Environmental Pollution xxx (2017) 1–10

Contents lists available at ScienceDirect

Environmental Pollution

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

Ecotoxicological evaluation of the risk posed by bisphenol A, triclosan, and 4-nonylphenol in coastal waters using early life stages of marine organisms (*Isochrysis galbana, Mytilus galloprovincialis, Paracentrotus lividus,* and *Acartia clausi*)☆

Tania Tato [a, b], Noelia Salgueiro-González [c], Víctor M. León [d], Sergio González [a, b], Ricardo Beiras [a, b, *]

[a] *Estación de Ciencias Mariñas de Toralla (ECIMAT), Universidade de Vigo, Illa de Toralla, 36331 Vigo, Galicia, Spain*
[b] *Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo, Campus Lagoas-Marcosende, 36200 Vigo, Galicia, Spain*
[c] *Grupo Química Analítica Aplicada, Departamento de Química, Instituto Universitario de Medio Ambiente (IUMA), Centro de Investigaciones Científicas Avanzadas (CICA), Facultade de Ciencias, Universidade da Coruña, Campus de A Zapateira, 15071 A Coruña, Galicia, Spain*
[d] *Instituto Español de Oceanografía, Centro Oceanográfico de Murcia, Apdo. 22, C/Varadero 1, 30740 San Pedro del Pinatar, Murcia, Spain*

A R T I C L E I N F O

Article history:
Received 12 December 2016
Received in revised form
4 September 2017
Accepted 10 September 2017
Available online xxx

Keywords:
Plastic additives
Personal care products
Endocrine disrupting chemicals
Environmental risk assessment
Alkylphenols
Embryo-larval bioassays

A B S T R A C T

This study assessed the environmental risk on coastal ecosystems posed by three phenolic compounds of special environmental and human health concern used in plastics and household products: bisphenol A (BPA), triclosan (TCS) and 4-nonylphenol (4-NP). These three chemicals are among the organic contaminants most frequently detected in wastewater. The most toxic compound tested was 4-NP, with 10% effective concentration at 11.1 μg L^{-1} for *Isochrysis galbana*, 110.5 μg L^{-1} for *Mytilus galloprovincialis*, 53.8 μg L^{-1} for *Paracentrotus lividus*, and 29.0 μg L^{-1} for *Acartia clausi*, followed by TCS (14.6 μg L^{-1} for *I. galbana*, 149.8 μg L^{-1} for *M. galloprovincialis*, 129.9 μg L^{-1} for *P. lividus*, and 64.8 μg L^{-1} for *A. clausi*). For all species tested, BPA was the less toxic chemical, with toxicity thresholds ranging between 400 and 1200 μg L^{-1} except for *A. clausi* nauplii (186 μg L^{-1}). The relatively narrow range of variation in toxicity considering the broad physiological differences among the biological models used point at non-selective mechanisms of toxicity for these aromatic organics. Microalgae, the main primary producers in pelagic ecosystems, showed particularly high susceptibility to the chemicals tested. When the toxicity thresholds experimentally obtained were compared to the maximum environmental concentrations reported in coastal waters, the risk quotients obtained correspond to very low or low risk for BPA and TCS, and from low to high for 4-NP.

1. Introduction

From the very beginnings of the plastics industry, it has been necessary to add materials to a basic polymeric matrix in order to improve its processing and end-use performance, and to achieve the desired properties in the final product (Deanin, 1975). Among the thousands of chemicals used as plastic components, phenolic compounds raise special environmental and human health concern.

Bisphenol A (BPA), with the chemical name 2,2bis(4-hydroxyphenyl) propane, is mainly used as a monomer for the production of epoxy resins, phenol resins, polycarbonates, polyesters and lacquer coatings, and even food cans (EU, 2010). This chemical is classified as an endocrine disrupting compound (EDC) because of its detectable estrogenic and/or antiandrogenic potency in humans and wildlife, and its deleterious effects on mammary glands, brain and behavioral development demonstrated in different organisms (reviewed by Flint et al., 2012). The large global production (4.5 million T/year), its environmental ubiquity and its toxicological effects, including concerns on human health effects (vom Saal et al., 2012), makes BPA a candidate to be included in the list of priority substances in water policy. Even though Directive

☆ This paper has been recommended for acceptance by Prof. von Hippel Frank A.

* Corresponding author. Estación de Ciencias Mariñas de Toralla (ECIMAT), Universidade de Vigo, Illa de Toralla, 36331 Vigo, Galicia, Spain.
E-mail address: rbeiras@uvigo.es (R. Beiras).

https://doi.org/10.1016/j.envpol.2017.09.031
0269-7491/

2008/105/EC considered BPA as 'possible future priority substance', this pollutant has not been already mentioned in the new water Directive 2013/39/EU. Nevertheless, the use of BPA in infant plastic feeding bottles and toys manufacture was restricted by Directive 2011/8/EU and Directive 2014/81/EU, respectively. Furthermore, its use as additive in plastics in contact with food was also regulated with a specific migration limit of 0.6 mg kg^{-1} (EU, 2011). On the other hand, the European Food Safety Authority has recently concluded BPA poses no health risk to humans because current exposure to this compound via ingestion is too low to cause damage (EFSA, 2015). Therefore, the ecotoxicological status of BPA is under strong debate and the environmental risk posed by this compound deserves further investigation.

Triclosan (TCS) is an halogenated biphenyl ether that has been mainly used as broad spectrum antimicrobial in pharmaceutical and personal-care products (Singer et al., 2002). Because of its thermal stability, TCS is increasingly used also as an antimicrobial material preservative in plastics and textile fibers (Dann and Hontela, 2011). Despite its moderate production (1500 T/y), TCS has been widely detected in influents, effluents and biosolids of WWTPs, in lakes, rivers, marine waters and sediments of several European countries, USA, Canada, Australia, Japan and Hong Kong (reviewed by EC, 2010; Dann and Hontela, 2011; see also Pintado-Herrera et al., 2014a,b). As the occurrence of TCS in the environment and human body (including breast milk) increased, emerging health concerns related to its use have also raised. Trace levels of TCS have been suggested to promote the development of cross-resistance to antibiotics among bacteria (Schweizer, 2001). Dermal irritations, higher incidence of allergies and altered thyroid hormone metabolism have also been associated to the exposure to this chemical (reviewed by Dhillon et al., 2015). Furthermore, TCS can be potentially transformed into more toxic chlorinated compounds such as dioxins in the environment (Latch et al., 2003).

In Europe, TCS is still regulated in Annex VI of the Cosmetics Directive 76/768/EEC, to a maximum concentration of 0.3%, and it is currently under evaluation in the context of both REACH and the Biocides Directive. Currently, Canada and Japan restrict the use of TCS in cosmetics (Health Canada, 2015; Japan Ministry of Health, 2000), and in 2010 TCS was removed from the EU list of provisional additives for use in plastic food-contact material (EC, 2010). Several European countries, including Denmark, Sweden, Norway and Finland, have issued national consumer advisories for the use of TCS (Lee and Chu, 2013). TCS is not regulated in the United States, however EPA is reevaluating the risks and the agency may consider new regulatory action if warranted (US-EPA, 2015).

Nonylphenols (NPs) are by far the most important alkylphenols, constituted by an alkyl chain with 9 C located at either *ortho*- (2-NP), *meta*- (3-NP), or mainly *para*- (4-NP) position on the phenolic ring. These compounds are the degradation products of nonylphenol ethoxylates (NPEs), one of the most common non-ionic surfactants used in detergents and cleaning products, with a total production of 500,000 T/y, according to Ying et al. (2002). Moreover, NPs are used as pesticides, as a monomer in phenol/formaldehyde resins and mainly as plasticizers for high density polyethylene, polyethyleneterephthalate, and polyvinylchloride (Loyo-Rosales et al., 2004). Other uses are intermediate in the production of tri-(4-nonylphenyl) phosphite, a plastic antioxidant, and as a catalyst in the curing of epoxy resins (Talmage, 1994).

Among all NPs, 4-NP stands out as an EDC because it mimics the female hormone 17-β-estradiol and inhibits the aromatase enzyme essential for the synthesis of testosterone, according with a variety of both *in vitro* and *in vivo* assays (David et al., 2009). Due to its persistence in the environment, its moderate bioaccumulation in organisms and its toxicity, 4-NP has been included in different EU and international regulations to preserve the environment and protect human health. Thus, product formulations marketed in the EU cannot contain more than 0.1% of NPE or NP (Directive 2003/53/EC). NPEs have been replaced by more expensive alcohol ethoxylates, which degrade more quickly in the environment. The EU has also included 4-NP on the list of priority hazardous substances for surface water and its chronic and acute environmental quality standards are 0.3 and 2 μg L^{-1}, respectively (Directive 2013/39/EU). In USA, EPA also recommends that 4-NP concentration should not exceed 6.6 μg L^{-1} in fresh water and 1.7 μg L^{-1} in saltwater (US-EPA, 2016a). In order to meet these criteria, EPA is encouraging a voluntary phase-out of 4-NP in industrial laundry detergents (US-EPA, 2010).

The aim of this study was to assess the environmental risk, on coastal ecosystems, posed by three phenolic compounds of special environmental and human health concern used by the plastics industry, BPA, TCS and 4-NP. These three chemicals are among the 30 organic contaminants most frequently detected in wastewater (Kolpin et al., 2002). For the toxicity assessment, a battery of internationally accepted bioassays with highly sensitive early life stages of marine organisms were used, including the microalgal growth test with *Isochrysis galbana*, the bivalve embryo test with *Mytilus galloprovincialis*, the sea-urchin embryo test with *Paracentrotus lividus*, and the copepod nauplii survival test with *Acartia clausi*. Afterwards, a standard risk characterization procedure was assessed, based on the maximum measured concentration of these plastics components from different areas of the world, using the thresholds of toxicity quantified in this work.

2. Materials and methods

2.1. Biological material and bioassays

All experiments were carried out using 0.22 μm-filtered sea water (FSW) of oceanic characteristics (34 ± 2 psu salinity, 8.2 ± 0.1 pH, 8.0 ± 0.1 mg L^{-1} dissolved oxygen) from an uncontaminated area in the outer part of Ría de Vigo (Galicia, NW Iberian Peninsula). A non-axenic culture of *I. galbana* provided by ECIMAT was grown in 500 mL flasks in a 10-fold diluted EDTA-free f/2 medium, and kept in an incubator at 20 °C with 24 h light cycle intensity 60 mE m^{-2} s^{-1} using cool daylight lamps (Osram L36W/865; emission spectrum range 380–780 nm). When reaching the exponential growth phase (after 5–6 days), an intermediate experimental culture, inoculated with the previous culture (density 7000 cells mL^{-1}), was carried out in 5 L Erlenmeyer flask with autoclaved FSW, with bubbling filtered air and enriched with the above-mentioned culture medium. The experimental design followed Pérez et al. (2010), using 250 mL borosilicate Erlenmeyer flasks in triplicate and three additional flasks as control cultures. No agitation was provided during incubation. Cell density, was measured after the 72 h of exposure and cell counts were carried out with a Z2 Coulter Counter particle size analyzer (Beckman-Coulter Particle Count and size analyzer USA). Growth rate (GR) was calculated as described by Pérez et al. (2010):

$$GR(day-1) = \frac{[ln(final\ number\ cells)] - [ln(initial\ number\ cells)]}{3}$$

(3 is the number of days).

Acceptability criteria for the test were 16-fold increase in cell density of controls in 72 h, and a coefficient of variation among replicates not exceeding 7% (ISO, 2006).

Mature mussels (*M. galloprovincialis*) and sea-urchins (*P. lividus*) were collected by scuba divers during the natural spawning season in the outer part of Ría de Vigo (NW Iberian Peninsula). Sea-urchins