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Environmental risk assessment of triclosan and ibuprofen in marine sediments using individual and sub-individual endpoints☆

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ABSTRACT

The guidelines for the Environmental Risk Assessment (ERA) of pharmaceuticals and personal care products (PPCP) recommend the use of standard ecotoxicity assays and the assessment of endpoints at the individual level to evaluate potential effects of PPCP on biota. However, effects at the sub-individual level can also affect the ecological fitness of marine organisms chronically exposed to PPCP. The aim of the current study was to evaluate the environmental risk of two PPCP in marine sediments: triclosan (TCS) and ibuprofen (IBU), using sub-individual and developmental endpoints. The environmental levels of TCS and IBU were quantified in marine sediments from the vicinities of the Santos submarine sewage outfall (Santos Bay, São Paulo, Brazil) at 15.14 and 49.0 ng g^{-1}, respectively. A battery (n = 3) of chronic bioassays (embryo-larval development) with a sea urchin (*Lytechinus variegatus*) and a bivalve (*Perna perna*) were performed using two exposure conditions: sediment-water interface and elutriates. Moreover, physiological stress through the Neutral Red Retention Time Assay (NRRT) was assessed in the estuarine bivalve *Mytella charruana* exposed to TCS and IBU spiked sediments. These compounds affected the development of *L. variegatus* and *P. perna* (75 ng g^{-1} for TCS and 15 ng g^{-1} for IBU), and caused a significant decrease in *M. charruana* lysosomal membrane stability at environmentally relevant concentrations (0.08 ng g^{-1} for TCS and 0.15 ng g^{-1} for IBU). Chemical and ecotoxicological data were integrated and the risk quotient estimated for TCS and IBU were higher than 1.0, indicating a high environmental risk of these compounds in sediments. These are the first data of sediment risk assessment of pharmaceuticals and personal care products of Latin America. In addition, the results suggest that the ERA based only on individual-level and standard toxicity tests may overlook other biological effects that can affect the health of marine organisms exposed to PPCP.

1. Introduction

Until recently pharmaceuticals and personal care products (PPCP) were not included in environmental monitoring programs mainly because of their low environmental concentrations and the absence of analytical methodologies to detect them. The concern about the environmental contamination by PPCP began to be part of the agenda of governments after the publication of studies showing fish feminization due to exposure to estrogenic substances (Harries et al., 1997; Jobling et al., 1998; Hinck et al., 2009) and the massive death of vultures caused by the ingestion of diclofenac (Green et al., 2004). These studies were important to trigger concern on the environmental risks of PPCP, which include antimicrobial, anti-inflammatory, contraceptives drugs, antidepressants and antiepileptic (USEPA, 2015). The preoccupation has involved especially - although not exclusively - the aquatic biota, since the water bodies are the final destination of many of these substances (Arnold et al., 2014).

The knowledge about the effects of PPCP on freshwater organisms has evolved significantly (e.g. Fent et al., 2006; Arnold et al., 2014), but there are few empirical data about the ecotoxicity of such compounds to marine organisms nowadays (Gaw et al., 2014).

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This information gap is especially important regarding contaminated sediments and marine or estuarine benthic biota (Brausch and Rand, 2011). Previous studies focusing on marine sediments showed that carbamazepine, ibuprofen, fluoxetine, 17α-ethynylestradiol and propranolol inhibit *Vibrio fischeri* bioluminescence at concentrations ranging from 36.1 to 163.9 ng g^{-1}, and affect the embryo-larval development of sea urchin and the growth rate of marine algae (Maranho et al., 2014, 2015a). Maranho et al. (2015b) also observed lethal and sublethal effects (alterations in cellular energy status, metabolism of monoamines, and inflammation properties) in polychaetes exposed to environmental concentrations of human pharmaceuticals in marine spiked sediments.

Currently, triclosan (TCS) and ibuprofen (IBU) belong to classes of emerging compounds of greatest concern to environmental protection agencies, such as the USEPA (2015) and Environment Canada (2011). Triclosan and ibuprofen have been commonly found in environmental matrices such as surface waters and sediments in concentrations ranging from pg L^{-1} to μg L^{-1} and pg g^{-1} to μg g^{-1}, respectively (Kolpin et al., 2002; Lindström et al., 2002; Agüera et al., 2003; Weigel et al., 2004; Xie et al., 2008; Zhao et al., 2010; Pintado-Herrera et al., 2013).

Given the considerable lack of information about the effects of human PPCP in sediments on marine organisms and considering that standard toxicity tests may not be sensitive enough to see the effects of human PPCP in aquatic biota (Aguirre-Martínez et al., 2015), it is important not only to increase the availability of ecotoxicological data but also to adopt new approaches to assess the environmental risks associated with PPCP in coastal areas (Fabbri and Franzellitti, 2016).

The procedure to conduct an environmental risk assessment (ERA) of PPCP within the regulatory scope by the European Medicine Agency (EMEA, 2006) is based on different types of evaluation. The first level of evaluation demands (i) the estimate of the biota exposure to the studied substance, either by direct measurements from environmental samples (Measured Environmental Concentrations - MEC), or indirectly through a prediction of its environmental concentrations (Predicted Environmental Concentrations - PEC). If environmental risks are expected, then the ERA framework leads to the second level of evaluation: (ii) identification of the final destination of the substance (based on its physical-chemical characteristics) and its ecotoxicological effects. From these data, the Predicted No Effect Concentrations (PNEC) can be estimated and at last (iii) the risk quotient (RQ) is calculated from the ratio between PEC (or MEC) and PNEC. If RQ < 1, further evaluations are not required; if RQ > 1, more refined evaluations are needed, including the evaluation of more sensitive endpoints and the performance of sediment bioassays.

Consequently, the evaluation of the potential effects of PPCP to aquatic organisms plays an important role in the ERA. The use of standardized ecotoxicological assays (e.g. OECD Guidelines for Testing Chemicals) is the most common ecotoxicological approach employed in the scope of the ERA in relation to PPCP (Hernando et al., 2006). Although such assays bring relevant information, they are unable to show a more realistic view of environmental risks of PPCP since the biological responses are quantified only at the individual level. The inclusion of chronic or sub-chronic endpoints, as well as sensitive responses at sub-individual levels are important to evaluate the risks of PPCP, since the most common environmental scenario is a continuous exposure to low concentrations in the marine environment. In addition, the evaluation of effects at lower levels of biological organization (i.e. sub-individual) can predict effects at higher levels (e.g. mortality, population decline, community structure) and may generate information about the mechanisms of action of PPCP in non-target organisms (Villalaín et al., 2001; Martin-Diaz et al., 2009; Pereira et al., 2014).

The current study evaluated the environmental risk of two widely used pharmaceutical substances (TCS and IBU) in three marine invertebrates used in sediment assessments (the mussel *Perna perna* and the sea urchin *Lytechinus variegatus*) including a new alternative sediment sentinel species, the mussel *Mytella charruana*. The biota exposure was firstly estimated by measuring environmental concentration of these substances in sediments from the vicinity of the sewage outfall of Santos Bay, Southeast Brazil. Then the concentration effect of TCS and IBU was established based on chronic and sub-individual endpoints measured in the organisms exposed to spiked sediments. At last, following the environmental risk assessment of EMEA (2006), it was estimated the RQ for each of the substances studied. This study is the first environmental risk assessment for PPCP in marine sediments from an area of the Latin America coast and will contribute to the knowledge of environmental risks associated with these substances in marine tropical ecosystems.

2. Materials and methods

2.1. Chemicals

The bactericidal triclosan (CAS number: 3380-34-5) has a molecular weight of 289.5 g mol^{-1}, water solubility of 10 mg L^{-1}, pKa value of 7.9 and log Kow 4.76 with half live of 40 days (Huang et al., 2015; TOXNET, 2016). The anti-inflammatory ibuprofen (CAS number: 15687-27-1) has a molecular weight of 206.28 g mol^{-1}, water solubility of 21 mg L^{-1}, pKa value of 4.91 and log Kow 3.97 with half live of 19 days (Conkle et al., 2012; TOXNET, 2016). All reagents used in the current study were purchased from Sigma-Aldrich® (purity was ≥98%).

2.2. Exposure assessment

The exposure assessment of TCS and IBU was performed by quantifying the concentration of these compounds in sediments (i.e. MEC estimative) from the vicinity of the sewage outfall of Santos Bay at the coast of São Paulo, Southeastern Brazil (Fig. 1).

2.2.1. Study area and sediment sampling

The Santos and São Vicente estuarine system is located in the coast of the state of São Paulo, Southeastern Brazil. This area comprises a dense urbanization area, the biggest industrial complex located in the Brazilian coastal zone, and also the major Latin American port.

Five sampling sites within the surroundings of the sewage outfall of Santos Bay were established for sediment collection (Fig. 1) to determine the environmental levels of TCS and IBU. These sites were established based on the same criteria adopted by Environmental Agency of São Paulo (CETESB, 2007) to monitor the quality of surface water from the vicinity of the sewage outfall of Santos Bay. The sediment used in the ecotoxicological assays was collected in a reference area (*Toque Toque Grande* beach, in the coast of *São Sebastião*, São Paulo, SE Brazil), located approximately 80 km away from relevant contamination sources (Fig. 1). The Environmental Agency of São Paulo monitored this area weekly in 2014, indicating optimal quality throughout the year (CETESB, 2014).

The sediments were collected in February 2015 using a *van Veen* grab sampler and maintained in plastic bags in a cooler box with ice during the transport to the laboratory, and then kept in the dark at the temperature of 4 °C.

2.2.2. Physical-chemical characterization

The sediments, both from the reference area and the surroundings of the submarine sewage outfall, were analyzed for

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