–

pesticides, all the most frequent compounds in LC, are included in level 3 of priority to monitoring (Table S1).

Six of the 25 identified pesticides (including DDT breakdown products) are banned compounds according to the Uruguayan legislation: (p,p′-DDT, p,p′-DDD, p,p′-DDE, atrazine, alpha-BHC and ethion). Of the remaining 19 pesticides, seven are products not approved for use in the European Union (EU): acetochlor, bifenthrin, chlorpyrifos, diazinon, metalaxyl, permethrin, and simazine (Pesticide Action Network, 2022). This represents a major challenge because these compounds have been banned for its adverse effects on the ecosystem and human health.

The total concentrations of detectable pesticides range from 1.4 (diazinon) to 1580 ng·L^{-1} (thiabendazole) (Table 2). The average concentrations for each pesticide per sample were basically below 100 ng·L^{-1} however a few compounds showed much higher values, such as the herbicides 2.4-D (1500 ng·L^{-1}and 590 ng·L^{-1} in July and April respectively), glyphosate (227,5 ng·L^{-1} to 1045 ng·L^{-1} in September and October), and AMPA (580 ng·L^{-1} to 890 ng·L^{-1} in July and June), and S-metolachlor (145 ng·L^{-1} in August). The fungicide thiabendazole (1500 L^{-1} in August) and insecticides were also detected, such as ethion (130 ng·L^{-1} in February).

The extreme values of thiabendazole (fungicide used on wheat, potato, and citrus crops) and 2.4-D (herbicide used in the region in the same crops that thiabendazole and in grassland), could be related to inefficient use or overdosing in the application of these products. This would not be the first record of irregular pesticides practices in the basin (Gonzalez-Fernández and Orcasberrro, 2018). The occurrence of 2.-4 D is easily explained due to the high mobility in soils of the ionized form of the acid (IUPAC Footprint, 2017).

The concentrations in water of p,p′-DDT (ranged between 9 ng·L^{-1} to 210 ng·L^{-1}), was in some cases two order of magnitude above the USEPA guideline for Ambient Water Criteria for this compound (1 ng·L^{-1}) to protect freshwater aquatic life (US EPA, 2018). Also p,p′-DDD and p,p′-DDE presented concentrations above these guidelines. Based on the Risk Quotients Assessment approach, the concentrations of DDT and DDE was much higher than those indicated as high risk for the aquatic environment by Zeng et al. (2018), showing that LC is severely contaminated by these compounds.

Like most OCs, DDT was banned for many countries, including Uruguay in the 1970s (Boroukhovitch, 1998), and globally by the Stockholm Convention in 2001 (Lallas, 2001). However, DDT residues are still regularly detected in aquatic systems worldwide (Ricking and Schwarzbauer, 2012; Sharma et al., 2019; Vasseghian et al., 2021; Montagner et al., 2022; de Souza et al., 2020). After decades of banning, the high concentration of DDT and its degradation products may be determined by a long-term application that can cause accumulation in soils. Their occurrence can be due to the physicochemical properties of DDT, such as its high persistence, and high Koc that can be transported adsorbed to suspended sediment from the catchment (Boul, 1995; Ricking and Schwarzbauer, 2012; Kurek et al., 2019). In this sense, the LC catchment lies on clay and silt soils, easily transportable and relatively impermeable (Crosa et al., 1990). This would suggest that surface runoff would play an important role in the transport of suspended sediment (Kurek et al., 2019; Gong et al., 2020). For p,p′-DDT relatively rapid sorption to clays has been reported, increasing the capacity for transport to superficial waters (Boul, 1995). Although highly soluble pesticides tend to be more transportable in runoff water, Andrade et al. (2021) established that high-persistence compound (like DDT) is longer available for the runoff process. Also, in LC the northern zone, where the tributaries drain, receives a large amount of organic matter (Crosa et al., 1990), which would increase DDT adsorption (Boul, 1995; Kurek et al., 2019). This result suggests the need to monitoring soil pollution in the agricultural zones of LC, and analyzes the influence of the rainfall events on pesticide concentrations in superficial water.

For all others pesticides, the concentration levels found were below the limits established for the protection of aquatic life (Canadian Council of Ministers of the Environment, 2011; European Environment Agency (EEA), 2016; USEPA. US Environmental Protection Agency, 2018), and those established for drinking water in Uruguay (OSE, 2012).

The dominant (>1.30 ng·L^{-1}) detected CUPs include chlorantraniliprole (number 8 in Fig. 2), S-metolachlor (23), glyphosate (14), and its metabolite AMPA (4) (Fig. 2). These results are in concordance with the extensive use of these compounds in Uruguay (DGSA, 2020) and in the region (Souza et al., 2022). Based on Ecological Risk Assessment carry out by Iturburu et al. (2019) and Pérez et al. (2021) in Pampas Region, low ecotoxicological risk is expected at the concentration detected in this study for glyphosate and AMPA. The same would be expected for metolachlor (Ccanccapa et al., 2016) and chlorantraniliprole (Song et al., 2019).

Chlorantraniliprole was the pesticide with the major FO (%) in superficial water, being the first report of this compound in aquatic systems in

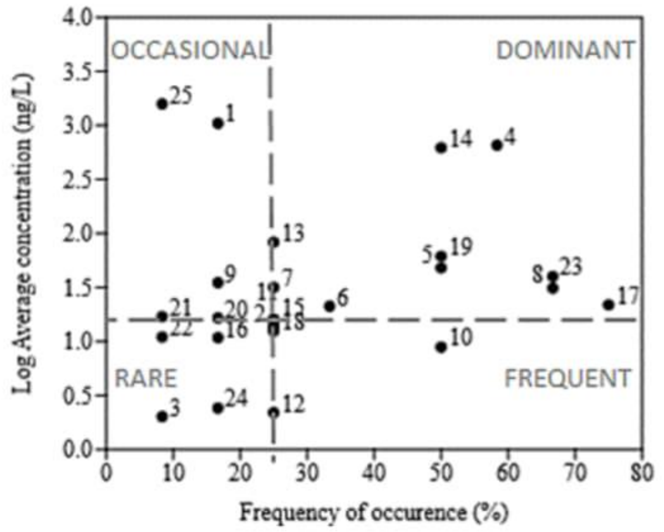

Fig. 2. Olmstead-Tukey diagram for the pesticides found at sampling sites. FO (%) vs the average concentration of the quantified pesticides (ng·L-1) for the whole data set. Dashed lines represent the median value of FO (%) and concentration and delimited four quadrants to classify substances into Dominant, Frequent, Rare and Occasional classes. For numbers that represent pesticides See Table 1.

Uruguay. This compound is a novel insecticide with low toxicity to mammals and beneficial arthropods (Boukouvala and Kavallieratos, 2021). However, it is highly toxic to aquatic invertebrates (Boukouvala and Kavallieratos, 2021; Nakanishi et al., 2021) and moderately toxic to fishes (Stinson et al., 2022), and aquatic plants (Abas et al., 2022). Also, their physicochemical properties like low aqueous solubility, low volatility, and highly persistent in soils (Bakker et al., 2020; Boukouvala and Kavallieratos, 2021) could determine a terrestrial persistence that can drift after rain events, reaching the lagoon by runoff and percolation (Pérez-Parada et al., 2018; Song et al., 2019). This new-generation diamide insecticide is now the most used pesticide globally (Bakker et al., 2020), and presents a common occurrence in aquatic ecosystems (Sandstrom et al., 2022; Stinson et al., 2022). The high presence observed in LC throughout the year would be related to farmers' intensive use. In Uruguay, was registered in 2011 to control of many Lepidoptera, Coleoptera, Diptera, Hemiptera, and Isoptera (SATA, 2011). Since its introduction in the market, its use has increased over the years being by 2018 the 14 % of total insecticides volume (L) imported in Uruguay (DGSA. Direccion de Servicios Agricolas, 2018), while in 2022, it was 29 % (DGSA, 2020). Initially, chlorantraniliprole was introduced as a replacement for pyrethroids in soybean and maize crops but is used in many summer and winter crops.

The dominant herbicide glyphosate is the most common pesticide applied in Uruguay, and in the region (Iturburu et al., 2019; Souza et al., 2022). In Uruguay represent 57,3 % of the total herbicides imported (DGSA, 2020), and they are part of the technological package associated with summer crops, especially soybeans (Table 1) (Maggi et al., 2020; Rizzo et al., 2021). In Uruguay, the detection of glyphosate appears associated with agricultural activities (Cespedes-Payret et al., 2009; Mañay et al., 2004; DINAMA, 2020; Cracco et al., 2022) even in protected areas (Nardo et al., 2015; Soutullo et al., 2020).

Metolachlor is the second most used herbicide in the country (Direccion de Servicios Agricolas, 2021). Is widely used in soybean, corn, and potato, to control weeds and clear vegetation cover before sowing (Rizzo et al., 2021; Zhang et al., 2022). This would explain its presence in the system from spring until the end of the study (summer crops) (Fig. 3). This compound is highly soluble in water but degrades slowly, with a half-life (DT50) from 15 to 182 days in soil and degradation metabolites with a high persistence rate (Rizzo et al., 2021). For this reason, metolachlor is the second most common herbicide detected in water worldwide (de Souza et al., 2020), as is the most frequently found compound in water samples collected throughout the US (Rose et al., 2018). The compound was also detected in high FO (%) in two Atlantic coastal lagoons from Uruguay (Griffero et al., 2019). A study carried out in different localities associated with rainfed agriculture enclosed to a RAMSAR sites showed that -metolachlor presents the highest FO (%) in fish muscle tissue (Ernst et al., 2018).

For LPs p-p′-DDT (number 17 in Table 1) (and their metabolites p,p′-DDD) and the recently banned atrazine (5) are the dominant pesticides in the system Atrazine is the most often detected herbicide in surface waters worldwide (de Souza et al., 2020) and at the regional level (Iturburu et al., 2019; Pérez et al., 2021). In Uruguay was banned in 2016, however, due to the lack of a substitute, and the large stocks declared by the importing companies, its use was allowed until March 2018 (Resolución N° 72/017 DGSA). Therefore, their presence in water could reflect the application of leftover stocks following the ban. This compound is highly persistent in the environment (Singh et al., 2018; de Albuquerque et al., 2020). Despite its moderate solubility in water, it has a high potential to contaminate ground and surface waters (Lerch et al., 2018; de Albuquerque et al., 2020), with evidence that it interferes with reproduction and development and may cause cancer (Rusiecki et al., 2004; Boffetta et al., 2013; Puvvula et al., 2021; Chen et al., 2021). Atrazine was banned in the European Union in 2004 (European Environment Agency (EEA), 2016), U.S. Environmental Protection Agency (EPA) approved its continued use in September 2020. In LC, atrazine is present from November to March possibly associated with the spring corn crop in the area. The concentrations were lower than international concentration level guidelines (Canadian Council of Ministers of the Environment, 2011; European Environment Agency (EEA), 2016; USEPA. US Environmental Protection Agency, 2018), and no ecotoxicological risk was expected at these levels (Ccanccapa et al., 2016; Iturburu et al., 2019; Pérez et al., 2021).

The other banned compound found in this study was alpha-BHC, classified as a rare pesticide in LC due to its low occurrence. This compound was added to the persistent organic pollutants (POPs) list at the Stockholm Convention (Vijgen et al., 2011) and was banned in Uruguay in 2014. This pesticide has a high persistence in soil, but not as much so as DDT (Srivastava et al., 2019; Adithya et al., 2021), so it may have been used before 2014 in LC. However, its occurrence in February 2019 could indicate a punctual use. This would be of concern since this compound is used in eucalyptus afforestation, and an increase in forest area has been observed in LC during the period 2010–2020 (Sum Sologaistoa, 2021).

Azoxystrobin (6) and chlorpyrifos (9) are "frequent" compounds (<1.30 ng·L^{-1}) with a higher occurrence in the summer months. In the case of "occasional" compounds, Bifenthrin (7), cypermethrin (10) and ethion (13) were observed during summer (December to March) and 2.4-D

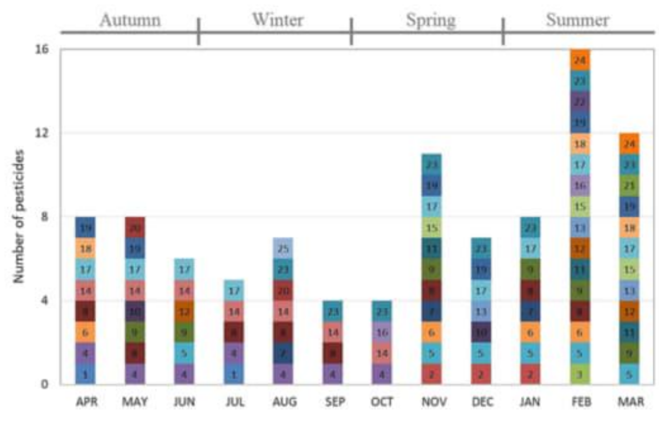

Fig. 3. Number of pesticides in surface water detected in Laguna del Cisne, each month includes seven samples in the LC from April 2018 to March 2019. The numbers included in each box represent the compound's name (see Table 2).

Table 3
Analysis of variance (ANOVA) showing p values for pesticide concentration in superficial water between seasons. Values with asterisk * are statistically different at probability values of $p \leq 0.05$.

	Autumn	Winter	Spring	Summer
Autumn		1	1	0,153
Winter	1		1	0,038*
Spring	1	1		0,59
Summer	0,153	0,038*	0,591	

(1) in autumn and winter (March to September) (Fig. 2). Eleven pesticides classified as "rare" were found in the system, mainly during the November–March period. The high occurrence of insecticides found in this study represents a high potential for chronic toxicity to aquatic organisms, especially invertebrates (Kumar et al., 2021).

The temporal distribution showed differences between months, with higher occurrences in spring and summer than in autumn and winter (Fig. 3). The lowest presence was observed during winter and early spring, with a minimum occurrence in September–October (4 compounds) and maximum in summer (February, 16 compounds). This higher occurrence in summer months could be related to the fact that the major crops in the area (soybean) are sowed in the early spring and harvested in autumn (Table 1) (Bálsamo, 2018; Gonzalez-Fernández and Orcasberrro, 2018). In winter, the "rare" pesticides found were compounds typically used in this season's crops, such as permethrin (used in fruit crops and forestry) and 2.4-D (used in wheat and as a grassland herbicide during rotation of crops) (Table 1). The relation of crops to the pesticides applied and their persistence was presented in Supplementary 1.

For pesticide concentrations, higher values were observed in winter (mean: 504 $ng{\cdot}L^{-1}$) and lower in summer (mean: 22 $ng{\cdot}L^{-1}$). In autumn and spring, the values obtained were similar (mean: 191 $ng{\cdot}L^{-1}$ and 131 $ng{\cdot}L^{-1}$ respectively) indicating moderate variability (Cv: 0.3) between these seasons. One-way ANOVA with Dunnett's T3 post hoc test showed a significant difference in pesticide concentrations between winter and summer seasons ($p < 0.01$). No significant differences between the other season were established ($p < 0.01$) (Table 3).

On a spatial scale, the distribution of pesticides showed a gradient across the lagoon (Supplementary Fig. 1). The occurrence of pesticides in the eastern part of the lagoon (W1 and W2) was more strongly associated with each other than with the rest of the sites. The two streams (Piedra del Toro and Cañada del Cisne) are in this zone and drain the northern catchment of LC. This result suggests a differential input of pesticides into the system related to the discharge of these tributaries from the surrounding farming area. However, due to the scarce information on land uses, the characterization of runoff-related pesticide input and the identification of land uses could not be determined. The same association pattern was observed in the sites located to the west, near the OSE water treatment plant. The pesticide concentrations tended to be higher on this part of the lagoon, although the levels in drinking water are below the acceptable levels (OSE, 2012). Although there is no research in Uruguay, international studies show that long-term exposure to low concentrations of CUPs and LPs has potential health risks to humans (Sjerps et al., 2019; Syafrudin et al., 2021). For safety measures, constant monitoring must be performed in this system and in potable water to study the pesticide contamination and their sources and the toxicity impacts on human health.

3.2. Pesticides occurrence in fish

The occurrence of pesticides was analyzed in muscle tissue of 51*C. voga* samples. Except for the compounds: chlorantraniliprole, glyphosate and AMPA, all pesticides classified as Dominant, Frequent and Occasional in surface water, were simultaneously analyzed in fish muscle. Twenty of these pesticides were not present in *C. voga*. On the other hand, all compounds analyzed in fishes were also searched in water. Aldrin, dieldrin, and propiconazole were detected only in fish samples.

Based on the physicochemical characteristics of the pesticides, the Kruskal-Wallis test showed significant differences in water solubility and log Kow of the compounds detected in water and fishes tissue ($p = 0.023$ and $p = 0.048$ respectively) (Supplementary Fig. 2). In superficial water were found pesticides with high water solubility values (mean 64,389 mgl^{-1}) than in fishes (mean 19 mgl^{-1}), while high log Kow pesticides are preferentially bioaccumulated in fish muscle (3.2 and 4.9 respectively). In this sense, was found that pesticides with low solubility, and high KOW are preferentially bioaccumulated in fish muscle. This is consistent with that reported by several authors who report that compounds with these properties are more able to accumulate in biological tissues (Pérez-Parada et al., 2018; Mazzoni et al., 2020; Kumar et al., 2021).

For all the five compounds present in both matrices, the concentration in muscle was higher than that detected in water. We detected the bioaccumulation of eight different compounds (four LPs and four CUP's). These findings represent a significant concern for the species in the system, and the aquatic community in general (Pérez-Parada et al., 2018; Mazzoni et al., 2020).

In fishes the maximum number of compounds found per month was eight (April 2018), while in December and March 2019, no pesticide residues was detected (Table 4). Propiconazole was detected in the highest concentrations, ranging from 24,933 $ng{\cdot}Kg^{-1}$ in July to 453,000 $ng{\cdot}Kg^{-1}$ in May (mean: 134,458 $ng{\cdot}Kg^{-1}$) followed by far by p,p′-DDE (maximum value 3600 $ng{\cdot}Kg^{-1}$ in May), and permethrin (maximum value 2900 $ng{\cdot}Kg^{-1}$ in April). (Table 4). The occurrence of propiconazole in fish is possibly determined by their high bioconcentration factor (116 $L{\cdot}Kg^{-1}$) (Table S1). This compound is used mainly in summer crops, which would explain its presence in a short period in muscle fish tissue (autumn- early winter) (Table 1) Even in low concentrations, propiconazole (banned in European Union since 2018) represents a risk for aquatic organisms, affecting the structure and function of communities (Bhagat et al., 2021). Several authors report in short-term exposure experiments that a wide molecular to population levels damage in fishes occurs (Souders et al., 2019; Valadas et al., 2019; Zhao et al., 2020; Henriques et al., 2021). This is of concern, because the concentrations of propiconazole estimated in this study were two orders of magnitude higher than those used in all these sublethal effects experiments. The long-term exposure of the individuals in LC, at concentrations above that typically detected in the environment (Souders et al., 2019), represents a high ecotoxicological risk to the fish community in the system. Additionally, propiconazole is known to function synergistically with several compounds, especially with pyrethroids (permethrin were detected in April 2018), representing a major risk for fish (Bhagat et al., 2021).

The most frequent compounds were organochlorines, indicating historical contamination, mainly associated with p,p′-DDT metabolites. In this sense, p,p′-DDE were present in 8 of the 12 months (Fig. 4). The high concentration of DDT metabolites in fish tissue is consistent with data collected worldwide (Sharma et al., 2019; Girones et al., 2020; Kumar et al., 2021; Montagner et al., 2022). The fact that p,p′-DDE represents the most stable metabolite of p,p′-DDT (Ricking and Schwarzbauer, 2012), could explain the higher occurrence of this compound over p-p′-DDD. The residue concentration in fish was in the major cases, three orders of magnitude higher than in superficial water, which evidences the highest potential of bioaccumulation of these compounds, due to its stability, high persistence, and hydrophobicity (Ricking and Schwarzbauer, 2012; Pérez-Parada et al., 2018; Mazzoni et al., 2020). Long and short-term effects of DDT and its derivatives represent a major hazard to aquatic organisms (Montagner et al., 2022). Many physiological and behavioral parameters have been affected by the high toxicity to fishes, like oxidative stress, neurotoxic effects, and death (Turusov et al., 2002; Martyniuk et al., 2020; Kumar et al., 2021).

Aldrin showed lower concentrations than its metabolite dieldrin (compounds analyzed only in fish) indicating the past use of aldrin in the basin, it is remarkable that these compounds were detected in only one sample. Possibly associated with its prohibition in Uruguay in 1970s (Boroukhovitch, 1998). Like the rest of LPs, their environmental persistence represents a high risk to fish health in LC (Bojarski and Witeska,

Table 4
Mean concentration (ng/Kg) of pesticides residues in *C. voga* samples from Laguna del Cisne during the period April 2018–March 2019.

n°	Pesticide	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR
		n = 6	n = 4	n = 6	n = 3	n = 3	n = 3	n = 3	n = 4	n = 4	n = 4	n = 5	n = 6
1	Aldrin	2650	-	-	-	-	-	-	-	-	-	-	-
2	Azoxystrobin	2205	-	-	-	-	-	-	-	-	-	-	-
3	Chlorpyrifos	1968	1833	-	-	-	2718	-	-	-	-	1600	-
4	Dieldrin	1720	-	-	-	-	-	-	-	-	-	-	-
5	p,p'-DDD	1694	-	1500	-	-	-	-	-	-	-	-	-
6	p,p'-DDE	3117	3600	2100	-	2109	2226	2406	2068	-	1000	-	-
7	Permethrin	2900	-	-	-	-	-	-	-	-	-	-	-
8	Propiconazole	219,500	453,000	59,450	24,933	-	-	-	-	-	-	-	-

2020; Martyniuk et al., 2020). Also, LPs can biomagnify in aquatic food webs (Mazzoni et al., 2020). In this sense, *C. voga* combines the consumption of inorganic sediment and detritus with benthic-associated algae and invertebrates (Corrêa and Piedras, 2008; Sagrario and Ferrero, 2013), so can function as a strong link between benthic and pelagic food webs (Sagrario and Ferrero, 2013).

This feeding habit of *C. voga* could indicate an intake of pesticides through sediments. In this sense, the persistent compounds, generally LPs, present high values of the Koc partition coefficient (Srivastava et al., 2019). This parameter is correlated with Kow and has been used to assess the exposure and risk of pesticides to organisms (Kurek et al., 2019; Srivastava et al., 2019).

The only study on *C. voga* that evaluated the accumulation of these contaminants in tissue was carried out by Barni et al. (2016). These authors observed histological damage (mainly in liver and gills), and vitellogenesis induction directly related to POPs exposure. Therefore, long-term exposure to these compounds represents a great concern for the species in the system and the aquatic community in general (Barni et al., 2016; Pérez-Parada et al., 2018; Mazzoni et al., 2020).

Despite the large number of CUPs detected in water, only three were found to accumulate in fish: azoxystrobin, chlorpyrifos, and permethrin (Table 4). CUPs are less hydrophobic, persistent in terrestrial and aquatic environments, and more readily metabolized than legacy compounds (Pérez-Parada et al., 2018; de Souza et al., 2022). In the case of insecticides, Organophosphates and Pyrethroid were introduced to replace Organochlorines (Huang et al., 2020; Yang et al., 2021; Montagner et al., 2022). However, is largely reported that these compounds can induce damage to the nervous system, oxidative stress, and endocrine disruption in exposed fishes (Pérez-Parada et al., 2018; Kumari, 2020; Bhagat et al., 2021 Derby et al., 2021; Yang et al., 2021). In this study, a Pyrethroid (permethrin) and a Organophosphate (chlorpyrifos) accumulated in muscle tissue.

Another significant concern is the presence of azoxystrobin, about 26 times the levels measured in superficial water. Like other strobirulin fungicides, this compound induces oxidative stress and adversely affects aquatic species (Ernst et al., 2018; Pérez-Parada et al., 2018; Kumari, 2020).

In Uruguay, chlorpyrifos was previously detected in fishes in three different localities associated with rainfed agriculture (Ernst et al., 2018). It was identified as an occasional substance with high concentrations in a detritivorous fish with similar feeding habits to *C. voga* (*Prochilodus lineatus*). Chlorpyrifos is the second insecticide used in Uruguay (DGSA, 2020), and like other organophosphate insecticides, these pesticides were banned by EU and USA due their persistence and toxicity. Is highly toxic to birds, fish, aquatic invertebrates, and honeybees and moderately toxic to aquatic plants, algae (Barron and Woodburn, 1995; X. Huang et al., 2020; H. Huang et al., 2020). Our results showed that this compound is present in several samples thought the year and the mean level is about 225 times the levels measured in superficial waters. This result suggests a long term exposure and a great accumulation over the years.

The GLM analysis of the biological data showed a significant relationship between chlorpyrifos and DDT metabolites (p-p′-DDE and p-p′-DDD) to the lipid content of the individuals (Table 5; Supplementary Fig. 3). These results are consistent with the correlation between lipids, and the octanol/water partition coefficient in the bioaccumulation of organic contaminants in aquatic organisms (Pérez-Parada et al., 2018; Mazzoni et al., 2020; Kumar et al., 2021). In this sense, all these compounds present high values of Kow (Table S1). Also, the positive relationship between body size and p-p′-DDE, could be evidenced that bioaccumulation tends to increase with the age of the individuals for this compound.

The high solubility of propiconazole possibly determines the no relationship between fat content and the bioaccumulation process. Likewise, these compounds showed a negative relation with HIS index (a liver lipid content indicator), and a positive relation with the Condition Factor. The K parameter reflects the general fish condition, among them, the feeding conditions of the individuals (Froese, 2006) these results could indicate that individuals with higher feeding rates would have more chances of incorporating it in muscle.

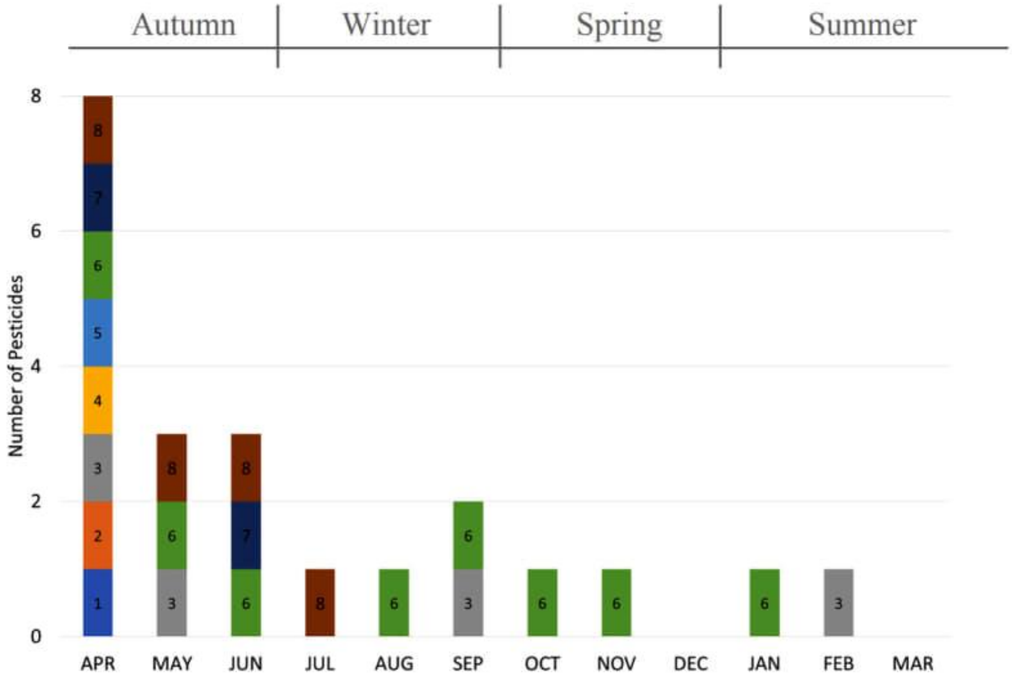

Fig. 4. Number of pesticide residues in *C. voga* per month from Laguna del Cisne from April 2018–March 2019. The numbers in each box represents the compound's name (see Table 4).

Due to the low frequency of detection (1 month) of aldrin, dieldrin, and permethrin, it was not possible to analyze the relationships between accumulation and biological parameters of fish.

The Redundancy analysis (RDA) carried out to analyze the relationships between the five pesticides present in both matrices did not show significant associations ($R^2 = 0.2898$; $F = 0.4897$; $p = 0.89$). The first three axes explained only 15.51 %, 9.88 %, and 3.47 % of the total variability, respectively. Mantel test was also not significant ($R = -0.2484$; $p = 0.74$). Also, an RDA was carried out with a 2-month delay in the concentration in fish muscle tissue to identify the possible effects of accumulative precipitations. This period corresponds to the impact of monthly cumulative precipitation in the Laguna del Cisne basin (Goyenola et al., 2011). However, no significant relationships were found ($R2 = 1.2181$; $F = 0.6704$; $p = 0.72$).

4. Conclusions

This work represents the first survey conducted in Uruguay to investigate the presence and interactions of pesticides in aquatic ecosystems including superficial water and a fish species. Although there are studies that monitor pesticides in different matrices, most of them do not consider a monthly sampling frequency over a year. The high sampling frequency in this study allows us to understand the influence of past and current agricultural practices on the dynamics of pesticides in freshwater ecosystems. In this case the sampling approach made it possible to identify 25 pesticides in surface waters and evaluate the seasonal occurrence with the calendar of farming practices throughout the year. We also detected bioaccumulation of eight different pesticides in the muscle tissue of the detritivorous fish *Cyphocharax voga*.

The mixture contained Triazoles, Strobilurins, Pyrethroid, Organophosphates, and mainly Organochlorines. This suggests a potential exposure of fish populations to legacy and current-use pesticides. Several of the detected compounds represent a significant risk to aquatic organisms. Because of this potential risk, studies are needed to evaluate the effects of these mixtures and concentrations on aquatic biota at an experimental level. In particular, the synergies and antagonisms between the different active principles deserve further study to establish the real importance of these findings from an ecotoxicological point of view.

Our results suggest that the precautionary measures aiming at an agroecological transition in the Laguna del Cisne are insufficient to solve the problem after one year of application.

The management of the exclusion zone should consider the hydrological conditions of the LC, as it includes the lagoon floodplain. In this sense would be necessary to enlarge this area to reduce pesticide input to surface waters.

The historical contamination by persistent organochlorines is still a problem, probably by the transport of suspended sediment from the catchment. Future studies should be analyzed soil pollution in the catchment and the influence of rainfall events on pesticide concentrations in superficial water.

The level of p,p′-DDT (and their metabolites) in superficial water, was above international guidelines, indicating a contamination source of this compound in the system. However, the concentrations found for all detected pesticides in superficial waters were below the limits established for drinking water in Uruguay.

For these reasons, a strict monitoring program in the lagoon is required, to remove contaminants before distribution to the population. Considering that Laguna del Cisne's catchment is under restrictions for pesticide use, there is a need to improve the pesticide policy framework in the country,

Table 5
Summary of the results of generalized linear models (GLM) performed to evaluate the relationship between pesticide residue concentration in muscle tissue, and biological factors of the individuals of *C. voga* in Laguna del Cisne. Asterisks represent significance levels of the input parameters.

GLM	Variable	Estimate ± SE	t	p-value
Chlorpyrifos	Intercept	0.81 ± 0.29	−2.894	<0.01*
	Lipid content	1.13 ± 0.25	4.639	<0.001*
	GSI	0.65 ± 0.23	2.822	<0.01*
Propiconazole	Intercept	3.07 ± 0.38	8.025	<0.001*
	Condition Factor	0.39 ± 0.23	1.695	0.098
	HSI	−1.96 ± 0.56	−3.537	<0.01*
p,p-DDD	Intercept	−2.05 ± 0.45	−4.54	<0.001*
	Lipid content	1.01 ± 0.28	3.656	<0.001*
	HSI	−1.50 ± 0.61	−2.453	<0.019*
p,p-DDE	Intercept	0.19 ± 0.20	0.951	0.347
	Lipid content	0.46 ± 0.17	2.836	0.007*
	Length	0.80 ± 0.21	3.869	<0.001*

to minimize the environmental concerns of harmful pesticides in this drinking water source. The characteristics of the Laguna del Cisne basin are not very different from all the agroproductive Uruguayan countryside, suggesting a generalized but not visualized environmental problem that should be assessed. It is important to highlight that LC is currently the only aquatic system with precautionary measures. In this sense, our results show how complex it can be to reduce pesticide contamination in aquatic systems. Furthermore, the results highlight the importance of monitoring multiresidues in different environmental matrices to generate adequate baselines for environmental management.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.162310.

CRediT authorship contribution statement

César Rodríguez-Bolaña: Conceptualization, Methodology, Data curation, Formal analysis, Visualization, Investigation, Writing - original draft, Writing - review & editing. **Andrés Pérez-Parada:** Conceptualization, Supervision, Data curation, Investigation, Methodology, Resources, Writing - review & editing. **Giancarlo Tesitore:** Data curation, Visualization, Investigation. **Guillermo Goyenola:** Investigation, Visualization, Writing – review & editing. **Alejandra Kröger:** Investigation, Writing – review & editing. **Martín Pacheco:** Investigation, Writing – review & editing. **Natalia Gérez:** Resources, Writing – review & editing. **Analia Berton:** Resources, Writing – review & editing. **Gianna Zinola:** Resources, Writing – review & editing. **Guillermo Gil:** Resources, Writing – review & editing. **Alejandro Mangarelli:** Resources, Data curation, Methodology, Writing - review & editing. **Fiamma Pequeño:** Resources, Writing – review & editing. **Natalia Besil:** Resources, Writing – review & editing. **Silvina Niell:** Resources, Writing – review & editing. **Horacio Heinzen:** Resources, Writing – review & editing, Data curation. **Franco Teixeira de Mello:** Conceptualization, Supervision, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing - review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

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

Acknowledgments

This work was supported by Food and Agriculture Organization of the United Nations (FAO) through project GCP/URU/031/GFF, and by Comisión Académica de Postgrado (CAP) (BFPD_2022_1_43490721). HH, AP-P and FTM thanks Sistema Nacional de Investigadores (SNI) and Programa de Desarrollo de las Ciencias Básicas (PEDECIBA).

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Supplementary Data 1

List of pesticides analyzed for each matrix. W: Superficial water; F: Fishes. Priority level for the National Monitoring Program carried out by the Uruguayan Ministry of Environment: 3: Maximum; 2: Significant. Classification in Occasional, Rare, Frequent and Dominant based on average concentration and frequency of occurrence using the water samples following the Olmsted-Tukey diagram (Figure 2). Physicochemical properties and environmental. Data obtained from IUPAC Footprint.

						Physicochemical properties					Environmental Fate		
Nº	Pesticide	Matrix analyzed	Use	Ministry of Environment Priority level	Classification in water	Water solubility (mg/L)	log Kow	Vapour pressure at 20 °C (mPa)	constant at 25 °C (Pa m^3 mol^{-1})	Interpretation Henry's law constant	degradation (days, aerobic) DT50	Interpretation DT50 (soil degradation)	Adsorption Kf (soil)
1	AMPA	W	Degradation product	3	DOMINANT	1466561	-1.63	No data	0.16	Moderately volatile	121.4	Persistent	197.5
2	Atrazine	W/F	Herbicide	3	DOMINANT	35	2.7	39	1.50E-04	Non-volatile	75	Moderately persistent	3.2
3	Chlorantraniliprole	W	Insecticide	3	DOMINANT	0.88	2.86	6.30E-09	3.20E-09	Non-volatile	597	Very persistent	2.95
4	Glyphosate	W	Herbicide	3	DOMINANT	10500	-3.2	131	2.10E-08	Non-volatile	15	Non-persistent	70.85
5	Metolachlor (S-Metolachlor)	W	Herbicide	3	DOMINANT	530	3.4	1.7	2.40E-03	Non-volatile	90	Moderately persistent	0.93
6	p,p´ DDD	W/F	Degradation product	2	DOMINANT	0.09	06.02	0.18	No data	No data	1000	Very persistent	No data
7	p,p´ DDT	W/F	Insecticide	2	DOMINANT	6	6.91	25	No data	No data	6200	Very persistent	No data
8	Azoxystrobin	W/F	Fungicide	3	FREQUENT	6.7	2.5	1.10E-07	7.4E-09	Non-volatile	78	Moderately persistent	7.35
9	Chlorpyrifos	W/F	Insecticide	3	FREQUENT	01.05	4.7	1.43	478	Non-volatile	50	Moderately persistent	91.6
10	2,4 D	W	Herbicide	3	OCCASIONAL	24300	-0.82	99	4.00E-06	Non-volatile	4.4	Non-persistent	0.45
11	Bifenthrin	W/F	Insecticide	3	OCCASIONAL	1	6.6	178	7.74E-05	Non-volatile	26	Non-persistent	No data
12	Cypermethrin	W/F	Insecticide	3	OCCASIONAL	9	5.55	678	0.31	Moderately volatile	22.1	Non-persistent	91.6
13	Ethion	W/F	Insecticide	2	OCCASIONAL	2	05.07	0.2	3.85E-02	Non-volatile	90	Moderately persistent	No data
14	Thiabendazole	W	Fungicide	2	OCCASIONAL	30	2.39	53	37	Non-volatile	500	Very persistent	35.5
15	Acetochlor	W	Herbicide	3	RARE	282	4.14	22	2.10E-02	Non-volatile	14	Non-persistent	4.5
16	alpha-HCH	W	Insecticide	2	RARE	2	3.82	5.99	No data	No data	175	Persistent	No data
17	Cyproconazole	W	Fungicide	3	RARE	93	03.09	26	5.00E-05	Non-volatile	142	Persistent	8.7
18	Diazinon	W/F	Insecticide	2	RARE	60	3.69	11.97	6.09E-02	Non-volatile	9.1	Non-persistent	4.1
19	Metalaxyl	W	Fungicide	2	RARE	8400	1.75	0.75	1.60E-05	Non-volatile	36	Moderately persistent	2.49
20	Metribuzin	W	Herbicide	3	RARE	10700	1.75	121	2.50E-05	Non-volatile	07.03	Non-persistent	874
21	p,p´ DDE	W/F	Degradation product	2	RARE	0.12	6.51	No data	No data	No data	5000	Very persistent	No data
22	Permethrin	W/F	Insecticide	2	RARE	0.2	6.1	7	1.89E-01	Moderately volatile	13	Non-persistent	1.87
23	Pyraclostrobin	W	Fungicide	2	RARE	1.9	3.99	2.6E-5	531	Non-volatile	41.9	Moderately persistent	145
24	Simazine	W/F	Herbicide	3	RARE	5	2.3	81	5.60E-05	Non-volatile	60	Moderately persistent	15.88
25	Tebuconazol	W	Fungicide	3	RARE	36	3.7	13	1.00E-05	Non-volatile	63	Moderately persistent	12.69
26	Acetamiprid	W	Incecticida	2		2950	0.8	173	5.30E-08	Non-volatile	1.6	Non-persistent	1.58
27	Alachlor	W/F	Herbicide	2		240	03.09	2.9	3.20E-03	Non-volatile	14	Non-persistent	16.5
28	Aldrin	W/F	Insecticide	2		27	6.5	8.6	1.72E+01	Moderately volatile	28	Non-persistent	No data
29	alpha-endosulfan	W/F	Insecticide	2		0.32	4.75	8.3	6.86E-06	Moderately volatile	50	Moderately persistent	No data
30	Ametryn	W	Herbicide	3		200	2.63	365	4.10E-04	Non-volatile	37	Moderately persistent	76.81
31	Amitraz	W	Insecticide	2		0.1	5.5	51	01.06	Moderately volatile	0.2	Non-persistent	No data
32	Azinphos-methyl	W/F	Insecticide	2		28	2.96	5	57	Non-volatile	10	Non-persistent	No data
33	beta-HCH	W/F	Insecticide	2		0.24	3.78	No data	4.50E-07	Non-volatile	No data	No data	No data
34	beta-endosulfan	W/F	Insecticide	2		0.45	3.83	No data	No data	No data	No data	No data	No data
35	Boscalid	W	Fungicide	2		4.6	2.96	72	5.18E-08	Non-volatile	484.4	Very persistent	12.6
36	Carbaryl	W	Insecticide	2		9.1	2.36	416	9.20E-05	Non-volatile	16	Non-persistent	2.6
37	Carbendazim	W	Fungicide	3		8	1.48	0.09	3.60E-03	Non-volatile	40	Moderately persistent	3.4
38	Carbofuran	W	Insecticide	2		322	1.8	0.08	5.00E-05	Non-volatile	29	Non-persistent	1.14
39	Chlorfenapyr	W	Insecticide	2		112	4.83	981	5.81E-04	Non-volatile	1.4	Non-persistent	No data
40	Chlorothalonil	W/F	Fungicide	3		0.81	2.94	76	2.50E-02	Non-volatile	3.53	Non-persistent	27,2
41	Chlorpyrifos-methyl	W	Insecticide	3		2.74	4	3	235	Moderately volatile	12	Non-persistent	44.9
42	Cis-chlordane	W/F	Insecticide	2		0.1	2.78	1.3	3.90E-04	Non-volatile	365	Very persistent	No data
43	Clomazone	W	Herbicide	3		1212	2.58	27	5.90E-03	Non-volatile	22.6	Non-persistent	02.08
44	Cyfluthrin	W/F	Insecticide	3		19	5.9	3	5.30E-02	Non-volatile	28	Non-persistent	01.02
45	Cyhalofop butil	W	Herbicide	3		0.44	3.32	53	9.51E-04	Non-volatile	0.2	Non-persistent	41.5
46	Cyhalothrin (lambda)	W/F	Insecticide	3		5	5.5	2	2.00E-02	Non-volatile	175	Persistent	4167
47	delta-HCH	W/F	Insecticide	2		1.1	4.14	3.5x10-5	2.10E-07	Non-volatile	No data	No data	No data
48	Deltamethrin	W/F	Insecticide	3		2	4.6	1.24E-5	3.10E-02	Non-volatile	58.2	Moderately persistent	17500
49	Dicamba	W	Herbicide	3		250000	-1.88	1.67	5.06E-05	Non-volatile	4	Non-persistent	10.16
50	Dieldrin	W	Insecticide	3		0.14	3.7	24	6.50E-02	Non-volatile	1400	Very persistent	1563
51	Difenoconazole	W/F	Fungicide	3		15	4.36	3.33E-5	9.00E-07	Non-volatile	130	Persistent	41
52	Endosulfan	W/F	Insecticide	2		0.32	4.75	0.83	1.48	Moderately volatile	50	Moderately persistent	0.28
53	Endrin	W/F	Insecticide	2		0.24	3.2	2.0E-7	1.48E-01	Moderately volatile	4300	Very persistent	No data
54	Epoxiconazole	W	Fungicide	3		7.1	3.3	35	4.70E-04	Non-volatile	354	Persistent	12.18
55	Fipronil	W/F	Insecticide	2		3.78	3.75	2	2.31E-04	Non-volatile	142	Persistent	11.85
56	Heptachlor	W/F	Insecticide	2		56	5.44	53	3.53E+02	Non-volatile	285	Persistent	No data
57	Heptachlor (epoxide B)	W	Degradation product	2		0.2	4.98	No data	No data	No data	No data	No data	No data
58	Hexachlorobenzene	W/F	Fungicide	2		47	3.93	1.45	1.03E+01	Moderately volatile	2000	Very persistent	No data
59	Imazalil	W	Fungicide	3		184	2.56	158	1.00E-04	Non-volatile	76.3	Moderately persistent	126.9
60	Imazapyr	W	Herbicide	3		9740	0.11	13	3.00E-07	Non-volatile	11	Non-persistent	1.23
61	Imazetapyr	W	Herbicide	3		1400	1.49	133	1.30E-02	Non-volatile	90	Moderately persistent	No data
62	Iprodione	W	Fungicide	3		6.8	3	5	7.00E-06	Non-volatile	36.2	Moderately persistent	16.36
63	Isoxadifen-ethyl	W	Herbicide	2		No data	No data	No data	No data	No data	No data	No data	No data
64	Kresoxim-methyl	W	Fungicide	3		2	3.4	23	3.60E-04	Non-volatile	16	Non-persistent	4.26
65	Lindane	W	Insecticide	2		8.52	3.5	4.4	1.48E-06	Non-volatile	980	Very persistent	15.9
66	Malaoxon	W	Degradation product	2		8.14	0.97	No data	No data	No data	No data	No data	No data
67	Malathion	W/F	Insecticide	2		148	2.75	3.1	1.00E-03	Non-volatile	0.17	Non-persistent	1.58
68	Metalaxyl-M (Mefenoxam)	W	Fungicide	2		26000	1.71	0.75	3.50E-05	Non-volatile	36	Moderately persistent	1.63
69	Methidathion	W	Insecticide	2		240	2.57	0.25	3.30E-04	Non-volatile	10	Non-persistent	No data
70	Methiocarb	W	Insecticide	2		27	3.18	15	1.20E-04	Non-volatile	2.94	Non-persistent	5.88
71	Methoxychlor	W/F	Insecticide	2		0.1	5.83	0.08	2.00E-02	Non-volatile	120	Persistent	No data
72	Metsulfuron Metil	W	Herbicide	3		2790	-1.87	1.4E-8	2.87E-06	Non-volatile	10	Non-persistent	0.77
73	Mirex	W/F	Insecticide	2		1	5.28	No data	8.30E+02	Volatile	300	Persistent	No data
74	Oxyfluorfen	W/F	Herbicide	2		116	4.86	26	2.30E-02	Non-volatile	35	Moderately persistent	99.37
75	Parathion	W/F	Insecticide	2		12.4	3.83	0.89	3.02E-02	Non-volatile	49	Moderately persistent	17
76	Parathion-methyl	W/F	Insecticide	2		55	3	0.2	8.57E-03	Non-volatile	12	Non-persistent	34.4
77	Penoxulam	W	Herbicide	2		408	-602	2.49E-11	2.95E-14	Non-volatile	32	Moderately persistent	1.27
78	Pirimiphos-methyl	W	Insecticide	2		11	4.2	2	608	Non-volatile	39	Moderately persistent	3.63
79	Prochloraz	W	Fungicide	2		26.5	3.5	0.15	1.64E-03	Non-volatile	120	Persistent	38
80	Profenofos	W	Insecticide	2		28	1.7	2.53	1.65E-03	Non-volatile	7	Non-persistent	No data
81	Propanil	W/F	Herbicide	3		95	2.29	193	4.40E-04	Non-volatile	0.4	Non-persistent	6.95
82	Propiconazole	W	Fungicide	2		150	3.72	56	9.20E-05	Non-volatile	71.8	Moderately persistent	15
83	Pyrimethanil	W	Fungicide	3		110	2.84	1.1	2.20E-03	Non-volatile	50.9	Moderately persistent	8.38
84	Thiamethoxam	W	Insecticide	3		4100	-0.13	6.6E-6	4.70E-10	Non-volatile	50	Moderately persistent	No data
85	trans-Chlordane	W/F	Insecticide	2		0.1	2.78	1.3	3.90E-04	Non-volatile	365	Very persistent	No data
86	Tricyclazole	W	Fungicide	3		596	1.4	27	5.86E-07	Non-volatile	450	Very persistent	3.1
87	Trifloxystrobin	W	Fungicide	3		0.61	4.5	34	2.30E-03	Non-volatile	0.34	Non-persistent	43.5
88	Trifluralin	W	Herbicide	3		221	5.27	9.5	10.2	Moderately volatile	133.7	Persistent	79.4

Supplementary Data 1 (Continued).

List of pesticides analyzed for each matrix. W: Superficial water; F: Fishes. Environmental fate and ecotoxicological data obtained from IUPAC Footprint. *Reference fish species: Oncorhynchus mykiss. For approved use: S:Soybean; M:Maize; W:Wheat; SGH:sorghum; F: Forestry; FR:Fruits; P:Potato; R: Rice; NR: Not Registered.

			Physicochemical properties						Ecotoxicology				Use
Nº	Pesticide	Matrix analyzed	Adsorption Kf (soil)	Interpretation Kf (soil)	Water-sediment DT 50 (days)	Interpretation WS DT50	Water phase only DT 50 (days)	Interpretation W DT50	Fish* Acute 96 hours (LC50 (mg/L)	Threshold for concern	concentration factor BCF (l kg^{-1})	Threshold for concern	Approval for use
1	AMPA	W	197.5	Non-mobile	132	Slow	5.47	Moderately fast	> 100	Low	Low risk*	No data	Metabolite
2	Atrazine	W/F	3.2	Moderately mobile	80	Moderately fast	No data	No data	4.5	Moderate	4.3	Low potential	M/SGH/F
3	Chlorantraniliprole	W	2.95	Moderately mobile	170	Slow	23.5	Slow	12	Moderate	15	Low potential	S/M/SGH/FR
4	Glyphosate	W	70.85	Non-mobile	20.8	Fast	9.9	Moderately fast	> 100	Low	0.5	Low potential	S/M/W/P
5	Metolachlor (S-Metolachlor)	W	0.93	Moderately mobile	365	Stable	88	Stable	3.9	Moderate	68.8	Low potential	S/M/SGH
6	p,p´ DDD	W/F	No data	No data	No data	No data	No data	No data	0.07	High	No data	No data	Metabolite
7	p,p´ DDT	W/F	No data	No data	No data	No data	No data	No data	2.5	Moderate	3173	Moderate	Banned
8	Azoxystrobin	W/F	7.35	Moderately mobile	205	Slow	6.1	Moderately fast	0.47	Moderate	Low risk*	No data	S/M/W/SGH
9	Chlorpyrifos	W/F	91.6	Slightly mobile	36.5	Moderately fast	5	Moderately fast	13	High	1374	Moderate	S/M/W/SGH/F/FR
10	2,4 D	W	0.45	Mobile	0.45	Fast	7.7	Moderately fast	100	Moderate	10	Low potential	M/W/SGH/P
11	Bifenthrin	W/F	No data	No data	161	Slow	8	Moderately fast	26	High	1703	Moderate	S/M/FR/P
12	Cypermethrin	W/F	91.6	Slightly mobile	36.5	Moderately fast	5	Moderately fast	151	High	331	Moderate	S/M/W/F/FR
13	Ethion	W/F	No data	No data	No data	No data	No data	No data	0.5	Moderate	586	Moderate	S/M/W/F
14	Thiabendazole	W	35.5	Slightly mobile	4	Fast	1.6	Moderately fast	0.55	Moderate	96.5	Low potential	M/W/R
15	Acetochlor	W	4.5	Moderately mobile	19.7	Fast	40.5	Stable	0.36	Moderate	20	Low potential	S/M/F
16	alpha-HCH	W	No data	No data	No data	No data	No data	No data	>0,82	Moderate	No data	No data	Banned
17	Cyproconazole	W	8.7	Moderately mobile	1000	Stable	No data	No data	19	Moderate	28	Low potential	W
18	Diazinon	W/F	4.1	Slightly mobile	10.4	Fast	4.3	Moderately fast	3.1	Moderate	500	Moderate	FR/P
19	Metalaxyl	W	2.49	Moderately mobile	56	Moderately fast	56	Stable	0.96	Moderate	7	Low potential	S/M/W/SGH
20	Metribuzin	W	874	Mobile	50	Moderately fast	41	Stable	74.6	Moderate	10	Low potential	S/P
21	p,p´ DDE	W/F	No data	No data	No data	No data	No data	No data	32	High	1800	Moderate	Metabolite
22	Permethrin	W/F	1.87	Moderately mobile	40	Moderately fast	23	Slow	125	High	300	Moderate	NR
23	Pyraclostrobin	W	145	Non-mobile	28	Fast	2	Moderately fast	6	High	706	Moderate	S/M/W/SGH
24	Simazine	W/F	15.88	Slightly mobile	33	Moderately fast	46	Stable	90	Moderate	221	Moderate	M/SGH/FR
25	Tebuconazol	W	12.69	Slightly mobile	365	Stable	42.6	Stable	4.4	Moderate	78	Low potential	S/M/W/SGH/F
26	Acetamiprid	W	1.58	Moderately mobile	No data	No data	4.7	Moderately fast	100	Low	No data	No data	S/M
27	Alachlor	W/F	16.5	Slightly mobile	2	Fast	No data	No data	1.8	Moderate	39	Low potential	Banned
28	Aldrin	W/F	No data	No data	No data	No data	No data	No data	46	High	3348	Moderate	Banned
29	alpha-endosulfan	W/F	No data	No data	No data	No data	No data	No data	2	High	2755	Moderate	Banned
30	Ametryn	W	76.81	Non-mobile	No data	No data	No data	No data	5	Moderate	33	Low potential	M/F/FR
31	Amitraz	W	No data	No data	No data	No data	No data	No data	0.74	Moderate	1838	Moderate	FR
32	Azinphos-methyl	W/F	No data	No data	No data	No data	No data	No data	0.02	High	40	Low potential	NR
33	beta-HCH	W/F	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	Banned
34	beta-endosulfan	W/F	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	Banned
35	Boscalid	W	12.6	Slightly mobile	No data	No data	No data	No data	2.7	Moderate	107	Moderate	S/FR/P
36	Carbaryl	W	2.6	Moderately mobile	5.8	Fast	3.1	Moderately fast	2.6	Moderate	No data	No data	S/M/W/F/FR
37	Carbendazim	W	3.4	Moderately mobile	33.7	Moderately fast	7.9	Moderately fast	0.19	Moderate	44	Low potential	S/M/W/FR/R
38	Carbofuran	W	1.14	Moderately mobile	9.7	Fast	6.1	Moderately fast	0.18	Moderate	25	Low potential	M/SGH/P/R
39	Chlorfenapyr	W	No data	No data	No data	No data	No data	No data	7	High	No data	No data	FR
40	Chlorothalonil	W/F	27,2	Slightly mobile	0.57	Fast	0.82	Fast	17	High	100	Moderate	FR/P
41	Chlorpyrifos-methyl	W	44.9	Slightly mobile	14	Fast	2.9	Moderately fast	0.41	Moderate	1800	Moderate	M/W/SGH
42	Cis-chlordane	W/F	No data	No data	No data	No data	No data	No data	0.09	High	8460	High potential	Banned
43	Clomazone	W	02.08	Moderately mobile	54	Moderately fast	No data	No data	14.4	Moderate	40	Low potential	S/R
44	Cyfluthrin	W/F	01.02	Moderately mobile	8	Fast	1	Moderately fast	47	High	506	Moderate	S
45	Cyhalofop butil	W	41.5	Slightly mobile	0.1	Fast	0.15	Fast	0.79	Moderate	7.5	Low potential	S/R/W/SGH
46	Cyhalothrin (lambda)	W/F	4167	Non-mobile	15.1	Fast	0.24	Fast	21	High	4982	Moderate	S/M/W/SGH/P
47	delta-HCH	W/F	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	Banned
48	Deltamethrin	W/F	17500	Non-mobile	65	Moderately fast	17	Slow	15	High	1400	Moderate	S/M/W/F/R
49	Dicamba	W	10.16	Very mobile	41	Moderately fast	40	Stable	>100	Moderate	15	Low potential	S/M/W/SGH
50	Dieldrin	W	1563	Non-mobile	No data	No data	No data	No data	12	High	35000	High	Banned
51	Difenoconazole	W/F	41	Slightly mobile	1053	Stable	3	Moderately fast	1.1	Moderate	330	Moderate	S/M/W/FR/R
52	Endosulfan	W/F	0.28	Mobile	No data	No data	No data	No data	2	High	2755	Moderate	Banned
53	Endrin	W/F	No data	No data	No data	No data	No data	No data	73	High	3970	Moderate	NR
54	Epoxiconazole	W	12.18	Slightly mobile	119.8	Slow	65.8	Stable	3.14	Moderate	70	Low potential	S/W
55	Fipronil	W/F	11.85	Slightly mobile	68	Moderately fast	54	Stable	248	Moderate	321	Moderate	M/W/F/R
56	Heptachlor	W/F	No data	No data	No data	No data	No data	No data	7	High	2430	Moderate	Banned
57	Heptachlor (epoxide B)	W	No data	No data	No data	No data	No data	No data	0.02	High	1440	Moderate	Banned
58	Hexachlorobenzene	W/F	No data	No data	No data	No data	No data	No data	0.03	High	35000	High potential	Banned
59	Imazalil	W	126.9	Non-mobile	117	Slow	7.8	Moderately fast	1.48	Moderate	56.3	Low potential	M/W
60	Imazapyr	W	1.23	Moderately mobile	No data	No data	No data	No data	>100	Low	2.54	Low potential	R
61	Imazetapyr	W	No data	No data	No data	No data	No data	No data	>340	Low	1.6	Low potential	S/M/R
62	Iprodione	W	16.36	Slightly mobile	4	Fast	2	Moderately fast	3.7	Moderate	70	Low potential	FR/P
63	Isoxadifen-ethyl	W	No data	No data	No data	No data	No data	No data	0.34	Moderate	No data	No data	NR
64	Kresoxim-methyl	W	4.26	Moderately mobile	1.3	Fast	0.85	Fast	0.19	Moderate	220	Moderate	S/W/FR/R
65	Lindane	W	15.9	Moderately mobile	394	Stable	21	Slow	29	High	1300	Moderate	Banned
66	Malaoxon	W	No data	No data	No data	No data	No data	No data	No data	No data	No data	No data	Metabolite
67	Malathion	W/F	1.58	Moderately mobile	0.4	Fast	0.4	Fast	18	High	103	Moderate	M/W/SGH/FR
68	Metalaxyl-M (Mefenoxam)	W	1.63	Moderately mobile	32.1	Moderately fast	24.8	Slow	> 100	Low	15	Low potential	Metabolite
69	Methidathion	W	No data	No data	70	Moderately fast	6	Moderately fast	0.01	High	12.6	Low potential	S/M
70	Methiocarb	W	5.88	Slightly mobile	4	Fast	1.6	Moderately fast	0.65	Moderate	75	Low potential	S/M/SGH
71	Methoxychlor	W/F	No data	No data	No data	No data	No data	No data	52	High	1622	Moderate	NR
72	Metsulfuron Metil	W	0.77	Very mobile	224.3	Slow	115	Stable	>110	Low	1	Low potential	W/R
73	Mirex	W/F	No data	No data	Stable	Stable	Stable	Stable	>100	Low	51400	High potential	NR
74	Oxyfluorfen	W/F	99.37	Non-mobile	No data	No data	No data	No data	0.25	Moderate	1637	Moderate	S/F/FR
75	Parathion	W/F	17	Slightly mobile	4.3	Fast	3.5	Moderately fast	1.5	Moderate	40	Low potential	Banned
76	Parathion-methyl	W/F	34.4	Moderately mobile	5	Fast	15	Slow	2.7	Moderate	71	Low potential	Banned
77	Penoxulam	W	1.27	Moderately mobile	No data	No data	No data	No data	>100	Low	100	Moderate	Rice
78	Pirimiphos-methyl	W	3.63	Moderately mobile	No data	No data	No data	No data	404	Moderate	741	Moderate	NR
79	Prochloraz	W	38	Slightly mobile	359	Slow	2	Moderately fast	1.5	Moderate	371	Moderate	M/W/R
80	Profenofos	W	No data	No data	No data	No data	No data	No data	>0,08	High	1186	Moderate	S/M
81	Propanil	W/F	6.95	Slightly mobile	1.25	Fast	1.2	Moderately fast	5.4	Moderate	111	Moderate	R
82	Propiconazole	W	15	Slightly mobile	561	Stable	6	Moderately fast	2.6	Moderate	116	Moderate	S/R/W/SGH
83	Pyrimethanil	W	8.38	Moderately mobile	81	Moderately fast	6.7	Moderately fast	10.56	Moderate	Low risk*	No data	FR
84	Thiamethoxam	W	No data	No data	40	Moderately fast	30.6	Stable	> 125	Low	Low risk*	No data	S/M/W/SGH/F
85	trans-Chlordane	W/F	No data	No data	No data	No data	No data	No data	0.09	High	8460	High potential	Banned
86	Tricyclazole	W	3.1	Moderately mobile	453	Stable	92	Stable	7.3	Moderate	3.1	Low potential	W/R
87	Trifloxystrobin	W	43.5	Slightly mobile	2.4	Fast	1.1	Moderately fast	22	High	431	Moderate	S/M/W/R
88	Trifluralin	W	79.4	Non-mobile	5.5	Fast	13	Moderately fast	88	High	15	Low potential	S/M/F

Supplementary Data 2

Period of analysis and Quantification limits (LOQ) of pesticides for each matrix analized.

		Water (n= 88)		Fish (n= 39)	
	Pesticide	LOQ (ng.L-1)	Period of analysis	LOQ (ng.kg-1)	Period of analysis
1	2.4-D	500	Apr 18-Mar 19	NA	
2	Acetamiprid	500	Apr 18-Mar 19	NA	
3	Acetochlor	8	Aug 18-Mar 19	NA	
4	Alachlor	8	Aug 18-Mar 19	5000	Apr 18-Mar 19
5	Aldrin	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
6	alpha-BHC	8	Aug 18-Mar 19	NA	
7	alpha-Endosulfan	50	Aug 18-Mar 19	5000	Apr 18-Mar 19
8	Ametryn	8	Jun 18-Mar 19	NA	
9	Amitraz	8	Aug 18-Mar 19	NA	
10	AMPA	500	Apr 18-Oct 19	NA	
11	Atrazine	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
12	Azinphos-methyl	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
13	Azoxystrobin	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
14	beta-HCH	8	Aug 18-Mar 19	5000	Apr 18-Mar 19
15	beta-Endosulfan	20	Aug 18-Mar 19	5000	Apr 18-Mar 19
16	Bifenthrin	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
17	Boscalid	8	Jun 18-Mar 19	NA	
18	Carbaryl	5000	Jun 18-Mar 19	NA	
19	Carbendazim	10000	Jun 18-Mar 19	NA	
20	Carbofuran	5000	Jun 18-Mar 19	NA	
21	Chlorantraniliprole	8	Apr 18-Mar 19	NA	
22	Chlorfenapyr	8	Aug 18-Mar 19	NA	
23	Chlorothalonil	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
24	Chlorpyrifos	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
25	Chlorpyrifos-methyl	8	Apr 18-Mar 19	NA	
26	cis-Chlordane	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
27	Clomazone	8	Aug 18-Mar 19	NA	
28	Cyfluthrin	20	Apr 18-Mar 19	5000	Apr 18-Mar 19
29	Cyhalofop-butyl	8	Aug 18-Mar 19	NA	
30	Cyhalothrin (lambda)	8	Aug 18-Mar 19	5000	Apr 18-Mar 19
31	Cypermethrin	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
32	Cyproconazole	8	Jun 18-Mar 19	NA	
33	delta-HCH	8	Aug 18-Mar 19	5000	Apr 18-Mar 19
34	Deltamethrin	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
35	Diazinon	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
36	Dicamba	5000	Apr 18-Mar 19	NA	
37	Dieldrin	8	Apr 18-Mar 19	NA	
38	Difenoconazole	500	Jun 18-Mar 19	5000	Apr 18-Mar 19
39	Endosulfan sulfate	20	Apr 18-Mar 19	5000	Apr 18-Mar 19
40	Endrin	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
41	Epoxiconazole	8	Jun 18-Mar 19	NA	
42	Ethion	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
43	Fipronil	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
44	Glyphosate	200	Apr 18-Oct 19	NA	
45	Heptachlor	8	Jun 18-Mar 19	5000	Apr 18-Mar 19
46	Heptachlor (epoxide B)	8	Jun 18-Mar 19	NA	
47	Hexachlorobenzene	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
48	Imazalil	8	Jun 18-Mar 19	NA	
49	Imazapyr	500	Apr 18-Mar 19	NA	
50	Imazetapyr	100	Apr 18-Mar 19	NA	
51	Iprodione	8	Aug 18-Mar 19	NA	
52	Isoxadifen-ethyl	8	Aug 18-Mar 19	NA	
53	Kresoxim-methyl	8	Aug 18-Mar 19	NA	
54	Lindane	8	Apr 18-Mar 19	NA	
55	Malaoxon	500	Jun 18-Mar 19	NA	
56	Malathion	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
57	Metalaxyl	500	Jun 18-Mar 19	NA	
58	Metalaxyl (Mefenoxam)	8	Jun 18-Mar 19	NA	
59	Methidathion	8	Aug 18-Mar 19	NA	
60	Methiocarb	5000	Jun 18-Mar 19	NA	
61	Methoxychlor	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
62	Metolachlor (S-Metolachlor)	8	Jun 18-Mar 19	NA	
63	Metribuzin	8	Aug 18-Mar 19	NA	
64	Metsulfuron Metil	500	Jun 18-Mar 19	NA	
65	Mirex	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
66	Oxyfluorfen	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
67	p,p'-DDD	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
68	p,p'-DDE	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
69	p,p'-DDT	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
70	Parathion	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
71	Parathion-methyl	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
72	Penoxulam	1000	Jun 18-Mar 19	NA	
73	Permethrin	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
74	Pirimiphos-methyl	8	Aug 18-Mar 19	NA	
75	Prochloraz	8	Aug 18-Mar 19	NA	
76	Profenofos	8	Aug 18-Mar 19	NA	
77	Propanil	8	Apr 18-Mar 19	5000	Apr 18-Mar 19
78	Propiconazole	8	Jun 18-Mar 19	NA	
79	Pyraclostrobin	8	Jun 18-Mar 19	NA	
80	Pyrimethanil	8	Aug 18-Mar 19	NA	
81	Simazine	8	Jun 18-Mar 19	5000	Apr 18-Mar 19
82	Tebuconazole	1000	Jun 18-Mar 19	NA	
83	Thiamethoxam	1000	Jun 18-Mar 19	NA	
84	Tiabendazole	500	Jun 18-Mar 19	NA	
85	trans-Chlordane	8	Aug 18-Mar 19	5000	Apr 18-Mar 19
86	Tricyclazole	1000	Jun 18-Mar 19	NA	
87	Trifloxystrobin	8	Jun 18-Mar 19	NA	
88	Trifluralin	8	Aug 18-Mar 19	NA	

Supplementary Data 3a

Optimized conditions and settings used in this study by LC-MS/MS. Q: Quantitative transition; q: qualitative transition; tR (min): retention time in minutes.

Pesticide	Transition	m/z		TR (min)
2,4-D	Q	215		7,5
	q	215	161	
	q		163	
Acetamiprid	Q	223	126	15,1
		223	99	
Acetoclor	Q	270	148	21,7
	q	270	224	
Alachlor	Q	270	162	21,8
	q	270	238	
Ametrina	Q	228	186	19,9
	q	228	96	
Amitraz	Q	294	122	24,6
	q	294	163	
Atrazine	Q	216	104	19,3
	q	216	174	
Azinphos Me	Q	318	132	20,1
	q	318	160	
Azoxystrobin	Q	404	344	20
	q	404	372	
Boscalid	Q	343	140	20,6
	q	345	271	
Carbaryl	Q	202	127	18,5
	q	202	145	
Carbendazim	Q	192	132	12,8
	q	192	160	
Carbofuran	Q	222	123	17,9
	q	222	165	
clorantraniliprole	Q	484	286	19,8
	q	484	453	
Clomazone	Q	240	125	19,9
	q	240	89	
	q	240	99	
Cyhalofop-butyl	Q	358	120	22,6
	q	358	256	
	q	358	302	
Ciproconazol	Q	292	125	21,3
	q	292	70	
Diazinon	Q	305	153	22,3
	q	305	169	
Dicamba	Q	219	175	6,8
	q	219	145	
Difenoconazol	Q	406	251	22,6
	q	406	337	
Epoxiconazol	Q	330	101	21,5
	q	330	121	
Fipronil	Q	435	250	9
	q	435	330	
Imazalil	Q	297	159	18,8
	q	297	201	
	q	299	161	
Imazapyr	Q	262	217	14,2
	q	262	220	
Imazethapyr	Q	290	177	17,2
	q	290	245	
Iprodione	Q	330	245	21,7
	q	330	288	
Malaoxon	Q	315	127	17,9
	q	315	99	
Malathion	Q	331	285	20,6
	q	331	99	
Metalaxyl	Q	280	192	19,5
	q	280	220	
Methidathion	Q	303	145	19,7
	q	303	85	
Methiocarb	Q	226	121	20,7
	q	226	169	
Metolachlor	Q	284	176	21,7
	q	284	252	
Metsulfuron	Q	382	141	17,8
	q	382	167	
Penoxsulam	Q	484	195	18,3
	q	484	326	
Pirimifos Me	Q	306	108	22,6
	q	306	164	
Procloraz	Q	376	266	21,8
	q	376	308	
Propanil	Q	218	127	20,8
	q	218	162	
Propiconazol	Q	342	159	22,3
	q	342	69	
Pyraclostrobin	Q	388	163	22,3
	q	388	194	
Tebuconazol	Q	308	125	22,1
	q	308	70	
Thiamethoxam	Q	292	181	12,6
	Q	292	211	
	q	292	246	
Tiabendazol	Q	202	131	14,2
	q	202	175	
Tricyclazole	Q	190	136	16,5
	q	190	163	
Trifloxystrobin	Q	409	186	22,5
	q	409	206	

Supplementary Data 3b

Optimized conditions and settings used in this study by GC-MS/MS. Q: Quantitative transition; q: qualitative transition; tR (min): retention time in minutes.

DA	Transitions	m/z	TR (min)
(E)-Chlorfenvinphos	Q	323.00>267.00	14.387
	q	323.00>295.00	
(Z)-Chlorfenvinphos	Q	323.00>267.00	14.696
	q	323.00>295.00	
2-Phenylphenol	Q	170.10>141.10	8.265
	q	170.10>115.10	
3-Hydroxycarbofuran	Q	180.10>137.00	7.749
	q	180.10>162.10	
Acephate	Q	136.00>94.00	7.647
	q	136.00>119.00	
Acetochlor	Q	223.10>132.10	12.077
	q	223.10>147.10	
Alachlor	Q	188.10>160.10	12.307
	q	188.10>132.10	
Aldrin	Q	262.90>193.00	13.46
	q	188.10>132.10	
alpha-BHC	Q	218.90>182.90	9.969
	q	218.90>144.90	
alpha-Endosulfan	Q	338.90>160.00	15.789
	q	338.90>266.90	
Ametryn	Q	227.10>170.10	12.514
	q	227.10>185.10	
Atrazine	Q	215.10>58.00	10.528
	q	215.10>200.10-	
Azinphos-methyl	Q	160.10>132.10	22.164
	q	160.10>77.00	
Azoxystrobin	Q	344.10>329.10	29.354
	q	344.10>183.10	
beta-BHC	Q	218.90>182.90	10.54
	q	218.90>144.90	
beta-Endosulfan	Q	338.90>160.00	17.723
	q	338.90>266.90	
Bifenthrin	Q	181.10>166.10	20.805
	q	181.10>153.10	
Boscalid	Q	342.10>140.10	26.002
	q	342.10>112.10	
Bromopropylate	Q	340.90>182.90	20.895
	q	340.90>184.90	
Bupirimate	Q	273.10>193.10	16.731
	q	273.10>150.10	
Buprofezin	Q	172.10>57.00	16.746
	q	172.10>131.10	
Cadusafos	Q	158.90>130.90	9.792
	q	158.90>97.00	
Captan	Q	149.10>105.10	14.932
	q	149.10>79.10	
Carbaryl	Q	144.10>116.10	12.472
	q	144.10>89.00	
Carbofuran	Q	164.10>149.10	10.391
	q	164.10>131.10	
Chlorantraniliprole	Q	278.00>249.00	21.076
	q	280.00>251.00	
Chlorothalonil	Q	265.90>168.00	11.092
	q	265.90>230.80	
Chlorpyrifos	Q	313.90>257.90	13.405
	q	313.90>285.90	
Chlorpyrifos-methyl	Q	285.90>93.00	12.102
	q	285.90>270.90	
cis-Chlordane	Q	372.80>263.90	15.759
	q	-372.80>336.80	
Clomazone	Q	204.10>107.00	10.574
	q	204.10>78.00	

DA	Transitions	m/z	TR (min)
Clothianidin	Q	132.00>71.00	8.065
	q	132.00>45.00	
Coumaphos	Q	362.00>226.00	24.425
	q	362.00>109.00	
Cyflutrhin-1	Q	226.10>206.10	25.248
Cyfluthrin-2	q	206.>199.10	25.443
Cyfluthrin-3			25.555
Cyfluthrin-4			25.65
Cyhalothrin-1	Q	197.00>141.00	22.34
Cyhalothrin-2	q	197>161	22.661
Cypermethrin-1	Q	181.10>152.10	25.88
Cypermethrin-2			26.083
Cypermethrin-3	q		26.178
Cypermethrin-4		181>127.00	26.272
Cyproconazole-1	Q	222.10>125.10	17.158
Cyproconazole-2	q	222.10≥76.10	17.204
Cyprodinil	Q	224.10>208.10	14.417
	q	224.10>197.10	
delta-BHC	Q	218.90>182.90	11.373
	q	218.90>144.90	
Deltamethrin-1	Q	252.90>93.00	28.651
Deltamethrin-2	q	252.90>171.90	29.022
Diazinon	Q	304.10>179.10	10.951
	q	304.10>162.10	
Dichlorvos	Q	185.00>93.00	6.004
	q	185.00>109.00	
Dicofol deg. (DCBP)	Q	250.00>139.00	13.843
	q	185.00>109.00	
Dieldrin	Q	276.90>241.00	16.64
	q	276.90>170.00	
Difenoconazole-1	Q	323.00>265.00	28.472
	q	323.00>202.00	
Difenoconazole-2	Q	323.00>265.00	28.575
	q	323.00>202.00	
Dimethoate	Q	125.00>79.00	10.266
	q	125.00>47.00	
Endosulfan sulfate	Q	386.80>252.90	19.019
	q	386.80>288.80	
Endrin	Q	262.90>191.00	17.326
	q	262.90>193.00	
Epoxiconazole	Q	192.00>138.00	20.112
	q	192.00>111.00	
Ethion	Q	230.90>174.90	17.876
	q	230.90>184.90	
Ethoprophos	Q	200.00>158.00	9.294
	q	200.00>114.00	
Etofenprox	Q	163.10>135.10	26.489
	q	163.10>107.10	
Famoxadone	Q	330.10>224.10	29.707
	q	330.10>196.10	
Fenazaquin	Q	160.20>145.10	21.506
	q	145.20>115.10	
Fenitrothion	Q	277.00>260.00	12.973
	q	277.00>109.10	
Fensulfothion	Q	293.00>153.00	17.662
	q	293.00>125.00	
Fenthion	Q	278.00>109.00	13.53
	q	278.00>125.00	
Fenvalerate-1	Q	419.10>225.10	27.602
Fenvalerate-2	q	419.10>167.10	28.002
Fipronil	Q	366.90>212.90	14.535
	q	366.90>254.90	
Fludioxonil	Q	248.00>182.00	16245

Supplementary Data 3b (Continued).

Optimized conditions and settings used in this study by GC-MS/MS. Q: Quantitative transition; q: qualitative transition; tR (min): retention time in minutes.

DA	Transitions	m/z	TR (min)
Flusilazole	Q	233.10>165.10	16.701
	q	233.10>152.10	
Flutriafol	Q	219.10>123.10	15.956
	q	219.10>95.00	
Fluvalinate-1	Q	250.10>55.00	27.828
Fluvalinate-2	q	250.10>200.0	27.962
Folpet	Q	259.90>130.00	15.123
	q	259.90>95.00	
gamma-BHC (Lindane)	Q	218.90>182.90	10.7
	q	218.90>144.90	
Heptachlor	Q	271.80>236.90	12.458
	q	271.80>117.00	
Heptachlor-exo-epoxide	Q	352.80>262.90	14.623
	q	352.80>281.90	
Hexaconazole	Q	214.00>159.00	16.199
	q	283.80>213.80	
Imazalil	Q	215.00>173.00	16.199
	q	215.00>159.00	
Iprodione	Q	314.00>245.00	20.534
	q	314.00>56.00	
Kresoxim-methyl	Q	206.10>116.10	16.777
	q	206.10>131.10	
Malathion	Q	173.10>99.00	13.224
	q	173.10>127.00	
Mepanipyrim	Q	222.10>221.10	15.805
	q	223.10>222.10	
Metalaxyl (Mefenoxam)	Q	249.20>190.10	12.514
	q	249.20>146.10	
Methamidophos	Q	141.00>95.00	5.931
	q	141.00>126.00	
Methidathion	Q	145.00>58.00	15.319
	q	145.00>85.00	
Methoxychlor	Q	227.10>169.10	21.076
	q	227.10>212.10	
Metolachlor (S-Metolachlor)	Q	238.10>162.10	13.349
	q	238.10>133.1	
Metribuzin	Q	198.10>82.00	12.128
	q	198.10>110.10	
Mirex	Q	271.80>236.80	22.878
	q	273.80>238.80	
Monocrotophos	Q	127.10>109.00	9.772
	q	127.10>95.00	
Myclobutanil	Q	179.10>125.00	16.655
	q	179.10>152.00	
Omethoate	Q	156.00>110.00	8.936
	q	156.00>141.00	
Oxadixyl	Q	163.10>132.10	17.845
	q	163.10>117.10	
Oxyfluorfen	Q	361.00>300.00	16.716
	q	361.00>252.0	
p.p'-DDD	Q	235.00>165.00	17.876
	q	235.00>199.00	
p.p'-DDE	Q	246.00>176.00	16.488
	q	246.00>211.00	
p.p'-DDT	Q	235.00>165.00	19.141
	q	235.00>199.00	
Parathion	Q	291.10>109.00	13.627
	q	291.10>137.00	
Parathion-methyl	Q	263.00>109.00	12.281
	q	263.00>136.00	
Pendimethalin	Q	252.10>162.10	14.373
	q	252.10>191.10	
Permethrin-1	Q	183.10>168.10	24.202
Permethrin-2	q	183.10>165.10	24.453
Phorate	Q	260.00>75.00	9.862
	q	260.00>231.00	
Phosmet	Q	160.00>133.00	20.76
	q	160.00>77.00	
Piperonyl butoxide	Q	176.10>131.10	19.886
	q	176.10>117.10	
Pirimicarb	Q	238.10>166.10	11.552
	q	238.10>72.00	
Pirimiphos-methyl	Q	305.10>180.10	12.889
	q	305.10>290.10	
Prochloraz	Q	180.10>138.10	24.578
	q	180.10>69.00	
Procymidone	Q	283.00>96.00	14.991
	q	283.00>255.00	
Propanil	Q	160.90>99.00	12.038
	q	160.90>90.00	
Propaquizafop	Q	443.10>299.10	31.898
	q	443.10>371.10	
Propiconazole-1	Q	259.00>69.00	18.912
	q	259.00>191.00	
Propiconazole-2	Q	259.00>69.00	19.11
	q	259.00>191.00	
Pyraclostrobin	Q	164.10>132.10	27.802
	q	164.10>77.00	
Pyrimethanil	Q	198.10>183.10	11.042
	q	198.10>158.10	
Pyriproxyfen	Q	136.10>78.00	22.34
	q	136.10>96.00	
Quintozene	Q	294.80>236.80	10.585
	q	294.80>264.80	
Quizalofop-ethyl	Q	372.10>299.10	26.218
	q	299.10>255.10	
Simazine	Q	201.10>173.10	10.437
	q	201.10>186.10	
Spiroxamine-1	Q	100.10>58.00	12.256
Spiroxamine-2	q		12.973
	q	100.10>72.00	
Tebuconazole	Q	250.10>125.10	19.629
	q	250.10>153.10	
Tefluthrin	Q	177.00>127.10	11.297
	q	177.00>137.10	
Terbacil	Q	161.00>144.00	11.309
	q	161.00>118.00	
Tetraconazole	Q	336.00>204.00	13.725
	q	336.00>218.00	
Tetradifon	Q	355.90>159.00	21.813
	q	355.90>228.90	
Tetramethrin-1	Q	164.10>107.10	20.639
	q	164.10>135.10	
Tetramethrin-2	Q	164.10>107.10	20.941
	q	164.10>135.10	
Thiabendazole	Q	201.10>174.10	14.932
	q	201.10>130.10	
Tolylfluanid	Q	238.00>137.10	14.652
	q	238.00>91.10	
Triadimenol-1	Q	168.10>70.00	15.006
			15.243
Triadimenol-2	q	168.10>112.10	
Trifloxystrobin	Q	222.10>190.10	18.836
	q	222.10>162.10	
Trifluralin	Q	306.10>264.10	9.573
	q	306.10>206.10	

Capítulo 2:

Comparación de métodos determinísticos y probabilísticos para la evaluación del riesgo ecológico acuático de los plaguicidas en una cuenca de uso mixto del suelo: Un estudio de caso en Uruguay

El contenido de este capítulo se encuentra publicado en la revista Science of the Total Environment y se presenta en el formato original del artículo:

Rodríguez-Bolaña, C., Pérez-Parada, A., Niell, S., Heinzen, H., & Teixeira de Mello, F. (2024). Comparative deterministic and probabilistic approaches for assessing the aquatic ecological risk of pesticides in a mixed land use basin: A case study in Uruguay. *Science of The Total Environment*, 910, 168704.

DOI: https://doi.org/10.1016/j.scitotenv.2023.168704

Science of the Total Environment 910 (2024) 168704

Contents lists available at ScienceDirect

Science of the Total Environment

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

Comparative deterministic and probabilistic approaches for assessing the aquatic ecological risk of pesticides in a mixed land use basin: A case study in Uruguay

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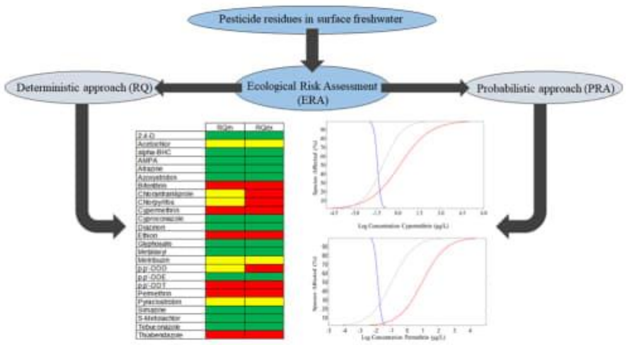
HIGHLIGHTS

- Deterministic and probabilistic risk assessment was conducted for 25 pesticides.
- Legacy and current-use insecticides indicated the highest ecological risk.
- High risk during spring and summer due to intensive agricultural production.
- Probabilistic approach provides an improvement over deterministic risk assessment.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Yolanda Picó

Keywords:
Deterministic risk assessment
Probabilistic risk assessment
Species sensitivity distributions
Legacy and current use pesticides
Drinking-water source

ABSTRACT

Environmental concentrations of 25 pesticides in superficial water were employed to conduct an ecological risk assessment (ERA) in a mixed land-use basin utilized as a drinking water source. A deterministic risk assessment (RQ) was utilized to evaluate the chronic risk to aquatic biota, while a probabilistic risk assessment (PRA) approach was applied to assess the acute and chronic risk in the most sensitive species and at the community level. A high risk was identified for insecticides (pyrethroids, organophosphates and organochlorines). RQs ranged from $4.0e^{-4}$ (2,4-D) to 105.3 (ethion) considering median concentrations and from $8.0e^{-4}$ (2,4-D) to 230 (p,p'-DDT) considering extreme concentrations. Temporal variation in ΣRQs showed the highest risk during spring and summer months, which is related to the crop calendar and land use in the Laguna del Cisne basin. For PRA, the probability of exceeding the hazardous concentration HC5 (5th percentile) was higher for the most

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https://doi.org/10.1016/j.scitotenv.2023.168704
Received 28 August 2023; Received in revised form 30 October 2023; Accepted 17 November 2023
Available online 20 November 2023

sensitive species in chronic exposure, especially for cypermethrin (38.9 %), permethrin (25.6 %), and chlorpyrifos (16.6 %). In the case of acute exposures, the probability of surpassing HC5 was higher for the entire freshwater biota, with the highest values observed for bifenthrin (28.3 %), cypermethrin (25.5 %), permethrin (11.75 %), and ethion (11.1 %). The advantages and disadvantages of PRA for assessing pesticide ecological risk were compared with the conventional deterministic RQs approach, highlighting that PRA offers improvements over the deterministic risk assessment, especially for organophosphate pesticides. Additionally, PRA provides a more comprehensive evaluation of risk for both short and long-term exposure, has the potential to incorporate others available toxicity data (e.g., LD50, Daily Intake), and utilizes different hazardous concentrations, such as HC5, HC10, and HC50. Our findings emphasize the urgent need to establish a national regulatory framework to evaluate and mitigate pesticide risks in aquatic ecosystems, especially in drinking water source like Laguna del Cisne.

1. Introduction

The intensification of agriculture and the subsequent extensive use of pesticides have been linked to several impacts on aquatic ecosystems like biodiversity loss and the disruption of ecosystem functions (Pérez-Parada et al., 2018; Sharma et al., 2019; de Souza et al., 2020). In this sense, once applied to the fields, the pesticide molecules may reach streams through different mechanisms, with surface runoff after rain events being a widely accepted pathway for the mobility of most pesticides (Triegel and Guo, 2018; Andrade et al., 2021; de Souza et al., 2022).

In aquatic systems, the physicochemical characteristics of pesticides, such as aqueous solubility, octanol-water partition coefficient, dissociation constant, organic carbon constant, and Henry's law constant, have a significant impact on their partitioning between environmental compartments (Pérez-Parada et al., 2018; Mac Loughlin et al., 2022; Rodríguez-Bolaña et al., 2023). In this sense, pesticides with higher water solubility are mainly present in the aqueous phase. In contrast, medium or low solubility are able to interact with suspended particles and sediments or accumulate in biological tissues (Pérez-Parada et al., 2018). This could pose a risk to aquatic biota, even at low concentrations, if toxicity is greater than water exposure (de Souza et al., 2022; Montagner et al., 2022).

Ecological Risk Assessment (ERA) of pesticides in aquatic ecosystems is often conducted using the Risk Quotients approach (RQs), involving the comparison of environmental concentrations (exposure) and reported ecological effects (toxicity) on sentinel species (Iturburu et al., 2019; Mac Loughlin et al., 2022; Mentzel et al., 2022). While RQs are commonly used for regulatory purposes in many countries, this deterministic approach is criticized for its conservative assumptions, leading to overestimated risks (Iturburu et al., 2019; Nagai, 2021; Mentzel et al., 2022).

In contrast, Probabilistic Risk Assessment (PRA) offers a more quantitative risk evaluation (Solomon et al., 2000; Maertens et al., 2022; Nagai, 2017; Mentzel et al., 2022; McNamara et al., 2023). PRA considers the variability in sensitivity among aquatic species and uses a cumulative distribution function to construct a species sensitivity distribution (SSD). Often, NOEC and EC50 values are utilized for chronic and acute toxicity assessments, respectively, to establish the predicted no-effect concentration (PNEC) (Posthuma et al., 2002; Nagai, 2017; Bertrand and Iturburu, 2023). In PRA, the intersection of the SSD and the probability distributions of environmental measurement concentrations (EC) determines the risk magnitude to aquatic life. Hazardous concentrations of 5 % of species (HC5) are commonly established to protect most species in the environment (Nagai, 2017; Maltby et al., 2005; Nagai, 2021; Maertens et al., 2022).

SSD calculation and PRA implementation are methods widely recognized within regulatory frameworks designed to assess the ecological risk of pesticides on aquatic ecosystems. PRA has been utilized by official agencies in the United States (USEPA, 2014), the European Union (EFSA (European Food Safety Authority), 2018), and Australia and Canada (Lee-Steere, 2009; Bhuller et al., 2021).

Latin American countries have their own pesticide regulations and surveillance programs. In this sense, half of the countries (Brazil, Bolivia, Colombia, Ecuador, Peru, and Venezuela) have incorporated risk assessment framework for aquatic pesticide risks into their legislations (Carriquiriborde et al., 2014; Casallanovo et al., 2021). However, even in these countries, their risk characterization is solely based on the worst-case deterministic approach, with no implementation of a probabilistic risk assessment (Carriquiriborde et al., 2014; Camargo et al., 2020). To advance in the assessment of aquatic environmental risk, regulatory authorities in Argentina, Brazil, and Chile have encouraged the use of toxicity bioassays in native species to predict the hazards of pesticides in local scenarios (Bertrand and Iturburu, 2023). These improvements aim to reflect and compare the potential effects of site-specific agricultural practices and environmental conditions on representative species exposed to different concentrations and mixtures of pesticides (Iturburu et al., 2019; Casallanovo et al., 2021; Bertrand and Iturburu, 2023). Nevertheless, this refinement does not yet include the implementation of PRA approaches.

In Uruguay, pesticide regulation dates to 1977 (Decree 149/977, https://www.impo.com.uy/bases/decretos/149-1977), making it one of the oldest in Latin America (Mañay et al., 2004; Carriquiriborde et al., 2014). The risk characterization process relies on the RQ approach and is applied only during the chemical registration or reevaluation process, utilizing acute and chronic toxicity data from internationally validated species. Additionally, no toxicity tests using native species are conducted, and no PRA is performed. While official monitoring of pesticides in water bodies is regularly performed, authorities are currently not conducting Ecological risk assessments. Only one risk assessment study has been published in Uruguay. Based on the RQ approach, the authors reported a high ecological risk in aquatic organisms for several pesticides and emerging contaminants in two protected lagoons (Griffero et al., 2019).

This is of great significance because the agricultural sector plays a significant role in the Uruguayan economy, representing one of the major contributions to the national Gross Domestic Product (GDP) (Soutullo et al., 2020; García-Préchac et al., 2022). In this context, Palladino et al. (2023) reported an increase in the amounts of herbicides applied per hectare, rising from 2.58 to 7.97 kg/ha. Additionally, in 2022, the country imported a total of 14.8 million kg of active pesticide ingredients (DGSA. Direccion de Servicios Agricolas, 2023).

Laguna del Cisne (LC) is one of the major sources of drinking water in Uruguay, serving over 100,000 people (Gonzalez-Fernández and Orcasberro, 2018; Rodríguez-Bolaña et al., 2023). In a previous study, we reported the occurrence and the annual variations of multiclass pesticides, including both legacy and current-use compounds in the superficial water of LC (Rodríguez-Bolaña et al., 2023). Furthermore, bioaccumulation of several pesticides in fish's tissues was documented (Rodríguez-Bolaña et al., 2023).

Considering that most of the detected compounds would pose a risk to aquatic organisms, and given the absence of an established framework for conducting risk assessments in Uruguay, the objectives of this study were (i) to perform an Ecological Risk Assessment (ERA) using RQ and PRA methods in LC based on our previously reported concentrations in superficial water, and (ii) to critically assess the advantages and disadvantages of these approaches, with the goal of establishing a national risk assessment approach for aquatic systems in Uruguay.

2. Materials and methods

2.1. Study area

Laguna del Cisne, located at 34°45′S; 55°49′W, is a shallow system with an average depth of 2.0 m and a total area of 127 ha (Rodríguez-Bolaña et al., 2023) (Fig. 1). The catchment area encompasses various land uses, including agriculture, afforestation, animal breeding, wetlands, and urbanized zones (Rodríguez-Bolaña et al., 2023). Land-use activities involve double cropping and crop-pasture rotations employing different management techniques. The primary summer crop is soybean, cultivated under continuous annual cropping with no-till (and maize to a lesser extent) (García-Préchac et al., 2022). Long-term crops in the area consist of exotic forest plantations, mainly *Eucalyptus* spp., vineyards, and a smaller scale of citrus, apple, and pear cultivation. To the south, it is bordered by the Salinas Norte urbanization; this housing development is located downstream in the drainage basin and is therefore not considered within the land uses of the basin.

The measured environmental concentrations (MECs) of pesticides in superficial water was obtained from Rodríguez-Bolaña et al. (2023). Experimental conditions and instrumentation used for pesticide analysis in water is described in Rodríguez-Bolaña et al. (2023). The average monthly concentration for each pesticide can be found in Supplementary Table 1.

2.2. Calculation of risk quotients

Pesticide physicochemical and toxicity properties were obtained from the Pesticide Properties Database (PPDB, 2022) (http://sitem.herts.ac.uk/aeru/projects/ppdb), and the US EPA ECOTOX database (https://cfpub.epa.gov/ecotox/).

Three trophic levels were taken into consideration: fish (*Oncorhynchus mykiss*), aquatic invertebrates (*Daphnia magna*), and algae (*Pseudokirchneriella subcapitata*, *Anabaena* sp., and *Scenedesmus* sp.).

Chronic risk in surface water was conducted based on the RQ approach, according to Eq. (1) Mac Loughlin, et al. (2022):

$$RQ = MEC/PNEC \quad (1)$$

where MEC is the measured environmental concentration of a pesticide in surface water, and PNEC is the predicted no-effect concentration calculated according to Eq. 2:

$$PNEC = CC/AF \quad (2)$$

where CC is the critical concentration and AF is the assessment factor.

The lowest NOEC (No-Observed-Effect Concentration) values for chronic exposure for fish, aquatic invertebrates, and algal species were employed to determine critical concentration (CC). In cases where NOEC values were not available, the lowest value of median lethal concentration (LC50) or half-maximal effective concentration (EC50) was used instead (Table S2).

The Assessment Factor (AF) was established following Iturburu et al. (2019). Specifically, an AF of 10 was used when three NOECs were available, 50 when two NOECs were available, 100 when only one NOEC value (for fish or invertebrates) was available, and 1000 when no NOEC values were available, and an L(*E*)C50 was used (Iturburu et al., 2019).

Median and maximum detected concentrations were utilized as the general and worst-case MEC for calculating the median RQ (RQm) and extreme RQ (RQex). Additionally, the monthly RQm was computed to identify temporal variations of pesticide levels within the system.

To categorize months based on each detected compound's cumulative individual RQ values, the sum of RQm, RQex, and the minimum RQ (RQmin) were calculated (Σ RQmonth).

The RQ values serve as risk indicators, where an RQ > 1 indicates a high risk of harmful effects; an RQ between 0.1 and 1 represents a

Fig. 1. Superficial water sampling sites in the Laguna del Cisne, Uruguay (34°44′46.32″ S, 55°49′18.12″ W) with the main tributaries Piedra del Toro, Cañada del Cisne and El Estero. Land uses in the basin range from natural pasture to intensive crops and afforestation. Red circle: drinking water facility.

medium expected risk (medium risk), RQ between 0.01 and 0.1 corresponds to low environmental risk, and an RQ < 0.01 signifies a negligible environmental risk (Iturburu et al., 2019).

2.2.1. Calculation of probabilistic risk assessment

For the probabilistic determination of ecological risk, both acute (EC50) and chronic (NOEC) toxicity values were used to establish species sensitivity distributions (SSDs). The toxicity data used in the SSDs were obtained from the ECOTOX database (https://cfpub.epa.gov/ecotox/).

Three freshwater taxonomic groups were selected to generate the SSD curves: primary producers (algae and macrophytes), aquatic invertebrates, and vertebrates (fishes). The criteria used for selecting the toxicity data included in the analysis are presented in Table 1. The data selection process followed the principles of reliability (Klimisch et al., 1997). We exclusively included data assessed as reliability class 1 or class 2.

To manage the dataset, we employed a data spreadsheet. Toxicity data reported as ppb or mol/L were converted to μg/L. When multiple toxicity values with similar endpoints and measure effects were reported for a species, the geometric mean was calculated (Xu et al., 2015). A minimum of ten species will be considered to generate the SSDs for each pesticide (Newman et al., 2000; Wheeler et al., 2002).

The toxicity data were fitted to a log-logistic distribution using the SSD toolbox program (Etterson, 2020), which was downloaded from https://www.epa.gov/endangered-species/provisional-models-endangered-species-pesticide-assessments. Confidence intervals of 2.5 % and 97.5 % were derived using a bootstrapping function with 1000 iterations. The Anderson-Darling goodness-of-fit test assessed whether the data followed a log-normally distributed.

Ecological risk refers to the probability of species being exposed to environmental concentrations of toxicants that exceed their effect concentrations (Posthuma et al., 2002). This probability can be calculated as the area under the curve (AUC) of the probability density function (PDF) of the measured concentrations and the cumulative density function (CDF) following the method described by Fedorenkova et al. (2012). The probability can be expressed by Eq. (3) as:

$$Pr(x1 > x2) = \int - \infty\infty(1 - \mathrm{CDFx1}(x)) \times \mathrm{PDFx2}(x)dx \quad (3)$$

where x1 is a random variable representing the logarithm of field concentrations, and x2 is a random variable representing the logarithm of species sensitivity concentrations (Fedorenkova et al., 2012).

Hazard concentrations (HC5) were estimated from each pesticide by determining the concentration corresponding to the 5th percentile. The evaluation endpoint was the protection of 95 % of species (Nagai, 2021; Maertens et al., 2022).

3. Results and discussion

3.1. Deterministic risk assessment

The median (MECm) and extreme (MECex) measured concentrations for the twenty-five pesticides (11 insecticides, 8 herbicides, and 6 fungicides) analyzed, along with their respective RQ values, are presented in Table 2. RQs based on MECm indicate that 24 % of the compounds exceeded the predicted non-effect concentration (PNEC) values of the most sensitive species. This percentage increases to 36 % for MECex. Ethion, cypermethrin, p,p'-DDT, permethrin, bifenthrin, and thiabendazole exhibited RQs higher than 1 for both median and maximum concentrations. Chlorantraniliprole, chlorpyrifos, and p'-DDD indicated a high risk only when considering the maximum concentration (Table 2).

All of these compounds are insecticides (except for the fungicide thiabendazole) that possess high potential toxicity not only for aquatic invertebrates but also for the entire aquatic community, leading to reduced diversity and biomass (Pérez-Parada et al., 2018; Bekele et al., 2021; Bertrand and Iturburu, 2023; Mali et al., 2023; Ranatunga et al., 2023).

The extremely high RQm value found by ethion (105.3) raises significant ecological concern in LC. Organophosphates (OP), including ethion, are known to be toxic to non-target organisms even at low concentrations and can be bioaccumulated in fish tissues, such as the liver and muscles, leading to immunotoxicological effects (Bekele et al., 2021; Gu et al., 2023). The toxicity of these pesticides is attributed to their irreversible inhibition of acetylcholinesterase activity, which affects the nervous system in aquatic and terrestrial fauna, including honeybees (*Apis mellifera*) (Sidhu et al., 2019). Additionally, these pesticides have been associated with an increased risk of cancer, neurotoxic, and genotoxic effects in humans (Sidhu et al., 2019; Mali et al., 2023). For this reason, ethion has been banned in the United States and many countries in the European Union (Rodríguez-Bolaña et al., 2023). However, in Uruguay, ethion is still used in cattle as an ectoparasite control for horn flies and ticks. The overuse of this compound prompted Uruguayan authorities to implement a temporary ban on ethion in 2016 due to the presence of high concentrations found in exported meat. This situation resulted in the rejection of the merchandise by the Food Safety and Inspection Service-USDA (https://www.impo.com.uy/bases/resoluciones-mgap/SN20160405001-2016).

The high RQ values of cypermethrin (16.6), permethrin (6.6) and bifenthrin (2.2) are especially concerning, as it has been extensively reported that pyrethroids can induce damage to the nervous system, oxidative stress, and endocrine disruption in aquatic insects and fishes (Schulz et al., 2021; Ranatunga et al., 2023; Xie et al., 2022; Zhu et al., 2020).

Bifenthrin has been banned by for agricultural use in the European Union (EFSA (European Food Safety Authority), 2022) and is classified as a restricted-use pesticide by the US Environmental Protection Agency (USEPA, 2017), it is still used in various types of crops in Uruguay, including soybean, maize, forestry, and fruits crops (Rodríguez-Bolaña et al., 2023).

Table 1
Data selection criteria used for the toxicity data included in the analysis. EC50 = median effect concentration; NOEC = no-observed-effect concentration.

	Acute toxicity data			Chronic toxicity data		
	Fish	Invertebrates	Primary producers	Fish	Invertebrates	Primary producers
Endpoint	EC50	EC50	EC50	NOEC	NOEC	NOEC
Measured effect	Mortality Immobilization	Mortality Immobilization	Growth rate Abundance	Mortality Immobilization Behavior Growth rate Development	Mortality Immobilization Feeding rate Growth rate Reproduction	Growth rate Abundance
Test duration (days)	2–4	2–4	1–4	>21	>21	macrophytes >6 algae >2

Table 2
Risk quotient (RQ) of pesticides detected in water samples from Laguna del Cisne during April 2018 – March 2019. based on median (MECm) and extreme (MECex) concentration. Green: lower risk; yellow: Moderate risk; red: high risk.

Pesticide	MECm (μg/l)	MECex (μg/l)	Assessment factor	PNEC (μg/l)	RQm	RQex
S-Metolachlor	0.019	0.183	100	7.07	0.003	0.026
Chlorantraniliprole	0.017	0.120	50	0.0894	0.19	1.34
p,p'-DDD	0.017	0.112	1000	0.07	0.249	1.59
AMPA	0.705	1.63	1000	200	0.003	0.008
p,p'-DDT	0.016	0.233	1000	0.001	16.2	230.0
Chlorpyrifos	0.005	0.015	10	0.001	0.56	1.54
Glyphosate	0.550	0.970	10	200	0.003	0.005
Atrazine	0.052	0.063	10	10	0.005	0.006
Azoxystrobin	0.031	0.037	10	4.4	0.007	0.008
Ethion	0.118	0.171	100	0,0011	105.3	153.3
Bifenthrin	0.008	0.104	10	0.0013	2.21	26.10
Cyproconazole	0.007	0.043	10	2.1	0.003	0.021
Metalaxyl	0.019	0.024	100	4.2	0.002	0.003
Acetochlor	0.016	0.019	10	0.059	0.27	0.32
p,p'-DDE	0.002	0.003	100	1.3	0.002	0.003
2.4-D	1.15	2.20	10	2720	0.0004	0.0008
Cypermethrin	0.050	0.061	10	3	16.6	20.0
Simazine	0.022	0.026	100	0.4	0.004	0.004
Permethrin	0.012	0.032	100	0.00093	6.66	17.77
Metribuzin	0.014	0.023	1000	0.42	0.032	0.054
Diazinon	0.002	0.005	10	0.056	0.037	0.087
Tebuconazole	0.002	0.003	10	1	0.003	0.003
Thiabendazole	1.61	1.87	10	1.2	1.34	1.56
Pyraclostrobin	0.017	0.017	100	0.04	0.21	0.21
alpha-BHC	0.003	0.002	100	1	0.001	0.002

Synergistic toxic effects between pyrethroids (Ranatunga et al., 2023; Ren et al., 2023) and between pyrethroids and OP insecticides are commonly observed in aquatic ecosystems (Cedergreen, 2014; de Souza et al., 2020; Martin et al., 2021; Zhan et al., 2022). Even Martin et al. (2021) reported potential joint toxic effects of combinations of herbicides (triazine), fungicides (azole), and pyrethroids at environmentally relevant doses. Azole fungicides inhibit CytP-450 enzymes, which is the major detoxification pathway of pyrethroids (Kasai, 2004; Ye et al., 2022). However, further toxicity tests must be conducted to confirm these interactive effects.

Noteworthy are the high RQm and RQex values for p,p′-DDT (16.2 and 230.5, respectively). Like other organochlorine pesticides (e.g., aldrin, dieldrin, endrin, and heptachlor), DDT was banned in Uruguay for pest control in natural and artificial fields in 1968, and globally by the Stockholm Convention in 2001 (Mañay et al., 2004). However, due to its physicochemical properties, such as high persistence, low water solubility, and high Kow, residues of DDT and its metabolites, DDD and DDE, are still frequently detected in aquatic systems worldwide (de Souza et al., 2020; Vasseghian et al., 2021; Montagner et al., 2022). Like other Persistent Organic Pollutants (POPs), their significant environmental impact on freshwater ecosystems is well documented (Barra et al., 2006; Sharma et al., 2019; Vasseghian et al., 2021; Krithiga et al., 2022). In Latin America, environmental contamination with DDT and its metabolites has been reported in various aquatic matrices (Barra et al., 2006; Souza et al., 2022; Montagner et al., 2022), including groundwater (Grondona et al., 2023), despite decades of bans. Other POPs such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and especially *per-* and polyfluoroalkyl substances (PFASs) have also been detected in Latin American aquatic systems (Souza et al., 2022). Most of these chemicals are included in the Stockholm Convention; however, they are still in use in many countries, and their occurrence in aquatic environments is not as well studied as DDT. In this sense, Latin America countries should create monitoring programs for these compounds to include in regulatory actions.

The values of monthly ΣRQ months are presented in Fig. 2. This approach assumes a concentration addition effect, where mixture toxicity is based on the cumulative effects of each pesticide individually (Iturburu et al., 2019). The results indicate a very high risk for RQmin, RQm, and RQex during the summer months (January to March), and a high risk during winter and autumn (Fig. 2). Only during October (spring), a medium expected risk was observed, with ΣRQ values ranging from 0.1 to 1.

The higher risk observed during the summer months could be attributed to the intensive use of pesticides in the basin during this period, greater number of detections and at higher concentration levels. However, ethion is the compound that most contributes to the pesticide mixture risk in these months, with 87 % and 79 % of the total ΣRQm value in February and March, respectively. On the other hand, the value of RQex for p,p'-DDT (RQex = 40) explained the peak observed in July (ΣRQex = 40.2). In this sense, the presence of DDT in the system, even after decades of banning, could be explained by its physicochemical properties, such as high environmental persistence and Koc, as well as an input from the basin through surface runoff during this month of heavy rainfall, influenced by the hydrological conditions of LC basin (Rodríguez-Bolaña et al., 2023).

These results provide insights into the risk posed by legacy and current-use pesticides to aquatic biota in LC. Additionally, the seasonal variability found in the risk level indicates that organisms are exposed to more complex mixtures of pesticides with high concentrations in spring-summer months. In subtropical freshwater ecosystems like LC, the maximum biological activity occurs during these months (Kruk et al., 2009), so this scenario would represent a major threat to the biological integrity of the system. In this context, it is crucial to emphasize the importance of the ΣRQ (sum of Risk Quotients), as it signifies the overall potential risk posed by pesticides. This perspective holds greater significance than the approach employed by government agencies, which

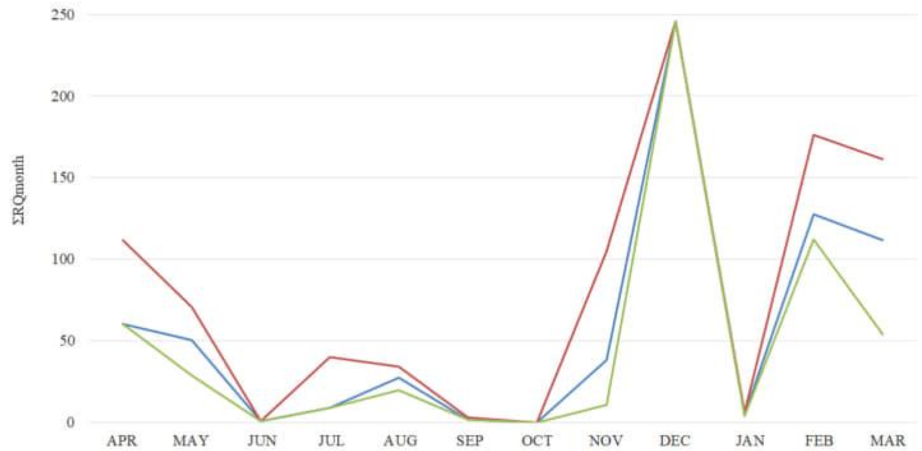

Fig. 2. Temporal values of monthly ΣRQmin (green line); ΣRQm (blue line) and ΣRQex (red line) in Laguna del Cisne during April 2018 – March 2019.

relies on the individual RQ analysis of each pesticide separately.

3.2. Probabilistic risk assessment

Due to the insufficient data for local representative species, the SDD curves in our study were constructed using freshwater species reported worldwide. The absence of toxicity data from native species represents a limitation in neotropical aquatic systems (Bertrand and Iturburu, 2023); however, it has been observed that for insecticides (Maltby et al., 2005; Bertrand and Iturburu, 2023) and herbicides (Van den Brink et al., 2006) SSDs constructed using species recommended in test guidelines did not show significant differences from those constructed using non-recommended species.

As species sensitivity is linked to the mode of action of pesticides (Maltby et al., 2005; Nagai, 2021), our article undertook a comparison of SDDs based on the most sensitive species with those derived from various taxonomic groups (at the community level) for both chronic and acute toxicity. The outcomes revealed that insecticides exhibit greater toxicity towards arthropods than other species groups, while primary producers frequently emerge as the most sensitive taxonomic group to herbicides (Fig. 3). Owing to the unclear disparity in sensitivity across taxonomic groups for fungicides (Nagai, 2017), we constructed the SDD curves using all available taxonomic groups.

To predict the effect of chronic exposure, the SDD was constructed using no observed effect concentrations (NOECs), while short-term exposure regimes were based on acute EC50s values. The hazardous concentrations for 5 % of the species (HC5s) derived for each pesticide with at least 10 species are summarized in Table 3. In the case of AMPA, azoxystrobin, and tebuconazole was not possible to generate an acute SSD due a lack of available information. In addition, for alpha-BHC, chlorantraniliprole, cyproconazole, p-p'DDD, p,p`-DDE, pyraclostrobin, and thiabendazole insufficient toxicity data were available to generate an acute and chronic SSD.

3.2.1. Chronic toxicity

Sensitive species data for chronic toxicity (NOEC) was limited; however, when available, the number of species at risk at the environmental concentration (EC) was higher for individual species compared to the community level. This indicates two different tolerance distributions related to the mode of action of the pesticides (Table 3).

Like the RQ approach, the PRA results showed that insecticides constitute the highest ecological risk in the system for both sensitive and all species. However, in this approach, pyrethroids pose a higher risk than organophosphates.

The fungicide metalaxyl was the only non-insecticide exceeding a 5 % risk at the environmental concentration (12.5 %).

At the community level, the probability of exceeding HC5 was highest for pyrethroids, with cypermethrin (21.6 %) and bifenthrin (19.8 %) having the highest probabilities. Followed by organophosphates, with ethion (16.1 %) and chlorpyrifos (12.1 %). The probability curve obtained by comparing the SSD and the distribution of the ECs for these compounds is presented in Fig. 4.

Permethrin exceeded the HC5 only when considering arthropod data, with 25.6 % of species at risk. These results suggest that non-arthropods have a low susceptibility to this compound. However, the ecological effect on arthropod in LC should be considered very high.

On the other hand, for p,p′-DDT, the HC values indicated an ecological risk for 10.6 % of aquatic community species (Table 3). In the case of its metabolite, p,p`-DDE, which showed a medium and high expected risk based on RQm and RQex, respectively, the calculation of HC5 value was not possible due to data unavailability, underscoring the limited alternatives to estimate ecological risk in such cases other than relying on RQ. The ecological risk calculation posed by the metabolite p,p`-DDE should not be skipped as it has a higher acute toxicity than its parent compound.

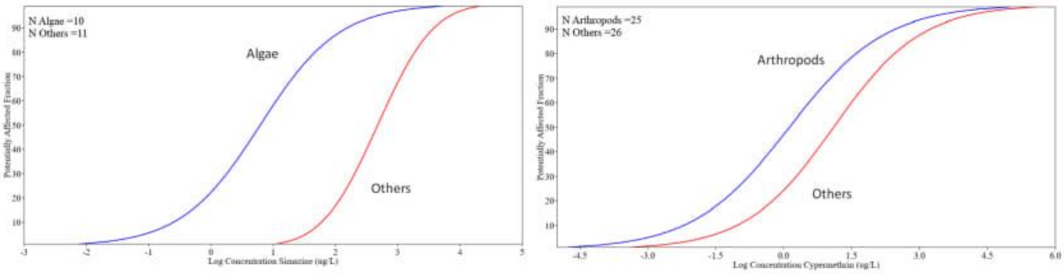

Fig. 3. Species sensitivity distributions for sensitive groups (Algae for herbicide Simazine, and Arthropods for insecticide Cypermethrin) and for others aquatic species based in long-term toxicity. N: Number of species used to construct SSDs. Toxicity values were obtained from the ECOTOX database.

Table 3
Hazardous concentrations for 5 % of species (HC5; µg/L) for chronic (A) and acute toxicity (B), based in Species Sensitivity Distributions and environmental pesticide concentrations (EC) in Laguna del Cisne. For sensitive species: Arthropods were considered for insecticides, primary producers for herbicides, and all taxonomic groups for fungicides. These concentrations were determined for all species at risk in relation to environmental pesticide concentrations (EC) in Laguna del Cisne Use: I: insecticide; H: herbicide; F: fungicide. ND: No data available. Pesticides are sorted by risk at EC for sensitive species. N: Number of species used to estimate HC5.

		Sensitive Species			All Species		
A. Chronic toxicity	Use	N	HC5 (95 % CI)	Species at risk at EC	N	HC5 (95 % CI)	Species at risk at EC
Cypermethrin	I	25	8.8e-4 (1.3e-4 - 5.8e-3)	38.9	51	9.9e-4 (1.4e-4 - 6.8e-3)	21.6
Permethrin	I	30	8.3e-4 (1.3e-4 - 5.0e-3)	25.6	45	5.9e-2 (1.3e-2 - 2.6e-1)	2.75
Chlorpyrifos	I	33	9.7e-4 (2.4e-4 - 3.7e-3)	16.6	67	1.9e-3 (6.8e-4 - 5.4e-3)	12.1
Bifenthrin	I		ND	ND	12	2.8e-3 (1.8e-04 - 4.4e-2)	19.8
Ethion	I		ND	ND	17	1.6e-1 (3.0e-2) - 8.9e-1	16.1
p,p'-DDT	I		ND	ND	11	8.0e-3 (1.0e-4 - 6.1e-1)	10.6
2.4-D	H	17	1.5e+1 (6.9e-1 - 3.3e+2)	1.2	46	2.8e+1 (5.3–1.4e+2)	0.9
Simazine	H	10	8.3e-2 (8.0e-3 - 8.8e-1)	2.1	21	3.6e-1 (5.1e-2 - 2.6)	3.1e-2
Atrazine	H	35	1.4e-2 (6.1e-1 - 3.6)	2.9e-2	73	1.4 (7.2e-1 - 3.0)	2.1e-1
Glyphosate	H	47	3.5e+1 (1.4e+1–8.6e+1)	1.6e-1	87	3.7e+1 (2.3e+1–6.0e+1)	4.8e-2
Metribuzin	H	31	5.9e-1 - 4.6)	3.0e-2	51	7.1e-1 (2.1e-1 - 2.3)	0.1
S-Metolachlor	H		ND	ND	10	2.0e-1 (4.3e-3 - 1.0e+1)	4.2
Acetochlor	H		ND	ND	10	9.9e-2 (3.2e-3 - 2.9)	2.3
AMPA	H		ND	ND	10	8.5 (2.7e-2 - 2.6e+5)	0.9
Metalaxyl	F	11	7.1e+1 (5.1–1.0e+3)	12.5	11	7.1e+1 (5.1–1.'e+3)	12.5
Tebuconazole	F	10	4.2e+2 (1.6e+2–1.1e+2)	1.1e-6	10	4.2e+2 (1.6e+2–1.1e+2)	1.1e-6
Azoxystrobin	F		ND	ND	13	1.4 (5.6e-1 - 3,9)	3.9e-2

		Sensitive Species			All Species		
B. Acute toxicity	Use	N	HC5 (95 % CI)	Species at risk at EC	N	HC5 (95 % CI)	Species at risk at EC
Bifenthrin	I	13	6.0 e-3 (1.6e-3 - 2-3e-2)	26.8	16	2.7e-3 (4.2e-4 - 1.6e-2)	28.3
Cypermethrin	I	16	1.7e-2 (6.1e-3 - 4.8e-2)	20.7	20	1.0e-3 (7.5e-5 - 1.4e-2)	25.5
Permethrin	I	19	1.2e-2 (3.3e-3 - 4.5e-2)	9.8	27	2.8e-3 (2.3e-4 - 3.3e-2)	11.7
Ethion	I	10	1.2 (1.0e-1 - 15.6)	5.8	14	2.3e-1 (1.4e-2 - 3.8)	11.1
Chlorpyrifos	I	31	5.6e-2 (2.3e-2 - 1.3e-1)	0.2	44	1.8e-2 (4.9e-3 - 7.1e-2)	3.4
Diazinon	I	11	2.5e-1 (4.0e-2 - 1.5)	7.1e-3	22	4.5e-1 (3.0e-2 - 6.7)	0.9
p,p'-DDT	I	15	3.6e-1 (1.0e-1 .- 1.2)	0.1	23	4.2e-1 (1.3e-1 - 1.3)	0.82
2.4-D	H	14	5.9e+1 (1.1e+1–3.1e+2)	2.1e-2	23	1.1e+2 (1.7e+1–6.9e+2)	1.7e-1
Simazine	H	26	2.4e+1 (9.8–61.8)	1.2e-3	33	2.6e+1 (10.2–67-2)	2.9e-3
Atrazine	H	55	7.9 (4.0–15.7)	2.2e-2	73	5.8 (2.7–12-5)	1.4e-2
Glyphosate	H	15	4.1e+1 (7.3–2.3e+2)	1.3e-2	37	2.3e+2 (6.2e+1–8.5e+2)	2.0e-3
Metribuzin	H	12	2.8e-1 (1.6e-2 - 4.9)	1.6	12	2.9e-1 (1.6e-2 - 5.1)	1.8
S-Metolachlor	H	11	6.5 (1.4e-1 - 2.9e+2)	0.6	13	4.8e+1 (5.4–4.3e+2)	3.0e-3
Acetochlor	H		ND	ND	8	1.0e+3 (1.1 e+1–8.4e+2)	2.0e-4
Metalaxyl	F	10	1.2e+4 (4.1e+3–3.8e+3)	1.1	10	1.2e+4 (4.1e+3–3.8e+3)	1.1

3.2.2. Acute toxicity

Once again, the pyrethroids insecticides bifenthrin (28.3 %), cypermethrin (25.5 %), permethrin (11.75 %), and the OP ethion (11.1 %) significantly exceeded the HC5 values for short-term exposure distribution in water (Table 3). The joint probability curve, obtained by comparing the SSD and the of the ECs for these compounds, is presented in Fig. 5.

Contrary to long-term exposures, the percentage of species affected in acute exposure was highest at the community level, except for the herbicide's glyphosate and metolachlor.

Therefore, the temporal variations of pesticides observed in LC could represent a high ecological risk to the entire freshwater community, particularly during the spring and summer months. However, it is important to note that the toxicity data used in PRA are generated by tests where organisms are exposed to specific concentrations for a certain period (Nagai, 2021; Bertrand and Iturburu, 2023).

To fully characterize the potential effect of short-term exposure or pulsed exposures to pesticides on the community, more frequent sampling is needed to capture the magnitude, frequency, and duration of pulsed exposures during the months of highest risk. Additionally, it is important to consider possible synergistic and antagonistic effects between different pesticide mixtures to comprehensively evaluate the risk to aquatic biota.

Both RQ and PRA approaches have highlighted the significant ecological risk generated by insecticides to the aquatic biota in LC. However, the methodological approach determined differences in the substance groups involved in the highest risk, related to the fact that RQ is based on the most sensitive species, and PRA on a range of toxicity values. Ethion exhibited the highest risk according to RQm, while in PRA, the major hazard is associated with pyrethroids. Ethion is recognized for its high toxicity to arthropods, presenting an EC50 of 0.056 µg/L for *Daphnia magna*. However, its impact on fish is relatively less severe, with an EC50 of 500 µg/L in *Pimephales promela* (PPDB, 2022). In contrast, the pyrethroids bifenthrin, cypermethrin, and permethrin exhibited EC50 values of 0.11, 0.21 and 0.6 µg/L for *D. magna*, but are highly toxic to fishes registering EC50 values of 0.26, 1.51 and 12.5 µg/L respectively.

Consequently, for a more precise Ecological Risk Assessment (ERA), it becomes imperative to incorporate sensitivities across multiple species. Relying solely on the most sensitive species tends to overstate the pesticide risks, particularly in the case of organophosphates.

In the case of pyrethroids, RQ tends to underestimate the risk. These compounds are extensively used worldwide, and pesticide residues are detected regularly in aquatic ecosystems (Souza et al., 2022; Xie et al., 2022). To assess the more realistic exposure impacts of these compounds on aquatic biota, a PRA approach is strongly recommended, as it includes the variability in toxicological data between species.

Also, using PRA allows the evaluation of chronic and acute impacts on aquatic communities. This impact was different according to the type of exposure and pesticide mode of action. For chronic exposure, the mode of action determines a higher risk for the most sensitive group (e.g., insecticides more toxic to invertebrates). On the contrary, in short-term exposure, the pesticide mode of action is less determinant since the higher risk was observed in the entire aquatic community. These results indicate the need to incorporate the agricultural calendar in aquatic risk evaluations since the community hazard is related to the

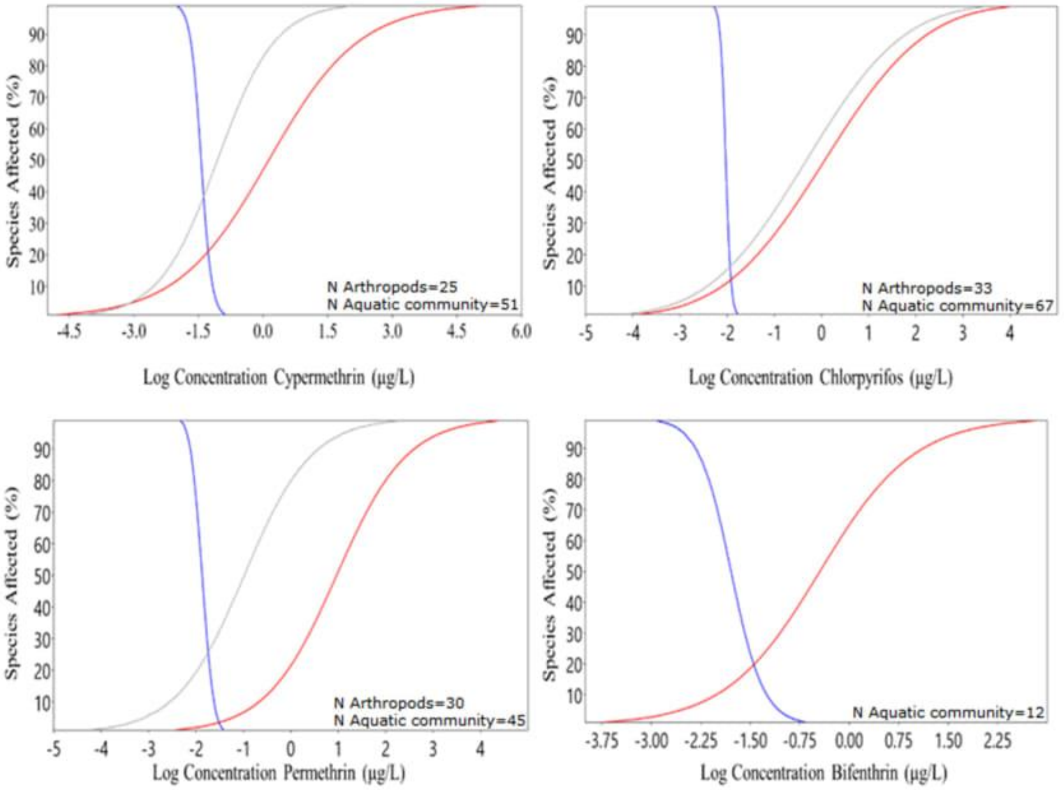

Fig. 4. Probabilistic Environmental Risk with Environmental Concentration distribution (blue line) and Species Sensitivity Distributions for arthropods (gray line) and aquatic community (red line) based on chronic toxicity values. N: Number of species used to construct SSDs.

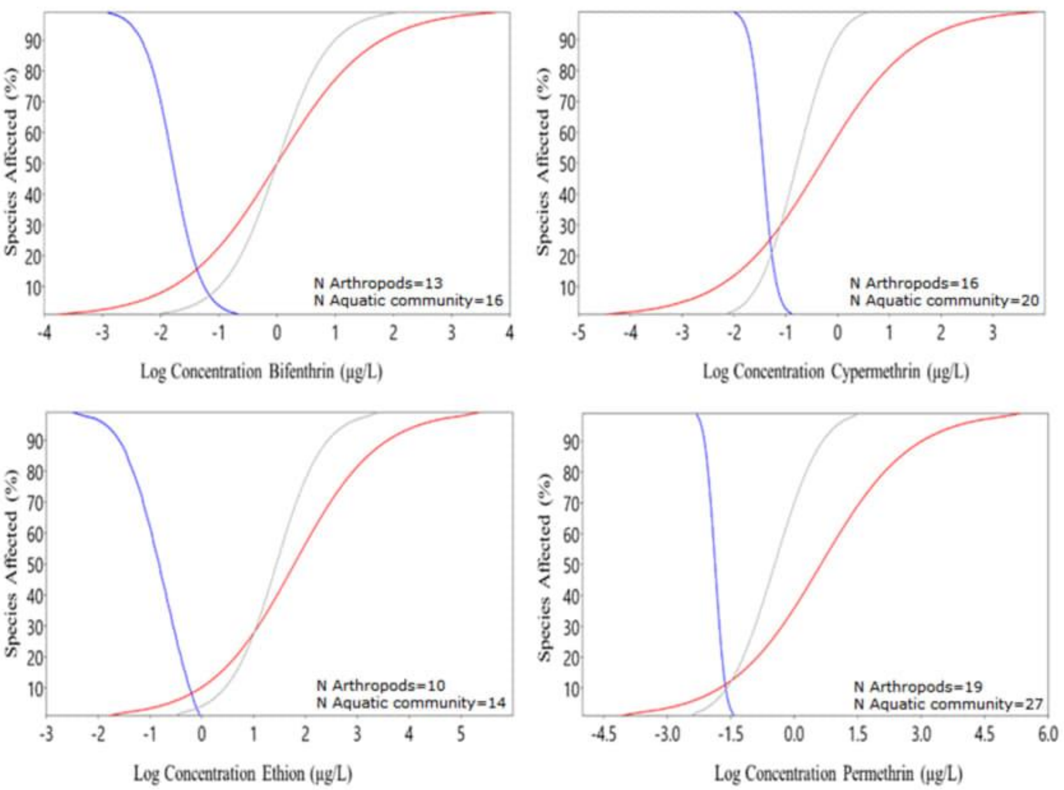

Fig. 5. Probabilistic Environmental Risk with Environmental Concentration distribution (blue line) and Species Sensitivity Distributions for arthropods (gray line) and aquatic community (red line) based on acute toxicity values. N: Number of species used to construct SSDs.

magnitude and duration of pesticide pulses.

Another improvement of the probabilistic assessment approach involves the utilization of various hazardous concentrations, such as HC5, HC10, and HC50, to provide information about the level of exposure for sensible species or an entire community. Additionally, this approach has the capacity to incorporate a range of available online toxicity data (e.g., LD50, Daily Intake), to enhance ecological risk assessment.

However, one of the most significant limitations of applying SSD is the insufficient availability of toxicity data necessary to construct the SDD curves (Posthuma et al., 2002; Maltby et al., 2005; Nagai, 2021; Maertens et al., 2022). In such cases, the deterministic approach becomes the only alternative for determining risk assessment, particularly for newly introduced compounds. In these cases, it is highly recommended to use toxicity data derived from native species as they possess site-specific characteristics essential for accurate risk evaluation (Demetrio et al., 2022).

In this sense, in Latin America, one of the major markets for pesticides, and contamination of aquatic environment ecosystems has risen dramatically (Carriquiriborde et al., 2014; Camargo et al., 2020; Rodríguez-Bolaña et al., 2023), several regional regulatory authorities have been promoting toxicity bioassays with local fauna as an input for the development of ERAs. Developing local scenarios will allow the impact of agricultural practices and environmental conditions like weather, soil, and hydrology on pesticide exposure/effect on aquatic organisms (Casallanovo et al., 2021; Demetrio et al., 2022; Bertrand and Iturburu, 2023).

In Uruguay, pyrethroids and OP were used at high rates (DGSA. Direccion de Servicios Agricolas, 2023), and in similar crops (Scarlato et al., 2022). Our findings suggest redundant application of insecticides in the basin, which is not the first occurrence of irregular pesticide practices in LC (Gonzalez-Fernández and Orcasberro, 2018; Rodríguez-Bolaña et al., 2023). In this context, we strongly recommend establishing a national regulatory framework that includes risk assessment guidelines to determine the level of contamination in aquatic ecosystems.

The challenges associated with pesticides in aquatic environments require the need to develop an ERA framework to evaluate and mitigate their impacts on aquatic biota. These toxicants are subject to regulation worldwide, which involves the conduct of an ERA for non-target organisms, and deterministic approach represent a common and traditional method. Based on our results, the adoption of PRA methods over RQ is strongly recommended. This approach account for variability in pesticide exposure and its effects, providing a more comprehensive evaluation of potential risks. However, the combined effects of compound mixtures remain largely unexplored. Therefore, future research should focus on unraveling the intricate toxicological interactions between pesticides and examining their potential impacts on aquatic organisms.

4. Conclusion

This study contributes to assessing the environmental risks associated with pesticides in aquatic ecosystems by comparing deterministic and probabilistic approaches using a mixed land-use basin as a case study.

The ecological risk assessment conducted for both approaches revealed a significant ecological risk linked to the concentrations of insecticides. However, while the use of the most sensitive species determined that OP ethion was the most dangerous compound, the consideration of several sensitivities allowed us to identify that pyrethroids were the riskiest compounds for the aquatic biota. Our result highlights that the application of the PRA approach provides a more comprehensive assessment of risk, especially for OPs which are highly toxic to arthropods but less harmful to other groups such as fish.

The use of species sensitivity distribution allows us to determine differences between the percentage of species affected by acute and chronic exposure. The higher probability of risk observed at the community level for acute shows the relevance of assessing the influence of crop calendar and rainfall events for environmental monitoring and risk characterization due to represent a significant concern for the aquatic biota, mainly in summer months.

A regular monitoring program and adaptive strategy based on environmental results are necessary for LC to prevent the impacts of pesticides on aquatic organisms and human health, especially considering its significance as a drinking water source. When determining the sampling frequency, the crop calendar should be considered, ensuring that it incorporates the magnitude and duration of pulsed exposures during intensive pesticide use in the basin.

In contrast, the mode of action characteristic of each pesticide determines that in long-term exposure, the most sensitive group is more affected.

This study represents the first report of PRA of pesticides in aquatic ecosystems and the first RQ based on annual monitoring in Uruguay, A regular monitoring program and adaptive strategy based in environmental results are necessary to LC to prevent the impacts of pesticides on aquatic organisms and human health, especially considering its significance as a drinking water source. When determining the sampling frequency, the crop calendar should be considered, ensuring that it incorporates the magnitude and duration of pulsed exposures during intensive pesticide use in the basin.

Given that Uruguay currently carries out monitoring surveys and lacks a government-led ERA, our results emphasize the urgent need to establish a national regulatory framework that includes risk assessment guidelines to determine the level of contamination in our aquatic ecosystems. To achieve this, Uruguay should update its legislation on pesticide use and not only encourage risk analysis but also promote toxicology testing on native species to obtain a more realistic picture of the risks entailed. This would allow for the development and refinement of ecological risk assessments, aligning Uruguay with other countries in the world.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.168704.

CRediT authorship contribution statement

César Rodríguez-Bolaña: Conceptualization, Investigation, Methodology, Writing – original draft, Data curation, Formal analysis, Visualization, Writing – review & editing. **Andrés Pérez-Parada:** Conceptualization, Writing – review & editing, Supervision. **Silvina Niell:** Resources, Writing – review & editing. **Horacio Heinzen:** Resources, Writing – review & editing. **Franco Teixeira de Mello:** Funding acquisition, Conceptualization, Investigation, Writing – review & editing, Supervision.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the Food and Agriculture Organization of the United Nations (FAO) through project GCP/URU/031/GFF, and by the Comisión Académica de Postgrado (CAP) (BFPD_2022_1_43490721). AP-P and FTM thank the Sistema Nacional de Investigadores and the PEDECIBA. CR-B, AP-P and FTM thanks the Programa de Doctorado en Ciencias Ambientales (Udelar).

The authors thank Dr. Guillermo Goyenola, MSc Giancarlo Tesitore, MSc Alejandra Kröger, and Lic. Martín Pacheco for their assistance in field sampling, as well as MSc. Samanta Stebniki for her technical assistance throughout the project.

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Capítulo 3:

Uso de multi-biomarcadores para evaluar la toxicidad del chlorantraniliprole en la especie nativa *Cnesterodon decemmaculatus* (Jenyns, 1842).

El contenido de este capítulo se encuentra bajo revisión en la revista Environmental Toxicology and Chemistry y se presenta en formato de manuscrito.

Rodríguez-Bolaña, C., Pérez-Parada, A., Hued, C., Bonifacio, A:F., Tagliaferro, M., Teixeira de Mello, F. (En revisión). Multibiomarkers approach to assess the acute toxicity of chlorantraniliprole in *Cnesterodon decemmaculatus* (Jenyns, 1842) (Cyprinodontiformes: Poeciliidae). Environmental Toxicology and Chemistry.

Multibiomarkers approach to assess the acute toxicity of chlorantraniliprole in *Cnesterodon decemmaculatus* (Jenyns, 1842) (Cyprinodontiformes: Poeciliidae)

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Highlights

- Effects of acute sublethal exposition chlorantraniliprole (CHL) were evaluated in *Cnesterodon decemmaculatus*
- Behavioral, morphological, and biochemical biomarkers were applied.
- Hormesis phenomena for AChE and CAT were found in organisms.
- The IBR index indicates disturbance levels increase with concentration.
- CHL may have significant consequences at individual and ecological levels.

Abstract

Diamide insecticides are among the most promising alternatives to older insecticides due to their efficacy and highly specific mode of action. Chlorantraniliprole (CHL) is the most widely used diamide worldwide, with South America being its primary market. In the present study, the sublethal effects of CHL were assessed in *Cnesterodon decemmaculatus* by acute exposure (96 h) to 1/10 (1.5 mg/L) and 1/100 (0.15 mg/L) of the LC50, using a multi-biomarker approach across different levels of biological organization. Locomotor activity (distance traveled, time immobile, average and maximum speeds), somatic index, enzymatic activities of acetyl-cholinesterase (AChE) in muscle and brain, catalase (CAT) in muscle, brain, gills and liver, glutathione-S-transferase (GST) in gills and liver, aspartate amino-transferase (AST), alanine amino-transferase (ALT), AST/ALT ratio and alkaline phosphatase (ALP) in the liver were measured. Locomotor activity decreased significantly in exposed fish compared to the control. No cholinergic effects were observed, possibly due to the mode of action of CHL. The muscles and brain were the organs most affected by oxidative stress. CAT activity showed a significant decrease in these tissues, while it was higher in the gills at low concentrations. GST showed a stimulatory response in the liver at the highest concentration, and no significant changes were recorded on transaminases and somatic index. The IBR index indicates that individuals' disturbance increase with concentration. CHL induced several defense mechanisms, including hormetic responses, to maintain homeostasis in the organisms. For muscle-targeted insecticides, locomotor activity serves as one of the most effective biomarkers for assessing the impact of exposure. This study represents the first report on the effects of a diamide insecticide in a native South American fish.

Keywords: Biomarkers, Behavioral toxicology, Chlorantraniliprole, *Cnesterodon decemmaculatus*, Toxicity mechanisms,

1. Introduction

Insecticides are widely used in agriculture and public health to eliminate insects that can harm crops, livestock, or human health (Rezende-Teixeira et al., 2022; Araújo et al., 2023). Widely used insecticides, such as organochlorines, organophosphates, carbamates, pyrethroids, and neonicotinoids, have been described as persistent in the environment, accumulate in soil and water, generate resistance in insect pests, and affect non-target organisms (Wijngaarden et al., 2005; Ansari et al., 2014; Le Goff & Giraudo, 2019; Arya et al., 2022; Araújo et al., 2023).

Diamide insecticides have recently been incorporated into the pesticide portfolio as one of the most promising alternatives to older insecticide classes since their ryanodine receptor target is a highly specific biochemical pathway for insects (Du & Fu, 2023; Li et al., 2023). Also, their broad-spectrum efficacy against a wide range of insect pests, including the orders Lepidoptera, Coleoptera, Diptera, and Hemiptera, as well as their relatively low resistance development, have made them an important asset for pest management strategies in various crop types (Du & Fu 2023; Sgarbi et al., 2023; He et al., 2024).

Chlorantraniliprole (CHL) is currently the most extensively utilized diamide insecticide globally (Li et al., 2023; He et al., 2024). This compound was introduced as an alternative to organophosphates and pyrethroids in various agricultural applications (Rezende-Teixeira et al., 2022). It shows shows low water solubility (Sw= 0.88 mg/L at 20°C) and moderate octanol-water partition coefficient (K_{OW}= 2.86); but remains fat-soluble; it is very persistent in soil (DT50=597 days) and exhibits slow water degradation (DT50= 170 days) (PPDB, 2024). Recently, several studies have reported that CHL is highly toxic to soil bacterial communities (Wu et al., 2021); it can adversely affect beneficial terrestrial insects (Tuelher et al., 2017; Sgarbi et al., 2023; Li et al., 2024) and induces oxidative stress to aquatic invertebrates (Wang et al., 2022; Sgarbiet al., 2023) and fishes (Meng et al., 2022; Mohamed et al., 2022; Stinson et al., 2022; AlMisherfi et al., 2023). Due to these characteristics, CHL has been included in the Highly Hazardous Pesticides (HHP) list compiled by the International Pesticide Action Network (PAN International List of Highly Hazardous Pesticides, 2021).

Since 2020, South America has led worldwide pesticide application (FAOStat, 2024) and has become the largest market for CHL, accounting for approximately 35% of global demand (Global Overview: Insecticides Market, 2024). This has recently led to increased occurrence reports of this pesticide in freshwater bodies across several Latin American countries (Francelino et al., 2023; Peresin et al., 2023; Rodríguez-Bolaña et al., 2023; Alana Dos Santos et al., 2024; Navarro et al., 2024).

In another study, Rodríguez-Bolaña et al. (2024) conducted an Ecological Risk Assessment (ERA) using both deterministic (RQ) and probabilistic (PRA) approaches, finding a moderate to high ecological risk for CHL to aquatic biota in surface waters of Uruguay. Despite these findings, toxicological evaluations of diamides on native aquatic organisms of South American are still lacking. Therefore, toxicity bioassays have gained prominence allowing for the evaluation of pesticide exposure and effects on aquatic systems resulting from regional agricultural practices (Bertrand & Iturburu, 2023; Rodríguez-Bolaña et al., 2024).

Aquatic ecosystems can be contaminated with legacy and current-use insecticides through runoff, leaching, and atmospheric deposition (Pérez-Parada et al., 2018; Latif et al., 2023). Their toxicity can vary depending on factors such as dosage, exposure duration, and the specific formulation of the insecticide. However, even at low doses, they could accumulate in aquatic organisms, affecting them at different biological levels of organization, including molecular, cellular, tissue, organ, population, community, and ecosystem levels (van der Oost et al., 2003; Bonifacio et al., 2016; Sharma & Verma, 2019; Gonçalves et al., 2021; Bertrand & Iturburu, 2023).

Among the most suitable organisms to be used in toxicological evaluation, fish present several advantages. They often have a shorter lifespan and faster reproductive cycle, allowing the assessment of chronic exposure effects; they have been frequently used as bioindicators due to their direct exposure to aquatic contaminants and their position in the food chain (Slaninova et al., 2009; Pérez-Parada et al., 2018; Bertrand & Iturburu, 2023). Also, fish can be easily maintained under laboratory conditions, and their different biological responses provide insights into their toxicity mechanisms.

Pesticides can induce oxidative stress in organisms, resulting from an imbalance between the production of reactive oxygen species (ROS) and the fish's antioxidant defense mechanisms, which may cause cell or organism damage or death (Slaninova et al., 2009; Gonçalves et al., 2022). The initial responses occur at the molecular level, and the most studied biomarkers at this level are related to enzymes involved in biotransformation and antioxidant defenses, such as catalase (CAT), superoxide dismutase (SOD), glutathione-S-transferases (GSTs), and glutathione peroxidases (van der Oost et al., 2003; Gonçalves et al., 2021). Other relevant enzymes for assessing pesticide contamination effects include cholinesterase, which is used to evaluate neurotoxic effects (Fulton & Key, 2001; Bernal-Rey et al., 2020; Gonçalves et al., 2022), and transaminases, which serve as indicators of hepatic damage in fish (De la Torre et al., 1999; Bonifacio et al., 2016; 2020). In addition to enzymatic activity, behavioral parameters like swimming velocity and immobility serve as useful biomarkers for evaluating effects on locomotion, survival, and social interactions in fish due to pesticide contamination (Scott & Sloman, 2004; Sharma et al., 2019; Bonifacio et al., 2020).

Integrating a multibiomarker approach at different organizational levels provides a more comprehensive view of how environmental stressors, such as pesticides, impact organisms. Significant changes in these biomarkers help identify damage or stress at both the individual and population levels (Mittelbach et al., 2014; Sharma et al., 2019).

Aiming to better understand the toxicity of CHL in fish, we evaluated the effects of its sub-lethal concentrations on *Cnesterodon decemmaculatus* by estimating several biomarkers at different levels of biological organization.

2. Materials and methods

Adult females of *C. decemmaculatus* were selected for the experiments due to the characteristics that make them suitable for toxicity assessments. These include their small size, wide distribution, high abundance in South American basins, and ease of collection and maintenance under laboratory conditions (Pautasso et al., 2023). They have been employed in several studies to evaluate the toxicity of several pesticides (Bonifacio et al., 2017; 2020; Bertrand & Iturburu, 2023; Ruiz de Arcaute et al., 2023; Pautasso et al., 2023) being the predominant species used for bioassays in the region (Bertrand & Iturburu, 2023).

Individuals were collected using a 1 mm mesh dip net from a site on Yuspe River (648320W; 318170S) (Córdoba, Argentina). This river serves as a reference site in environmental studies because it is an unpolluted site (Rautenberg et al., 2022; Zambrano et al., 2023).

Fish were transported to the laboratory and acclimated for 15 days in 120 L tanks in a temperature-controlled room at 21 ± 1°C and a 12-hour light-to-dark cycle. Throughout this period, individuals were fed twice daily with commercial fish food.

2.1 Acute toxicity test

Short-term (96-h) static toxicity tests were performed to evaluate the toxicity of Chlorantraniliprole (CHL) (>99%) purchased from HPC Standards GmbH (Cunnersdorf, Germany). Stock solutions were prepared in methanol (MeOH) supplied by J.T. Baker (Darmstadt, Germany) and diluted with dechlorinated tap water to reach the work concentration. Previous studies estimated that the 96 h LC50 of CHL for *Channa punctatus* and *Cirrhinus mrigala* was 14.424 mg/L and 16.465 mg/L respectively (Bantu & Vakita, 2013; Rathnamma & Nagaraju, 2014a). Based on these values, fish of *C. decemmaculatus* were exposed to sub-lethal doses at the following concentrations for 96 hours: 0 mg/L (Control), 0.15 mg/L (T1=1/100 of the 96 h LC50), and 1.5 mg/L (T2= 1/10 of the 96 h LC50). Ten individuals per treatment were randomly assigned to 2 L aerated glass aquaria. All the bioassays were performed by duplicate. Individuals were not fed during the experiment. The water in each aquarium was renewed every 24 hours. The concentrations of CHL were measured in the aquarium water at 0 and 24 h based on a liquid-liquid extraction (LLE) described in Rodríguez-Bolaña et al., (2023). Fish without respiratory movements and no response to tactile stimulus were considered dead and were removed immediately.

2.2. Behavioral parameters

After the exposure period, each fish was individually transferred to a recording aquarium (25 cm width x 9 cm depth x 25 cm height) containing 2 liters of dechlorinated tap water.

Once the fish settled at the bottom, their activity was recorded for 10 minutes. To assess whether CHL affects locomotor activity, we determined the mean speed of mobile episodes (m/s), maximum speed (m/s), duration of immobility of each individual (s), and distance traveled (m). All these parameters were obtained at the end of each trial from video recordings using Animal Tracker, an ImageJ-based tracking API (Gulyás et al., 2016).

2.3. Somatic indexes

Fish were euthanized by severing the spinal cord behind the operculum and dissected. Standard length (mm) and body weight (g) were determined. Fulton condition factor (K) was calculated for each fish according to the equation (Froese, 2006):

(1) $K=100^{*} W/L^{3}$. W is the body weight (g), and L is the standard length (cm).

To measure enzyme activity, Gills, muscles, liver, and brain were removed from individuals in each treatment and control group.

Each liver was weighted to calculate the hepatosomatic index (HSI) according to the equation (Chellappa et al., 1995):

(2) $HSI= 100^{*} Wl/W$. Where Wl is the liver's weight (g), and W is the body weight.

2.4. Enzyme activities

Enzyme extracts for each organ were prepared from individual fish, according to Bonifacio et al. (2017). For acetylcholinesterase (AChE), catalase (CAT) and glutathione-S-transferase (GST) activities, organs were homogenized in 0.1 M potassium phosphate buffer, pH 6.5 glycerol 20%, 1mM ethylene diamine tetraacetic acid (EDTA) and 1.4mM dithioerythritol (DTE) using a glass homogenizer (Potter Elvehjem). Samples were

198 centrifuged at 6900g and 4° C for 10 min to separate cell debris from the supernatant
199 using a refrigerated centrifuge. AChE activity was measured only in brain and muscle
200 homogenates, according to Ellman et al. (1961). Briefly, this technique is based on the
201 acetylcholine degradation by AChE to acetate and thiocholine. The latter, with
202 dithiobisnitrobenzoic acid (DTNB), generates a yellow compound (ε =1.36×104 M−1
203 cm−1) with ab- absorbance at 412 nm. Absorbance was measured during the first 3 min,
204 every 15 seconds.

205 Catalase activity (CAT) was determined according to Beutler (1982) following
206 the decrease in absorbance at 240 nm due to the consumption of the substrate (H2O2).
207 The activity was measured for 2 minutes following Tagliaferro et al. (2018).

208 The activity of GST was determined only in the liver and gills using 1-chloro-2,4-
209 dinitrobenzene (CDNB) as substrate at 340 nm, according to Habig et al. (1974). Briefly,
210 this substrate conjugates with glutathione (GSH) and generates a thioester with
211 absorbance at 340 nm. The absorbance increase was measured for 3 min at 25 °C
212 (Tagliaferro et al., 2018).

213 For transaminases and alkaline phosphatase, livers from each individual fish were
214 homogenized in phosphate buffer (pH 7.4) and centrifuged at 15,000g at 4°C for 10 min
215 to separate cell debris from the supernatant. The activities of aspartate aminotransferase
216 (AST) (L-Aspartate-2-oxaloglutarate aminotransferase) and alanine aminotransferase
217 (ALT) (L-Alanine-2-oxaloglutarate aminotransferase) were estimated according to
218 Reitman & Frankel (1957). The reaction mixture comprised 2 mmol/L of alpha-
219 ketoglutarate along with specific substrates for AST and ALT (100 mmol/L of aspartate
220 and 200 mmol/L of alanine, respectively), dissolved in a phosphate buffer (100 mM, pH
221 7.4). The reaction started with the addition of aliquots to the supernatant. After a 30-
222 minute incubation period, the 2,4-dinitrophenylhydrazine reagent was added, and the

resulting colored product was quantified via spectrophotometric measurement at 505 nm. The ratio of aspartate aminotransferase to alanine aminotransferase activity (AST/ALT ratio) was estimated for each treatment.

Alkaline phosphatase (ALP) orthophosphoric monoester phosphohydrolase activity was determined colorimetrically using a commercial kit (Wiener Lab 1361003) (Bonifacio et al., 2017). The enzymatic activity was calculated in terms of the protein content of the sample (Bradford, 1976) and reported in nkat (mg prot)-1.

2.4. Integrated Biomarker Response index

Results of AChE, CAT, GST, transaminases, and behavioral parameters were analyzed using the Integrated Biomarker Response (IBR) index, following the method described by Beliaeff and Burgeot (2002) and modified by Devin et al. (2014) to integrate all these biomarkers for assessing the health status of individuals exposed to CHL. The IBR index was calculated using the CALculate IBR Interface (Calibri, https://shiny.otelo.univ-lorraine.fr/calibri/R/) developed by the Laboratory for Continental Environments (LIEC) at Lorraine University (France).

2.5. Statistical analysis

Data distributions were analyzed using the Shapiro-Wilks index (Sokal and Rohlf, 1999). ANOVA test was performed to compare the biological parameters among different treatments, followed by Tukey's post-hoc test, and Kruskal–Wallis test, followed by the Dunn test, was used to compare nonparametric data (Sokal & Rohlf, 1999).

Differences were considered significant at $p < 0.05$. Statistical analyses were performed using Paleontological Statistics (PAST) software v4.13 (Hammer et al., 2001).

3. Results

3.1. Analytic concentrations of chlorantraniliprole

The chlorantraniliprole concentration in water from control groups was below the detection limits (DL) at 0 and 24 h. The lowest and highest concentrations showed drops of 5.31% and 2.01% at 24 h, respectively (**Table 1**). Since the water was renewed every 24 hours, this result indicates that concentrations remained relatively constant throughout the experiment.

TABLE 1

3.2. Behavioral parameters

The locomotor activity of individuals for the control group and treatments is shown in **Figure 1**. Exposure to CHL significantly reduces the distance traveled, average speed, and maximum speed while significantly increasing the time immobile of the individuals (ANOVA, $p < 0.05$).

Mean distance differed significantly between treatments, with the lowest activity at the highest concentration (Tukey post hoc test, C vs. T1; $p < 0.01$, C vs. T2; $p < 0.001$, T1 vs. T2; $p < 0.05$).

The effects on average speed and time immobile were quite similar between exposure treatments but significantly different for the control group (Tukey post hoc test, C vs. T1; $p < 0.05$, C vs. T2; $p < 0.05$, T1 vs. T2; $p=0.07$ and C vs. T1; $p < 0.05$, C vs. T2; $p < 0.05$, T1 vs. T2; $p=0.31$ respectively).

Regarding maximum speed, the only significant differences were observed between the control group and individuals exposed to the highest CHL concentration (Tukey post hoc test, C vs. T1; $p=0.11$, C vs. T2; $p < 0.05$, T1 vs. T2; $p=0.09$).

FIGURE 1

3.3. Somatic indexes

The death of one individual was recorded in the T2 group. No mortality was recorded in either the control group or the T1 group.

After exposure to CHL, the Fulton Condition Factor (K) of individuals and the hepatosomatic index (HSI) showed no significant differences among treatments and the control group ($F=0.16$, $p=0.89$ and $F=0.28$, $p=0.56$, respectively). (**Table 2**).

TABLE 2

3.4. Enzyme activities

Brain AChE activity showed significant inhibition in fishes exposed to T1, while no statistical differences were registered between T2 and the control group. On the other hand, none of the treatments significantly affected the AChE activity in muscle (**Table 3**).

Catalase activity in the brain and muscle was significantly inhibited in individuals exposed to the highest CHL concentration. In contrast, CAT activity was significantly inhibited at the lowest concentration in gills. In this organ, fish exposed to T2 presented the highest values; however, no statistical differences were registered between T2 and the control group.

Liver CAT activity tended to increase with concentration, but these differences were not significant. Similarly, no significant differences were found in AST, ALT, the AST/ALT ratio, or ALP activity in this organ. In contrast, GST activity in the liver showed a significant difference between T2 and the other groups. However, no differences were observed between treatments in the gills (Table 3).

TABLE 3

3.5. Integrated Biomarker Response index

The integrated biomarker response (IBR) values show that CHL exposure affected individuals differently. The higher values of the IBR index (that integrates all the responses) were higher in individuals exposed to T2, followed by T1 treatment. For the AChE, CAT, and GST enzymes (**Figure 2A**), T2 presented an IBR of 6.16, with CAT as the most discriminant biomarker in the liver, gills, and muscle, GST in the gills, and AChE in the brain. These results suggest that the response of *C. decemmaculatus* to the highest CHL concentration is characterized by an increase in CAT activity and the gills being the most severely affected organ.

In T1 (IBR=2.55), CAT in the brain and muscle and AChE in the brain exhibited values lower than the control group, while CAT in the brain, AChE in muscle, and GST in liver, indicated the highest stress levels among all treatments.

For transaminases (**Figure 2B**), individuals exposed to T2 (IBR= 1.71) presented the highest impact on the liver, where the AST and ALT activities indicated hepatic damage. On the other hand, for T1 (IBR= 0.82), the liver-specific enzyme (ALP) presented the highest response.

For behavioral parameters (**Figure 2C**), the IBR index was higher in T2 (IBR=6.33), followed by T1 (IBR=3.89) and the control group (IBR=0.03). Individuals exposed to 1.5 mg/L of CHL exhibited the highest impact, with the shortest distance traveled, the lowest average and maximum speed, and the longest duration of immobile episodes. Conversely, the control group was the least affected across all parameters.

FIGURE 2

4. Discussion

Our results show that exposure to sublethal concentrations of CHL induces multiple effects in *C. decemmaculatus* across different levels of biological organization. This study represents the first report on the toxicity of CHL diamide insecticide on this key aquatic South American bioindicator species. Although the concentrations used in our bioassays exceed typical environmental levels, which range from trace detections to a maximum of 10.2 μg/L (Stinson et al., 2022; Rodríguez Bolaña et al., 2023; Navarro et al., 2024), they are crucial for identifying effects not apparent at lower concentrations and for simulating extreme contamination scenarios.

Pesticides can affect locomotor activity in fish through physiological and neurological processes, such as imbalances in energy metabolism, disruption of nerve signal transmission, muscle dysfunction, and alterations in sensory sensitivity (Scott & Sloman, 2004; Sharma et al., 2019). The sensitivity of behavioral parameters as toxicity biomarkers in *C. decemmaculatus* exposed to pesticides has been previously described by Bonifacio et al. (2016, 2020). Although some authors have suggested that CHL may impact behavioral patterns and neuromuscular health in fish (Rathnamma & Nagaraju, 2014a; Stinson et al., 2022), our work is the first study that evaluates behavior changes by recording locomotor activity. Individuals exposed to the high concentration of CHL reduced their distance traveled by three times and their average and maximum speeds by two times. They also increased the time immobile of individuals by almost three times compared to the control group. These results suggest that CHL exposure could have relevant consequences for fishes inhabiting their natural environments since their altered behavioral patterns may affect their feeding efficiency, reproductive fitness, and predator avoidance, putting their growth and survival at risk.

The most frequently observed associations with behavioral disruption involve cholinesterase (ChE) (Scott & Sloman, 2004). When AChE is inhibited, the rate at which it degrades acetylcholine decreases, promoting excessive stimulation of cholinergic receptors that may result in symptoms such as muscle spasms, weakness, paralysis, and a decrease in survival rates (Slaninova et al., 2009; Bernal-Rey et al., 2020). AChE activity in *C. decemmaculatus* has shown high sensitivity to organophosphates, pyrethroids, and carbamates (De la Torre et al., 2005; Bonifacio et al., 2016; Bonifacio et al., 2017; Bernal-Rey et al., 2020). However, in our study, AChE activity in muscle tissue was similar between the control group and the treatments, indicating no significant anticholinergic effect in this tissue.

In the brain, acetylcholinesterase activity decreased only in individuals exposed to 0.15 mg/L. The neurotoxic effects of CHL in fish are unknown; however, due to its mode of action, which involves the activation of ryanodine receptors (RyRs) in muscle cells, direct neurotoxic effects are not expected. In this context, AChE inhibition at T1 might be linked to compensatory or adaptive mechanisms rather than the activation of acetylcholine receptors by the compound. Graude et al. (2024) reported that CHL affects the transcription of genes involved in Ca^{2+} signaling, leading to homeostasis alteration in *Perca flavescens*. Although diamides have higher selectivity for insect RyRs, Hasenbein et al. (2018) observed significant activation of RyRs in *Pimephales promelas* at 2.40 mg/L of CHL (a concentration close to T2). Our findings suggest that locomotor activity may be a more sensitive biomarker than AChE activity for muscle-targeting insecticides.

For detoxification and biotransformation enzymes, CAT activity varied significantly in almost all tissues compared to controls. This enzyme plays a crucial role in protecting cells from oxidative damage by catalyzing the decomposition of hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2), thereby neutralizing reactive oxygen species (ROS) and preventing oxidative stress (Santana et al., 2022). Our results indicate that the muscle and brain are the most susceptible organs to oxidative stress. In both the muscle and brain, the inhibition of CAT activity at high concentrations suggests a reduced capacity to manage oxidative stress, which may lead to increased cellular damage and toxic effects. Additionally, CAT shows a stimulatory response in the brain at low concentrations, indicating a potential hormetic effect. In this context, an increase in enzymatic activity may indicate that cells respond to oxidative stress by upregulating CAT activity to mitigate damage. However, at higher concentrations, the threshold for hormesis may be exceeded, leading to the inhibition of enzyme activity and compromising the organism's ability to manage oxidative stress. This inhibition could result from an overload of reactive oxygen species (ROS), direct inhibition of the enzyme, or damage to cellular systems. This hormesis phenomenon has been described in fish exposed to insecticides (Fulton & Key, 2001; Rodrigues et al., 2015; Bonifacio et al., 2017; Amenyogbe et al., 2021), but for CHL, it has only been reported in insects and anurans (Tuelher et al., 2017; Wang et al., 2022; Fonseca Peña & Brodeur, 2023).

A hormetic response was also observed for CAT in the gills, with individuals exposed to low concentrations exhibiting the most severe effects. For short-term exposures in *Labeo rohita*, the gills have been identified as having the highest levels of CHL residues (Bantu et al., 2016). Also, due to their direct contact with the environment and their role in respiratory exchange through a thin epithelium with a large surface area, the gills are particularly sensitive to changes in water quality.

In this context, the decline in locomotor activity observed in this study could be attributed not only to muscular distress but also to additional physiological costs, such as respiratory dysfunction. This can lead to diminished endurance, slower swimming speeds, and increased resting periods. However, to better understand the complex interactions between exposure and biomarker activity, future research should include histopathological examinations of gills to fully assess the effects at the cellular level.

Chlorantraniliprole did not exhibit significant effects on CAT activity, transaminases, or the hepatosomatic index in the liver. However, an increase in GST activity was observed in fish exposed to the highest concentration of CHL in this organ. This enzyme is involved in the detoxification of xenobiotics (van der Oost et al., 2003; Gonçalves et al., 2021), so the observed elevation in GST activity suggests an adaptive response aimed at mitigating oxidative stress and potential toxic effects induced by CHL in individuals.

The liver is a target organ for various pollutants due to its role in detoxification and metabolism (Mohamed et al., 2022). Previously, it has been reported that CAT and transaminases biomarkers in *C. decemmaculatus* are very sensitive to many insecticides (Bonifacio et al., 2016; Bonifacio & Hued, 2019; Ossana et al., 2019). However, the non-significant results in these parameters suggest that *C. decemmaculatus* could have a lower sensitivity to CHL than to organophosphorus and pyrethroid compounds (more toxic and bioaccumulative).

While the IBR index is most used in biomonitoring programs to compare the contamination at different sites (Beliaeff and Burgeot, 2002; Devin et al., 2014), its use in bioassays provides a versatile and straightforward method to evaluate the health condition of organisms. The IBR index allows us to integrate the broad variability responses of the biomarkers analyzed into a single value. Individuals exposed to T2 exhibited more than twice the IBR value compared to those exposed to T1 and almost four times the value of the control group. This indicates a greater physiological or biochemical response in these individuals, suggesting they are the most severely affected.

Current research on the impact of CHL on fish is limited, mainly focusing on a few species from Southeast Asia. Our work is the first report in South American native fishes, where this compound is widely used. Acute exposure experiments are helpful tools for evaluating the immediate effects on organisms. In this sense, this study provides valuable insight into CHL potential impacts on exposed organisms. We encourage future studies to include chronic toxicity tests to assess the impact of CHL at both individual and ecological levels. We also recommend conducting studies with environmentally relevant concentrations and evaluating toxicological interactions with other insecticides, such as organophosphates, which frequently co-occur in South American aquatic environments. These studies can provide crucial information on toxicity under more realistic scenarios and may be critical for establishing regulatory limits and environmental management measures for CHL.

4. Conclusion

Our study concludes that sublethal acute exposure to CHL is toxic to *C. decemmaculatus*, as evidenced by alterations in locomotor activity and dose-response patterns in antioxidant and detoxification enzyme activities. This is the first report on the impacts of a diamide insecticide on this fish species, which is considered a key South American aquatic bioindicator. Due to the mode of action of the compound, the decrease in locomotor activity does not appear to be directly related to AChE inhibition (cholinergic effects) but rather to other mechanisms of toxicity. In this context, the muscles and brain are the organs most affected by oxidative stress, as evidenced by the inhibition of CAT activity at high concentrations. In the liver, only GST showed a stimulatory effect at the highest concentration, indicating an increase in the detoxification process of individuals. Several adaptive responses involving AChE and CAT were observed, demonstrating the organism's ability to manage oxidative stress. To gain a more comprehensive understanding of these mechanisms, further studies should include additional exposure concentrations and dose-response assessments. The relatively low impact on the liver suggests that CHL is less toxic to *C. decemmaculatus* than organophosphates and pyrethroids. For muscle-targeted insecticides like diamides, locomotor parameters may be one of the most effective biomarkers to assess the impact of exposure. Since CHL is the most widely used diamide insecticide globally and has a high potential for runoff into water bodies, these results underscore the importance of establishing regular monitoring programs. Such programs are crucial for assessing and mitigatingCHL risks to non-target organisms.

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Table 1. Concentration of Chlorantraniliprole in exposure media at 0 hours and 24 hours (n=3).

	Chlorantraniliprole (mg/l)	
	T0	T24
Control	<DL	<DL
0.15 mg/l	0.158 ± 0.008	0.1496 ± 0.004
1.5 mg/l	1.842 ± 0.003	1.805 ± 0.021

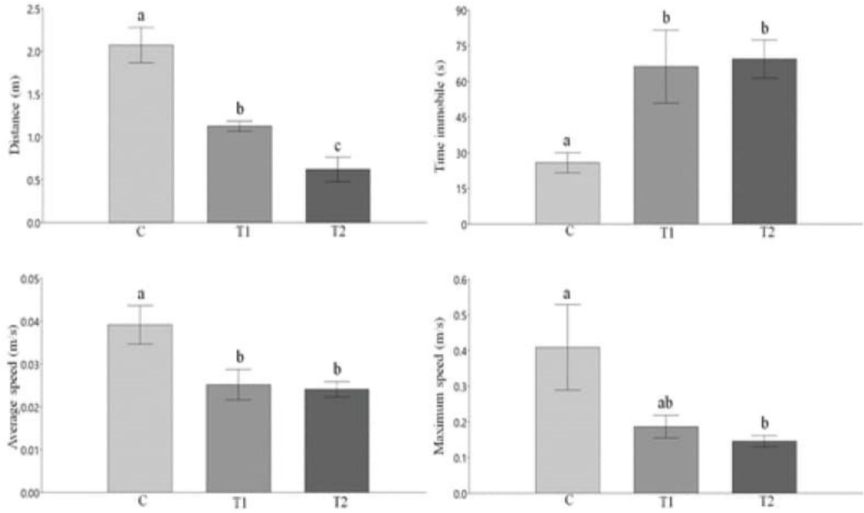

Figure 1. Behavioral parameters of locomotor activity recorded in *C. decemmaculatus* exposed to chlorantraniliprole. C: control group, T1: 0.15 mg/L, T2: 1.5 mg/L. Different letters indicate significant differences among treatments and between treatments and control group ($p < 0.05$).

22x13mm (600 x 600 DPI)

Table 2. Fulton condition factor (K) and hepatic somatic index (HSI) of *C. decemmaculatus* exposed to Chlorantraniliprole (n = 29). C: control group, T1: 0.15 mg/L, T2: 1.5 mg/L. The values are expressed as means ± standard error.

Treatment	K	HSI
Control	1.77 ± 0.21	1.76 ± 0.25
T1	2.41 ± 0.28	0.94 ± 0.25
T2	1.82 ± 0.11	1.39 ± 0.39

Table 3. Enzyme activities (nkat/mg prot) in different tissues of *C. decemmaculatus* exposed to Chlorantraniliprole. C: control group, T1: 0.15 mg/L, T2: 1.5 mg/L. The values are expressed as means ± standard error. Different letters indicate significant differences (ANOVA p<0.05, Tukey's test p < 0.05).

Treatment		Control	T1	T2
Biomarker	Organ			
CAT	Gills	5.94 ± 0.91 (ab)	2.56 ± 0.51 (a)	8.32 ± 1.92 (b)
	Liver	74.74 ± 32.01	124.26 ± 45.21	193.34 ± 44.85
	Brain	13.36 ± 2.35 (a)	18.89 ± 3.01 (b)	7.33 ± 2.97 (c)
	Muscle	3.58 ± 0.35 (a)	2.89 ± 0.21 (ab)	1.8 ± 0.13 (b)
AChE	Brain	9.66 ± 0.72 (a)	6.51 ± 0.52 (b)	10.47 ± 0.44 (a)
	Muscle	0.55 ± 0.21	1.93 ± 0.44	1.01 ± 0.11
GST	Gills	0.04 ± 0.009	0.04 ± 0.003	0.23 ± 0.01
	Liver	0.50 ± 0.25 (a)	0.90 ± 0.44 (a)	3.71 ± 0.18 (b)
AST	Liver	10.47 ± 1.64	11.69 ± 0.59	12.75 ± 2.31
ALT	Liver	2.03 ± 0.71	1.97 ± 0.75	3.35 ± 0.53
AST/ALT	Liver	5.15 ± 2.26	5.94 ± 0.75	3.38 ± 2.76
ALP	Liver	17.78 ± 8.71	32.99 ± 13.09	18.7 ± 5.37

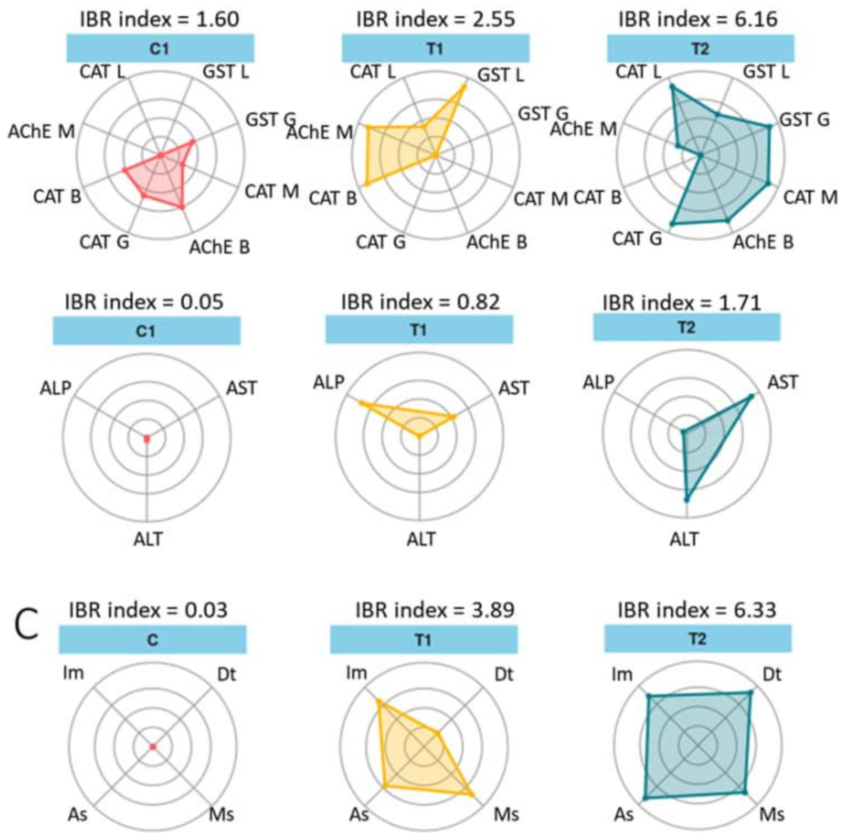

Figure 2. Radar plots representing the Integrated biomarker response (IBR) for C: control group, T1: 0.15 mg/L and T2: 1.5 mg/L. A: CAT: Catalase. (L): liver; (B): brain; (G): gill; AChE: Acetylcholinesterase. (B): brain; (M): muscle; GST: Glutathione S-Transferase. (L): liver; (G): gill. (B). B: ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; ALT: alanine aminotransferase. C: (Im): duration of immobility; (Dt): distance traveled; (As): average speed; (Ms): maximum speed.

36x36mm (600 x 600 DPI)

Discusión general

El enfoque de monitoreo llevado adelante en este trabajo, que combina un muestreo mensual en agua; la evaluación de bioacumulación en peces; la implementación de análisis de riesgo, y que además incluyo la realización de bioensayos de toxicidad del compuesto más frecuente en el sistema, representa uno de los trabajos más completos sobre la investigación ambiental de plaguicidas a nivel nacional (Figura 6). Aunque este tipo de enfoque puede ser costoso debido a la alta demanda de infraestructura, logística y personal capacitado, es fundamental para obtener una visión completa sobre el posible impacto de los contaminantes en el sistema. Permite además, ajustar frecuencias de colecta, matrices representativas y compuestos potencialmente peligrosos, facilitando así una gestión y protección ambiental adecuada de los recursos hídricos.

En Uruguay, la mayor cantidad de información de plaguicidas proviene de instituciones gubernamentales, los cuales basan sus planes de monitoreo en la matriz agua y con una frecuencia estacional o menor. Si bien los datos obtenidos son insumos muy valiosos para la gestión, la baja temporalidad de los muestreos muchas veces no permite determinar la presencia de aquellos compuestos que se degradan rápidamente, ni los eventos de alta concentración y/o diversidad de plaguicidas que llegan asociados a eventos de precipitación o a un mal manejo (Lefrancq et al., 2017; Chow et al., 2020). Asimismo, con la excepción de un nuevo programa recientemente ejecutado en la cuenca del río San Salvador, la más productiva del país (Ministerio de Ambiente, 2023) ninguno de los planes de monitoreo analiza la matriz agua y peces simultáneamente.

A nivel mundial, las principales agencias gubernamentales promueven y presentan en su normativa, programas de monitoreo en ecosistemas acuáticos que involucran el análisis en más de una matriz ambiental. En este sentido, el "Water Quality Monitoring Program" de la EPA (USEPA, 2023) y el "Water Framework Directive", principal marco regulatorio de la Unión Europea (European Parliament & Council, 2000) incorporan una alta frecuencia de muestreo (especialmente en cuerpos de agua prioritarios como fuentes de agua potable) y la inclusión de biota como herramienta complementaria de evaluación ambiental.

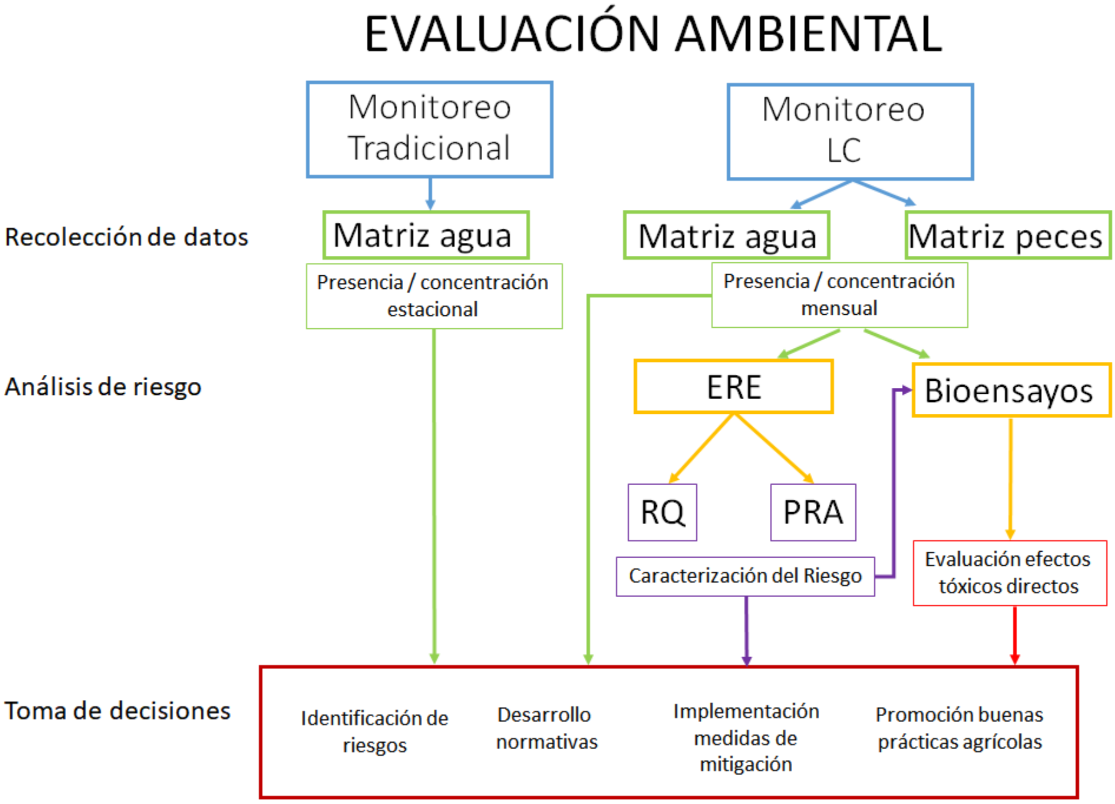

Figura 6. Evaluación ambiental de la calidad del agua mediante monitoreo tradicional de residuos de plaguicidas desarrollado a nivel nacional y la estrategia de monitoreo llevada adelante en este trabajo en Laguna del Cisne (LC). El monitoreo continuo de matrices ambientales (agua y peces), junto con la evaluación de riesgo ecotoxicológico (ERE) y la realización de bioensayos permiten una evaluación integral del impacto de los plaguicidas en el medio ambiente, contribuyendo de una manera más informada a la toma de decisiones sobre la gestión de los recursos.

La estrategia aplicada en esta tesis adoptó este enfoque, lo que permitió determinar la variabilidad temporal de los plaguicidas en agua y peces, y sus relaciones con los usos del suelo y las características de los compuestos (Objetivo 1).

Esta tesis también contribuye a la aplicación de metodologías alternativas de análisis de riesgo ecotoxicológico en ecosistemas acuáticos en función de la rapidez, complejidad y representatividad necesaria para la toma de decisiones (Objetivo 2).

En este sentido, una característica de los planes de monitoreo actuales de varios países de Sudamérica, incluido Uruguay, es que los monitoreos tienen como objetivo la obtención y análisis de datos sobre la presencia y concentración de plaguicidas en agua pero no contemplan la realización de evaluaciones de riesgo ecotoxicológico. La ERE se debe considerar un componente esencial de la gestión ambiental, ya que permite caracterizar y cuantificar los riesgos asociados con la exposición a los contaminantes por parte de la biota acuática (Carazo-Rojas et al., 2018; Maertens et al., 2022). Por esta razón, existen varios esfuerzos para incorporar evaluaciones de riesgo asociados a los programas de monitoreo, ejemplo de ello son la EPA (USEPA, 2014) y Unión Europea (EFSA, 2018) que tienen incorporados un marco de evaluación de riesgos avanzado en su legislación. En Latinoamérica, Brasil, Bolivia, Colombia, Ecuador, Perú y Venezuela también lo incluyen, aunque en estos casos solamente se basan en el peor escenario posible (Worst-Case Approach) para caracterizar el riesgo (Carriquiriborde et al., 2014; Casallanovo et al., 2021).

Complementario a los ERE, los bioensayos de toxicidad representan una herramienta fundamental en los programas de monitoreo debido a su capacidad para evaluar de manera directa los efectos biológicos de los plaguicidas en la biota (van der Oost et al. 2003; Bonifacio et al., 2020; Bertrand and Iturburu, 2023). En el capítulo 3 de este trabajo se evidenció el impacto negativo de la exposición de peces al insecticida CHL al medir diferentes biomarcadores en varios niveles de organización. Esto supone una contribución importante sobre la toxicidad de uno de los compuestos más utilizados en Uruguay sobre una especie representativa de nuestros ecosistemas.

Variabilidad temporal de plaguicidas en agua superficial y evaluación de riesgo ecotoxicológico: Herramientas para la gestión ambiental

En un contexto de medidas cautelares y de protección implementada en 2016 para la cuenca de la Laguna del Cisne, el análisis de muestras de agua permitió detectar la presencia de 25 plaguicidas. La ocurrencia de algunos de estos es claramente heredada de prácticas agrícolas pasadas, como el caso de los plaguicidas organoclorados. En este sentido, el uso masivo de estos compuestos en el pasado, así como sus características físico químicas (e.g. alta persistencia y K_{oc}) determinan que sigan encontrándose regularmente en sistemas acuáticos de todo el mundo, incluso décadas después de su prohibición (Sharma et al., 2019; Souza et al., 2020; Montagner et al., 2022).

Sin embargo, llamativamente la gran mayoría de los compuestos encontrados presentaron, o una marcada estacionalidad (e.g. metolaclor, atrazina, ciproconazole), o fueron compuestos de “baja” persistencia ambiental en suelo y agua (DT50 menor a 30 días, PPDB, 2024) (e.g. 2,4-D, bifentrina, cipermetrina), pudiendo indicar en ambos casos un uso reciente en la cuenca.

Debido a que no contamos con información previa, el impacto de las medidas cautelares aún se desconoce. Sin embargo, la presencia de múltiples plaguicidas en el sistema sugiere que deberían incorporarse medidas extras de gestión. Esto pone de manifiesto la importancia de adoptar un manejo adaptativo de la cuenca, que permita ajustar y optimizar las estrategias de gestión a través de evidencia constante que logre identificar rápidamente el estado del sistema y su evolución (Lindenmayer & Likens, 2009; Henrys et al., 2024).

Basados en nuestros resultados, una de las medidas que deberían considerarse es la ampliación de la zona de amortiguación. La regulación actual que define 20 metros de exclusión en los afluentes y 100 metros en la laguna, parecería no estar siendo suficiente, ya que los plaguicidas continúan llegando al sistema. Fundamentalmente debería considerarse el área de inundación de la laguna como sitio de exclusión, ya que el espejo de agua puede pasar de 20 a 190 hectáreas según el régimen hidrológico y la demanda de abastecimiento en la planta potabilizadora (Goyenola et al., 2024); y la escorrentía superficial parece jugar un papel importante en la carga de plaguicidas en el sistema. Por otro lado, la contaminación por organoclorados, principalmente DDT y sus metabolitos (con niveles superiores a los valores guía para protección de biota acuática) estaría asociado tanto a su elevada persistencia ambiental, como a este continuo transporte desde la cuenca. Por consiguiente, para garantizar la calidad del agua se debería también adoptar un plan de monitoreo considerando estos eventos de lluvia y que analicen además el grado de contaminación del suelo de la cuenca para una correcta mitigación de los impactos.

La estrategia utilizada, basada en una alta frecuencia de muestreo permitió determinar la presencia casi continua de plaguicidas de uso extendido en Uruguay, como el caso del glifosato y el clorantraniliprole (DGSA, 2024) reafirmando la importancia de estos compuestos en la actual producción agrícola del país (Cespedes-Payret et al., 2009; Soutullo et al., 2020; Rizzo et al., 2021) y en particular de la cuenca estudiada. También se lograron detectar compuestos de uso ganadero, como los organofosforados diazinon y etión. En este sentido, luego de las medidas cautelares, se ha registrado un aumento en la actividad ganadera de la cuenca, sobre todo en zonas cercanas a la laguna (Gonzalez-Fernández, & Orcasberrro, 2018).

Asimismo, la metodología empleada hizo posible la identificación de un patrón estacional relacionado con el calendario de los cultivos. Anteriormente al establecimiento de las medidas cautelares, los principales cultivos de la cuenca, al igual que en resto del país, se sembraban en primavera y se cosechaban en otoño (Bálsamo, 2018; González-Fernández y Orcasberrro, 2018), los cuales se mantienen actualmente. Todos los compuestos encontrados en este período están registrados para su uso en cultivos estivales (soja y maíz principalmente) y ganadería. El uso inapropiado de plaguicidas incluye además compuestos con restricciones de comercialización (diazinon, etión, permetrina y clorpirifós) y prohibidos para su uso (atrazina).

La atrazina, presente de noviembre a marzo, fue prohibida en 2016 por MGAP (Res. 104 12/16). Su ocurrencia post prohibición no es exclusiva de esta cuenca, ya que ha sido reportada recientemente en otros cuerpos de agua del país (Ministerio de Ambiente, 2023). Debido a que es un herbicida muy eficaz para un amplio rango de cultivos, antes de su prohibición era uno de los compuestos más utilizados. Posiblemente los residuos de atrazina sufren un proceso similar a los plaguicidas organoclorados y su presencia en el ecosistema provenga de una llegada tardía al sistema o de que este actúa como sumidero de residuos que hace años se encuentran presentes en los sedimentos. Sin embargo, su escasa vida media (DT50 suelo= 75 días; DT50 agua = 80 días; PPDB, 2024) hace suponer que su presencia podría relacionarse a un uso actual en la cuenca. Para una mejor comprensión de la dinámica ambiental en el sistema, futuros trabajos deberían incluir información hidrológica y de usos del suelo. Esto último representó limitante importante en este trabajo, ya que el acceso a la información fue muy restringida y dependiente de la voluntad de algunos productores.

Con respecto a las concentraciones, la reglamentación nacional establece límites en agua potable fundamentalmente para plaguicidas organoclorados, obsoletos desde el punto de vista productivo, encontrándose referencia normativa únicamente para seis de los compuestos encontrados. En todos estos casos, las concentraciones estuvieron por debajo de los estándares establecidos en agua potable. Actualmente, estos parámetros de control se basan en la norma UNIT 833:2008. Si bien esta normativa se encuentra alineada con regulaciones internacionales (OMS, EPA, Unión Europea) algunos compuestos no están incluidos y para otros los límites permitidos son mayores (como en el caso de la atrazina) por lo que debería discutirse su actualización. En este sentido, en el caso de la Unión Europea, los plaguicidas que no presentan un valor máximo permisible, la normativa establece un criterio general de 0,1 µg/L para plaguicidas individuales y sus metabolitos y de 0,5 µg/L para una sumatoria de plaguicidas totales presentes en la muestra (Unión Europea, 2020). En este contexto, debe considerarse que el marco regulatorio nacional también es obsoleto en relación a los compuestos y niveles permitidos. En este trabajo se encontraron en agua ocho plaguicidas de uso prohibido en la Unión Europea: atrazina, bifentrina, permetrina, clorpirifós, diazinon, acetoclor, simazina y metalaxyl (Pesticide Action Network, 2022).

El riesgo que representan estos plaguicidas para el ambiente quedó evidenciado en la ERE implementada en este trabajo. Los plaguicidas de las familias piretroides y organofosforados presentaron un riesgo ecotoxicológico muy alto mediante las dos aproximaciones utilizadas. Asimismo, compuestos restringidos en Uruguay como permetrina y etión; y las altas concentraciones de organoclorados detectadas, también representaron un riesgo potencial para la biota acuática a corto y largo plazo. En el análisis PRA, la concentración perjudicial para el 5% de las especies (HC5) fue mayor para la especie más sensible en exposición a largo plazo, mientras que en exposiciones agudas el riesgo es mayor para todas las especies, evidenciando el efecto de los eventos de pulsos en toda la comunidad.

La evaluación de riesgo ambiental es una parte fundamental del control regulatorio de los plaguicidas por parte de las agencias gubernamentales en todo el mundo. En Uruguay hay 81 plaguicidas altamente peligrosos autorizados para su uso, de los cuales 43 están prohibidos en la Unión Europea y Estados Unidos (Cárcamo, 2020). En este contexto, y considerando los resultados obtenidos, la implementación de este tipo de análisis en sistemas acuáticos a nivel nacional, resultan fundamentales para brindar respuestas rápidas ante eventos de contaminación aguda (picos de contaminación) y como herramienta para evaluar la normativa actual sobre la gestión de plaguicidas.

En este sentido, como primera medida se sugiere adoptar un análisis RQ de manera rutinaria junto a los planes de monitoreo llevados adelante en el país. Este método es sencillo y proporciona una evaluación rápida de los riesgos ambientales. Permite identificar no sólo los compuestos más riesgosos para el ambiente, sino también épocas del año con mayor riesgo potencial. Sin embargo, para escenarios complejos y de alto riesgo como las fuentes de abastecimiento de agua potable, el desarrollo de metodologías probabilísticas representa la mejor opción, ya que ofrece un análisis más detallado y realista.

Complementariedad de matrices agua superficial y peces: Evaluación integral de la calidad ambiental

El paquete tecnológico actual, asociado a los diferentes cultivos abarca una gran variedad de plaguicidas con diferentes características fisicoquímicas. Esto determina que el uso de diferentes matrices que capturen plaguicidas con diferentes características físico químicas sea fundamental para conocer el estado del sistema (Gonzalez et al., 2013; Montagner et al., 2022; Slaby et al., 2022). En este sentido, la integración de biota permite evaluar no solamente la bioacumulación/bioconcentración/biomagnificación de contaminantes y su efecto potencial en organismos, sino también detectar compuestos que presenten una mayor afinidad por esta matriz debido a sus propiedades químicas (e.g. solubilidad, lipofilia, persistencia).

En este trabajo a lo largo del año se detectó en agua la presencia de múltiples compuestos de diferentes clases químicas (e.g. organoclorados, piretroides, organofosforados, neonicotinoides, triazinas, estrobilurinas). El análisis de la matriz peces por su parte, permitió detectar la bioacumulación de ocho plaguicidas en músculo de *Cyphocharax voga*. La mezcla de pesticidas también incluyó triazoles, estrobilurinas, piretroides, organofosforados y principalmente organoclorados, sugiriendo una exposición de las poblaciones de peces a pesticidas heredados y de uso actual.

Este enfoque multimatríz permitió observar una complementariedad entre ambas matrices. Basados en las propiedades fisicoquímicas de los compuestos, los análisis estadísticos revelaron que los plaguicidas de baja solubilidad y alto K_{OW} tienden a encontrarse presentes en la matriz peces. Así, compuestos dominantes en agua como los herbicidas atrazina y metolacloro caracterizados por una alta solubilidad no estuvieron presentes en peces. Por otro lado, compuestos de baja solubilidad como el propiconazole y POP´s como el aldrin y dieldrin que tienden a bioacumularse en los tejidos debido a su alta persistencia y solubilidad en grasas, solamente fueron encontrados en peces.

Por otro lado, insecticidas de uso actual como el clorpirifós y la permetrina; el fungicida azoxystrobin y POP´s como como p,p´-DDE y p,p´-DDD presentaron niveles muy superiores en biota a los encontrados en agua, demostrando el potencial de bioacumulación y una dinámica aun relevante en el sistema. Para el clorpirifós y la permetrina, esto reafirma los resultados obtenidos en la ERE, destacando aún más la necesidad de actualizar la normativa actual sobre su uso.

Además del contenido lipídico, la bioacumulación estuvo significativamente relacionada con la talla de los individuos.
El hábito alimenticio de *C. voga* podría indicar una ingesta de plaguicidas a través de los sedimentos. En este sentido, la especie combina el consumo de sedimentos inorgánicos y detritus, con invertebrados bentónicos (Corrêa y Piedras, 2008; Sagrario y Ferrero, 2013; Pacheco et al., en revisión).

El único antecedente sobre bioacumulación en *C. voga* evidenció la existencia de daños histológicos, principalmente a nivel de hígado y branquias, debido a la exposición a compuestos organoclorados (Barni et al. 2016). Por lo tanto, la exposición a largo plazo representa una gran preocupación para la especie y la comunidad acuática en general, ya que esta es un importante nexo entre las redes tróficas bentónicas y pelágicas (Sagrario y Ferrero, 2013) cumpliendo un rol particular en este sistema (Pacheco, en revisión). Sin embargo, para una mejor compresión del destino final de los contaminantes, futuros estudios deberán enfocarse en analizar la posible presencia de patrones de biomagnificación y el impacto en la biodiversidad en LC.

Para futuros trabajos en la cuenca sería importante mejorar las capacidades analíticas para la detección de plaguicidas en sedimentos a fin de incluirla de forma satisfactoria en la evaluación ambiental. Esta matriz fue analizada en LC y más recientemente en el río San Salvador (Teixeira de Mello et al., 2019; Ministerio de Ambiente ministerio de Ambiente 2023), sin embargo debido a los elevados límites de cuantificación que se manejan con las técnicas actuales en ningún caso se logró determinar la presencia de plaguicidas. Los sedimentos, al igual que los peces, podría proporcionar información acerca de la contaminación histórica de la cuenca y sobre la disponibilidad biológica y transporte en el ecosistema acuático (Carazo-Rojas et al., 2018; Pérez-Parada et al., 2018; Chow et al., 2020). Hasta alcanzar las capacidades para evaluar sedimentos, una estrategia sería continuar utilizando especies detritívoras de peces como *Cyphocharax voga*.

Bioensayos como indicadores de la toxicidad de plaguicidas en peces

Para comprender mejor cómo los plaguicidas afectan a los organismos acuáticos no solamente se debe analizar la exposición, sino también su peligrosidad. En este sentido, los bioensayos son herramientas fundamentales para evaluar la toxicidad, bioacumulación y efectos subletales de sustancias químicas en los ecosistemas acuáticos van der Oost et al. 2003; Slaninova et al., 2009; Ullah & Zorriehzahra 2015).

La elección de las especies a utilizar en este tipo de estudios representa un punto clave, ya que puede definir la relevancia de los resultados obtenidos. En este sentido, para fines regulatorios comúnmente se utilizan especies modelo, las cuales presentan un manejo en laboratorio estandarizado y permiten la comparación de datos entre diferentes estudios, pero que en su mayoría son ajenas a nuestros ecosistemas. Esto determina que raramente los resultados puedan generalizarse, dada las diferentes características de las especies y de los ambientes (Carriquiriborde et al., 2014; Bertrand & Iturburu, 2023). Debido a esto es que recientemente se ha venido promoviendo el uso de especies nativas por parte de diversas agencias reguladoras de América Latina, debido a que su mayor relevancia biológica y ecológica (Bertrand & Iturburu 2023). En Uruguay, el uso de especies nativas en bioensayos de toxicidad continúa siendo escasa, lo que limita el conocimiento sobre los efectos de estos compuestos en nuestra biota.

Desde su incorporación en el mercado nacional en 2014, el CHL ha sido uno de los insecticidas más aplicados en el país, alternando año a año el primer puesto en volumen de importación con el clorpirifós, existiendo además un incipiente mercado de formulación local (DGSA, 2024). En 2018 representaba el 15% del mercado de insecticidas (DGSA, 2019), lo que sumado a su uso en un amplio rango de cultivos explicarían el hecho de ser el compuesto más frecuente en agua durante este estudio. El análisis de RQ evidenció que las concentraciones máximas encontradas representaron un riesgo alto para la biota acuática, mientras que el análisis PRA no pudo ser realizado debido a la ausencia de datos, poniendo de manifiesto la escasa información existente sobre este compuesto. Otra característica, que lo convirtió en candidato ideal para los bioensayos, es el hecho que su presencia en peces no fue analizada (no formó parte del scope para esta matriz), por lo que se desconoce su potencial de bioacumulación y efectos en esta matriz.

A nivel regional, diversos bioensayos con peces nativos reportan efectos subletales de piretroides (Carriquiriborde et al., 2007; Mugni et al., 2012; Brodeur et al., 2017) y organofosforados (Bonifacio et al., 2017, López Aca et al., 2018; Marques et al., 2021; Lombardero et al., 2024) compuestos de uso actual con alto riesgo en el sistema. Si bien el CHL es uno de los insecticidas más usados en América Latina desde hace varios años, esta tesis representa el primer antecedente en la evaluación del efecto para la región.

Los resultados demuestran sensibilidad de *Cnesterodon decemmaculatus* al CHL. La exposición subletal promovió estrés oxidativo en los individuos en diferentes niveles de organización, destacándose alteraciones en la actividad locomotora y en las actividades de las enzimas de detoxificación.

El principal modo de acción del compuesto es la interferencia con el sistema de contracción muscular de los insectos a través de la inhibición de los canales de calcio en los músculos (Du & Fu, 2023). Esto explicaría que a diferencia de insecticidas organofosforados, carbamatos y piretorides (neurotóxicos), no se haya observado efectos colinérgicos en los individuos expuestos a altas concentraciones. Por lo tanto, para el caso de las diamidas, la actividad locomotora representaría una herramienta más eficaz para evaluar el impacto de la exposición.

La disminución de la actividad locomotora estaría relacionada con una alteración de la función muscular, debido a impactos en las enzimas antioxidantes y detoxificantes. El tejido muscular y el cerebro fueron los órganos más afectados por el estrés oxidativo, evidenciado por la inhibición de la actividad de la CAT a concentraciones elevadas.

Se observaron diversas respuestas adaptativas de los organismos a bajas concentraciones, lo que demuestra la capacidad de la especie para manejar el oxidativo. Este fenómeno se ha descrito en peces expuestos a insecticidas (Fulton & Key, 2001; Rodrigues et al., 2015; Bonifacio et al., 2017; Amenyogbe et al., 2021), pero para CHL solo se ha reportado en insectos y anuros (Tuelher et al., 2017; Wang et al., 2022; Fonseca Peña & Brodeur, 2023). Sin embargo, para obtener una comprensión más completa de estos mecanismos, se sugiere que futuros estudios incluyan una mayor cantidad de concentraciones de exposición para realizar una mejor evaluacion de la relación dosis-respuesta.

Por otro lado, la aplicación del índice IBR, comúnmente utilizado en biomonitoreo de calidad de aguas (Devin et al., 2014), representó una herramienta muy útil para comparar el estado de salud de los organismos expuestos a las diferentes concentraciones. Este método, ofrece una representación gráfica integrada del impacto y es fácil de interpretar, por lo que se recomienda su aplicación en futuros estudios similares.

En función de los resultados se sugiere realizar una evaluación de riesgo más detallada del CHL, así como ajustar su uso hasta que no se conozca profundamente sus impactos en el ambiente. Con respecto a esto último, Uruguay ha experimentado una fuerte expansión del cultivo de colza, el cual se ha duplicado cada año desde 2019, alcanzando en 2023 las 345.000 hectáreas (1/3 del área sembrada con cultivos de invierno) (Observatorio Oleaginosos Uruguay, 2023). Si bien cada año aumenta la cantidad de productos nuevos para uso en colza, no hay registros de ingredientes activos para insectos de suelo (hormiguicida), por lo que se agregó el CHL, y el cyantraniliprole (otra diamida) como paquetes para combatir ese tipo de plaga (DGSA 2023), aunque no están específicamente diseñados para el control de hormigas.

En este sentido, estudios adicionales deberían evaluar los efectos subletales de la exposición crónica en la condición de los peces. Asimismo, para analizar el efecto de concentraciones ambientalmente relevantes se podrían desarrollar estrategias de biomonitoreo de peces, tanto activo (e.g. peces enjaulados) como pasivo (muestreo in situ) y obtener así una visión más realista del impacto sobre los ecosistemas.

En los últimos años, Uruguay ha impulsado exitosamente, mediante diferentes vínculos interinstitucionales, el desarrollo de capacidades analíticas y de formación técnica para el monitoreo de plaguicidas en sus sistemas acuáticos. Como siguiente paso debería adoptar nuevas estrategias de evaluación ambiental que proporcionen la mayor cantidad de información posible y que permitan una gestión más eficaz de los recursos hídricos. En un contexto de continua expansión en el uso del suelo y la introducción de nuevos paquetes tecnológicos relacionados al uso de plaguicidas la gestión adaptativa y la implementación de protocolos para el desarrollo de ERE resultan esenciales.

Conclusiones finales

La información generada en esta tesis ha contribuido a mejorar los planes de monitoreo de plaguicidas, con el potencial de trascender más allá de las fronteras de Uruguay. Una evaluación integral cómo la realizada puede permitir ajustar estrategias (e.g. frecuencias, matrices), definir compuestos prioritarios y optimizar recursos sin comprometer la representatividad del ecosistema.

Contribuye además al desarrollo de metodologías que permitan implementar diferentes alternativas de ERE, que ayuden a la toma de decisiones informadas para la gestión ambiental.

La evaluación de riesgos sugiere priorizar la vigilancia ambiental de compuestos organofosforados y piretroides, tanto en LC como a nivel nacional.

La normativa uruguaya sobre uso de plaguicidas debe ser actualizada sobre una base empírica. Se identificaron plaguicidas altamente peligrosos (e.g. clorpirifós, etión, bifentrin) y compuestos prohibidos para su uso (e.g. atrazina). El 35% de los plaguicidas de uso actual encontrados están prohibidos por la legislación europea.

Se evidenciaron efectos tóxicos del CHL en los peces. Aunque es un sustituto de compuestos más tóxicos, como los organofosforados, su uso creciente en la producción agrícola resalta la necesidad de profundizar en el conocimiento sobre este plaguicida y las diamidas en general. Para comprender mejor sus efectos, se recomienda realizar bioensayos más detallados con especies acuáticas nativas.

Es importante destacar que LC es actualmente el único sistema acuático del país con medidas cautelares. Los resultados muestran la complejidad de reducir la contaminación por pesticidas en sistemas acuáticos que estuvieron históricamente expuestos aun en condiciones restrictivas.

El mantenimiento de la calidad ambiental del sistema resulta indispensable para asegurar que LC pueda continuar siendo una fuente segura de agua potable. Para reforzar las medidas cautelares se sugiere: 1) La adopción de un manejo adaptativo de la cuenca que permita ajustar y optimizar continuamente las medidas de gestión. 2) Ampliar la zona de amortiguamiento actual, prohibiendo actividades de agricultura y ganadería principalmente en el área de inundación de la laguna. 3) Fortalecer la fiscalización de las medidas cautelares y mejorar el conocimiento de los plaguicidas empleados en el territorio. 4) Realizar una gestión adecuada de los plaguicidas empleados y continuar promoviendo el uso de buenas prácticas agrícolas (BPA).

Producto de la contaminación por plaguicidas en desuso como ser organoclorados y atrazina, futuros estudios deberán analizar la evolución de estos hallazgos, asi como la contaminación del suelo de la cuenca, y el efecto de la escorrentía superficial con la ocurrencia de estos compuestos en agua.

Una limitante de este trabajo ha sido la imposibilidad de acceder a puntos ubicados dentro de predios privados, y a la información sobre usos del suelo y aplicación de plaguicidas. La búsqueda de mecanismos normativos que permitan resolver estos obstáculos debería ser considerado por las autoridades pertinentes.

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ANEXO 1. Criterio de clasificación de los principales plaguicidas basadas en tipo, modo de acción, persistencia ambiental (días), vida media (días) y toxicidad oral (rango de LD50 (mg/kg) de peso corporal). Adaptado de PPBD, 2024.

Criterio	**Categoría**	**Descripción**	**Ejemplo**
Tipo de Plaguicida	Insecticida	Controla insectos.	Clorpirifós
	Herbicida	Controla plantas no deseadas.	Glifosato
	Fungicida	Controla hongos patógenos.	Propiconazole
Modo de Acción	Contacto	Entra por contacto directo en el organismo objetivo	Permetrina
	Sistémico	Se transporta a través de la planta o el organismo	Acetamiprid
	Ingestión	Entra por ingestión en el organismo objetivo	Clorpirifós
Persistencia en ambiente	Baja	Se degrada rápidamente < 30 días	Carbendazim
	Moderada	Degradación intermedia 30 - 365 días	Permetrina
	Alta	Persistente > 365 días	DDT
Vida Media	Corto plazo	Vida media en el ambiente < 30 días	Clorotalonil
	Medio plazo	Vida media en el ambiente entre 30 y 180 días	Imidacloprid
	Largo plazo	Vida media en el ambiente > 180 días	DDT
Toxicidad	Extremadamente Tóxico	Rango de LD50 (mg/kg) Oral < 5	Paratión
	Altamente Tóxico	Rango de LD50 (mg/kg) Oral 5 - 50	Diazinón
	Moderadamente Tóxico	Rango de LD50 (mg/kg) Oral 50 - 500	Glifosato
	Ligeramente Tóxico	Rango de LD50 (mg/kg) Oral 500 - 2000	Clorotalonil
	Prácticamente No Tóxico	Rango de LD50 (mg/kg) Oral >2000	Difenoconazol