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Assessment of multiple hormone activities of a UV-filter (octocrylene) in zebrafish (*Danio rerio*)

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HIGHLIGHTS

- Octocrylene (OCT) can accumulate in fishes up to sufficiently high levels to cause adverse effects on the endocrine system.
- The accelerated ovary development indicates that OCT has the effect on sex-endocrinology.
- The gene alterations address that OCT has the multiple hormone activities.

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ABSTRACT

In this study, zebrafish (*Danio rerio*) were exposed to a UV-filter-octocrylene (OCT) with elevated concentrations for 28 d. The total body accumulation of OCT in zebrafish was found to reach 2321.01 ("L" level), 31,234.80 ("M" level), and 70,593.38 ng g^{-1} ("H" level) when the average OCT exposure concentration was controlled at 28.61, 505.62, and 1248.70 μg L^{-1}, respectively. Gross and histological observations as well as RT-qPCR analysis were conducted to determine the effects of OCT accumulation on zebrafish. After exposure, the gonad-somatic index and percentage of vitellogenic oocytes were found to increase significantly in the ovaries of female zebrafish at the H accumulation level. Significant up-regulation of *esr1* and *cyp19b* were observed in the gonads, as well as *vtg1* in the livers for both female and male zebrafish. At M and H accumulation levels, apparent down-regulation of *ar* was observed in the ovaries and testis of the female and male zebrafish, respectively. Although the extent of the effects on zebrafish differed at different accumulation levels, the induction of *vtg1* and histological changes in the ovaries are indications of estrogenic activity and the inhibition of *esr1* and *ar* showed antiestrogenic and antiandrogenic activity, respectively. Thus, as OCT could easily accumulate in aquatic life such as zebrafish, one of its most of concern hazards would be the disturbance of the histological development and its multiple hormonal activities.

1. Introduction

By virtue of the increased public awareness of the hazards associated with overexposure to ultraviolet (UV) radiation, UV filters are now commonly added to products such as creams, lipsticks, and in the UV-protection of numerous materials and products (Balmer et al., 2005; Fent et al., 2010). It is estimated that about 10,000 tons of UV filters are produced annually for the global market (Danovaro et al., 2008). There are two main categories of UV filters:1) Inorganic filters composed of titanium dioxide and/or zinc oxide for scattering and reflecting UV light, and 2) organic compounds such as 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC) or benzophenone-3 (BP-3), which work by absorbing UV-light. In the European Union, 28 UV filters have been registered in total (Schlumpf et al., 2008). All have been identified as potentially dangerous pollutants to the aquatic environment.

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UV filters can easily enter the aquatic system via direct or indirect pathway such as being washed off the skin during bathing and swimming. Because the molecules of most UV filters are very stable, it is difficult for them to be completely removed by conventional wastewater treatment processes. The treated effluents may thus be the primary source of UV filters entering the aquatic environment (Buser et al., 2006; Fent et al., 2010). High concentrations of EHMC (up to 20,070 ng L^{-1}) and 4-methylbenzylidene camphor (up to 960 ng L^{-1}) have been measured from domestic wastewaters (Kupper et al., 2006), and, if they are not sufficiently removed by treatment, may significantly contaminate the receiving water bodies due to effluent discharge. In surface waters, benzophenone-4 (BP-4) has been measured at levels as high as 849 ng L^{-1} (Rodil et al., 2008). EHMC was measured at 26–5610 ng L^{-1} in the source water for drinking water supply (Loraine and Pettigrove, 2006).

Of the most commonly used UV filters, octocrylene (OCT) has attracted significant research attention in the environmental protection field because it is more refractory and hydrophobic than other UV filters (Rodil et al., 2009). OCT has been found in several different water bodies across the globe. Seawater collected from Hong Kong, for example, can be contaminated with OCT at up to 103–6812 ng L^{-1} (Tsui et al., 2015). It has also been reported that the concentration of OCT in lake water ranges from 2 to 27 ng L^{-1} (Poiger et al., 2004). OCT has even been detected in tap water at levels as high as 170 ng L^{-1} (Díaz-Cruz et al., 2012). Authors have also monitored the presence of OCT in the effluent of domestic wastewater treatment plant to find that its concentration can reach 0.56–1.8 μg L^{-1} (unpublished data).

The Kow of OCT is as high as about 10^7, which allows it to easily accumulate in aquatic life. In a Swiss lake, OCT was detected from the mussel of brown trout as 2400 ng g^{-1} lipid weight (l.w.) (Buser et al., 2006). In a remote Brazilian coastal area, the accumulated OCT in Franciscana dolphins was as high as 89–782 ng g^{-1} (l.w.) (Gago-Ferrero et al., 2013). OCT accumulations of 25–11,875 ng g^{-1} body weight (b.w.) were also reported in the liver of cod in Oslofjord (Langford et al., 2015). Unfortunately, relatively little information is available regarding the toxic effect of OCT accumulation in aquatic organisms.

There has been growing concern regarding the endocrine-disrupting effect of UV filters on aquatic life, for example, the alterations in gonad histology of mature fathead minnows caused by benzophenone-2 (BP-2) (Weisbrod et al., 2007). Many UV filters have been found to be hormonally active and show agonistic and/or antagonistic effects towards nuclear receptors, estrogen responsive genes, and steroidogenesis (Zucchi et al., 2010; Christen et al., 2011; Kim et al., 2014). Accordingly, any study on the potential risk associated with OCT may also need to be concentrated on its possible endocrine disrupting effect when it is accumulated in the bodies of aquatic animals. Zebrafish (*Danio rerio*) makes an excellent vertebrate model for assessing the toxicity of this manner of chemicals *in vivo,* especially when analyzing the action mode (Reimers et al., 2004). Previous studies have also shown that in order to evaluate the ultimate toxicological effect of a target chemical on aquatic life, experimental conditions should be established appropriately for the chemical to be accumulated in the test aquatic animal to appreciably high levels during a certain duration of exposure (Zucchi et al., 2010; Blüthgen et al., 2012). In the case of zebrafish, OECD guidelines specify that 28 d is a reasonable exposure time (OECD, 1996) to control target chemical in the experimental solution to sufficiently elevated concentration.

The objective of this study was to gain knowledge on the endocrine disrupting potential of OCT on aquatic life by using zebrafish for *in vivo* bioassay. We conducted gross and histological observations as well as RT-qPCR analysis to monitor the multiple hormone activities of OCT after the chemical was accumulated to a certain level.

2. Materials and methods

2.1. Chemicals

2-Ethylhexyl-2-cyano-3,3-diphenylpropenoate (OCT, CAS No:6197-30-4, purity ≥ 95%) was purchased from TCI (Tokyo, Japan). Dimethylsulfoxide (DMSO), methanol, and dichloromethane of HPLC grade were purchased from Fisher Scientific (Shanghai, China). Stock solutions of OCT (1, 10, 30 g L^{-1}) were prepared by dissolution in DMSO and stored in a dark environment at 4 °C between uses. Before exposure, the stock solutions were diluted and DMSO solvent concentration maintained at ≤ 0.01% (v/v).

2.2. Maintenance of zebrafish

Juvenile zebrafish (about five months old, mean body length 3.22 cm, mean body weight 0.28 g) was obtained from an animal lab (Hebei, Shijiazhuang) and transferred to an ultra-white fish tank. Before conducting the experiment, female and male zebrafish was separately acclimatized for one month in reconstituted tap water with a total hardness of 125 mg L^{-1} as $CaCO_3$ and an electrical conductivity of 270 μS cm^{-1}. The water temperature was kept constant at 27 ± 1 °C with a photoperiod set to 14:10 h light/dark. Zebrafish was fed twice daily with a combination of brine shrimp and flake fish food. Water parameters (nitrate, nitrite, pH) were measured daily and oxygen saturation was maintained at ≥80%.

2.3. Exposure of zebrafish to OCT

Adult zebrafish was exposed to OCT solutions including a blank control, solvent control (DMSO, ≤0.01%), and a series of prepared OCT solution (100, 1,000, 3000 μg L^{-1}) for 28 d. The concentrations and duration of exposure were determined based on previous studies on other UV filters (Christen et al., 2011) and OECD guidelines (OECD, 1996). A static-renewal procedure was conducted during exposure. Zebrafish of similar size was removed from the culture tank randomly and placed into 5 L glass beakers covered with gauze. Every 48 h, zebrafish was transferred to new exposure solutions and the surplus food and faeces were immediately removed by siphoning. Throughout the entire exposure period, zebrafish was fed as previously described. Their mortality, development, and abnormal behaviors were recorded daily.

2.4. Analysis of OCT in exposure water and zebrafish

To determine the actual OCT concentrations during exposure, aliquots of 200 mL exposure water from each treatment group were taken at the beginning (0 h), after 24 h, and prior to water renewal (48 h). The adsorption and accumulation of OCT in the zebrafish may not have been consistent during the whole exposure, resulting in variations in OCT level in the water; thus, in order to accurately obtain actual OCT concentrations, samples were taken on days 1–3,10–12, and 19–21, respectively.

About 200 mL aliquots of each nominal concentration were taken for analysis at 0 h, 24 h, and 48 h. The water samples were stored at −20 °C in brown glass bottles until analysis. Watersample extraction and chemical analysis were performed as follows. Before solid-phase-extraction, the pH of exposure samples was adjusted to 3 with hydrochloric acid. Agilent C18 (500 mg and 3 cc) cartridges were conditioned with 10 mL dichloromethane, 10 mL methanol, and 10 mL Milli-Q water, then water samples were passed through