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THE ANTINEOPLASTIC DRUGS CYCLOPHOSPHAMIDE AND CISPLATIN IN THE AQUATIC ENVIRONMENT – REVIEW

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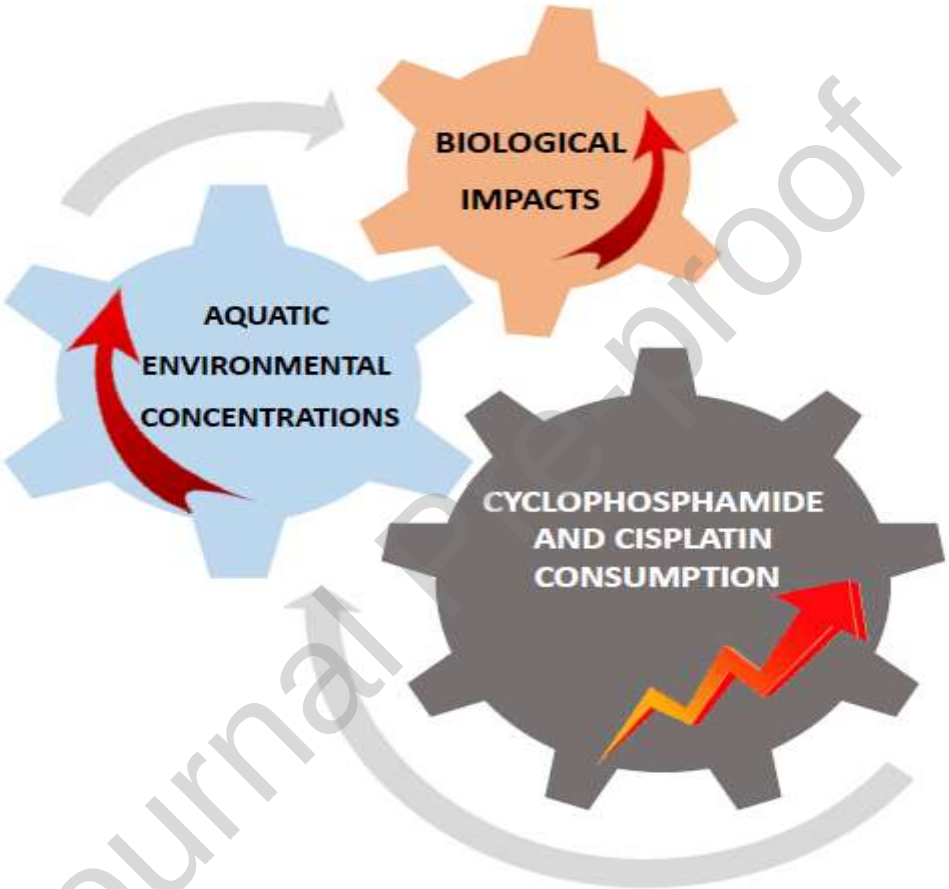
Abstract

Cyclophosphamide (CP) and Cisplatin (CDDP) are antineoplastic drugs widely used in the treatment of neoplastic diseases that have been detected in the aquatic environment. This review summarizes the current knowledge on the presence in the aquatic environment of these two drugs and their effects on freshwater and marine invertebrates, which include good model species in ecotoxicology and risk assessment programs. The consumption levels, occurrence in freshwater and marine ecosystems, and the impacts exerted on aquatic organisms, even at low concentrations, justifies this review and the selection of these two drugs. Both pharmaceuticals were detected in different aquatic environments, with concentrations ranging from ng L^{-1} up to 22.1 μg L^{-1} (CP) and 250 μg L^{-1} (CDDP). The available studies showed that CP and CDDP induce individual and sub-individual impacts on aquatic invertebrate species. The most common effects reported were changes in the reproductive function, oxidative stress, genotoxicity, cytotoxicity and neurotoxicity. The literature used in this review supports the need to increase monitoring studies concerning the occurrence of antineoplastic drugs in the aquatic environment since negative effects have been reported even at trace

concentrations (ng L^{-1}). Furthermore, marine ecosystems should be considered as a priority since less is known on the occurrence and effects of antineoplastic drugs in this environment comparing to freshwater ecosystems.

Graphical abstract



Keywords: Pharmaceuticals, anticancer drugs, cytotoxic drugs, invertebrates, biological impacts, accumulation levels.

1. ANTINEOPLASTIC DRUGS IN THE AQUATIC ENVIRONMENT

In the last decades, several substances have been continuously released into the aquatic ecosystems, including Pharmaceutically Active Compounds (PhACs) (Desbiolles et al., 2018; Mezzelani et al., 2018). Among these chemical substances are the antineoplastic drugs (also identified as anticancer drugs, cytotoxic drugs or cytostatic drugs), which have received little attention up to date, especially when compared to other classes of pharmaceuticals, such as antibiotics, anti-inflammatory drugs and anti-depressants (Desbiolles et al., 2018; Kosjek and Heath, 2011; Mezzelani et al., 2018). These drugs belong to a major class identified as "antineoplastic and immunomodulating agents", which are classified by the World Health Organization (WHO, 2019) as class L of the Anatomical Therapeutic Chemical (ATC) classification, and includes four sub-classes: antineoplastic agents (L01), endocrine therapy (L02), immunostimulants (L03), and immunosuppressants (L04). Antineoplastic drugs are used in cancer treatments (chemotherapy) and several of them are characterized for being cytotoxic, genotoxic, mutagenic and teratogenic, acting not only on target tumor cells but also on healthy cells (Besse et al., 2012; Białk-Bielińska et al., 2017; Filipič et al., 2020; Kosjek and Heath, 2011; Mater et al., 2014). The antineoplastic drugs main mode of action includes the inhibition or alteration of the DNA transcription, interfering directly with the DNA synthesis, or the interaction with proteins that regulate biological processes of the cells to disrupt or control cellular proliferation (Besse et al., 2012; Gómez-Canela et al., 2020; Santos et al., 2017).

According to the World Cancer Report (2020) by the International Agency for Research on Cancer (IARC), cancer is the second most common cause of death worldwide and has caused approximately 9.6 million deaths in 2018. The estimations for the future points out that new cancer cases will surpass 27 million per year by 2040, a 50% increase in the estimated 18.1 million new cancer cases in 2018 (Wild et al., 2020). As a consequence, the amount of antineoplastic drugs consumed per year and the development of new ones is also expected to increase during the next years across the globe. In Europe, countries such as France, Spain, Germany and Portugal reported consumption of antineoplastic drugs in the order of tons per year (Besse et al., 2012; Franquet-Griell et al., 2017b; Kümmerer et al., 2016; Santos et al., 2017). In Spain, for example, the mean annual

consumption was approximately 23 tons per year between 2010 and 2015 (Franquet-Griell et al., 2017b).

After consumption, these drugs can be excreted through the urinary and/or digestive system as metabolites or the parent compound, being discharged into the hospital and household wastewaters, eventually reaching wastewater treatment plants (WWTPs) (Isidori et al., 2016; Valcárcel et al., 2011; Zhang et al., 2013). However, low or inefficient removal rates of these pharmaceuticals in WWTPs have been reported (Česen et al., 2015; Franquet-Griell et al., 2017b; Martín et al., 2014; Negreira et al., 2014b; Zhang et al., 2013). In fact, coastal zones and ultimately the marine ecosystems are commonly the final destination for treated and non-treated wastewaters, and several pharmaceutical classes have already been reported in these environments (Álvarez-Muñoz et al., 2018; Fernández-Rubio et al., 2019; Kötke et al., 2019; Sathishkumar et al., 2020), presenting a threat to non-target aquatic organisms derived from their bioactive behaviour. Currently, antineoplastic drugs can be found in aquatic ecosystems in the ng L^{-1} to μg L^{-1} range (Buerge et al., 2006; Elizalde-Velázquez and Gómez-Oliván, 2020; Zhang et al., 2013). Even at a lower concentration such as ng L^{-1}, these pharmaceuticals may pose a risk to aquatic organisms (Fonseca et al., 2018, 2017; Trombini et al., 2016). However, there is still a knowledge gap regarding the real effects of environmentally relevant concentrations of antineoplastic drugs in non-target organisms, especially in marine species (Bebianno and Garcia da Fonseca, 2020; Mater et al., 2014).

The fact that the concentration of antineoplastics in aquatic ecosystems is in the ng L^{-1} range, or even below, represents a challenge to the investigation of their effects. In particular, the lack of highly sensitive analytical methods and equipment justifies the scarce information regarding concentration levels of these pharmaceuticals (Heath et al., 2016; Kosjek and Heath, 2011). Moreover, the research developed has been focused on the ecotoxicological effects in freshwater species, with a high discrepancy between the number of studies with freshwater and marine species (Bebianno and Garcia da Fonseca, 2020), a trend also verified for other pharmaceutical classes (Almeida et al., 2020a, 2020b; Gaw et al., 2014; Mezzelani et al., 2018). Furthermore, a limited range of aquatic species belonging to different trophic levels, such as algae, bivalves, crustaceans, and fish, has been selected

for the evaluation of antineoplastic drugs toxic effects (Brezovšek et al., 2014; Jureczko and Przystaś, 2019; Parrella et al., 2015; Trombini et al., 2016).

In this way, the present paper constitutes an updated review concerning concentration levels of anticancer drugs in aquatic environments and their impacts towards aquatic species. Aiming to identify research topics of interest and knowledge gaps, the terms "Anticancer drugs" and "Aquatic" were selected and introduced in the platform Scopus. It is important to highlight that the term "Anticancer drugs" was used instead of "Antineoplastic drugs" since by using this last option fewer articles were identified. To create visualization maps, the author's keywords from all the obtained research articles were chosen as an item of interest and the relation (link) between keywords was analysed by the co-occurrence between the different keywords (Cheng et al., 2018). This analysis was carried out using the VOSviewer 1.6.15, a software tool for constructing and visualizing bibliometric networks (van Eck and Waltman, 2010). In the maps, each link is characterized by the number of publications in which two keywords occurred together. Keywords were grouped into clusters, represented by different colours. The search conducted, using "Anticancer drugs" and "Aquatic" terms, resulted in a total of 107 scientific articles found, published between 1990 and 2020. From these articles, 373 keywords were obtained. Only connected keywords with a minimum threshold of co-occurrence (2) were considered to obtain a significant number of keywords and, at the same time, more focused research with representative clusters. A total of 67 keywords meet these criteria and are represented in Figures 1 and 2. In Figure 1 it is possible to identify circles with the same colours, which means keywords belonging to the same cluster. The size of the circle is proportional to the weight of keyword occurrence, being the weight related to the number of research articles in which the keywords occur. From this representation, it is possible to understand that the research topics such as the risk assessment, predicted environmental concentrations, genotoxicity, and cytotoxicity have been receiving attention from the scientific community. In Figure 2, the average publication year of the documents in which the keywords occurred is represented by different colours and, taking into account the criteria already described above for the keywords selected to be analysed, only research articles from 2014 to 2020 were considered. As in Figure 1, the size of each circle is proportional to the weight of keyword occurrence.

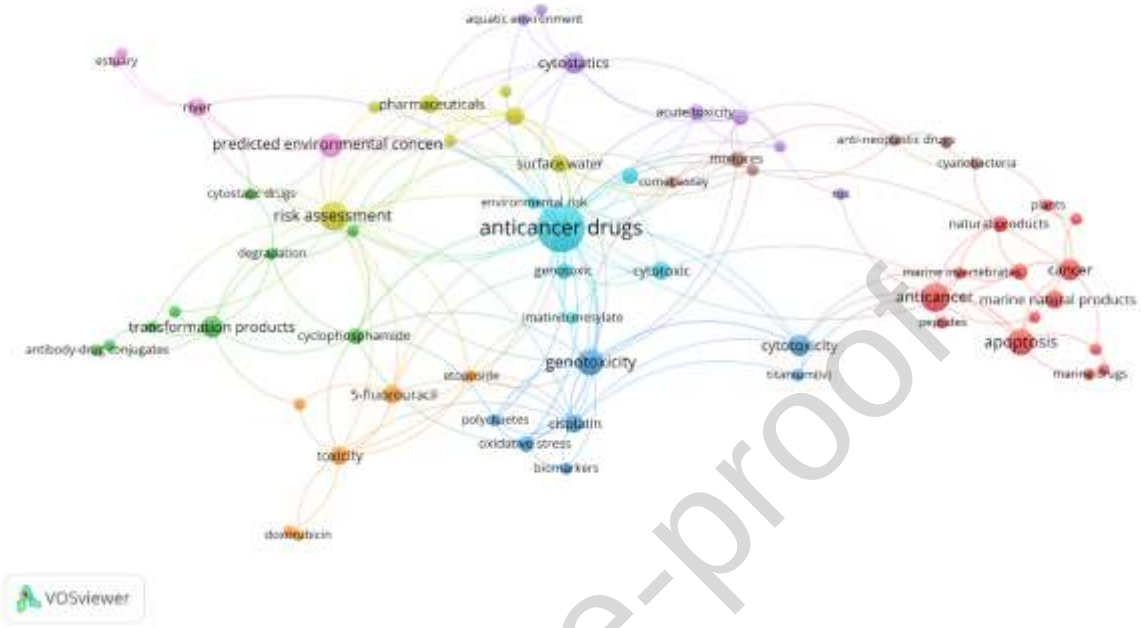


Figure 1: Keywords co-occurrence cluster analysis related to the "Anticancer drugs" and "Aquatic" researched terms.

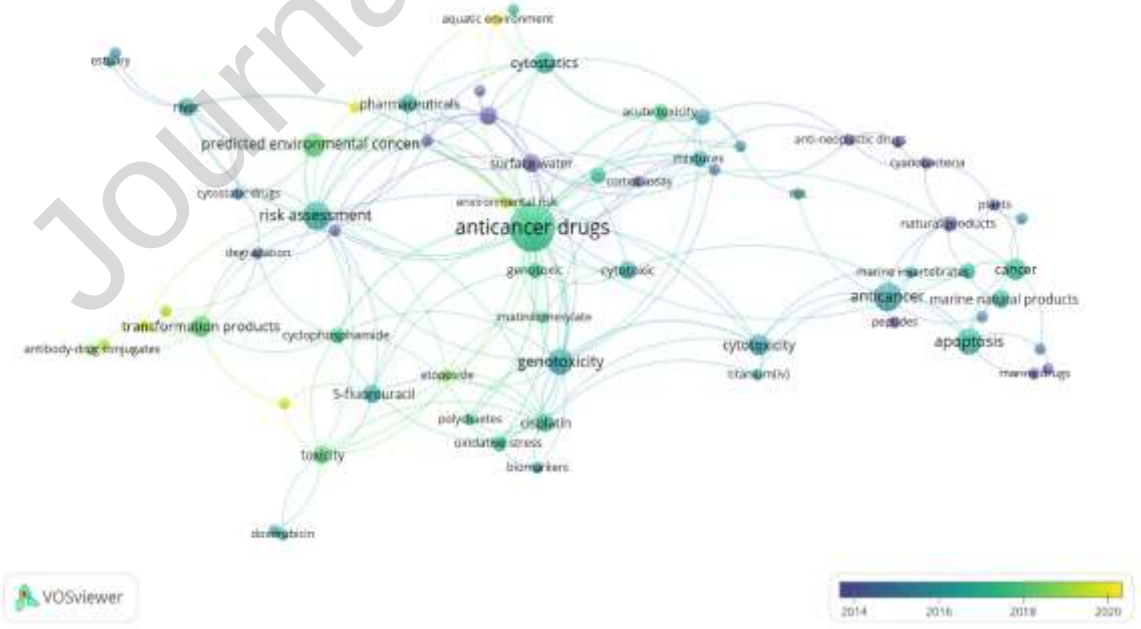


Figure 2: Keywords co-occurrence temporal analysis related to the "Anticancer drugs" and "Aquatic" researched terms. The colours represent the average publication year of the documents in which the keywords occurred.

From Figures 1 and 2, it is possible to observe that the anticancer drugs 5-Fluorouracil, cisplatin (CDDP) and cyclophosphamide (CP) have been raising concerns in the last few years in the scientific community, especially since 2017. Considering the increasing number of publications, but also their high consumption levels, their presence in different aquatic systems and risks to aquatic wildlife, CP and CDDP pharmaceuticals were the target of the present review (Besse et al., 2012; Buerge et al., 2006; Gouveia et al., 2019).

Aiming to summarize the current knowledge on the environmental levels of CP and CDDP and their effects on marine and freshwater invertebrate species after laboratory exposures, Google Scholar, Scopus, ScienceDirect and Web of Science were selected as databases for bibliographic searching. The methodological approach used to identify research articles of interest was based on a combination of keywords, related to CP and CDDP effects in aquatic invertebrates, such as: antineoplastic drugs, cytotoxic drugs, cyclophosphamide, cisplatin, aquatic environment, freshwater environment, field concentrations, marine invertebrate species, freshwater invertebrate species, effects, biomarkers, toxicity. From this bibliographic search 78 research articles were obtained and used to build the entire review paper, 49 of them related to CP and CDDP presence in freshwater and marine environments, determination of their predicted environmental concentrations (PECs), and effects towards invertebrate aquatic species.

Invertebrate species have been among the most used models to study the effects of antineoplastic drugs, such as CP and CDDP, with fewer studies using vertebrate species. Invertebrates have the potential to be used as bioindicators of environmental disturbances due to some important characteristics, including sedentary and filter-feeding behaviour, wide spatial distribution and easy sampling (Parmar et al., 2016; Scalici et al., 2020; Strehse and Maser, 2020). In particular, invertebrate species, such as *Daphnia magna* (crustacean) and *Mytilus galloprovincialis* (bivalve), are worldwide abundant and distributed, sensitive to a wide range of stressors, and widely used in laboratory experiments to study physiological, cytotoxic, and genotoxic alterations, for example, caused by pharmaceuticals (Almeida et al., 2018b; Freitas et al., 2019a; Mastroianni et al., 2020; Parrella et al., 2014b; Russo et al., 2018a). Moreover, some of these species, namely bivalves, are also sessile

organisms and present a filter-feeding behaviour, being able to bioaccumulate several substances, namely emerging contaminants such as pharmaceuticals, and reflect their effects (Almeida et al., 2018a; Costa et al., 2020; Freitas et al., 2019b; Vaughn and Hoellein, 2018). For these reasons, the present review focused on the impacts caused by CP and CDDP on invertebrate species, including freshwater and marine species. From the 49 research articles related to CP and CDDP, only 14 aimed to assess CP and CDDP effects towards invertebrate aquatic species. The majority (57%) of these studies used crustaceans as model species, in particular *D. magna*, and only 3 used bivalve species. The effects observed were mostly related to genotoxicity, survival capacity, and alterations on reproductive behavior (Parrella et al., 2015, 2014b; Russo et al., 2018a; Zounková et al., 2007).

2. CISPLATIN AND CYCLOPHOSPHAMIDE IN THE AQUATIC ENVIRONMENT

Cyclophosphamide is an alkylating agent (L01A group, ATC classification), more specifically a nitrogen mustard analogue (L01AA sub-group). Alkylating agents are the oldest and a major class of chemotherapeutic drugs that act by attaching an alkyl group onto the DNA helix causing the inhibition of the DNA replication and ultimately of the cell division (Fu et al., 2012; Kondo et al., 2010; Kümmerer et al., 2016). Since the late 1950s, CP has been applied in the treatment of several neoplastic diseases such as lymphomas, leukemia, ovary, and breast cancer, among other solid tumors, being one of the most efficient and commonly used antineoplastic drugs, and is also identified as an immunosuppressant (e.g.: autoimmune diseases) (Emadi et al., 2009; Lutterbeck et al., 2020). Cisplatin is also an alkylating agent and belongs to a group of drugs defined as platinum-based compounds (L01XA sub-group). This drug has been widely used as a chemotherapy agent since the end of the 1970' to treat several cancers, namely sarcomas, ovarian, lung, and especially testicular cancer (Dasari and Bernard Tchounwou, 2014).

Although the consumption of antineoplastic drugs is known to be in the order of tonnes per year in European countries, the consumption data for individual drugs is highly variable worldwide and across countries (Besse et al., 2012; Gómez-Canela et al., 2020). Nonetheless, Gómez-Canela et al.

(2020) compiled the consumption data of the most widely used antineoplastic drugs, reporting for CP the European mean value of 10.4 μg/inhab/day. Although Gómez-Canela et al. (2020) report did not include the European mean value for CDDP, other authors have identified consumption values ranging from 0.65 to 1.090 μg/inhab/day in France, United Kingdom and Portugal (Besse et al., 2012; Gómez-Canela et al., 2020; Rowney et al., 2009; Santos et al., 2017). After consumption, these drugs are discharged into wastewaters effluents at different concentration levels. In hospital wastewater effluents (HWWs), for example, to the best of our knowledge, the maximum value reported for CP was 687,000 ng L^{-1} (Gouveia et al., 2019; Hamon et al., 2018). On the other hand, the maximum value reported for CDDP in HWWs was 266,000 ng L^{-1} (Gouveia et al., 2019). The biodegradation of CP is very low since it is a polar compound, highly soluble in water, with a low octanol/water partition coefficient (log K_{ow} of 0.63) and a half-live in the order of years, showing thus the capacity to persist in the aquatic environment (Buerge et al., 2006; Heath et al., 2016). Cisplatin also presents some concerning physico-chemical properties. Being a polar compound and highly soluble in water (with a very low log K_{ow} of -2.19), CDDP is rapidly hydrolyzed into different new transformation products that constitute new, stable, and potentially more toxic mixtures (Fonseca et al., 2017; Ghafuria et al., 2018; Parrella et al., 2014b; Russo et al., 2020). The hydrophilic properties of CP and CDDP suggest that they can persist into the water phase, being unlikely their elimination by sorption onto sewage sludge in WWTPs and, thus, increasing the potential risk of releasing these drugs into the environment through wastewater streams discharge (Ghafuri et al., 2018; Kosjek and Heath, 2011). In fact, the removal rates of these drugs in the WWTPs is highly variable and inconsistent across the literature, with values of removal ranging from 10% to 100% for CP, depending on the treatment applied (Gómez-Canela et al., 2012; Ioannou-Ttofa and Fatta-Kassinos, 2020; Isidori et al., 2016). The removal rates may also vary according to the specific quantification levels of each facility, with different literature values. As an example, Ferrando-Climent et al. (2013) reported a limit of quantification (LOQ) of 3.6 ng L^{-1} when analyzing CP whereas in a study conducted by Llewellyn et al. (2011) the LOQ obtained ranged between 0.11 ng L^{-1} and 0.4 ng L^{-1} for the same drug. For CDDP, Lenz et al. (2007) reported a removal rate of 51% using a membrane bioreactor as an advanced biological treatment, an alternative to the conventional activated sludges in WWTPs.

As a consequence of the limitations to detect these drugs under environmental conditions, PECs have been previously determined by different authors for CP and CDDP, as identified in Table 1. However, information reported in the literature concerning the PECs for antineoplastic drugs is still scarce and variable, with values ranging from 2.94 to 70.2 ng L^{-1} and from 0.1 to 0.8 ng L^{-1} for CP and CDDP, respectively (Franquet-Griell et al., 2015; Gómez-Canela et al., 2020; Mišík et al., 2019).

Published PECs, which are presented in Table 1, were calculated based on the available consumption data of the pharmaceutical selected, excretion rates, elimination rates in WWTPs, and a dilution factor into the receiving waters, aiming to estimate the concentrations of the pharmaceutical in the aquatic environment, namely influents and effluents of WWTPs and rivers (Gómez-Canela et al., 2020). Most of the PECs presented in Table 1 were calculated according to the European Medicine Agency (EMA) guidelines, using Eq. 1:

$$PEC = \frac{\text{consumption} \times \text{Fexc} \times (1 - \text{Fwwtp})}{\text{WWinhab} \times \text{inhab} \times \text{DF} \times 365}$$

where, consumption is the total amount of each pharmaceutical consumed per year in a defined zone; Fexc is the excreted fraction of the parent drug through the urinary and/or digestive system; Fwwtp is the fraction of the pharmaceuticals removed in the WWTPs, so (1-Fwwtp) corresponds to the fraction of pharmaceutical's release from WWTPs to surface waters; WWinhab is the volume of wastewater produced per inhabitant per day; Inhab is the number of inhabitants of the selected study zone, and DF is the dilution factor from the WWTP effluents to the surface waters and a default value of 10 was assumed by the authors, as suggested by EMA guidelines. This equation is considered the most accurate to estimate PECs (Gómez-Canela et al., 2020).

Although for most of the studies PECs were obtained by using this equation, some authors used slightly different equations. For example, Kümmerer and Al-Ahmad (2010) did not consider the excreted fraction of the parent drug through the urinary and/or digestive system and the fraction of the pharmaceuticals removed in the WWTPs as independent values and so, these authors considered losses by the metabolism as well as due to adsorption and biodegradation in the same value. Martín et al. (2014) also calculated the PECs without considering the excretion rates of the drugs through the

urinary and/or digestive system. Moreover, some authors have defined specific assumptions in order to be able to calculate the PECs for some regions. Among them, Besse et al. (2012) and Ghafuria et al. (2018) have considered an Fwwtp value of 1 in the cases where the WWTP removal rates were not available. This value corresponds to a scenario where no removal of the drugs occurs at the WWTPs, corresponding to the worst-case scenario.

Despite the limitations on drugs quantification, the presence of CP and CDDP in different aquatic compartments from different countries worldwide has been detected by different authors, as summarized in Table 2. When analysing Table 2 it is possible to conclude that freshwater ecosystems have received higher attention comparing to marine environments, a trend also verified for other pharmaceutical classes (Almeida et al., 2020a; Fabbri and Franzellitti, 2016; Mezzelani et al., 2018). To the best of our knowledge, there are no studies regarding the presence of CP and CDDP in the marine environment.

From Table 2 it is possible to observe that CP and CDDP are found in freshwater ecosystems in concentrations up to concentrations of 22100 ng L^{-1} and 250 μg L^{-1}, respectively. However, it is also possible to observe that these two compounds can be at concentrations lower than the limit of detection (LOD). Concerning the CDDP concentrations found in the literature, it should be taken into account that the majority of the data are related to the total concentrations of platinum (Pt) based cytotoxic drugs, which includes not only CDDP but may also include carboplatin and oxaliplatin, among others. Pt-based compounds can be used as coordination complexes to treat 50% to 70% of cancer patients (Vyas et al., 2014) and, as a consequence, research has been focused on the detection of these drugs as complexes. Moreover, the difficulties in the detection and identification of individual Pt-based drugs in the environment, namely CDDP, can also justify the lack of results for this drug (Turner and Mascorda, 2015).

Higher concentrations of CP and CDDP were found in HWW which can be explained by the fact that hospitals are one of the main sources of antineoplastics drugs since the majority of cancer patients are treated at these facilities (Olalla et al., 2018). Along with the HWW, WWTP influents also present high concentration levels of CP and CDDP, a direct consequence of the increase in outpatient treatments for cancer patients (Böhlandt et al., 2017; Ferrando-Climent et al., 2013). Regarding the

concentrations found in WWTP effluents, the range of concentrations is very similar to the ones found in WWTP influents, reinforcing the inefficient removal of these drugs by WWTP.

Most of the studies related to the CDDP and CP quantification were made in Europe, mainly in Spain (Ferrando-Climent et al., 2014, 2013; Franquet-Griell et al., 2017a; Gómez-Canela et al., 2014; Isidori et al., 2016; Negreira et al., 2014a; Olalla et al., 2018; Vidmar et al., 2015) (Table 2). Asia and North America have also reported the presence of these drugs in surface waters (Azuma et al., 2016, 2015; Rabii et al., 2014; Usawanuwat et al., 2014; Yin et al., 2010) (Table 2). However, information regarding the presence of CP and CDDP in Africa and South America surface waters was not found. Fewer research efforts have been made to study the presence of pharmaceuticals in Africa and South America when comparing to other parts of the world, being the majority of research concentrated in well-developed countries (Hughes et al., 2013; Wilkinson et al., 2019). In Africa, in a study conducted by Fekadu et al. (2019), several pharmaceuticals, belonging to different classes, have been detected in surface waters with maximum concentrations 20,000 times higher than in European countries, reinforcing the urgency for advanced WWTP in these regions and the necessity to prioritize the monitoring of pharmaceuticals such as antineoplastic drugs for which, and to the best of our knowledge, there is no data regarding their presence.

From Table 2, it is also possible to verify that the presence of CP and CDDP concentrations found are in the same range, regardless of the country and the freshwater system, from low to high ng L^{-1}, across countries. In Spain, for example, Isidori et al. (2016) reported a CP concentration ranging from 1080 ng L^{-1} to 22100 ng L^{-1} in Barcelona hospital effluents. Also, in effluents from a hospital located in Barcelona values ranging from 0.004 μg L^{-1} and up to 4.72 μg L^{-1} were reported by Gómez-Canela et al. (2014). In another study conducted by Olalla et al. (2018), CP was detected in concentrations up to 3000 ng L^{-1} in Valencia hospital effluents. In Slovenia, Vidmar et al. (2015) reported CDDP concentrations up to 0.352 ng mL^{-1} when studying hospital effluents and WWTP influents and effluents. Contrarily, Isidori et al. (2016) found concentrations of this drug up to 352 ng L^{-1} in Slovenian HWWs and WWTPs.

Comparing with predicted values of CP and CDDP (Table 1), it is possible to conclude that the measured environmental concentrations (MECs) of these two drugs are in the same concentration

range (Table 2). The highest PEC for CP, with a value of 70.2 ng L^{-1}, was reported by Rowney et al. (2009) in WWTPs effluents in the United Kingdom. As for rivers, the highest PECs were reported in Spain by Martín et al. (2014) and Ortiz de García et al. (2013), with very similar values. All MECs in WWTPs effluent and surface waters in Spain were higher than the PECs calculated for this country (Tables 1 and 2). The same observation was made in other countries, namely in Portugal where a study conducted by Gouveia et al. (2020) with WWTPs effluents, revealed higher MECs values than the PECs calculated by Santos et al. (2017) and Cristóvão et al. (2020). Regarding the case of Spain, Franquet-Griell et al. (2017a) calculated the PECs for several antineoplastic drugs according to consumption data for northeast Spain, and taking into account the results obtained the most widely reported drugs were further monitored in Besòs River (Barcelona). In this study, CP was among the drugs selected to be monitored, having a calculated PEC of 2.57 ng L^{-1} and MEC ranging from 5.0 - 13.7 ng L^{-1}. Although the PEC was underestimated, the PEC/MEC was considered reliable for this drug. Regarding the PEC obtained for CDDP, the results were also in the same range as the measured concentrations presented in Table 2. The highest PEC of this drug reported, 0.00278 μg L^{-1}, was obtained by Ghafuria et al. (2018) in Iran for HWW. The same authors have calculated the PEC/MEC ratio, after quantification of this drug in the HWW of the study area (Table 1), being the results considered within the acceptable range.

Despite the PECs of CP and CDDP in freshwater and marine environments, to the best of our knowledge, there is no information regarding concentrations of CP and CDDP in the marine environment. However, in a freshwater environment, the maximum concentration detected was of 22100 ng L^{-1} and 250 μg L^{-1}, respectively. Furthermore, there are no studies published on CP and CDDP concentration levels in wild freshwater and marine aquatic invertebrate species. Moreover, the effects of these drugs on aquatic invertebrate species were evaluated only after laboratory exposures, being the effects on field organisms still unknown. Data regarding the effects of CP and CDDP on aquatic invertebrate organisms is still scarce and there is no information regarding the potential for bioaccumulation of these drugs in these organisms. In the following section, the effects on aquatic invertebrate species exposed, under laboratory conditions, to CP and CDDP are discussed.

3. LABORATORY STUDIES: CYCLOPHOSPHAMIDE AND CISPLATIN EFFECTS IN FRESHWATER AND MARINE INVERTEBRATES

Several laboratory studies have been performed in order to assess CP and CDDP toxic effects on aquatic invertebrates and are summarized in Table 3. The minimum concentration tested for CP and CDDP was 0.1 ng L^{-1} and the maximum concentration tested was 2000 mg L^{-1} and 10 mg L^{-1}, respectively. Acute and chronic assays were performed in different species with both drugs. The minimum exposure period using CP and CDDP was 24 h and the maximum was 14 days. The effects observed in freshwater and marine species are described in the following sections.

3.1.1. Freshwater species

To the best of our knowledge, only two studies were performed addressing the impacts of CP on freshwater invertebrate species, namely with *Daphnia magna* (Russo et al., 2018b; Zounková et al., 2007), *Brachionus calycifloru*, *Thamnocephalus platyurus* and *Ceriodaphnia dubia* (Russo et al., 2018b) (Table 3). In the study conducted by Zounková et al. (2007), *D. magna* was exposed for 48 h to CP and an acute immobilization test was performed to assess the toxic effects of this drug. The 50% effective concentration (EC50) value was calculated to evaluate the toxicity of CP and the EC50 obtained was 930 mg L^{-1}. According to these results, CP presented low toxicity to *D. magna*. On the other hand, Russo et al. (2018b) performed a study that aimed to evaluate the acute and chronic ecotoxicity of CP in rotifer (*B. calyciflorus*) and crustacean (*T. platyurus* and *C. dubia*) species, by assessing the mortality and offspring reduction, respectively. The LC50 (concentration causing 50% of mortality) was calculated for all species among with the EC50 values, except for *T. platyurus* for which the EC50 was not calculated. For *B. calyciflorus* (LC50 = 1924 mg L^{-1}, EC50 = 89.84 mg L^{-1}), *T. platyurus* (LC50 = 1396 mg L^{-1}), and *C. dubia* (LC50 = 986.6 mg L^{-1}, EC50 =58.03 mg L^{-1}) the results showed that the LC50 and EC50 values obtained were several orders of magnitude higher (mg L^{-1}) than CP environmental relevant concentrations, which are in the ng-µg L^{-1} range.

Regarding CDDP, six studies were performed to assess the effects of this drug in different freshwater invertebrate species (Table 3). Zounková et al. (2007) has also tested the effects of CDDP (among other 4 cytotoxic drugs) in *D. magna*, *P. putida* and *P. subcapitata* following the same methodology described above. Results showed that CDDP was the most toxic antineoplastic drug for

D. magna with an EC50 of 0.64 mg L^{-1}, while for *P. putida* (EC50 = 1.2 mg L^{-1}) and *P. subcapitata* (EC50 = 2.3 mg L^{-1}) was one of the most toxic drugs. In another study conducted by Kolarević et al. (2016), the ecotoxicological effects of CDDP were assessed in two bivalve species, the mussels *Unio pictorum* and *Unio tumidus*, by exposing these species to concentrations ranging from 0.004 (1.20 µg L^{-1}) to 4 µM (1202 µg L^{-1}), during 72 h. The authors used bivalves' hemocytes to investigate the level of DNA damage and the results showed that CDDP did not induce significant DNA damage. As the authors explained, the main mode of action of CDDP includes the formation of crosslinks on the DNA and, as a consequence, this drug blocks replication and transcription, interfering with the cell cycle and causing damages in the organisms. Nonetheless, DNA damage should not be caused by the formation of DNA crosslinks and a negative correlation should occur between the rate of crosslink formation and DNA damage level. In order to assess the crosslink formation, the authors performed a comet assay, and in the treatment of the *in vivo* assay with a concentration of 4 µM (1202 µg L^{-1}) the formation of crosslinks was detected. Other studies have assessed the mortality and inhibition of reproduction in *D. magna* and *C. dubia* (Parrella et al., 2014a, 2014b) as well as in *B. calycifloru* and *T. platyurus* after exposure to CDDP (Parrella et al., 2014b) (Table 3). Parrella et al. (2014b) assessed mortality by performing acute toxicity tests, where the species *B. calycifloru*, *T. platyurus*, and *C. dubia* were exposed to CDDP for 24 h and *D. magna* for 48 h. Chronic toxicity tests were also performed to evaluate inhibition of reproduction, with a duration of 21 days and 7 days for *D. magna* and *C. dubia*, respectively, while growth was also investigated in *B. calycifloru* after a 48 h exposure period. The authors concluded that according to the acute toxicity tests results *D. magna* (LC50 = 0.94 mg L^{-1}) and *C. dubia* (LC50 = 2.50 mg L^{-1}) were more sensitive to CDDP than *T. platyurus* (LC50 = 8.44 mg L^{-1}) and *B. calyciflorus* (LC50 = 6.52 mg L^{-1}). As for the chronic toxicity tests results revealed that *D. magna* (EC50 = 1.63 µg L^{-1}) and *C. dubia* (EC50 = 16.83 µg L^{-1}) were more sensitive to CDDP exposure than *B. calyciflorus* (EC50 = 440 µg L^{-1}). Parrella et al. (2014a) further evaluated the inhibition of reproduction of CDDP acting as binary mixtures with other antineoplastic drugs in *D. magna* and *C. dubia*, revealing that mixtures assays showed a synergic tendency on the treatment with imatinib (IM) and CDDP on *C. dubia* at the lowest concentration tested (IM/CDDP = 7.0/2.1 µg L^{-1}). Moreover, the same effect levels produced by single exposures were observed at lower concentrations

when acting as mixtures. For example, the single exposure to CDDP at a concentration of 12.3 µg L^{-1}caused an offspring reduction of 43.8% in *C.dubia* while the mixture IM/CDDP at a concentration of 7.0/2.1 µg L^{-1} caused an offspring reduction 57.1%. Regarding the genotoxicity induced in *D. magna* and *C. dubia* by CDDP exposure, three studies have been published (Kundi et al., 2016; Parrella et al., 2015; Russo et al., 2018a) (Table 3). Parrella et al. (2015) performed *in vivo* comet assay with *D. magna* and *C. dubia* to evaluate the DNA damage produced by CDDP. The results obtained showed that CDDP induced DNA damage in both species, with a no observed effect concentration (NOEC) value of 0.001 µg L^{-1} and 4.57 µg L^{-1}, and a lowest observed effect concentration (LOEC) value of 0.01 µg L^{-1} and 14.65 µg L^{-1}, for *D. magna* and *C. dubia*, respectively. The results obtained by Kundi et al. (2016) when testing mixtures of antineoplastic drugs to assess genotoxic effects were also in accordance with the results reported by Parrella et al. (2014a), where mixtures of drugs induced effects at a lower concentration than those effective in single drugs exposures.

3.1.2. Marine species

Only four invertebrate species have been used to assess the toxic effects of CP in marine organisms and two to assess the toxic effects of CDDP (Table 3). Canty et al. (2009) used the bivalve species *Mytilus edulis* and the sea star *Asterias rubens* to evaluate the clearance rate (CR) and righting time (RT), respectively, along with genotoxicity, and cytotoxicity induced by CP in both species. The organisms were exposed to a concentration range between 18 mg L^{-1} and 180 mg L^{-1} for 7 days. Regarding the behavioral responses in *A. rubens*, a significant increase in the RT was observed at 32 and 56 mg L^{-1}. Contrarily, cytotoxic effects were not detected at any concentration tested. However, significant effects were observed regarding the induction of micronuclei (Mn assay) in the coelomocytes and hemocytes and DNA strand breaks (assessed using the comet assay) at 32 and 56 mg L^{-1}. In *M. edulis*, although behavioral (evaluated by the determination of the CR rate) and cytotoxic effects were not observed at the concentrations tested, significant differences in the induction of micronuclei as well as DNA strand breaks were detected between exposed and non-exposed mussels to CP. The authors also conclude that the results evidenced good correlations

between behavioral and physiological responses and genetic damage in *M. edulis* and *A. rubens* after exposure to CP.

The mussel *M. galloprovincialis* has also been used in ecotoxicological studies to assess the CP (Fernandes et al., 2020) and CDDP (Trombini et al., 2016). Fernandes et al. (2020) exposed this species to CP for 14 days to a 1000 ng L^{-1} concentration and oxidative stress, neurotoxicity, cytotoxicity and genotoxicity were evaluated. Results showed that CP induced oxidative stress and damage along with DNA damage. Moreover, cytotoxicity and DNA damage were also detected in the hemocytes from the exposed mussels. Taking into account the results obtained by Fernandes et al. (2020), *M. galloprovincialis* seems to be a more sensitive species than *M. edulis* since cytotoxicity was observed by this author in *M. galloprovincialis* at concentrations of CP much lower than the concentrations tested in *M. edulis* by Canty et al. (2009), where cytotoxicity was not observed. Similar results to Fernandes et al. (2020) were obtained by Trombini et al. (2016) when exposing *M. galloprovincialis* to CDDP for 14 days to a concentration of 100 ng L^{-1}. This drug, at the concentration tested, induced oxidative stress and alterations in the antioxidant capacity, assessed through the quantification of the antioxidant enzyme activities (superoxide dismutase, SOD; catalase, CAT; total glutathione peroxidases, T-GPx; and selenium-dependent glutathione peroxidase, Se-GPx), in *M. galloprovincialis*. Furthermore, neurotoxicity and DNA damage in mussels hemocytes were also reported by the authors.

Investigating the impacts of antineoplastic drugs in polychaetes, Fonseca et al. (2018) evaluated the toxic effects of CP in *Hediste diversicolor,* after 14 days of exposure, at the following concentrations: 10, 100, 500 and 1000 ng L^{-1}. The endpoints evaluated were behavioral impairment, neurotoxicity, oxidative stress, biotransformation metabolism, oxidative damage, and genotoxicity. As for behavioral impairment, the burrowing behavior did not show a clear pattern over increasing CP concentrations. Neurotoxicity was evaluated through the quantification of acetylcholinesterase (AChE) activity and no significant differences were observed between treatments. Regarding antioxidant enzyme activities analyzed (SOD; CAT; Se-GPx, T-GPx; glutathione S-transferases, GSTs) there were no significant differences between the controls and the concentrations tested, although authors reported an increase in SOD activity followed by a decrease along with increasing concentrations

under the experimental conditions. The same authors have reported inhibition of T-GPx and GSTs activities comparing to the controls and along with increasing CP concentrations. The results obtained for the lipid peroxidation (LPO) showed that cellular damage occurred at higher concentrations. DNA damage was also observed in all CP exposed conditions. On the other hand, Fonseca et al. (2017) evaluated the toxic effects of CDDP in *H. diversicolor*. The behavioral impairment, neurotoxicity, oxidative stress, biotransformation metabolism, oxidative damage, metal exposure and genotoxicity were used as endpoints after a14 days exposure period to CDDP at a concentration of 0.1, 10 and 100 ng L^{-1}. At the highest concentration tested significant burrowing impairment along with neurotoxicity occurred, since inhibition of the AChE activity was observed. At 100 ng L^{-1} of CDDP, the activity of antioxidant and biotransformation enzymes (SOD, CAT, GSTs) were also inhibited, contrarily to the significant increase verified in the metallothionein-like proteins. CDDP also induced oxidative damage at the highest concentration tested. Genotoxicity was not observed at any concentration tested. In another study performed by Fonseca et al. (2019) mixtures of antineoplastic drugs, including CP and CDDP, were used to evaluate the toxic effects of these drugs in the same polychaete species (*H. diversicolor*), by analyzing almost the same endpoints as Fonseca et al. (2018, 2017). In Fonseca et al. (2019), the most relevant results were related to the observed synergetic effect of tamoxifen (TAM) and CP, in one of the tested mixtures (CDDP – 100 ng L^{-1}, CP – 1000 ng L^{-1}, TAM – 100 ng L^{-1}), which caused an additive interaction between CP and CDDP and, as consequence, the inhibition of CAT activity. When CP was tested alone, at the same concentration), the antioxidant capacity was not inhibited (Fonseca et al., 2018). Cellular damage was observed in all mixtures (1: CDDP - 0.1 ng L^{-1} + CP - 10 ng L^{-1} + TAM - 0.1 ng L^{-1}; 2: CDDP - 10 ng L^{-1} + CP -100 ng L^{-1} + TAM - 10 ng L^{-1}; 3: CDDP - 100 ng L^{-1} + CP - 500 ng L^{-1} + TAM - 25 ng L^{-1}; 4: CDDP - 100 ng L^{-1} + CP - 1000 ng L^{-1} + TAM - 100 ng L^{-1}), including in the mixture 1, where the concentrations of CP and CDDP were 0.1 ng L^{-1} and 10 ng L^{-1}, respectively, reinforcing that results from drugs acting alone may not be the same as the ones resulting from mixtures, since cellular damage was not observed at these concentrations when drugs were acting alone (Fonseca et al., 2018, 2017). Moreover, mid-grade DNA damage was also observed in all mixtures.

Overall, the present review shows that there is a lack of knowledge concerning the impact of CP and CDDP on aquatic species, especially on marine species, with only 3 research articles using marine species (*M. edulis, H. diversicolor* and *M. galloprovincialis*). The studies found were performed using concentrations considered environmentally relevant (up to 1000 ng L^{-1}) when considering chronic exposures. Moreover, the majority of laboratory studies published have been focused on the evaluation of specific biomarkers, such as genotoxicity and neurotoxicity, considering drugs acting alone, contrarily to what frequently occurs in the aquatic environment. Consequently, the effects of these drugs on these species are almost unknown when acting as contaminant mixtures, and the real impacts may be underestimated. Furthermore, due to the reduced number of research articles, the biological effects of CP and CDDP on these organisms are not yet fully understood, although effects related to oxidative stress, neurotoxicity, cytotoxicity and genotoxicity were reported for the species already mentioned.

4. FINAL REMARKS

The environmental consequences of pharmaceuticals and other pollutants in the aquatic environment are an issue of rising concern, with impacts that can range from molecular alterations to effects at the population level. For this review, two antineoplastic drugs, CP and CDDP, were selected based on their consumption values, occurrence in the environment, and the lack of toxicological data available for aquatic organisms. Invertebrate freshwater and marine species were selected to review the toxic effects of these to pharmaceuticals due to their importance as bioindicator species and value as biological resources.

This review on the CP and CDDP occurrence in freshwater and marine environment showed concentrations ranging from ng L^{-1} up to 22.1 μg L^{-1} and 250 μg L^{-1}, respectively, in different water compartments. However, considering the limitations of the analytical techniques to measure this class of pharmaceuticals in surface water and the variability reported, more studies are needed to better understand their spatial and temporal concentrations. Predicted environmental concentrations were also assessed within this review, being the values here presented similar to the detected environmental concentrations and in the range of ng L^{-1}. Nonetheless, the lack of data, such as the consumption

values, excretion rates, and removal rates of these drugs in WWTPs, is a major barrier to calculate accurate PECs, which are an important and useful tool that allows to better prioritize pharmaceuticals, more specifically antineoplastic drugs, that have a high probability to be detected in the aquatic environment. The studies assessing the impacts of CP and CDDP on aquatic invertebrate species used concentrations ranging from 0.1 ng L^{-1} to 2000 mg L^{-1} and from 0.1 ng L^{-1} to 10 mg L^{-1}, respectively. Oxidative stress, genotoxic and neurotoxic responses, as well as alterations on reproduction, were detected at a minimum concentration of 100 ng L^{-1}, acting alone and as a mixture of drugs. It is also important to notice that, although toxic effects can occur at higher concentrations than the PECs, higher concentrations can potentially be found in specific aquatic environments, such as hospital effluents, posing at risk aquatic species along with possible chronic exposure to these pharmaceuticals. Moreover, although the majority of studies were performed with relevant environmental concentration, none of them included a recuperation/depuration phase, which is important to understand if the organisms are capable to re-establish their basal biological performance when the drugs are no longer present in the environment. In addition, most of the studies did not consider the drugs acting as mixtures and/or stressful climate change scenarios, which differs from what occurs in the environment. Furthermore, this review supports the importance of increasing the monitoring studies, especially on the marine environment where the lack of information is evident.

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6. REFERENCES

Almeida, Â., Calisto, V., Esteves, V.I., Schneider, R.J., Soares, A.M.V.M., Figueira, E., Freitas, R., 2018a. Effects of single and combined exposure of pharmaceutical drugs (carbamazepine and cetirizine) and a metal (cadmium) on the biochemical responses of *R. philippinarum*. Aquat. Toxicol. 198, 10–19. https://doi.org/10.1016/j.aquatox.2018.02.011

Almeida, Â., Freitas, R., Calisto, V., Esteves, V.I., Schneider, R.J., Soares, A.M.V.M., Figueira, E., Campos, B., Barata, C., 2018b. Effects of carbamazepine and cetirizine under an ocean acidification scenario on the biochemical and transcriptome responses of the clam *Ruditapes philippinarum*. Environ. Pollut. 235, 857–868. https://doi.org/10.1016/J.ENVPOL.2017.12.121

Almeida, Â., Silva, M.G., Soares, A.M.V.M., Freitas, R., 2020a. Concentrations levels and effects of 17alpha-Ethinylestradiol in freshwater and marine waters and bivalves: A review. Environ. Res. 185, 109316. https://doi.org/10.1016/j.envres.2020.109316

Almeida, Â., Solé, M., Soares, A.M.V.M., Freitas, R., 2020b. Anti-inflammatory drugs in the marine environment: Bioconcentration, metabolism and sub-lethal effects in marine bivalves. Environ. Pollut. https://doi.org/10.1016/j.envpol.2020.114442

Álvarez-Muñoz, D., Rodríguez-Mozaz, S., Jacobs, S., Serra-Compte, A., Cáceres, N., Sioen, I., Verbeke, W., Barbosa, V., Ferrari, F., Fernández-Tejedor, M., Cunha, S., Granby, K., Robbens, J., Kotterman, M., Marques, A., Barceló, D., 2018. Pharmaceuticals and endocrine disruptors in raw and cooked seafood from European market: Concentrations and human exposure levels. Environ. Int. 119, 570–581. https://doi.org/10.1016/j.envint.2018.07.006

Azuma, T., Arima, N., Tsukada, A., Hirami, S., Matsuoka, R., Moriwake, R., Ishiuchi, H., Inoyama, T., Teranishi, Y., Yamaoka, M., Mino, Y., Hayashi, T., Fujita, Y., Masada, M., 2016. Detection of pharmaceuticals and phytochemicals together with their metabolites in hospital effluents in Japan, and their contribution to sewage treatment plant influents. Sci. Total Environ. 548–549, 189–197. https://doi.org/10.1016/j.scitotenv.2015.12.157

Azuma, T., Ishiuchi, H., Inoyama, T., Teranishi, Y., Yamaoka, M., Sato, T., Mino, Y., 2015. Occurrence and fate of selected anticancer, antimicrobial, and psychotropic pharmaceuticals in an urban river in a subcatchment of the Yodo River basin, Japan. Environ. Sci. Pollut. Res. 22,

18676–18686. https://doi.org/10.1007/s11356-015-5013-6

Bebianno, M.J., Garcia da Fonseca, T., 2020. Fate and effects of C«cytostatic pharmaceuticals in the marine environment, *in*: Fate and effects of anticancer drugs in the environment. Springer International Publishing, pp. 295–330. https://doi.org/10.1007/978-3-030-21048-9_13

Besse, J.P., Latour, J.F., Garric, J., 2012. Anticancer drugs in surface waters. What can we say about the occurrence and environmental significance of cytotoxic, cytostatic and endocrine therapy drugs? Environ. Int. 39, 73–86. https://doi.org/10.1016/j.envint.2011.10.002

Białk-Bielińska, A., Mulkiewicz, E., Stokowski, M., Stolte, S., Stepnowski, P., 2017. Acute aquatic toxicity assessment of six anti-cancer drugs and one metabolite using biotest battery – Biological effects and stability under test conditions. Chemosphere 189, 689–698. https://doi.org/10.1016/j.chemosphere.2017.08.174

Böhlandt, A., Sverdel, Y., Schierl, R., 2017. Antineoplastic drug residues inside homes of chemotherapy patients. Int. J. Hyg. Environ. Health 220, 757–765. https://doi.org/10.1016/j.ijheh.2017.03.005

Booker, V., Halsall, C., Llewellyn, N., Johnson, A., Williams, R., 2014. Prioritising anticancer drugs for environmental monitoring and risk assessment purposes. Sci. Total Environ. 473–474, 159–170. https://doi.org/10.1016/j.scitotenv.2013.11.145

Brezovšek, P., Eleršek, T., Filipič, M., 2014. Toxicities of four anti-neoplastic drugs and their binary mixtures tested on the green alga P*seudokirchneriella subcapitata* and the cyanobacterium *Synechococcus leopoliensis*. Water Res. 52, 168–177. https://doi.org/10.1016/J.WATRES.2014.01.007

Buerge, I.J., Buser, H.-R., Poiger, T., Müller, M.D., 2006. Occurrence and fate of the cytostatic drugs cyclophosphamide and ifosfamide in wastewater and surface waters. https://doi.org/10.1021/ES0609405

Canty, M.N., Hutchinson, T.H., Brown, R.J., Jones, M.B., Jha, A.N., 2009. Linking genotoxic responses with cytotoxic and behavioural or physiological consequences: Differential sensitivity of echinoderms (*Asterias rubens*) and marine molluscs (*Mytilus edulis*). Aquat. Toxicol. 94, 68–76. https://doi.org/https://doi.org/10.1016/j.aquatox.2009.06.001

Česen, M., Kosjek, T., Laimou-Geraniou, M., Kompare, B., Širok, B., Lambropolou, D., Heath, E., 2015. Occurrence of cyclophosphamide and ifosfamide in aqueous environment and their removal by biological and abiotic wastewater treatment processes. Sci. Total Environ. 527–528, 465–473. https://doi.org/10.1016/J.SCITOTENV.2015.04.109

Cheng, F.F., Huang, Y.W., Yu, H.C., Wu, C.S., 2018. Mapping knowledge structure by keyword co-occurrence and social network analysis: Evidence from Library Hi Tech between 2006 and 2017. Libr. Hi Tech 36, 636–650. https://doi.org/10.1108/LHT-01-2018-0004

Costa, S., Coppola, F., Pretti, C., Intorre, L., Meucci, V., Soares, A.M.V.M., Solé, M., Freitas, R., 2020. Biochemical and physiological responses of two clam species to Triclosan combined with climate change scenario. Sci. Total Environ. 724, 138143. https://doi.org/10.1016/j.scitotenv.2020.138143

Cristóvão, M.B., Janssens, R., Yadav, A., Pandey, S., Luis, P., Van der Bruggen, B., Dubey, K.K., Mandal, M.K., Crespo, J.G., Pereira, V.J., 2020. Predicted concentrations of anticancer drugs in the aquatic environment: What should we monitor and where should we treat? J. Hazard. Mater. 392, 122330. https://doi.org/10.1016/j.jhazmat.2020.122330

da Fonseca, T.G., Abessa, D.M.S., Bebianno, M.J., 2019. Effects of mixtures of anticancer drugs in the benthic polychaete *Nereis diversicolor*. Environ. Pollut. 252, 1180–1192. https://doi.org/10.1016/j.envpol.2019.05.095

Dasari, S., Bernard Tchounwou, P., 2014. Cisplatin in cancer therapy: Molecular mechanisms of action. Eur. J. Pharmacol. https://doi.org/10.1016/j.ejphar.2014.07.025

Desbiolles, F., Malleret, L., Tiliacos, C., Wong-Wah-Chung, P., Laffont-Schwob, I., 2018. Occurrence and ecotoxicological assessment of pharmaceuticals: Is there a risk for the Mediterranean aquatic environment? Sci. Total Environ. 639, 1334–1348. https://doi.org/10.1016/J.SCITOTENV.2018.04.351

Elizalde-Velázquez, G.A., Gómez-Oliván, L.M., 2020. Occurrence, toxic effects and removal of metformin in the aquatic environments in the world: Recent trends and perspectives. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2019.134924

Emadi, A., Jones, R.J., Brodsky, R.A., 2009. Cyclophosphamide and cancer: Golden anniversary. Nat.

Rev. Clin. Oncol. https://doi.org/10.1038/nrclinonc.2009.146

Fabbri, E., Franzellitti, S., 2016. Human pharmaceuticals in the marine environment: Focus on exposure and biological effects in animal species. Environ. Toxicol. Chem. 35, 799–812. https://doi.org/10.1002/etc.3131

Fekadu, S., Alemayehu, E., Dewil, R., Van der Bruggen, B., 2019. Pharmaceuticals in freshwater aquatic environments: A comparison of the African and European challenge. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2018.11.072

Fernandes, E., Fonseca, T.G., Carriço, T., Mestre, N., Tavares, Á., Bebianno, M.J., 2020. Cytotoxic responses of the anticancer drug cyclophosphamide in the mussel *Mytilus galloprovincialis* and comparative sensitivity with human cells lines. Chemosphere 127678. https://doi.org/10.1016/j.chemosphere.2020.127678

Fernández-Rubio, J., Rodríguez-Gil, J.L., Postigo, C., Mastroianni, N., López de Alda, M., Barceló, D., Valcárcel, Y., 2019. Psychoactive pharmaceuticals and illicit drugs in coastal waters of North-Western Spain: Environmental exposure and risk assessment. Chemosphere 224, 379–389. https://doi.org/10.1016/j.chemosphere.2019.02.041

Ferrando-Climent, L., Rodriguez-Mozaz, S., Barceló, D., 2014. Incidence of anticancer drugs in an aquatic urban system: From hospital effluents through urban wastewater to natural environment. Environ. Pollut. 193, 216–223. https://doi.org/10.1016/j.envpol.2014.07.002

Ferrando-Climent, L., Rodriguez-Mozaz, S., Barceló, D., 2013. Development of a UPLC-MS/MS method for the determination of ten anticancer drugs in hospital and urban wastewaters, and its application for the screening of human metabolites assisted by information-dependent acquisition tool (IDA) in sewage samples, *in*: Analytical and bioanalytical chemistry. Springer, pp. 5937–5952. https://doi.org/10.1007/s00216-013-6794-4

Filipič, M., Novak, M., Žegura, B., 2020. Genotoxicity of the Residues of Anticancer Drugs: A Hazard for Aquatic Environment, *in*: Fate and effects of anticancer drugs in the environment. Springer International Publishing, pp. 403–420. https://doi.org/10.1007/978-3-030-21048-9_16

Fonseca, T.G., Auguste, M., Ribeiro, F., Cardoso, C., Mestre, N.C., Abessa, D.M.S., Bebianno, M.J., 2018. Environmental relevant levels of the cytotoxic drug cyclophosphamide produce harmful

effects in the polychaete *Nereis diversicolor*. Sci. Total Environ. 636, 798–809. https://doi.org/10.1016/J.SCITOTENV.2018.04.318

Fonseca, T.G., Morais, M.B., Rocha, T., Abessa, D.M.S., Aureliano, M., Bebianno, M.J., 2017. Ecotoxicological assessment of the anticancer drug cisplatin in the polychaete *Nereis diversicolor*. Sci. Total Environ. 575, 162–172. https://doi.org/10.1016/J.SCITOTENV.2016.09.185

Franquet-Griell, H., Cornadó, D., Caixach, J., Ventura, F., Lacorte, S., 2017a. Determination of cytostatic drugs in Besòs River (NE Spain) and comparison with predicted environmental concentrations. Environ. Sci. Pollut. Res. 24, 6492–6503. https://doi.org/10.1007/s11356-016-8337-y

Franquet-Griell, H., Gómez-Canela, C., Ventura, F., Lacorte, S., 2017b. Anticancer drugs: Consumption trends in Spain, prediction of environmental concentrations and potential risks. Environ. Pollut. 229, 505–515. https://doi.org/10.1016/J.ENVPOL.2017.06.011

Franquet-Griell, H., Gómez-Canela, C., Ventura, F., Lacorte, S., 2015. Predicting concentrations of cytostatic drugs in sewage effluents and surface waters of Catalonia (NE Spain). Environ. Res. 138, 161–172. https://doi.org/10.1016/j.envres.2015.02.015

Freitas, R., Coppola, F., Costa, S., Pretti, C., Intorre, L., Meucci, V., Soares, A.M.V.M., Solé, M., 2019a. The influence of temperature on the effects induced by Triclosan and Diclofenac in mussels. Sci. Total Environ. 663, 992–999. https://doi.org/10.1016/J.SCITOTENV.2019.01.189

Freitas, R., Leite, C., Pinto, J., Costa, M., Monteiro, R., Henriques, B., Di Martino, F., Coppola, F., Soares, A.M.V.M., Solé, M., Pereira, E., 2019b. The influence of temperature and salinity on the impacts of lead in *Mytilus galloprovincialis.* Chemosphere 235, 403–412. https://doi.org/10.1016/j.chemosphere.2019.05.221

Fu, D., Calvo, J.A., Samson, L.D., 2012. Balancing repair and tolerance of DNA damage caused by alkylating agents. Nat. Rev. Cancer. https://doi.org/10.1038/nrc3185

Gaw, S., Thomas, K. V., Hutchinson, T.H., 2014. Sources, impacts and trends of pharmaceuticals in the marine and coastal environment. Philos. Trans. R. Soc. B Biol. Sci. https://doi.org/10.1098/rstb.2013.0572

Ghafuria, Y., Yunesian, M., Nabizadeh, R., Mesdaghinia, A., Dehghani, M.H., Alimohammadi, M., 2018. Environmental risk assessment of platinum cytotoxic drugs: a focus on toxicity characterization of hospital effluents. Int. J. Environ. Sci. Technol. 15, 1983–1990. https://doi.org/10.1007/s13762-017-1517-6

Gómez-Canela, C., Cortés-Francisco, N., Oliva, X., Pujol, C., Ventura, F., Lacorte, S., Caixach, J., 2012. Occurrence of cyclophosphamide and epirubicin in wastewaters by direct injection analysis-liquid chromatography-high-resolution mass spectrometry. Environ. Sci. Pollut. Res. 19, 3210–3218. https://doi.org/10.1007/s11356-012-0826-z

Gómez-Canela, C., Santos, M.S.F., Franquet-Griell, H., Alves, A., Ventura, F., Lacorte, S., 2020. Predicted Environmental Concentrations: A useful tool to evaluate the presence of cytostatics in surface waters, in: Fate and effects of anticancer drugs in the environment. Springer International Publishing, pp. 27–54. https://doi.org/10.1007/978-3-030-21048-9_2

Gómez-Canela, C., Ventura, F., Caixach, J., Lacorte, S., 2014. Occurrence of cytostatic compounds in hospital effluents and wastewaters, determined by liquid chromatography coupled to high-resolution mass spectrometry. Anal. Bioanal. Chem. 406, 3801–3814. https://doi.org/10.1007/s00216-014-7805-9

Gouveia, T.I.A., Alves, A., Santos, M.S.F., 2019. New insights on cytostatic drug risk assessment in aquatic environments based on measured concentrations in surface waters. Environ. Int. 133, 105236. https://doi.org/10.1016/j.envint.2019.105236

Gouveia, T.I.A., Silva, A.M.T., Ribeiro, A.R., Alves, A., Santos, M.S.F., 2020. Liquid-liquid extraction as a simple tool to quickly quantify fourteen cytostatics in urban wastewaters and access their impact in aquatic biota. Sci. Total Environ. 740, 139995. https://doi.org/10.1016/j.scitotenv.2020.139995

Hamon, P., Moulin, P., Ercolei, L., Marrot, B., 2018. Oncological ward wastewater treatment by membrane bioreactor: Acclimation feasibility and pharmaceuticals removal performances. J. Water Process Eng. 21, 9–26. https://doi.org/10.1016/j.jwpe.2017.11.012

Hann, S., Stefánka, Z., Lenz, K., Stingeder, G., 2005. Novel separation method for highly sensitive speciation of cancerostatic platinum compounds by HPLC-ICP-MS. Anal. Bioanal. Chem. 381,

405–412. https://doi.org/10.1007/s00216-004-2839-z

Heath, E., Filipič, M., Kosjek, T., Isidori, M., 2016. Fate and effects of the residues of anticancer drugs in the environment. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-016-7069-3

Hughes, S.R., Kay, P., Brown, L.E., 2013. Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. Environ. Sci. Technol. https://doi.org/10.1021/es3030148

Ioannou-Ttofa, L., Fatta-Kassinos, D., 2020. Cytostatic drug residues in wastewater treatment plants: Sources, removal rfficiencies and current challenges, *in*: Fate and effects of anticancer drugs in the environment. Springer International Publishing, pp. 103–138. https://doi.org/10.1007/978-3-030-21048-9_6

Isidori, M., Lavorgna, M., Russo, C., Kundi, M., Žegura, B., Novak, M., Filipič, M., Mišík, M., Knasmueller, S., de Alda, M.L., Barceló, D., Žonja, B., Česen, M., Ščančar, J., Kosjek, T., Heath, E., 2016. Chemical and toxicological characterisation of anticancer drugs in hospital and municipal wastewaters from Slovenia and Spain. Environ. Pollut. 219, 275–287. https://doi.org/10.1016/j.envpol.2016.10.039

Jureczko, M., Przystaś, W., 2019. Ecotoxicity risk of presence of two cytostatic drugs: Bleomycin and vincristine and their binary mixture in aquatic environment. Ecotoxicol. Environ. Saf. 172, 210–215. https://doi.org/10.1016/j.ecoenv.2019.01.074

Kolarević, S., Gačić, Z., Kostić, J., Sunjog, K., Kračun-Kolarević, M., Paunović, M., Knežević-Vukčević, J., Vuković-Gačić, B., 2016. Impact of Common Cytostatics on DNA Damage in Freshwater Mussels *Unio pictorum* and *Unio tumidus*. CLEAN - Soil, Air, Water 44, 1471–1476. https://doi.org/10.1002/clen.201500482

Kondo, N., Takahashi, A., Ono, K., Ohnishi, T., 2010. DNA Damage Induced by Alkylating Agents and Repair Pathways. J. Nucleic Acids 2010, 543531. https://doi.org/10.4061/2010/543531

Kosjek, T., Heath, E., 2011. Occurrence, fate and determination of cytostatic pharmaceuticals in the environment. TrAC Trends Anal. Chem. 30, 1065–1087. https://doi.org/10.1016/j.trac.2011.04.007

Kötke, D., Gandrass, J., Xie, Z., Ebinghaus, R., 2019. Prioritised pharmaceuticals in German estuaries and coastal waters: Occurrence and environmental risk assessment. Environ. Pollut. 255, 113161.

https://doi.org/10.1016/j.envpol.2019.113161

Kümmerer, K., Al-Ahmad, A., 2010. Estimation of the cancer risk to humans resulting from the presence of cyclophosphamide and ifosfamide in surface water. Environ. Sci. Pollut. Res. 17, 486–496. https://doi.org/10.1007/s11356-009-0195-4

Kümmerer, K., Haiß, A., Schuster, A., Hein, A., Ebert, I., 2016. Antineoplastic compounds in the environment—substances of special concern. Environ. Sci. Pollut. Res. 23, 14791–14804. https://doi.org/10.1007/s11356-014-3902-8

Kümmerer, K., Helmers, E., Hubner, P., Mascart, G., Milandri, M., Reinthaler, F., Zwakenberg, M., 1999. European hospitals as a source for platinum in the environment in comparison with other sources. Sci. Total Environ. 225, 155–165. https://doi.org/10.1016/S0048-9697(98)00341-6

Kundi, M., Parrella, A., Lavorgna, M., Criscuolo, E., Russo, C., Isidori, M., 2016. Prediction and assessment of ecogenotoxicity of antineoplastic drugs in binary mixtures. Environ. Sci. Pollut. Res. 23, 14771–14779. https://doi.org/10.1007/s11356-015-4884-x

Lenz, K., Mahnik, S.N., Weissenbacher, N., Mader, R.M., Krenn, P., Hann, S., Koellensperger, G., Uhl, M., Knasmüller, S., Ferk, F., Bursch, W., Fuerhacker, M., 2007. Monitoring, removal and risk assessment of cytostatic drugs in hospital wastewater. Water Sci. Technol. 56, 141–149. https://doi.org/10.2166/wst.2007.828

Llewellyn, N., Lloyd, P., Jürgens, M.D., Johnson, A.C., 2011. Determination of cyclophosphamide and ifosfamide in sewage effluent by stable isotope-dilution liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1218, 8519–8528. https://doi.org/10.1016/j.chroma.2011.09.061

Lutterbeck, C.A., Machado, Ê.L., Kümmerer, K., 2020. Degradation and elimination of anticancer drugs by water and wastewater treatment – Toxicity and biodegradability before and after the treatment, *in*: Fate and effects of anticancer drugs in the environment. Springer International Publishing, pp. 139–168. https://doi.org/10.1007/978-3-030-21048-9_7

Martín, J., Camacho-Muñoz, D., Santos, J.L., Aparicio, I., Alonso, E., 2014. Occurrence and ecotoxicological risk assessment of 14 cytostatic drugs in wastewater. Water. Air. Soil Pollut. 225, 1–10. https://doi.org/10.1007/s11270-014-1896-y

Mastroianni, G., Scognamiglio, M., Russo, C., Fiorentino, A., Lavorgna, M., 2020. Environmental

metabolomics: A powerful tool to investigate biochemical responses to drugs in nontarget organisms, *in*: Fate and effects of anticancer drugs in the environment. Springer International Publishing, pp. 441–465. https://doi.org/10.1007/978-3-030-21048-9_18

Mater, N., Geret, F., Castillo, L., Faucet-Marquis, V., Albasi, C., Pfohl-Leszkowicz, A., 2014. In vitro tests aiding ecological risk assessment of ciprofloxacin, tamoxifen and cyclophosphamide in range of concentrations released in hospital wastewater and surface water. Environ. Int. 63, 191–200. https://doi.org/10.1016/j.envint.2013.11.011

Mezzelani, M., Gorbi, S., Regoli, F., 2018. Pharmaceuticals in the aquatic environments: Evidence of emerged threat and future challenges for marine organisms. Mar. Environ. Res. 140, 41–60. https://doi.org/10.1016/j.marenvres.2018.05.001

Mišík, M., Filipic, M., Nersesyan, A., Kundi, M., Isidori, M., Knasmueller, S., 2019. Environmental risk assessment of widely used anticancer drugs (5-fluorouracil, cisplatin, etoposide, imatinib mesylate). Water Res. 164, 114953. https://doi.org/10.1016/J.WATRES.2019.114953

Moldovan, Z., 2006. Occurrences of pharmaceutical and personal care products as micropollutants in rivers from Romania. Chemosphere 64, 1808–1817. https://doi.org/10.1016/j.chemosphere.2006.02.003

Negreira, N., de Alda, M.L., Barceló, D., 2014a. Cytostatic drugs and metabolites in municipal and hospital wastewaters in Spain: Filtration, occurrence, and environmental risk. Sci. Total Environ. 497–498, 68–77. https://doi.org/10.1016/j.scitotenv.2014.07.101

Negreira, N., López de Alda, M., Barceló, D., 2014b. Study of the stability of 26 cytostatic drugs and metabolites in wastewater under different conditions. Sci. Total Environ. 482–483, 389–398. https://doi.org/10.1016/J.SCITOTENV.2014.02.131

Olalla, A., Negreira, N., López de Alda, M., Barceló, D., Valcárcel, Y., 2018. A case study to identify priority cytostatic contaminants in hospital effluents. Chemosphere 190, 417–430. https://doi.org/10.1016/j.chemosphere.2017.09.129

Ortiz de García, S., Pinto Pinto, G., García Encina, P., Irusta Mata, R., 2013. Consumption and occurrence of pharmaceutical and personal care products in the aquatic environment in Spain. Sci. Total Environ. 444, 451–465. https://doi.org/10.1016/j.scitotenv.2012.11.057

Parmar, T.K., Rawtani, D., Agrawal, Y.K., 2016. Bioindicators: the natural indicator of environmental pollution. Front. Life Sci. 9, 110–118. https://doi.org/10.1080/21553769.2016.1162753

Parrella, A., Kundi, M., Lavorgna, M., Criscuolo, E., Russo, C., Isidori, M., 2014a. Toxicity of exposure to binary mixtures of four anti-neoplastic drugs in *Daphnia magna* and *Ceriodaphnia dubia.* Aquat. Toxicol. 157, 41–46. https://doi.org/10.1016/j.aquatox.2014.09.012

Parrella, A., Lavorgna, M., Criscuolo, E., Russo, C., Fiumano, V., Isidori, M., 2014b. Acute and chronic toxicity of six anticancer drugs on rotifers and crustaceans. Chemosphere 115, 59–66. https://doi.org/10.1016/j.chemosphere.2014.01.013

Parrella, A., Lavorgna, M., Criscuolo, E., Russo, C., Isidori, M., 2015. Eco-genotoxicity of six anticancer drugs using comet assay in daphnids. J. Hazard. Mater. 286, 573–580. https://doi.org/10.1016/j.jhazmat.2015.01.012

Rabii, F.W., Segura, P.A., Fayad, P.B., Sauvé, S., 2014. Determination of six chemotherapeutic agents in municipal wastewater using online solid-phase extraction coupled to liquid chromatography-tandem mass spectrometry. Sci. Total Environ. 487, 792–800. https://doi.org/10.1016/j.scitotenv.2013.12.050

Rowney, N.C., Johnson, A.C., Williams, R.J., 2009. Cytotoxic drugs in drinking water: A prediction and risk assessment exercise for the thames catchment in the United Kingdom. Environ. Toxicol. Chem. 28, 2733. https://doi.org/10.1897/09-067.1

Russo, C., Isidori, M., Deaver, J.A., Poynton, H.C., 2018a. Toxicogenomic responses of low level anticancer drug exposures in *Daphnia magna*. Aquat. Toxicol. 203, 40–50. https://doi.org/10.1016/j.aquatox.2018.07.010

Russo, C., Lavorgna, M., Česen, M., Kosjek, T., Heath, E., Isidori, M., 2018b. Evaluation of acute and chronic ecotoxicity of cyclophosphamide, ifosfamide, their metabolites/transformation products and UV treated samples. Environ. Pollut. 233, 356–363. https://doi.org/10.1016/j.envpol.2017.10.066

Russo, C., Lavorgna, M., Piscitelli, C., Isidori, M., 2020. Toxicity of Anticancer Drug Residues in Organisms of the Freshwater Aquatic Chain, *in*: Fate and effects of anticancer drugs in the environment. Springer International Publishing, Cham, pp. 379–401.

https://doi.org/10.1007/978-3-030-21048-9_15

Santos, M.S.F., Franquet-Griell, H., Lacorte, S., Madeira, L.M., Alves, A., 2017. Anticancer drugs in Portuguese surface waters – Estimation of concentrations and identification of potentially priority drugs. Chemosphere 184, 1250–1260. https://doi.org/10.1016/J.CHEMOSPHERE.2017.06.102

Sathishkumar, P., Meena, R.A.A., Palanisami, T., Ashokkumar, V., Palvannan, T., Gu, F.L., 2020. Occurrence, interactive effects and ecological risk of diclofenac in environmental compartments and biota - a review. Sci. Total Environ. 698, 134057. https://doi.org/10.1016/j.scitotenv.2019.134057

Scalici, M., Colamartino, M., Spani, F., Traversetti, L., Persichini, T., Maisano, M., Fasulo, S., Colasanti, M., 2020. Integrated early warning systems in marine bivalves reveal detrimental alterations of coastal habitats. Hydrobiologia 847, 2573–2585. https://doi.org/10.1007/s10750-020-04275-1

Steger-Hartmann, T., Kümmerer, K., Hartmann, A., 1997. Biological degradation of cyclophosphamide and its occurrence in sewage water. Ecotoxicol. Environ. Saf. 36, 174–179. https://doi.org/10.1006/eesa.1996.1506

Steger-Hartmann, T., Kümmerer, K., Schecker, J., 1996. Trace analysis of the antineoplastics ifosfamide and cyclophosphamide in sewage water by two-step solid-phase extraction and gas chromatography-mass spectrometry. J. Chromatogr. A 726, 179–184. https://doi.org/10.1016/0021-9673(95)01063-7

Strehse, J.S., Maser, E., 2020. Marine bivalves as bioindicators for environmental pollutants with focus on dumped munitions in the sea: A review. Mar. Environ. Res. https://doi.org/10.1016/j.marenvres.2020.105006

Trombini, C., Garcia da Fonseca, T., Morais, M., Rocha, T.L., Blasco, J., Bebianno, M.J., 2016. Toxic effects of cisplatin cytostatic drug in mussel *Mytilus galloprovincialis*. Mar. Environ. Res. 119, 12–21. https://doi.org/10.1016/j.marenvres.2016.05.004

Turner, A., Mascorda, L., 2015. Particle-water interactions of platinum-based anticancer drugs in river water and estuarine water. Chemosphere 119, 415–422.

https://doi.org/10.1016/j.chemosphere.2014.06.074

Usawanuwat, J., Boontanon, N., Boontanon, S.K., 2014. Analysis of three anticancer drugs (5-fluorouracil, cyclophosphamide and hydroxyurea) in water samples by HPLC-MS/MS. Int. J. Adv. Agr. Environ. Eng 1, 72–76.

Valcárcel, Y., González Alonso, S., Rodríguez-Gil, J.L., Gil, A., Catalá, M., 2011. Detection of pharmaceutically active compounds in the rivers and tap water of the Madrid Region (Spain) and potential ecotoxicological risk. Chemosphere 84, 1336–1348. https://doi.org/10.1016/j.chemosphere.2011.05.014

van Eck, N.J., Waltman, L., 2010. Software survey: VOSviewer, a computer program for bibliometric mapping. Scientometrics 84, 523–538. https://doi.org/10.1007/s11192-009-0146-3

Vaughn, C.C., Hoellein, T.J., 2018. Bivalve Impacts in Freshwater and Marine Ecosystems. Annu. Rev. Ecol. Evol. Syst. 49, 183–208. https://doi.org/10.1146/annurev-ecolsys-110617-062703

Vidmar, J., Martinčič, A., Milačič, R., Ščančar, J., 2015. Speciation of cisplatin in environmental water samples by hydrophilic interaction liquid chromatography coupled to inductively coupled plasma mass spectrometry. Talanta 138, 1–7. https://doi.org/10.1016/J.TALANTA.2015.02.008

Vyas, N., Turner, A., Sewell, G., 2014. Platinum-based anticancer drugs in waste waters of a major UK hospital and predicted concentrations in recipient surface waters. Sci. Total Environ. 493, 324–329. https://doi.org/10.1016/j.scitotenv.2014.05.127

Wild, C.P., Weiderpass, E., Stewart, B.W., 2020. World Cancer report 2020: Cancer Research for Cancer Prevention, Cancer Control. Lyon, France: International Agency for Research on Cancer. ISBN 978-92-832-0448-0. Available from: http://publications.iarc.fr/586.

Wilkinson, J., Boxall, A., Kolpin, D., 2019. A Novel Method to Characterise Levels of Pharmaceutical Pollution in Large-Scale Aquatic Monitoring Campaigns. Appl. Sci. 9, 1368. https://doi.org/10.3390/app9071368

Yin, J., Shao, B., Zhang, J., Li, K., 2010. A preliminary study on the occurrence of cytostatic drugs in hospital effluents in Beijing, China. Bull. Environ. Contam. Toxicol. 84, 39–45. https://doi.org/10.1007/s00128-009-9884-4

Zhang, J., Chang, V.W.C., Giannis, A., Wang, J.Y., 2013. Removal of cytostatic drugs from aquatic

environment: A review. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2012.12.061

Zounková, R., Odráška, P., Doležalová, L., Hilscherová, K., Maršálek, B., Bláha, L., 2007. Ecotoxicity and genotoxicity assessment of cytostatic pharmaceuticals. Environ. Toxicol. Chem. 26, 2208. https://doi.org/10.1897/07-137R.1

Zuccato, E., Calamari, D., Natangelo, M., Fanelli, R., 2000. Presence of therapeutic drugs in the environment. Lancet 355, 1789–1790. https://doi.org/10.1016/S0140-6736(00)02270-4

Tables

Table 1: Predicted environmental concentrations (PEC) of Cyclophosphamide (CP) and Cisplatin (CDDP) in the aquatic environment. PEC_{WWTPE}, PEC in wastewater treatment plants effluents; PEC_{river}, PEC in river.

Antineoplastic drug	Location	PEC_{WWTPE}	PEC_{river}	Reference
CP	**United Kingdom**	**70.2 ng L^{-1}**		**(Rowney et al., 2009)**
	Germany		**0.6 ng L^{-1}**	**(Kümmerer and Al-Ahmad, 2010)**
	France		**>1.75 ng L^{-1}***	**(Besse et al., 2012)**
	Spain		**4.35 ng L^{-1}**	**(Ortiz de García et al., 2013)**
	Spain		**4.56 ng L^{-1}[a]**	**(Martín et al., 2014)**
	United Kingdom	**40.9 ng L^{-1}**	**4.1 ng L^{-1}**	**(Booker et al., 2014)**
	Spain		**0.11 ng L^{-1}***	**(Franquet-Griell et al., 2015)**
	Germany		**0.01 µg L^{-1}**	**(Kümmerer et al., 2016)**
	Spain		**2.57 ng L^{-1}***	**(Franquet-Griell et al., 2017a)**
	Portugal		**1.36 – 1.49 ng L^{-1}***	**(Santos et al., 2017)**
	India		**3.45 ng L^{-1}***	**(Cristóvão et al., 2020)**
	Portugal		**0.837 ng L^{-1}***	**(Cristóvão et al., 2020)**
	Belgium		**3.06 ng L^{-1}***	**(Cristóvão et al., 2020)**
CDDP	**United Kingdom**	**0.6 ng L^{-1}**		**(Rowney et al., 2009)**
	France		**0.52 ng L^{-1} [b]***	**(Besse et al., 2012)**
	Iran	**0.00278 µg L^{-1}***		**(Ghafuria et al., 2018)**
	India		**0.0141 ng L^{-1}***	**(Cristóvão et al., 2020)**
	Portugal		**0.147 ng L^{-1}***	**(Cristóvão et al., 2020)**
	Belgium		**0.680 ng L^{-1}***	**(Cristóvão et al., 2020)**

*PECs calculated based on Eq.1.

[a] Value recalculated by Franquet-Griell et al., (2015).

[b] Data presented correspond to the PEC without considering excretion rates.

Table 2: Concentrations of Cyclophosphamide (CP) and Cisplatin (CDDP) in the aquatic environment. HWW, Hospital Wastewaters; WWTP Influent, Wastewater Treatment Plant Influent; WWTP Effluent, Wastewater Treatment Plant Effluent.

Antineoplastic drug	Location	Environment				Reference
		HWW	WWTP Influent	WWTP Effluent	Surface water	
CP	**Germany**	**146 ng L^{-1}**				**(Steger-Hartmann et al., 1996)**
	Germany	**19 ng L^{-1} - 4.5 µg L^{-1}**	**< 6 - 143 ng L^{-1}**	**< 6 - 15 ng L^{-1}**		**(Steger-Hartmann et al., 1997)**
	Italy				**2.2 - 10.1 ng L^{-1}**	**(Zuccato et al., 2000)**
	Switzerland		**2 - 11 ng L^{-1}**	**2 - 10 ng L^{-1}**	**0.05 - 0.17 ng L^{-1}**	**(Buerge et al., 2006)**
	Romania				**up to 65 ng L^{-1}**	**(Moldovan, 2006)**
	Germany			**56 ng L^{-1}**		**(Kümmerer and Al-Ahmad,**
	China	**100 ng L^{-1}**				**(Yin et al., 2010)**
	United Kingdom			**3.5 - 3.7 ng L^{-1}**		**(Llewellyn et al., 2011)**
	Thailand				**1.907 µg L^{-1} L^{-1}**	**(Usawanuwat et al., 2014)**
	Spain		**4.6 ng L^{-1}**	**8.9 ng L^{-1}**		**(Negreira et al., 2014a)**
	Spain	**< LOQ – 200.7 ng L^{-1}**	**ND – 25.5 ng L^{-1}**			**(Ferrando-Climent et al., 2013)**
	Spain			**ND - 20 ng /L**		**(Ferrando-Climent et al., 2014)**
	Spain	**< 0.004 - 4.72 µg L^{-1}**	**< 0.004 - 0.01 µg L^{-1}**	**< 0.004 - 0.005 µg L^{-1}**		**(Gómez-Canela et al., 2014)**
	Japan			**up to 20 ng L^{-1}**	**up to 20 ng L^{-1}**	**(Azuma et al., 2015)**
	Japan	**384 ng L^{-1}**				**(Azuma et al., 2016)**
	Spain	**1080 - 22100 ng L^{-1}**	**19 - 27 ng L^{-1}**	**17 ng L^{-1}**		**(Isidori et al., 2016)**
	Slovenia	**< LOD - 32 ng L^{-1}**	**< LOD - 6 ng L^{-1}**	**< LOD**		**(Isidori et al., 2016)**
	Spain				**5.0 - 13.7 ng L^{-1}**	**(Franquet-Griell et al., 2017a)**
	Spain	**46 – 3000 ng L^{-1}**				**(Olalla et al., 2018)**
	Portugal		**ND – 80 ng /L**	**up to 45 ng /L**		**(Gouveia et al., 2020)**
CDDP	**Germany**	**up to 3500 ng L^{-1} [a]**				**(Kümmerer et al., 1999)**
	Austria	**1.7 µg L^{-1}**				**(Hann et al., 2005)**
	Austria		**3 - 250 µg L^{-1} [a]**	**2 - 150 µg L^{-1} [a]**		**(Lenz et al., 2007)**
	Canada		**17 - 22 ng L^{-1}**	**18 - 21 ng L^{-1}**		**(Rabii et al., 2014)**
	United Kingdom	**0.02 - 137.83 µg L^{-1} [b]**				**(Vyas et al., 2014)**
	Slovenia	**0.352 µg L^{-1} [c]**	**0.0233 µg L^{-1} [c]**	**0.0128 µg L^{-1} [c]**		**(Vidmar et al., 2015)**
	Spain	**0.0144 µg L^{-1} [c]**	**0.0079 µg L^{-1} [c]**	**0.0059 µg L^{-1} [c]**		**(Vidmar et al., 2015)**

Slovenia	**226 - 352 ng L^{-1} [c]**	**23 - 27 ng L^{-1} [c]**	**< LOQ [c]**	**(Isidori et al., 2016)**
Spain	**< LOQ [c]**	**< LOQ [c]**	**< LOQ [c]**	**(Isidori et al., 2016)**
Iran	**47.2 - 146.2 µg L^{-1}**			**(Ghafuria et al., 2018)**

LOQ, limit of quantification; ND, non-detected; LOD, limit of detection.
[a] Data presented correspond to the total Platinum-based cytotoxic drugs, cisplatin and carboplatin.
[b] Data presented correspond to the total Platinum-based cytotoxic drugs, cisplatin, carboplatin and oxaliplatin.
[C] Data present corresponds to total platinum concentrations.

Table 3: Laboratory studies testing the effects of Cyclophosphamide (CP) and Cisplatin (CDDP)in aquatic invertebrate species. Assay duration in hours (h) or days (d).

Antineoplastic drug	Reference	Environment	Laboratory studies			
			Species	Concentrations tested	Assay duration	Endpoints
CP	**(Canty et al., 2009)**	**Marine**	***Mytilus edulis***	**18 – 180 mg L^{-1}**	**7 d**	**Clearance rate Genotoxicity Cytotoxicity**
			Asterias rubens	**18 – 180 mg L^{-1}**	**7 d**	**Righting time Genotoxicity Cytotoxicity**
	(Fonseca et al., 2018)	**Marine**	***Nereis diversicolor***	**10 - 1000 ng L^{-1}**	**14 d**	**Behavioral impairment Neurotoxicity Oxidative stress Biotransformation Oxidative damage Genotoxicity**
	(Fonseca et al., 2019)	**Marine**	***N. diversicolor***	**0.1 -100 ng L^{-1} [a]**	**14 d**	**Behavioural impairment Neurotoxicity Biotransformation metabolis Oxidative stress Genotoxicity**
	(Fernandes et al., 2020)	**Marine**	***Mytilus galloprovincialis***	**1000 ng L^{-1}**	**14 d**	**Oxidative stress Neurotoxicity Cytotoxic Genotoxic**
	(Zounková et al., 2007)	**Freshwater**	***Daphnia magna***	**N/a***	**48 h**	**Mobility**
	(Russo et al., 2018b)	**Freshwater**	***Brachionus calycifloru***	**250 – 2000 mg L^{-1}**	**24 h 48 h**	**Mortality Reproduction**
			Thamnocephalus platyurus	**6.25 – 200 mg L^{-1}**	**24 h**	**Mortality**
			Ceriodaphnia dubia	**125 – 2000 mg L^{-1} 250 – 2000 mg L^{-1} 12.5 – 200 mg L^{-1}**	**24 h 7 d**	**Mortality Reproduction**
CDDP	**(Trombini et al., 2016)**	**Marine**	***M. galloprovincialis***	**100 ng L^{-1}**	**14 d**	**Oxidative stress Genotoxicity Neurotoxicity**
	(Fonseca	**Marine**	***N.***	**0.1 - 100 ng L^{-1}**	**14 d**	**Behavioural**

et al., 2017)					**impairment** **Neurotoxicity** **Oxidative** **Metal** **Biotransformation** **Oxidative damage** **Genotoxicity**
(Fonseca et al., 2019)	**Marine**	***N. diversicolor***	**10 – 1000 ng L^{-1} [a]**	**14 d**	**Behavioural impairment** **Neurotoxicity** **Oxidative** **Genotoxicity**
(Zounková et al., 2007)	**Freshwater**	***D. magna***	**N/a***	**48h h**	**Mobility**
(Kolarević et al., 2016)	**Freshwater**	***Unio*** ***Unio tumidus***	**0.004 - 4 µM (1,20 - 1202 µg L^{-1}**	**72 h**	**Genotoxicity**
(Parrella et al., 2014b)	**Freshwater**	***B. calycifloru*** ***T. platyurus*** ***C. dubia*** ***D.magna***	**N/a***	**24 h** **48 h** **24 h** **24 h** **7 d** **48 h** **21 d**	**Mortality** **Growth** **Mortality** **Mortality** **Reproduction** **Mobility** **Reproduction**
(Parrella et al., 2014a)	**Freshwater**	***D. magna*** ***C. dubia***	**0.5 – 2.0 µg L^{-1}** **0.08 - 5.00 µg L^{-1} [b]** **12.3 – 125.3 µg L^{-1}** **2.1 - 50.20 µg L^{-1} [b]**	**21 d** **7 d**	**Reproduction** **Reproduction**
(Parrella et al., 2015)	**Freshwater**	***D. magna*** ***C. dubia***	**0.001 – 100 µg L^{-1}** **0.03 – 300 µg L^{-1}**	**24 h** **24 h**	**Genotoxicity**
(Kundi et al., 2016)	**Freshwater**	***D. magna*** ***C. dubia***	**0.1 – 100 µg/ L^{-1}** **0.07 – 3.6 µg L^{-1} [b]** **0.3 – 300 µg L^{-1}** **0.0002 – 0.01 µg L^{-1} [b]**	**24 h** **24 h**	**Genotoxicity**
(Russo et al., 2018a)	**Freshwater**	***D. magna***	**10 ng L^{-1}**	**24h**	**Genotoxicity**

*Not available
[a]Concentrations tested in ternary mixtures with other antineoplastic drugs.
[b]Concentrations tested in binary mixtures with other antineoplastic drugs.

Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered
as potential competing interests:

HIGHLIGHTS

- Up to 22.1 µg/L (CP) and 250 µg/L (CDDP) were detected in the aquatic environment.
- Predicted and measured environmental concentrations are at the ng/L and µg/L range.
- 0.1–2x10^9 ng/L (CP) and 0.1–1x10^7 ng/L (CDDP) ranges were used in ecotoxic assays.
- Under lab conditions toxicity occurred in aquatic species at a minimum of 100 ng/L."
- In the literature more studies addressed the freshwater than the marine environment.