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Toxicities of four anti-neoplastic drugs and their binary mixtures tested on the green alga *Pseudokirchneriella subcapitata* and the cyanobacterium *Synechococcus leopoliensis*

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ABSTRACT

The residues of anti-neoplastic drugs are new and emerging pollutants in aquatic environments. This is not only because of their increasing use, but also because due to their mechanisms of action, they belong to a group of particularly dangerous compounds. However, information on their ecotoxicological properties is very limited. We tested the toxicities of four anti-neoplastic drugs with different mechanisms of action (5-fluorouracil [5-FU], cisplatin [CDDP], etoposide [ET], and imatinib mesylate [IM]), and some of their binary mixtures, against two phytoplankton species: the alga *Pseudokirchneriella subcapitata*, and the cyanobacterium *Synechococcus leopoliensis*. These four drugs showed different toxic potential, and the two species examined also showed differences in their susceptibilities towards the tested drugs and their mixtures. With *P. subcapitata*, the most toxic of these drugs was 5-FU (EC_{50}, 0.13 mg/L), followed by CDDP (EC_{50}, 1.52 mg/L), IM (EC_{50}, 2.29 mg/L), and the least toxic, ET (EC_{50}, 30.43 mg/L). With *S. leopoliensis*, the most toxic was CDDP (EC_{50}, 0.67 mg/L), followed by 5-FU (EC_{50}, 1.20 mg/L) and IM (EC_{50}, 5.36 mg/L), while ET was not toxic up to 351 mg/L. The toxicities of the binary mixtures tested (5-FU + CDDP, 5-FU + IM, CDDP + ET) were predicted by the concepts of 'concentration addition' and 'independent action', and are compared to the experimentally determined toxicities. The measured toxicity of 5-FU + CDDP with *P. subcapitata* and *S. leopoliensis* was higher than that predicted, while the measured toxicity of CDDP + ET with both species was lower than that predicted. The measured toxicity of 5-FU + IM with *P. subcapitata* was higher, and with *S. leopoliensis* was lower, than that predicted. These data show that these mixtures can have compound-specific and species-specific synergistic or antagonistic effects, and they suggest that single compound toxicity data are not sufficient for the prediction of the aquatic toxicities of such anticancer drug mixtures.

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1. Introduction

During recent years, scientific and public concern regarding the occurrence of residues of pharmaceuticals in the environment has been increasing, as their presence has been demonstrated across a wide variety of hydrological, climatic and land-use settings, where they have been consistently detected (Hughes et al. 2013; Rodriguez-Mozaz and Weinberg, 2010; McClellan and Halden, 2010; Hernando et al., 2006; Kümmerer, 2001; Fent et al., 2006; Miège et al., 2009). Pharmaceuticals can be excreted through feces and urine as mixtures of metabolites and the unchanged parent compounds. Predominantly, these then enter the aquatic environment via the effluent from hospital and municipal wastewater treatment plants, landfill leakages, and to a minor extent, in the discharge from the pharmaceutical industry. Due to their ubiquitous presence in the environment that arises from their continual input into the aquatic compartment, they are considered as 'pseudo'-persistent pollutants (Hernando et al., 2006). The concentrations of the residues of pharmaceuticals in the environment are relatively low compared to other pollutants, which has led to the belief that these compounds have no environmental impact on living organisms.

At the same time, ecotoxicological data are often based merely on acute toxicity determinations, and therefore they use high concentrations that are far from the actual concentrations in the environment. However, based on their therapeutic functions and mechanisms of action, certain groups of pharmaceuticals are believed to represent a risk for non-target organisms, even at concentrations of a few nanograms per liter, and particularly under conditions of chronic exposure (Fent et al., 2006).

One such group of pharmaceuticals is the anti-neoplastic (cytostatic or cancer chemotherapeutic) drugs that are used for the treatment of cancers. Their main purpose is to prevent uncontrolled proliferation of cancer cells, and thus they act by inhibition of cell growth or by killing cancer cells via their interactions with DNA function and cell signaling (Mahnik et al., 2004). According to their mechanisms of action, anti-neoplastic drugs can be classified as alkylating agents, antimetabolites, platinum complexes, intercalating agents, cytotoxic antibiotics, mitotic spindle inhibitors, topoisomerase inhibitors, protein kinase inhibitors, and monoclonal antibodies (Besse et al., 2012). However, these agents attack not only tumor cells, but also normal growing cells and tissues, which is the cause of their side effects during chemotherapy. Due to their mechanisms of action, many anti-neoplastic drugs are classified as mutagenic, carcinogenic, teratogenic and/or toxic to reproductive systems, and it can be assumed that they can elicit these effects also in exposed, non-target, aquatic organisms (Kümmerer et al., 2000; Lenz et al., 2007).

Compared to a number of other pharmaceuticals anti-neoplastic drugs are consumed in much lower quantities but, recent studies have indicated that their consumption is increasing, and thus the probability of the occurrence of their residues in the environment is also increasing (Yin et al., 2010; Besse et al., 2012). Data on the occurrence of these drugs in the environment remain relatively limited, although the presence of several of them has been shown in hospital discharges, in wastewater treatment plant influents and effluents, and even in surface waters (Kosjek and Heath, 2011; Kosjek et al., 2013). Studies have also shown that most of these drugs are polar and persistent, which gives them high aquatic mobility and promotes their dissipation in surface waters (reviewed in Kosjek and Heath, 2011).

Data on the potentially toxic effects of such anti-neoplastic drugs towards aquatic organisms are even scarcer than these environmental occurrence data. The data available are mainly acute ecotoxicity data; however, such data do not allow for the prediction of any adverse effects of life-cycle exposure to these compounds in aquatic organisms. In addition, the residues of pharmaceuticals in the environment can occur as complex mixtures, and therefore even though the concentrations of the individual compounds might be low, their effects in mixtures might be of ecotoxicological significance (Brain et al., 2004). Thus the question is: what are the effects of mixtures of anti-neoplastic drugs on aquatic organisms?

The relevance of this question is derived also from the fact that in many cancer treatment regimens, combinations of anti-neoplastic drugs are used to achieve better cancer-treatment effects (Ocvirk, 2009). This increased efficiency of combinations of anti-neoplastic drugs for the killing of the target tumor cells can therefore be defined as a potentially higher threat in the environment for non-target organisms than that posed by the individual compounds.

The aim of this study was thus to evaluate the toxicities of four anti-neoplastic drugs and some of their binary mixtures for the green alga *Pseudokirchneriella subcapitata* and the cyanobacterium *Synechococcus leopoliensis*. The compounds studied are 5-fluorouracil (5-FU), cisplatin (or *cis*-diamminedichloroplatinum; CDDP), etoposide (ET) and imatinib mesylate (IM). According to their mechanisms of action, these compounds represent different classes of anticancer drugs.

5-Fluorouracil is a pyrimidine analog that belongs to the group of antimetabolites. It exerts its anticancer effects through a block of DNA synthesis and replication, by inhibition of thymidylate synthase and incorporation of its metabolites into DNA and RNA (Longley et al., 2003). Such antimetabolites that are mainly represented by 5-FU and its prodrug capecitabin are among the most consumed anticancer drugs in developed countries (Besse et al., 2012).

Cisplatin belongs to the group of platinum complexes. It forms DNA and protein adducts and crosslinks that block DNA transcription and replication, which leads to cell death (Gonzalez et al., 2001).

Etoposide is a topoisomerase inhibitor that inhibits topoisomerase II and causes an increase in DNA and chromosomal breakage and cell death (Pommier, 2013). As a cancer treatment, ET is most often used in combination with other anti-neoplastic drugs, including 5-FU and CDDP (Valkov and Sullivan, 2003).

Imatinib mesylate was the first of the protein kinase inhibitors, which are anti-neoplastic drugs that were developed for targeted chemotherapy. It selectively inhibits the BCR-ABL tyrosine kinase, and has become the therapy of choice for Philadelphia-chromosome-positive leukemia (Moen et al., 2007). Imatinib mesylate also inhibits some other tyrosine kinase activities (e.g., c-KIT, the PDGF receptor), which indicates its potential use for treatment of other cancers