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Accumulation and effects of the UV-filter octocrylene in adult and embryonic zebrafish (*Danio rerio*)

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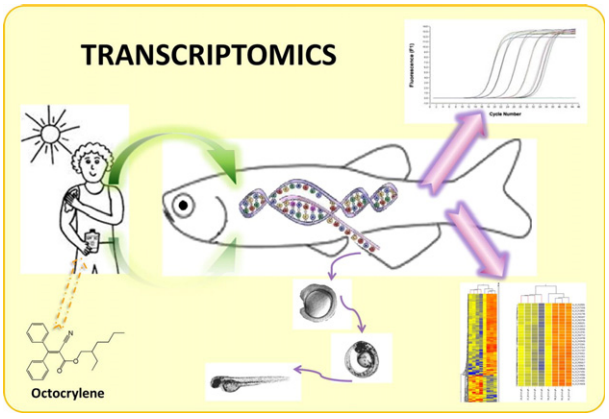
HIGHLIGHTS

- UV filter octocrylene (OC) is largely used but poorly investigated.
- This is the first transcription characterization of OC in zebrafish.
- Mechanism of action of OC in brain and testes is proposed.
- OC becomes accumulated in fish.

GRAPHICAL ABSTRACT



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ABSTRACT

Wide application of the UV-filter octocrylene (OC) in cosmetics leads to contamination of the aquatic environment, but effects of OC remain unclear. Here we determine bioaccumulation and molecular effects of OC. Adult male zebrafish were exposed to 22, 209 and 383 μg/L and embryos to 69, 293 and 925 μg/L OC. OC accumulated in fish up to 17 μg/g. Calculated BCF varied between 41 and 136. Microarray analysis in brain and liver following exposure to 383 μg/L OC revealed alteration of 628 and 136 transcripts, respectively. Most prominent GO processes included developmental processes, organ development, hematopoiesis, formation of blood vessels, blood circulation, fat cell differentiation and metabolism. Validation by RT-qPCR in brain and liver of adult fish and embryos included a series of genes. Blood levels of 11-ketotestosterone were not altered. The transcriptomics data suggest that OC mainly affects transcription of genes related to developmental processes in the brain and liver as well as metabolic processes in the liver.

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1. Introduction

UV-filters are ingredients in many personal care products such as sunscreens, shampoos, soaps, creams, lotions, perfumes, lipsticks and other cosmetics (Hauri et al., 2003). In addition, they are also present in food contact materials including plastics and cartons (Muncke, 2010). UV-filters are mainly (aromatic) organic molecules, which absorb harmful UV-irradiation by their conjugated pi-systems (chromophores), and thus protect human skin and commercial products from degradation. Many UV-filters are high production volume chemicals and enter the aquatic environment either directly, during bathing activities, or indirectly via incomplete removal in wastewater treatment plants. UV-filters are mostly lipophilic, found in all aquatic compartments of fresh and sea water and in biota (Fent et al., 2010; Balmer et al., 2005) and are sometimes increased in summer months (Magi et al., 2013; Buser et al., 2006).

Octocrylene (OC) is one of the most widely and increasingly used UV-filters (Avenel-Audran et al., 2010). Thus, the major input source in the environment occurs via application in cosmetics. OC has been detected up to 4400 ng/L in rivers and lakes (Rodil et al., 2009a) and 167 ng/L in tap water (Díaz-Cruz et al., 2012). An average of 38 μg/L OC was found in (household) gray water (Hernández-Leal et al., 2010). Concentrations in the ng/L range (12–390 ng/L) up to 12 μg/L OC have been detected in wastewater (Magi et al., 2013; Balmer et al., 2005). Due to the high lipophilicity of OC ($\log K_{ow} = 6.9$) and its low biodegradability (Rodil et al., 2009b; Hernández-Leal et al., 2010), OC has a considerable tendency for bioaccumulation. Up to 2400 ng/g lipid weight (lw) and 30 ng/g dry weight (dw) of OC were found in river fish in Switzerland (Buser et al., 2006) and Spain (Gago-Ferrero et al., 2013a, 2013b), respectively. In marine mussels at shores, up to 7100 ng/g dw was found (Bachelot et al., 2012). Higher sediment concentrations were reported in lakes (up to 642 μg/kg dw) compared to rivers (25 μg/kg dw) (Kaiser et al., 2012; Rodil and Moeder, 2008). Recently, 89–782 ng/g lw OC were measured in the liver of marine mammals along the Brazilian coast (Gago-Ferrero et al., 2013a, 2013b).

Previously, we have shown that some UV-filters possess hormonal activities in vitro and in fish (Blüthgen et al., 2012; Zucchi et al., 2011a, 2011b; Christen et al., 2011; Kunz and Fent, 2006). Benzophenone-1 and benzophenone-2 show estrogenic activity and lead to reproductive effects in fish (Kunz and Fent, 2006; Weisbrod et al., 2007). For most UV-filters biological activities and modes of action are poorly understood. Benzophenone-3 (Blüthgen et al., 2012) and EHMC (Zucchi et al., 2011a) alter the transcription profile in fish, including genes involved in the sex hormone system and steroidogenesis.

To date, the toxicological profile and modes of action of OC are poorly known. OC possesses anti-estrogenic and (anti)-androgenic activity in vitro (Kunz and Fent, 2006). However, it is not known whether OC has endocrine activity in fish in vivo as well. Moreover, chronic effects in fish have not been investigated, nor its molecular effects. Therefore, the aim of the present study was to elucidate the modes of action of OC in zebrafish (*Danio rerio*) after chronic exposure as revealed by transcriptomics. We evaluated the transcription profiles in brain and liver of adult zebrafish males and eleuthero-embryos, respectively, by microarrays and RT-qPCR at different exposure times. We also tested the hypothesis, whether the in vitro hormonal activity was reflected in vivo by means of RT-qPCR and 11-ketotestosterone level in blood of males. The transcriptomics approach was chosen in order to get first insights into the molecular mechanisms of OC in fish and to estimate potential (eco) toxicological impacts of OC.

2. Materials and methods

2.1. Chemicals

Octocrylene (OC, 2-Ethylhexyl 2-cyano-3,3-diphenylacrylate, CAS no: 6197-30-4, purity >97%), dimethylsulfoxide (DMSO), pentane and formic acid were purchased from Sigma-Aldrich (Buchs, Switzerland). The solvents used, ethyl acetate, acetonitrile, methanol, ethanol and diethyl ether (anhydrous), were of HPLC grade and obtained from J.T. Baker (Stehelin AG, Basel, Switzerland). Water used for sample preparation and HPLC was of HPLC grade and in-house prepared (Nanopure Diamond, Barnstead, Switzerland). SPE columns Strata-X (200 mg/6 mL) were purchased from Phenomenex (Brechbühler AG, Schlieren, Switzerland) and Oasis HLB (6 cc 200 mg) from Waters (Waters AG, Baden-Dättwil, Switzerland), respectively. Syringe filters Titan 2 HPLC Filter (17 mm, 0.45 μm, PVDF membrane) were purchased from ThermoScientific (US). KoiMed Sleep (Ethylenglycolmonophenylether) was purchased from KOI&BONSAI Zimmermann (Bühlertann, Switzerland). Heparin ammonium salt (100 kU) was obtained from Sigma-Aldrich (Buchs, Switzerland), and BD Micro-Fine+Innen sterile insulin syringes (0.5 mL, 0.33 mm (29G) × 12.7 mm) from Becton Dickinson (Allschwil, Switzerland). The kits used for RNA extraction (RN*easy* Mini Kit 74104 and RN*ase*-Free DN*ase* Set 79254) and enzymes for cDNA synthesis (M-MLV Reverse Transcriptase (M1705)) were purchased from Qiagen (Basel, Switzerland) and Promega (Dübendorf, Switzerland), respectively.

2.2. Zebrafish

Juvenile zebrafish were obtained from Harlan Laboratories (Itingen, Switzerland) and cultivated to adulthood in culture tanks (300 L). Fish of both sexes were held in reconstituted deionized water (salt concentrations: $CaCl_2 \times 2H_2O$ 147.0 mg/L, KCl 2.9 mg/L, $MgSO_4 \times 7H_2O$ 61.6 mg/L, $NaHCO_3$ 32.4 mg/L) with a conductivity of 350–360 μS/cm, and applying a static-water renewal weekly. Water temperature was 26 ± 1 °C and the photoperiod used was 16:8 h light/dark. Fish were fed twice daily with a combination of frozen brine shrimps (*Artemia salina*), mosquito larvae and *Daphnia magna*. Water parameters, such as nitrate, nitrite and pH were controlled regularly using Test strips (Easy Test, JBL) and oxygen was ensured ≥ 80%.

2.3. Exposure (study design)

The study design was chosen based on the OECD guideline No. 204 (Fish, prolonged toxicity test). Adult male zebrafish (about 2 years old, mean body length 3.9 cm, mean weight 441 mg) were selected from the culture tank and randomly placed into 10 L stainless steel tanks in well-aerated reconstituted water (15 fish/tank). The fish loading rate in the different dose groups was between 0.64–0.68 g/L in the first 8 days of exposure and 0.47–0.50 g/L between 8 and 16 days of exposure. The experimental design consisted of five groups with five replicates each, including water control, solvent control (0.005% DMSO), 100, 1000 and 2000 μg/L OC (nominal concentration, dissolved in DMSO). The solvent concentration was 0.005% DMSO in all tanks, except in water controls. Exposure concentrations have been chosen to address both environmental levels and effect concentrations. Depending on what is regarded as environmental concentration, our lowest nominal concentration is about 4-times (household gray water, Hernández-Leal et al., 2010), 8-times (wastewater, Balmer et al., 2005) or 23-times higher (river water, Rodil et al., 2009a). However, real concentrations were much lower. In addition, the higher but non-toxic concentrations were chosen to address metabolism of OC by determination of metabolites.

Thus, the lowest OC concentration can be regarded as representative of a ten-times higher than worst-case environmental concentration (Magi et al., 2013; Balmer et al., 2005), and higher concentrations were used to elucidate metabolism and molecular mechanism of OC toxicity. Oxygen (≥80%), pH value (6.4–6.8), nitrate and nitrite concentration (0 mg/L) were continuously monitored to ensure high water quality, temperature was 26 ± 1 °C, and light cycle was 16:8 h light/dark. Appearance, mortality and abnormal behavior of fish were recorded daily, and fish were fed twice daily.