Chemosphere 189 (2017) 689–698

Contents lists available at ScienceDirect

Chemosphere

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

Acute aquatic toxicity assessment of six anti-cancer drugs and one metabolite using biotest battery – Biological effects and stability under test conditions

Anna Białk-Bielińska [a, *], Ewa Mulkiewicz [a], Marcin Stokowski [a, b], Stefan Stolte [a, c], Piotr Stepnowski [a]

[a] Department of Environmental Analysis, Faculty of Chemistry, University of Gdańsk, ul. Wita Stwosza 63, 80-308 Gdańsk, Poland
[b] Institute of Oceanology, Polish Academy of Sciences, ul. Powstańców Warszawy 55, 81-712 Sopot, Poland
[c] UFT - Center for Environmental Research and Sustainable Technology, Faculty 4, University of Bremen, Leobener Straße, D-28359 Bremen, Germany

HIGHLIGHTS

- New ecotoxicity data for six anticancer drugs and one metabolite were obtained.
- EC_{50} values were at μg L^{-1} level – comparable to environmental concentrations.
- The most sensitive organism was *L. minor*.
- Metabolite/potential degradation products toxicities were preliminarily assessed.
- 5-FU proved to be the most toxic to *R. subcapitata*, while MET and TAM to *L. minor*.

ARTICLE INFO

Article history:
Received 20 June 2017
Received in revised form
29 August 2017
Accepted 30 August 2017
Available online 21 September 2017

Handling Editor: Jim Lazorchak

Keywords:
Aquatic organisms
Cytostatic drugs
Ecotoxicity
Risk assessment
Stability

ABSTRACT

Available ecotoxicological data for anti-cancer drugs and their metabolites are incomplete, and only some studies have been accompanied by chemical analysis. Therefore, the main aim of this study was to evaluate the acute toxicity of the six most commonly used cytostatics, namely cyclophosphamide (CF), ifosfamide (IF), 5-fluorouracil (5-FU), imatinib (IMT), tamoxifen (TAM) and methotrexate (MET) and its metabolite — 7-hydroxymethotrexate (7-OH-MET), towards selected aquatic organisms, namely bacteria *Vibrio fischeri*, algae *Raphidocelis subcapitata*, crustaceans *Daphnia magna* and duckweed *Lemna minor*. All ecotoxicological tests were accompanied by chemical analysis to determine the differences between nominal and actual concentrations of investigated compounds and their stability under test conditions. For unstable compounds, tests were performed in static and semi-static conditions. It was observed that *L. minor* was the most sensitive organism. The compounds that were most toxic to aquatic organisms were 5-FU (highly toxic to algae, EC_{50} = 0.075 mg L^{-1}), MET and TAM (very toxic to highly toxic to duckweed depending on the test conditions; EC_{50MET} 0.08–0.16 mg L^{-1}, EC_{50TAM} 0.18–0.23 mg L^{-1}). It is suspected that MET and 5-FU mainly affected algae and plants most probably because the exposure time was long enough for them to cause a specific effect (they inhibit DNA replication and act predominantly on actively dividing cells). Furthermore, the obtained results also suggest that the toxicity of the metabolites/potentially produced degradation products of MET towards duckweed is lower than that of the parent form, whereas the toxicity of TAM degradation products is in the same range as that of TAM.

1. Introduction

Pharmaceutical residues in the environment have become a focus of great concern in recent years. Considerable attention has been paid to popular "over the counter" and prescription medicines and veterinary drugs. However, anti-cancer drugs (anti-neoplastic or cytostatic agents) used in chemotherapy have received less attention, even though they have high pharmacological potency. As they have been designed to disrupt or prevent cellular proliferation,

* Corresponding author.
E-mail address: a.bialk-bielinska@ug.edu.pl (A. Białk-Bielińska).

https://doi.org/10.1016/j.chemosphere.2017.08.174
0045-6535/

usually by interfering with DNA synthesis (Brooker et al., 2014; Fernando-Climent et al., 2014), they have been shown to have potent cytotoxic, genotoxic, mutagenic, carcinogenic, endocrine-disrupting and/or teratogenic effects in several organisms (Česen et al., 2016; Gajski et al., 2016; Kovács et al., 2015; Kundi et al., 2016; Mišík et al., 2016; Novak et al., 2016; Parrella et al., 2014a; Zounkova et al., 2010, 2007). Therefore, there is a grave concern that cytostatic drugs, which interfere with the structure and functions of DNA, will affect not only tumour cells but also normal cells; hence, all living organisms might be susceptible to their toxicity (Besse et al., 2012; Parrella et al., 2014a).

As anti-cancer drugs and their metabolites are released from hospital and domestic wastewater and have been shown to be recalcitrant in wastewater, they are introduced continuously into the aquatic environment. Because of their high polarity and resistance to degradation (Besse et al., 2012; Brooker et al., 2014), they have already been detected in different compartments (including wastewater, surface water and drinking water) at concentration levels ranging from ng L^{-1} to μg L^{-1} (Besse et al., 2012; Brooker et al., 2014; Ferrando-Climent et al., 2014; Martin et al., 2011; Negreira et al., 2014, 2013; Rabii et al., 2014; Xie, 2012). Although the concentrations of anti-cancer drugs in the environment are lower than those of other general classes of pharmaceuticals, every living organism may potentially be affected by their peculiar molecular mode of action and because they are presumed to cause effects even when present at very low concentrations (Parrella et al., 2014b). Furthermore, a major concern is the excretion of metabolites of cytostatic drugs, which can also reach the aquatic environment and cause toxic effects (as was already proved for tamoxifen and its metabolites (Borgatta et al., 2015; Negreira et al., 2014) and recently for the metabolites/transformation products of cyclophosphamide and ifosfamide (Česen et al., 2016)).

Because the number of patients receiving chemotherapy has been considerably increasing in the last few decades and the incidence of cancer is predicted to increase in the forthcoming years, the demand for cytostatic drugs is also increasing (Negreira et al., 2014). Therefore, the presence of these compounds in the aquatic environment, and their potential consequences for both humans and wildlife, are receiving increasing attention. However, studies on the ecotoxicological effects of these compounds and associated risks due to their presence in the aquatic environment remain limited. Although, according to the existing requirements for performing the Environmental Risk Assessment (ERA) of human pharmaceuticals, chronic toxicity data are recognised as the most desired one, the basic knowledge about pharmaceuticals' acute toxicity is crucial (CHMP, 2006; Crane et al., 2006; Schmitt et al., 2010; Tarazona et al., 2010).

Therefore, the main aim of this study was to evaluate the acute toxicity of the six most commonly used chemotherapeutic agents (consumption data are presented in Table 1A, Appendix) (Besse et al., 2012; Brooker et al., 2014; Martin et al., 2011)—anti-cancer drugs differing in their physicochemical properties and mode of action (Table 1): cyclophosphamide (CF), ifosfamide (IF), 5-fluorouracil (5-FU), imatinib (IMT), tamoxifen (TAM) and methotrexate (MET) and its metabolite (7-hydroxymethotrexate (7-OH-MET)), towards selected aquatic organisms representing different levels of biological organisation, namely the marine bacteria *Vibrio fischeri*, green algae *Raphidocelis subcapitata* (formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*), crustaceans *Daphnia magna* and duckweed *Lemna minor*. Available ecotoxicological data for these pharmaceuticals are incomplete, and only some studies have been accompanied by chemical analysis (Table 2); therefore, valid ecotoxicological data are required for a sound environmental risk assessment of these compounds. To determine differences between nominal and actual concentrations of barely water-soluble compounds and the stability of invesigated compounds under test conditions, all ecotoxicological tests were accompanied by chemical analysis. An assessment of the stability and biological effects will support the environmental risk assessment of anti-cancer drugs.

2. Materials and methods

2.1. Chemicals

CF, IF, 5-FU, TAM and MET were purchased from Sigma–Aldrich (Germany), IMT and 7-OH-MET were supplied by Santa Cruz Biotechnology Inc. (Germany) and Biozol (Germany), respectively. Acetonitrile and methanol (MeOH), both HPLC grade, were supplied by POCH (Poland). Salts used for preparing culture media and dimethyl sulfoxide (DMSO) were supplied by Sigma–Aldrich.

2.2. Standard stock solutions

Stock solutions of the compounds were freshly prepared on the day of each test in the test medium. To improve the low water solubility of TAM, IMT, MET (Table 1) and 7-OH-MET, organic solvents (DMSO for IMT, MET and 7-OH-MET; MeOH for TAM) were added at the highest final concentrations, as given in Table 3.

2.3. Ecotoxicological tests

The ecotoxicological studies were conducted in accordance with the internationally accepted guidelines. Selected details and conditions of the performed tests are presented in Table 3. Each test was repeated three times for each substance with at least two parallel replicates, and a minimum of four dilutions in each range-finding test and six dilutions in each main test were applied. The quality of each test was verified by testing the reference substances.

The highest tested concentration of each analyte was either limited by the compound's water solubility or was set at 100 mg L^{-1} for well water-soluble compounds because, according to the classification of EC-Directive 93/67/EEC (1996), compounds with EC_{50} higher than 100 mg L^{-1} are not considered harmful for aquatic organisms.

Furthermore, because of the lower water solubility of TAM, MET, 7-OH-MET and IMT, the addition of an organic solvent was necessary to perform the ecotoxicological test. Solvent controls containing the highest concentration of the organic solvent applied in each test were used. These experiments showed no significant differences in the response of the tested organisms in the case of solvent controls compared to standard (water) controls.

All ecotoxicological tests were accompanied by chemical analysis (detailed description is provided in Section 2.4) to enable the observed effects to be linked to the concentration and nature of the studied compounds. If the obtained analytical results proved that a specific analyte was not stable (degradation higher than 20%, as recommended by the OECD 221 (2006)) during the test with *L. minor*, additional ecotoxicological tests were performed under semi-static conditions in two manners: (*i*) with a solution exchange at days 3 and 5 and (*ii*) with a daily solution exchange. Only the test with *L. minor* under semi-static conditions was performed as it is very easy to realise in contrast to other ecotoxicological tests (e.g. test with algae).

Dose–response curve parameters and plots were obtained using the drfit package (version 0.05–95) for the R language and environment for statistical computing (www.r-project.org) (R Development Core Team, 2005).