Environ Sci Pollut Res
DOI 10.1007/s11356-016-7069-3

EDITORIAL

Fate and effects of the residues of anticancer drugs in the environment

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Received: 1 June 2016 / Accepted: 9 June 2016

Pharmaceuticals have brought enormous benefits to humanity in terms of healthier and longer lives. They continue to be prescribed in increasing amounts and it is not surprising that they are considered as environmental contaminants of emerging concern. Pharmaceuticals are excreted through faeces and urine as a mixture of unaltered parent compounds and metabolites and can enter the aquatic environment via hospital and wastewater treatment plant effluents, landfill leachates and, to a minor extent, in the discharge from the pharmaceutical industry. Their continual input into the aquatic compartment means that they are continuously present in the environment. Anticancer drugs (antineoplastic or cytostatic agents) are a group of highly active chemotherapy agents designed to prevent or disrupt the proliferation of tumour cells. These drugs interfere directly or indirectly with the structure and functions of DNA, which affect besides tumour cells also non-target cells and tissues of exposed organisms. When compared to many other groups of pharmaceuticals (e.g. nonsteroidal anti-inflammatory drugs, antibiotics and lipid regulators), they are prescribed in much lower quantities, and their levels in the aquatic environment are either below or at the current limits of analytical detection (sub ng/L). Many of these drugs are mutagenic, carcinogenic, teratogenic and/or toxic to reproductive systems and are classed as highly hazardous compounds. The question remains as to whether chronic exposure to anticancer drug residues at present levels could have a detrimental effect on the environment and human health.

In 2011, a review paper by Kosjek and Heath (2011) revealed the need for enhanced analytical methods that form the basis for a true evaluation of the environmental impact of cytostatic pharmaceutical residues. The recently completed EU's 7th FP project "CytoThreat" was set up to investigate the effects of anticancer drugs in the environment. It also addressed the need to develop new analytical methods for determining environmental exposure to cancer drug residues (parent compounds, metabolites and transformation products) and possible biomarkers, all of which, can be used to provide the necessary ecotoxicity data for making an accurate environmental risk assessment. This special issue brings together 11 papers from 25 research institutions in 12 countries dealing with the occurrence, fate and adverse effects of anticancer drug residues in the environment.

The papers are grouped under three thematic headings:

(i) Developing analytical methods for determining the occurrence and fate of cytostatic drug residues including their metabolites and transformation products during water treatment and in the environment

Three publications fall under this heading. The first paper describes an interlaboratory comparison for determining selected anticancer drugs in aqueous samples (Heath et al. 2016). The exercise involved the commonly prescribed drugs cyclophosphamide, ifosfamide, 5-fluorouracil, gemcitabine, etoposide, methotrexate and cisplatinum in aqueous matrices

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Published online: 28 June 2016

including hospital wastewater, wastewater treatment plant effluent and surface waters. Given the small number of laboratories analysing cytostatic drug residues, only four compounds (cyclophosphamide, ifosfamide, methotrexate and etoposide) fulfilled the necessary criteria of having a statistical minimum of five independent laboratory measurements. Among these compounds, methotrexate yielded the highest within-laboratory repeatability for all three matrices. Overall, interlaboratory reproducibility was poor, and the smallest absolute differences between the spiked and measured values were determined in river water.

Besides the parent drugs (cyclophosphamide, ifosfamide, etoposide), the fate of their metabolites and transformation products in a lab-scale wastewater treatment plant was also addressed. The findings are reported in two publications. In the first paper, Česen et al. (2016) describe the development of an analytical method for determining cyclophosphamide, ifosfamide and their selected metabolites/transformation products: carboxy-cyclophosphamide, keto-cyclophosphamide and N-dechloroethyl-cyclophosphamide in wastewaters. The LOQ of the developed method were in low nanograms per liter. The method was applied to hospital wastewater and influent and effluents from a receiving wastewater treatment plant. In hospital effluent, levels up to 3 $\mu g\ L^{-1}$ were detected, while in influent and effluent from the water treatment plant were below the LOQ. The authors also describe the formation of transformation products during UV and UV/H_2O_2 treatments and tentatively identified three novel transformation products. In a second paper by Kosjek et al. (2016), the fate of etoposide during microbiological breakdown is described with the primary focus on identifying biotransformation products. In total, five transformation products were proposed; among them, four etoposide transformation products are described for the first time. Even though the chemical structures of these new compounds cannot be confirmed due to the lack of authentic compounds, their molecular formulae can be used to target them in monitoring studies.

(ii) Assessing the ecotoxicological and genotoxic properties of selected anticancer drugs and their mixtures

Seven publications fall under this heading. The paper by Kovacs et al. (2016) reports the findings of acute toxicity studies involving four cytostatic drugs: 5-fluorouracil (5-FU), cisplatin (CDDP), etoposide (ET) and imatinib mesylate (IM) in zebrafish (*Danio rerio*) embryos and in adult fish and sub-chronic toxicity of 5-FU and IM in the early-life stage toxicity test. The tested drugs were characterized by low acute and sub-chronic toxicity, which indicates low susceptibility of fish towards these drugs. However, a previously published study by Kovacs et al. (2015) reports how chronic two-generation exposure of zebrafish to 5-FU at environmentally relevant concentrations (10 $ng\ L^{-1}$) caused histopathological changes in the liver and kidney, impaired their DNA integrity and induced massive whole-transcriptome changes. It can be concluded that standard acute and sub-acute toxicity tests, recommended by EMA guidelines (EMA 2006), are not adequate for predicting of potential delayed adverse effects of anticancer drugs. For predicting the adverse effect of DNA reactive anticancer drugs in vertebrates, chronic exposure toxicity studies including the detection of a genotoxic effect are recommended.

Mišík et al. (2016a) reports the impact of 5-FU, CDDP and ET on the fertility of higher plants using pollen abortion experiments. All compounds increased the frequencies of abortive grains with the lowest effective doses between 1 and 10 mg/kg of dry soil. Pichler et al. (2014) reported that IM also induces pollen abortion, however at a higher effective dose (>150 mg/kg). In higher plants (*Tradescantia* and *Allium*), the induced genotoxic effects of the four drugs indicates that induction of DNA damage is one mechanism that accounts for the induction of abortive pollen (Misik et al., 2014). However, the doses at which induction of abortive grains as well as genotoxic effect were detected are four to six orders of magnitude higher than the concentrations predicted in the environment.

In two papers, the cytotoxic and genotoxic effects of the selected anticancer drugs in in vitro test systems are described. Gajski et al. (2016) reports the findings of a comparative in vitro toxicological characterization of 5-FU, CDDP and ET towards zebrafish liver (ZFL) cells, human hepatoma (HepG2) cells and human peripheral blood lymphocytes (HPBLs). Their cytotoxic and genotoxic potential were different in different cell lines with ZFL being the most sensitive and HPBLs the least sensitive. The authors concluded that ZFL cells provide a relevant and sensitive tool to screen genotoxic potential of environmental pollutants in the frame of environmental hazard assessment. Novak et al. (2016) applied the model with HepG2 cells to explore the differences in the mechanisms of genotoxic effects of 5-FU, CDDP, ET and IM. The analysis of changes in the expression of genes involved in response to DNA damage, apoptosis and oncogenesis revealed that 5-FU, CDDP and ET, but not IM, de-regulated expression of these genes suggesting that IM has a different mechanism of action. Contrary to 5-FU, CDDP and ET, IM most likely does not interact directly with DNA. Importantly, the genotoxic effects of the tested anticancer drugs were observed at their therapeutic concentrations, which may lead to increased risk of delayed adverse side effects in patients. Their findings also indicate that exposure to 5-FU, CDDP and ET, that interact with DNA directly, may represent a higher risk for delayed effects such as cancer, reproductive effects and heritable disease than exposure to IM.

The residues of pharmaceuticals in the environment typically occur as complex mixtures, and therefore, even though the concentrations of an individual compound might be low, a

so-called cocktail effect might be of ecotoxicological significance. Genotoxicity of binary mixtures of 5-FU, CDDP, ET and IM were studied in *Tradescantia* micronucleus assay by Mišík et al. (2016b). The authors found clear evidence for synergism in experiments with mixtures with IM and antagonism in a high-dose experiment with a mixture of 5-FU and ET. The effects of the mixtures were observed at concentrations several orders higher than the predicted environmental concentrations, and it is unlikely that the residues of anticancer drugs in the environment causes adverse effects in higher plants.

Kundi et al. (2016) investigated the genotoxicity of binary mixtures of 5-FU, CDDP, ET and IM in *Daphnia magna* and *Ceriodaphnia dubia* using the comet assay. The results obtained for *D. magna* showed independent action that produced additive effects for mixtures. The exception is IM + 5-FU that has an antagonistic interaction. In *C. dubia*, most mixtures had antagonist interactions except for IM + 5-FU and IM + CDDP that showed Bliss independence. This corroborate the findings of a previous reproductive toxicity study with *D. magna* and *C. dubia* revealing that the majority of these binary mixtures exerted an independent action (Parrella et al., 2014a). When comparing the effective concentrations of a binary mixture to those of the single compounds (Parrella et al., 2014b), combinations of anticancer drugs could be of environmental concern because their effects occur at very low concentrations that are in the range of concentrations encountered in aquatic systems.

Eleršek et al. (2016) tested the effect of a mixture of 5-FU + IM + ET on the growth inhibition of green alga *Pseudokirchneriella subcapitata* and cyanobacterium *Synechococcus leopoliensis*. At low effect concentrations, the effect was in *P. subcapitata* clearly synergistic, while in *S. leopoliensis* it was close to additive, and *P. subcapitata* was more sensitive than *S. leopoliensis*. In addition, a previous study of binary mixtures of the anticancer drugs revealed that algae were more sensitive than cyanobacteria, and that these mixtures can have compound-specific and species-specific synergistic or antagonistic effects (Brezovšek et al., 2014). These data provide additional confirmation that single compound toxicity data are not sufficient for predicting the aquatic toxicity of anticancer drug mixtures.

(iii) Developing guidance on improving the environmental and human risk assessment of cytostatics released into the environment

In the paper by Kümmerer et al. (2016), the authors question whether or not risks associated with the presence of antineoplastic drugs are underestimated based on a predicted environmental concentration trigger value of 0.01 μg L^{-1} stated in the EMA and 1 μg L^{-1} in the FDA guidelines. The authors identified 102 active antineoplastic agents, which are environmentally relevant. Based on consumption analysis, they calculated that the share of drugs with DNA-damaging properties increased during the period 2006 to 2012 from 24 to 67 %. As for the compounds that interact with DNA directly, no safe action limit can be assumed and the authors propose that DNA-damaging drugs are exempt from the action limit set by the EMA and FDA guidelines for performing an environmental risk assessment and recommend a case-by-case evaluation of the risk associated with their presence in the environment.

The publications in this Special Issue of Environmental Science and Pollution Research represent an important contribution to our limited knowledge and understanding of the risk posed by anticancer drug residues in the environment. They cover the development of sensitive analytical methods for characterizing cytostatic drug residues that will contribute towards understanding their fate in the aquatic environment. They also provide valuable information concerning the ecotoxicity and genotoxicity of selected anticancer drugs and reveal that their residues, although they occur at very low concentrations, represent a possible threat to the aquatic environment. These publications begin to fill in the many knowledge gaps that exist and will contribute towards a more reliable environmental and human health risk assessment. In the future, for the exposure assessment, research should focus on systematic environmental monitoring to obtain data on the distribution of anticancer drug residues including metabolites and transformation products, whereas for hazard assessment, targeted ecotoxicological studies of existing and new anticancer drugs and their mixtures are necessary.

Acknowledgments The guest editors sincerely thank Prof. Walter Giger, a Scientist Emeritus at Eawag, the Swiss Federal Institute of Aquatic Science and Technology, and a Professor Emeritus at ETH Zurich for his support during the CytoThreat project and in the preparation of this Special Issue. We also acknowledge the support and help by the ESPR Editor-in-Chief Prof. Philippe Garrigues and Ms. Géraldine Billerot for their support.

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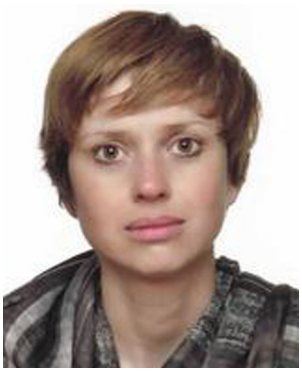

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