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Study of bioconcentration of oxybenzone in gilt-head bream and characterization of its by-products

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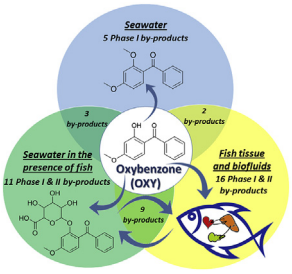
HIGHLIGHTS

- Uptake and biotransformation study with oxybenzone-exposed gilt-head bream.
- Oxybenzone and its by-products were observed in all the tissue/biofluids.
- Up to 20 by-products (12 novel structures) were annotated using HRMS.
- Different oxybenzone degradation was found in the presence and absence of fish.
- Besides oxybenzone, by-products should be considered for risk assessment studies.

GRAPHICAL ABSTRACT



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ABSTRACT

The widespread occurrence of UV filters such as oxybenzone (OXY) in the aquatic ecosystems has raised social and scientific concern due to their high bioaccumulation potential and possible adverse effects in organisms. Within this context, the aim of the present work was to study the uptake, distribution, metabolization and elimination of OXY in different tissues (liver, gill and muscle) and biofluids (bile and plasma) of gilt-head bream (*Sparus aurata*) in a controlled seawater ecosystem (50 ng/mL OXY) within a 14-day exposure. The highest OXY concentrations in all the tissue/biofluids were found at the end of the experiment. The highest OXY levels were found in bile (1.8–17 μg/mL). In the case of liver, the concentrations found (9–160 ng/g) were lower than those expected for a lipidic matrix, which could be explained by a high OXY metabolization. Up to 20 Phase I and Phase II by-products of OXY were annotated by means of liquid chromatography–high resolution mass spectrometry, of which 12 were reported for the first time. In addition to OXY, its by-products might also cause adverse effects and their biomonitoring is advisable in order to fully characterize OXY exposure.

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1. Introduction

Pharmaceuticals and personal care products (PPCPs) have raised scientific and public concern since they may reach the aquatic ecosystems at detectable and potentially harmful concentrations (Arpin-Pont et al., 2016; Boxall et al., 2012). UV filters are among the PPCPs which have focused much attention due to their wide range of applications in sunscreen products and daily use cosmetics, skin creams, body lotions and hair sprays and dyes (Balmer et al., 2005; Brausch and Rand, 2011), as well as food contact materials, including plastics and cartons, and textiles (Muncke, 2011). 28 UV light reflecting and scattering inorganic (TiO_2, ZnO) and UV light absorbing organic UV filters are listed in the EU Cosmetics Directive for commercial cosmetic products, giving good protection against UVB and even UVA radiation (European Parliament and Council, 2009). One of the most widely used organic UV filter is (2-hydroxy-4-methoxyphenyl)-phenylmethanone, commonly known as oxybenzone (OXY) or benzophenone-3 (Balmer et al., 2005). The environmental concern of OXY has increased due to its lipophilicity (log $K_{ow} = 3.6$) and bioaccumulation capability, as well as its potential adverse effects, including slight estrogenic and both strong antiestrogenic and antiandrogenic *in vitro* effects (Kunisue et al., 2012; Kunz et al., 2006; Kunz and Fent, 2006), induction of vitellogenin and reduction of hatching rated in fish (Coronado et al., 2008), and alterations in liver, kidney and reproductive organ in rodents (Nakamura et al., 2015).

Both direct (swimming and bathing) and indirect (mainly wastewater) inputs are responsible for the high levels of UV filters found in the aquatic ecosystems (Balmer et al., 2005; Brausch and Rand, 2011). Wastewater inputs of UV filters to the environment are an indicative of a lack of efficiency of the commonly used removal techniques in wastewater treatment plants (Balmer et al., 2005; Gago-Ferrero et al., 2012). OXY has been found in raw and treated wastewater (Balmer et al., 2005; Hernández Leal et al., 2010; Li et al., 2007; Negreira et al., 2009; Snyder et al., 2006) and in sewage sludge (Gago-Ferrero et al., 2011; Rodil et al., 2009), but also in lake and river water (Fent et al., 2010; Kameda et al., 2011; Poiger et al., 2004), river sediments (Gago-Ferrero et al., 2011; Zhang et al., 2011) and fish (Al-Salhi et al., 2012; Balmer et al., 2005; Fent et al., 2010; Molins-Delgado et al., 2018; Nagtegaal et al., 1997).

Although accumulation of UV filters in biota has been scarcely studied, current knowledge was reviewed by Gago-Ferrero and coworkers, and they reported that fish are important bioindicators in order to understand the possible adverse effects of lipophilic contaminants such as OXY (Gago-Ferrero et al., 2013b). Additionally, transformation and metabolization of UV filters is of great relevance since some of their by-products might be even more toxic than the parent compound (Kim and Choi, 2014; Kunisue et al., 2012; Kunz and Fent, 2006, 2006; Nakagawa and Suzuki, 2002; Nashev et al., 2010; Suzuki et al., 2005). Furthermore, the concentration of intermediates 2,4-dihydroxybenzophenone (DHB), 2,2′-dihydroxy-4-methoxybenzophenone (DHMB) and 2,3,4-trihydroxybenzophenone (THB) in rat blood decreased much more slowly over time compared to the parent compound OXY, indicating that degradation products might have more significant adverse effects than OXY over the long term (Jeon et al., 2008).

In this context, the aim of the present work was to study the uptake, distribution and metabolization of OXY in tissues (liver, gill, muscle) and biofluids (plasma, bile) of juvenile gilt-head bream (*Sparus aurata*) under controlled dosing experiments. Furthermore, by-products generated from OXY under different environmental conditions (in the presence and absence of fish in seawater) were annotated using a suspect screening strategy involving high-resolution mass spectrometry (HRMS) analysis of the different matrices.

2. Materials and methods

2.1. Standards and reagents

OXY (98%) and oxybenzone-(phenyl-d_5) ([$^2H_5$]-OXY, 98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of OXY and [$^2H_5$]-OXY were prepared at 5 g/L in methanol (MeOH, 99.9%, Fisher Scientific, Loughborough, UK), and diluted with MeOH to prepare standard solutions for calibration curve and spiking purposes. Stock dosing solution of OXY was prepared at 25 g/L in ethanol (EtOH, 99.9%, Scharlab, Barcelona, Spain) and diluted down to 0.83 mg/L in Milli-Q water. The final concentration of EtOH in the dosing solution was <0.005%. All stock solutions were stored at −20 °C prior to use.

MeOH (HPLC grade, 99.9%), ethyl acetate (EtOAc; 99.8%) and *n*-hexane (95%) were supplied by LabScan (Dublin, Ireland), formic acid (HCOOH, ≥98%) by Scharlab, and ethyl 3-aminobenzoate methanesulfonate (tricaine, >98%), ethylendiaminetetraacetic acid (EDTA, >99%) and ammonium hydroxide (NH_4OH, 25%) by Panreac (Barcelona, Spain). Ultra-pure water was obtained using a Milli-Q water purification system (<0.05 μS/cm, Milli-Q model Integral 5, Millipore, Bedford, MA, USA).

Oasis HLB (200 mg, 6 mL) and Florisil (2 g, 12 mL) cartridges used for preconcentration and clean-up purposes were purchased from Waters Corporation (Milford, MA, USA) and Supelco (Walton-on-Tames, UK), respectively.

MeOH (Fisher Scientific) was used as mobile phase solvent and formic acid (HCOOH, Optima, Fischer Scientific, Geel, Belgium) for mobile phase modification. High purity nitrogen gas (>99.999%) supplied by Air Liquide (Madrid, Spain) was used for evaporation purposes in the XcelVap Automated Evaporation and Concentration System (Horizon Technology, Salem, New Hampshire, USA) and in the electrospray ionization and collision cell.

2.2. By-product identification experiments

For uptake and by-product identification experiments, juvenile gilt-head bream, weighing ~40 g and measuring ~13 cm in length, were used. Fish were obtained from Groupe Aqualande (Roquefort, France) and shipped to the Research Centre for Experimental Marine Biology and Biotechnology of Plentzia (UPV/EHU), where the exposure experiments were performed. The laboratory was maintained at a constant temperature of 18 °C and a 14:10 h light:dark cycle. Fish were acclimatized for 3 months upon arrival and stabilized for an additional 1 week in the dosing tank before starting the exposure. The water was continuously aerated using aquarium oxygenators and fish were fed daily with 0.10 g pellets/fish. Water temperature (13 °C) and pH (7.4 ± 0.3) were constant during the exposure. Dissolved oxygen, nitrite, nitrate and ammonium content were periodically monitored.

A 14-day exposure experiment was designed both to study OXY uptake and to identify by-products of OXY. Four 1000 × 700 × 650 mm polypropylene tanks containing 250 L of seawater were used: two of them contained 100 fish each and the other two only seawater. One tank containing fish and one tank without fish were continuously fortified at 50 ng/mL (OXY nominal concentration), whereas the other two tanks were used as control (one with fish, one without fish).

OXY dosing was performed using a continuous flow-through system with a peristaltic pump delivering 10 L seawater/h and another pump infusing OXY dosing solution (0.83 mg/L) at 0.6 L/h to exposure tanks. OXY dosing solutions were refilled every 24 h with fresh solution. Control tanks were maintained at identical conditions over the duration of the experiment but only seawater was delivered.