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REVIEW

Occurrence of Pharmaceutical and Pesticide Transformation Products in Freshwater: Update on Environmental Levels, Toxicological Information and Future Challenges

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Abstract
Pharmaceuticals and pesticides are recognized micropollutants in freshwater systems. Their ever-increasing frequency of detection, levels found and little information available about their effects on non-target organisms, make them emerging contaminants. However, parental compounds are not the only substances of concern. Their metabolites and degradation products, hereby referred to as transformation products, are increasingly detected in freshwater samples and wastewater effluents. In the past years, a wealth of publications provided concentration levels detected in freshwater and some toxicological data, which required critical systematization. This review identified concentrations for 190 transformation products (92 from pesticides and 98 from pharmaceuticals) in water bodies and wastewater effluents. A concentration heatmap was produced to easily spot the substances found at higher levels and plan future research. The very limited available toxicological data link exposure to transformation products to adverse outcomes in humans (genotoxicity and alteration in detoxification processes) and aquatic species (mostly related to apical endpoints). Overall, environmental levels of these transformation products may pose a severe threat to aquatic organisms and need to be further investigated in sound experimental designs, testing for the effects of the single substances as well as of their mixtures. Such toxicological information is highly needed to improve both water treatment technologies and monitoring programmes.

Pesticide and Pharmaceutical Transformation Products as Environmental Contaminants

Over the past decades, scientists produced a wealth of information about the toxic effects of pesticides and pharmaceuticals on freshwater species. Both groups of compounds are widely used in the world, with recognized benefits for human health and welfare (Santos et al. 2010; Mcknight et al. 2015). Moreover, their use is globally escalating, owing to i) today's social habits, ii) the increase in life expectancy, iii) the human population growth and iv) the consequent increase in food demand. More so under the actual pandemia caused by the SARS-CoV-2 coronavirus. Despite the need for them, these classes of chemicals have also been associated with severe human and environmental health risks and are common micropollutants of freshwater systems (Corcoran et al. 2010; Santos et al. 2013; Reemtsma et al. 2013; Ortiz de García et al. 2014; Mcknight et al. 2015). Pesticides and pharmaceuticals are designed to have specific biological activity, exerting the desired effect before undergoing excretion and/or degradation. These same characteristics also make them persistent in the environment, ultimately causing toxicity to non-target fauna and flora (Fent et al. 2006; McKnight et al. 2015). Due to these characteristics and the still limited information available, they are the most representative classes included in the watch list of substances for Union-wide monitoring of the Water Framework Directive (WFD). Currently, they represent over 88% of the compounds listed in the WFD (European Union 2022).

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Pesticides occur in natural water, mainly by run-off from the agricultural fields where they are applied and through industrial wastewater. Although soils can store a good amount of pesticides due to the high affinity of these compounds to organic matter, surface water and groundwater are susceptible to pesticide contamination because of the existing soil–water interconnections, mainly adsorption (Sharma et al. 2019). Pesticides that are highly adsorbed to soil particles are less likely to infiltrate deep but can easily be carried via run-off of and reach surface water (Syafrudin et al. 2021). Due to their increased use, detection of pesticides in different water compartments is becoming more and more frequent (Corcoran et al. 2010; Reemtsma et al. 2013; Ortiz de García et al. 2014; Evgenidou et al. 2015; Vryzas, 2018). On the other hand, the distribution of pharmaceutical substances in the environment is predominantly made by aqueous transport of compounds contained in discharged wastewater effluents, which persisted through the conventional treatment processes (Khan et al. 2020). Contamination by pharmaceutical compounds may also occur by terrestrial run-off from agricultural fields and aquaculture activities (Hong et al. 2018). Sorption is also an important process for the transport of pharmaceuticals in an aquatic environment. This process is responsible for the partitioning of pharmaceuticals between the water and the sediment phase (Bavumiragira et al. 2022).

Once pesticides and pharmaceuticals reach the aquatic environment, they undergo a series of abiotic and biotic transformation and degradation processes. Hydrolysis, photodegradation and biodegradation are considered the most important mechanisms involved in their transformation or degradation (Syafrudin et al. 2021; Khan et al. 2020). Hydrolysis is an abiotic degradation process that creates products more polar than the parental compounds. These reactions are mainly catalysed by hydrogen or hydroxide molecules (Bavumiragira et al. 2022). Photolysis or photochemical degradation of pesticides and pharmaceuticals occurs by decomposition of these compounds in the presence of ultraviolet (UV) light. When exposed to sunlight, pesticides and pharmaceuticals containing chemical functional groups able to absorb solar radiation are prone to photolysis. The reaction transforms parental compounds into transformation products that are usually more biodegradable and hydrolysable (Wilkinson et al. 2017; Bavumiragira et al. 2022). Biodegradation is a biotic process that can result in the partial or complete transformation of pesticides and pharmaceuticals by microorganisms, such as certain fungi, bacteria, protozoans and microalgae. These microorganisms are present in wastewater treatment plants (WWTPs) or occur naturally in suspended solids, sediments and within animals (i.e. gut microbiota) (Wilkinson et al. 2017; Jaffar et al. 2022). Microbial degradation is recognized in the literature as having an important role in the degradation of several pharmaceuticals in a wide range of water compartments (Christensen and Li, 2014). For pesticides, microbial degradation includes the mineralisation process, which consists in the break of a parental pesticide into carbon dioxide and co-metabolization where microbial-catalysed reactions break pesticides into other chemical forms (Syafrudin et al. 2021). Surface waters receiving wastewater effluents rich in microorganisms are usually prone to show higher biodegradation effectiveness. High rates of biodegradation are typically observed along the sediment–water interface in water bodies and wetlands (Li et al. 2016). Degradation effectiveness varies according to biotic and abiotic factors, such as temperature, pH, UV light, presence of dissolved organic matter, suspended material and micro- and macrobiota (Vryzas, 2018). Low turbidity, small depth, low total organic carbon content and sandy sediments favour the degradation of pesticides and pharmaceuticals (Baena-Nogueras et al. 2017). On the other hand, higher depths, low temperature and higher turbidity can lower the degradation effectiveness (Syafrudin et al. 2021; Bavumiragira et al. 2022). Nevertheless, the described processes originate transformation products that enter in natural water by a panoply of different sources. In recent years, several works have reported the detection of these transformation products in the range of ng to µg/L, sometimes at concentrations even higher than those found for the parental compounds (le Cor et al. 2021). However, the focus of the reports was primarily on the detection and quantification of the parental compounds. Concern about their transformation products, with the involvement of more groups in this research, took off mostly in the last decade, especially for pharmaceutical transformation products.

Investigation about the occurrence and fate of transformation products in the aquatic environment skyrocketed in recent years, mainly due to advances reached in the chemical analytical methods (Fent et al. 2006; Valls-Cantenys et al. 2016). New instruments and methods with higher separation efficiencies, ability to find more polar compounds and deal with confounding matrix effects, appeared allowing scientists to detect trace concentrations in environmental compartments (Fent et al. 2006; Celiz et al. 2009; Valls-Cantenys et al. 2016). Previous excellent reviews have been dedicated to this topic, although mostly to pharmaceutical and personal care products or emerging contaminants of concern and less so to pesticides (La Farre et al. 2008; Celiz et al. 2009; Mompelat et al. 2009; Evgenidou et al. 2015; Picó & Barceló, 2015; le Cor et al. 2021; Ibáñez et al. 2021; Mosekiemang et al. 2021; Madikizela et al. 2022). Furthermore, the number of works produced about this theme suffered a remarkable increase in recent years. Many of these compounds, parental or transformation products, are however little known in terms of potential detrimental effects and not included in the regulatory monitoring frameworks. Hence, they are nowadays recognized as emerging contaminants of

concern (Murray et al. 2010; Evgenidou et al. 2015; NORMAN network, www.norman-network.net). Pesticides and pharmaceuticals are the two main classes of chemicals continuously represented in the watch list of the WFD and are thus the focus of this review.

The aim of this literature review was to identify ecotoxicological knowledge gaps limiting the risk assessment of transformation products of pesticides and pharmaceuticals found in aquatic samples. We present and discuss updated information about quantification methods, occurrence, fate and the effects of transformation products of these two classes of chemicals. Over recent years, information has been published that needed to be systematized and appraised to bring understanding about their potential impacts on human health and aquatic biota. An important aspect, still enigmatic, is whether these transformation products are more harmful to non-target organisms than their parental compounds and which other factors may influence their potential toxicity. Another problem is the concern raised by transformation products not only as sole compounds per se but also in complex mixtures; mixtures of different metabolites of the same substance and mixtures of different substances, including parental compounds and transformation products.

Applied Methodology

The literature review carried out focused on the global occurrence and fate of the target contaminants in freshwater (i.e. surface-, ground- and influent/effluent wastewater), as well as on the available toxicological and ecotoxicological data. It covers articles published between 1997 and 2022, which have been searched in SCOPUS, Web of Science, PubMed and Google Scholar databases. The terms "pesticides" or "pharmaceuticals" were searched for in combination with "transformation products" or "degradation products", "metabolites", "freshwater", "quantification", "human health" or "aquatic species". The search fields were the "article title", "abstract" and "keywords". Criteria for inclusion of articles in the review were related to the detail provided by the studies (i.e. quantification of the transformation products identified, suitable information about the species employed in the biotests, the age of the exposed organisms, relevant exposure design and endpoints assessed), as well as authors' awareness and control of essential experimental conditions that may bias the results. All analytical methods of quantification have been included, rather than focusing on the most widespread techniques. Adding to this, most articles available in the literature are directed to parental compounds. Some of these works identify a few metabolites. Others do not include terms related to transformation products in the search fields and so they may not been detected. Some articles identify transformation products but do not quantify them, preventing prediction of their concentration in environmental samples (i.e. Mosekiemang et al. 2021; Madikizela et al. 2022). Articles about degradation experiments of pesticides and pharmaceuticals under controlled conditions have also been included, since such transformation processes can occur in natural conditions.

Sources and Fate of Environmental Contamination

Transformation Products of Pesticides

Pesticides have been used since ancient times. Most of them were mainly inorganic compounds or substances of natural origin. However, the development and synthesis of organic pesticides after the second world war increased exponentially its use, making pesticides the second most used group of substances in the environment, only behind fertilizers (Davis 2014; Stokstad and Grullon 2013). Millions of tons of pesticides are applied each year, predominantly in agriculture, which is the main activity responsible for the leaking of pesticides and their sub-products into freshwater ecosystems (Fenner et al. 2013). Although applied in the soil, pesticides can reach aquatic ecosystems through diffuse/non-point-source or point-source pollution sites (Vryzas 2018; Fig. 1).

Diffuse or non-point-source pollution is related with the movement of pesticides from large areas across the watersheds that reach the aquatic environment. Point-source pollution is related to a specific identifiable source which can include chemical run-off during storage, loading, disposal, as well as the misapplication of pesticides to water bodies (Syafrudin et al. 2021). Groundwater is heavily impacted by pesticides, and their metabolites or degradation products (i.e. N,N-dimethylsulfamide; aminomethylphosphonic acid; 2,6-Dichlorobenzamide), mainly resulting from point-source pollution (Postigo and Barcelo 2015). Leaching of landfill and septic tanks or industrial leakage are amongst the main sources of groundwater contamination by pesticides and metabolites (Postigo and Barcelo 2015). Pesticide characteristics (i.e. solubility and vapour pressure) are responsible for higher or lower rates of leaching to groundwater (Park et al. 2020). They also play an essential influence on the degradation of pesticides and the formation of transformation products, including active metabolites. Sorption–desorption, volatilization, chemical and biological degradation, uptake by plants, soil infiltration and leaching are some processes responsible for the appearance of new metabolites and their transportation into groundwater (Arias-Estévez et al. 2007). Groundwater tends, however, to be less affected by contamination than other water bodies, due to the natural attenuation capacity of aquifers and their large capacities (Postigo

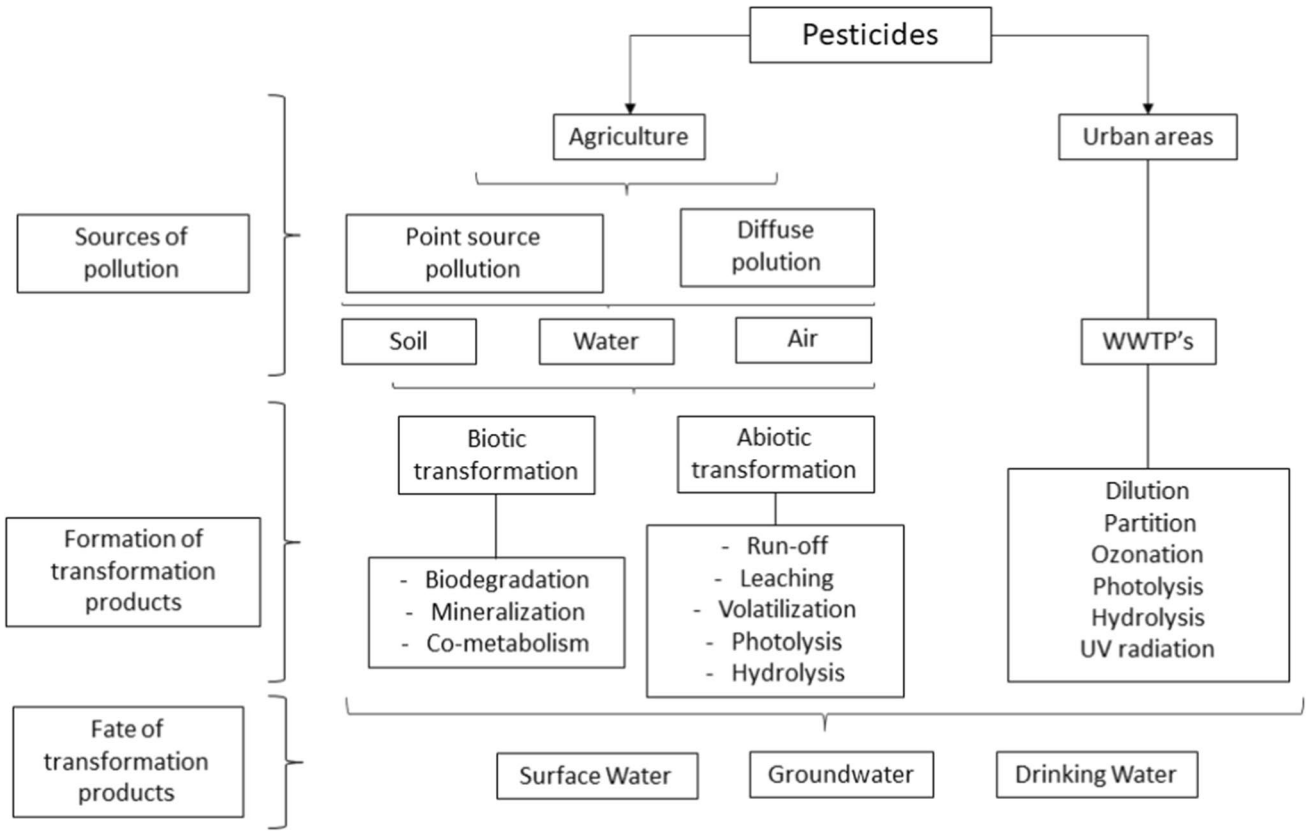


Fig. 1 Sources of pollution, as well as formation and fate of transformation products deriving from pesticides

and Barcelo 2015). Nonetheless, recent monitoring studies show that pesticides, mainly herbicides, and their metabolites/degradation products (i.e. desethylatrazine; cyanazine amide) are present in aquifers (Lapworth and Gooddy 2006; Reemtsma et al. 2013).

Surface waters are mainly contaminated by diffuse pollution sources. Run-off of pesticides and metabolites from agricultural fields after heavy rains are the main pathways of transportation of these substances to surface waters (Vryzas 2018). Moreover, during rainfall pesticides and degradation products imprisoned in soil or sediments can reach surface waters due to movements of those sediments (Vryzas 2018). Application of pesticides using sprays or even the plantation of seeds can be a source of surface water contamination, thanks to wind dispersal (Vryzas 2018). In these waters, photodegradation is the main process responsible for the degradation of pesticides. The formation of such metabolites can reach higher concentrations and show higher toxicity than the parental compounds (Reddy and Kim 2015). Wastewater treatment plants are also amongst the main sources of point-source pollution. Pesticides applied in urban areas (i.e. in maintenance of green areas or ponds) tend to finish in WWTPs, where traditional wastewater treatment methods are ineffective for the removal of these compounds (Rousis et al. 2017; Munze et al. 2017). Moreover, WWTPs effluents show in some cases higher concentrations of pesticides and their transformation products, as well as more toxicity, than the influents. Owing to all this, pesticides and their metabolites can ultimately reach drinking water, exposing humans, as indicated by their detection in the serum and blood of some patients in clinical and scientific studies (Chau et al. 2015; Tyagi et al. 2015).

Transformation Products of Pharmaceuticals

According to Daughton (2016), the first studies regarding the presence of pharmaceuticals in the environment date back to the 1940s. Later on, between the 60 s and 70 s, several works were produced about the possibility of contamination of drinking and surface water by pharmaceuticals, through the discharge of wastewater effluents (i.e. Stumm-Zollinger and Fair 1965; Hignite and Azarnoff 1977). Nowadays, pharmaceutical products are continuously released into the environment, although in small quantities (Fig. 2).

After consumption by humans, pharmaceuticals pass through the liver where they are directly effluxed from the organism (phase 0) or enter phase I and phase II of drug metabolism (Fig. 3). In phase I, more polar metabolites,

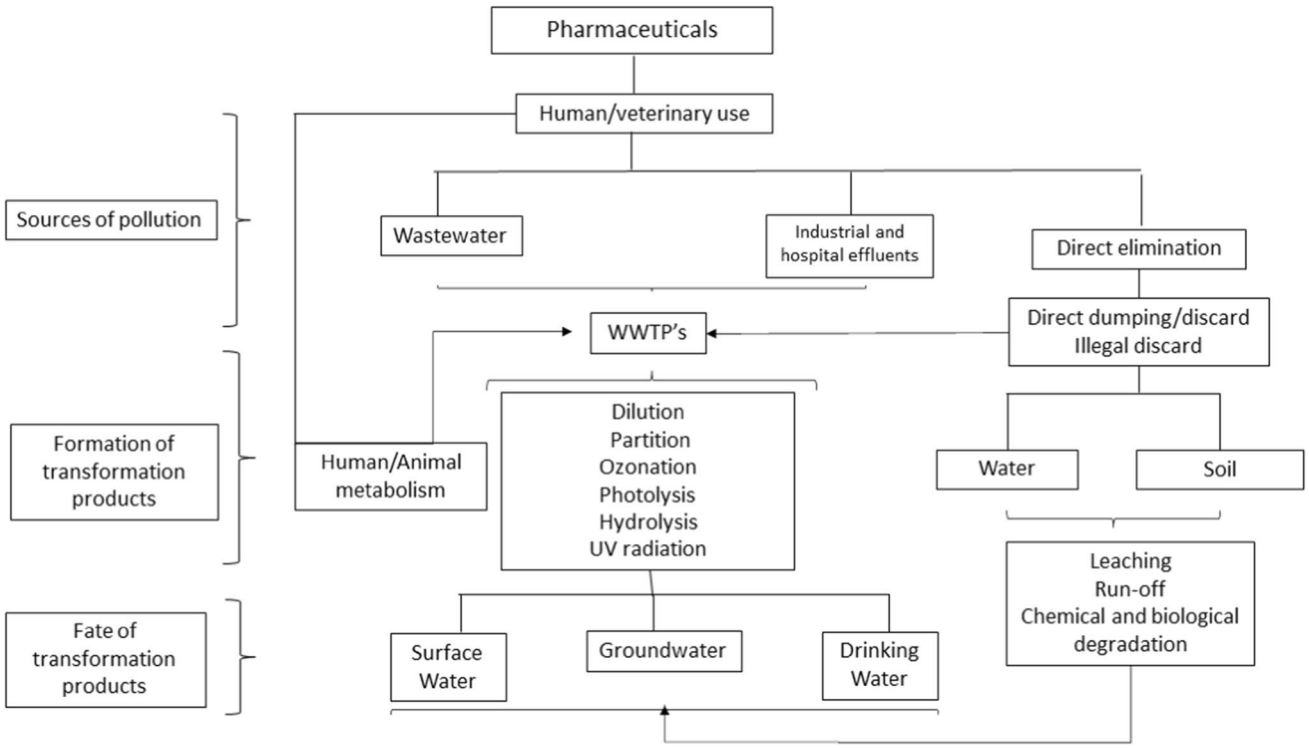


Fig. 2 Sources of pollution as well as formation and fate of transformation products deriving from pharmaceuticals

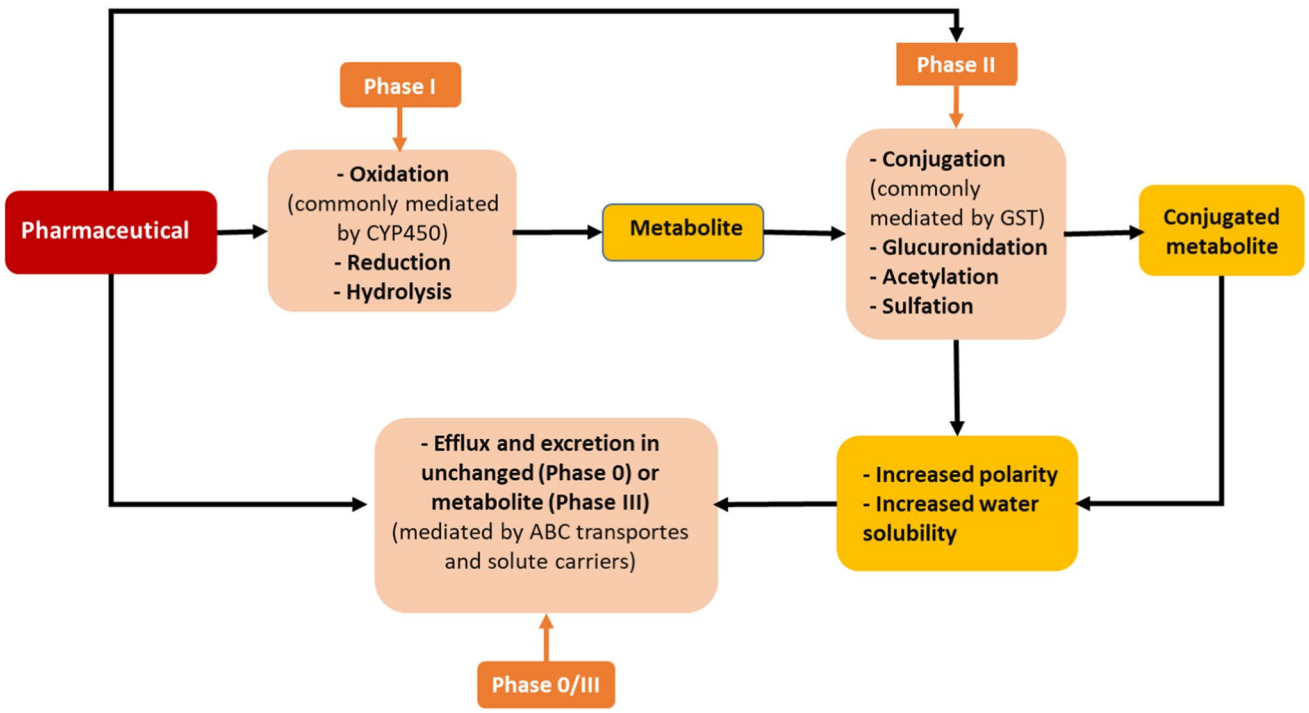


Fig. 3 Schematic representation of pharmaceutical's biotransformation and excretion

often still active, are produced through oxidation, reduction or hydrolysis reactions. These reactions are commonly mediated by different CYP450 genes (i.e. CYP1A; CYP2B; CYP3A). Many of these transformation products become

substrates of phase II, where endogenous hydrophilic groups are added through methylation, glucuronidation, acetylation, sulfation or conjugation with glutathione or amino acids such as glycine, taurine and glutamic acid to form water-soluble inactive compounds that can be excreted by the body in phase III (Fig. 3). Phase III excretion is mediated by ABC transporters and different solute carriers. Due to such reactions, pharmaceuticals can thus be excreted by humans in different forms: unchanged (small proportion) or as active or inactive metabolites (Jjemba 2006; Brown et al. 2015).

Hospital effluents and direct elimination (i.e. through inadequate sanitary disposal) of unused pharmaceuticals in sewage are therefore amongst the most important sources of water contamination (Santos et al. 2010) by pharmaceutical transformation products. Influents are treated in WWTPs (Wastewater Treatment Plants) by three main processes of pollutant removal. In the first treatment, the removal of suspended solids occurs. This treatment has a low degree of efficiency in the removal of micropollutants, like pharmaceuticals (parental compounds or metabolites). In the second treatment, several types of reactions occur, such as dilution, partition, biotic and abiotic transformation (Luo et al. 2014). In this treatment, the level of efficiency is variable depending on the substance or metabolite in question, as well as their physicochemical properties (Luo et al. 2014). The third treatment is related to health questions to humans or specific uses of the treated water. It consists in further removal of substances, like nitrogen or phosphorus, and it is not mandatory, in general (Guardabassi et al. 2002; Luo et al. 2014). After this processing, in some cases, the total load of pharmaceutical compounds or metabolites in the effluent can be higher than that in the influent (Luo et al. 2014). This can be explained by the degradation of parental compounds into several metabolites or degradation products and the transformation of metabolites back into the parental compounds that can occur during the biological treatment in the WWTPs (Luo et al. 2014). Parental compounds and metabolites can also be imprisoned in faecal matter and released into the water during the biological treatment, thus increasing the overall concentration of those substances (Luo et al. 2014). This shows that the treatments available in WWTPs are still not fully efficient in the removal of these micropollutants. Hence, discharge of contaminated effluents introduces into natural waters the parental compounds and many more metabolites or transformation products (i.e. venlafaxine, tramadol, O-desmethyltramadol) (Santos et al. 2010; Luo et al. 2014). Additionally, some of these pharmaceutical metabolites are expected to be more toxic than parental compounds and consequently more dangerous to the wildlife (Celiz et al. 2009). The use of medicines is not exclusive to humans. These are also used in agriculture and aquaculture to treat diseased animals. As in humans, they are also excreted mostly as metabolites in the urine and faeces of animals or through adsorption in dirt pounds and after tanks cleaning, thus entering the environment without any kind of treatment and contaminating the soil and water (Santos et al. 2010). This contamination contributes to further input of transformation products into natural waters via run-off and leaching from the affected soils (Kemper 2008). Other anthropological activities also act as sources of contamination. Industry discharges (sometimes illegally), the use of WWTPs sludge contaminated with all kinds of pharmaceutical compounds as fertilizer, or leakage of septic tanks from households still not connected to the sewage systems, are examples of these (Carrara et al. 2008; Santos et al. 2010).

Detection of Pesticide and Pharmaceutical Transformation Products in Water Compartments

Analytical Methods of Pesticide and Pharmaceutical Transformation Products

As previously mentioned, knowledge about contamination of the aquatic environment by pesticides and pharmaceutical transformation products has increased, mainly due to advances reached in analytical methods (Fent et al. 2006; Valls-Cantenys et al. 2016). New methods, with higher separation efficiencies and the ability to find more polar compounds, appeared. This allowed scientists to detect concentrations in environmental compartments in the order of ng/L and µg/L and consequently raised awareness and concern about their potential hazardousness (Fent et al. 2006; Santos et al. 2010; Valls-Cantenys et al. 2016). However, these advances in analytical methods are not efficient if the correct sample preparation is not performed. The extraction of the analytes from an environmental water sample is a crucial step before the instrumental analysis. Extraction techniques are based on the passage of an analyte by different solvents, which must be the most suitable for the type of analytical tool to be employed (Rutkowska et al. 2019; Campanale et al. 2021). This step can highly influence the analytical process, mainly for quantitative analysis, since the analyte volume must be increased, whilst any interferences must be eliminated (Campanale et al. 2021). Several sample preparation techniques are already described in the literature. For analysis of water samples, SPE (solid-phase extraction) is the most extensively used technique (Dimpe and Nomngongo 2016; Campanale et al. 2021). This method uses columns or disks able to retain the active compounds present in water samples and posteriorly release them by washing with small quantities of suitable solvents (Dimpe and Nomngongo 2016; Campanale et al. 2021). This provides an extract with few interferences, suitable for different analytical methodologies, such as High-Pressure Liquid

Chromatography-Mass Spectrometry (HPLC–MS) and Gas Chromatography-Mass Spectrometry (GC–MS). More recently, a new SPE-based approach has been tested: Solid-Phase MicroExtraction (SPME). This newer method is faster and requires fewer quantity of solvents and is well described as suitable for Gas chromatography (GC) analysis (Campanale et al. 2021). Liquid–liquid extraction (LLE) is a simple method widely used for water samples that is also applied in the analysis of pesticide and pharmaceuticals (Dimpe and Nomngongo 2016; Campanale et al. 2021). This method has the advantage to be well established amongst different governmental agencies, but it also is time-consuming and requires the use of organic solvents that are harmful to the environment and even the handler (Dimpe and Nomngongo 2016; Campanale et al. 2021). Though the techniques described are the most well established for pharmaceuticals and pesticides, they have some disadvantages. One is the loss of more volatile analytes during the extraction process, which can affect the result of the analysis; another is the use of toxic solvents (Dimpe and Nomngongo 2016; Campanale et al. 2021). Much more methods are available in the literature, although less widespread. The development of new cost-effective and green methodologies for fast extraction is the next challenge in need to be addressed to improve and analytical determination. Regarding the analytical methods and instrumentation, GC and/or liquid chromatography (LC) coupled to mass spectrometry (MS) are, nowadays, the most applied methods to detect pesticides and pharmaceutical compounds. For LC, some variations to this method are well established in the literature, such as high-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC) (Gumustas et al. 2013). Gas chromatography is the most suitable method to separate non-polar parental compounds and their transformation products (Sparkman et al. 2011). This happens because of the inclusion of a derivatization process during GC that increases volatility and sensitivity, but also increases the duration of the procedure (Subramaniam et al. 2013). Coupling the GC with the MS has the advantage to offer a specific mass spectrum for a certain compound when an electron ionization (EI) is also performed (Foltz et al. 2016). Polar pharmaceutical or pesticide and their transformation products are mainly separated by LC (Martín-Pozo et al. 2019). Most of the pesticides and pharmaceuticals in their unchanged form or as transformation products are usually quantified at low concentrations in environmental water samples (ng to µg/L). This makes liquid chromatography with tandem mass spectroscopy (LC–MS/MS) a widely used method for determination of these compounds. This is due to its higher discrimination between the analyte and matrix signal, coupled to robustness and relative ease of use (Kaufmann et al. 2012). However, the selectivity and sensitivity of the MS vary with the selected ionization. Electrospray ionization (ESI) is the most chosen technique for detecting pharmaceuticals, since it is the most potent ionization method for the target compounds (Huang et al. 2019).

Levels in Different Water Compartments

Transformation Products of Pesticides

As mentioned above, advances in the detection techniques led to an increase in knowledge about the occurrence of pharmaceutical and pesticide transformation products in different water compartments. Nevertheless, the quantification of transformation products is still not a focus in scientific investigation, as often studies only present new methods of detection and their validation, but not the concentrations found in real samples, even for parental compounds (Wode et al. 2015; Boix et al. 2016). Overall, the search carried out in the scientific databases returned 87 articles providing concentrations of pesticide and pharmaceutical transformation products in environmental water samples. Only one article quantified both pesticide and pharmaceutical transformation products (Huntscha et al. 2012). Of these, 29 articles were dedicated to pesticides, presenting concentrations obtained for 92 transformation products resulting from 43 parental compounds (Fig. 4, Table S1 in the Support Information). The most assessed pesticide transformation products (69%) belonged to three functional classes: organochlorine and chloroacetanilide herbicides/pesticides; triazine herbicides; and organophosphate and carbamate pesticides (Fig. 4, Table S1). Data about the quantification of pesticide transformation products found in different water samples, and respective detection methods, are presented in Fig. 5 and Table S1 (supplementary data). Transformation products of triazine herbicides were frequently reported in different studies, with a special focus on atrazine and terbuthylazine (Fig. 5, Table S1). Concentrations of these varied widely from 0.046 µg/L for desethylatrazine to 124,01 µg/L for hydroxy-terbuthylazine. The high concentration found for hydroxy-terbuthylazine resulted from an experiment where the parental compound was applied in a constructed wetland planted with *Typha latifolia* (Papadopoulos et al. 2007). According to the authors, the maximum concentration of the metabolite was within the highest concentration range found for the parental compound. This is of main concern, given that these concentrations are in the order of µg/L. Though knowledge about the environmental impact of this transformation product is sparse, recent works highlighted negative effects on the early developmental stages of fish species even at concentrations found in natural water samples (Velisek et al. 2014).

Chloroacetanilide herbicides are widely used for grass control in several crops. Compounds of this class are structurally similar and were extensively used from the mid-1990s

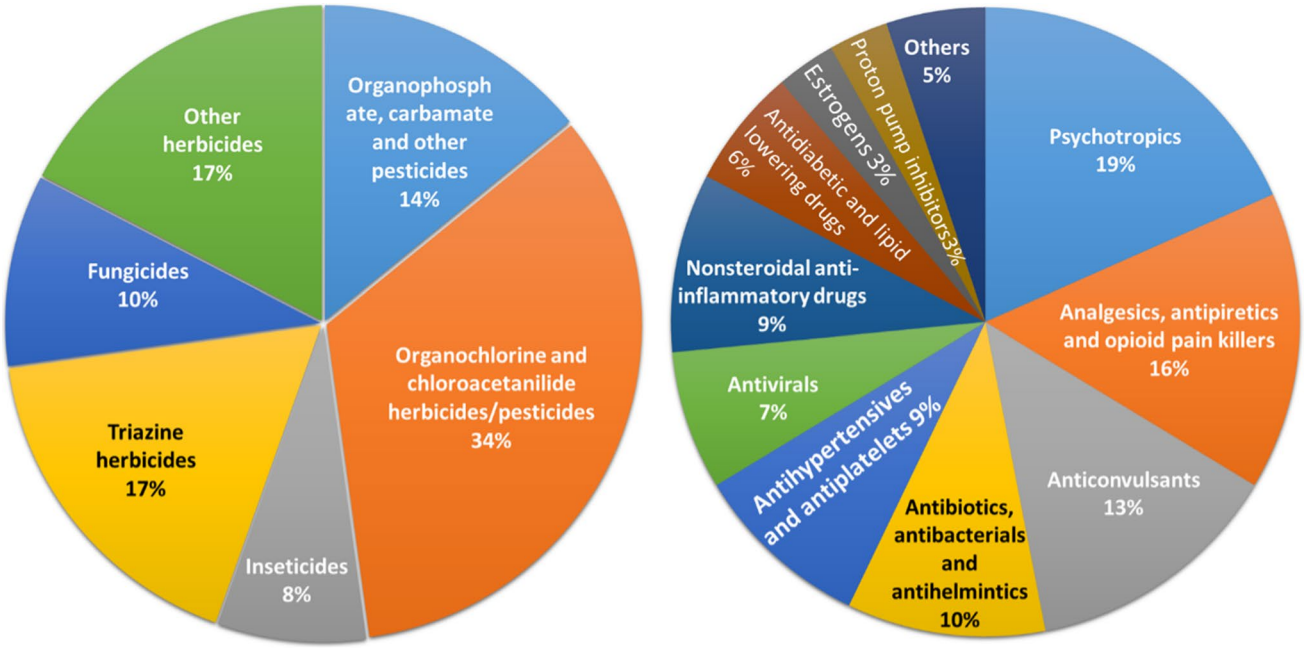


Fig. 4 Relative frequency of transformation products quantified in environmental water samples per functional class of pesticides or pharmaceuticals. Concentrations for 92 pesticide transformation products derived from 43 parental compounds and 98 pharmaceutical transformation products derived from 64 parental compounds were found in the scientific literature published between 2000 and 2020

until recently (Elsayed et al. 2015). The main transformation products ethane sulfonic acid (ESA) and oxalinic acid (OXA), alongside the parental compound, are easily transported to water bodies and usually detected in both surface and groundwater (Table S1), contributing to the degradation of water quality (Baran and Gourcy 2013). Another interesting observation is the concentration level of metolachlor OXA and ESA in relation to the parental compound. Studies reported that metabolites of metolachlor, mainly ESA, were found in groundwater at higher concentrations than the parental compound (White et al. 2009; Baran and Gourcy 2013). This may occur because metabolites adsorb less to soil particles, compared to the parental compound and are thus more prone to infiltration to aquifer recharge (Baran and Gourcy 2013). This highlights the importance of monitoring programmes not only for pesticides alone but also for their transformation products.

One of the first mass-produced pesticides in the world was DDT (Dichlorodiphenyltrichloroethane). It is an inexpensive and highly efficient short-term insecticide, but in the long term, it is problematic to human and animal health (Kezios et al. 2013). This pesticide was systematically banned in developed countries since the 1970s and a global ban of DDT, for non-vector control use, was exerted in the Stockholm Convention on Persistent Organic Pollutants, which took effect in 2004. However, this substance as well as several of its transformation products are still found in natural water bodies (Table S1) and tissues of different organisms (Veljanoska-Sarafiloska et al. 2013). A study conducted in African lakes showed that 4,4-DDE, a DDT metabolite, was biomagnified in fish species of the lake (Deribe et al. 2013). This was worrying, as those fish were consumed by local populations, possibly impacting human health. It is also of high concern the fact that metabolites of DDT, as well as the parental compound, are still commonly found in the environment even after an almost total ban worldwide, showing the great persistence of this substance and its transformation products in the ecosystems.

Carbofuran is one of the carbamate pesticides most toxic to vertebrates, including humans, but knowledge about its main transformation products is still sparse. Otieno and colleagues (2010) reported the presence of very high concentrations of 3-ketocarbofuran and carbofuran-3-hydroxy (Table S1) in surface waters highly impacted by agrochemical procedures. Concentrations found (> 890 µg/L) were well above the standard water concentrations allowed by the USA and European authorities for safe drinking and human use (Otieno et al. 2010). Also, these two compounds appeared to be more persistent and were detected in higher concentrations than the parental compound (Otieno et al. 2010). Considering this, it would be crucial to gain a higher level of knowledge about the possible effects of these substances on non-target organisms, including humans, that could help infer about the need for more strict monitoring routines aiming at minimizing potential impacts on water quality and populations' health.

Transformation Products of Pharmaceuticals

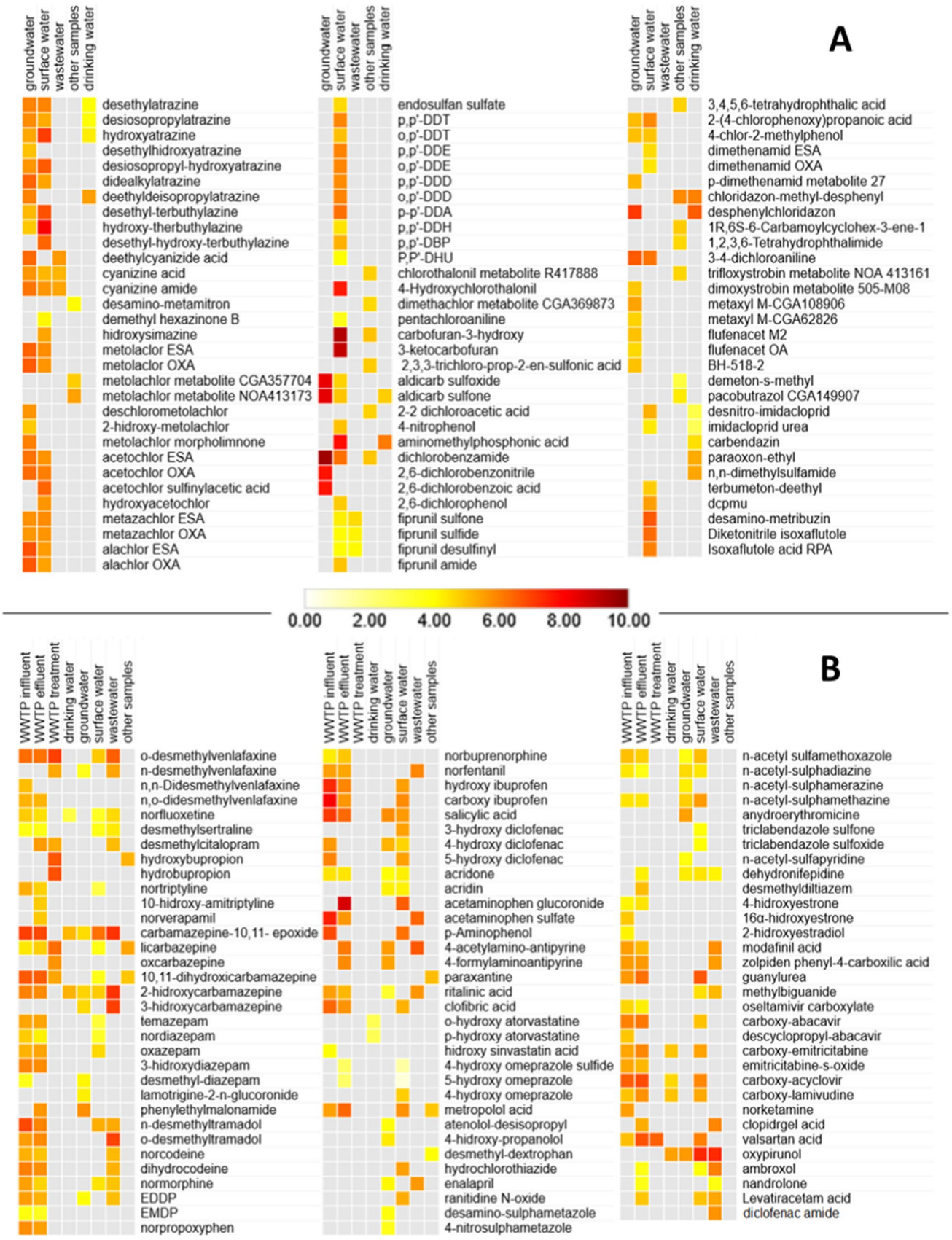
Fifty-eight articles were dedicated to pharmaceuticals, presenting concentrations obtained for 98 transformation products resulting from 64 parental compounds (Fig. 4, Table S2 in the Support Information). The most investigated transformation products belonged also to three functional classes: psychotropic drugs; analgesics, antipyretics and opioid painkillers and anticonvulsants (Fig. 4, Table S2). Carbamazepine transformation products, alongside metabolites of selective monoamine reuptake inhibitors and of ibuprofen, were the ones most reported in the literature (Fig. 5). The range of concentrations found varied from < 0.50 ng/L to 462000 ng/L, showing that very high concentration values of pharmaceutical metabolites are already found in the natural environment. Acetaminophen metabolites were the ones with higher reported concentrations. Sunkara and Wells (2010) reported concentrations higher than 400000 ng/L for acetaminophen glucuronide and sulphate in WWTP effluents. Those values were obtained in samples collected after application of conventional treatment processes in WWTP, pointing out the inefficiency of these treatments for the removal of micropollutants. Moreover, the authors refer that sometimes, metabolite concentrations were higher in the effluent than in the influent and one of the reasons for that was the bioconversion that may occur during the biological treatment, as mentioned previously. However, following UV treatment, none of the metabolites was found. This could be soothing, but the UV treatment is not always applied in WWTP; it is an optional treatment used mainly in water for human consumption (Luo et al. 2014; Guardabassi et al. 2002). Water without UV treatment loaded with transformation products can thus re-enter the water cycle, potentially risking aquatic fauna and flora. Also, it can be reused in agricultural practices and therefore contaminate crops, making metabolites enter the food chain with risk to human health. Carboxy ibuprofen was also reported at a very high concentration, higher than 100000 ng/L, in WWTP influents according to Paíga and colleagues (2016). Samples were collected in a relatively small WWTP designed to serve a little less than 50,000 people. Receiving wastewaters were mainly domestic and conventional treatments with activated sludge were applied (Paíga et al. 2016). Carboxy ibuprofen is one of the most representative ibuprofen metabolites. Ibuprofen is a commonly used non-steroidal anti-inflammatory (NSAID) drug and in 2016 it was the most used NSAID in Portugal, where the study was conducted (Monteiro et al. 2017). It is thus important to have a stricter monitoring routine for these substances to better evaluate the possible effects of metabolites on human and non-human health. Also, carbamazepine-10,11-epoxide was reported to occur at concentrations higher than 10000 ng/L in WWTP influents (Gros et al. 2012) and municipal wastewater (Petrovic et al. 2014). This is one of the main carbamazepine metabolites and one of the most detected in natural water samples (Table S2). An interesting fact in the study of Petrovic et al. (2014) is that carbamazepine-10,11-epoxide was found at a much higher concentration than the parental compound. This was also reported previously by Lopez-Serna et al. (2012) in a study conducted in the Ebro River in Spain. Those data reinforce the necessity of an extensive assessment and monitoring routine for metabolites, once they can be more prevalent in water compartments, compared to their parental compounds.

Risks of Pharmaceutical and Pesticide Transformation Products

Human Health

Although the available data are sparse, freshwater contamination does not affect only organisms living in those systems. Ultimately, humans can also suffer negative effects from exposure to transformation products. Humans are exposed to pesticide and pharmaceutical transformation products in different ways. Data presented in Tables S1 and S2 show levels of those transformation products detected in drinking water and groundwater as well, which is a common source of drinking water in cities around the world (Guimarães et al. 2019). As previously mentioned, exposure can occur via contaminated recreational water and/or consumption of contaminated freshwater organisms or other food produced with water originating from contaminated sites. Knowledge about human health risks caused by transformation products of pesticides and pharmaceuticals is still sparse, compared to parental compounds. Studies available in the scientific literature are presented in Table 1.

The adverse effects that pesticides can cause on human health are a long-known problem. This discussion gained bigger attention and impact since the publication of the book Silent Spring in 1962. In this publication, Rachel Carson described not only the environmental impacts coinciding with the widespread use of DDT in agriculture in the USA, but also the potential of DDT to cause cancer in exposed workers. In the book, other pesticides were also surveyed, such as 2,4-D (2,4-Dichlorophenoxyacetic acid), chlordane and heptachlor. More recently different environmental agencies, including EPA (United States Environmental Protection Agency) and ECHA (European Chemicals Agency) or international conventions are banning the use of some pesticides that were described as hazardous to human health. Amongst the pesticide metabolites that can elicit problems,


A
groundwater
surface water
wastewater
other samples
drinking water
desethylatrazine
desiosopropylatrazine
hydroxyatrazine
desethylhidroxyatrazine
desiosopropyl-hydroxyatrazine
didealkylatrazine
deethyldeisopropylatrazine
desethyl-terbuthylazine
hydroxy-therbuthylazine
desethyl-hydroxy-terbuthylazine
deethylcyanizide acid
cyanizine acid
cyanizine amide
desamino-metamitron
demethyl hexazinone B
hidroxysimazine
metolaclor ESA
metolaclor OXA
metolachlor metabolite CGA357704
metolachlor metabolite NOA413173
deschlorometolachlor
2-hidroxy-metolachlor
metolachlor morpholimnone
acetochlor ESA
acetochlor OXA
acetochlor sulfinylacetic acid
hydroxyacetochlor
metazachlor ESA
metazachlor OXA
alachlor ESA
alachlor OXA
endosulfan sulfate
p,p'-DDT
o,p'-DDT
p,p'-DDE
o,p'-DDE
p,p'-DDD
o,p'-DDD
p-p'-DDA
p,p'-DDH
p,p'-DBP
P,P'-DHU
chlorothalonil metabolite R417888
4-Hydroxychlorothalonil
dimethachlor metabolite CGA369873
pentachloroaniline
carbofuran-3-hydroxy
3-ketocarbofuran
2,3,3-trichloro-prop-2-en-sulfonic acid
aldicarb sulfoxide
aldicarb sulfone
2-2 dichloroacetic acid
4-nitrophenol
aminomethylphosphonic acid
dichlorobenzamide
2,6-dichlorobenzonitrile
2,6-dichlorobenzoic acid
2,6-dichlorophenol
fiprunil sulfone
fiprunil sulfide
fiprunil desulfinyl
fiprunil amide
3,4,5,6-tetrahydrophthalic acid
2-(4-chlorophenoxy)propanoic acid
4-chlor-2-methylphenol
dimethenamid ESA
dimethenamid OXA
p-dimethenamid metabolite 27
chloridazon-methyl-desphenyl
desphenylchloridazon
1R,6S-6-Carbamoylcyclohex-3-ene-1
1,2,3,6-Tetrahydrophthalimide
3-4-dichloroaniline
trifloxystrobin metabolite NOA 413161
dimoxystrobin metabolite 505-M08
metaxyl M-CGA108906
metaxyl M-CGA62826
flufenacet M2
flufenacet OA
BH-518-2
demeton-s-methyl
pacobutrazol CGA149907
desnitro-imidacloprid
imidacloprid urea
carbendazin
paraoxon-ethyl
n,n-dimethylsulfamide
terbumeton-deethyl
dcpmu
desamino-metribuzin
Diketonitrile isoxaflutole
Isoxaflutole acid RPA
0.00
2.00
4.00
6.00
8.00
10.00
B
WWTP influent
WWTP effluent
WWTP treatment
drinking water
groundwater
surface water
wastewater
other samples
o-desmethylvenlafaxine
n-desmethylvenlafaxine
n,n-Didesmethylvenlafaxine
n,o-didesmethylvenlafaxine
norfluoxetine
desmethylsertraline
desmethylcitalopram
hydroxybupropion
hydrobupropion
nortriptyline
10-hidroxy-amitriptyline
norverapamil
carbamazepine-10,11- epoxide
licarbazepine
oxcarbazepine
10,11-dihydroxicarbamazepine
2-hidroxycarbamazepine
3-hidroxycarbamazepine
temazepam
nordiazepam
oxazepam
3-hidroxydiazepam
desmethyl-diazepam
lamotrigine-2-n-glucoronide
phenylethylmalonamide
n-desmethyltramadol
o-desmethyltramadol
norcodeine
dihydrocodeine
normorphine
EDDP
EMDP
norpropoxyphen
norbuprenorphine
norfentanil
hydroxy ibuprofen
carboxy ibuprofen
salicylic acid
3-hydroxy diclofenac
4-hydroxy diclofenac
5-hydroxy diclofenac
acridone
acridin
acetaminophen glucoronide
acetaminophen sulfate
p-Aminophenol
4-acetylamino-antipyrine
4-formylaminoantipyrine
paraxantine
ritalinic acid
clofibric acid
o-hydroxy atorvastatine
p-hydroxy atorvastatine
hidroxy sinvastatin acid
4-hydroxy omeprazole sulfide
5-hydroxy omeprazole
4-hydroxy omeprazole
metropolol acid
atenolol-desisopropyl
4-hidroxy-propanolol
desmethyl-dextrophan
hydrochlorothiazide
enalapril
ranitidine N-oxide
desamino-sulphametazole
4-nitrosulphametazole
n-acetyl sulfamethoxazole
n-acetyl-sulphadiazine
n-acetyl-sulphamerazine
n-acetyl-sulphamethazine
anydroerythromicine
triclabendazole sulfone
triclabendazole sulfoxide
n-acetyl-sulfapyridine
dehydronifepidine
desmethyldiltiazem
4-hidroxyestrone
16α-hidroxyestrone
2-hidroxyestradiol
modafinil acid
zolpiden phenyl-4-carboxilic acid
guanylurea
methylbiguanide
oseltamivir carboxylate
carboxy-abacavir
descyclopropyl-abacavir
carboxy-emitricitabine
emitricitabine-s-oxide
carboxy-acyclovir
carboxy-lamivudine
norketamine
clopidrgel acid
valsartan acid
oxypirunol
ambroxol
nandrolone
Levatiracetam acid
diclofenac amide

◂**Fig. 5** Maximum concentrations of pesticide (**A**) and pharmaceutical (**B**) transformation products found in different water compartments. The heatmaps were done with the log-transformed values (pg/L) (Tables S1 and S2). Grey squares represent situations for which no information could be found

mitotane was proven to be a selective toxicant to humans and is used as an adjuvant drug to treat adrenocortical tumours (Wajchenberg et al. 2000). Mitotane or o,p'-dichlorodiphenyldichloroethane (o-p'-DDD) is a DDT metabolite and apparently the only chemical able to inhibit corticoid synthesis and at the same time destroy cortical cells (Wajchenberg et al. 2000). However, despite the therapeutic use, mitotane was already reported in the literature to have side effects at hormonal levels in patients who were treated with this compound (Daffara et al. 2008). The authors analysed the blood cells and the saliva of the patients and found that mitotane treatment was linked to the inhibition of cortisol and DHEAS (Dehydroepiandrosterone sulphate). Also, perturbations of the thyroid function were described. Moreover, for males, an inhibition of testosterone secretion was also found. However, these side effects were usually reversible with the adequate treatment. Another DDT metabolite, DDE (dichlorodiphenyldichloroethylene) was reported to induce apoptosis of human peripheral blood mononuclear cells, both in vitro and in vivo (Perez-Maldonado et al. 2006). The authors studied blood collected from 61 healthy children during the year 2004 and from 57 children from southern Mexico. Exposure to both DDT, DDD and DDE was found in the tested children. However, significant correlations between apoptosis and exposure to pesticides were only found for DDE blood levels, ($p = 0.010$ and 0.040 for 2003 and 2004, respectively). This causes great concern since DDE is the most persistent DDT metabolite and thus exposure tends to be chronic, and apoptosis of the cells could result in an impairment of the immune system (Perez-Maldonado et al. 2006). Both p,p′-DDE chloroethane and p,p′-DDD (dichlorodiphenyldichloroethane) were reported to induce DNA damage in human lymphocytes, even at low concentrations (Geric et al. 2012). In this study, in vitro human lymphocytes were exposed for 1, 6 and 24 h to p,p'-DDE (4.1 µg/mL) or p,p'-DDD (3.9 µg/mL) and genotoxic effects were assessed using the cytokinesis-block micronucleus assay and the comet assay. Results showed an increase in the number of cells containing micronucleus, in relation to the control, in the 24-h exposures. Also, according to the comet assay, the percentage of DNA damages increased, in relation to the control. It is important to notice that the concentrations used are in the range found in human fluids, suggesting that these effects are already occurring in humans exposed to the metabolites (Geric et al. 2012).

The metabolite 2,4-dichlorophenol, from the herbicide 2,4-D, was reported to cause effects on antioxidant enzymes and glutathione levels in human erythrocytes in vitro (Bukowska, 2003): the activity of superoxide dismutase decreased whilst that of glutathione peroxidase increased in a dose-dependent (10–500 ppm) manner. Moreover, exposure to 250-ppm 2,4-dichlorophenol also decreased the level of reduced glutathione in erythrocytes by 32%, in relation to the control. These effects are similar, though more pronounced, to those resulting from exposure to the parental compound 2,4-D, pointing to a major need for monitoring pesticide metabolites in natural samples. Dialkylquinoneimine metabolites of chloroacetanilide herbicides like alachlor and acetochlor were reported to induce in vitro sister chromatid exchanges in human lymphocytes (Hill et al. 1997). This study was performed to test the hypothesis that the oncogenicity of chloroacetanilide herbicides previously described was caused by genotoxic intermediates, like diethylbenzoquinoneimine, an alachlor metabolite. The investigation was done with cultured human peripheral lymphocytes, mostly T cells. At 0.3-µM high variability was observed, with effects elicited by N-dealkyl-alachlor, aniline metabolites and their 4-hydroxy derivatives and diethylbenzoquinone, in only half of the cases. At 0.1–0.3 µM the ratio between treated and control cells for sister chromatid exchange was always higher in exposures to diethylbenzoquinoneimine than to dimethyl- and ethylmethylbenzoquinoneimines. The study showed that all the compounds assessed were toxic to lymphocytes and provided the first evidence that metabolites of chloroacetanilide herbicides were genotoxic to humans and could significantly affect the immune system (Hill et al. 1997). Glyphosate metabolites were also reported to have cyto- and hematotoxicity in humans. Aminomethylphosphonic acid (AMPA) is the main metabolite of glyphosate. This transformation product is recognized to have similar levels of toxicity comparing to its parental compound, and human exposure was already described (Benachour and Séralini, 2009; Kwiatkowska et al. 2014). The embryonic kidney, HUVEC primary neonate umbilical cord vein and JEG3 placental cell lines were exposed to 18 different AMPA concentrations varying from 10 ppm to 10% for 24 h (Benachour and Séralini, 2009). The authors reported that AMPA exposure induced succinate dehydrogenase and adenylate kinase effects on human cells and thus mortality. AMPA exposure resulted in the destruction of the cell membrane, in all cell types. More recently, another study was performed to determine AMPA hematotoxicity in human erythrocytes (Kwiatkowska et al. 2014). The authors exposed human erythrocytes to 0.01–5 mM AMPA, during 1, 4 or 24 h and evaluated the exposure effects in haemolysis, haemoglobin oxidation, ROS formation and the erythrocytes morphology. Results showed that AMPA induced haemolysis at concentrations equal or higher than 0.05 mM and haemoglobin oxidation (≥ 0.25 mM) after 24 h of incubation. An increase in ROS production was also registered

Table 1 Toxicological studies about the human health risks of pesticide and pharmaceutical transformation products

Transformation product [Parental compound] Reference	Concentrations	Sample	Exposure duration	Endpoints	Effects
Pesticides					
Chloroacetanilide, aniline; hydroxychloroacetanilide; and diethylquinoneimine [Alachlor] (Hill et al. 1997)	0; 0.03; 0.1; and 0.3 µM	Lymphocyte cells	72 h	Oncogenicity	Induction of chromatid exchange at 0.1 µM for hydroxychloroacetanilide, 0.3 µM for chloroacetanilide and aniline
Mitotane [DDT] (Daffara et al. 2008)	Distinct values for each sample	Blood cells and saliva	not applicable	Hormonal levels and organ toxicity	Inhibition of cortisol and DHEAS. Induction of thyroid function perturbations. Inhibition of testosterone secretion
2–4-dichlorophenol [2–4-D] (Bukowska, 2003)	10 to 500 ppm	Blood cells	1 h	Antioxidant enzymes	Increase of superoxide dismutase and increase of gluthathione peroxidase activities
DDE [DDT] (Perez-Maldonado et al. 2006)	Distinct values for each sample	Blood cells	not applicable	Genotoxicity	Induction of peripheral blood mononuclear cells
p-p' DDE [DDT] (Geric et al. 2012)	4.1 µg/ml	Lymphocyte cells	1; 6 and 24 h	Genotoxicity	Induction of DNA damage
p-p' DDE [DDT] (Geric et al. 2012)	3.9 µg/ml	Lymphocyte cells	1; 6 and 24 h	Genotoxicity	Induction of DNA damage
AMPA [glyphosate] (Benachour and Séralini, 2009)	18 concentrations from 10 ppm to 10%	Embryonic kidney HUVEC primary neonate umbilical cord vein, embryonic kidney. and JEG3 placental cell lines	24 h	Cytotoxicity	Increased cellular mortality. Destruction of the membrane of all cell types
AMPA [glyphosate] (Kwiatkowska et al. 2014))	0.01–5 mM	Erythrocytes	1, 4 and 24 h	Haemolysis, haemoglobin oxidation, ROS formation and morphology	Induction of haemolysis (0.05 to 5 mM) and haemoglobin oxidation (0.25 to 5 mM) at 24-h incubation. Increase in ROS production at concentrations starting from 0.25 Mm
Methylsulphonic acid [glyphosate] (Kwiatkowska et al. 2014))	0.01–5 mM	Erythrocytes	1, 4 and 24 h	Haemolysis, haemoglobin oxidation, ROS formation and morphology	Induction of haemolysis (0.1 to 5 mM) and haemoglobin oxidation (0.5 to 5 mM) at 24-h incubation. Increase in ROS production at 0.5 and 5 mM
Pharmaceuticals					
Gemfibrozil 1-O-β- glucoronide [gemfibrozil] (Ogilvie et al. 2006)	0.25 to 64 µM	Liver microsomes	2 to 40 min	CYP2C8 activity	Potent inhibitor of CYP2C8

Table 1 (continued)

Transformation product [Parental compound] Reference	Concentrations	Sample	Exposure duration	Endpoints	Effects
				Genotoxicity and mitogenicity	
2-hidroxyestrone and 16-α hydroxyestrone [estrogens] (Eliassen et al. 2008)	not applicable	Blood cells	not applicable		Levels of 2-hydroxyestrone, and the ratio between 2-hydroxyestrone and 16-α hydroxyestrone were linked with certain types of breast cancer tumours in woman
Morphine-3-glucoronide [morphine] (Dozio et al. 2022)	1, 10 and 100 µM	Astrocytes	12, 24, 48 and 96 h	Proteomics	96-h exposure lead to dysregulation of biological pathways linked with extracellular matrix organization, antigen presentation, cell adhesion and glutamate homeostasis
Morphine-6-glucoronide [morphine] (Dozio et al. 2022)	1, 10 and 100 µM	Astrocytes	12, 24, 48 and 96 h	Proteomics	Acute exposure increased the levels of proteins involved in cell adhesion and decreased the levels of extracellular matrix

at concentrations starting from 0.25 mM. The same study also investigated the hematotoxic effects of other glyphosate metabolite: methylphosphonic acid. The results were similar to those obtained for AMPA, although at a different concentration range. Induction of haemolysis and haemoglobin oxidation occurred at concentrations ≥ 0.1 and 0.5 mM, respectively. In addition, ROS production was found at concentrations ≥ 0.5 mM (Kwiatkowska et al. 2014).

Pharmaceutical metabolites are not usually expected to represent an exposure concern to humans. However, biotransformation and detoxification reactions can lead to the formation of active pharmaceutical metabolites potentially more toxic than the respective parental compounds (Celiz et al. 2009). For example, gemfibrozil 1-O-β-glucuronide, the major gemfibrozil metabolite, was found to be a more potent inhibitor of CYP2C8 than the parental compound in human liver microsomes (Ogilvie et al. 2006). Also, Ogilvie and colleagues found that gemfibrozil glucuronide, contrarily to the parental compound gemfibrozil, was found to be a CYP2C8 selective inhibitor acting in a metabolism-dependent way. To depict such differences, the authors evaluated both the parental compound and its main metabolites as inhibitors of the main drug metabolizing CYP450 enzymes (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4) in human liver microsomes. Compounds inhibiting the activity of the CYP450 complex can affect the metabolism of other drugs and lead to accumulation and potential toxic effects, exerting an undesired effect in the exposed person (Ogilvie et al. 2006). In fact, the chemical reactivity of glucuronide metabolites has been linked with toxic properties. These metabolites can reach appreciable concentrations in human tissues and blood. They can also undergo hydrolysis and pH-dependent intramolecular acyl migration, irreversibly reacting with human tissues. This can cause chemical alterations leading to drug toxicity expressed by alterations in functional properties of the modified molecules or hypersensitivity and other immunotoxic reactions (Shipkova et al. 2003).

Pharmaceutical endocrine disruptors have been linked to several adverse effects on human health (Safe 2000). A wide range of parental compounds have been associated with hazardous effects on human reproduction and cancer development, amongst others, and metabolites are not excluded. Estrogen metabolites are reported as possible mitogenic and genotoxic substances. Investigating blood samples collected between 1989 and 1990 in subjects taking oestrogens and in controls not taking them, Eliassen et al. (2008) found a significant positive association in women of the plasma levels of 2-hydroxyestrone, and the ratio between 2-hydroxyestrone and 16-α hydroxyestrone, with certain types of breast cancer tumours. The authors recognized, nevertheless, the need for replicating the study and increasing research about the relationship between estrogen metabolites and estrogen and

progesterone receptors related to breast tumours. Morphine is a strong painkiller, which is widely prescribed worldwide. However, this opiate was described to be potentially toxic to humans, not only the parental compound but also its metabolites' morphine-3-glucoronide and morphine-9-glucoronide (Dozio et al. 2022). In a recent study, Dozio and colleagues performed a deep proteomic study in human astrocytes to investigate the role of central nervous system glial cells in the mechanisms originating the side effects of morphine administration in humans. For that, they exposed astrocytes during 12, 24, 48 and 96 h to 1-, 10- and 100-µM morphine, morphine-3-glucoronide and morphine-6-glucoronide. The proteomic analysis showed the 96-h exposure to morphine-3-glucoronide lead to dysregulation of biological pathways linked with extracellular matrix organization, antigen presentation, cell adhesion and glutamate homeostasis. For morphine-6-glucoronide (12-24-h exposure), increased levels of proteins involved in cell adhesion and decreased levels of extracellular matrix were observed.

Aquatic Biota

Transformation Products of Pesticides

Knowledge about toxic effects caused by pesticide transformation products is still sparse, compared to parental compounds. Studies available in the scientific literature are presented in Table 2.

One of the most controversial pesticides is DDT, which was reported to cause health issues to humans and living organisms in general. Moreover, studies are available in the literature linking exposure to DDT metabolites to negative effects on the health of aquatic organisms. Donohoe and Curtis (1996) injected juvenile rainbow trout with o,p'-DDT, o,p'-DDE or p,p'-DDE with doses ranging from 5 to 30 mg/kg at 0, 14 and 28 days and sampling was done at 14 and/or 42 days. They reported that o,p'-DDT and o,p'-DDE had estrogenic activity, because of the elevated plasma vitellogenin levels they can elicit in vivo and their interaction with hepatic estrogenic binding sites (Donohoe and Curtis, 1996). A study conducted in freshwater amphipods (*Hyalella azteca* and *Diporeia* spp.) reported that the metabolites DDD and DDE are less lethal than DDT (Lotufo et al. 2000). *Hyalella azteca* and *Diporeia* spp. were exposed to a wide range of concentrations of DDD for 10 days and DDT and DDE for 28 days. Besides mortality, median lethal residue (LR50), mean effect concentration (EC50) and mean effect residue (ER50) in tissues were also assessed. Although metabolites were less lethal, mortality of *H. azteca* was significantly higher in DDD and DDE treatments than in the control at 0.692 µg/L and 2.258 µg/L, respectively (Lotufo et al. 2000). This raises high concern, once concentrations of DDD in this range have already been reported in freshwater ecosystems. The endocrine-disrupting activity of o,p'-DDE was also evaluated more recently (Davis et al. 2009). In this study, the authors investigated the effects of this metabolite and other compounds on the expression of the vitellogenin gene from the tilapia *Oreochromis mossambicus* and the growth hormone insulin-like growth factor-I axis. Injection of 100 µg/g o,p'-DDE in fish increased the expression of vitellogenin A and B, as well as the transcription of estrogen receptors α and β and the expression of the putative somatolactin receptor and insulin-like growth factor (Davis et al. 2009). This once again reinforces the potential endocrine disruption that DDT metabolites may cause in freshwater fish. As previously mentioned, metabolites of triazine herbicides are amongst the most frequently found in freshwater systems. Moreover, there is evidence in the literature linking these substances to negative effects on living organisms. The main degradation product of diuron is 3,4-dichloroaniline for which the toxic potential towards freshwater organisms is described in the literature. In zebrafish, a sub-chronic exposure (11 days) to this metabolite caused deformations at ≥ 0.25 mg/l, whilst locomotor activity and mortality were impaired at ≥ 0.5 mg/l (Scheil et al. 2009). A recent work investigated the effects of 3,4-dichloroaniline on biotransformation enzymes and the oxidative stress response in the liver and gills of the Nile tilapia (*Oreochromis niloticus*) (Felicio et al. 2018). The authors found that in fish exposed for seven days to 40 and 200 ng/L the levels of several biotransformation and antioxidant enzymes were altered often in a non-monotonic response, except for ethoxyresorufin-O-deethylase (EROD) activity that exhibited a dose-dependent increase. Moreover, the multixenobiotic resistance (MXR) activity and the activity of glutathione S-transferase (GST) enzymes were decreased in gills after exposure to 3–4-dichloroaniline. Because the MXR mechanism is crucial for the protection of aquatic organisms against xenobiotics aggression (Ferreira et al. 2014), this suggests that exposure to this metabolite is endangering the health of fish and the contaminated aquatic systems. A reduction in this mechanism can lead to higher susceptibility of animals to xenobiotics by impairing homeostatic processes.

The acute and chronic toxicity of deethylatrazine and deisopropylatrazine, metabolites of atrazine, were investigated in two amphipod species and in the microalgae *Pseudokirchneriella subcapitata* (Ralston-Hooper et al. 2009). *Hyalella azteca* and *Diporeia* spp. were exposed to concentrations ranging from 0.55 to 15 mg/L for 96 h and from 0.03 to 3000 µg/L for 21 days. Results showed the median lethal concentrations (LC50) and median growth inhibition concentration (IC50) for algae were ≥ 1.5 mg/L, i.e. higher than the levels found in the environment (Ralston-Hooper et al. 2009). In a recent study, marbled crayfish (*Procambarus fallax* f. *virginalis*) were exposed for 62 days to four concentrations of terbuthylazine-2-hydroxy: 0.75 µg/L

Table 2 Ecotoxicological studies about the effects of pesticide transformation products on aquatic species

Transformation product [parental compound] Reference	*Species*	Concentrations	Exposure duration	Endpoints	Effects
o-p' DDT [DDT] (Donohoe and Curtis, 1996)	*Oncorhynchus mykiss*	0, 0.1, 1, 5, 10 and 30 mg/kg	42 days	Determination of vitellogenin levels	Increased levels of vitellogenin in plasma and interaction with hepatic estrogenic binding sites in vivo
o-p' DDE [DDT] (Donohoe and Curtis, 1996)	*Oncorhynchus mykiss*	0, 0.1, 1, 5, 10 and 30 mg/kg	42 days	Determination of vitellogenin levels	Increased levels of vitellogenin in plasma and interaction with hepatic estrogenic binding sites in vivo
p-p' DDE [DDT] (Donohoe and Curtis, 1996)	*Oncorhynchus mykiss*	0, 0.1, 1, 5, 10 and 30 mg/kg	42 days	Determination of vitellogenin levels	No differences found in vitellogenin levels, relative to controls
DDD [DDT] (Lotufo et al. 2000)	*Hyalella azteca*	0.095, 0.178, 0.366, 0.692 and 1.381 µg/L	10 days	Mortality and lethal residues in tissues	DDD was less lethal than the parental compound (DDT) but its lethality was higher than that of the control at > 0.69 µg/L
	Diporeia spp.	0.944, 2.791, 7.420 and 17.056 µg/L	28 days		No significant effects found
DDE [DDT] (Lotufo et al. 2000)	*Hyalella azteca*	1.117, 2.258, 4.947, 8.208 and 22.021 µg/L	10 days	Mortality and lethal residues in tissues	DDE was less lethal than the parental compound (DDT) but its lethality was higher than that of the control at > 2.258 µg/L
	Diporeia spp.	2.293, 4.726, 9.141 and 20.194 µg/L	28 days		No significant effects found
o-p' DDE [DDT] (Davis et al. 2009)	*Oreochromis mossambicus*	5 µg/g	35 days	Determination of Vitellogenin levels and hormone/insulin-like growth factor i-axis	Increase in plasma levels of insulin growth factor
		100 µg/g	5 days		Increase in expression of both vitellogenin A and B, estrogen receptors α and β and also in insulin growth factor
3–4-dichloroaniline [diuron] (Scheil et al. 2009)	*Danio rerio*	0.005, 0.01, 0.1 0.25, 0.5 and 1 mg/L	8 and 11 days	Mortality and locomotor activity	Locomotor activity and mortality were impaired at ≥ 0.5 mg/l
		0.05, 0.1, 0.15, 0.2 and 0.2 5 mg/L	168 h	Hsp70 levels	A significant increase in relation to control was found at 0.25 mg/L
		0.5, 0.7, 1, 1.5 and 2 mg/L	11 days	Embryonic and larval development	By-product caused larvae deformations at ≥ 0.25 mg/l

Table 2 (continued)

Transformation product [parental compound] Reference	*Species*	Concentrations	Exposure duration	Endpoints	Effects
3–4-dichloroaniline [diuron] (Felicio et al. 2018)	*Oreochromis niloticus*	40 and 200 ng/L	7 days	Antioxidant and biotransformation biomarkers	By-product caused significant alterations in antioxidant and biotransformation biomarkers, with ethoxyresorufin-O-deethylase (EROD) activity showing a dose-dependent response
Deethylatrazine [atrazine] (Ralston-Hooper et al. 2009)	*Hyalella azteca*	550, 1000, 2500, 5000, 10,000, 15000 µg/L	96 h, 21 and 42 days	Mortality and sex ratio	LC50 values were 5100 µg/L at 96 h and higher than 3000 µg/L at 21 days; no change in the sex ratio was found
	Diporeia spp.	0.03, 0.3, 3, 30, 300, 3000 µg/L	96 h, 21 and 42 days	Mortality and sex ratio	LC50 values were 7200 µg/L at 96 h and higher than 3000 µg/L at 21 days; no change in the sex ratio was found
	Pseudokirchneriella subcapitata	No reported	96 h	Growth inhibition	Growth inhibition occurred at concentrations > 2000 µg/L
Deisopropylatrazine [atrazine] (Ralston-Hooper et al. 2009)	*Hyalella azteca*	550, 1000, 2500, 5000, 10,000, 15000 µg/L	96 h, 21 and 42 days	Mortality and sex ratio	LC50 values were > 3000 µg/L at 96 h and 330 µg/L at 21 days; no change in the sex ratio was found
	Diporeia spp.	0.03, 0.3, 3, 30, 300, 3000 µg/L	96 h, 21 and 42 days	Mortality and sex ratio	LC50 values were > 3000 µg/L at 96 h and 300ug/L at 21 days; no change in the sex ratio was found
	Pseudokirchneriella subcapitata	No reported	96 h	Growth inhibition	Growth inhibition occurred for concentrations higher than 3000 µg/L
Therbuthylazine-2-hydroxy [therbuthylazine] (Koutnik et al. 2017)	*Procambarus fallax f. virginalis*	0.75, 75, 375 and 750 µg/L	62 days	Mortality, growth, oxidative balance, antioxidant defences, ontogeny and histology	Lower weight at 75 µg/L; delayed ontogenic development and lowered antioxidant defences in exposed animals

Table 2 (continued)

Transformation product [parental compound] Reference	*Species*	Concentrations	Exposure duration	Endpoints	Effects
Desethyl-terbuthylazine [terbuthylazine] (Velisek et al. 2016)	*Cyprinus carpio*	1.80, 180, 900 and 1800 µg/L	7, 14, 20, 27 and 31 days	Growth, LC50, histology, oxidative stress, mortality	LC50 of 441.6 µg/L at 31 days; lower weight and length in fish exposed to 1800 µg/L for 7 days and 900 µg/L for 20 days; delayed ontogenetic development at > 1.8 µg/L; decreased antioxidant enzyme activity in all concentrations
AMPA [glyphosate] (Guilherme et al. 2014)	*Anguilla anguilla*	11.8 and 23. µg/L	1 and 3 days	DNA and chromosome damage	Significant genotoxic effect in relation to control group
Fiprunil sulphide and sulfone [fipronil] (Weston and Lydy, 2014)	*14 macroinvertebrate species*	4 – 7 concentration steps separated by a factor of 2	48 and 96 h	Mortality and ability to swim, cling or crawl, depending on the species	Mean 96-h EC50 of 7 – 10 ng/L
Fiprunil sulphide [fipronil] (Gong et al. 2021)	*Danio rerio*	0.1 to 10 mg/L	72 h	Mortality and oxidative stress	LC50 = 0.36 mg/L. Significant decreased of SOD activity at 5 mg/L
	Chlorella pyrenoidosa			Algae growth inhibition rate; content of pigment	EC50: 0.10 mg/L; chlorophyll content significantly decreased in dose–response relationship;
Fiprunil sulfone [fipronil] (Gong et al. 2021)	*Danio rerio*	0.1 to 10 mg/L	72 h	Mortality and oxidative stress	LC50 = 0.21 mg/L. Significant decreased of SOD activity at 5 mg/L
	Chlorella pyrenoidosa			Algae growth inhibition rate; content of pigment	EC50: 0.13 mg/L; chlorophyll content significantly decreased in dose–response relationship;
Fiprunil desulfinyl [fipronil] (Gong et al. 2021)	*Danio rerio*	0.1 to 10 mg/L	72 h	Mortality and oxidative stress	LC50 = 1.13 mg/L. Significant decreased of SOD activity at 5 mg/L
	Chlorella pyrenoidosa			Algae growth inhibition rate; content of pigment	EC50: 0.43 mg/L; chlorophyll content significantly decreased in dose-– response relationship;
Metolachlor OXA [metolachlor] (Velisek et al. 2018)	*Procambarus fallax f. virginalis*	4.2, 42 and 420 µg/L	45 days	Growth rate, behaviour, oxidative stress, histology and mortality	Decreased growth and activity of antioxidant enzymes in all tested concentrations; delayed ontogenetic development and lower levels of reduced glutathione and lipid peroxidation

Table 2 (continued)

Transformation product [parental compound] Reference	*Species*	Concentrations	Exposure duration	Endpoints	Effects
Metolachlor OXA [metolachlor] (Rozmánková et al. 2020)	*Danio rerio*	1, 30, 100 and 300 µg/L (single exposure); 1 and 30 µg/L (mixture)	120 h	Mortality, hatching success, embryonic malformations, locomotion, spontaneous movements, heartbeat and gene expression	Increased craniofacial, non-inflated gas bladder and yolk sac malformations at 100 µg/L or higher. Induction of *p53* gene at 100 µg/L
Metolachlor ESA [metolachlor] (Rozmánková et al. 2020)	*Danio rerio*	1, 30, 100 and 300 µg/L (single exposure); 1 and 30 µg/L (mixture)	120 h	Mortality, hatching success, embryonic malformations, locomotion, spontaneous movements, heartbeat and gene expression	Increased craniofacial, non-inflated gas bladder and yolk sac malformations at 100 µg/L or higher. Induction of p53 gene at 100 µg/L. Induction of *p53 and thyroid system regulation (dio2, thra, thrb)* at 30 and 1 µg/L, respectively
3-trifluoromethyl-4-aminophenol [3-trifluoromethyl-4-nitrophenol] Huerta et al. 2020)	*Petromyzon marinus*	0, 5, 50 and 200 µM	Undefined exposure time	Respiratory control ratio, mitochondrial oxygen consumption and mitochondrial transmembrane potential	No significant effects found
4-nitro-3-methyl-phenol [3-trifluoromethyl-4-nitrophenol] (Huerta et al. 2020)	*Petromyzon marinus*	0, 5, 50 and 200 µM	Undefined exposure time	Respiratory control ratio, mitochondrial oxygen consumption and mitochondrial transmembrane potential	No significant effects found
4-amino-3-methylphenol [3-trifluoromethyl-4-nitrophenol] (Huerta et al. 2020)	*Petromyzon marinus*	0, 5, 50 and 200 µM	Undefined exposure time	Respiratory control ratio, mitochondrial oxygen consumption and mitochondrial transmembrane potential	Decreased respiratory control ratio at 50 µM; decreased oxygen consumption at 200 µM
4-nitroso-3-methyl-phenol [3-trifluoromethyl-4-nitrophenol] (Huerta et al. 2020)	*Petromyzon marinus*	0, 5, 50 and 200 µM	Undefined exposure time	Respiratory control ratio, mitochondrial oxygen consumption and mitochondrial transmembrane potential	No significant effects found
3-phenoxybenzyl alcohol [permethrin] (Hernández-Moreno et al. 2022)	*Oncorhynchus mykiss*	0.78, 3.15, 12.5, 50 and 100 mg/L	96 h	Mortality	Moderately toxic (LC50 = 1.93 mg/L)
Benzenesulfonamide [asulam] (Hernández-Moreno et al. 2022)	*Oncorhynchus mykiss*	0.78, 3.15, 12.5, 50 and 100 mg/L	96 h	Mortality	Non-toxic (LC50 > 100 mg/L)
benzimidazol [carbendazim] (Hernández-Moreno et al. 2022)	*Oncorhynchus mykiss*	0.78, 3.15, 12.5, 50 and 100 mg/L	96 h	Mortality	Slightly toxic (LC50 = 66.19 mg/L)

Table 2 (continued)

Transformation product [parental compound] Reference	*Species*	Concentrations	Exposure duration	Endpoints	Effects
cyanoacetamide [DBNPA] (Hernández-Moreno et al. 2022)	*Oncorhynchus mykiss*	0.78, 3.15, 12.5, 50 and 100 mg/L	96 h	Mortality	Slightly toxic (LC50 = 68 mg/L)
cis-2,6-dimethylmorpholine [fenpropimorph] (Hernández-Moreno et al. 2022)	*Oncorhynchus mykiss*	0.78, 3.15, 12.5, 50 and 100 mg/L	96 h	Mortality	Non-toxic (LC50 > 100 mg/L)
ethiprole sulfone [ethiprole] (Gao et al. 2021)	*Danio rerio*	100, 300, 800, 2000, 5000 µg/L	4 days	Mortality; oxidative stress; development	LC50 value was 1750 µg/L; induction of antioxidant enzymes and the developmental anomalies at 100 µg/L
ethiprole sulphide [ethiprole] (Gao et al. 2021)	*Danio rerio*	100, 110, 120, 150, 180 µg/L	4 days	Mortality; oxidative stress; development	LC50 value was 111 µg/L; induction of antioxidant enzymes and the developmental anomalies at 10 µg/L or higher
rac-ethiprole amide [ethiprole] (Gao et al. 2021)	*Danio rerio*	100, 500, 2500, 10,000, 50,000 µg/L	4 days	Mortality; oxidative stress; development	LC50 > 50,000 µg/L
ethiprole sulfone amide [ethiprole] (Gao et al. 2021)	*Danio rerio*	100, 500, 2500, 10,000, 50000 µg/L	4 days	Mortality; oxidative stress; development	LC50 > 50,000 µg/L
desethylsulfinyl ethiprole [ethiprole] (Gao et al. 2021)	*Danio rerio*	500, 800, 1500, 2500, 5000 µg/L	4 days	Mortality; oxidative stress; development	LC50 = 1728 µg/L

(environmentally relevant), 75, 375 and 750 µg/L (Koutnik et al. 2017). Antioxidant defences, oxidative balance, histology, early ontogeny, growth and mortality were the parameters assessed to depict possible effects of this metabolite. Concentrations over 75 µg/L caused lower weight compared to the control group. The outcome of the study showed that terbuthylazine-2-hydroxy delayed ontogenetic development. Also, levels of thiobarbituric acid and antioxidant enzymes were significantly ($p < 0.01$) lower in groups exposed to the metabolite. This shows the potential danger of this metabolite to freshwater species, although the alterations found occurred in the groups exposed to non-environmental concentrations (Koutnik et al. 2017). The toxicity of terbuthylazine-desethyl, another metabolite of triazine herbicides, was assessed in the early stages of development of the common carp (*Cyprinus carpio*) (Velisek et al. 2016). Carp embryos were exposed to 1.80 µg/L (environmentally relevant), 180 µg/L, 900 µg/L and 1800 µg/L and samples were collected on days 7, 14, 20, 27 and 31. The 31d LC50 of terbuthylazine-desethyl was estimated to be 441.6 µg/L. Animals also exhibited lower weight and length at 7 (1800 µg/L) and 20 (900 µg/L) days of exposure. Terbuthylazine-desethyl at non-environmental concentrations also delayed the ontogenetic development, in relation to control. However, antioxidant enzyme activity was significantly lower in all test concentrations, including the environmentally relevant one, indicating that contamination by this metabolite should be compromising feral aquatic populations.

The main metabolite of glyphosate, AMPA is one of the most controversial pesticides nowadays, due to its potential hazard to wildlife and human populations. Moreover, AMPA by itself was reported as hazardous to *Anguilla anguilla* by Guilherme et al. (2014). The eels were exposed for 1 and 3 days to environmentally relevant concentrations (11.8 and 23.6 µg/L) and genotoxicity was investigated by assessing damage to DNA through the Comet assay and erythrocytic nuclear abnormalities. These results showed a genotoxic effect of AMPA at concentrations already found in aquatic systems. About organophosphates, a recent study was conducted with the parasitic sea lamprey (*Petromyzon marinus*) to address possible effects on cardiac mitochondrial bioenergetics of the lampricide 3-trifluoromethyl-4-nitrophenol and its metabolite 3-trifluoromethyl-4-aminophenol, as well as 4-nitro-3-methyl-phenol (Huerta et al. 2020). The latter has a similar molecular structure and is a known transformation product of fenitrothion and its metabolites 4-amino-3-methylphenol and 4-nitroso-3-methyl-phenol. Mitochondria were extracted from the hearts of animals captured on the great lakes and incubated with 0, 5 and 50 µM of the test compounds to assess the respiratory control ratio and mitochondrial oxygen consumption or with 0, 5, 50 and 200 µM to assess the mitochondrial transmembrane potential. Results showed that 4-amino-3-methylphenol significantly lowered the respiratory control ratio (88% at 50 µM) and oxygen consumption by 64% (at 200 µM and with the addition of high concentrations of ADP) and by 45% (at 200 µM and addition of substrate for complex II). At last, for mitochondrial transmembrane potential, none of the tested transformation products caused significant alterations.

Fipronil is a phenylpyrazole insecticide with crescent use in urban areas. The toxicity of its sulphide and sulfone metabolites was not recognized until 2014 when Weston and Ludy carried out a study determining EC50 values for 14 macroinvertebrate species. Results indicated a mean 96 h EC50 of 7−10 ng/L for fipronil metabolites in *Chironomus dilutus* (Weston and Lydy 2014). The same study also reported that creeks receiving urban stormwater run-off in California contained metabolite concentrations twice the EC50 found for C. *dilutus* and approximately one-third of the EC50 found for other aquatic macroinvertebrates (Weston and Lydy 2014). A recent study evaluated the toxicity of different fipronil metabolites: fipronil sulphide, fipronil sulphone and fipronil desulfinyl (Gong et al. 2021). In this work, the authors analysed the effects of 72-h exposure to these metabolites at concentrations ranging from 0.1 to 10 mg/L on zebrafish embryos and the green algae *Chlorella pyrenoidosa*. In zebrafish, LC50 values of 0.36, 0.31 and 1.13 mg/L were found for fipronil sulphide, sulfone and desulfinyl, respectively. Moreover, at 5 mg/L all metabolites significantly increased SOD activity, in relation to control. In *C. pyrenoidosa* growth inhibition, EC50 values of 0.10, 0.13 and 0.43 mg/L were found for fipronil sulphide, sulfone and desulfinyl, respectively. The metabolites investigated also caused a significant decrease in chlorophyll content, in relation to control, in a dose–response manner (Gong et al. 2021).

Metabolites of chloroacetanilide herbicides are highly prevalent in aquatic ecosystems, mainly in oxalinic and endosulfonic acid forms. Metolachlor OXA was reported to negatively affect the early life stages of marbled crayfish (Velisek et al. 2018). Animals were exposed for 45 days to 4.2 µg/L (environmentally relevant), 42 µg/L and 420 µg/L and several endpoints were assessed. Metolachlor OXA caused significantly lower growth and decreased activity of antioxidant enzymes at all tested concentrations. The highest tested concentrations delayed ontogenetic development and decreased the levels of reduced glutathione and lipid peroxidation (Velisek et al. 2018). More recently, a study was performed to evaluate the impacts of single and combined exposure of metolachlor and its metabolites metolachlor ESA and metolachlor OXA on zebrafish embryos (Rozmánková et al. 2020). In this study, zebrafish embryos were exposed for 120 h to 1, 30, 100 and 300 µg/L of the single compounds or to 1 and 30 µg/L of a compound mixture and sublethal endpoints such as malformations, hatching rate, larval length, spontaneous movements, heartbeat and locomotion, as well

as expression levels of eight genes linked to different critical pathways, were monitored. Increased craniofacial, non-inflated gas bladder and yolk sac malformations at 100 µg/L or higher were reported for both metabolites. For metolachlor OXA, a significant induction of p53 gene was found at 100 µg/L, compared to control, whilst for metolachlor ESA, a significant induction of p53 gene at 30 and 100 µg/L and thyroid system regulation (dio2, thra, thrb) was observed at 1 µg/L, in comparison to the control group. The disruption of the thyroid system represented a plausible danger for population maintenance, since it occurred at low environmental concentrations (Rozmánková et al. 2020). A recent study evaluated the acute toxicity of several biocide metabolites using the rainbow trout (*Oncorhynchus mykiss*) as a test model (Hernández-Moreno et al. 2022). The author exposed juvenile trout according to OECD TG203, for 96 h to 0.78, 3.15, 12.5, 50 and 100 mg/L of the following metabolites: 3-phenoxybenzyl alcohol, benzenesulfonamide, benzimidazole, cyanoacetamide and cis-2,6-dimethylmorpholine. The most toxic metabolite was 3-phenoxybenzyl alcohol, with an LC50 value of 1.93 mg/L, considered moderately toxic by the authors. Benzimidazole and cyanoacetamide with LC50 values of 66.19 and 68 mg/L, respectively, were reported as slightly toxic, whilst benzenesulfonamide and cis-2,6-dimethylmorpholine with LC50 values higher than 100 mg/L were considered non-toxic (Hernández-Moreno et al. 2022).

Ethiprole is a non-systemic phenylpyrazole compound widely used as an insecticide. Recently, a study was performed to evaluate zebrafish embryotoxicity and effects on antioxidant enzymes (catalase, CAT and superoxide dismutase, SOD, activities) and oxidative stress (lipid peroxidation) of its main metabolites, *i.e.* ethiprole sulfone, ethiprole sulphide, ethiprole amide, ethiprole sulfone amide and desethylsulfinyl ethiprole (Gao et al. 2021). Results showed that only ethiprole sulfone and sulphide had effects on antioxidant defences and embryonic development. Ethiprole sulfone had an LC50 value of 1750 µg/L, induced antioxidant enzymes and increased developmental anomalies at 100 µg/L. Ethiprole sulphide had an LC50 value of 111 µg/L, induced antioxidant enzymes and increased developmental anomalies at 10 µg/L or higher. Rac-ethiprole amide and ethiprole sulfone amide had LC50 values higher than 5000 µg/L, whilst the LC50 value for desethylsulfinyl ethiprole was 1728 µg/L (Gao et al. 2021).

Transformation Products of Pharmaceuticals

Nowadays, one main challenge to the scientific community is to understand the effects of these substances on non-target organisms. There are, already, several reports about this topic. However, knowledge about the toxic effects caused by pharmaceutical transformation products is still scarce. A summary of the works found in the literature is shown in Table 3.

As mentioned above, metabolites can be formed during wastewater treatment in WWTPs. In fact, this situation is reported for photodegradation products of both prednisone and dexamethasone (DellaGreca et al. 2004). In this study, photoproducts of both pharmaceuticals were isolated, from an initial solution of 100 mL of both compounds mixed with 500 mL of water and their toxicity to different species was evaluated: the rotifer *Brachionus calyciflorus* and the crustaceans *Thamnocephalus platyurus* and *Daphnia magna* for acute toxicity and the microalgae *Pseudokirchneriella subcapitata* and the crustacean *Ceriodaphnia dubia* for chronic toxicity. Acute assays lasted for 24 h and were based on mortality (LC50). In chronic assays, growth inhibition was the endpoint assessed for algae (72-h duration) and population growth was the endpoint for *C. dubia* (7-day duration). Some photodegradation products of prednisone and dexamethasone were found to be more toxic than the parental compounds. However, the LC50 values obtained by the authors were considerably higher than the concentrations generally found in surface waters. The chronic exposures decreased the population growth in *C. dubia* (DellaGreca et al. 2004). A similar study was conducted for the non-steroidal anti-inflammatory drug naproxen and its photodegradation products (Isidori et al. 2005). In this work, acute toxicity tests were conducted with *B. calyciflorus*, *T. platyurus* and *C. dubia*. Chronic toxicity was assessed (reproduction and/or growth) in *B. calyciflorus*, *C. dubia* and the microalgae *P. subcapitata*. Results showed that photodegradation products were more acutely toxic than the parental compound, although at levels (mg/L range) well above those found in freshwater systems. Chronic exposure reduced the population growth in *C. dubia* at low concentrations (µg/L) for some photoproducts (Isidori et al. 2005). This situation warns of the need to improve treatment methodologies, for better removal of both the parental compounds and their transformation products. A more recent study also reported that diclofenac metabolites formed through UV photolysis treatments were more toxic than their parental compound (Diniz et al. 2015) (Table 2).

Lienert and colleagues (2007) developed a study where the ecotoxicological risk of 42 pharmaceuticals and their metabolites was evaluated. In the study, both parental compounds and their respective metabolites were treated as a mixture of toxicants of similar action. When relevant data were not available in the literature, the authors estimated them from quantitative structure–activity relationships (QSAR). Moreover, from their known pharmaceutical information, they figured out the removal efficiency of these contaminants from urine. The results of this evaluation showed that mixtures of ibuprofen and its metabolites could represent an ecotoxicological risk for aquatic organisms.

Table 3 Ecotoxicological studies about the effects of pharmaceutical transformation products on freshwater species

Transformation product [parental compound] Reference	*Species*	Concentrations	Exposure duration	Endpoints	Effects
Prednisone, dexamethasone and their undisclosed photodegradation products [prednisone, dexamethasone] (Della Greca et al. 2004)	*Brachionus calyciflorus*	5 different test concentrations without known value. Results are reported as median effective concentrations in ppm	24 h	Mortality	5-prednisone and 2-dexamethasone photoderivates had lower LC50 values than parent compounds but at levels not found in environmental samples (mg/L range)
	Thamnocephalus platyurus		24 h	Mortality	All photoderivates had lower LC50 values than parental compounds (higher toxicity), but at non environmentally relevant concentrations (> 710 ppm)
	Daphnia magna		24 h	Mortality	All photoderivates had lower EC50 values than parental compounds (higher toxicity), but at non environmentally relevant concentrations (mg/L range)
	Pseudokirchneriella subcapitata		72 h	Growth inhibition	Toxic effects similar to those found for the other species, except Ceriodaphnia dubia
	Ceriodaphnia dubia		7 days	Population growth	Both the photoderivatives of prednisolone and dexamethasone showed higher toxic effects on *C. dubia* growth after 7 days

Table 3 (continued)

Transformation product [parental compound] Reference	*Species*	Concentrations	Exposure duration	Endpoints	Effects
naproxen and its undisclosed photodegradation products [naproxen](Isidori et al. 2005)	*Brachionus calyciflorus*	Concentration values are not given. All test solutions were dissolved in DMSO (0.01% v/v). 5 different concentration were tested, as well as, a negative control	24 /48 h	Mortality and reproduction	All photoderivates had lower LC50 values than parental compounds, but at levels not found in environmental samples (mg/L range) for acute assay. In the chronic reproduction assay only one photoderivate was less toxic than the parental compound
	Thamnocephalus platyurus		24 h	Mortality	All photoderivates had lower LC50 values than parental compounds, but at levels not found in environmental samples (mg/L range)
	Ceriodaphnia dubia		24 h and 7 days	Mortality and reproduction	All photoderivates had lower LC50 values than parental compounds, but at levels not found in environmental samples (mg/L range). For reproduction, only one photoderivate was less toxic than the parental drug
	Pseudokirchneriella subcapitata		96 h	Growth	All photoderivatives of naproxen showed higher toxic effects on *P. subcapitata* growth
diclofenac, ketoprofen, atenolol and their photodegradation products (undisclosed) [diclofenac, ketoprofen, atenolol] (Diniz et al. 2015)	*Danio rerio*	1 mg/L	7 days	Oxidative stress	Diclofenac metabolites formed through UV photolysis treatments were more toxic than their parental compounds. Activity of antioxidant enzymes and lipid peroxidation levels were higher for by-products than the parental drugs. Overall, oxidative stress response causing toxicity was observed for all pharmaceuticals and by-products

Table 3 (continued)

Transformation product [parental compound] Reference	*Species*	Concentrations	Exposure duration	Endpoints	Effects
norfluoxetine [fluoxetine] (Stanley et al. 2007)	*Pimephales promelas*	1 to 250 µg/L	7 days	survival and growh	The authors related higher toxicity in fish exposed to s-fluoxetine, which in mammals is expected to be more potent than R-norfluoxetine
	Daphnia magna	10 to 1000 µg/L	21 days	immobilization, reproduction and grazing rate	No observed effects
Norfluoxetine [fluoxetine] (Fong and Molnar, 2008)	*Dreissena polymorpha*	100 nM to 50 µM	4 h	spawning	Increased spawning in zebra mussels at 1–50 µM
	Mytilopsis leucophaeata	100 nM to 50 µM	4 h	spawning	Increased spawning in zebra mussels at 1–50 µM
	Sphaerium striatinum	100 nM to 10 µM	4 h	parturition	Significant increase in parturition induced at 10 µM
norfluoxetine [fluoxetine] (Rodrigues et al. 2020)	*Danio rerio*	0.64, 3.2, 16, 80 and 400 ng/L	80 h	Embryonic development, gene expression and sensorimotor responses	Increase of embryonic anomalies in relation to control, mainly for pigmentation. No effects found for gene expression and sensomotory response
Norfluoxetine [fluoxetine] (Atzei et al. 2021)	*Danio rerio*	0.03 to 10 µM	5 days	Embryonic development, gene expression and light/dark movement	Inhibition of light/dark, zebrafish locomotory activity, mainly in dark. Responses followed a dose–response relationship
norfluoxetine [fluoxetine] (Rodrigues et al. 2022)	*Danio rerio*	400 ng/L	80 h	Embryonic development and gene expression	Increase in pigmentation anomalies of embryos and larvae, relative to the parental compound
n-desmethylsertraline [sertraline] (Lajeunesse et al. 2011)	*Salvelinus fontinalis*	WWTP water samples (undisclosed concentrations)	3 months	Tissue bioaccumulation and Na/K-ATPase activity	Bioaccumulation in several tissues, including (brain and liver). Na/K-ATPase activity negatively correlated with brain bioaccumulation desmethylsertraline-exposed brain tissue
o-desmethylvenlafaxine [venlafaxine] (Stropnicky, 2017)	*Orconectes obscurus*	0, 1 and 8 µg/L	14 days	Aggressive behaviour	Increase in the number of attacks per minute at the highest concentration tested
	Procambarus clarkii	0, 1 and 8 µg/L	14 days	Aggressive behaviour	Increase in the number of attacks per minute at the highest concentration tested

Table 3 (continued)

Transformation product [parental compound] Reference	*Species*	Concentrations	Exposure duration	Endpoints	Effects
o-desmethylvenlafaxine [venlafaxine] (Atzei et al. 2021)	*Danio rerio*	0.03 to 300 µM	5 days	Embryonic development, gene expression and light/dark movement	Inhibition of light/dark, zebrafish locomotory activity, mainly in dark. Responses followed a dose–response relationship
Clofibric acid [clofibrate] (Nunes et al. 2008)	*Gambusia holbrooki*	176.4, 211.6, 253.92, 304.71 and 365.65 mg/L	96 h	Oxidative damage	Decrease in the amount of oxidized glutathione content in the liver and gills in exposed fish
n- and o-desmethyltramadol [tramadol] (Zhuo et al. 2012)	*Danio rerio*	Intraperitoneal injection of tramadol (65 mg/kg)	1 h	Weight, mitochondrial changes and behaviour	Detection of n- (mostly) and o-desmethyltramadol in brain tissue. Fish exposed to tramadol exhibited weight loss, abnormal behaviour and mitochondrial structural changes, possibly mediated by its by-products
Oxazepam [temazepam] (Huerta et al. 2016)	*Pimephales promelas*	0.8, 4.7 and 30.6 µg/L	28 days	Behaviour and bioaccumulation	Brain was the tissue with higher accumulation rates; behavioural effects detected in the novel tank diving test were observed in fish exposed to 4.7 µg/L
Oxazepam [temazepam] (Fahlman et al. 2021)	*Perca fluviatilis*	15 µg/L	14 days	anti-predator behaviour	Stimulation of anti-predator behaviour (decreased activity, decreased distance to conspecifics and increased littoral habitat use)
Oxcarbamazepine [carbamazepine] (Desbiolles et al. 2020)	*Lemna minor*	27 ng/L	17 days	Phytometabolites	Increase in nitrogen compounds. Chlorophyll index was higher in relation to control
	Hydra circumcincta	900 ng/L	14 days	Reproduction, morphological changes and oxidative stress biomarkers	Single exposure impacted the total antioxidant capacity
Acridine 9-carboxylic acid [oxcarbazepine] (Desbiolles et al. 2020)	*Lemna minor*	27 ng/L	17 days	Phytometabolites	Alterations of the nitrogen balance and chlorophyll indices at environmental concentrations
Oseltamivir carboxylate [oseltalmivir] (Chen et al. 2020)	Oryzias latipes	0, 0.06, 0.3, 90 and 300 µg/L	14, 21 and 56 days	median survival, growth, reproduction and hatchability	Long-term parental exposure to by-products affected the embryonic development of fish hatchability at 300 µg/L and development 90 µg/L

Table 3 (continued)

Transformation product [parental compound] Reference	*Species*	Concentrations	Exposure duration	Endpoints	Effects
Oseltamivir ethyl ester [oseltamivir] (Chen et al. 2020)	*Oryzias latipes*	0, 0.06, 0.3, 90 and 300 µg/L	14, 21 and 56 days	median survival, growth, reproduction and hatchability	Long-term parental exposure to by-products affected the embryonic development of fish hatchability at 300 µg/L and development 90 µg/L
Fenofibric acid [fenofibrate] (Jung et al. 2021)	*Danio rerio*	5, 10, 20, 30 and 40 mg/L	72 h	Mortality	LC_{50} = 53.32 mg/L
Carbamazepine-10,11-epoxide [carbamazepine] (Bars et al. 2021)	*Danio rerio*	250 µg/L	120 h	embryonic development	Delay in swim bladder inflation at 120hpf
5-(4-hydroxyphenyl)-5-phenylhydantoin [phenytoin] (Bars et al. 2021)	*Danio rerio*	250 µg/L	120 h	embryonic development	No effects found

Likewise, acetylsalicylic acid, bezafibrate, carbamazepine, diclofenac, fenofibrate and paracetamol in a mixture with their respective metabolites could be of potential risk for aquatic organisms, however, to a lesser extent than ibuprofen. In Table S2, ibuprofen metabolites detected in environmental samples reach concentrations > 120 000 ng/l that, together with the results of Lienert et al. (2007), suggests that this contamination is jeopardizing affected aquatic ecosystems and their populations. Whilst QSAR models have some limitations that may generate not fully accurate data, the information presented by those authors established a relevant basis for highly needed subsequent research and risk assessment studies.

Norfluoxetine, the main fluoxetine metabolite, was reported to cause enantiospecific sublethal effects in *Pimephales promelas* and *Daphnia magna* (Stanley et al. 2007). In this study, *P. promelas* juveniles were exposed for seven days to 1, 10, 50, 100 and 250 µg/L of R-, rac- and S-fluoxetine. The enantiomer S-fluoxetine showed higher toxicity to growth, survival and feeding rate. The authors related their results to the fact that S-norfluoxetine is more potent to mammals than R-fluoxetine. But this pattern was not found for *D. magna*. For this microcrustacean, a 21-day toxicity test was performed to determine immobilization, reproduction and grazing rate. Less than 24-hpf individuals were exposed to 10, 50, 100, 250, 500 and 1000 µg/L of R-, rac- and S-fluoxetine. The results obtained were similar for the three compounds, and the taxa differences were attributed to the higher homology between fish and mammals than between crustaceans and mammals. Norfluoxetine was also reported to induce spawning and parturition in bivalves (Fong and Molnar 2008). The authors exposed zebra mussels to 100 nM–50 µM, dark false mussels to 100 nM–50 µM and finger-nail clams to 100 nM–10 µM. Norfluoxetine increased spawning in both zebra mussels and dark false mussels, relative to the respective controls, at concentrations in the range of 1–50 µM. In finger-nail clams, norfluoxetine induced significant parturition only at 10 µM, relative to controls. Recently, Rodrigues and colleagues (2022) found that norfluoxetine could affect the embryonic development of zebrafish larvae. In the study, newly hatched embryos were exposed for 80hpf to norfluoxetine (0.0014 µM) and fluoxetine (0.0015 µM). Larvae exposed to norfluoxetine showed an increased frequency of pigmentation anomalies, in relation to the parental compound (Rodrigues et al. 2022).

Still concerning the SSRI (selective serotonin reuptake inhibitors) type of depressants, the primary metabolite of sertraline, n-desmethylsertraline, was found to affect Na/K-ATPase activity in the trout brain (Lajeunesse et al. 2011). The authors studied the distribution of selected SSRI in several tissues of brook trout, as well as the Na/K-dependent ATPase pump activity in the brain. Fish were exposed for 3 months to a WWTP-treated effluent (primary treatment)

before and after ozonation. The metabolite n-desmethylsertraline was one of the main substances found in various tissues. Also, Na/K-ATPase activity was negatively correlated with the accumulation of n-desmethylsertraline in the brain. Within the group of serotonin and norepinephrine reuptake inhibitors (SNRI), o-desmethylvenlafaxine (the active metabolite of venlafaxine) was implicated in behavioural changes of freshwater organisms (Stropnicky, 2017). The author exposed two species of crayfish, *Orconectes obscurus* and *Procambarus clarkii* to 0, 1 or 8 µg/L of o-desmethylvenlafaxine. The aggression behaviour of the crayfish, measured by the number of attacks per minute of exposed animals, was the endpoint assessed. An increase in the number of attacks was found for both species at 8 µg/L (Stropnicky, 2017). A more recent study related o-desmethylvenlafaxine exposure to behavioural changes in freshwater species (Atzei et al. 2021). The authors exposed zebrafish embryos to this metabolite in a concentration range of 0.03–300 µM, for 5 days. Embryonic development was monitored and a light/dark behavioural assay was performed. No significant developmental anomalies were elicited by o-desmethylvenlafaxine. However, a dose–response inhibition on locomotory function, mainly under dark conditions, was found (Atzei et al. 2021).

Clofibric acid, a metabolite of clofibrate, is another metabolite with reported negative effects on fish species. This compound caused modifications of biomarkers related to antioxidant defences and oxidative stress in *Gambusia holbrooki* (Nunes et al. 2008). In their work, the authors exposed the fish for 96 h to 176.34, 211.60, 253.92, 304.71 and 365.65 mg/L of clofibric acid. This metabolite caused a decrease in the activity of several antioxidant enzymes and in particular the levels of oxidized glutathione, in both the liver and gills. The effects of chronic tramadol exposure were studied in the zebrafish brain (Zhuo et al. 2012). Following intramuscular injections (25 or 65 mg/kg), both n- and o-desmethyltramadol were detected in brain tissue, mainly n-desmethyltramadol. This is important, since fish chronically exposed to tramadol exhibited weight loss, abnormal behaviour and mitochondrial structural changes. Considering that the two metabolites were present in the brain tissue, it may be possible that both can exert their effects on the exposed animals. Nevertheless, further studies focused on their administration and specific effects are needed to support this.

Oxazepam is one of the main metabolites of diazepam, a widely used benzodiazepine that is prescribed as an anticonvulsant, amongst other functions. In a recent study, specimens of *Pimephales promelas* were exposed to 0.8, 4.7 and 30.6 µg/L oxazepam for 28 days and the relationship between its internal concentrations and effects on fish behaviour was investigated with two types of tests: novel tank diving test and shelter-seeking test (Huerta et al. 2016b). The authors concluded the brain was the tissue with higher accumulation rates and significant behavioural effects in the novel tank diving test were observed in fish exposed to 4.7 µg/L. Although 4.7 µg/L is a concentration higher than found in freshwater bodies, it raises concern about the effects this metabolite can exert on fish behaviour and ultimately endanger populations impacted by this substance. Another study with the same compound revealed behavioural changes on *Perca fluvialis* (Fahlman et al. 2021). The results showed that anti-predation behaviour was stimulated in exposed animals, characterized by decreased activity and distance to conspecifics, as well as increased littoral habitat use (Fahlman et al. 2021).

Carbamazepine is one of the most used anticonvulsants worldwide. Recently, some of its transformation products were a matter of study by Desbiolles et al. (2020). Their study focused on the chronic effects of oxcarbamazepine and acridine 9-carboxylic acid, in single or combined exposure with carbamazepine, in two different models: the duckweed *Lemna minor* and the cnidarian *Hydra circumcinta*. Tested concentrations were the same for both models; 600, 27 and 900 ng/L for carbamazepine, oxcarbamazepine and acridine 9-carboxylic acid, respectively. For *L. minor*, exposure lasted 17 days and different phytometabolites were monitored. Exposure to the transformation products separately and in a mixture with the parental compound caused alterations of nitrogen balance, namely an increase in nitrogen compounds. The chlorophyll index was also higher in oxcarbamazepine groups than in the control. Nevertheless, the phenols index varied deeply without any specific trend or alteration relative to the control group. *Hydra circumcinta* individuals were exposed to the compounds for 14 days and different endpoints were assessed, such as reproduction, morphological changes and evaluation of antioxidant and oxidative stress biomarkers. The results showed that oxcarbamazepine exposure had implications in the total antioxidant capacity of *H. circumcincta* increasing two-fold in relation to control. Exposure to acridine 9-carboxylic acid affected all tested endpoints, except the reproduction. Combined exposure assays resulted in an increase in malformations on cnidarians and a decrease in the budding rate (Desbiolles et al. 2020). Another carbamazepine metabolite (carbamazepine-10,11-epoxide) was recently addressed for its possible effects on zebrafish embryonic development (Bars et al. 2021). The authors exposed zebrafish embryos from ~ 3 to 120hpf to a concentration of 250 µg/L of this metabolite, i.e. considerably higher than the maximum concentration found in the environment. Embryonic development was monitored through the exposure period and anomalies were registered. Results showed that swim bladder inflation was significantly delayed in carbamazepine-10,11-epoxide-exposed larvae, compared to the control

(Bars et al. 2021). This is important since inflation of the swim bladder allows larvae to stay in the water column and have more chances of survival.

A recent study focused on the metabolites of the well-known antiviral oseltamivir (Tamiflu) and their chronic effects on the medaka *Oryzias latipes* (Chen et al. 2020). Results showed that long-term parental exposure to both oseltamivir carboxylate and oseltamivir ethyl ester affected embryonic development and fish hatchability at 300 µg/L and embryonic development at 90 µg/L. Fenofibric acid, a metabolite of the anti-lipidemic agent fenofibrate, was also evaluated for its toxicity to zebrafish embryos (Jung et al. 2021). An LC50 value of 53.32 mg/L was found at 72 h, which is considerably higher than the normally occurring concentration in the environment.

The Way Forward

This review gives an updated perspective on freshwater contamination by pharmaceuticals and pesticide transformation products and the available information about the toxicity of these substances. Detection of pharmaceuticals and pesticides is increasing in freshwater ecosystems, and concentrations in the range of ng to µg/L have been widely reported. Moreover, this same trend is described for their metabolites and transformation products. This occurrence made this field one of the most studied by the scientific community in the last years, with a number of published works addressing the potentially hazardous effects of such previously overlooked substances. The present research identified concentrations of 190 metabolites and transformation products (92 from pesticides and 98 from pharmaceuticals) in water bodies and wastewater effluents, none of them included in monitoring programmes set to achieve the good environmental status of freshwater ecosystems. Their formation processes, environmental fate in aquatic ecosystems and effects on humans and biota, summarized in Fig. 6, are varied and a considerable cause of concern. Reported concentrations are mainly in the order of ng to µg/L. The concentration heatmap produced in this work allows us to easily spot the substances found at higher levels.

Although the information presented herein about the quantification of pesticides and pharmaceutical transformation products is extensive (almost 200 compounds), this may just represent the tip of the iceberg. Worldwide there are more than 1500 pesticides approved for use in agriculture and about 4000 pharmaceutical compounds approved for human consumption (aus der Beek et al. 2016; Anagnostopoulou et al. 2022). These parental compounds can have one or several transformation products, which brutally increases the potential number of these pollutants in the aquatic environment. Also, transformation products of pesticides banned for several decades now are still found

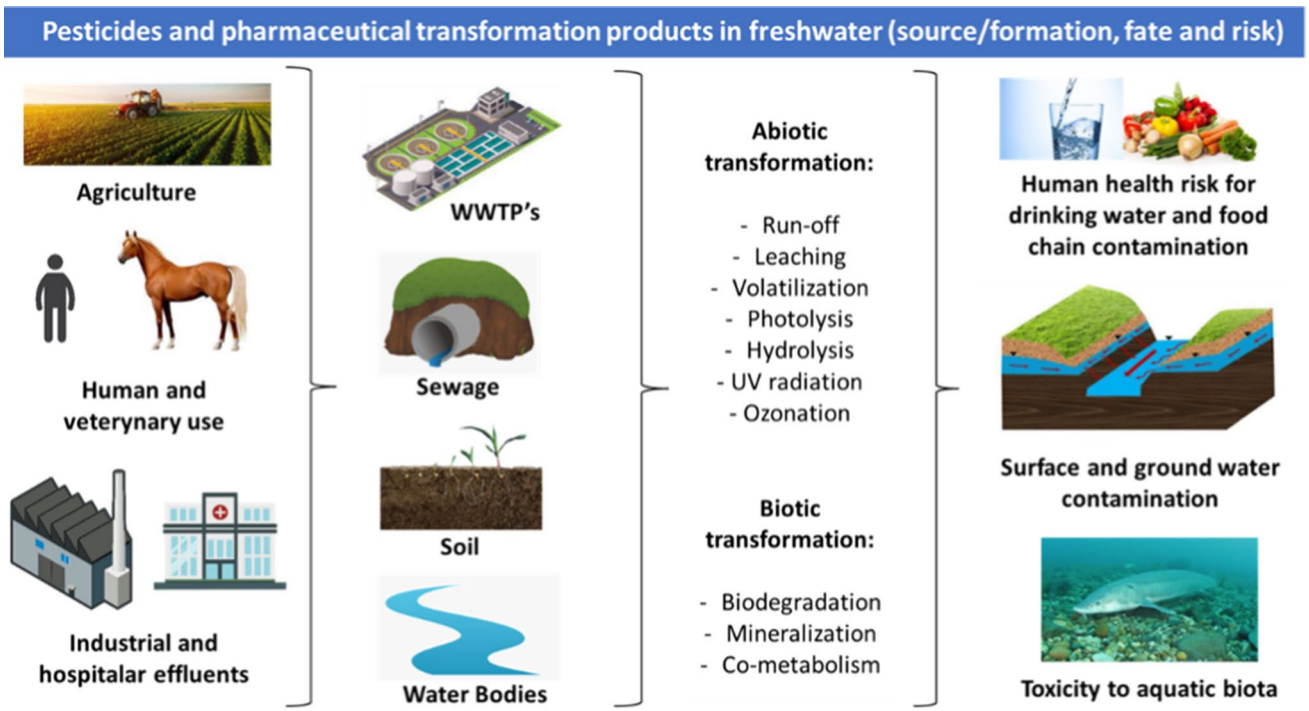


Fig. 6 Overall representation of pesticides and pharmaceutical transformation products aquatic contamination and risks for human and aquatic species

in freshwater. Transformation products are in several cases more stable in the environment and consequently reach concentrations higher than their parental compounds (Schuhmann et al. 2019; Celiz et al. 2009). All these numbers and characteristics reinforce the need to increase the monitoring of these compounds in aquatic systems and evaluate their impact on human and environmental health.

The toxicological information available for the transformation products identified is very little and scattered, with no strategic approach underlying data collection for risk assessment and monitoring prioritization. Concerning the risk to humans, less than twenty metabolites (of the two groups combined) were investigated in in vitro studies. Several of these were found to elicit genotoxicity and effects on biotransformation and antioxidant processes. In aquatic organisms, only about 34% of the transformation products originating from pesticides and 14% of those originating from pharmaceuticals were evaluated for their potentially hazardous effects on biota. Most of these studies evaluated effects on only one (majority) or two trophic levels and more than half of them on vertebrates. Effects on plants and algae were rarely assessed. For pesticides, over 50% of the assessments were about acute and subacute toxicity effects, whilst for pharmaceuticals only about 20% of the assessments concerned chronic toxicity. Adding to this, for pharmaceutical metabolites various studies tested very high exposure levels, reporting effects at concentrations higher than those found in the environment. Nevertheless, for pesticide metabolites, several reports described a considerably wide range of negative effects on freshwater organisms, occurring at environmentally relevant concentrations. For pharmaceutical metabolites, different classes of drugs were proven to cause hazardous effects and jeopardize the homeostasis of freshwater species.

All in all, the data presented herein clearly demonstrate that pesticide and pharmaceutical transformation products pose a threat to aquatic fauna and flora. Concerning the relative toxicity of transformation products, compared to the parental compounds, the available data prevent a clear global conclusion. In some cases, the transformation products are in fact less toxic. In other cases, some transformation products can be more active and toxic than the parental substance. Nowadays, there is increasing evidence that pesticide transformation products can be more toxic and persistent than their parental compounds (Iwafune 2018). In silico assays, performed with the ECOSAR (Ecological Structure Activity Relationships) software, which predicts the toxicity of different compounds, showed that the transformation products of several pesticides have a high toxicity potential to aquatic fauna and flora (Anagnostopoulou et al. 2022). Transformation products resulting from penoxsulam, pyrimethanil, imidacloprid, acetamiprid, thiacloprid and carbendazim were predicted to be more toxic than their parental compounds. In contrast, transformation products of fipronil present equal levels of toxicity, relative to fipronil itself (Anagnostopoulou et al. 2022). For pharmaceutical transformation products, there is a general idea that these compounds are less active and, consequently, less toxic than their parental compounds. However, there is evidence that some transformation products may be more toxic than the parental compounds. In humans, metabolites such as morphine and o-desmethyltramadol are more active than the parental compound (codeine and tramadol, respectively) (Rodieux et al. 2018). There are also reports of potential toxic effects elicited in patients, *i.e.* pethidine and dextroptopoxyphene (Coller et al. 2009). On the other hand, photodegradation products of prednisone, dexamethasone, naproxen, diclofenac, ketoprofen and atenolol formed in watercourses or even in WWTPs were reported to be toxic to different aquatic species at higher magnitude than their parental compounds (DellaGreca et al. 2004; Isidori et al. 2005; Diniz et al. 2015). Nonetheless, for most of the transformation products identified, the information is still scarce to draw sound conclusions.

Something that is still not accounted for in most of the ecotoxicological works is the metabolism of parental substances in the test media. During exposure, parental compounds are metabolized and transformed by the exposed organisms. This is a process, influenced by media abiotic factors, which originates different transformation products. Such compounds can cause negative effects on the organisms, by themselves or in mixture with the respective parental compound. A previous study reported that fish exposed to tramadol exhibited weight loss, abnormal behaviour and structural mitochondrial changes that could be linked to the metabolites formed during the exposure, which accumulated in the animals' brains and muscular tissue (Zhuo et al. 2012). The possibility that several negative impacts reported on aquatic species exposed to pharmaceuticals may derive not only from those compounds, but also from the mixture with their metabolites or even exclusively from the metabolites needs to be addressed soon.

Overall, the results warn of the need to continue improving treatment methodologies, for better removal of transformation products, not only to avoid their discharge to the aquatic environment but also to assure a better quality for water reuse. From a toxicological viewpoint, it is also striking the lack of mechanistic information useful to improve predictive toxicology and the risk assessment of these chemicals. Most works focused on assessing classical apical endpoints employing standard testing approaches. Whilst this is always fruitful to obtain a quick grasp of the severity of a contamination scenario, more studies investigating the modes of action of these compounds are urgently needed. Also, the limited availability of reference standards for several transformation products makes it difficult to test the toxicity of these compounds to living organisms

(Anagnostopoulou et al. 2022). However, this obstacle can be surpassed using in silico approaches, which reduce the need for animals and chemicals and can be valuable tools for toxicity and risk assessment.

Future toxicological investigations should be based on the framework of Adverse Outcome Pathways (AOP) (Ankley et al. 2010). This concept identifies various key events and relationships between them, linking a molecular initiating event to an adverse outcome of significance to risk assessment. The adverse outcome is usually considered at the organ level or higher, preferably the ecological level. It indicates a morphological or physiological alteration occurring in an organism or its systems that elicits functional impairment or impairs its ability to compensate for chemical stress and achieve homeostasis. The AOP framework is recognized as useful to support regulatory decision-making and the prioritization of chemicals for risk assessment (Vinken et al. 2017; Perkins et al. 2019), a most important aspect for the contamination scenario described herein. Present-day high-throughput technologies (i.e. proteomic sequencing) allowing for the rapid and cost-effective generation of data should be used to identify key events and key event relationships through which the initiating event(s) will reflect on adverse outcomes to apical endpoints. Guidance documents for the development of AOPs were made available (OECD, 2013, 2018), as well as supporting databases and tools, such as the e.AOP.portal (http://aopkb.org), the AOP Wiki (http://aopwiki.org), the Effectopedia (http://effectopedia.org) and the Wikipathways (https://www.wikipathways.org/index.php/WikiPathways), the Harmonized Template 201: Intermediate effects (https://www.oecd.org/ehs/templates/harmonised-templates-intermediate-effects.htm) and the AOP Xplorer (http://datasciburgoon.github.io/aopxplorer. Collaborative networks based on resource and knowledge sharing, and rational effort application, should be made at a global level to establish and implement a structured strategy rapidly allowing to fulfil these gaps whilst avoiding unnecessary experimental redundancy (Martens et al. 2018).

The present work emphasizes the need to reinforce the existing knowledge about contamination by pharmaceutical and pesticide transformation products in freshwater systems. This report compiled and analysed a significant amount of information linking exposure to transformation products to adverse outcomes in aquatic species and humans. Technological needs and knowledge gaps were identified and discussed, delineating future research steps on the topic, ultimately aiming at improving water management and monitoring programmes.

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Data Availability Authors confirm that all relevant data are included in the article or its supplementary file.

Declarations

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

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