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Tamoxifen ecotoxicity and resulting risks for aquatic ecosystems

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HIGHLIGHTS

- First ecotoxicity tests of tamoxifen on algae.
- A PNEC of tamoxifen for continental aquatic ecosystems of 81 ng L^{-1}.
- Risk quotients of tamoxifen calculate for four situations.
- Tamoxifen could present a significant environmental risk for aquatic ecosystems.

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ABSTRACT

Tamoxifen, a drug used to treat cancer, is regularly found in hydrosystems at concentrations of several hundred ng L^{-1}. To characterize its ecotoxicity, we implemented a battery of bioassays on organisms belonging to 3 different trophic levels: *Pseudokirchneriella subcapitata*, *Chlorella vulgaris* and *Chlamydomonas reinhardtii*, for primary producers, *Daphnia magna* (immobilization, grazing and reproduction) for primary consumers, and *Danio rerio* for secondary consumers (embryotoxicity test). In view of the results obtained and the ecotoxicity values of tamoxifen available in the literature, we established a PNEC (Predictive No Effect Concentration) equal to 81 ng L^{-1} for continental water. This PNEC allowed us to calculate Risk Quotients (RQ) for 4 continental hydrosystems in 4 different countries in which measures of tamoxifen had already been performed on surface waters. In two of the situations studied, RQs were higher than 1, reaching a maximum of 2.6. These results show the need to deepen the characterization of ecotoxicological risks linked to the discharge of tamoxifen in surface waters. In addition, we propose applying this approach to other drug residues detected in the environment.

1. Introduction

Drugs and their residues are now recognized as ubiquitous in ecosystems: the hydrosphere (surface water (Heath et al., 2010), deep water (Loos et al., 2010), drinking water (Gibs et al., 2007)), the geosphere (Silva et al., 2011; Yang et al., 2011) and the biosphere (via bioconcentration, bioaccumulation and bioamplification phenomena) (Lajeunesse et al., 2011; Zenker et al., 2014). The main sources of these generalized contaminations are: (i) urban effluents (domestic and hospital) (Kümmerer, 2009; Verlicchi et al., 2010) treated by sewage plants (WWTP) that are often poorly adapted to ensure the total degradation of these compounds ((Ternes, 1998); (Heberer, 2002); (Joss et al., 2005)); (ii) pharmaceutical plants (Larsson et al., 2007; Collado et al., 2014); and (iii) domestic livestock breeding and aquaculture (Halling-Sørensen et al., 1998; Lalumera et al., 2004).

Among the many drugs emitted into the environment, our attention was drawn to tamoxifen. It is an anti-estrogenic compound used to treat hormone-dependent breast cancer. This compound has been detected in hospital (Langford and Thomas, 2009) and urban effluents (Zhou et al., 2009) at concentrations reaching 740 ng L^{-1}, as well as in surface waters at concentrations reaching 212 ng L^{-1} (Zhang et al., 2013). This compound has also been found in the sediment compartment (212–431 ng g^{-1}; (Yang et al., 2011)). However, only few ecotoxicity data are available concerning tamoxifen (see Table 4).

So, in the first part of this work, tamoxifen toxicity was characterized for organisms representing different trophic levels of aquatic ecosystems (primary producers, primary consumer and secondary consumer): (i) three different algae species (growth inhibition tests); (ii) A microcrustean (acute, sub-chronic

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and chronic ecotoxicity tests); (iii) A fish species (embryotoxicity test).

Then, the PNEC (Predictive No Effect Concentration) of tamoxifen was calculated for continental hydrosystems on the basis of the toxicity results obtained in this study and the data available in the literature.

Lastly, the evaluation of ecotoxicological risks related to the presence of tamoxifen in the environment was then established by comparing the PNEC established with Measured Environmental Concentrations (MEC) in different situations described in the literature (Roberts and Thomas, 2006; Coetsier et al., 2009; Yang et al., 2011; López-Serna et al., 2012), which allowed formulating a Risk Quotient (RQ) for each situation studied.

2. Materials and methods

2.1. Algal cultures

Pseudokirchneriella subcapitata (Ps), *Chlorella vulgaris* (Cv) and *Chlamydomonas reinhardtii* (Cr), 3 green algae (*Chlorophyceae*), were cultivated under 3000 lux (16 h d^{-1}) at 20 ± 1 °C in oligo L.C. medium (AFNOR, 1980) (for Ps and Cv) and in TAP (TRIS acetic acid phosphate) medium (for Cr) (Gorman and Levine, 1965). Media and flasks were autoclaved (120 °C, 1 bar, 20 min) before inoculation.

2.2. Rearing of daphnids

Daphnids (*Daphnia magna*) were reared in M4 medium (Elendt and Bias, 1990). Thirty individuals were kept in 2 L glass flasks at 20 ± 1 °C under 500 lux (16 h d^{-1}); they were fed with a solution of *P. subcapitata* (10^6–10^7 cells/daphnid) added daily to the culture flasks. Neonates were collected daily and used in tests or discarded. Half of the medium was renewed once a week. Mother daphnids were discarded after 1 month and new cultures were initiated with neonates obtained by parthenogenesis.

2.3. Chemicals

Tamoxifen (CAS Number 10540-29-1) and all the chemicals used in this study were purchased from Sigma–Aldrich Chemical (ST. QUENTIN FALLAVIER, France). A stock solution of tamoxifen was prepared in DMSO (50 mM or 18.575 g L^{-1}) and kept at −20 °C. For each exposure, test solutions were freshly prepared by proportional successive dilutions of an aliquot of the stock solutions so that DMSO concentration was always less than 0.01% (v/v) in the tested solutions.

2.4. Algal growth tests

The growth tests performed on the 3 algae were selected according to OECD guideline N° 201 (OECD, 2006). The algae were seeded on 48 well microplates at a concentration of 10^4 cells per mL (Cv, Ps) or 10^3 cells per mL (Cr) in their specific culture medium in contact with different concentrations of tamoxifen after 3 d preconditioning under the test conditions, i.e. in a climatic chamber (Aralab Fitoclima D 1200), 20 ± 1 °C under continuous lighting of 10000 lux. The range of tamoxifen concentrations tested was as follows: 0, 10, 50, 100, 200, 300, 400, 500, 600, 800 and 1600 µg L^{-1}. Three replicates were prepared for each concentration. In parallel, a control with the highest concentration of DMSO introduced in the wells (0.01%) was prepared to ensure the absence of effect of the solvent on algal growth. To limit evaporation in the wells during the experiment, susceptible to modify the tamoxifen concentration, ultrapure water was placed in the outer wells of the plate. Seventy-two hours after inoculation the content of the wells was homogenized and algal density was measured using a Malassez cell counter (2 counts per well) and a Zeiss microscope, magnification ×400. Inhibition concentrations 50% (IC50) were then calculated using the RegTox software.

2.5. Daphnid toxicity tests

2.5.1. Acute toxicity: immobilization test

The daphnid immobilization test was carried out according to the ISO standard protocol (ISO, 1995). Five neonate daphnids (<24 h) were transferred into a glass test tube containing 10 ml of a tamoxifen solution freshly prepared in the rearing medium. The concentration range tested was: 0, 100, 200, 400, 800, 1600, 3200, 6400, and 12800 µg L^{-1}. Four replicate tubes were tested per concentration (20 daphnids). In order to prevent algal growth, tubes were incubated in the dark during the exposure period. After 24 h and 48 h of exposure, the number of immobilized daphnids in each tube was recorded and 48 h-EC50 was calculated using a probit model.

2.5.2. Subchronic toxicity: grazing activity

Daphnia grazing activity was measured under static conditions (no algal growth) over a period of 48 h, according to Clément and Zaid (2004). The experiment was carried out in 250 ml glass beakers containing 150 ml of rearing medium, five daphnids (aged between 3 and 4 d at the start of test) and 500000 *P. subcapitata* cells/ml. Fives replicates per treatment were tested with the following tamoxifen concentration range: 0, 5, 25, 50, 100 and 150 µg L^{-1}. For each concentration tested, two beakers without daphnids were used as controls to evaluate possible algal growth. All the beakers were placed in the dark at 20 ± 1 °C and were gently shaken three times a day to ensure mixing of the algal suspensions in order to improve cell availability for daphnids. Residual algal densities were measured after 24 and 48 h using a particle counter (Coultronics, model Z1, threshold size 3.6 µm). Daphnid grazing activity was evaluated by calculating the filtration rate (F, in ml/daphnid/h) according to Gauld (Gauld, 1951):

$$F = (V/n)[\ln(C_0) - \ln(\mathrm{Ct})/t - A]$$

$$A = [\ln(C_0) - \ln(C'_t)]/t$$

where V (ml) is the total volume of medium, n the number of daphnids in volume V, C_0 and Ct are initial and final algal densities (cells/mL) and t the exposure duration time (duration of experiment in hours). A is a correction factor used to take into account changes in the algae density controls (algae without daphnids) expressed as the final algal density C'_t.

No mortality was observed during the experiments, whatever the tamoxifen concentration.

2.5.3. Chronic toxicity: reproduction test

To test the chronic toxicity of tamoxifen on the reproduction of *D. magna*, we referred to guideline 211 of the OECD (OECD, 1998). The daphnids aged less than 24 h were placed for 21 d in 100 mL beakers containing 80 mL of rearing medium (1 daphnid per beaker and 10 beakers per concentration) contaminated as a function of the following range of tamoxifen concentrations: 0, 5, 15, 30, 60 and 120 µg L^{-1}. The daphnids were fed once a week with an algal suspension of *P. subcapitata* so that algal density was in the region of 10^6 cells mL^{-1}. The medium was renewed twice a week. The number of young daphnids produced was counted daily.

2.6. Fish embryo test

The fish embryo test used here as an alternative to the 96 h fish acute toxicity assay was carried out according to OECD guidelines,