County Clerk

From: Joe DiNardo <jmjdinardo@aol.com>
Sent: Sunday, November 26, 2017 6:47 AM
To: IEM Committee; County Clerk
Cc: cadowns@haereticus-lab.org
Subject: Dermatology Paper - Oxybenzone Review 3 of 4
Attachments: 16 Downs_et_al 2015 Arch_Env_Contam_Tox_SS.pdf; 17 Brausch and Rand Chemosphere 82t.pdf; 18 ABC-Review biota UV F_publicado.pdf; 19 and 20 Chlorinated Oxybenzone Info.docx; 21 Occurrence UVF Drinking Water Sao Paulo Brazil.pdf

Aloha Chair and Maui County Council,

Mahalo Nui Loa for inviting us to provide testimony before your committee. It was an honor and privilege.

We are submitting the attached studies to you, as requested by the Infrastructure and Environmental Management committee after our presentations on Oxybenzone and Octinoxate's effects on marine life and human health. After reviewing the attached studies, we are confident that you will all feel comfortable with your vote to support the legislation to ban the sale of SPF Sunscreen products containing Oxybenzone and/or Octinoxate.

Mahalo,

Craig Downs – Executive Director – Haereticus Environmental Laboratory

Joe DiNardo – Retired Personal Care Industry Toxicologist & Formulator

Notes:

- Because of the size of the files there will be several Emails sent per topic; all will be numbered appropriately.

- The first Email on the topic will contain the main article (Dermatology Paper – Oxybenzone Review, Oxybenzone HEL Monograph or Octinoxate HEL Monograph) and the references used to support the main article will be included.

- Chemical names used in the attached research papers may vary – please feel free to ask us to clarify any concerns you may have associated with terminology:

1) Oxybenzone = Benzophenone-3 (BP-3) and metabolites maybe noted as Benzophenone-1 and 4-Methylbenzophenone

2) Octinoxate = Ethylhexyl Methoxycinnamate (EHMC) = Octyl Methoxycinnamate (OMC)

Arch Environ Contam Toxicol
DOI 10.1007/s00244-015-0227-7

Toxicopathological Effects of the Sunscreen UV Filter, Oxybenzone (Benzophenone-3), on Coral Planulae and Cultured Primary Cells and Its Environmental Contamination in Hawaii and the U.S. Virgin Islands

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Abstract Benzophenone-3 (BP-3; oxybenzone) is an ingredient in sunscreen lotions and personal-care products that protects against the damaging effects of ultraviolet light. Oxybenzone is an emerging contaminant of concern in marine environments—produced by swimmers and municipal, residential, and boat/ship wastewater discharges. We examined the effects of oxybenzone on the larval form (planula) of the coral *Stylophora pistillata,* as well as its toxicity in vitro to coral cells from this and six other coral species. Oxybenzone is a photo-toxicant; adverse effects are exacerbated in the light. Whether in darkness or light, oxybenzone transformed planulae from a motile state to a deformed, sessile condition. Planulae exhibited an increasing rate of coral bleaching in response to increasing concentrations of oxybenzone. Oxybenzone is a genotoxicant to corals, exhibiting a positive relationship between DNA-AP lesions and increasing oxybenzone concentrations. Oxybenzone is a skeletal endocrine disruptor; it induced ossification of the planula, encasing the entire planula in its own skeleton. The LC_{50} of planulae exposed to oxybenzone in the light for an 8- and 24-h exposure was 3.1 mg/L and 139 µg/L, respectively. The LC_{50}s for oxybenzone in darkness for the same time points were 16.8 mg/L and 779 µg/L. Deformity EC_{20} levels (24 h) of planulae exposed to oxybenzone were 6.5 µg/L in the light and 10 µg/L in darkness. Coral cell LC_{50}s (4 h, in the light) for 7 different coral species ranges from 8 to 340 µg/L, whereas LC_{20}s (4 h, in the light) for the same species ranges from 0.062 to 8 µg/L. Coral reef

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contamination of oxybenzone in the U.S. Virgin Islands ranged from 75 μg/L to 1.4 mg/L, whereas Hawaiian sites were contaminated between 0.8 and 19.2 μg/L. Oxybenzone poses a hazard to coral reef conservation and threatens the resiliency of coral reefs to climate change.

Oxybenzone (BP-3; benzophenone-3; 2-hydroxy-4-methoxphenyl phenylmethanone; CAS No. 131-57-7) often is used as an active ingredient in sunscreen lotions and personal-care products, such as body fragrances, hair-styling products, shampoos and conditioners, anti-aging creams, lip balms, mascaras, insect repellants, as well as dishwasher soaps, dish soaps, hand soaps, and bath oils/salts (CIR 2005; http://www.goodguide.com/ingredients/184390-oxybenzone). BP-3 and other benzophenone derivatives often are found as contaminants in boating, residential, and municipal wastewater effluents and are considered "emerging environmental contaminants of concern" by the U.S. Environmental Protection Agency (Eichenseher 2006; Richardson 2006, 2007; Blitz and Norton 2008; Gago-Ferrero et al. 2011; Kameda et al. 2011; Rodil et al. 2012; Aquero et al. 2013).

Between 6000 and 14,000 tons of sunscreen lotion, many of which contain between 1 and 10 % BP-3, are estimated to be released into coral reef areas each year, putting at least 10 % of the global reefs at risk of exposure, and approximately 40 % of coral reefs located along coastal areas at risk of exposure (Shaath and Shaath 2005; UNWTO 2007; Danovaro et al. 2008; Wilkinson 2008). In Okinawa, BP-3 levels on coral reefs that were 300–600 m away from public swimming beaches ranged from 0.4 to 3.8 pptrillion (Tashiro and Kameda 2013); in South America, sediments near coral communities/reefs contained BP-3 concentrations between 54 and 578 pptrillion (Baron et al. 2013). Schlenk et al. (2005) discovered through a Toxicity Identification Evaluation that BP-3 was unequivocally identified as the source of estrogenic activity in marine sediments near wastewater outfalls. Although the half-life in seawater is several months, BP-3 can act as a pseudo-persistent pollutant; its contamination of a site may be constantly renewed, resulting in ecological receptors experiencing persistent exposure (Vione et al. 2013). Concerns regarding the adverse impacts of exposure to BP-3 on coral reefs and other marine/aquatic ecosystems have led to either banning oxybenzone-containing products in marine-managed areas (e.g. Mexico's marine ecoparks; Xcaret 2007; Xel-há 2007) or public relations campaigns by management agencies to encourage reduction of environmental contamination of sunscreen lotions by swimmers (e.g. "Protect Yourself, Protect the Reef" Bulletin U.S. NPS 2012).

BP-3 exhibits a number of toxicological behaviors ranging from the molecular level to multi-organ system pathologies (Gilbert et al. 2012). Benzophenones, including BP-3, are documented mutagens that increase the rate of damage to DNA, especially when exposed to sunlight (Popkin and Prival 1985; Zeiger et al. 1987; Knowland et al. 1993; NTP 2006). BP-3 produced a positive mutagenic response by inducing the *umu* operon (genotoxicity assay Nakajima et al. 2006). Benzophenones, and especially BP-3, either can act directly as genotoxicants or become genotoxicants by bioactivation via cytochrome P450 enzymes (Takemoto et al. 2002; Zhao et al. 2013). The types of damage to genetic material by benzophenones include oxidative damage to DNA, formation of cyclobutane pyrimidinic dimers, single-strand DNA breaks, cross-linking of DNA to proteins, and an increase in the formation of DNA abasic sites (Cuquerella et al. 2012). Benzophenones also exhibit pro-carcinogenic activities (Kerdivel et al. 2013). BP-3 can generate reactive oxygen species, which are potential mutagens, when applied topically to the skin followed by UV light exposure (Hanson et al. 2006).

BP-3 is a reproductive toxicant whose mechanisms of action and its pathological effects are poorly characterized in various model species. In mice studies, BP-3 exposure significantly affected fecundity, as well as inducing unexplained mortality in lactating mothers (Gulati and Mounce 1997). Studies in both mice and rats demonstrated that generational exposure to BP-3 reduced body weight, increased liver (>50 %) and kidney weights, induced a 30 % increase in prostate weight, a reduction in immunocompetence, and significantly increased uterine weight in juveniles (Gulati and Mounce 1997; French 1992; Schlumpf et al. 2008; Rachon et al. 2006). In mammals, BP-3 is renowned for having estrogenic and anti-androgenic activities, causing activation of estrogen receptor proteins and inhibition of androgen receptors (Morohoshi et al. 2005; Suzuki et al. 2005; Kunz et al. 2006; Molina–Molina et al. 2008; Nashez et al. 2010). Topical application of BP-3 to the skin has been shown to be absorbed and transferred to breast milk, creating risk to breast-fed neonates (Hany and Nagel 1995). In addition, an association between exposure to benzophenones and an increased occurrence of endometriosis in women was recently found by Kunisue et al. (2012).

In fish, BP-3 actions are similar to those in mammals, causing an endocrine disruption by modulating estrogen receptor signaling pathways, inducing reproductive pathologies, and reducing reproductive fitness (Kunz et al. 2006; Coronado et al. 2008; Cosnefroy et al. 2011; Bluthgen et al. 2012). Chronic exposure to BP-3 in fish resulted in reduced egg production, induction of vitellogenin protein in males, and a significant reduction in egg hatchings (Nimrod and Benson 1998; Coronado et al. 2008). These findings raise the possibility of "gender shifts" in fish exposed to BP-3 during the entirety of their

life history or during "windows of sensitivity" (Coronado et al. 2008).

A few studies exist that have evaluated the effects of BP-3 exposure in invertebrates. In insects, BP-3 inhibited expression of the *usp* gene (ultraspiracle protein)—a protein that combines with the EcR protein to form the ecdysone receptor, which controls aspects of developmental and reproductive processes (Ozáez et al. 2013). Gao et al. (2013) found that BP-3 exposure resulted in oxidative injuries, reduced glutathione, and adversely affected cell viability in the protozoan ciliate, *Tetrahymena thermophila.*

Since the 1970s, coral reefs have been devastated on a global scale. Regional weather and climate events often are responsible for acute events of mass-mortality of coral reefs (Carpenter et al. 2008). However, the long-term causative processes of sustained demise often are locality specific (Edinger et al. 1998; Rees et al. 1999; Golbuu et al. 2008; Smith et al. 2008; Downs et al. 2011, 2012; Omori 2011). Records of coral recruitment in many areas of the Caribbean, Persian Gulf, Red Sea, Hawaiian Islands, and elsewhere have exhibited precipitous declines (Richmond 1993, 1997; Hughes and Tanner 2000; Rogers and Miller 2006; Williams et al. 2008). This is most apparent in the deterioration of juvenile coral recruitment and survival rates along coastal areas (Dustan 1977; Miller et al. 2000; Abelson et al. 2005; Williams et al. 2008). As with other invertebrate species, coral larvae (i.e., planula) and newly settled coral (i.e., recruits) are much more sensitive to the toxicological effects of pollution compared with adults (Kushmaro et al. 1997). Hence, even small impacts to larval development and survival can have significant effects on coral demographics and community structure (Richmond 1993, 1997). To manage BP-3 pollution and mitigate its effect on the ecological resilience of coral reefs, the toxicological effects of BP-3 on larval survival and development need to be characterized (Fent et al. 2010; US EPA 2012; NRC 2013).

In this study, we examined the toxicological effects of exposures to varying concentrations of BP-3 on the larval form (planula) of the scleractinian coral *Stylophora pistillata,* the most abundant coral species in the northern Gulf of Aqaba, Red Sea (Loya 1972). Many chemical pollutants affect organisms differently when exposed to light, a process known as chemical-associated phototoxicity (Yu 2002; Platt et al. 2008). Because reef-building corals are photosynthetic symbiotic organisms, and many coral species have planulae that are photosynthetically symbiotic (e.g., *S. pistillata*), we examined the effects of BP-3 exposure in planulae subjected to either darkness or to environmentally-relevant light conditions. Histopathology and cellular pathology, planula morphology, coral bleaching, DNA damage as the formation of DNA abasic lesions, and planula mortality were measured in response to BP-3 exposure. Median lethal concentration (LC_{50}), effect concentration (EC_{20}), and no observable effect concentrations (NOEC) were determined for coral planulae exposed to BP-3 in both darkness and in light. Coral planulae are a relatively difficult resource to procure for toxicological studies. Therefore, primary coral cell cultures were used in in vitro toxicological tests of BP-3 to examine their validity as a surrogate model for coral planulae in generating an effect characterization as part of an Ecological Risk Assessment. The confidence in this model was examined by comparisons of the LC_{50} results of BP-3-exposed planulae to the BP-3 LC_{50} of coral cells (calicoblasts) from adult *S. pistillata* colonies. Coral-cell toxicity testing was conducted on six other species that originate from either the Indo-Pacific or Caribbean Sea/Atlantic Ocean basins to provide in vitro data on the species' sensitivity distribution of BP-3. To determine the environmentally relevant concentration of BP-3 in seawater on coral reefs, we measured BP-3 concentrations at various locations in the U.S. Virgin Islands and the U.S. Hawaiian Islands.

Materials and Methods

Planula Collection and Toxicity Exposures

Planula collection and planula-toxicity exposures were conducted at the Inter-University Institute of Marine Sciences (IUI) in Eilat, Israel. *Stylophora pistillata* (Esper 1797) planulae were collected from the wild within the IUI designated research area by placing positively buoyant planula traps over *Stylophora* colonies measuring more than 25 cm in diameter. Permit for collection was given to Y. Loya by the Israel National Park Authority. Traps were set between 17:00 and 18:00 h, and then retrieved at 06:00 h the next morning. Planulae were inspected and sorted by 07:15 h, and toxicity exposure experiments began at 08:00 h.

Experimental design and culture conditions were based on modified (for coral) guidelines set forth in OECD (2013) and described in Downs et al. (2014). This experiment for BP-3 was conducted concurrently with the study conducted in Downs et al. (2014).

All seawater (ASW) was made artificially using Fisher Scientific Environmental-Grade water (cat#W11-4) and Sigma-Aldrich sea salts (cat#S9883) to a salinity of 38 parts per thousand at 22 °C. Benzophenone-3 (BP-3; 2-Hydroxy-4-methoxyphenyl-phenylmethanone; Aldrich cat#T16403) was solubilized in dimethyl sulfoxide (DMSO) and then diluted with ASW to generate stock solutions and exposure solutions. Solutions of BP-3 for toxicity exposures each contained 5 microliters of DMSO

per one liter and were of the following concentrations: 1 mM BP-3 (228 parts per million), 0.1 mM BP-3 (22.8 mg/L; parts per million), 0.01 mM BP-3 (2.28 mg/L; parts per million), 0.001 mM BP-3 (228 μg/L; parts per billion), 0.0001 mM (22.8 μg/L; parts per billion), and 0.00001 mM (2.28 μg/L; parts per billion). For every exposure time-period, there were two control treatments with four replicates each: (a) planulae in ASW, and (b) planulae in ASW with 5 microliters of DMSO per 1 L. There was no statistical difference between the two controls for any of the assays.

Planulae were exposed to different BP-3 concentrations during four different time-period scenarios: (a) 8 h in the light, (b) 8 h in the dark, (c) a full diurnal cycle of 24 h, beginning at 08:00 in daylight and darkness from 18:00 in the evening until 08:00 h the next day, and (d) a full 24 h in darkness. For the 24-h exposure, planulae from all treatments were transferred to new 24-well microplates with fresh ASW/BP-3 media at the end of the 8-h daylight exposure before the beginning of the 16 h dark exposure.

At the end of the 8 and 24-h time points, chlorophyll fluorescence, morphology, planula ciliary movement, and mortality were measured, while at least one planula from each replicate of each treatment was chemically preserved, and the remaining living planulae were flash frozen in liquid nitrogen for the DNA apyrimidinic (AP) site assay.

Chlorophyll Fluorescence as an Estimate of Bleaching

Chlorophyll fluorescence was measured using a Molecular Dynamics microplate fluorometer with an excitation wavelength of 445 nm and an emission wavelength of 685 nm. Fluorescence measurements were taken at the end of the 8-h light and dark periods of BP-3 exposure. All ten planulae in each replicate well were measured in aggregate. Each well was measured independently of the other wells. Justification and caveats for this assay are described in Downs et al. (2014).

DNA Abasic Lesions

DNA abasic or apurinic/apyrimidinic lesions (DNA AP sites) were quantified using the Dojindo DNA Damage Quantification Kit-AP Site Counting (DK-02-10; Dojindo Molecular Technologies, Inc.) and conducted as described in Downs et al. (2014). Four individual planulae (one from each well) from each treatment were individually assayed. Only planulae that were relatively intact were assayed, even if scored as dead. Planulae from 228 ppm BP-3 at 8 h in the light were not collected, because there were no coherent planulae.

Transmission Electron Microscopy

Transmission electron microscopy was used for tissue and cellular pathomorphology assessment on three planulae from each treatment. Methodology for this technique was described in Downs et al. (2014). At least three planula from each treatment were collected and fixed for analysis.

Coral Cell Toxicity Assay

Cultured colonies of *S. pistillata* (Esper 1797) were obtained from Exotic Reef Imports (www.exoticreefimports.com) and did not need a permit for possession. Cultured colonies of *Pocillopora damicornis* (Linnaeus 1758) was provided by the National Aquarium and did not need a permit for possession. *Montastrea annularis*, *Montastrea cavernosa* (Linnaeus 1766), and *Porites astreoides* (Lamarck 1816) were obtained from the Florida Keys National Marine Sanctuary under permit# FKNMS-2011-139. Cultured colonies of *Acropora cervicornis* (Lamarck 1816) and *Porites divaricata* (Lesueur 1821) were provided by Dr. Cheryl Woodley of the U.S. National Oceanic and Atmospheric Administration and did not need a permit for possession. Corals were maintained in glass and Teflon-plumbed aquaria in 36 ppt salinity artificial seawater (Type 1 water using a Barnstead E-Pure filter system that included activated carbon filters) at a temperature of 24 °C. Corals were grown under custom LED lighting with a peak radiance of 288 photosynthetic photon flux density μmol/m^2/s. Light Spectra ranged from 380 to 740 nm. Light was measured using a Licor 250A light meter and planar incidence sensor. Description of coral cell isolation from each species is described in Downs et al. (2010, 2014).

Exposure experiments of cells were conducted in PTFE-Telfon microplates. Cells of all species except *Acropora cervicornis* were exposed to BP-3 concentrations in cell culture media of 570 parts per trillion to 228 parts per million for 4 h in the light, whereas *Stylophora* cells also were exposed for 4 h in the dark. *Acropora cervicornis* cells were exposed to BP-3 concentrations in cell culture media of 570 ng/L (parts per trillion) to 228 mg/L (parts per million) for 4 h in the light. Lighting was from custom LED fixtures that had wavelength emissions from 390 to 720 nm with a light intensity of 295 μmol/m^2/s of photon flux density.

Viability was confirmed using the trypan blue exclusion assay. There were four replicate wells with cells per treatment. Duplicate aliquots of cells from each replicated wells were collected into a microcentrifuge tube, centrifuged at 300×*g* for 5 min, and the supernatant aspirated. Cells were gently resuspended in culture media that contained 0.5–1.5 % (w/v) of filtered trypan blue (Sigma-

Aldrich, cat#T6146), and incubated for 5 min. Viable versus dead cells were counted using a modified Neubauer hemocytometer (Hausser-Levy Counting Chamber).

Sampling and analysis of benzophenones in seawater samples via gas chromatography-mass spectrometry (GC–MS) and liquid chromatography-mass spectrometry (LC–MS). Dichloromethane, methanol, acetone are pesticide-grade solvents (Fisher Scientific). Analytical standards were purchased from Sigma Aldrich and included: Benzophenone (cat# B9300), Benzhydrol (cat#B4856), 4-hydroxybenzophenone (cat#H20202), 2-hydroxy-4-methoxy benzophenone (cat#H36206), 2,4-dihydroxy benzophenone (cat# 126217), 2-2′-dihydroxy-4-methoxy benzophenone (cat# 323578), 2,3,4-trihydroxy benzophenone (cat# 260576), 2,2′,4,4′-tetrahydroxy benzophenone (cat#T16403). Internal standard solutions (phenanthrene-d10 and chrysene-d12) were purchased from AccuStandard Inc. (New Haven, CT).

Field personnel collecting samples were subject to an Alconox Liqui-Nox detergent decontamination immediately before entering the sampling site and did not apply any sunscreen lotion or nonorganic personal-care products to their body for at least 21 days before sampling. Between 100 and 500 mL of seawater were collected approximately 35 cm below the surface of the water into EPA-certified clean, amber jars. In the field, water samples were extracted using Phenomenex C18 solid phase extractions columns that were first activated with methanol. All columns were capped and then shipped and stored frozen at −80 °C or colder.

Extraction of analytes from seawater samples collected in the U.S. Virgin Islands (under a U.S. National Park Service permit, STT-045-08) followed the methodology described in Jeon et al. (2006). Seawater samples were collected using precleaned 1-L amber glass bottles with Teflon lined lids (I-Chem, 300 series, VWR). Seawater samples were extracted using C-18E cartridges (500 mg, 6 mL Phenomenex Inc.) on a vacuum manifold (Phenomenex Inc.). Cartridges were conditioned with 5 mL of methanol and then 5 mL of water, after which the seawater samples were then added to the column. Following extraction, the cartridges were dried for 10 min, capped, and frozen until processed. The cartridges were eluted with 2 mL of acetone followed by 2 × 5 mL dichloromethane. The extracts were evaporated to dryness under a gentle stream of nitrogen. Then, 50 µL of MSTFA (*N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide, Sigma-Aldrich) was added, capped, vortexed for 30 s, and heated at 80 °C for 30 min. Extracts were transferred to gas chromatography vials with a rinse step to a final volume of 1 mL and the internal standard was added. Percentage recovery for all 8 target analytes using this method with seawater was >95 %.

Seawater samples from Hawaii were collected using precleaned one liter amber glass bottles with Teflon lined lids (I-Chem, 300 series, VWR). Samples were extracted using C-18E cartridges (500 mg, 6 mL Phenomenex Inc.) on a vacuum manifold (Phenomenex Inc.). Cartridges were conditioned as indicated in the previous paragraph and eluted with 5 mL of methanol. For LC–MS analysis, samples were run on an AB_SCIEX 5500 QTRAP Triple Quadrupole Hybrid Linear Ion Trap Mass Spectrometer with a Spark Holland Symbiosis HPLC for analytical separation. The analytes were measured with MRM (multiple reaction monitoring) followed by switching to ion trap functionality (Q3- LIT) to confirm the fragmentation pattern of the MRMs. The source was set at 700 °C and the gasses were set to 60 arbitrary units of nitrogen. The curtain gas was set at 45 arbitrary units, and all MRMs were optimized using infusion based introduction of analytical standards. Analytical separation was performed using a Phenomenex Hydro RP 4.6 × 50 2.6 µm particle size stationary phase, with the mobile phase composed of methanol and water with the addition of 0.1 % formic acid and 5 mM of ammonium acetate in both phases. The flow rate was set at 0.9 mL per min, and a ballistic gradient and re-equilibration was run over 5 min. Percentage recovery for target analytes was >85 %, Limit of Detection was 100 pptrillion, and Quantitative Limit of Measurement was 5 ppbillion (µg/L).

Statistical Methods

OECD (2006) was used as a guidance document for our approach in the statistical analysis of the data. To address different philosophies and regulatory criteria, Effect Concentration response (EC_{20} and EC_{50}) and median Lethal Concentration response (LC_{50}) were determined using three initial methods: PROBIT analysis (Finney 1947), linear or quadratic regression (Draper and Smith 1966), and spline fitting (Scholze et al. 2001). Data were analyzed using linear or quadratic regression and PROBIT methods individually for each experiment, based on model residuals being random, normally distributed, and independent of dosing concentrations (Crawley 1993, Fig. 5.1), as well as having good fit, statistically significant, and biologically interpretable regressors (Agresti 2002; Newman 2013). Spline fitting did not meet these criteria. In several analyses, BP-3 concentrations as $\log_{10}(x + 1)$ were transformed to conform to model assumptions.

Data were tested for normality (Shapiro–Wilk test) and equal variance. When data did not meet the assumption of normality and homogeneity, the no-observed-effect concentration (NOEC) was determined using Kruskal–Wallis one-way analysis of variance, using Dunnett's Procedure (Zar 1996) to identify concentrations whose means differed

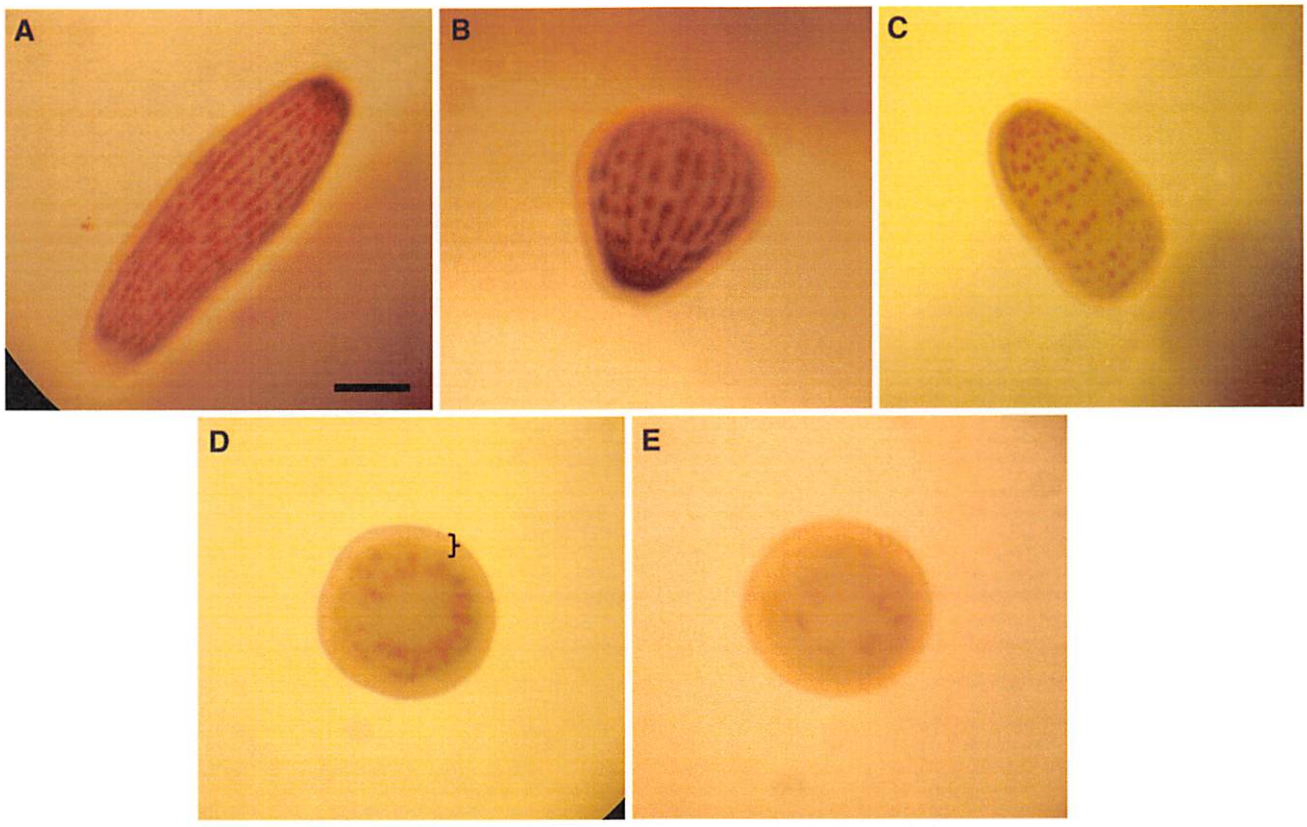

Fig. 1 *Stylophora pistillata* planulae exposed to various treatments of benzophenone-3 (BP-3). **a** Control planula exposed for 8 h in light. **b** Planula exposed to 22.8 parts per billion (μg/L) BP-3 for 8 h in the light. **c** Planula exposed to 228 parts per billion (μg/L) BP-3 for 8 h in the light. **d** Planula exposed to 2.28 parts per million (mg/L) BP3 for 8 h in the light. **e** Planula exposed to 28.8 parts per million (mg/L) BP3 for 8 h in the light. *Scale bar* is 0.5 mm

significantly from the control (Newman 2013). When variances among treatments were heterogeneous, we verified these results using a Welch ANOVA. In cases where responses were homogeneous within the control treatment (i.e., all planulae survived) or another concentration (i.e., all planulae died or were deformed), the Steel Method (Steel 1959) was substituted, which is the nonparametric counterpart to Dunnett's Procedure (Newman 2013). Four replicates of each experimental concentration provided good statistical power for parametric analyses, but it is cautioned that the relatively small sample size for the nonparametric Steel Method (Steel 1959) made results of this test less powerful. To facilitate comparisons among other treatment means, figure legends include results of Newman–Keuls Method post hoc test, which compares each concentration to all others.

Parametric (Pearson's r) or nonparametric (Spearman's ρ) regression analyses were used to determine the relationship between mortality of coral planulae and coral cells. Coral planulae are available only immediately after spawning and a strong association between these two responses would allow mortality of coral cells to serve as a surrogate for this reproductive response. JMP version 9.0 or 10.0 (SAS Institute, Inc., Cary, NC), SAS version 9.3 and SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA) were used for analyses.

Results

Toxicopathology

Planulae under control conditions have an elongated, "cucumber-like" morphology with organized rows of zooxanthellae-containing gastrodermal cells running from the aboral pole to the oral pole (Fig. 1a; "brown dots" in the rows are individual zooxanthella cells). Normal planulae are in near-constant motion, being propelled by cilia that cover the elongated body. Within the first 4 h of exposure of planulae to BP-3 in both light and darkness, planulae showed a significant reduction in ciliary movement and the morphology had significantly changed from the elongated form to a deformed "dewdrop" (Fig. 1b). At 228 μg/L BP-3, planulae contain noticeably less zooxanthellae (brown spots) indicative of "bleaching" (Fig. 1c). The mouth of the planula at the oral pole began to increase three- to fivefold in diameter at the end of the 8-h exposure

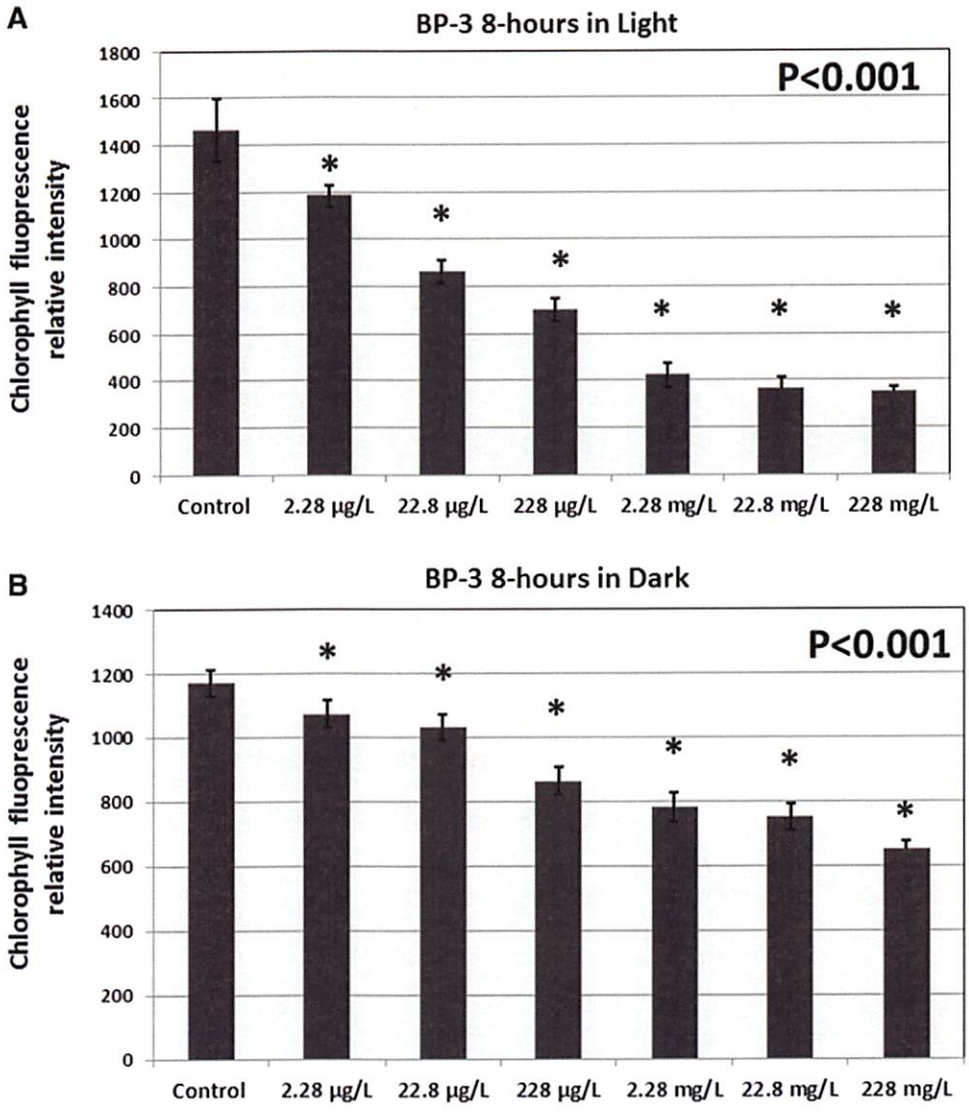

Fig. 2 Relative chlorophyll fluorescence emission at 685 nm with excitation at 445 nm of planulae of *Stylophora pistillata* exposed to various treatments of benzophenone-3 (BP-3). *Bars* show treatment means with whiskers representing ±1 standard error of the mean. $N = 4$ replicates per treatment. **a** Planulae exposed to various BP-3 concentrations for 8 h in the light. Treatment means with *different letters* differed significantly from the control at $\alpha = 0.05$, based on Kruskal–Wallis one-way analysis of variance on ranks followed by a Dunnett's Method post hoc test against a control. **b** Planulae exposed to various BP-3 concentrations for 8 h in the dark. Treatment means with different *superscript letters* differed significantly from the control at $\alpha = 0.05$, based on one-way analysis of variance followed by a Dunnett's Method post hoc test against control

(Fig. 1d). By the end of the 8 h of exposure for all BP-3 concentrations, the oral pole was recessed into the body in deformed planulae (Fig. 1b) and the epidermis of all the deformed planulae took on a white opaque hue. For planulae exposed to the higher concentrations of BP-3, it was apparent that the epidermal layer had lost its typical transparency and become opaque (Fig. 1, bracket indicates opaqueness of epidermal layer).

At the end of the 8-h exposure, all planulae exposed to all of the concentrations of BP-3 became sessile. Additionally, there was a positive relationship between exposure to increasing concentrations of BP-3 and planula bleaching (Figs. 1a–e, 2). Bleaching is the loss of symbiotic dinoflagellate zooxanthellae, photosynthetic pigments, or both. Chlorophyll fluorescence as an indicator of the concentration of chlorophyll *a* pigment corroborated these visual observations; exposure to BP-3, whether in light or darkness, caused planulae to bleach (Fig. 2). The Lowest Observable Effect Concentration for inducing chlorophyll-defined bleaching is 2.28 µg/L in the light ($P < 0.001$, Dunnett's Method) and 22.8 µg/L in the dark ($P < 0.01$, Dunnett's Method).

Normal planulae have four layers of organization. At the surface of the planula is the epidermis (Fig. 3a–c). The outer aspect of the epidermis has densely packed ciliated cells (Fig. 3a), spirocysts and nematocysts/blasts (Fig. 3b), and cells containing chromogenic organelles. Between the epidermis and the gastrodermal tissue layers is the mesoglea (Fig. 3c–d). Within the gastrodermal tissue are cells that contain symbiotic dinoflagellate zooxanthellae within an intracellular vacuole (Fig. 3e). Figure 3e depicts a healthy morphology, with the presence of starch granules, coherent chloroplasts, and the presence of a pyrenoid body that interfaces with chloroplasts. Figure 3f illustrates the integrity of chloroplasts (cp) within the dinoflagellate, especially the structure of the tri-partite rows of the

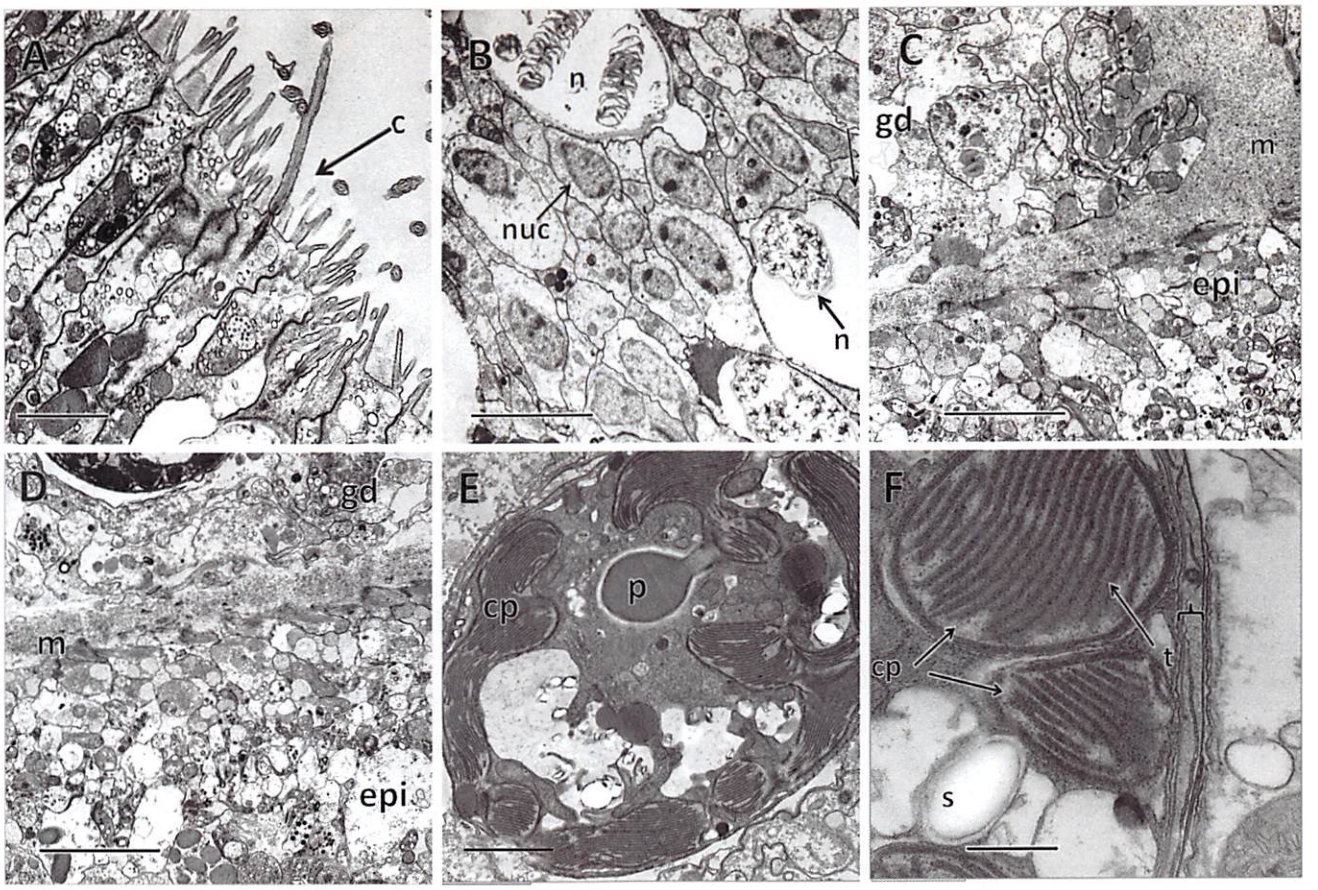

Fig. 3 Transmission electron microscopy of *Stylophora pistillata* planula control treatment. **a** Epidermal surface, indicating the presence of functional cilia (c) and tightly adjoined epidermal cells; *bar* indicates 2000 nm. **b** Epidermal surface indicates intact nematocysts (n) and nuclei (nuc); *bar* indicates 5000 nm. **c** Mesoglea (m) demarks the epidermal tissue (epi) from the gastrodermal tissue (gd); *bar* indicates 5000 nm. **d** Micrograph indicates the interface of the gastroderm (g), mesoglea (m), and epidermis (epi); *bar* indicates 5000 nm. **e** Zooxanthella in the gastrodermal tissue of planula, indicating the presence of intact chloroplasts (cp) and pyrenoid body (p). Notice the absence of a vacuolar space between the coral vacuolar membrane and the thecal plates/membrane of the zooxanthella; *bar* indicates 2000 nm. **f** Close-up of cytosolic structure of zooxanthella. Chloroplasts (cp) exhibit intact chloroplastic membrane and coherent, parallel rows of thylakoid membranes. *Bracket* (]) indicates the absence of vacuolar space between the coral vacuolar membrane and the zooxanthella's thecal plate/membrane; *bar* indicates 500 nm

thylakoid (t) membranes. Dinoflagellates from control planulae contained an abundance of starch granules (S), as well as the absence of vacuolated space between the dinoflagellate's thecal plate and the host's symbiophagic membrane (indicated by "{"; Fig. 3f).

Transmission electron microscopy of planula exposed to 288 parts per billion BP-3 for 8 h in the light (Fig. 4) showed that the planulae experienced catastrophic tissue lysis and cellular degradation in both the epidermis and gastrodermis, as well as partial collapse of the mesoglea (Figs. 3 vs. 4). At the surface of the epidermis, there was a complete loss of ciliated cells (Fig. 4a). The development and extent of cell death and tissue deterioration was greatest at the surface of the epidermis and became less pronounced at the center of the planula. In the middle area of the epidermal tissue, between the outer surface of the epidermis and its boundary with the mesoglea, the incidence of autophagic cell death became more pronounced (Fig. 4b; Tsujimoto and Shimizy 2005; Samara et al. 2008). Individual cells were dense with autophagic bodies, and many of the nuclei exhibited delamination of the nuclear bilayer membrane and vacuolization of the inner nuclear membrane containing chromatin (Fig. 4c; "}" indicates vacuolization; Eskelinin et al. 2011). None of the nuclei observed in the micrographs exhibited any signs of apoptosis, such as condensation of chromatin (Kerr et al. 1972; White and Cinti 2004; Taatjes et al. 2008). Specialized cells, such as spirocysts, also exhibited deterioration (Fig. 4d). The mesoglea exhibited structural deterioration; this vascular space contained an abundance of debris, including detached cells (Fig. 4e). The gastrodermis also exhibited extensive trauma (Fig. 4e–g). Many gastrodermal cells exhibited considerable dense autophagic bodies (Fig. 4f), although there were a few instances of

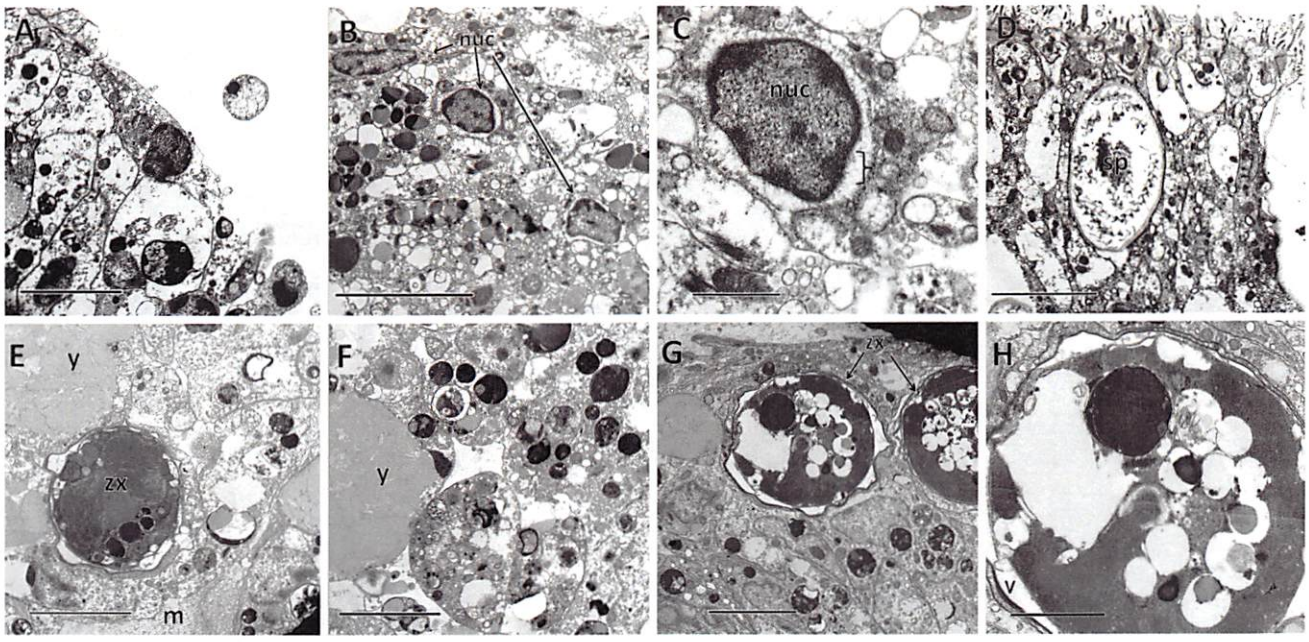

Fig. 4 Transmission electron microscopy of *Stylophora pistillata* planula exposed to 228 parts per billion (μg/L) benzophenone-3 for 8 h in the light. **a** Surface of the epidermal layer; indicating a lack of cilia and cells dying either via necrosis or autophagic cell death; *bar* indicates 5000 nm. **b** Epidermal tissue where cells exhibit an abundance of vacuolated bodies, especially the presence of vacuolated nuclei (nuc); *bar* indicates 5000 nm. **c** Magnification of vacuolated nuclei (nuc) that completely lacks nuclear blebbing (a sign of apoptosis). "}" indicates vacuolization of delaminated nuclear double membrane; *bar* indicates 1000 nm. **d** Epidermal layer with vacuolated ciliated cells, spirocysts (sp) and nematocysts; *bar* indicates 5000 nm. **e** Micrograph depicts intersection of mesoglea (m) and gastrodermal tissue containing both zooxanthella (zx) gastrodermal cells and yolk (y); *bar* indicates 5000 nm. **f** Epidermal tissue adjacent to yolk exhibits extensive autophagic vacuolization; *bar* indicates 5000 nm. **G** Gastrodermal cells containing symbiophagic zooxanthellae. Zooxanthellae have undergone extensive internal vacuolization; *bar* indicates 5000 nm. **h** Increased magnification focused on vacuolated zooxanthella, (v) indicates symbiophagic vacuole; *bar* indicates 2000 nm

nuclear autophagy. Gastrodermal cells containing symbiotic zooxanthella exhibited the early stages of symbiophagy, with vacuolization occurring around the zooxanthella (Fig. 4e–g). None of the zooxanthellae showed "normal" morphologies. They instead displayed extensive internal vacuolization, homogenization of chromatin density, and chloroplast degradation, especially of the thylakoid membranes (Fig. 4g–h).

Transmission electron microscopy of planulae exposed to 228 μg/L BP-3 for 8 h in darkness (Fig. 5) exhibited a similar gradient of cell death and tissue deterioration from the surface of the planula to its center as seen in planulae exposed to BP-3 in the light, although the progression of cellular deterioration was not as severe (Fig. 5a–h). Along the surface of the epidermal tissue layer, ciliated cells were undergoing cellular degradation (Fig. 5a). The cell layer immediately below the ciliated cells was degraded, characterized by an abundance of vacuolated bodies and loss of the plasma membrane (Fig. 5b, c). Many of the nuclei exhibited partial delamination of the bilayer nuclear membrane, but unlike the nuclei observed in planulae exposed to BP-3 in the light, vacuolization was not complete and the bilayer was still partially anchored by nuclear pores (Fig. 5b, c). Deeper into the epidermal layer, along the boundary with the mesoglea, cellular degradation persisted, especially of the spirocysts (Fig. 5d). There is an extracellular matrix that acts as a barrier between the epidermal tissue and mesoglea, and again between the gastrodermal tissue and mesoglea. Under these conditions, the integrity of the boundary layer between the epidermis and mesoglea had severely deteriorated, whereas the boundary layer between the gastrodermis and mesoglea remained intact (Fig. 5e). Within the gastrodermis, a vast majority of the cells were alive, but exhibiting signs of massive autophagy (Fig. 5f; Klionsky et al. 2012). It should be noted that there were almost no instances of delamination of the nuclear membrane in the gastrodermal cells; nuclei looked healthy (Fig. 5f). Many of the cells were dense with autophagosomic bodies, and most of the zooxanthellae were undergoing symbiophagy, as indicated by the vacuolization around the dinoflagellate cell (Fig. 5f; Downs et al. 2009). In zooxanthellae that were not significantly degraded (Fig. 5f vs. h), thylakoids exhibited a pathomorphology similar to that found in zooxanthellae of corals exposed to heat stress (32 °C) in darkness; thylakoid lamellae were diffuse (Fig. 5g; Downs et al. 2013), suggesting that the zooxanthellae were directly affected by the BP-3 exposure. In contrast to the findings of Danovaro

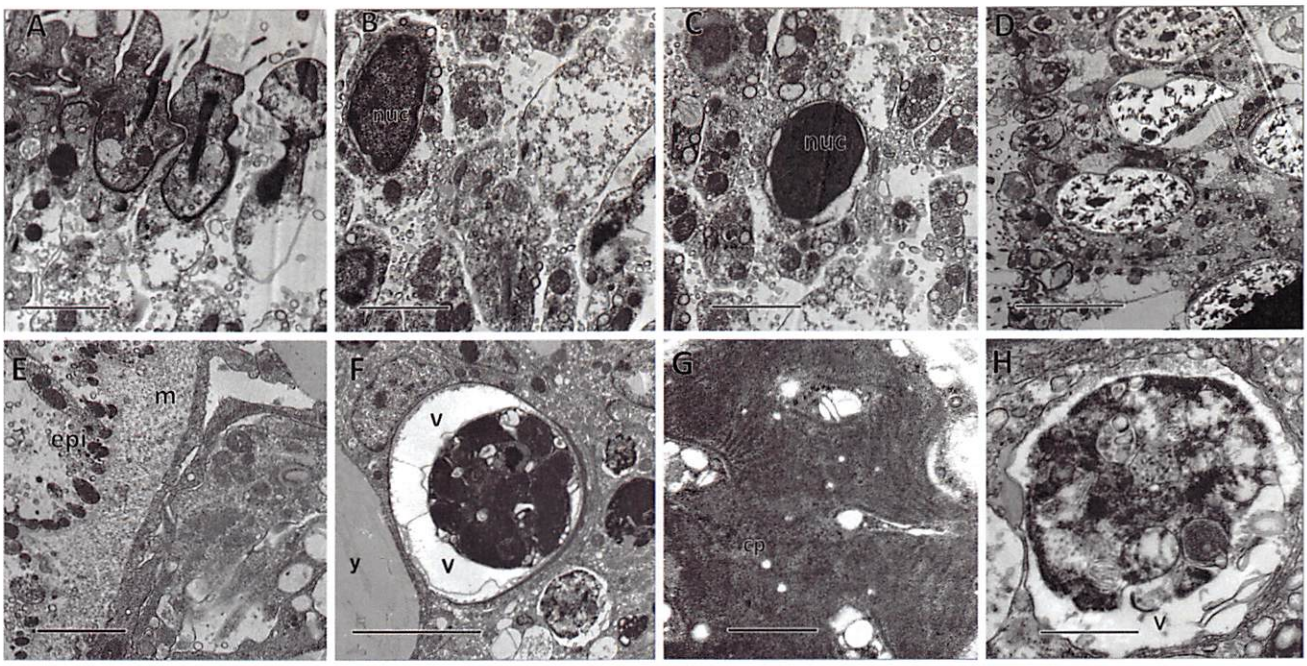

Fig. 5 Transmission electron microscopy of *Stylophora pistillata* planula exposed to 228 parts per billion (μg/L) benzophenone-3 for 8 h in the dark. **a** Surface of the epidermal layer; ciliated cells are present, but undergoing early stages of autophagic cell death. Cells beneath the cilia layer exhibiting late stage autophagic cell death and necrosis. Note scratches in the micrograph; *bar* indicates 2000 nm. **b** Epidermal tissue area between cilia and nematocyst layer showing extensive vacuolization. Early stages of nuclear vacuolization (nuc). Note scratches in the micrograph; *bar* indicates 2000 nm. **c** Epidermal tissue in area exhibiting advanced stages of cell death; nucleus vacuolization (nuc). Note scratches in the micrograph; *bar* indicates 2000 nm. **d** Extensive vacuolization of cells surrounding nematocysts. Note scratches in the micrograph; *bar* indicates 5000 nm. **e** Mesoglea (m), gastrodermal and epidermal tissues. Symbiophagy occurring to zooxanthella (zx) surrounded by extensive vacuolization in neighboring cells; *bar* indicates 2000 nm. **f** Gastrodermal tissue and yolk (y). All cells exhibiting extensive vacuolization (v), especially within the gastrodermal cell surrounding the zooxanthella. Coral cells showing increased level of autophagosome content but no signs of autophagic cell death or necrosis; *bar* indicates 5000 nm. **g** Zooxanthella chloroplast with thylakoid dispersion-pathomorphologies. Chloroplast (cp); *bar* indicates 1000 nm. **h** Zooxanthella exhibiting extensive pyknosis; symbiophagic vacuole (v); *bar* indicates 1000 nm

et al. (2008), viral inclusion bodies were not observed in our electron microscopy examination.

During the initial examination of the planulae using transmission electron microscopy, scratches in the micro-sections under observation were readily apparent (Figs. 5a–c and 6). Scratches to the microsection can arise as a result of hardened particles from the sample that scrape between the diamond blade and micro-sectioned sample (Carson 1997; Crang and Klomparens 1988). This is a common occurrence in biological samples that contain $CaCO_3$ skeleton (coral or vertebrates). These scratches are preventable if the samples are first decalcified before embedding in a resin and sectioned (Crang and Klomparens 1988). Coral planula samples do not normally need to be decalcified, because they should contain no aragonite skeletal matrix. An Alizarin red stain confirmed the presence of a $CaCO_3$ crystal matrix on the surface of the planula (data not shown; Barnes 1972). Decalcifying the fixed coral planulae with EDTA before embedding the sample in resin alleviated the "scratch" artifact and the remaining samples that were processed using a decalcification step were devoid of scratches.

Increasing concentrations of BP-3 induced significantly higher levels of DNA AP lesions in planulae exposed to the light compared to the controls (Fig. 7a, b), as well as planulae exposed to BP-3 in the dark (Fig. 7c, d).

No-Observed-Effect Concentration

Estimating Lowest-observed-effect Concentration (NOECs) for planulae exposed to BP-3 for 8 h was problematic because responses in the control treatment were homogeneous (Shapiro–Wilk; $P < 0.05$); all planulae survived and were not deformed, so analyses defaulted to the less powerful, nonparametric method (Steel 1959). The NOEC for both the proportion of live coral planulae and nondeformed planulae exposed to BP-3 for 8 h in either the light or the dark was 228 ppmillion (mg/L) (Steel Method (Steel 1959), all $Z > 2.32$, $P < 0.0809$; Fig.8a, c). In contrast to the Steel Method, the NOEC for planulae in the light determined by a Kruskal–Wallis One-Way Analysis of Variance on Ranks was 228 μg/L (H Statistic = 21.903; $P \leq 0.001$; Dunnett's Procedure). The NOEC for planulae

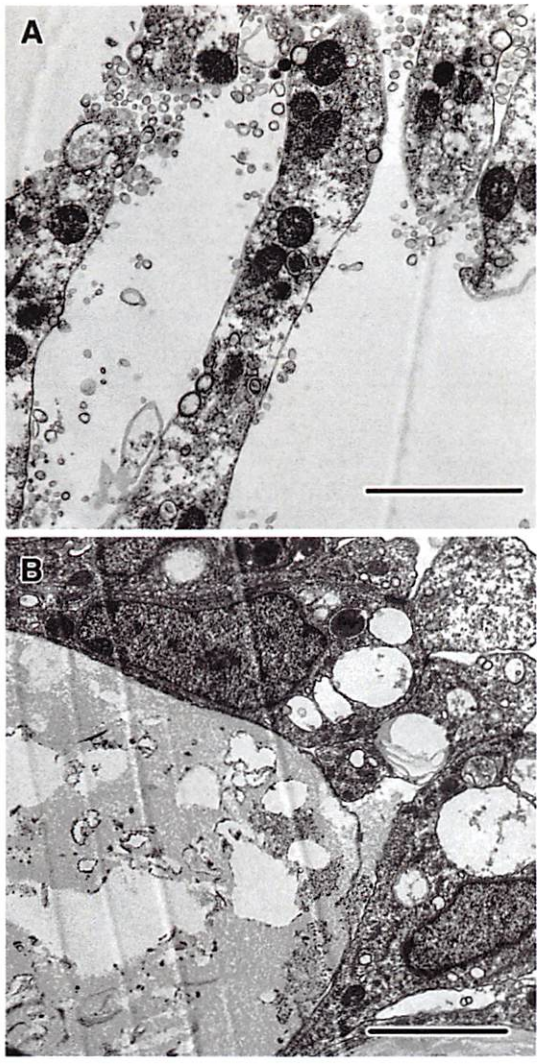

Fig. 6 "Scratch" artifacts in transmission electron microscopy micrographs of *Stylophora pistillata* planula exposed to 288 parts per billion (μg/L) benzophenone-3. When microsectioning planula embedded in a plastic resin without first decalcifying the sample, scratches can manifest on the mounted ultrathin sections. The scratches form as a result of the diamond blade fracturing the aragonite skeleton and pieces of the skeleton adhering to the edge of the diamond blade. As the contaminated blade cuts through the sample block, it scratches the ultrathin sections of the sample. These scratches can be alleviated by cleaning the diamond blade and removing aragonite skeleton in the sample through decalcification before embedding the sample in a resin. **a** Scratches apparent in ultrathin section of epidermal section of a planula; *bar* indicates 2000 nm. **b** Scratches apparent in ultrathin section of gastrodermal section of a planula; *bar* indicated 5000 nm

in the dark determined by a Kruskal–Wallis One-Way Analysis of Variance on Ranks was 228 μg/L (H Statistic = 22.402; $P \leq 0.001$; Dunnett's Procedure).

Estimates for NOECs for planulae exposed to BP-3 for 24 h in light or darkness also were problematic because responses in the control and at all concentrations greater than 22.8 μg/L (in certain cases, ≥2.28 μg/L) were homogeneous (Fig. 8b, d); all planulae survived and were not deformed in the control but died at the higher concentrations (Laskowski 1995). Using the nonparametric Steel Method, we determined the NOEC as 2.28 μg/L for the proportion of coral planulae alive after 24 h of exposure to BP-3 in the light and 22.8 μg/L in the dark (both $Z = 2.48$, $P = 0.0543$). The corresponding NOECs for non-deformed planulae were identical to these values (Fig. 9a, c). In contrast, the NOEC for planulae exposed for 24 h in the light, determined by a Kruskal–Wallis One-Way Analysis of Variance on Ranks, was 228 μg/L (Fig. 9b; H Statistic = 22.084; $P \leq 0.001$; Dunnett's Procedure). The NOEC for planulae exposed for 24 h in darkness, determined by a Kruskal–Wallis One-Way Analysis of Variance on Ranks, was 228 μg/L (Fig. 9d; H Statistic = 22.112; $P \leq 0.001$; Dunnett's Method).

The NOEC for DNA abasic sites in planulae met ANOVA assumptions and was determined as 22.8 μg/L (100 nM; one-way ANOVA $F_{4,15} = 73.1$, $P < 0.0001$, $R^2 = 0.95$; Dunnett's Method for this comparison, $P < 0.0001$) when exposed in the light, and 22.8 μg/L (100 nM) when exposed in the dark (Welch ANOVA $F_{5,7.67} = 142.1$, $P < 0.0001$; Dunnett's Method for this comparison, $P < 0.0001$). The NOEC for mortality of *S. pistillata* calicoblast cells was below the 570 ng/L concentration for cells exposed to the dark for 4 h (Fig. 10a, b). The NOEC for mortality of *S. pistillata* calicoblast cells was 570 ng/L for cells exposed to the light for 4 h (Fig. 10c, d).

LC_{50}, EC_{50}, and EC_{20} Values

Regression models used to estimate median LC_{50} (concentration expected to cause death in 50 % of the population), EC_{20} and median EC_{50} (effective concentrations, which adversely affect 20 and 50 % of the population, respectively) after 8 h of exposure to BP-3 had coefficients of determination (R^2) between (0.91 and 0.97). Using regression models, the median LC_{50} for the proportion of live coral planulae exposed in the light was 3.1 mg/L, whereas for planulae exposed in the dark, the LC_{50} was 5.4 times higher: 16.8 mg/L (Table 1; Supplemental Fig. 1a, c). PROBIT analysis for LC_{50} in the light was 2.876 mg/L (mg/L), whereas LC_{50} in the dark was 12.811 mg/L (Table 1; Supplemental Fig. 2a, c).

Models used to estimate LC_{50} and EC_{50}, of coral planulae after 24 h of exposure to BP-3 continued to explain the substantial variation ($0.86 < R^2 \leq 0.997$). The 24 h-LC_{50} for the proportion of live coral planulae, after exposure in the light, was just 103.8 μg/L (ppbillion) compared with 873.4 μg/L in the dark exposure (Table 1; Supplemental Fig. 1b, d). PROBIT analysis for 24-h LC_{50} in the light was 139 μg/L, whereas LC_{50} in the dark was 799 μg/L (Table 1; Supplemental Fig. 2b, d).

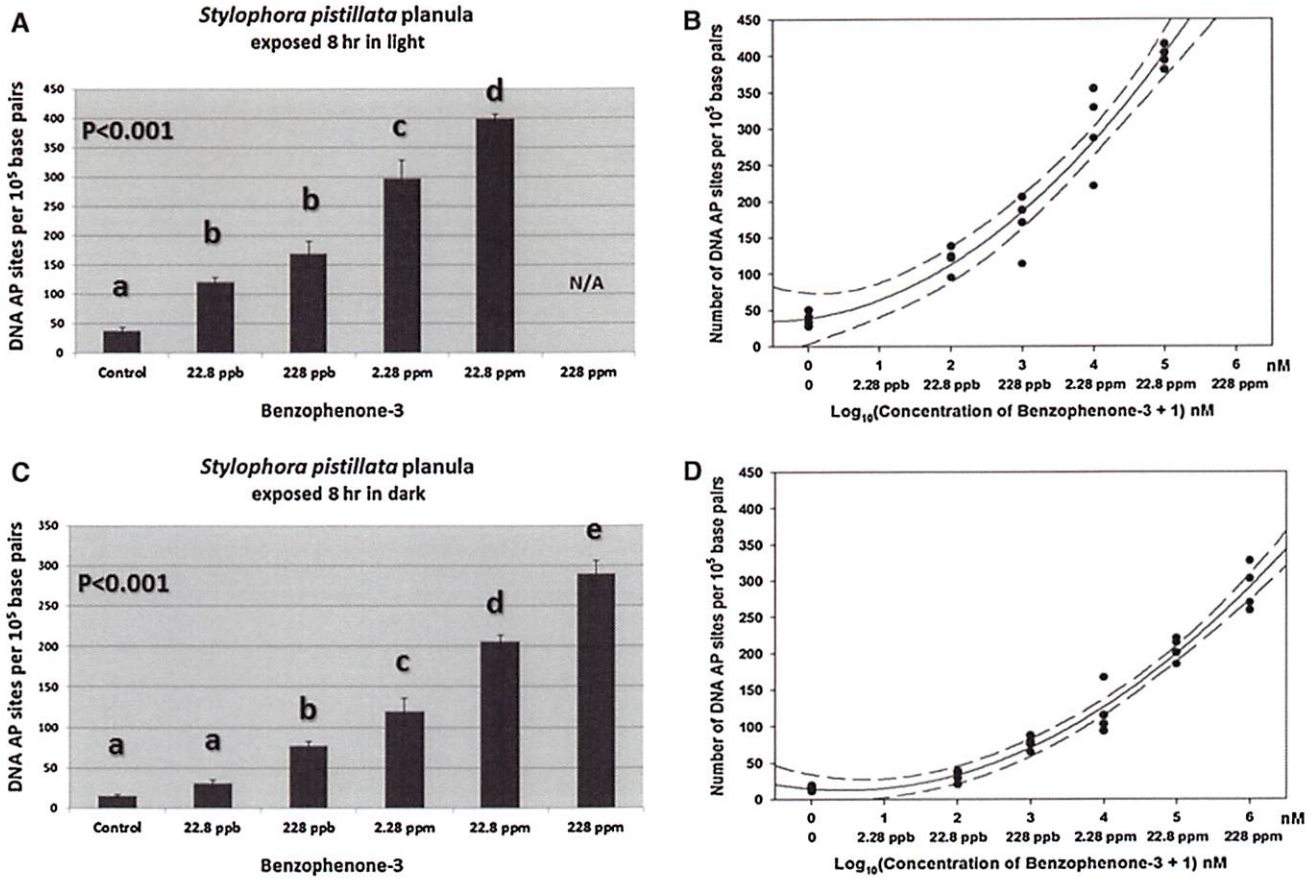

Fig. 7 Number of DNA apyrimidinic lesions in planulae of *Stylophora pistillata* exposed to various concentrations of benzophenone-3 (BP-3). *Bars* show treatment means of four replicates with whiskers representing ±1 standard error of the mean. Treatment means with *different letters* differed significantly at $\alpha = 0.05$, based on Kruskal–Wallis one-way analysis of variance on ranks followed by a Student–Newman–Keuls Method post hoc test. **a** Planulae exposed for 8 h in the light. **b** Log-linear regression between DNA AP lesions of coral planulae of *Stylophora pistillata* exposed to concentrations of BP-3 for 8 h in the light. Quadratic regression line (*solid*) and 95 % confidence intervals (*dashed lines*) are shown. **c** Planulae exposed for 8 h in the dark. **d** Log-linear regression between DNA AP lesions of coral planulae of *Stylophora pistillata* exposed to concentrations of BP-3 for 8 h in the dark

The 8-h EC_{50} for nondeformed planulae exposed to BP-3 in the light and dark were much lower: 107 and 436 µg/L, respectively using regression modeling (Table 1; Supplemental Fig. 3a, c). PROBIT analysis for 8-h EC_{50} in the light was 133 ppbillion (µg/L), whereas EC_{50} in the dark was 737 µg/L (Table 1; Supplemental Fig. 4a, c). PROBIT analysis for 8-h EC_{20} in the light was 6.3 µg/L, whereas EC_{20} in the dark was 15.5 µg/L (Table 1; Supplemental Fig. 4a, c). The 24-h EC_{50} for nondeformed planulae exposed in the light and dark were much lower: 17 ppbillion and 105 µg/L, respectively using regression modeling (Table 1; Supplemental Fig. 3b, d). PROBIT analysis for 24-h EC_{50} in the light was 49 µg/L, whereas LC_{50} in the dark was 137 µg/L (Table 1; Supplemental Fig. 4a, d). PROBIT analysis for 24-h EC_{20} in the light was 6.5 µg/L, whereas EC_{50} in the dark was 10.4 µg/L (Table 1; Supplemental Fig. 4b, d).

The number of DNA abasic sites increased approximately tenfold across the BP-3 concentration gradient in the light, but nearly 20-fold in the dark (Fig. 7b, d). Similarly, the percentage of dead coral cells increased dramatically with increasing concentrations of BP-3, but the LC_{50} was much lower in the light at 39 µg/L than in the dark at 842 µg/L. PROBIT analysis for 4-h LC_{50} coral cells in the light was 42 ppbillion, whereas LC_{50} in the dark it was 679 µg/L (Table 2; Supplemental Fig. 5a, b).

Species Sensitivity Distribution Using Coral Cell Toxicity Assay

To provide a perspective of the differences in sensitivities of various species of Indo-Pacific and Caribbean coral reefs, the LC_{50}s and LC_{20}s with their corresponding upper

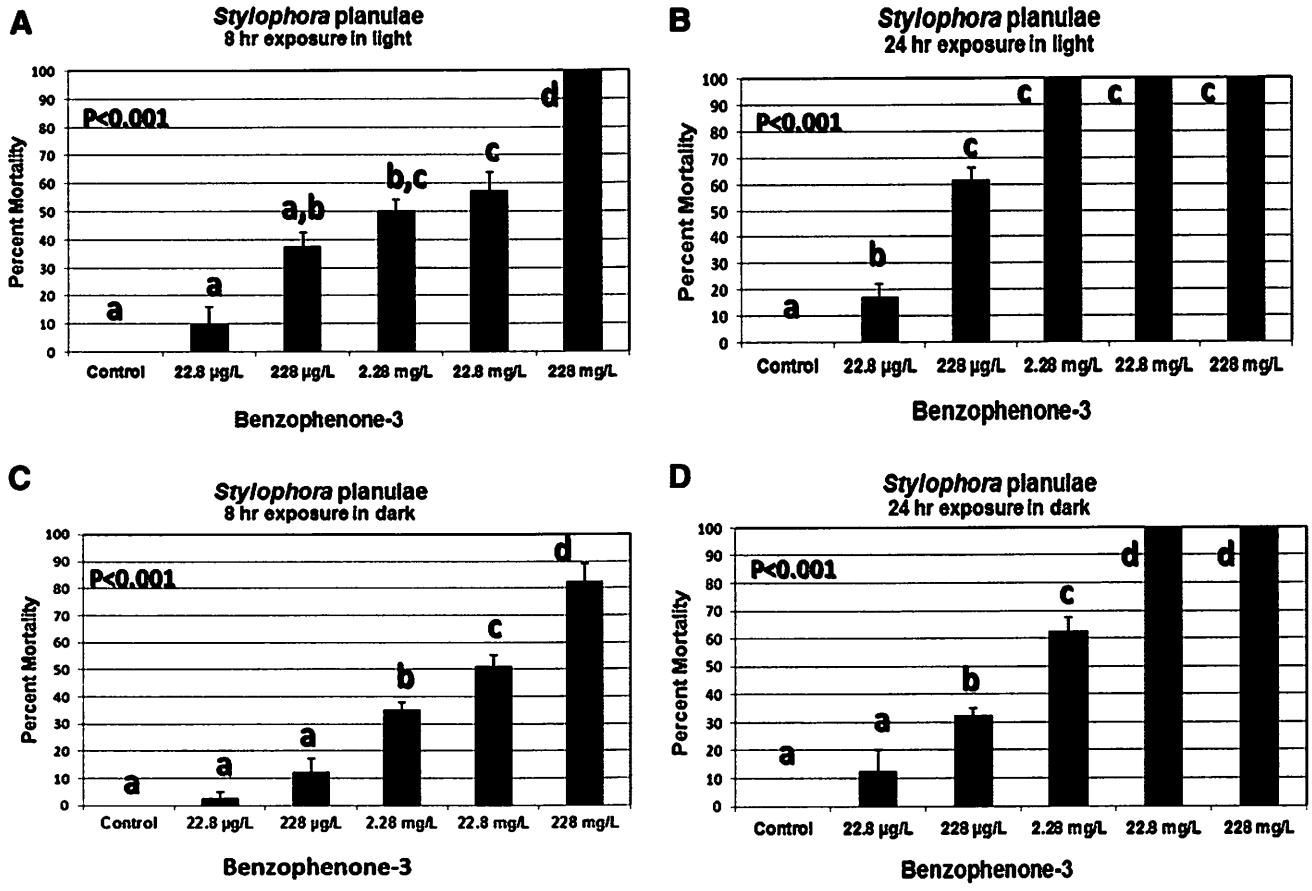

Fig. 8 Percent mortality of planula of *Stylophora pistillata* exposed to various concentrations of benzophenone-3. *Bars* show treatment means with whiskers representing ±1 standard error of the mean. Treatment means with *different letters* differed significantly at $\alpha = 0.05$, based on Kruskal–Wallis one-way analysis of variance on ranks followed by a Student–Newman–Keuls Method post hoc test. **a** Planulae exposed for 8 h in the light. **b** Planulae exposed for 8 h in the light and then 16 h of darkness. **c** Planulae exposed for 8 h in the dark. **d** Planulae exposed for 24 h in the dark

and lower 95 % confidence intervals for the two Indo-Pacific and five Caribbean species are provided in Table 1.

Correction Factor Between Mortality of Coral Planulae and Coral Cells

Coral cells were much more sensitive than coral planulae across a wide range of BP-3 concentrations, which makes cell mortality a potential indicator of reproductive and recruitment failures. To estimate the correction factor needed to translate coral cell mortality into potential mortality of coral planulae, one option is the use of a quadratic regression model to estimate these relationships: In the light ($F_{2,21} = 43.8$, $P < 0.0001$, $R^2 = 0.81$) % mortality of planulae = 2.26 − 0.28 (% mortality of cells) + 0.0107 (% mortality of cells)^2 In the dark ($F_{2,21} = 84.5$, $P < 0.0001$, $R^2 = 0.89$) % mortality of planulae = 0.86 − 0.0007 (% mortality of cells) + 0.0078 (% mortality of cells)^2

Environmental Chemistry Analysis

The purpose of the chemical analysis was to conduct a cursory survey of BP-3 concentrations on coral reefs. Seawater samples were collected from bays in St. John Island, U.S. Virgin Islands: Caneel Bay, Hawksnest Bay, and Trunk Bay in April 2007 (Fig. 11a, b). Caneel Beach is managed by the resort, Caneel Bay. Samples were collected at approximately 16:30 h near the dive platform that adjoins the Caneel Beach and along a large coral community that spans from the edge of Caneel Beach to the edge of Honeymoon Beach. There were 17 swimmers in Caneel Bay in the 48-h period before sampling. Swimmers were monitored from the shore of the resort from dawn to dusk. No benzophenones could be detected in either of the samples collected in Caneel Bay.

Hawksnest Bay is a densely visited beach within the U.S. National Park system on St. John Island. In general, more than 1000 visitors per day can enter into this bay. On the day of sampling, more than 230 people entered the

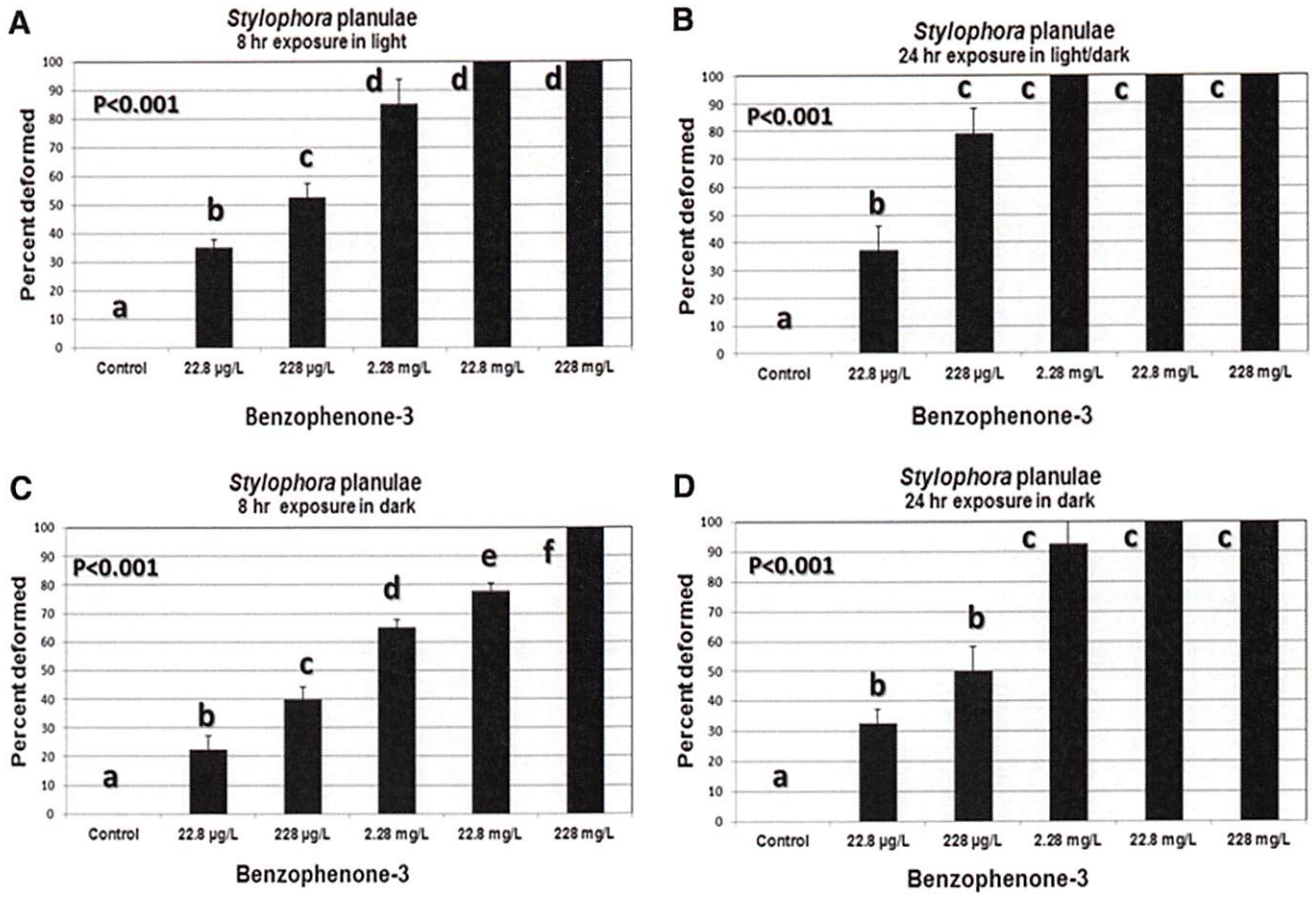

Fig. 9 Percentage of deformed planulae of *Stylophora pistillata* exposed to various concentrations of benzophenone-3. *Bars* show treatment means with whiskers representing ±1 standard error of the mean. Treatment means with *different letters* differed significantly at $\alpha = 0.05$, based on Kruskal–Wallis one-way analysis of variance on ranks followed by a Student–Newman–Keuls Method post hoc test. **a** Planulae exposed for 8 h in the light. **b** Planulae exposed for 8 h in the light, then 16 h of darkness. **c** Planulae exposed for 8 h in the dark. **d** Planulae exposed for 24 h in the dark

water and swam within 20 m of the three large *Acropora palmata* spurs (coral reefs) indicated in Fig. 11c; the majority swam in the sandy grooves that lie between the coral-reef spurs. These spurs are very shallow (1–3 m deep), with live coral often protruding above the surface of the water during low tide. The concentration of BP-3 in the western groove was 75 ppbillion (µg/L), whereas the larger, eastern groove had a BP-3 level of 95 ppbillion (µg/L). Samples were collected between 17:00 and 17:40 h.

Trunk Bay is an iconic landscape and a highly managed natural resource area. Before 2009, there could be more than 3000 visitors on the beach and in the water at Trunk Bay. After 2009, National Park Service policy reduced the number to 2000 visitors per day (personal communication, Rafe Boulon, retired, USVI NP Chief, Resource Management). A coral community surrounds the island in Trunk Bay, as well as an abundance of gorgonians to the west of the island, and there was once a very extensive stand of *A. palmata* corals to the east of the island. At a site near the edge of the Trunk Island coral community, BP-3 levels were 1.395 ppmillion (mg/L) (Fig. 11d). A sampling site 93 m east of the first sampling site contained 580 ppbillion (µg/L) BP-3 (Fig. 11d). Samples were collected at 11:00–11:24 h with more than ~180 swimmers in the water and ~130 sunbathers on the beach within 100 m of the two sampling sites.

Seawater samples were collected at five sites in Maunalua Bay, Oahu Island, Hawai'i on May 30, 2011 between 11:00 and 15:00 h (Fig. 12a, b). ASW samples were collected in public swimming areas in waters that were 1.3 m in depth and 35 cm from the surface of the water. Sites 1–4 had detectable levels of BP-3 (>100 pptrillion; ng/L) but were below the quantitative range of measurement (5 ppbillion (µg/L); Fig. 12b). Site 5 contained measurable levels of BP-3—19.2 ppbillion (µg/L) (Supplemental Fig. 6).

Samples were collected at two sites on June 3, 2011, along the northwest coast of Maui Island, Hawai'i (Fig. 12c). Kapalua Bay is a protected cove and has a

public beach that can often see >500 swimmers/day in the peak tourism season (personal communication, Kapalua Dive Co.; Fig. 12d). A seawater sample was collected 40 m from shore near the center of the bay, immediately above remnants of a coral reef at 09:30 h. The Kapalua sample had detectable levels of BP-3 but was below the quantitative range of measurement (5 ppbillion, 5 µg/L). From 06:30 to 09:30 h on the day of sampling, 14 swimmers had entered Kapaula waters. A seawater sample also was collected at Kahekili Beach Park, Maui Island, Hawai'i (Fig. 12e). Kahekili Beach is a public beach that also serves visitors from a number of nearby hotels and resorts. The sample was collected 30 m from shore, immediately above a coral reef. Unlike Kapalua, Kahekili is an exposed shoreline not protected within a bay, and retention time of contaminants is thought to be minimal because of the prevailing currents. The Kahekili sample had detectable levels of BP-3 but was below the quantitative range of measurement (5 ppbillion). Kahekili is a heavily visited beach and had 71 swimmers within 200 m of the sampling site at the time of sampling (11:45 h).

Table 1 Regression and PROBIT determination of LC_{50} for planulae mortality when exposed to BP-3 in the light and dark, and the EC_{50} for planulae deformity when exposed to BP-3 in the light and the dark

Planulae mortality	LC_{50}
Regression to estimate LC_{50} 8-h light	3.1 mg/L
PROBIT to estimate LC_{50} 8-h light	2.9 mg/L
Regression to estimate LC_{50} 8-h dark	16.8 mg/L
PROBIT to estimate LC_{50} 8-h dark	12.8 mg/L
Regression to estimate LC_{50} 24-h light	103.8 µg/L
PROBIT to estimate LC_{50} 24-h light	1.39 µg/L
Regression to estimate LC_{50} 24-h dark	873.4 µg/L
PROBIT to estimate LC_{50} 24-h dark	799 µg/L
Planulae deformation	**EC_{50}**
Regression to estimate EC_{50} 8-h light	107 mg/L
PROBIT to estimate EC_{50} 8-h light	133 mg/L
Regression to estimate EC_{50} 8-h dark	436 mg/L
PROBIT to estimate EC_{50} 8-h dark	737 mg/L
Regression to estimate EC_{50} 24-h light	17 µg/L
PROBIT to estimate EC_{50} 24-h light	49 µg/L
Regression to estimate EC_{50} 24-h dark	105 µg/L
PROBIT to estimate EC_{50} 24-h dark	137 µg/L
Planulae deformation	**EC_{20} (µg/L)**
PROBIT to estimate EC_{20} 8-h light	6.3
PROBIT to estimate EC_{20} 8-h dark	15.5
PROBIT to estimate EC_{20} 24-h light	6.5
PROBIT to estimate EC_{20} 24-h dark	10.4

PROBIT determination of EC_{20} for planulae deformity when exposed to BP-3 in the light and the dark

Discussion

Toxicopathology

Benzophenone-3 is a phototoxicant and induces different toxicities depending on whether the planulae are exposed to the chemical in light or in darkness. Corals will usually release brooded planulae at night or spawn gametes at night (Gleason and Hofmann 2011). Planulae of broadcasting species (those that spawn eggs and sperm that are fertilized in the water column) are positively buoyant and planktonic, residing at or near the surface of the ocean for 2–4 days before they are able to settle (Fadlallah 1983; Shlesinger and Loya 1985; Harii et al. 2007; Baird et al. 2009). Light levels on a clear sunny day in tropic latitudes can be as high as or higher than 2000 µmol/m^2/s of photosynthetically active radiation—five times more than what the corals experienced in this study, suggesting that actual environmental conditions may aggravate the phototoxicity. Whether the BP-3 pollution comes from swimmers, or from point and nonpoint wastewater sources, planulae will be at

Table 2 Differences in sensitivities of various species of Indo-Pacific and Caribbean coral reefs, the LC_{50}s and LC_{20}s of calicoblast cells exposed in vitro to benzophenone-3 with their corresponding upper and lower 95 % confidence intervals for the two Indo-Pacific and five Caribbean species. (µg/L) = to parts per billion. (ng/L) = parts per trillion

Coral species	LC_{50} (µg/L)	95 % CI	LC_{20}	95 % CI
Indo-Pacific species				
Stylophora pistillata (light)	42	28; 60	2 µg/L	1.14; 3.61
Stylophora pistillata (dark)	671	447; 984	14 µg/L	7; 26
Pocillopora damicornis	8	4.96; 12.15	62 ng/L	24; 136
Caribbean-Atlantic species				
Acropora cervicornis	9	5.4; 14.5	63 ng/L	22; 150
Montastrea annularis	74	40; 126	562 ng/L	166; 1459
Montastrea cavernosa	52	36; 72	502 ng/L	247; 921
Porites astreoides	340	208; 534	8 µg/L	3; 16
Porites divaricata	36	21; 57	175 ng/L	60; 420

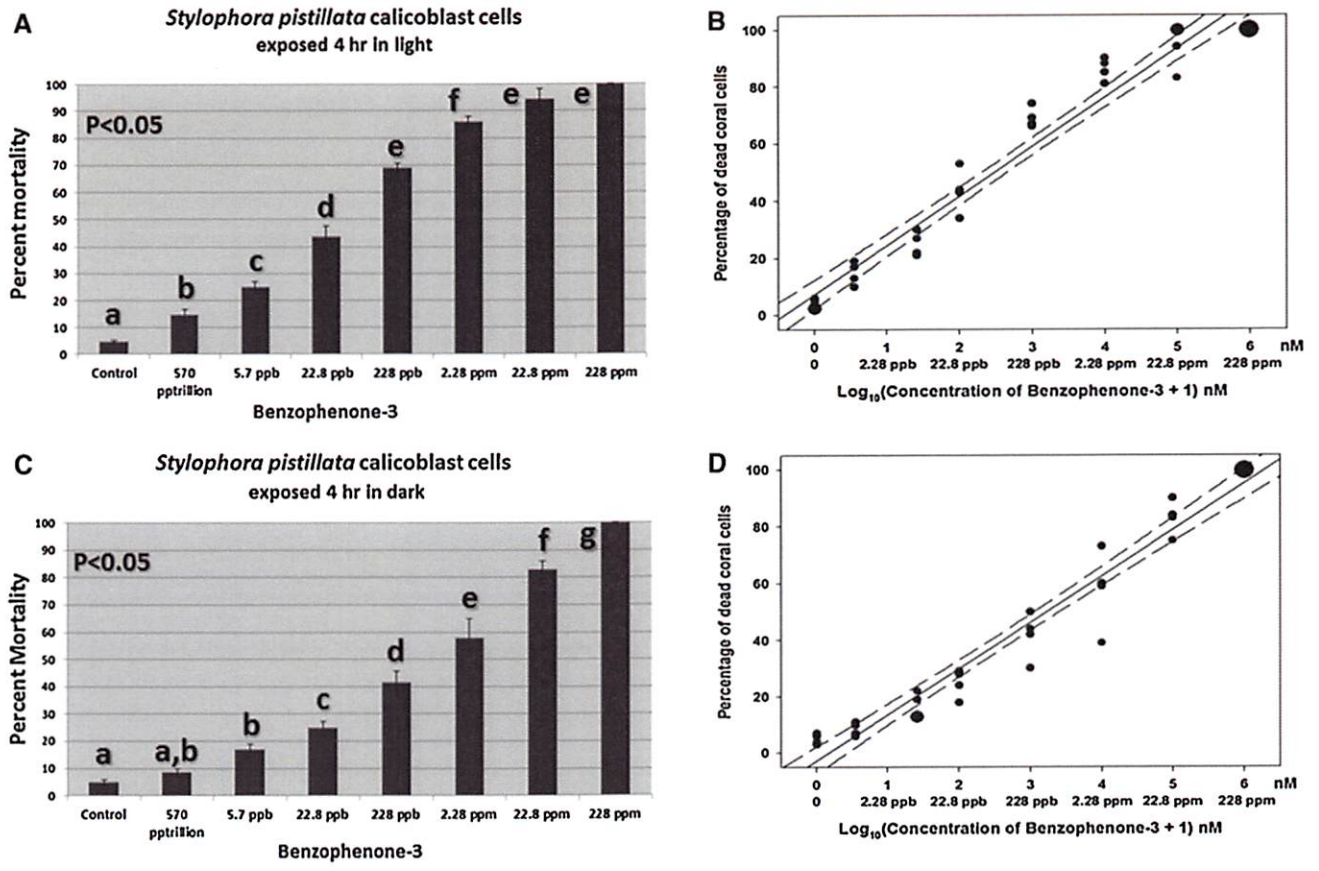

Fig. 10 Percentage mortality of calicoblast cells of *Stylophora pistillata* exposed to various concentrations of benzophenone-3. *Bars* show treatment means ($n = 4$) with whiskers representing ± 1 standard error of the mean. Treatment means with *different letters* differed significantly at $\alpha = 0.05$, based on one-way analysis of variance followed by a Tukey's Honestly Significant Difference Test. **a** Calicoblast cells exposed for 4 h in the light. **b** Log-linear regression between coral cell mortality and concentrations of BP-3 for 4 h in the light. Quadratic regression line (*solid*) and 95 % confidence intervals (*dashed lines*) are shown. *Larger symbols* represent multiple coincident data points, with symbol area proportional to the number of replicates with the same value. **c** Calicoblast cells exposed for 4 h in the dark. **d** Log-linear regression between coral cell mortality and concentration of BP-3 for 4 h in the dark

risk from both forms of toxicities (Brooks et al. 2009; Futch et al. 2010; Pitarch et al. 2010).

As with our previous paper examining benzophenone-2 (Downs et al. 2014), the data in this paper are consistent with the observation by Danovaro et al. (2008) that "sunscreens compounds" cause coral bleaching. In the light, BP-3 caused injury directly to the zooxanthellae, independent of any host-regulated degradation mechanism. Based on the pathomorphology of the thylakoids within the chloroplasts, the most probable interpretation is that BP-3 induces photo-oxidative stress to the molecular structures that form the thylakoid membranes (Downs et al. 2013). In darkness, bleaching resulted from the symbiophagy of the symbiotic zooxanthellae; a process whereby the coral gastrodermal cell "digests" the zooxanthella (Downs et al. 2009). Nesa et al. (2012) demonstrated that following exposure to light, the algal symbionts of corals increased the DNA damage to coral cells in coral planulae. Consistent with the Oxidative Theory of Coral Bleaching (Downs et al. 2002), Nesa et al. hypothesized that the sources of this damage was the production of oxygen radicals. If this is the case, then darkness-associated, BP-3-induced bleaching may reduce the exacerbated morbidity experienced by "bleached" planulae that would occur during the periods of daylight. Regardless of the toxicological mechanism, managing exposure of corals to BP-3 corals will be critical for managing coral reef resilience in the face of climate-change pressures associated with coral bleaching (West and Salm 2003).

Autophagy was the dominant cellular response to BP-3 exposure (Figs. 4a–f, 5b–d; Yla-Antilla et al. 2009). Micro-autophagosomes were abundant in all cell types and larger vacuolated bodies of specific organelles were readily observed. None of the nuclei in any coral cell-types exhibited any of the classic signs of apoptosis, such as pyknosis or karyorrhexis of the nucleus (Krysko et al.

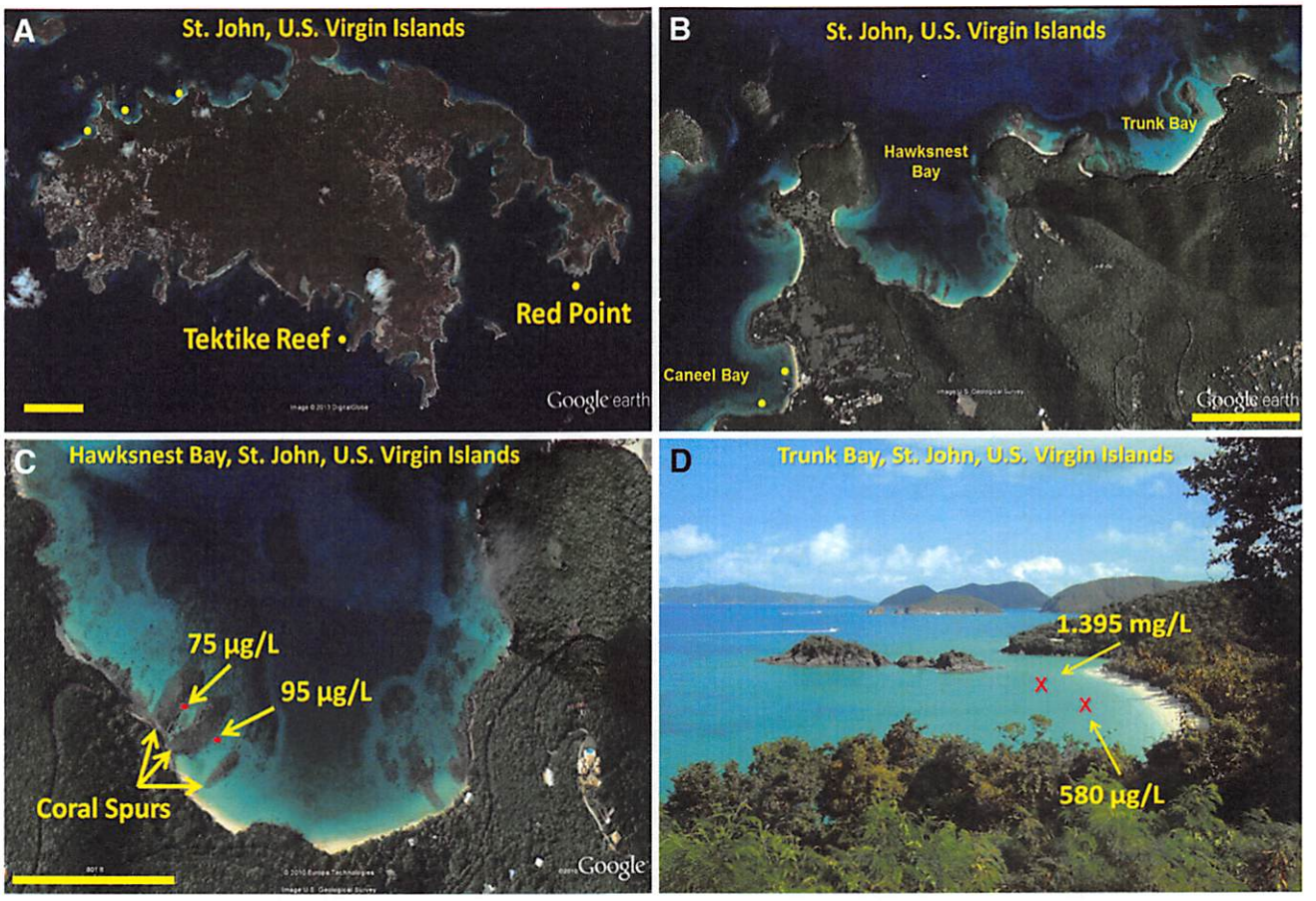

Fig. 11 Seawater analysis of benzophenone-3 (BP-3) in coral reef areas in St. John Island, U.S. Virgin Islands. **a** Aerial view of St. John indicating the five sampling sites, indicated by a *yellow dot*. No benzophenones were detected in samples from *Red Point* or at Tektite Reef. All samples were taken between 12:00 and 14:00 h. *Scale bar* is 1.5 km. **b** Aerial view of the three northwestern sites within St. John National Park: Trunk Bay, Hawksnest Bay, and Caneel Bay. The two sampling sites at Caneel Bay are indicated by *yellow dots*. No benzophenones were detected in samples from Caneel Bay. *Scale bar* is 500 m. **c** Aerial view of the two sampling sites in Hawksnest Bay, St. John Island. *Yellow arrows* indicate three coral reef spurs that are dominated by the U.S. Threatened Species, *Acropora palmata*. *Yellow arrows* pointing at *red dots* indicate the sample site. *Values* indicate the concentration of BP-3 in the water column. *Scale bar* is 245 m. **d** Elevated view of Trunk Bay, St. John Island. *Yellow arrows* pointing to *red* "X" indicate the sample site. The *values* indicate the concentration of BP-3 in the water column at those two sites

2008). The most fascinating aspect of these autophagic events were the delamination of the nuclear bilayer membrane (Figs. 4b, c, 5b, c), a classic hallmark of autophagic cell death and further evidence arguing against apoptosis as a regulated mechanism of cnidarian cell death (Tasdemir et al. 2008; Yla-Antilla et al. 2009; Klionsky et al. 2012). In both the light and the dark, there was a gradation of vitiated cells beginning at the surface of the epidermis to "non-morbid" cells in the gastrodermis that surrounded the yolk. In Figs. 4a and 5a, the cells are severely degraded; it is difficult to distinguish any mechanism of cell death, and the cells could easily be labeled as necrotic. Going 20,000 nm into the planula from the surface, cells exhibited the hallmarks of autophagic cell death. This tissue transect of the gradation of cell death is evidence for a model of cell death, first demonstrated in *C. elegans*, that requires autophagic degradation of cells for the manifestation of necrosis (Samara et al. 2008; Eskelinin et al. 2011).

BP-3 is a genotoxicant to corals, and its genotoxicity is exacerbated by light. Based on the current literature, this was not unexpected, but our data do underscore the threat that BP-3 may pose to not only corals but also to other coral-reef organisms (Hanson et al. 2006; Cuquerella et al. 2012). DNA AP lesions can be produced in response to oxidative interaction or alkylation events (Fortini et al. 1996; Drablos et al. 2004). Accumulation of DNA damage in the larval state has implications not only for the success of coral recruitment and juvenile survival, but also for reproductive effort and success as a whole (Anderson and Wild 1994; Depledge and Billinghurst 1999). Surviving planulae exposed to BP-3 may settle, metamorphose, and

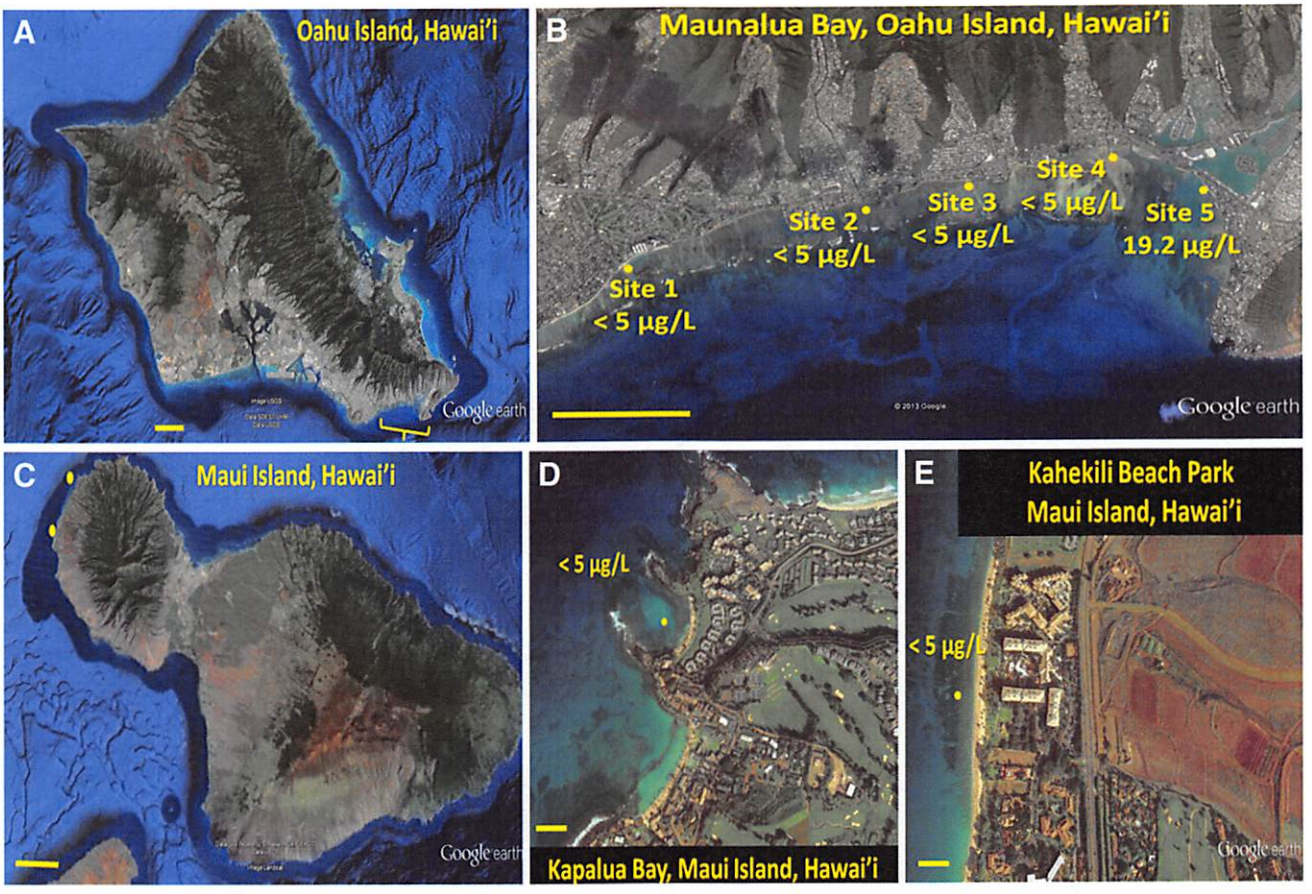

Fig. 12 Seawater analysis of benzophenone-3 (BP-3) in coral reef areas in Oahu and Maui islands, Hawai'i. *Yellow dots* indicate the sampling location in the panels. **a** Aerial view of Oahu indicating the five sampling sites. *Scale bar* is 5 km. **b** Aerial view of the five sampling site along the coast of Maunalua Bay, Oahu. Sites 1–4 had levels of BP-3 that were detectable, but below the quantitative range. *Scale bar* is 1.5 km. **c** Aerial view of the two sampling sites in Maui, Hawai'i. *Scale bar* is 6 km. **d** Elevated view of Kapalua Bay, Maui. *Scale bar* is 100 m. **e** Elevated view of Kahekili Beach, Maui. *Scale bar* is 100 m

develop into colonial adults, but they may be unfit to meet the challenges of other stressor events, such as increased sea-surface temperatures. Cnidarians are rather unusual in the animal kingdom in that the germline is not sequestered away from the somatic tissue in early stages of development; the somatic tissue gives rise directly to the germline during seasonal reproductive cycles. Damage to the genomic integrity of coral planulae therefore may have far-reaching and adverse impacts on the fitness of both the gametes in adults.

The ossification of the planulae from exposure to BP-3 is one of the strangest cases of developmental endocrine disruption to wildlife, although skeletal endocrine disruption in vertebrates is only now being recognized (Colburn et al. 1993; Depledge and Billinghurst 1999; Golub et al. 2004; Lind et al. 2004; Doherty et al. 2004; Agas et al. 2013). In mammals, estrogen and estrogenic compounds may influence different estrogen and thyroid hormone receptors, which affect bone growth and composition (Rickard et al. 1999; Lindberg et al. 2001; Golub et al. 2004). In classic vertebrate physiology, estrogen plays a complex role in ossification and skeletal maintenance, affecting both bone anabolism and catabolism (Simmons 1966; Väänänen and Härkönen 1996). In vertebrates, exposure to high levels of estrogen can result in skeletal hyperossification (Pfeiffer et al. 1940; Rickard et al. 1999). For "classic" endocrine disruptors, such as tributyltin and dioxin, ossification is inhibited, not induced (Birnbaum 1995; Jamsa et al. 2001; Tsukamoto et al. 2004; Finnila et al. 2010; Agas et al. 2013). Osteo-endocrine disruption is both complex and complicated; different compounds affect different cell types within the skeletal tissue differently (Hagiwara et al. 2008a, b; Agas et al. 2013). Benzophenones as endocrine disruptors are no exception; BP-3 and BP-2 showed contradictory effects on estrogen and aryl hydrocarbon receptors, and both compounds induced "…a kind of endocrine disruption that is not assessed by 'classical' estrogenic markers" (Schlecht et al. 2004; Seidlová-Wuttke et al. 2004; Ziolkowaska et al. 2006).

The ossification-induced opacity of the epidermal tissue layer of planulae was readily observed at the three highest

concentrations of BP-3 exposure but was not visually obvious at the lower concentrations, although we know from the electron microscopy sample processing that ossification was present to a lesser extent in the lower BP-3 exposures. Many endocrine disruptors do not exhibit a "classic" monotonic exposure–response curve, but instead exhibit nonmonotonic behaviors (vom Saal et al. 1995; Conolly and Lutz 2004; http://epa.gov/ncct/edr/non-monotonic.html). Ossification of planulae can be assayed by a variety of methods, including alizarin staining and calcein fluorescence. This study was not designed to be an exhaustive characterization of exposure–response behavior (i.e., regulatory toxicology); hence lower BP-3 exposure concentrations were not attempted. More comprehensive studies that examine the ossification response of both acute and chronic exposure of BP-3 in the lower pptrillion and ppquadrillion need to be conducted to determine accurately this endocrine behavioral response.

Ecotoxicology and Species Sensitivity

To conduct a relevant and accurate ecological risk or threat assessment, it is imperative that the species chosen reflects the structure of the specific coral-reef ecosystem being affected (Suter 2007). *Stylophora pistillata* used in this study, is indigenous to specific regions in the Indo-Pacific basins, and hence may not be a valid representative for coral-reef communities in Hawaii or the Atlantic/Caribbean basins. The use of coral planulae in research studies is a relatively difficult resource to obtain. It requires access to healthy coral colonies that are reproductively viable, spawning in specific dates and specific moon phases, and in addition, obtaining the necessary collection and transport permits. We therefore applied an in vitro primary cell toxicity methodology using a specific coral cell type that has been proposed as a surrogate for either planula or colonial polyp studies (Downs 2010). Comparison of LC_{50}s of coral cells in the light (42 ppbillion; µg/L) and coral planula in the light for 8 and 24 h [2.876 ppmillion (mg/L) and 139 ppbillion (µg/L), respectively] exhibits a similar response. The increased sensitivity of in vitro cell models versus whole organism models is a common phenomenon and accepted principle (Blaauboer 2008; Gura 2008). Diffusion of BP-3 across the epidermal boundary layer and reaching concentrations that are toxic in the interior of the planula (e.g., gastroderm) versus direct exposure by cultured cells could likely be the major factor influencing the variation in LC_{50} rate. Although there are obvious caveats to using in vitro models, this may be the only way to conduct ecotoxicological research and ecological risk assessments on coral species that are currently endangered with extinction, such as the species on the IUCN's Red List or species proposed/listed for protection under the U.S. Endangered Species Act.

When an environmental stressor impacts a community of organisms, different species may respond (tolerate) dissimilarly to one another; some species may tolerate the stressor at a particular level, whereas other species may succumb (Johnston and Roberts 2009; Maloney et al. 2011). This species sensitivity distribution is a crucial concept for ecological risk assessments and a predictor of the species composition of a community (community phase-shift) in reacting to a pollution stressor, as well as defining the probability of success for community/ecological restoration (Posthuma et al. 2002; van Woesik et al. 2012). This concept readily applies to corals and coral reefs. Coral bleaching in response to heat stress or freshwater input is an excellent example of this community behavior; some species have high tolerance to stress-induced bleaching, whereas others are highly susceptible, resulting in species-specific extinctions in localized areas (Goreau 1990; Loya et al. 2001; Jimenez and Cortes 2003). Species sensitivity distribution in response to pollutants in corals is also well documented, including synergisms between pollutants and heat stress (Loya 1975; Brown 2000; Fabricius 2005). For the Caribbean, the species sensitivity to BP-3 toxicity is consistent with the model for coral tolerance to general stress as set forth by Gates and Edmunds (1999): corals with slower growth rates, such as massive or boulder coral species, are inherently more tolerant than coral species with higher growth rates (e.g., branched species such as *A. cervicornis* and *P. divaricata*). In fringing reefs that have been impacted by anthropogenic stressors, especially fringing reefs near tourist beaches, *Acropora* species are the first to experience localized extinction. Species that tentatively endure a decade or longer of sustained stress, but are intermediate in their persistence, are the large boulder corals found in the genus *Montastrea* (synomym *Orbicella*). Coral cell toxicity data indicated that *P. astreoides* was at least 4.5× more tolerant to BP-3 toxicity than the second more tolerant coral species and at least 38× more tolerant than the most sensitive species. This is consistent with observations that *P. astreoides* is usually the last to become extinct in a polluted-impacted locality and one of the first to recruit once water quality parameters reach a minimum level of habitability (Peters 1984; Lirman et al. 2003; Alcolado-Prieto et al. 2012). From a management perspective, these data can be used to predict the changes in coral-reef community structure when challenged with BP-3, regarding which species will become extinct, as well as the species that will persist in areas that are adjacent to tourist beaches, popular mooring sites, or near sewage discharges. These data also can be integrated directly into reef resilience management

plans against climate change, acting as both a measurable endpoint for management effectiveness and as a target (concentration of BP-3 in seawater on a reef) for establishing action values for reef management.

Management of BP-3 Pollution for Coral Reef Conservation and Restoration

What do these pathological toxicities induced by BP-3 mean demographically and ecologically for corals and coral reefs? Trunk Bay in St. John Island, the U.S Virgin Islands, may represent an example of this effect. Ecologically, this area has been severely degraded in the past 25 years, despite the limited input from human activities in the watershed and from marine sources. The most obvious input is recreational swimming at Trunk (Downs et al. 2011). During our monitoring of this site from 2005 to 2010, settlement of planulae and recruitment/survival of juvenile coral was almost 0 %. Established coral colonies in this area were assayed for regeneration of tissue over experimentally induced lesions (laceration-regeneration assay, a single diagnostic test for the general health of a coral; Fisher et al. 2007); not a single colony exhibited any regeneration of any of the lesions during the 5-year investigation (Downs et al. 2011). This was in contrast with Caneel Bay, which had undetectable levels of BP-3 resulting from a much lower density/rate of swimmers and has a flourishing coral community on its southern bank with an abundance of recruitment. These demographic-level pathologies are consistent with the pathologies that manifest from BP-3 exposure. The pathologies exhibited at this site can be seen at other coral reef swimming areas the world over: Eilat, Israel (degraded with an abundance of sunscreen lotion users) versus Aqaba, Jordan (thriving coral reefs with swimmers that do not use sunscreen lotion; Fuad Al-horani, personal communication), Honolua Bay in Maui, Hawaii, Hanauma Bay Beach in Oahu, Hawaii, Seven Mile Beach in Grand Cayman, Bathway Beach in Grenada, Playa Langosta, and Playa Tortugas Beaches in Cancun, Mexico. At Okinawa, Tashiro and Kameda (2013) demonstrated that BP-3 contamination from beaches can travel over 0.6 km in distance from the pollution source. The threat of BP-3 to corals and coral reefs from swimmers and point and non-point sources of waste-water could thus be far more extensive than just a few meters surrounding the swimming area.

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Compliance with Ethical Standards

Conflict of Interest The authors can identify no potential conflicts of interest, neither financial nor ethically, involved in the writing or publication of this manuscript.

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Review

A review of personal care products in the aquatic environment: Environmental concentrations and toxicity

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ABSTRACT

Considerable research has been conducted examining occurrence and effects of human use pharmaceuticals in the aquatic environment; however, relatively little research has been conducted examining personal care products although they are found more often and in higher concentrations than pharmaceuticals. Personal care products are continually released into the aquatic environment and are biologically active and persistent. This article examines the acute and chronic toxicity data available for personal care products and highlights areas of concern. Toxicity and environmental data were synergized to develop a preliminary hazard assessment in which only triclosan and triclocarban presented any hazard. However, numerous PCPs including triclosan, paraben preservatives, and UV filters have evidence suggesting endocrine effects in aquatic organisms and thus need to be investigated and incorporated in definitive risk assessments. Additional data pertaining to environmental concentrations of UV filters and parabens, *in vivo* toxicity data for parabens, and potential for bioaccumulation of PCPs needs to obtained to develop definitive aquatic risk assessments.

Contents

1. Introduction

Personal care products (PCPs) are a diverse group of compounds used in soaps, lotions, toothpaste, fragrances, and sunscreens, to name a few. The primary classes of PCPs include disinfectants (e.g. triclosan), fragrances (e.g. musks), insect repellants (e.g. DEET), preservatives (e.g. parabens) and UV filters (e.g. methylbenzylidene camphor). Unlike pharmaceuticals which are intended for internal use, PCPs are products intended for external use on the human body and thus are not subjected to metabolic alterations; therefore, large quantities of PCPs enter the environment unaltered through regular usage (**Ternes et al., 2004**). Many of these compounds are used in large quantities, and recent studies have indicated many are environmentally persistent, bioactive, and have the potential for bioaccumulation (**Peck, 2006; Mackay and Barnthouse, 2010**).

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PCPs are among the most commonly detected compounds in surface water throughout the world (Peck, 2006); however, in comparison to pharmaceuticals, relatively little is known about PCP toxicity (Daughton and Ternes, 1999). Numerous reviews have been published examining pharmaceutical toxicity and occurrence (Halling-Sorensen et al., 1998; Crane et al., 2006; Fent et al., 2006; among others), but less attention has been placed on determining potential risk of PCP release into aquatic environments. The objective of this review was to summarize recent publications regarding environmental concentrations (surface water) and aquatic toxicity of PCPs in order to identify research needs and to estimate hazard as a result of PCP release into the aquatic environment.

2. Disinfectants

Triclosan (TCS) and triclocarban (TCC) are biphenyl ethers used as antimicrobials in soaps, deodorants, skin creams, toothpaste and plastics (McAvoy et al., 2002). TCS and TCC are among top 10 most commonly detected organic wastewater compounds for frequency and concentration (Kolpin et al., 2002; Halden and Paull, 2005). TCS has been identified in wastewater treatment plant (WWTP) effluent at concentrations greater than 10 $\mu g\,L^{-1}$ (Lopez-Avila and Hites, 1980). A USGS study monitoring 95 compounds in surface water throughout the United States, found TCS to be one of the most frequently detected compounds with surface water concentrations as high as 2.3 $\mu g\,L^{-1}$ (Kolpin et al., 2002). For all published studies conducted to-date TCS has been detected in 56.8% of surface water samples with a median concentration of about 50 $ng\,L^{-1}$ (Table 1). TCS has been detected in surface water worldwide (United States-Boyd et al., 2004; Loraine and Pettigrove, 2006; Benotti et al., 2009; Dougherty et al., 2010; Romania – Moldovan, 2006; United Kingdom – Kasprzyk-Horden et al., 2008; South Korea – Kim et al., 2009; Yoon et al., 2008, to name a few) with a number of studies in Switzerland having identified TCS and also its methyl derivative methyl triclosan (M-TCS) in WWTP effluent, surface water, and fish tissue. In this study, TCS was detected in surface water at concentrations higher than M-TCS and found as high as 74 $ng\,L^{-1}$ (Lindstrom et al., 2002) whereas concentrations in WWTP effluent were considerably higher for both compounds (up to 650 $ng\,L^{-1}$ TCS and 11 $ng\,L^{-1}$ M-TCS). M-TCS is relatively stable and lipophilic and thus is likely to bioaccumulate in biota. The highest reported concentrations of M-TCS were found in fish with concentrations as high as 2100 $ng\,g^{-1}$ of lipid (Buser et al., 2006). Interestingly, numerous studies investigating bioaccumulation have found that M-TCS is bioaccumulated whereas TCS is not based on data with semi-permeable membrane devices (Poiger et al., 2001; Lindstrom et al., 2002). Furthermore, bioaccumulation of TCS has not been observed in aquatic plants although M-TCS has been observed to bioaccumulate after 28 d in *Sesbania herbacea* (Stevens et al., 2009). However, other studies have contradicted these findings demonstrating TCS bioaccumulates to a much greater degree in algae than M-TCS (Coogan et al., 2007). It is currently unknown whether phylogenetic differences in physiology across trophic groups can influence bioaccumulation and what factors are most important for uptake and accumulation to occur. One possible explanation for differences in TCS bioaccumulation is due to potential ionization of TCS. In typical environmental conditions TCS ranges from completely protonated (pH = 5.4) to totally deprotonated (pH = 9.2) based on a p*Ka* values of 7.8 at normal pH ranges in surface waters (Young et al., 2008). These variations in ionization cause differences in *Dow* values relating to differences in bioaccumulation. Although this has not been investigated for TCS, studies with the pharmaceutical fluoxetine (Prozac®) indicate vast differences in bioaccumulation at different pH's (Nakamura et al., 2008). Based on these results at higher pH's TCS would be expected to accumulate more due to its pKa value of 7.8 whereas at lower pH's M-TCS would be expected to accumulate to higher levels.

TCC has been used in PCPs since 1957 and has been observed in surface water at concentrations up to 6.75 $\mu g\,L^{-1}$ (Halden and Paull, 2005). It is believed that TCC occurs as frequently in WWTP effluent and surface water as TCS; however, until 2004 TCC could not be detected at low levels ($ng\,L^{-1}$) (Halden and Paull, 2004; Coogan et al., 2007). However, TCC has been detected at higher concentrations and more frequently in WWTP effluent and surface water than TCS or M-TCS over the last 5 years (Coogan et al., 2007). Additionally, TCC has demonstrated a propensity to bioaccumulate more than either TCS or M-TCS in aquatic organisms. Other disinfectants (phenol, 4-metyhl phenol, and biphenylol) are also commonly used in households and have the potential to be released into aquatic environments. These compounds have been identified in surface water or WWTP effluent (Kolpin et al., 2002, 2004; Glassmeyer et al., 2005) with phenol found more often than 4-methyl phenol and biphenylol, as well as in greater concentrations (as high as 1.3 $\mu g\,L^{-1}$) (Kolpin et al., 2002).

Acute toxicity of TCS and biphenylol has been examined in invertebrates, fish, amphibians, algae, and plants. TCS is more toxic to similarly studied trophic groups in comparison to other disinfectants. For all disinfectants studied, invertebrates are only slightly more sensitive than fish for acute time periods (Table 2). Additionally for longer-term exposure, fish and vascular plants appear to be less sensitive to TCS exposure whereas algae and invertebrates are more sensitive (Table 3). High TCS sensitivity in algae is likely due to TCS antibacterial characteristics, through disruption of lipid synthesis through the FabI (fatty acid synthesis) and FASII (enoyl acyl carrier protein reductase) pathways (McMurry et al., 1998; Lu and Archer, 2005), membrane destabilization (Lyrge et al., 2003; Franz et al., 2008), or uncoupling of oxidative phosphorylation (Newton et al., 2005) which are similar between algae and bacteria (Coogan et al., 2007). Toxicity of TCS to animal species is likely due to non-specific narcosis as no common receptors are known to exist (Lyndall et al., 2010). Acute toxicity to amphibians has been studied in four different amphibians using a modified FETAX assay (Palenske et al., 2010). Amphibians were more sensitive than fish however they were not as sensitive as algae during short-term exposures. For longer exposure duration algae appears to be the most sensitive trophic group. Algal growth was the most sensitive endpoint and was affected at concentrations less than 1 $\mu g\,L^{-1}$ (Orvos et al., 2002). Aquatic plants, invertebrates, and fish were not highly sensitive to chronic exposure of TCS (Table 3). Only minimal aquatic toxicity data exists for TCC, but recent studies indicate TCC is slightly more toxic to aquatic invertebrate and fish for both

Table 1
Summary of measured concentration of personal care products in surface water ($ng\,L^{-1}$).

Compound	Class	n[a]	Range ($ng\,L^{-1}$)	Median ($ng\,L^{-1}$)
Triclosan	Disinfectant	710	<0.1–2300	48
Methyltriclosan	Disinfectant	4	0.5–74	–
Triclocarban	Disinfectant	29	19–1425	95
Musk ketone	Fragrance	178	4.8–390	11
Musk xylene	Fragrance	93	1.1–180	9.8
Celestolide	Fragrance	73	3.1–520	3.2
Galaxolide	Fragrance	282	64–12 470	160
Tonalide	Fragrance	245	52–6780	88
DEET	Insect repellant	188	13–660	55
Paraben[b]	Preservative	6	15–400	–
4MBC	UV filter	19	2.3–545	10.2
BP3	UV filter	18	2.5–175	20.5
EHMC	UV filter	21	2.7–224	6.1
OC	UV filter	22	1.1–4450	1.9

[a] n = Number of samples.
[b] Includes all parabens.

Table 2
Acute toxicity data for personal care products.

Compound	Category	Species	Trophic group	Endpoint/duration	LC50 (mg L^{-1})	Additional tox. values	References
Biphenylol	Antimicrobial	*Daphnia magna*	Invert.	48 h Mobility	3.66		1
		D. magna	Invert.	48 h Survival	3.66		2
		Tetrahymena pyriformis	Invert.	48 h Survival	5.7–8.26		3
		T. pyriformis	Invert.	60 h Survival	5.7–8.26		4
		Cyprinus carpio	Fish	44 h Survival	157–292		5
Triclosan	Antimicrobial	*D. magna*	Invert.	48 h	0.39		6
		Ceriodaphnia dubia	Invert.	24, 48 h (pH = 7.0)	0.2, ~125		6
		Pimephales promelas	Fish	24, 48, 72, 96 h	0.36, 0.27, 0.27, 0.26		6
		Lepomis macrochirus	Fish	24, 48, 96 h	0.44, 0.41, 0.37		6
		Oryzias latipes	Fish	96 h	0.602 (larvae), 0.399 (embryos)		7
		Xenopus laevis	Amphibian	96 h	0.259		8
		Acris blanchardii	Amphibian	96 h	0.367		8
		Bufo woodhousii	Amphibian	96 h	0.152		8
		Rana sphenocephala	Amphibian	96 h	0.562		8
		Pseudokirch-neriella subcapitata	Algae	72 h Growth	0.53 (μg L^{-1})		9
Triclocarban	Antimicrobial	*D. magna*	Invert.	48 h	0.01		10
		C. dubia	Invert.	48 h	0.0031		10
		Mysidopsis bahia	Invert.	48, 96 h	0.015, .01		10
		Salmo gairdneri	Fish	96 h	0.120		10
		L. macrochirus	Fish	96 h	0.097		10
		Scenedesmus subspicatus	Algae	72 h Growth	0.02		10
		P. subcapitata	Algae	72 h Growth	0.017 (μg L^{-1})		9
Benzophenone	Fixative	*Caenorahbditis elegans*	Nematode	24 h	56.8		11
		P. promelas	Fish	96 h	10.89		12
1,4-dichlorobenzene[a]	Insect repellant	*D. magna*	Invert.	24, 48 h Immobilization	1.6, 0.7		13, 14
		Artemia salina	Invert.	24 h	14		15
		Palaemonetes pugio	Invert.	96 h	60		16
		M. bahia	Invert.	96 h	1.99		17
		Danio rerio	Fish	24 h, 96 h	4.25, 2.1		18,19
		Jordanella floridae	Fish	96 h	2.05		20
		P. promelas	Fish	96 h	4.2		21
		O. mykiss	Fish	24 h	1.18		19
		L. macrochirus	Fish	96 h	4.3		22
		Cyprinodon variegatus	Fish	96 h	7.4		23
		Selenastrum capricornutum	Algae	96 h Growth	0.57		14
		Scenedesmus pannonicus	Algae	72 h Growth	31		13
		S. subspicatus	Algae	48 h Growth, Biomass	38, 28		22
		Skeletonema costatum	Algae	96 h Growth	59.1		17
N,N-diethyl-*m*-toluamide (DEET)[b]	Insect repellant	*D. magna*	Invert.	48 h, 96 h	160, 108		25
		Gammarus fasciatus	Invert.	96 h	100		26
		P. promelas	Fish	96 h	110		27
		Gambusia affinis	Fish	24–48 h	235		28
		Oncorhynchus mykiss	Fish	96 h	71.3		29
		Chlorella protothecoides	Algae	24 h Photosynthesis	388		30
Musk ambrette (MA)	Nitro musk	*Vibrio fischeri*	Bacteria	Microtox	>Sol.[c]		31
		Pseudokirch-neriella subcapitata	Algae	72 h	>Sol.		31
Musk ketone (MK)	Nitro musk	*V. fischeri*	Bacteria	Microtox	>Sol.		31
		Nitocra spinipes	Invert.	96 h	>1.0		32
		Acartia tonsa	Invert.	48 h	1.32	LC10 = 0.40	33
		D. magna	Invert.	24, 48 h	>Sol., 5.6		28
		D. magna	Invert.	48 h	>0.46		31
		D. rerio	Fish	96 h Survival, Hatching	>0.4		28
		P. subcapitata	Algae	72 h	>Sol.		31
Musk moskene (MM)	Nitro musk	*V. fischeri*	Bacteria	Microtox	>Sol.		31
		D. magna	Invert.	24 h	>Sol.		31
		Danio rerio	Fish	96 h Survival, Hatching	>0.4		34
		P. subcapitata	Algae	72 h	>Sol.		31
Musk Tibetene (MT)	Nitro musk	*V. fischeri*	Bacteria	Microtox	>Sol.		31
		P. subcapitata	Algae	72 h	>Sol.		31

Table 2 (*continued*)

Compound	Category	Species	Trophic group	Endpoint/duration	LC50 (mg L^{-1})	Additional tox. values	References
Musk xylene (MX)	Nitro musk	*V. fischeri*	Bacteria	Microtox	>Sol.		31
		D. magna	Invert.	24, 48 h Mobility	EC50 ⩾ Sol.		35
		Oncorhynchus. mykiss	Fish	96 h	>1000		36
		L. macrochirus	Fish	96 h	1.2		37
		D. rerio	Fish	96 h Survival, Hatching	>0.4		34
		P. subcapitata	Algae	72 h	>Sol.		31
Celestolide (ADBI)	Polycyclic musk	*N. spinipes*	Invert.	96 h	>2.0		38
		A. tonsa	Invert.	48 h	>2.0	LC10 > 2.0	39
		D. rerio	Fish	96 h Survival, Hatching	>1.0		38
		D. rerio	Fish	96 h Malformation	LOEC~0.65		39
		O. latipes	Fish	96 h Survival	1.97		40
Galaxolide (HHCB)	Polycyclic musk	*N. spinipes*	Invert.	96 h	1.90		28
		A. tonsa	Invert.	48 h	0.47	LC10 = 0.12	39
		Lampsilis cardium	Benthic invert.	24, 48 h	1.0, 0.99		41
		D. rerio	Fish	96 h Survival, Hatching	>0.67		39
		D. rerio	Fish	96 h Malformations	LOEC ~ 0.45		39
		O. latipes	Fish	96 h Survival	0.95		40
Tonalide (AHTN)	Polycyclic musk	*N. spinipes*	Invert.	96 h	0.61		31
		A. tonsa	Invert.	48 h	0.71	LC10 = 0.45	32
		L. cardium	Benthic invert.	24, 48 h	0.45, 0.28		41
		D. rerio	Fish	96 h Malformation	LOEC ~ 0.1		39
		D. rerio	Fish	96 h Survival, Hatching	>0.67		38
		O. latipes	Fish	96 h Survival	1.00		40
Traseolide (ATII)		*O. latipes*	Fish	96 h Survival	0.95		40
Phantolide (AHMI)		*O. latipes*	Fish	96 h Survival	1.22		40
Cachmeran (DPMI)		*O. latipes*	Fish	96 h Survival	11.6		40
Benzylparaben	Preservative	*T. thermophila*	Protozoa	24 h, 28 h	4.3, 5.7	LOEC = 0.48	42
		V. fisheri	Bacteria	15 min, 30 min Illuminescence	0.11, 0.11	LOEC = 0.02	42
		Photobacterium leiognathi	Bacteria	15 min, 30 min Illuminescence	1.3, 1.6	LOEC = 0.25	42
		D. magna	Invert.	48 h	4.0		43
		D. magna	Invert.	24 h, 48 h Mobility	5.2, 6	LOEC = 1.2	42
		P. promelas	Fish	48 h	3.3		43
Butylparaben	Preservative	*T. thermophila*	Protozoa	24 h, 28 h	5.3, 7.3	LOEC = 2.5	42
		V. fisheri	Bacteria	15 min, 30 min Illuminescence	2.5, 2.8	LOEC = 0.7	42
		P. leognathi	Bacteria	15 min, 30 min Illuminescence	3.7, 4.3	LOEC = 1.12	42
		D. magna	Invert.	48 h	5.3		43
		D. magna	Invert.	24 h, 48 h Mobility	6.2, 6	LOEC = 3.2	42
		P. promelas	Fish	48 h	4.2		43
Ethylparaben	Preservative	*T. thermophila*	Protozoa	24 h, 28 h	25, 30	LOEC = 10.7	42
		V. fisheri	Bacteria	15 min, 30 min Illuminescence	2.5, 2.7	LOEC = 0.55	42
		P. leognathi	Bacteria	15 min, 30 min Illuminescence	19, 24	LOEC = 5.5	42
		D. magna	Invert.	48 h	18.7		43
		D. magna	Invert.	24 h, 48 h Mobility	25,23	LOEC = 12	42
		P. promelas	Fish	48 h	34.3		43
Isobutylparaben	Preservative	*D. magna*	Invert.	48 h	7.6		43
		P. promelas	Fish	48 h	6.9		43
Isopropylparaben	Preservative	*D. magna*	Invert.	48 h	8.5		43
		P. promelas	Fish	48 h	17.5		43
Methylparaben	Preservative	*T. thermophila*	Protozoa	24 h, 28 h	54, 58	LOEC = 11.5	42
		V. fisheri	Bacteria	15 min, 30 min Illuminescence	9.6, 10	LOEC = 2.9	42
		P. leognathi	Bacteria	15 min, 30 min Illuminescence	31,35	LOEC = 8.5	42
		D. magna	Invert.	48 h	24.6		42
		D. magna	Invert.	24 h, 48 h Mobility	32, 21	LOEC = 15	42
		P. promelas	Fish	48 h	>Sol.		43

(*continued on next page*)

Table 2 (*continued*)

Compound	Category	Species	Trophic group	Endpoint/duration	LC50 (mg L^{-1})	Additional tox. values	References
Propylparaben	Preservative	*T. thermophila*	Protozoa	24 h, 28 h	9.7, 12.5	LOEC = 2.6	42
		V. fisheri	Bacteria	15 min, 30 min Illuminescence	2.5, 2.6	LOEC = 0.9	42
		P. leognathi	Bacteria	15 min, 30 min Illuminescence	21, 25	LOEC = 4.5	42
		D. magna	Invert.	48 h	12.3		43
		D. magna	Invert.	24 h, 48 h Mobility	13, 7	LOEC = 6	42
		P. promelas	Fish	48 h	9.7		43
Benzophenone-3	UV filter	*D. magna*	Invert.	48 h Immobility	1.9		44
Benzophenone-4	UV filter	*D. magna*	Invert.	48 h Immobility	50		44
4-Methylbenzy-lidene camphor	UV filter	*D. magna*	Invert.	48 h Immobility	0.56		44
2-Ethyl-hexyl-4-trimethoxy-cinnamate	UV filter	*D. magna*	Invert.	48 h Immobility	0.29		44

References: (1) Kopperman et al. (1974), (2) Carlson and Caple (1977), (3) Schultz et al. (1989), (4) Schultz and Riggin (1985), (5) Loeb and Kelly (1963), (6) Orvos et al. (2002), (7) Ishibashi et al. (2004), (8) Palenske et al. (2010), (9) Yang et al. (2008), (10) TCC Consortium (2002), (11) Ura et al. (2002), (12) Marchini et al. (1992), (13) Canton et al. (1985), (14) Calamari et al. (1982), (15) Abernathy et al. (1986), (16) Curtis and Ward (1981), (17) USEPA (1978), (18) Roederer (1990), (19) Calamari et al. (1983), (20) Smith et al. (1990), (21) Carlson and Kosian (1987), (22) Buccafusco et al. (1981), (23) Heitmuller et al. (1981), (24) Kuhn and Pattard (1990), (25) Seo et al. (2005), (26) Mayer and Ellersieck (1986), (27) Brooke et al. (1984), (28) Michael and Grant (1974), (29) Office of Pesticides Program (2000), (30) Costanzo et al. (2007), (31) Schramm et al. (1996), (32) Breitholtz et al. (2003), (33) Wollenberger et al. (2003), (34) Tas et al. (1997), (35) Hughes and Krishnaswami (1985), (36) MITI (1992), (37) Adema and Langerwerf (1985a,b), (38) Van der Plassche and Balk (1997), (39) Dietrich and Chou (2001), (40) Yamauchi et al. (2008), (41) Gooding et al. (2006), (42) Bazin et al. (2010), (43) Dobbins et al. (2009), (44) Fent et al., 2009.

[a] 1,4-dichlorobenzene table is modified from Boutonnet et al. (2004).

[b] DEET information is modified from Table presented by Costanzo et al. (2007).

[c] No effects found at concentrations exceeding water solubility.

Table 3
Chronic toxicity data for personal care products.

Compound	Category	Species	Trophic level	Endpoint/duration	LOEC (μg L^{-1})	NOEC (μg L^{-1})	References
Triclosan	Antimicrobial	*D. magna*	Invert.	21 d Survival, Reproduction	Repro. = 200 (LOEC)	Surv. = 200 (NOEC)	1
		C. dubia	Invert.	7 d Survival, Reproduction		50, 6	1
		C. dubia	Invert.	7 d Survival, Reproduction	IC25 = 170		2
		Chironomus riparius	Invert.	28 d Survival, Emergence		440	3
		Chironomus tentans	Invert.	10 d Survival, Growth	LC25 = 100		4
		Hyalella azteca	Invert.	10 d Survival, Growth	LC25 = 60		4
		O. mykiss	Fish	96 d ELS[c] Hatching, Survival	No Effect, 71.3		1
		O. latipes	Fish	14 d Hatching	213		5
		O. latipes	Fish	21 d Growth, Fecundity, HSI and GSI[d], VTG[e]	200, No Effect, 200, 20		5
		O. latipes	Fish	14 d Hatchability	IC25 = 290		2
		Gambusia affinis	Fish	35 d Sperm Count, VTG	101.3		6
		Danio rerio	Fish	9 d Hatchability	IC25 = 160		2
		Xenopus laevis	Amphibian	21 d Metamorphosis	No effect (200)		7
		Rana catesbeiana	Amphibian	18 d Development	300		8
		Rana pipiens	Amphibian	24 d Survival, Growth	230, 2.3		9
		Bufo americanus	Amphibian	14 d Survival, Growth	No effect (230)		10
		S. capricornutum	Algae	96 h Growth	EC50 = 4.46	EC25 = 2.44	1
		S. subspicatus	Algae	96 h Biomass, Growth Rate	EC50 = 1.2, 1.4	EC50 = 0.5, 0.69	1
		S. costatum	Algae	96 h Growth Rate	EC50 ⩾ 66	EC25 > 66	1
		A. flos-aquae	Algae	96 h Biomass	EC50 = 0.97	EC25 = 0.67	1
		P. subcapitata	Algae	72 h Growth	EC25 = 3.4	0.2	2, 11
		N. pelliculosa	Algae	96 h Growth Rate	EC50 = 19.1	EC25 = 10.7	1
		Natural algal assemblage	Algae	96 h Biomass	0.12		12
		Closterium ehrenbergii	Algae	96 h Growth		250	13
		Dunaliella tertiolecta	Algae	96 h Growth		1.6	14
		L. gibba	Plant	7 d Growth	EC50 ⩾ 62.5	EC25 ⩾ 62.5	1
		S. herbacea	Plant	28 d Seed Germination, Morphology	100 germination, 10 morphology		15
		E. prostrata	Plant	28 d Seed Germination, Morphology	No effect, 1000		15
		B. frondosa	Plant	28 d Seed Germination, Morphology	100, 10		15

Table 3 (*continued*)

Compound	Category	Species	Trophic level	Endpoint/duration	LOEC (μg L^{-1})	NOEC (μg L^{-1})	References
Triclocarban	Antimicrobial	*D. magna*	Invert.	21 d Growth	4.7	2.9	16
		M. bahia	Invert.	28 d Reproduction	0.13	0.06	16
		P. subcapitata	Algae	14 d Growth	10 000	EC50 = 36 000	16
Benzophenone	Fixative	*Pimephales promelas*	Fish	7 d Survival, Growth	9240, 3100	5860, 2100	17
		P. promelas	Fish	7 d ELS (Survival, Growth)	6400, 1800	3300, 1000	17
1,4-dichlorobenzene	Insect repellant	*D. magna*	Invert.	28 d Growth		0.22	18
		D. magna	Invert.	21 d Reproduction		0.3	19
		Jordanella floridae	Fish	28 d Growth		>0.35	20
		O. mykiss	Fish	60 d Growth		>0.122	18
		P. promelas	Fish	33 d Growth		0.57	21
		D. rerio	Fish	28 d Growth		1.0	22
Musk ketone (MK)	Nitro musk	*D. magna*	Invert.	21 d Development, Reproduction	340		23
		D. magna	Invert.	21 d Survival	LC50 = 338–675		24
		A. tonsa	Invert.	5 d Developmental Rate	EC50 = 66	EC10 = 10	25
		A. tonsa	Invert.	5 d Juvenile Survival	2000	800	25
		N. spinipes	Invert.	7 d Developmental Rate, Survival	30		23
		N. spinipes	Invert.	26 d Population Growth Rate	100		23
		D. rerio	Fish	ELS 24–48 h Tail Extension, Coagulated Eggs, Edema, Circulation	1000	330	26
		D. rerio	Fish	ELS 24–48 h Movement, Tail Extension	330	100	26
		D. rerio	Fish	ELS 48 h Heart Rate	10	3.3	26
		D. rerio	Fish	ELS 48 h Survival	33	10	26
		O. mykiss	Fish	21 d Reproduction	EC50 = 169–338		27
		L. macrochirus	Fish	21 d Survival	LC50 ⩾ 500		28
		D. rerio	Fish	8w Reproduction	33		28
		P. promelas	Fish	96 h Teratogenesis	EC50 ⩾ 400		28
		X. laevis	Amphibian	96 h FETAX[b]	>4000		29
		P. subcapitata	Algae	72 h Growth, Biomass	EC50 = 244, 118		30
Musk moskene (MM)	Nitro musk	*D. magna*	Invert.	21 d Survival	LC50 ⩾ Sol.		31
		O. mykiss	Fish	21 d Reproduction	EC50 ⩾ Sol.		29
		X. laevis	Amphibian	96 h FETAX	EC50 ⩾ 400		30
Musk xylene (MX)	Nitro musk	*D. magna*	Invert.	21 d Survival	LC50 = 680		32
		D. rerio	Fish	ELS 24–48 h Tail Extension, Coagulated Eggs, Edema, Circulation, Movement	1000	330	27
		D. rerio	Fish	ELS 48 h Heart Rate, Survival	330	10	27
		D. rerio	Fish	14 d Survival	LC50 = 400		33
		X. laevis	Amphibian	96 h FETAX[b]	>400		30
		P. subcapitata	Algae	72 h Growth, Biomass	EC50 ⩾ Sol.[a]		34
		M. aeruginosa	Algae	5 d Cell Count	>10 000		34
Celestolide (ADBI)	Polycyclic musk	*N. spinipes*	Invert.	7 d Developmental Rate, Survival	100		24
		A. tonsa	Invert.	5 d Developmental Rate	EC50 = 160	EC10 = 36	28
		A. tonsa	Invert.	5 d Juvenile Survival	600	240	28
		X. laevis	Amphibian	96 h FETAX	EC50 ⩾ 1000		30
Galaxolide (HHCB)	Polycyclic musk	*D. magna*	Invert.	21 d Development, Reproduction	282 (EC50)		24
		D. magna	Invert.	21 d Growth, Survival	205	11	35
		D. magna	Invert.	21 d Survival	LC50 = 293		36
		A. tonsa	Invert.	5 d Developmental Rate	EC50 = 59	EC10 = 37	26
		A. tonsa	Invert.	5 d Juvenile Survival		300	26
		N. spinipes	Invert.	7 d Developmental Rate, Survival	20		24
		L. cardium	Benthic invert.	96 h Growth	EC50 = 153–563		36
		Capitella sp.	Benthic invert.	119 d Survival, Growth, Development	123 mg kg^{-1}, No effect, 168 mg kg^{-1}		37
		Potamopyrgus antipodarum	Benthic invert.	94 d Adult and Juvenile Survival, Growth, Reproduction	100 Time to 1st reproduction, 10 number of offspring		38
		L. macrochirus	Fish	21 d Growth, Survival	182 LC50 = 452	182	39
		P. promelas	Fish	36 d Hatch, Survival,	>140, 68, 68, 68	>140, 140, 140,	40

(*continued on next page*)

Table 3 (*continued*)

Compound	Category	Species	Trophic level	Endpoint/duration	LOEC (μg L^{-1})	NOEC (μg L^{-1})	References
				Growth, Development		140	
		O. mykiss	Fish	21 d Reproduction	EC50 = 282		36
		D. rerio	Fish	21 d Survival	LC50 = 452		36
		O. latipes	Fish	72 h VTG, ERα[f]	500		41
		X. laevis	Amphibian	96 h FETAX	EC50 ⩾ 100		30
		X. laevis	Amphibian	32 d Survival	LC50 ⩾ 140		30
		P. subcapitata	Algae	72 h Growth, Biomass	466	201	42
		P. subcapitata	Algae	72 h Growth, Biomass	EC50 ⩾ 854, 723		43
Tonalide (AHTN)	Polycyclic musk	*D. magna*	Invert.	21 d Growth, Survival	184–401	89–196	39
		D. magna	Invert.	21 d Development, Reproduction	244 (EC50)		24
		A. tonsa	Invert.	5 d Developmental Rate	EC50 = 26	EC10 = 7.2	26
		A. tonsa	Invert.	5 d Juvenile Survival	160	60	26
		N. spinipes	Invert.	7 d Developmental Rate, Survival	>60		24
		L. cardium	Benthic invert.	96 h Growth	EC50 = 108–708		36
		D. rerio	Fish	ELS 24–48 h Heart Rate	33	10	27
		L. macrochirus	Fish	21 d Growth, Survival	184 LC50 = 314	89	36
		P. promelas	Fish	36 d Hatch, Survival, Growth, Development	>140, 140, 67, 67	>140, 67, 35, 35	40
		O. mykiss	Fish	21 d Reproduction	EC50 = 244		36
		D. rerio	Fish	21 d Survival	LC50 = 314		36
		O. latipes	Fish	72 h VTG, ERα[f]	500		43
		X. laevis	Amphibian	96 h FETAX	EC50 ⩾ 1000		30
		X. laevis	Amphibian	32 d Survival	LC50 = 100		30
		P. subcapitata	Algae	72 h Growth, Biomass	797–835	204–438	42
		P. subcapitata	Algae	72 h Growth, Biomass	EC50 ⩾ 797, 468		43
Benzylparaben	Preservative	*D. magna*	Invert.	7 d Growth, Reproduction	200, 2600		44
		P. promelas	Fish	7 d Growth	1700		44
Butylparaben	Preservative	*D. magna*	Invert.	7 d Growth, Reproduction	200, 2600		44
		P. promelas	Fish	7 d Growth	1000		44
		S. trutta	Fish	10 d VTG	134	76	45
Ethylparaben	Preservative	*D. magna*	Invert.	7 d Growth, Reproduction	9000, 2300		44
		P. promelas	Fish	7 d Growth	17 000		44
Isobutylparaben	Preservative	*D. magna*	Invert.	7 d Growth, Reproduction	300, 2000		44
		P. promelas	Fish	7 d Growth	3500		44
Isopropylparaben	Preservative	*D. magna*	Invert.	7 d Growth, Reproduction	4000, 2000		44
		P. promelas	Fish	7 d Growth	9000		44
Methylparaben	Preservative	*D. magna*	Invert.	7 d Growth, Reproduction	6000, 1500		44
		P. promelas	Fish	7 d Growth	25 000		44
Propylparaben	Preservative	*D. magna*	Invert.	7 d Growth, Reproduction	400, 6000		44
		P. promelas	Fish	7 d Growth	2500		44
		O. latipes	Fish	7 d VTG	99 00[g]		46
Benzophenone-1	UV filter	*P. promelas*	Fish	14 d VTG	4919.4		46
		O. mykiss	Fish	14 d VTG, Growth	4919		47
Benzophenone-2	UV filter	*P. promelas*	Fish	14 d VTG	8782.9		48
		O. mykiss	Fish	14 d VTG, Growth	8783		47
Benzophenone-3	UV filter	*O. mykiss*	Fish	14 d Growth	3900		47
Benzophenone-4	UV filter	*O. mykiss*	Fish	14 d Growth	4897		47
3-benzylidene camphor	UV filter	*Potamopyrgus antipodarum*	Benthic invert.	56 d Reproduction	0.28 mg kg^{-1} sediment		48
		Lumbriculus variegatus	Benthic invert.	28 d Reproduction	6.47 mg kg^{-1} sediment		48
		P. promelas	Fish	14 d VTG, Reproduction, Gonad Histology	434.6, 74, 74		49
		P. promelas	Fish	14, 21 d VTG	435, 74		50, 51
		O. mykiss	Fish	14 d VTG, Growth	453		47
		O. mykiss	Fish	10 d Injection	68 mg kg^{-1}		51
		X. laevis	Amphibian	35 d Metamorphosis	No effect		52
3-(4′-methylbenzy-lidene camphor)	UV filter	*Potamopyrgus antipodarum*	Benthic invert.	56 d Reproduction	1.71 mg kg^{-1} sediment		48
		Lumbriculus variegatus	Benthic invert.	28 d Reproduction	22.3 mg kg^{-1} sediment		48

Table 3 (*continued*)

Compound	Category	Species	Trophic level	Endpoint/duration	LOEC ($\mu g\ L^{-1}$)	NOEC ($\mu g\ L^{-1}$)	References
		O. mykiss	Fish	14 d Growth	415		47
Oxybenzone	UV filter	*O. mykiss*	Fish	14 d VTG	749		54
		O. latipes	Fish	21 d VTG, Hatching	620		54
Ethyl-4-aminobenzoate	UV filter	*P. promelas*	Fish	14 d VTG	4394		49

References: (1) Orvos et al. (2002), (2) Tatarazako et al. (2004), (3) Memmert (2006), (4) Dussault et al. (2008), (5) Ishibashi et al. (2004), (6) Raut and Angus (2010), (7) Fort et al. (2010), (8) Veldhoen et al. (2006), (9) Fraker and Smith (2004), (10) Smith and Burgett (2005), (11) Yang et al. (2008), (12) Wilson et al. (2003), (13) Ciniglia et al. (2005), (14) DeLorenzo and Fleming (2008), (15) Stevens et al. (2009), (16) TCC Consortium (2002), (17) Marchini et al. (1992), (18) Calamari et al. (1982), (19) Kuehn et al. (1989), (20) Smith et al. (1990), (21) Carlson and Kosian (1987), (22) Adema and de Ruiter (1987), (23) Breitholtz et al. (2003), (24) Grutzner (1995b), (25) Wollenberger et al. (2003), (26) Carlsson and Norrgren (2004), (27) Grutzner (1995c), (28) Tas et al. (1997), (29) Chou and Dietrich (1999), (30) Grutzner (1995a), (31) Schramm et al. (1996), (32) Adema and Langerwerf (1985a,b), (33) Sousa and Suprenant (1984), (34) Payne and Hall (1979), (35) Wuthrich (1996a), (36) Gooding et al. (2006), (37) Ramskov et al. (2009), (38) Pedersen et al. (2009), (39) Wuthrich (1996b), (40) Croudace et al. (1997), (41) Yamauchi et al. (2008), (42) Van der Plassche and Balk (1997)), (43) Van Dijk (1997), (44) Dobbins et al. (2009), (45) Bjerregaard et al. (2008), (46) Inui et al. (2003), (47) Kunz et al. (2006c), (48) Schmitt et al. (2008), (49) Fent et al. (2008), (50) Kunz et al. (2006a), (51) Kunz et al. (2006b), (52) Holbech et al. (2002), (53) Kunz et al. (2004), (54) Coronado et al. (2008).

[a] No effects found at concentrations exceeding water solubility.
[b] Frog Embryo Teratogenesis Assay – Xenopus.
[c] Early Life Stage.
[d] Hepatosomatic Index and Gonadosomatic Index.
[e] Vitellogenin.
[f] Estrogen receptor.
[g] Only concentration tested.

short- and long-term exposures than TCS (TCC Consortium, 2002) (Table 2, Table 3). M-TCS toxicity to aquatic organisms (*Daphnia magna* and *Scenedesmus subspicatus*) exposed for short time periods is considerably less than the parent compound TCS (Batscher, 2006a,b). In addition to ionization state affecting bioaccumulation, ionization state is also important when examining toxicity. Orvos et al. (2002) determined un-ionized TCS was slightly more toxic at pH's ranging between 8.17 and 8.21 than ionized TCS at higher pH's. This effect is not observed for TCC as TCC only ionizes at extreme pH's outside environmentally relevant ranges (Young et al., 2008).

In addition to typical acute and chronic studies, a number of studies have investigated effects of TCS exposure on swimming behavior of fish. TCS has induced alterations in swimming performance of *Oncorhynchus mykiss*, *Danio rerio*, and *Oryzias latipes* at concentrations as low as 71 $\mu g\ L^{-1}$ which is considerably greater than other endpoints indicating behavior is not a sensitive endpoint for identifying TCS effects (Orvos et al., 2002; Oliveira et al., 2009; Nassef et al., 2010).

Evidence suggests TCS is weakly estrogenic, likely due to its similarities in structure to the non-steriodal estrogen diethylstilbestrol (Foran et al., 2000; Ishibashi et al., 2004). TCS exposure has been implicated in changes in fin length and sex ratios of medaka (*O. latipes*) (Foran et al., 2000). TCS has also been demonstrated to induce VTG synthesis in male *O. latipes* after 21 d exposure (Ishibashi et al., 2004), and decreased sperm counts and VTG synthesis after 35 d exposure in *Gambusia affinis* (Raut and Angus, 2010). TCS has also been investigated for endocrine effects in *Xenopus laevis* and *Rana catesbeiana* (Veldhoen et al., 2006; Fort et al., 2010) due to similarities in structure to thyroid hormone (TH) (Veldhoen et al., 2006). TCS had no effects on *X. laevis* metamorphosis (Fort et al., 2010) and produced only slight effects in *R. catesbeiana* (Veldhoen et al., 2006) suggesting TCS only minimally affects thyroid hormone (TH) and development in amphibians. To-date, no studies have looked at potential endocrine disruption of other disinfectants with similar structures (i.e. TCC and M-TCS) (Table 3).

Based on toxicity data, algae appear to be the most sensitive trophic group to environmental concentrations of TCS and other disinfectants. However, it is possible that TCS, M-TCS, and TCC could affect benthic invertebrates at environmentally relevant concentrations due to disinfectants potential sorption to sediment although, heretofore, no studies have investigated acute or chronic effects in benthic invertebrates (Orvos et al., 2002). Additionally, TCS has been observed to cause development of antimicrobial strains of resistant bacteria as well as antibiotic resistant bacteria through development of cross-resistance (Braoudaki and Hilton, 2004). The potential environmental impacts of antimicrobial resistance in aquatic ecosystems are low although it could have major implications on human health and aquaculture.

Results of a probabilistic risk assessment indicate minimal effects of TCS on aquatic ecosystems as the 95th percentile of environmental concentrations is below the 5th percentile of sensitive species (Lyndall et al., 2010). Numerous uncertainties remain including effects on benthic organisms and effects of ionization and dissociation on partitioning, toxicity, and bioaccumulation that need to be determined to conduct a comprehensive risk assessment on TCS.

3. Fragrances

Fragrances are perhaps the most widely studied class of PCPs and are believed to be ubiquitous contaminants in the environment (Daughton and Ternes, 1999). The most commonly used fragrances are synthetic musks. Synthetic musks are fragrances used in a wide-range of products including deodorants, soaps, and detergents. Synthetic musks are either nitro musks, which were introduced in the late 1800s, or polycyclic musks, introduced in the 1950s (Daughton and Ternes, 1999). The most commonly used nitro musks are musk xylene (MX) and musk ketone (MK) whereas musk ambrette (MA), musk moskene (MM), and musk tibetene (MT) are used less frequently (Daughton and Ternes, 1999). Nitro musks however, are slowly being phased out due to their environmental persistence and potential toxicity to aquatic species (Daughton and Ternes, 1999). Polycyclic musks are currently used in higher quantities than nitro musks with celestolide (ABDI), galaxolide (HHCB) and toxalide (AHTN) used most commonly and traseloide (ATII), phantolide (AHMI), and cashmeran (DPMI) used less often (Daughton and Ternes, 1999). HHCB and AHTN production alone has been estimated at about 1 million pounds

per year and has thus been placed on the High Production Volume List by the USEPA (Peck, 2006).

Yamigashi et al. (1981, 1983) first identified nitro musks in the environment and conducted the first major monitoring study on MX and MK. MX and MK were found in greater than 80% of samples from river water, WWTP effluent, freshwater fish, and shellfish in Japan. Concentrations were highest in WWTP effluent ranging from 25 to 36 ng L^{-1} and 140–410 ng L^{-1} for MX and MK, respectively (Yamigashi et al., 1981, 1983). For all studies conducted in which fragrance concentrations were reported, MX and MK have been detected in 83–90% of WWTP effluents and approximately 50% of surface waters. Furthermore, Winkler et al. (1998) and Moldovan (2006) identified both nitro musks and polycylic musks in Elbe River (Germany) and Somes River (Romania) water samples ranging between 2 and 10 ng L^{-1} for nitro musks and 2–300 ng L^{-1} for polycyclic musks, with the polycyclic musk HHCB being detected at the highest concentration. Polycyclic musks have been observed more often in surface water (78.3% and 84.6% of samples for AHTN and HHCB, respectively) as well as in greater concentrations than nitro musk compounds worldwide. Kalferlein et al. (1998) and Geyer et al. (1994) published extensive reviews of musks found in the environment, and more specifically in biological compartments. The highest concentrations of polycylclic musks reported to-date has occurred in surface waters in Berlin, Germany that receive substantial input from WWTP at concentrations approaching 10 μg L^{-1} (Table 1) (Heberer et al., 1999).

Nitro and polycyclic musks are water soluble, but high octanol–water coefficients (log Kow = 3.8 for MK and 5.4–5.9 for polycyclic musks) (Schramm et al., 1996; Balk and Ford, 1999) indicate high potential for bioaccumulation in aquatic species (Geyer et al., 1994; Winkler et al., 1998). This potential has been realized by numerous researchers having identified high concentrations of musks in lipids from fresh- and saltwater fish and mollusks (Schramm et al., 1996). Median concentrations of synthetic musks in biota range from approximately 0.1 to 3 mg kg^{-1} of lipid for MK and AHTN, respectively. Dietrich and Hitzfield (2004) compiled bioconcentration (BCF) and bioaccumulation factors (BAF) for synthetic musks and found nitro musks, specifically MX, bioconcentrate and bioaccumulate more than polycyclic musks. MX has been observed to bioconcentrate up to 6700x in common carp (Gatermann et al., 2002), whereas AHTN bioconcentrates much less with BCF values ranging between 597 and 1069 in aquatic species (Dietrich and Chou, 2001; Fromme et al., 2001).

Table 4
Estimated hazard quotients based on the most sensitive endpoint investigated to date and highest observed environmental concentration.

Compound	Trophic group	Toxicity endpoint	Hazard quotient[a]
Triclosan	Invertebrate	EC25 – growth	0.038
	Fish	LOEC – ELS survival	0.032
	Amphibian	LOEC – development	0.130
	Algae	LOEC – growth	19.167
	Plant	LOEC – morphology	0.230
Triclocarban	Invertebrate	LOEC – reproduction	10.962
Musk ketone	Invertebrate	EC10 – development	0.039
	Fish	LOEC – ELS survival	0.039
Musk xylene	Fish	LOEC – ELS survival	0.001
Celestolide	Invertebrate	EC10 – development	0.014
Galxolide	Invertebrate	LOEC – development	0.624
	Fish	LOEC – development	0.183
Tonalide	Invertebrate	EC10 – development	0.942
	Fish	LOEC – ELS survival	0.205
Butylparaben	Fish	LOEC – VTG	0.005

[a] Hazard quotient is calculated by environmental concentration/effects concentration.

Nitro musks have relatively low or no propensity to cause acute toxicity to aquatic taxa studied to-date. Furthermore, only three studies examining MK and MX found acute toxicity to occur at levels below water solubility limits (0.15 mg L^{-1} for MX, 0.46 for MK) (EC, 2003a,b) (Table 2). However, nitro musks are potentially toxic to aquatic organisms over longer time periods with *D. rerio* (zebrafish) early life stage (ELS) studies being most sensitive (Table 3). Additionally, *D. rerio* in general are the most sensitive species studied to-date, whereas amphibians do not appear sensitive to nitro musk exposure (Chou and Dietrich, 1999; Carlsson and Norrgren, 2004). It has been suggested nitro musk transformation products have potential to be highly toxic to aquatic organisms although only minimal data exists (Daughton and Ternes, 1999). Polycyclic musks are more acutely toxic than nitro musks based on published literature. HHCB and AHTN are toxic to aquatic invertebrates at ppb to low ppm levels although they are relatively non-toxic to fish (Table 2), and for longer exposure periods, invertebrates also appear more sensitive to polycyclic musks than fish (Table 3). Similar to nitro musks, polycyclic musks are non-toxic to amphibians. Developmental rates of invertebrates and growth and development for ELS of fish are the most sensitive endpoints studied to date for polycyclic musks. Vitellogenin (VTG) synthesis, indicating potential endocrine effects, was not a sensitive endpoint suggesting musks do not induce estrogenic effects (Dietrich and Chou, 2001).

Based on the highest reported concentrations of synthetic musks in aquatic environments, only AHTN would have the potential to cause adverse effects in wildlife. EC10 values, based on 5 d developmental rates, of the saltwater copepod *Acartia tonsa* are just slightly below the highest environmental concentrations observed (Heberer et al., 1999; Wollenberger et al., 2003) resulting in a hazard quotient of close to 1 (Table 4). However, limited research exists on effects of musks on algae and benthic invertebrates, and therefore, potential risk cannot be accurately determined. Because synthetic musks possess high octanol–water coefficients benthic invertebrates are likely exposed to high concentrations of synthetic musks in sediment and should be tested to evaluate potential toxicity of musks released in WWTP effluent. Only a handful of studies have investigated synthetic musk toxicity to sediment/soil organisms indicating there is potential risk of musk exposure to benthic invertebrates (Balk and Ford, 1999). The polychaete species *Capitella* and snail species *Potamopyrgus antipodarum* were exposed to HHCB for two weeks and 96 d, respectively (Pedersen et al., 2009; Ramskov et al., 2009). Both studies found adult organisms to be insensitive to HHCB although juveniles were much more sensitive for both species. Both studies also examined potential effects for population and both studies concluded there were no population level effects at environmentally relevant concentrations.

Up to eight additional fragrances (acetophenone, camphor, d-limonene, ethyl citrate, indole, isoborneol, isoquinolone, and skatol) have been observed in surface water; however, all fragrances except ethyl citrate have been detected in only a small number of samples (Kolpin et al., 2002; Glassmeyer et al., 2005). Ethyl citrate is a tobacco additive that has frequently been detected in surface water throughout the US (Kolpin et al., 2004; Glassmeyer et al., 2005; among others). Acute and chronic toxicity is not expected to occur with any of these compounds tested individually; however, additional research needs to be conducted.

4. Insect repellants

N,N-diethyl-*m*-toluamide (DEET) is the most common active ingredient in insect repellants (Costanzo et al., 2007) and is routinely detected in surface waters throughout the United States (Glassmeyer et al., 2005). DEET was developed in the 1940s and

functions by interfering with insects ability to detect lactic acid on hosts (Davis, 1985). DEET is currently registered for use in 225 products in the US and it is estimated annual usage exceeds 1.8 million kg (USEPA, 1998).

DEET has been detected in WWTP effluent (Glassmeyer et al., 2005; Sui et al., 2010) and surface water (Kolpin et al., 2002; Glassmeyer et al., 2005; Sandstrom et al., 2005; Quednow and Puttmann, 2010) worldwide. DEET is relatively persistent in the aquatic environment, but unlike many other PCPs (i.e. fragrances and UV filters) DEET has a low BCF and is likely not accumulated into aquatic organisms (Costanzo et al., 2007). DEET has been regularly detected in effluent (95% of analyzed samples) and surface water (65% of all analyzed samples) with median concentrations of approximately 0.2 $\mu g\,L^{-1}$ and 55 $ng\,L^{-1}$, respectively. The only other insect repellant detected in WWTP effluent or surface water is 1,4-dichlorobenzene. 1,4-dichlorobenzene has been detected in surface water (40% of surface water screened) receiving significant inputs of WWTP effluent throughout the US at concentrations up to 0.28 $\mu g\,L^{-1}$ (Table 1) (Glassmeyer et al., 2005).

To-date very little data exists pertaining to acute toxicity of DEET to aquatic organisms. Costanzo et al. (2007) summarizes all data published through 2006 and since its publication no additional information has been reported. Data indicates DEET is only slightly toxic to aquatic organisms (Table 2) (Michael and Grant, 1974; Office of Pesticide Programs, 2000). Although DEET is relatively resistant to breakdown and commonly found in surface water, no known studies exist that have examined chronic toxicity of DEET exposure to aquatic organisms. DEET has been observed to inhibit cholinesterase in rats (Chaney et al., 2000), and it is possible similar effects could be observed in fish even though no research has been conducted. Additionally, no studies have been conducted to examine potential endocrine effects on aquatic organisms although studies have been conducted in rats. These studies indicated DEET has no effect on sperm count, morphology, or viability in male rats after 9 week exposure (Lebowitz et al., 1983) thus indicating little potential effects in aquatic species. Based on available information, Costanzo et al. (2007) performed a preliminary risk assessment and concluded DEET is not likely to produce biological effects at environmentally relevant concentrations in aquatic ecosystems; however, due to lack of information on chronic toxicity a definitive assessment could not be made. Similar conclusions are still applicable today as chronic toxicity of DEET to aquatic organisms remains undetermined.

Similar to DEET, a preliminary risk assessment has also been conducted on the moth repellant 1,4-dichlorobenzene. Invertebrates, specifically *D. magna*, appear the most sensitive from short-term exposure (Table 2) whereas fish appear to be most sensitive to long-term exposures (Table 3) (Boutonnet et al., 2004). Based on observed environmental concentrations, it is unlikely acute or chronic effects are occurring to freshwater and marine organisms (Boutonnet et al., 2004). Additionally, there is little potential for bioaccumulation of 1,4-dichlorobenzene (Boutonnet et al., 2004) and to-date there is no indication that it can cause endocrine effects.

5. Preservatives

Parabens (alkyl-*p*-hydroxybenzoates) are antimicrobial preservatives used in cosmetics, toiletries, pharmaceuticals, and food (Daughton and Ternes, 1999). There are currently seven different types of parabens in use (benzyl, butyl, ethyl, isobutyl, isopropyl, methyl, and propyl). In 1987 over 7000 kg of parabens were used in cosmetics and toiletries alone (Soni et al., 2005) and that number has been expected to increase over the last 20 years. Methyl- and propylparaben are the most commonly used in cosmetics and are typically co-applied to increase preservative effects (Peck, 2006). To-date only a handful of studies have examined paraben concentrations in WWTP and surface water. Greatest concentrations of parabens have been identified in surface water with concentrations ranging from 15 to 400 $ng\,L^{-1}$ depending on paraben, whereas effluent had lower concentrations ranging from 50 to 85 $ng\,L^{-1}$ (Table 1) (Benijts et al., 2004; Lee et al., 2005; Gregory and Mark, 2006; Kasprzyk-Hordern et al., 2008; Jonkers et al., 2010).

Of the seven different types of parabens currently in use, benzylparaben appears to be most acutely toxic (Madsen, 2009; Terasaki et al., 2009; Bazin et al., 2010) (Table 2). Methyl- and ethylparaben appear to be least acutely toxic with LC50 values approximately 3× greater than benzylparaben for all trophic groups studied (Table 3) (Bazin et al., 2010). It has previously been reported increasing chain length of parabens' substituents can increase paraben acute toxicity to bacteria (Dymicky and Huhtanen, 1979; Eklund, 1980) and this appears to be true for other trophic groups as well. There is currently a lack of information on the chronic effects of parabens to aquatic organisms with only a single known study examining toxicity in *D. magna* and *Pimephales promelas* (Dobbins et al., 2009). These authors found benzyl- and butylparaben were most toxic to invertebrates and fish whereas methyl- and ethylparaben appeared least toxic. This corresponds directly with results of acute studies, as well as previous studies indicating increased chain length of parabens increases toxicity. In addition to increasing chain length, chlorination also substantially increases toxicity of parabens to both bacteria and *D. magna* (Terasaki et al., 2009).

Based on limited environmental concentration and toxicity data, it appears benzyl-, butyl- and propylparaben could potentially cause adverse effects to aquatic organisms. Dobbins et al. (2009) concluded parabens only pose limited hazard to aquatic organisms; however, parabens, specifically benzyl-, butyl- and propylparaben, can elicit low-level estrogenic responses. *In vitro* studies conducted with fish MCF-7 cell lines and yeast estrogenic screening assays demonstrated parabens can elicit estrogenic responses at low levels (Routledge et al., 1998; Darbre et al., 2002, 2003). Furthermore, Inui et al. (2003) and Bjerregaard et al. (2008) demonstrated parabens can cause VTG synthesis in fish when exposed to low concentrations. Therefore, low level exposure to parabens could potentially cause estrogenic effects at environmentally relevant concentrations. Additional studies have been conducted examining effects of parabens on sexual endpoints including spermatogenesis and serum testosterone in male rats (Oishi, 2002). Both butyl- and propylparaben significantly inhibited spermatogenesis, but did not affect serum testosterone (Oishi, 2002). Golden et al. (2005) reviewed paraben endocrine activity in rats and determined butyl-, isobutyl-, and benzylparaben demonstrate estrogenic activity although their potency is much less than estrogen itself (Golden et al., 2005). These results indicate there are potential affects in aquatic organisms continually exposed to parabens. Preliminary data on environmental concentrations, however, suggest only minimal risk to aquatic organisms as effect concentrations are generally 1000x higher than what has been observed in surface water.

6. UV filters

Growing concern over effects of ultraviolet (UV) radiation in humans has caused an increased usage of UV filters. UV filters are used in sunscreen products and cosmetics to protect from UV radiation and can be either organic (absorb UV radiation, e.g. methylbenzylidene camphor) or inorganic micropigments (reflect UV radiation, e.g. ZnO, TiO_2); however, in this review only organic compounds will be discussed. Typically, three to eight separate UV filters are found in sunscreens and cosmetics and can make

up greater than 10% of products by mass (Schreurs et al., 2002). There are 16 compounds that are currently certified for use as sunscreen agents (SSA's) (Reisch, 2005) and 27 certified UV filters in cosmetics, plastics, among others in the US (Fent et al., 2008). UV filters enter the environment in two ways, either indirectly via WWTP effluent or directly from sloughing off while swimming and other recreational activities. A study in Switzerland estimated the input of four commonly applied UV filters into WWTPs to be as high as 118 g of 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC), 49 g of 4-methyl-benzilidine-camphor (4MBC), 69 g of benzophenone-3 (BP3), and 28 g of octocrylene (OC) per 10 000 people per day in high use times (Balmer et al. (2004)). Additionally, Poiger et al. (2004) estimated up to 1263 mg of UV filters are applied per person daily resulting in up to 966 kg of UV filters released directly into a small lake in Switzerland per year. Although UV filters are used at high levels and are likely to enter into aquatic environments, very little is known about their environmental concentrations due to a lack of analytical methods.

Balmer et al. (2004) examined presence of four UV filters (4MBC, BP3, EHMC, and OC) in wastewater effluent, surface water, and fish tissue in Switzerland. WWTP effluent had the greatest concentrations of UV filters with 4MBC being detected at the highest concentrations (2.7 $\mu g\,L^{-1}$) (Balmer et al., 2004) and was also detected at the highest concentrations in surface water (35 $ng\,L^{-1}$) and fish tissue (123 $ng\,g^{-1}$ lipid tissue). Poiger et al. (2004) found similar results in Swiss lakes with BP3 being detected at the highest concentrations (5–125 $ng\,L^{-1}$) (Table 1). Overall, 4MBC has been detected most frequently in WWTP effluent and surface water worldwide (95% and 86% of samples, respectively) whereas OC has been detected much less frequently in both (77% of WWTP effluent samples and 14% of surface water samples). The majority of additional environmental concentration data that exists pertains to bioaccumulation of UV filters in aquatic organisms. UV filters are known to bioaccumulate in fish at levels similar to PCBs and DDT (Daughton and Ternes, 1999) due to their high lipophilicity (log Kow = 3–7) and stability in the environment (Balmer et al., 2004; Poiger et al., 2004). UV filters have been found in lipid tissue in fish at concentrations up to 2 ppm (Nagtegaal et al., 1997). Additionally, UV filters were identified to have bioaccumulation factors of greater than 5000 in fish (21 $\mu g\,kg^{-1}$ in whole fish versus 0.004 $\mu g\,L^{-1}$ in water) (Hany and Nagel, 1995). In the only known laboratory study, 3-benzylidene camphor (3BC) was found to have a bioconcentration factor of 313 in *P. promelas* exposed for 21 d (Kunz et al., 2006a).

A single study indicates UV filters do not appear to be acutely toxic to aquatic organisms (Fent et al., 2009). *D. magna* were observed to be most sensitive over short-term exposures (48 h) to EHMC whereas they were least sensitive to benzophenone-4 (BP4) (Fent et al., 2009) (Table 2). The majority of studies pertaining to UV filters have focused on long-term exposures. Schmitt et al. (2008) investigated effects in benthic invertebrates and observed a significant reduction in *P. antipodarum* reproduction and increased mortality when exposed to 3BC and 4MBC for 56 d and significant decreases in reproduction and increased mortality in *Lumbriculus variegates* when exposed for 28 d to the same compounds (Table 3).

UV filters are well known to bioaccumulate and recent studies have also indicated the potential for estrogenic activity. *In vitro* assays using fish MCF-7 cell lines indicate five UV-A and UV-B sunscreens (BP3, homosalate (HMS), 4MBC, octyl-methoxycinnamate and octyl-dimethyl-PABA) have potential to cause estrogenic effects (Schlumpf et al., 2001; Kunz and Fent, 2006). Additionally, Kunz et al. (2006c) identified 10 UV filters had estrogenic effects using a recombinant yeast assay with rainbow trout ERα. Benzophenone-1 (BP1) was the most potent UV filter with 4′ hydroxybenzophenone (4HB) being the only other compound that demonstrated estrogenicity below 1 $mg\,L^{-1}$ exposure (Kunz et al., 2006c). Aquatic studies using fish (*P. promelas* and *O. mykiss*) indicate numerous UV filters have the potential to cause estrogenic effects and also adversely affect fecundity and reproduction (Table 3). 3BC appears to be the most estrogenic compound inducing VTG in *O. mykiss* and *P. promelas* after 14 d and 21 d exposure, respectively (Kunz et al., 2006a,b; Fent et al., 2008). Other UV filters (BP1 and BP2) also induce VTG in male fathead minnows but at concentrations 10-fold higher than 3BC. Oxybenzone induces VTG production in both *O. mykiss* and *O. latipes* at similar concentrations as 3BC and also significantly decreases fertilized eggs hatchability in *O. latipes* (Coronado et al., 2008). Based on a single study, amphibians do not appear as sensitive as fish as a result of 3BC exposure (Kunz et al., 2004). Recombinant yeast assays using fish hERα also indicate some UV filters also possess antiestrogenic activity (e.g. 4MBC and 3BC), androgenic activity (e.g. BP2 and HMS), and/or antiandrogenic activity (e.g. 4-hydroxy benzophenone [4HB]) (Kunz and Fent, 2006); however, no studies have investigated these effects *in vivo* in aquatic organisms. *In vivo* studies using rats have indicated 4-MBC affected the hypothalamus-pituitary–gonadal system in male rats thus altering gonadal weight and steroid hormone production. 3-BC also affected development of sex organs in male rats after 12 week exposure (Schlumpf et al., 2004). This data substantiates the *in vitro* data and indicates potential risk in aquatic species; however, the extent of risk of UV filters in WWTP effluent and surface water is currently unknown based on the scarcity of environmental concentration data. Additionally, the number of species used to identify toxic effects is minimal and therefore does not allow for a comprehensive risk profile to be developed.

7. Additional compounds

Three additional PCPs have been identified in surface water in the US by United States Geologic Survey researchers. The fixative benzophenone was detected most frequently (67.5% of samples in one study) whereas the flavorant menthol was detected at the highest concentrations (1.3 $\mu g\,L^{-1}$) (Kolpin et al., 2002; Glassmeyer et al., 2005). Benzophenone acute and chronic toxicity has been examined for *Caenorahbditis elegans* and *P. promelas* and both studies indicate benzophenone is relatively non-toxic to aquatic organisms (Marchini et al., 1992; Ura et al., 2002) (Table 2; Table 3). The other compound detected in surface water is methyl salicylate (wintergreen flavoring and liniment) although it has only been detected at low concentrations and in few environmental samples (Glassmeyer et al., 2005).

8. Hazard assessment

A preliminary hazard assessment was conducted for PCPs with sufficient chronic toxicity and environmental concentration data using hazard quotients (Table 4). A hazard quotient is the ratio of the exposure concentration divided by the toxicological benchmark concentration that can give a preliminary assessment of potential adverse effects (Suter, 2007). Hazard quotients exceeding 1 indicate potential effects. Based on published reports of toxicity and environmental concentrations, only triclosan and triclocarban have the potential to cause chronic effects based on hazard quotients greater than 1 (Table 4). Growth of a natural algal assemblage from a stream in Kansas, USA was significantly decreased after 96 h exposure to a concentration of 0.12 $\mu g\,L^{-1}$ TCS (Wilson et al., 2003) which resulted in a hazard quotient of 19. TCC also had a hazard quotient greater than 1 (10.9) for *Americamysis bahia* in response to 28 d exposure to 0.13 $\mu g\,L^{-1}$ (TCC Consortium, 2002). However, both of these hazard quotients could be consid-

ered worst-case scenarios as the environmental concentrations used were the maximum observed (approximately 20× higher than median concentrations (Table 1)) and the most sensitive species and biological endpoint was also considered (Table 3). Therefore, actual hazard is likely much lower than what is predicted here, which coincides with preliminary risk assessments conducted elsewhere (Costanzo et al., 2007; Lyndall et al., 2010).

Insufficient long-term *in vivo* data exists for UV filters and therefore hazard quotients were not capable of being calculated. Additionally, hazard quotients were not calculated for potential endocrine effects due to the dearth of *in vivo* studies on aquatic organisms; however, mammalian studies indicate UV filters are the most likely to cause endocrine effects.

9. Discussion

Based on published data, environmental concentrations and toxicity of PCPs have been largely overlooked in comparison to pharmaceutical compounds. Current published literature for environmental concentrations is fairly substantial for some PCP compounds (ex. TCS, DEET, fragrances) but relatively little is available for others (ex. TCC, UV filters, preservatives). Additional research for surface water concentrations and uptake into biota needs to be conducted in order to develop aquatic risk assessments for PCPs released in WWTP effluent. There are also substantial data gaps in published literature addressing potential toxicity of PCPs to aquatic organisms. The most overlooked trophic group is benthic invertebrates although they are likely exposed to the greatest concentrations of PCPs based on chemical properties of many PCP compounds. Additionally, toxicity of PCPs in algae and vascular plants are also not well represented in PCP literature.

Data developed thus far indicate most PCPs are relatively nontoxic to aquatic organisms at expected environmental concentrations. However, the primary concern for PCPs is their potential to cause estrogenic effects at relatively low concentrations. Preservatives and UV filters are known endocrine active compounds (Routledge et al., 1998; Schlumpf et al., 2001; Darbre et al., 2002, 2003; Kunz and Fent, 2006) with triclosan also suspected to cause endocrine effects (Foran et al., 2000; Ishibashi et al., 2004). Although numerous studies have identified effects in fish, only a handful of studies have examined these effects in non-vertebrate aquatic species (Veldhoen et al., 2006; Fort et al., 2010).

Besides potential for endocrine effects, the other major concern with PCPs is their potential to bioaccumulate in aquatic organisms. UV filters, disinfectants, and fragrances have all been shown to bioaccumulate in biota (Geyer et al., 1994; Winkler et al., 1998; Daughton and Ternes, 1999; Coogan et al., 2007), and can potentially biomagnify in higher trophic levels. To-date, no known studies have examined the potential for biomagnification and potential effects on higher trophic level organisms and subsequent effects on aquatic ecosystems.

As mentioned previously, PCPs have little potential to cause acute or chronic effects in aquatic organisms; however, most studies conducted have investigated compounds individually and not as environmentally relevant effluent mixtures. The more realistic exposure scenario would be through whole effluent testing (WET) to gain information on environmentally relevant mixtures. So far no known studies have investigated PCPs as part of whole effluent on aquatic organisms although it is likely additional effects could be observed using WET testing procedures.

Based on all data published through April 2010 a definitive risk assessment cannot be conducted to determine potential effects of PCP release into aquatic environments. Numerous preliminary risk assessments (TCS, fragrances, DEET, and parabens) have been performed yet no definitive assessment has been made due to incomplete data sets. Specifically the incomplete data include in order of importance are: (1) environmental concentrations of M-TCS, TCC, preservatives, and UV filters, (2) chronic data for DEET and preservatives, (3) endocrine effects of fragrances, (4) bioaccumulation and biomagnification of UV filters, and (5) acute data for M-TCS and UV filters. As these questions continued to be addressed, more definitive aquatic risk assessments can be developed and a better understanding of potential risk of PCP release into aquatic environments will be realized.

10. Conclusions

Personal care products are released into the environment unaltered through normal human usage. Although they can be released at levels greater than many other compounds, including pharmaceuticals, relatively little research has been conducted to identify environmental concentrations and potential toxicity. PCPs are also continually replenished through normal usage in the environment and are thus persistent compounds that warrant acute and chronic studies. Additional investigations into both acute and chronic toxicity need to be conducted in order to understand potential effects and risk of PCP release into surface water. Similar to pharmaceuticals, studies investigating PCP effects on benthic invertebrates is severely lacking. Most studies conducted to-date indicate little short- and long-term toxicity and therefore the primary issues of concern with PCPs are their ability to bioaccumulate to high levels as well as the propensity to cause estrogenic and endocrine effects. This review indicates additional research is needed to understand environmental concentrations, potential toxicity of mixtures, endocrine effects, and potential for bioaccumulation and biomagnification of PCPs in order to accurately identify potential risk of PCP release into the aquatic environment.

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REVIEW

An overview of UV-absorbing compounds (organic UV filters) in aquatic biota

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Abstract The purpose of this article is to summarize biological monitoring information on UV-absorbing compounds, commonly referred as organic UV filters or sunscreen agents, in aquatic ecosystems. To date a limited range of species (macroinvertebrates, fish, and birds), habitats (lakes, rivers, and sea), and compounds (benzophenones and camphors) have been investigated. As a consequence there is not enough data enabling reliable understanding of the global distribution and effect of UV filters on ecosystems. Both liquid chromatography and gas chromatography coupled with mass spectrometry-based methods have been developed and applied to the trace analysis of these pollutants in biota, enabling the required selectivity and sensitivity. As expected, the most lipophilic compounds occur most frequently with concentrations up to 7112 ng g^{-1} lipids in mussels and 3100 ng g^{-1} lipids (homosalate) in fish. High concentrations have also been reported for 4-methylbenzilidenecamphor (up to 1800 ng g^{-1} lipids) and octocrylene (2400 ng g^{-1} lipids). Many fewer studies have evaluated the potential bioaccumulation and biomagnification of these compounds in both fresh and marine water and terrestrial food webs. Estimated biomagnification factors suggest biomagnification in predator–prey pairs, for example bird–fish and fish–invertebrates. Ecotoxicological data and preliminary environmental assessment of the risk of UV filters are also included and discussed.

Keywords UV filters · Biota · Chromatography · Mass spectrometry · Bioaccumulation · Toxicity

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Introduction

UV filters, including both inorganic and organic sunscreen agents, constitute a group of emerging environmental pollutants, potentially hazardous compounds that have been receiving steadily growing attention over the last decade as society has become aware of the dangerous effects of UV solar radiation. These chemicals can be found not only in cosmetics but also in other personal care products, food packaging, pharmaceuticals, plastics, textiles, and vehicle-maintenance products to prevent photodegradation of polymers and pigments [1, 2].

Incomprehensibly, there are scarce data about, and limited understanding of, the environmental occurrence, fate, distribution and effects of many UV filters and their metabolites and other transformation products, despite their extensive use. According to market studies, sunscreen product sales were higher than half a billion US dollars in 2005, and it is estimated that 10,000 tons of UV filters are produced annually for the global market [3].

It is likely that usage of sunscreen agents is going to increase in the future, because of the recommendations of health authorities on the prevention of skin cancer. One of the main reasons for the scarcity of data was the lack of suitable analytical methods capable of detecting emerging

pollutants at trace levels in, usually, complex environmental matrices. However, as a consequence of increasing concern about the potential effects of sunscreens on ecosystems and humans, in last five years environmental analytical scientists have developed sensitive and selective analytical methods.

Eco-toxicological data on both UV filters and their degradation products is also missing. Despite the small amount of information available about their toxicity, the low environmental concentrations reported so far suggest a low potential risk. However, the long-term risk associated with the pseudo-persistence of these chemicals in the environment is largely unknown.

The purpose of this review is to summarize scarce and scattered information about the profiles of UV filters in aquatic organisms, analytical methods, bioaccumulation/biomagnification, ecotoxicity, and environmental analysis and risk assessment (ERA). Finally, the article identifies current gaps in our knowledge and potential future research needs in ERA.

Physicochemical properties

UV filters are substances with almost null absorption of visible radiation but important light absorption in the UVA (315–400 nm) and UVB (280–315 nm) ranges [4]. Sunscreens can be classified into organic (chemical) absorbers and inorganic (physical) blockers on the basis of their mechanism of action. Organic UV filters absorb UV radiation with excitation to a higher energy state. Excess energy is dissipated by emission of higher wavelengths or relaxation by photochemical processes, for example isomerization and heat release. They include camphors, benzophenones, cinnamates, triazines, among others. Inorganic sunscreens, i.e. titanium dioxide and zinc oxide, protect the skin by reflecting and scattering UV radiation.

The focus of this review is on organic UV filters. A feature common to all of these is the presence of an aromatic moiety with a side-chain with different degrees of unsaturation. Their structures and other physicochemical properties are listed in Table 1. Some, for example 4-methylbenzylidene camphor (4MBC), ethylhexylmethoxy cinnamate (EHMC), and octocrylene (OC), are chiral compounds. Although the enantiomers of these compounds are not expected to have different physicochemical properties, isomers and enantiomers may differ in biological behavior. Commercial formulations contain mainly geometrical (*E*) isomers, although some UV filters (e.g., methoxycinnamates) contain both the (*E*) and the (*Z*) isomers. Because of the high lipophilicity and poor biodegradability of many UV filters (mostly with log K_{ow} 4–8) they end up in sewage sludge during wastewater treatment [5–8], and accumulate in river sediments [9–12] and biota [13–20].

Analytical methodology

Sampling and sample preparation

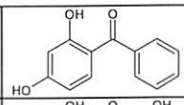
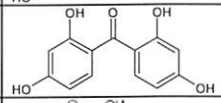
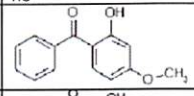
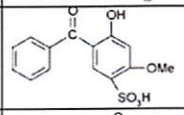
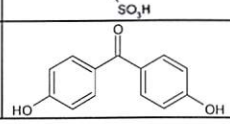
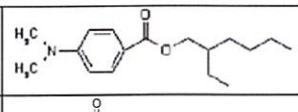
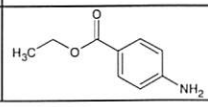
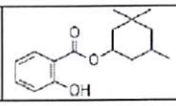
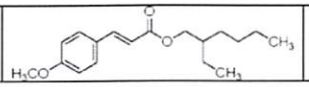
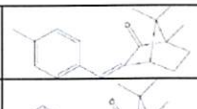
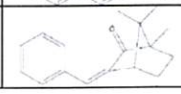
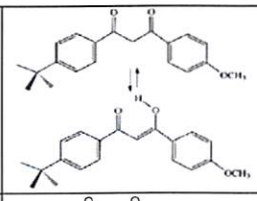
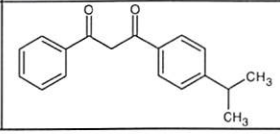
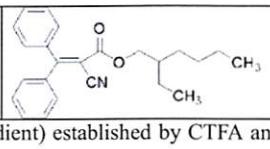
Sampling procedures for analysis of residues of UV filters in aquatic biota mainly involve traditional fishing, either by native fishers or by electric fishing, for which special permission is often needed. Unlike other matrices, there is the added difficulty of the availability of samples of the desired species, which often depends on external factors which are difficult to control. Moreover, the variability between individuals of the same species (size and living cycle) hinders comparison of results. Most studies have focused on fish, a representative matrix of the aquatic environment assumed to be able to retain and bioaccumulate UV filters because of the lipophilicity of the compounds. The most usual sample analyzed is muscle, probably because of its low lipid content in comparison with other tissues and because it is part of the human diet. Studies have also been conducted on macrozoobenthos, mussels, and birds. Selected tissues are homogenized by blending and often freeze-dried before extraction.

Extraction and clean up

Extraction of UV filters from tissues has been achieved by conventional Soxhlet extraction (which has become less attractive because of the time and solvent consumed) [16, 18], pressurized-liquid extraction (PLE) [14], solid–liquid extraction [13–15, 17, 19], and microwave-assisted extraction (MAE) [20]. These techniques lead to coextraction of a lipid fraction that must be removed before determination of the UV filters. Clean-up of biota sample extracts is usually a two-stage process. The sample extracts can first be subjected to gel-permeation chromatography (GPC), primarily to remove lipids, followed by adsorption chromatography on silica or Florisil columns. Quite often RP-HPLC has also been used for extraction and purification.

In the first work published on UV filter levels in biota [18], benzophenone-3 (BP3), 4MBC, homosalate (HMS), EHMC, ethylhexyl dimethyl PABA (OD-PABA), isopropyl dibenzoyl methane (IDM), and butyl methoxy dibenzoyl methane (BM-DBM) were extracted from fish tissue by Soxhlet extraction. The tissue was first homogenized and dried with sodium sulfate, then extracted with petroleum ether–ethyl acetate 2:1 (*v*/*v*). Lipids and other potential matrix interferences were removed by GPC (Bio Beads SX-3) with cyclohexane–acetone 3:1 (*v*/*v*) as mobile phase. For analysis of IDM and BM-DBM, CH_3I/NaH was added to half of the extract to form their derivatives for further GC–MS analysis. This half of the extract was then purified on a silica column (elution with hexane–ethyl acetate 7:3 (*v*/*v*)). The other half was also loaded on to a silica column and

Table 1 Physicochemical properties of the organic UV filters addressed in this review. In parentheses, the key system adopted herein

Name (INCI nomenclature)[a]	CAS no.	Structure	Molecular weight (g mol^{-1})	Log K_{ow}	Solubility (g L^{-1})[b]
Benzophenones					
Benzophenone-1 (BP1)	131-56-6		214.22	3.15[c]	0.39[c]
Benzophenone-2 (BP2)	131-55-5		246.22	2.78[d]	0.98[c]
Benzophenone-3 (BP3)	131-57-7		228.24	3.79[d]	0.10[c]
Benzophenone-4 (BP4)	4065-45-6		308.31	0.993[c]	11[c]
4,4′-Dihydroxybenzophenone (4DHB)	611-99-4		214.22	2.19[d]	0.6[c]
p-Aminobenzoic acid derivatives					
Ethylhexyldimethyl PABA (OD-PABA)	21245-02-3		277.4	5.412[c]	4.7×10^{-3}[c]
Ethyl-PABA (Et-PABA)	94-09-7		165.19	1.86[d]	1.31[d]
Salicylates					
Homosalate (HMS)	118-56-9		262.35	5.947[c]	0.021[c]
Cinnamates					
Ethylhexyl methoxycinnamate (EHMC)	5466-77-3		290.4	5.8	6.4×10^{-3}[c]
Camphor derivatives					
4-Methylbenzylidene camphor (4MBC)	36861-47-9		254.37	4.95	0.017
3-Benzylidene camphor (3BC)	15087-24-8		240.34	2.84	0.034
Dibenzoylmethane derivatives					
Butylmethoxydibenzoylmethane (BM-DBM)	70356-09-1		310.39	4.191	4.3×10^{-3}[c]
Isopropyldibenzoylmethane (IDM)	63250-25-9		266.33	4.382	0.027
Crylenes					
Octocrylene (OC)	6197-30-4		361.49	6.88	3.6×10^{-4}[d]

[a] INCI (International Nomenclature for Cosmetic Ingredient) established by CTFA and COLIPA

[b] In water at 25 °C

[c] Calculated by use of Advanced Chemistry Development (ACD/Labs) Software V11.02 (1999–2011 ACD/Labs)

[d] Experimental values from database of physicochemical properties; Syracuse Research Corporation: http://www.syrres.com/esc/physdemo.htm

the same solvent mixture in a different proportion (91:9 *v/v*) was used for elution.

A similar method was developed by Meinerling and Daniels [16] for analysis of 4MBC, BP3, EHMC, and OC in the muscle of rainbow trout. In this case Soxhlet extraction with *n*-hexane–acetone 9:1 (*v/v*) was followed by GPC (Bio Beads SX-3) with cyclohexane–ethyl acetate 1:1 (*v/v*) as eluent. In a further clean-up step, a Florisil column was used to remove polar compounds.

In the procedure followed by Balmer et al. [14] for analysis of 4MBC, BP3, EHMC, and OC, fish samples were homogenized with sodium sulfate and column extracted or PLE extracted with dichloromethane (DCM)–cyclohexane 1:1 (*v/v*). The extracts were then cleaned by GPC on a Biobeads S-X3 column with DCM–cyclohexane 35:65 (*v/v*) as eluent, followed by silica purification. Buser et al. [15] extracted 4MBC and OC by successive extraction with potassium oxalate (2 mL, 35 %), ethanol (100 mL), diethyl ether (50 mL), and *n*-pentane (70 mL). After extraction, matrix components were removed by GPC and silica purification.

The methods described above are only suitable for extracting UV filters with similar physicochemical properties. A method for simultaneous determination of nine UV filters, from polar to lipophilic, in fish has been reported by Zenker et al. [17]. Mid-polar and lipophilic UV filters were extracted from homogenized tissue by solvent extraction with ethyl acetate–*n*-heptane–water 1:1:1 (*v/v*) and further purified by reversed-phase RP-HPLC. The fraction containing mid-polarity UV filters was analyzed by HPLC–MS whereas the fraction containing the lipophilic ones was determined by GC–MS. Polar and medium-polarity UV filters were extracted with a mixture of methanol (MeOH) and acetonitrile (ACN), followed by HPLC–MS analysis. This is the procedure requiring the smallest amount of sample (4 g); good limits of detection are achieved for most compounds. The same method proved to be suitable for analysis of macrozoobenthos and bird samples also.

Bachelot et al. [20] developed a method for determination of EHMC, OC, and OD-PABA in marine mussels. MAE was performed with 25 mL acetone–heptane 1:1 (*v/v*). After extraction, the liner was rinsed with the same solvent mixture. The extracts were percolated through anhydrous sodium sulfate. Further purification was performed by RP-HPLC on a RP Spherisorb ODS2 column (4.6 mm×150 mm, 5.0 μm) following a procedure adapted from Zenker et al. [17].

GPC or column purification with silica or Florisil is useful whenever compounds with similar physicochemical properties must be separated from interfering matrix substances, for example lipids, present in the sample. When these methods are used for a mixture of compounds with different physicochemical properties they are less effective. RP-HPLC is a suitable alternative when UV filters with a large range of physicochemical properties must be analyzed.

Instrumental analysis

LC is the technique of choice for the analysis of UV filters in cosmetic products. In contrast, GC is preferred for their environmental analysis. Nevertheless, both techniques have been applied to the analysis of biological samples. The low concentration of the target analytes in biota samples requires high sensitivity and selectivity. Therefore, mass spectrometric (MS) detection is the most suitable technique for determination of these compounds in such complex matrices. Relevant data on analytical methods are summarized in Table 2.

GC–MS

UV filters are, with very few exceptions (e.g., octyl triazone (OT) and BM-DBM), amenable to GC. Matrix effects are not critical for the ionization modes, e.g. electron impact (EI) or chemical ionization (CI), typically used in GC–MS. As a consequence, method detection limits (MDL) are usually quite low [21]. On the other hand, this technique can only be successfully applied to a limited number of non-polar and volatile compounds. For more polar or thermally unstable compounds an additional derivatization step is required; here differences in matrix components may result in quite different derivatization efficiencies which may affect both precision and accuracy of the analysis.

Analysis has always been performed in electron-impact mode (GC–EI-MS). Quantification is achieved by operating in selected ion monitoring mode (SIM). The fragment ions usually selected for the quantification and confirmation of the analytes are listed in Table 2.

BM-DBM, IDM, 4MBC, OD-PABA, HMS, EHMC, and BP3 have been analyzed by GC–MS on a SE-54-CB column (50 m×55 mm, 0.25-μm film), working in SIM mode [18]. Balmer et al. [14] analyzed 4MBC, BP3, EHMC, and OC in fish by GC–EI-MS using two different columns a BGB-5 (30 m×0.25 mm; 0.25 μm) and an SE54 (25 m×0.32 mm; 0.25 μm). In that study $^{13}C_{12}$-PCB 77 was used as surrogate standard. Under the same GC–EI-MS conditions Buser et al. [15] analyzed 4MBC and OC in fish tissue, using $^{15}N_3$-musk xylene as internal standard. Zenker et al. and Fent et al. [17, 19] analyzed nine UV filters with a large range of physicochemical properties (log K_{ow} from 0.9 to 5.7) in fish, macrozoobenthos, and bird tissue. Four out of the nine UV filters investigated, BP3, 3BC, 4MBC, and EHMC (the most lipophilic) were detected by GC–EI-MS on an Optima-5-MS (50 m×0.2 mm; 0.35 μm) column. In this study benzophenone-d_{10} was used as surrogate standard.

Mottaleb et al. [13] analyzed 4MBC and OC with 10 other personal care products in fish tissue by both GC–EI-MS and GC–EI-IT (with an ion trap mass spectrometer). The GC–EI-MS analysis was carried out with a XTI-5 capillary column (30 m×0.25 mm; 0.25 μm) operating in

Table 2 Analytical methodology and occurrence data for the UV filters addressed in this review

Matrix	Specie	Tissue	UV filter	Sample amount	Extraction	Purification	Technique
Fish	Bluegill (*Lepomis macrochirus*)	Muscle	4MBC, OC	1 g	Rotatory extraction with acetone	Silica	GC-EI-MS
	Sonora sucker (*Catostomus insignis*)	Muscle, belly flap and skin	4MBC, OC	1 g	Sonication with acetone	GPC Silica	GC-EI-IT
Fish	White fish (*Coregonus sp.*)	Muscle	4MBC, BP3, EHMC, OC	5 g	ASE extraction: Homogenized with diatomaceous earth 3 cycles DCM/ cyclohexane (1:1, v/v) at room temperature	GPC (EnviroSep-ABC or Biobeads S-X3) Silica	GC-EI-MS
	Roach (*Rutilus rutilus*)			20 g	Homogenized with sodium sulphate		
	Perch (*Perca fluviatilis*)				Column extracted with DCM/cyclohexane (1:1, v/v))		
Fish	Brown trout (*S.Trutta fario*)	Muscle plus adipose tissue under the skin	4MBC, OC	10–25 g	Homogenized in 100 ml water with hand blender Solvent Extraction using potassium oxalate (2 mL, 35 %), ethanol (100 mL), diethyl ether (50 mL) and n-pentane (70 mL)	GPC (EnviroSep-ABC or Biobeads S-X3) Silica	GC-EI-MS
Fish	Rainbow trout (*Oncorhynchus mykiss*)	Muscle	4MBC, BP3, EHMC, OC	10 g	Homogenized with sodium sulphate Soxhlet extracted with n-hexane/acetone (9/1, v/v)	GPC (Biobeads S-X3) Florisil	LC-ESI-MS/MS
Fish	Barb (*Barbus barbus*) and Chub (*Leucisus cephalus*)	Muscle plus adipose tissue under the skin	4MBC, 3BC, BP1, BP2, 4DHB, BP3, BP4, EHMC, Et-PABA	4 g	Solvent extraction using ethyl acetate, n-heptane and HPLC water (1:1:1, v/v/v) or Solvent extraction with MeOH:ACN (1:1, v/v)	RP-HPLC (RP Spherisorb ODS2 column (4.6 mm × 150 mm, 5.0 µm)	LC-ESI-MS/MS and GC-EI-MS
	Barb (*Barbus barbus*) and Chub (*Leucisus cephalus*)	Muscle plus adipose tissue under the skin	BP4, 4DHB, BP1, BP2, Et-PABA	1 g	Solvent extraction with MeOH/ACN (1:1, v/v)	Syringe filtration	LC-ESI-MS/MS
Fish	Roach (*Rutilus rutilus*)	Muscle, offal, rest and whole fish	IDM, BM-DBM, 4MBC, OD-PABA, HMS, EHMC, BP3		Homogenized with sodium sulphate	GPC (Biobeads S-X3)	GC-EI-MS
	Perch (*Perca fluviatilis*)				Soxhlet extracted with petroleum ether:Etyl acetate (1:1, v/v)		

Table 2 (continued)

Matrix	Specie	Tissue	UV filter	Sample amount	Extraction	Purification	Technique
macrozoobenthos	Mussels (*Dreissena polymorpha*)	Whole macroinvertebrate	BP1, BP2, BP3, BP4, 4DHB, Et-PABA, EHMC, 4MBC, 3BC	4 g (fraction 1) and 1 g (fraction 2)	Fraction 1: Solvent extraction using ethyl acetate, n-heptane and HPLC water (1:1:1, v.v:v) or Solvent extraction with MeOH: ACN (1:1, v/v)	Fraction 1: RP-HPLC (RP Spherisorb ODS2 column (4.6 mm × 150 mm, 5.0 µm) Fraction 2: Syringe filtration	LC-ESI-MS/MS and GC-EI-MS
	Gammarus sp				Fraction 2: Solvent extraction with MeOH: ACN (1:1, v/v)		
Fish	Chub (*Leuciscus cephalus*) Brown trout (*Salmo trutta*) Barb (*Barbus barbus*) Eel (*Anguilla anguilla*)	Muscle plus adipose tissue under the skin					
Bird	Cormorants (*Phalacrocorax sp*)	Muscle					
Mussel[a]	*Mytilus edulis* and *Mytilus galloprovincialis*	Soft tissue	EHMC OC OD-PABA	3 g	MAE extraction with acetone:heptane (1:1, v/v)	Filtered (0.2 µm)) through 10 g anhydrous sodium sulphate RP-HPLC (RP Spherisorb ODS2 column (4.6 mm × 150 mm, 5.0 µm)	GC-EI-IT

Matrix	Chromatographic Column	MS/MS transition or SIM ions	Recovery (%)	MLOD	Concentrations (ng/g lipid)	Reference
Fish	XTI-5 capillary column (30 m × 0.25 mm; 0.25 µm)	4MBC: 115, 211, 254 OC: 177, 249, 361	98–99	5.3–17 ng/g	nd	13
	VF-5 MS capillary column (30 m × 0.25 mm; 0.25 µm)	4MBC: 211>169,155 OC: 250>248, 221	57–79	36–120 ng/g	nd	
Fish	BGB-5 (30 m × 0.25 mm; 0.25 µm) or SE54 (25 m × 0.32 mm; 0.25 µm)	4MBC: 254, 239 BP3: 228, 229 EHMC: 178, 290	93–115	7–380 ng/g lipid	72 (OC)	14
		OC: 249, 361		3–37 ng/g lipid	44–94 (4MBC), 66–118 (BP3), 64 (EHMC)	
				10–56 ng/g lipid	166 (4MBC), 123 (BP3), 25(OC)	
Fish	BGB-5 (30 m × 0.25 mm; 0.25 µm) or SE54 (25 m × 0.32 mm; 0.25 µm)	4MBC: 254, 239 OC: 249, 361	No data	5–20 ng/g lipid	50–1800 (4MBC) 40–2400 (OC)	15
Fish	PerfectSil 120 ODS-2 (125 mm × 3 mm, 3.5 µm)	4MBC: 255>105 EHMC: 291>161	86–108	2.4 ng/g muscle	214 (4MBC), 193–525 (BP3), 414 (EHMC), 300 (OC)	16

Table 2 (continued)

Matrix	Chromatographic Column	MS/MS transition or SIM ions	Recovery (%)	MLOD	Concentrations (ng/g lipid)	Reference
		BP3: 229>151 OC: 362>250				
Fish	Zorbax SB-C18 (150 mm × 3.0 mm, 3.5 μm) and OPTIMA-5-MS (50 m × 0.2 mm; 3.5 μm)	BP1: 213>213 BP2: 245>245 BP4: 307>307 4DHB: 213>213 Et-PABA: 166>138 EHMC: 178, 290 3BC: 240, 197 4MBC: 254, 237 BP3 : 228, 227	76–99 (BP4 no extracted)	8–205 ng/g lipid	45–700 (EHMC)	17
	Zorbax SB-C18 (150 mm+3.0 mm, 3.5 μm)	BP1: 213>213 BP2: 245>245 BP4: 307>307 4DHB: 213>213 Et-PAB: 166>138	80–99	1.8–10.7 ng/Kg body weigh		17
Fish	SE-54-CB (50 m × 0.55 mm; 0.25 μm)	IDM: 105, 147, 294 BM-DBM: 135, 161, 338 4MBC: 211, 239, 254 OD-PABA: 148, 165, 277 HMS: 109, 138, 262 EHMC: 161, 178,248 BP3: 165, 225, 242	89–106	50–90 ng/Kg body weigh	Muscle: 810 (4MBC), 310 (EHMC), 298 (BP3), 3100 (HMS); offal: 880 (4MBC), 283 (BP3), 185 (HMS); rest: 990 (4MBC), 50 (EHMC), 40 (BP3), 79 (HMS) whole fish: 930 (4MBC), 120 (EHMC), 150 (BP3), 791 (HMS); Muscle: 161 (4MBC), 41 (EHMC), 230 (BP3), 720 (HMS), 150(IDM); offal: 106 (4MBC), 270 (BP3), 970 (HMS), 210 (BM-DBM); rest: 60 (4MBC), 16 (EHMC), 22 (BP3), 41 (HMS), 9(IDM), 18 (TDM); whole fish: 78 (4MBC), 20 (EHMC), 78 (BP3), 237 (HMS), 29 (IDM), 44 (BM-DBM)	18
macrozoobenthos	Zorbax SB-C18 (150 mm+3.0 mm, 3.5 μm) and OPTIMA-5-MS (50 m × 0.2 mm; 0.35 μm)	BP1: 213>213 BP2: 245>245 BP4: 307>307 4DHB: 213>213 Et-PABA: 166>138 EHMC: 178, 290	70–105	6–50 ng/g lipid	22–150 (EHMC)	19

Table 2 (continued)

Matrix	Chromatographic Column	MS/MS transition or SIM ions	Recovery (%)	MLOD	Concentrations (ng/g lipid)	Reference
		3BC: 240, 197				
		4MBC: 254, 237				
		BP3: 228, 227				
Fish					91–133 (EHMC)	
					23–79 (EHMC)	
					91–151 (BP3), 11–173 (EHMC)	
					9–337 (EHMC)	
					<LOQ (BP3), 30 (EHMC)	
Bird					16–701 (EHMC)	
Mussel[a]	SGE-BPX5 capillary column (30 m × 0.25 mm, 0.25 μm)	EHMC: 178>121, 132, 161	89–116	2 ng/g dw	3–256 ng/g (EHMC)	20
		OC: 248>220, 219, 176			2–7112 ng/g (OC)	
		OD-PABA: 165>91, 118, 148				

[a] Concentrations expressed in ng/g (not ng/g lipid), *nd* not detected

SIM mode, and with benzophenone-d_{10} and $^{13}C_6$-*p*-*n*-nonylphenol as surrogates. Analysis performed on samples of bluegill (*Lepomis macrochirus*) muscle, with only 0.4 % lipid content, provided good results. When this method was applied to samples of sonora sucker (*Catostomus insignis*), with an average of 4.9 % lipid content, GC–EI-MS failed to provide acceptable results. Analysis of spiked sample extracts that had not previously been subjected to GPC purification resulted in substantially compromised chromatographic performance. In this case, addition of a GPC step was essential. This additional purification enabled continuous analysis of sonora sucker samples with no compromise in chromatographic performance compared with GC–EI-MS analysis of spiked bluegill tissue. However, dramatic increases in background signal and/or reduction in analyte sensitivity were observed for several analytes. As a consequence some compounds, including 4MBC, were indistinguishable from the background. To increase the sensitivity and reduce the background signal observed, the method was improved by the application of tandem mass spectrometric detection. GC–EI-IT analysis was performed with a VF-5 MS capillary column (30 m×0.25 mm; 0.25 μm) under the same chromatographic conditions and with detection in selected reaction monitoring mode (SRM). The optimized transitions used are listed in Table 2. Nevertheless, this approach only slightly improved detection of 4MBC and a few other compounds, but to much less an extent than expected (this aspect will be further discussed in the section "Limits of detection").

Similarly, GC–EI-IT with an ion-trap mass spectrometer was used by Bachelot et al. [20] for determination of EHMC, OC and OD-PABA. In this work an SGE-BPX5 capillary column (30 m×0.25 mm, 0.25 μm) was used for the separation. Data were acquired in SIM mode for the isotopically labeled internal standard chrysene-d_{12} and in SRM mode for the UV filters.

LC–MS

This technique enables analysis of a wide range of compounds and significantly increases the possibility of analysis of metabolites, which are usually more polar that the parent compounds, without the need for derivatization.

For ionization of the UV filters three different techniques have been used—electrospray ionization (ESI) (which is by far the most commonly used for trace analysis of these pollutants in environmental samples), atmospheric-pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI). All the approaches used for LC–MS analysis of UV filters in biota use ESI mode, which achieves efficient ionization of the analytes even though ESI is assumed to be susceptible to signal suppression or signal enhancement by components of sample matrix, as shown

by previous studies on UV filters in complex matrices such as sewage sludge [8]. Isotopically labeled compounds should be used as internal standards to compensate for the matrix effect. Quantification of UV filters in biota samples by external standard calibration is not recommended.

Meinerling and Daniels [16] developed an LC–MS–MS method for analysis of 4MBC, OC, BP3, and EHMC. Chromatographic separation was performed on a PerfectSil 120 ODS-2 (125 mm×3 mm) column with MeOH and water (each containing 0.05 % acetic acid) as mobile phase. Analysis was performed with a triple-quadrupole mass spectrometer fitted with electrospray interface operated in positive mode under SRM conditions (LC–ESI(+)–MS–MS). In this study external standard calibration was used.

Zenker et al. [17] analyzed nine UV filters by LC–ESI–MS–MS. Chromatographic separation was achieved on a Zorbax SB-C18 column (150 mm×3.0 mm, 3.5 μm) with a binary gradient prepared from a mixture of 0.1 % (*v*/*v*) formic acid in HPLC-grade water and 0.1 % (*v*/*v*) formic acid in ACN. Data acquisition was performed in SRM mode. Benzoic-d_5 acid was used as internal standard.

Method performance

Method recovery

High recovery was achieved in all the methods reported, especially when the lipid content of the biological sample analyzed was low.

Nagtegaal et al. [18] achieved recovery from 89 % to 96 % when extracting compounds with a Soxhlet-based procedure. Most studies analyzing lipophilic UV filters used solvent extraction and further clean up by GPC, and usually achieved good recovery (86–115 %). Mottaleb et al. [13] analyzed 4MBC and OC in fish tissue with low lipid content, with recovery of 98 and 99 %. In analysis of samples with higher lipid content lower recovery (57–79 %) was achieved.

Zenker et al. [17] used a mixture of ethyl acetate and *n*-heptane as extraction solvent; this enabled efficient extraction of eight of the nine UV filters with a wide range of polarity. Average recovery ranged from 76 to 99 % (SD from 0.3 to 4 %). However, extraction of the polar filter BP4 was feasible only with ACN and MeOH. Bachelot et al. [20] achieved even higher recovery, from 89 to 116 %, in analysis of lipophilic UV filters in mussel soft tissue extracted by MAE then further RP-HPLC purification.

Limits of detection

Method limits of detection were calculated by extraction of samples of fish spiked with low concentrations of analytes which can be detected in the presence of possible matrix effect. For biota samples MLODs are in the sub-ng g^{-1} range, although authors normalize their results differently, depending on the matrix, and express them in ng g^{-1} lipid or simply ng g^{-1}. The presence of UV filters in blanks is eventually reflected by higher MLODs.

MLODs are highly dependent on the matrix analyzed. Biological matrices may be quite different depending on the organism selected, the species, and the tissue chosen; even so, there is still great variability. As an example, Balmer et al. [14], in analysis of four lipophilic UV filters, obtained three significantly different MLODs ranges, 3–37, 10–56, and 7–380 ng g^{-1} lipid, as a function of the fish species analyzed. To compare MLODs between different methods and for different matrices is complicated. MLODs are usually lower when analysis is performed by GC–MS because matrix effects are usually smaller. Table 2 summarizes the MLODs obtained in each study.

Zenker et al. [17] developed a method for analysis of nine UV filters by GC–EI–MS and LC–ESI–MS–MS. In the first of these the limits of detection ranged between 8 and 36 ng g^{-1} lipid. For UV filters analyzed by LC–MS–MS limits of detection were between 86 and 205 ng g^{-1} lipid. These different MLODs are because of the greater matrix effect in analysis using electrospray interfaces, which can affect analyte ionization.

Mottaleb et al. [13] analyzed bluegill tissue (with low lipid content, 0.4 %) by GC–EI-MS and sonora sucker tissue (high lipid content, 4.9 %) by GC–EI-IT, which is, a priori, a more sensitive and selective technique. MLODs for most compounds in the GC–EI-IT study were higher than those obtained for bluegill tissue by use of GC–EI-MS (especially for 4MBC, 23-fold higher). Differences in detectability between the two approaches cannot be explained solely by differences in extraction efficiency. The authors suggest inefficient fragmentation of precursor ions in the ion trap. Because all MS–MS precursor ions are produced by EI, a relative hard ionization technique, it is likely that generation of product ions via collision-induced dissociation may eventually be problematic (precursor ions may be sufficiently stable, which makes further fragmentation unlikely). The MLODs afforded by GC–EI-IT exceeded the corresponding environmentally relevant concentration range identified in the literature. OC and 4MBC were not detected in any sample in the study.

Critical aspects in the analysis of UV filters

Background contamination is a common problem in the determination of UV filters at environmentally relevant levels. Therefore, several measures must be taken to prevent this problem. All glassware should be carefully cleaned. A typical procedure consists in washing and heating at 380 °C, then sequentially rinsing with different high-purity organic

solvents. Furthermore, gloves should be worn during sample preparation; separate solvents and only previously unopened packages of solvents, chemicals and other supplies, and glassware should be used.

In addition, a set of at least two operational blanks should be processed together with each batch of samples. Because many of the compounds analyzed undergo photodegradation, stock standard solutions should always be covered with aluminium foil and stored in the dark.

The presence of matrix effects has the potential to lead to compromised results, so precautions should be taken to minimize this effect. Measurements are further hindered by the lack of appropriate commercially available reference standards. Currently, only BP3-d_5 and 4MBC-d_4 are commercially available; none of the studies reported herein used these. Other isotopically labeled compounds, namely benzophenone-d_{10}, $^{15}N_3$-musk xylene, and $^{13}C_6$-*p*-*n*-nonylphenol were used for quantification. Development and further marketing of a wider range of isotopically labeled compounds for use as surrogate and internal standards is an important need for analysis of sunscreen agents in complex matrices.

Biota levels

UV filters enter the aquatic environment directly, as a result of swimming and other recreational activities or indirectly via wastewater treatment plants (WWTPs). Thus, it is expected that, because of the lipophilic properties of these compounds, they can reach and accumulate in tissues of aquatic organisms. Several fish species have been investigated together with, although to a lesser extent, mollusks, crustaceans, and birds. Table 2 summarizes UV filter occurrence data in biota.

A study carried out by Nagtegaal et al. [18] provided the first data on the occurrence of UV filters in fish. Perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) from Maarfelder Lake (Eifel, Germany) were analyzed and the presence of seven UV filters with total concentrations of approximately 2000 ng g^{-1} lipid and 500 ng g^{-1} lipid, respectively, in whole fish were reported. This early study, besides providing the first data on bioaccumulation of UV filters in fish, reported prevalence profiles in different fish tissues (muscle, offal, the rest, and whole fish). Results indicated that 4MBC and HMS can be selectively accumulated depending on the species; perch accumulates 4MBC in muscle and HMS in offal. In contrast, roach had higher levels of 4MBC in offal and of HMS in muscle. In contrast, EHMC and BP3 had similar bioaccumulation profiles in both species.

In Switzerland similar concentrations were found in lake fish. 4MBC, BP3, EHMC, and OC were detected in white fish (*Coregonus sp.*), roach and perch in the range 25–166 ng g^{-1} lipid, and from 45 to 700 ng g^{-1} lipid for EHMC in barb (*Barbus barbus*) and chub (*Leucisus cephalus*) [17]. Meinerling et al. [16] reported concentrations from 193 to 525 ng g^{-1} lipid in rainbow trout (*Oncorhynchus mykiss*).

Higher levels for 4MBC and OC (up to 1800 and 2400 ng g^{-1} lipid, respectively) were found in fish (brown trout, *S. Trutta fario*) from rivers downstream from a WWTP discharge [15], revealing its impact on the ecosystem. Buser et al. [28] demonstrated that the enantiomeric composition of 4MBC in perch was much different from that observed in the surrounding lake water. In contrast, the enantiomeric composition of 4MBC in roach was similar to that of the lake water indicating that bioconcentration or metabolism of a compound can be quite different from one species to another. The factors responsible for the differences in the enantiomeric composition of 4MBC found in fish remain unclear.

Concerning organisms other than fish, Fent et al. detected EHMC in crustaceans (*Dammarus sp.*) and mollusks (*Dreissena polymorpha*) at concentrations between 22 and 150 ng g^{-1} lipid. EHMC was also detected in different fish species at concentrations up to 337 ng g^{-1} lipid and in cormorants (*Phalacrocorax sp.*), at levels above 700 ng g^{-1}. BP3 was also detected, but at lower concentrations, in brown trout (*Salmo trutta*) and eel (*Anguilla anguilla*). These results suggest that biomagnification occurs through the food web; this aspect will be further discussed in the section "Bioaccumulation and biomagnification") [19].

Bachelot et al. proved the presence of UV filters residues in marine mussels (*Mytilus edulis* and *Mytilus galloprovincialis*) of the Mediterranean French coast [20]. In that study all mussel samples contained EHMC, at concentrations up to 256 ng g^{-1} dw. In addition, 55 % of the samples contained OC also. In particular one of these samples had the highest concentration reported so far for an UV filter in biota, 7,112 ng g^{-1} dw.

Besides WWTP discharges, another important factor affecting accumulation in aquatic biota samples is the season of the year, with summer being the period when peak concentrations of sunscreens are observed. As an example, the concentrations reported for OC in fish in September were found to be three to five-fold higher than those corresponding to May, before the swimming period [15]. Similarly, a study carried out by Fent et al. showed that concentrations in freshwater mussels collected in a lake with recreational activity were higher after the summer [19].

UV filter 4MBC and other benzotriazole compounds have been analyzed in a great variety of aquatic species, for example tidal flat organisms, fish, coastal birds, and even hammerhead sharks of the Japan coast, by Nakata et al. [22]. 4MBC was not detected in any of the samples analyzed, even though this common sunscreen agent has been detected in samples in different studies in Europe. The authors of that

study suggested these results were evidence of the different production and usage profiles of UV filters among countries.

As shown in Table 2, of all the sunscreen agents investigated, EHMC is the most frequently found, but at lower concentrations that those usually observed for UV filters of similar log K_{ow}, e.g. HMS (log K_{ow} 6.16) and OC (log K_{ow} 4.95). On the other hand, other sunscreens with log K_{ow} in the same range, OD-PABA (log K_{ow} 6.15) and 3BC (log K_{ow} 4.49) were never detected, suggesting fast and effective metabolism.

Bioaccumulation and biomagnification

The net accumulation of a chemical by an organism from its combined exposure to water, food, and sediment is known as bioaccumulation. Species higher in the food web can be exposed to all the chemicals that lower-order species accumulate (biomagnification). Bioaccumulation models are useful tools for understanding the biomagnification of such substances [23]. The extent of biomagnification for a given contaminant is expressed in terms of the biomagnification factor (BMF), the ratio of the concentration of the contaminant in a predator to its concentration in prey. A BMF above 1 indicates biomagnification of the contaminant. However, the most conclusive evidence of the accumulation of chemicals by organisms and biomagnification in food webs is expressed by the trophic magnification factor (TMF) [23, 24], which is used to estimate the accumulation of contaminants through trophic levels of different food webs, for instance between fresh water and marine water systems.

Because of the lipophilic character (low water solubility) of most UV filters they may be expected to accumulate in biota and in humans and be stored rather faster than they are metabolized or excreted. A recent study by León-González et al. [25] revealed that the metabolites of OD-PABA were detected in human urine after 8 days of single cutaneous application of a cream, indicating slow metabolism. Despite this, only one field-based study has examined biomagnification through food webs [19]. EHMC bioconcentration was proven in macrozoobenthos, fish from different trophic levels, and cormorants (*Phalacrocorax sp.*) a species of fish-eating birds, in Switzerland. For example, estimated BCF for fish were far above unity, in the range 167–1500. Biomagnification was also assessed, but higher concentrations of EHMC in cormorants than in fish were not statistically significant. Despite this, estimated BMF, occasionally slightly higher than unity, were indicative of possible positive trophic magnification of EHMC. Nevertheless, a possible explanation of this finding may be found in feeding ecology; because birds and mammals are homeotherms their rates of feeding higher than for fish and invertebrates (poikilotherms) [26]. Higher BCFs were, however, reported by Balmer et al. [14] for 4-MBC in fish from Lake Zürich. In particular, BCFs for roach ranged from 2,300 to 9,700.

Biological and chemical factors, for example size, sex, age, life cycle, and metabolic activity, are important when assessing bioaccumulation and trophic transfer of contaminants in food webs. The lipid and protein content of tissue, which vary according to season, reproduction, migration, feeding rate, diet composition, growth rate, and food chain length, should be taken into account in analysis of UV filters [27].

The phenomenon of chirality exists in all biological systems. All proteins, enzymes, and carbohydrates are chiral. Because biological processes may be stereoselective (favor one structural form over the other), enantiomers of chiral compounds, both parent compounds and transformation products (metabolites, products of photodegradation, and disinfection byproducts) must be investigated separately to obtain reliable information about their bioavailability and biomagnification through the food web. It must be taken into account that metabolites of achiral compounds can also be chiral. Buser et al. [28] investigated the enantiomeric pattern of 4-MBC in lake fish. This compound exists as two stereoisomers, (*Z*) and (*E*), as a consequence of an exocyclic C=C bond. Both are chiral compounds with two stereocenters provided by the camphor moiety of their chemical structure. First, HPLC separation of the (*E*) and (*Z*) isomers of 4MBC was performed on a Nucleosil 120–5 C-18 reversed-phase column (250 mm×4 mm) with ACN–water 60:40 (*v/v*) as mobile phase. Enantiomeric separation of 4MBC was subsequently achieved by GC on a laboratory-prepared column containing 2,6-bis-(*tert*-butyldimethylsilyl)-γ-cyclodextrin in 70 % PS086. Detection was performed in full scan and SIM (GC–MS) or SRM (GC–EI-IT) modes.

Although the study did not reach a sound conclusion, the isomer ratio observed seemed to indicate preferential accumulation of the (*E*) isomer. With regard to potential enantioselective fish metabolism of 4-MBC, results were completely different for roach and perch; roach accumulates both enantiomers whereas perch is unable to metabolize the [1*R*,4*S*-(*E*)-4-MBC] stereoisomer only.

Ecotoxicological considerations

The ecotoxicological implications of exposure of biota to sunscreens have been addressed quite frequently. Despite this, the sparse studies available are conclusive [29]. Fish have long been regarded as tracers for assessing the extent of lipophilic contamination of aquatic ecosystems. As a consequence most ecotoxicological studies on the effect of UV filters have been conducted on different fish species for "in vivo" testing. Several sunscreens have been found to

have estrogenic hormonal activity, in particular, two which are extensively used 4-MBC and OMC; moreover, some have been shown to have multiple endocrine-disrupting activity, for example androgenicity or antiestrogenicity. Adverse effects on fecundity and reproduction have also been observed for BP3, benzophenone 2 (BP2), and 3 benzylidene camphor (3BC). Analysis of vitellogenin (VTG) in rainbow trout and Japanese medaka (*Oricias latipes*) after aqueous exposure to BP3 indicated, however, that high effective concentrations in the range 620–749 $\mu g\ g^{-1}$ were needed to induce these effects [30]. These concentrations are greater than the reported level (19 $ng\ L^{-1}$) of BP3 in estrogenic fractions of effluent wastewater extracts [31]. When a similar test was conducted on 4MBC and EHMC [32], 4MBC had high estrogenic potency.

In male fathead minnows (*Pimephales promelas*), concentrations of BP2 of 1.2 $mg\ L^{-1}$ and higher were found to induce VTG, modify gonad histology, and emasculate secondary sex characteristics; oocyte production in female fish was also inhibited significantly [33, 34]. In the same fish species, 3BC had high estrogenic potency, inducing VTG at doses of 435 $\mu g\ L^{-1}$ and higher [35]; at concentrations near predicted environmental levels (3 $\mu g\ L^{-1}$) significant VTG induction, loss of secondary sexual characteristics, and inhibition of spermatogenesis were observed for male specimens. Loss of gender-specific mating behavior and cessation of milt production were, moreover, observed at 74 $\mu g\ L^{-1}$ and 285 $\mu g\ L^{-1}$, respectively [34, 35]. In females, at the same concentrations, first oogenesis was inhibited, followed by cessation of egg production and release of mature oocytes.

Effects on algae have also been assessed. In a test on inhibition of reproduction of the green alga *Scenedesmus vacuolatus* OC and 4MBC had no activity whereas EHMC, BP3, and OD-PABA at predicted no-effects concentrations (PNEC; calculated from EC_{50} with a safety factor of 1000) in the range 0.17–0.76 $\mu g\ L^{-1}$ were found to significantly inhibit algal growth [36]. In a similar study, exposure to BP3, 3BC, 4MBC, and EHMC resulted in inhibition of the growth of *Desmodesmus suspicatus*, with 72-h EC10 values in the range 0.21–0.56 $mg\ L^{-1}$ [37]. Potential endocrine and toxic effects of BM-DBM, EHMC, and OC on infaunal and epibenthic invertebrates and zebra fish (*Danio rerio*) embryos was recently investigated by the same authors [38]. Test results revealed toxicity of these sunscreens was low (Table 3), with effect concentrations far higher than those reported in the environment. In particular, EHMC was found to have a toxic reproductive effect on *Potamopyrgus antipodarum* and on *Melanoides tuberculata* with no-observed effect concentrations (NOECs) of 0.08 $mg\ kg^{-1}$ and 2 $mg\ kg^{-1}$, respectively; it also had sub-lethal effects on zebra fish with even higher NOEC, 100 $mg\ kg^{-1}$. Toxic effects on reproduction of the crustaceans *Daphnia magna* [19] and *Acartia tonsa* [39] have also been reported for BP1, BP3, and BP4.

In an early study by Donavaro and Corinaldesi [40] the affect of sunscreen agents on marine ecosystems was also demonstrated. These authors observed that UV filters increased virus production via prophage induction in marine bacterioplankton. Most recently the same authors also provided scientific evidence of the effect of these chemicals on hard corals and their symbiotic algae in the Celebes Sea, the Caribbean Sea, the Andaman Sea, and the Red Sea, by inducing rapid and complete coral bleaching even at extremely low concentrations [41]. Coral bleaching, the loss of intracellular endosymbionts (symbiodinium, also known as zooxanthellae, which impart specific colors, depending on the particular clade) as a result of expulsion under stress situations, has a negative effect on biodiversity and functioning of the great reef ecosystems of tropical seas.

Despite studies are mainly focused on solely one chemical, an organism is exposed not to single environmental chemicals but to mixtures of many. According to the literature, assessment of the effects of mixtures of chemicals has attracted increasing attention in recent decades. With regard to UV filters additive effects of mixtures are largely unknown, and are an important concern in environmental studies, because these substances are usually formulated as complex mixtures to achieve the high sun protection factors (SPF) currently demanded. Taking into account the large number of sunscreens used, and other endocrine-disrupting compounds, hormonally-active UV filters, may act additively. Indeed, cumulative interactions have been shown in a few studies [34, 42–44]; in particular, these papers report significant synergistic effects of combinations of UV filters mixed at NOECs of the individual compounds.

The lack of environmental occurrence and ecotoxicological data for most UV filters and matrices hinders reliable and integral environmental risk assessment for comprehensive protection of the environment. Moreover, for complete risk assessment metabolites produced by the organisms should also be considered and their prevalence and their ecotoxicity be. Nevertheless, preliminary ERA has recently been conducted by Fent et al. [34, 45] using the limited data available. According to calculated hazard quotients, a potential risk to aquatic ecosystems may be posed by 3BC, 4MBC, and EHMC.

Conclusions and future research perspectives

The biomonitoring data so far available have been provided by a limited number of research groups. Different sample characteristics, for example location, species, season, tissue, target analytes, and the analytical methods used, hinders comparison among studies, which in turn also hinders

Table 3 Summary of toxicity data available in the literature for UV filters

UV filter	Fish LOEC (mg L^{-1})	Aquatic invertebrates EC_{50} (mg L^{-1})	Algae EC_{50} (mg L^{-1})	Ref.
BP1	–	0.49–1.5[a] (AT)	–	[38]
	0.005[f]	–	–	[34]
BP2	0.001[f]	–	–	[34]
BP3	–	1.67 (DM)	–	[37]
	–	1.9 (DM)	–	[45]
	0.75[d]	–	–	[30]
	0.62[e]	–	–	[30]
	–	–	0.36 (EV)	[36]
BP4	–	50 (DM)	–	[45]
EHMC	–	0.57 (DM)	–	[37]
	–	–	0.19 (EV)	[36]
	–	0.29 (DM)	–	[45]
	9.87[c]	–	–	[32]
3BC	–	3.61 (DM)	–	[37]
	–	26.9–5.95[b,c] (LV)	–	[48]
	0.003[f]	–	–	[34]
4MBC	–	0.80 (DM)	–	[37]
	–	4.6[b] (PA)	–	[46]
	–	0.56 (DM)	–	[45]
	9.9[c]	–	–	[32]
IAMC	–	–	0.76 (EV)	[36]
OD-PABA	–	–	0.17 (EV)	[36]
Et-PABA	0.004[f]	–	–	[34]

[a]Under different experimental conditions

[b]μmol L^{-1}

[c]Different endpoints

[d]Vitellogenin induction in rainbow trout

[e]Vitellogenin induction in medaka

[f]Vitellogenin induction in fathead minnow

DM, *Daphnia magna*; AT, *Acartia tonsa*; EV, *Scenedesmus vacuolatus*; LV, *Lumbriculus variegatus*; PA, *Potamopyrgus antipodarum*; LOEC, lowest observed effect concentration

reliable assessment of the fate and effects of UV-absorbing compounds in aquatic ecosystems. Expression of the data in different units (body weight, lipids-basis) should be standardized, enabling comparison among similar studies. Researchers have used quite different analytical approaches, although most are based on solvent extraction, GPC clean-up, and analysis by GC–MS. The sensitivity and selectivity afforded are suitable for environmental trace analysis and recovery is also very good, with values close to 100 % when the lipid content of tissues is not high. Fish and, specifically, muscle has been the preferred sample for analysis, despite results which seem to indicate that individual compounds are selectively accumulated in muscle or offal, depending on the species.

Besides smart experimental design, for accurate comparison of contamination levels among different tissues, species, and locations, reporting of range and median values may be quite useful. Moreover, reporting of sunscreen concentrations in a specific organ, instead of a whole burden estimate approach, may lead to overestimation of BMFs and TMFs. Stable isotope analysis should be conducted to properly identify the trophic position of every species for further biomagnification considerations.

Ecotoxicological assessment of exposure to UV filters is a challenging task. Despite being scattered and limited, current ecotoxicological data indicate that the potential risk posed by these widely used chemicals requires further investigation. The estrogenic activity of most of the commonly used sunscreen agents is in the range of other well-characterized estrogenic chemicals. Findings indicate that some UV filters have endocrine-disrupting activity in, and/or affect reproduction of several species, although at concentrations higher than those measured in the environment. However, a propensity for rapid accumulation and temporal effects at environmentally relevant concentrations and the potential of mixture effects indicate the need for further studies to evaluate the effects of long-term exposure of biota to UV filters. Moreover, ecotoxicological studies indicate the need to consider multicomponent mixtures when evaluating hormonal activity of UV filters in aquatic organisms, for use in risk assessment to consider potential synergistic and/or antagonistic effects.

There should be greater emphasis on measurement of ecological, biological, and physicochemical variables in field studies conducted to analyze contaminants in species, and, more importantly, when comparing data between studies. In particular, the chemical characteristics of the UV filters should be considered on the basis of their different structural forms, including isomers and enantiomers. Preliminary findings indicate that stereochemical aspects of sunscreens should be included in future environmental and

toxicological research for proper characterization of their global prevalence in the environment and for elucidation of the processes of biodegradation of theses contaminants, because these properties may result in different potential for accumulation, as observed for other contaminants (perfluorinated compounds, halogenated flame retardants, etc).

Marine and, particularly, terrestrial environments should be more widely investigated in future studies to better understand the fate and effects of UV filters. The effect of climate change should also be considered, because seasonal changes in ice formation, temperature, drought–flood episodes, or food webs might have important effects on bioaccumulation and/or biomagnification of contaminants. For UV filters this is especially relevant, because higher levels of sunlight radiation, a consequence of increased depletion of the ozone layer, would increase the use of such chemicals.

Combining monitoring field studies with work on species biology, behavioral science, and exposure biomarkers, among others, would significantly contribute to improving our knowledge about these compounds.

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19) Altered UV absorbance and cytotoxicity of chlorinated sunscreen agents
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Abstract
Sunscreens are widely utilized due to the adverse effects of ultraviolet (UV) radiation on human health. The safety of their active ingredients as well as that of any modified versions generated during use is thus of concern. Chlorine is used as a chemical disinfectant in swimming pools. Its reactivity suggests sunscreen components might be chlorinated, altering their absorptive and/or cytotoxic properties. To test this hypothesis, the UV-filters oxybenzone, dioxybenzone, and sulisobenzone were reacted with chlorinating agents and their UV spectra analyzed. In all cases, a decrease in UV absorbance was observed. Given that chlorinated compounds can be cytotoxic, the effect of modified UV-filters on cell viability was examined. Chlorinated oxybenzone and dioxybenzone caused significantly more cell death than unchlorinated controls. In contrast, chlorination of sulisobenzone actually reduced cytotoxicity of the parent compound. Exposing a commercially available sunscreen product to chlorine also resulted in decreased UV absorbance, loss of UV protection, and enhanced cytotoxicity. These observations show chlorination of sunscreen active ingredients can dramatically decrease UV absorption and generate derivatives with altered biological properties.

20) Chemosphere. 2016 Jul;154:521-7. doi: 10.1016/j.chemosphere.2016.03.116. Epub 2016 Apr 14.
Chlorination of oxybenzone: Kinetics, transformation, disinfection byproducts formation, and genotoxicity changes.
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Author information

Abstract
UV filters are a kind of emerging contaminant, and their transformation behavior in water treatment processes has aroused great concern. In particular, toxic products might be produced during reaction with disinfectants during the disinfection process. As one of the most widely used UV filters, oxybenzone has received significant attention, because its transformation and toxicity changes during chlorine oxidation are a concern. In our study, the reaction between oxybenzone and chlorine followed pseudo-first-order and second-order kinetics. Three transformation products were detected by LC-MS/MS, and the stability of products followed the order of tri-chloro-methoxyphenoyl > di-chlorinated oxybenzone > mono-chlorinated oxybenzone. Disinfection byproducts (DBPs) including chloroform, trichloroacetic acid, dichloroacetic acid and chloral hydrate were quickly formed, and increased at a slower rate until their concentrations remained constant. The maximum DBP/oxybenzone molar yields for the four

compounds were 12.02%, 6.28%, 0.90% and 0.23%, respectively. SOS/umu genotoxicity test indicated that genotoxicity was highly elevated after chlorination, and genotoxicity showed a significantly positive correlation with the response of trichloro-methoxyphenoyl. Our results indicated that more genotoxic transformation products were produced in spite of the elimination of oxybenzone, posing potential threats to drinking water safety. This study shed light on the formation of DBPs and toxicity changes during the chlorination process of oxybenzone.

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RESEARCH ARTICLE

The occurrence of UV filters in natural and drinking water in São Paulo State (Brazil)

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Abstract Ultraviolet (UV) filters are widely used in the formulation of personal care products (PCPs) to prevent damage to the skin, lips, and hair caused by excessive UV radiation. Therefore, large amounts of these substances are released daily into the aquatic environment through either recreational activities or the release of domestic sewage. The concern regarding the presence of such substances in the environment and the exposure of aquatic organisms is based on their potential for bioaccumulation and their potential as endocrine disruptors. Although there are several reports regarding the occurrence and fate of UV filters in the aquatic environment, these compounds are still overlooked in tropical areas. In this study, we investigated the occurrence of the organic UV filters benzophenone-3 (BP-3), ethylhexyl salicylate (ES), ethylhexyl methoxycinnamate (EHMC), and octocrylene (OC) in six water treatment plants in various cities in Southeast Brazil over a period of 6 months to 1 year. All of the UV filters studied were detected at some time during the sampling period; however, only EHMC and BP-3 were found in quantifiable concentrations, ranging from 55 to 101 and 18 to 115 ng L^{-1}, respectively. Seasonal variation of BP-3 was most clearly noticed in the water treatment plant in Araraquara, São Paulo, where sampling was performed for 12 months. BP-3 was not quantifiable in winter but was quantifiable in summer. The levels of BP-3 were in the same range in raw, treated and chlorinated water, indicating that the compound was not removed by the water treatment process.

Keywords UV filters · Endocrine disruptors · Brazil · River water · Drinking water · Occurrence

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Introduction

Because of concern regarding the depletion of the ozone layer and the consequential increase of UV radiation in the stratosphere that results in a higher skin cancer risk, UV filters have become an important constituent in everyday product formulations (e.g., skin moisturizing cream, body lotion, spray and hair dye, shampoo, and lipstick; Brausch and Rand 2011). UV filters have other applications, such as stabilizers in plastics, paints, textiles, and in other materials, to prevent the photodegradation of polymers and pigments (Ozáez et al. 2013). These products can act as an environmental source of UV filters, primarily from degradation in landfills (Fent et al. 2008).

UV filters are colourless or yellow substances that absorb almost no visible light but that significantly absorb light in the UVA-UVB range. A common feature of all UV filters is the presence of an aromatic ring with side chains that have various degrees of unsaturation (Díaz-Cruz and Barcelo 2009). The majority of UV filters are lipophilic; however, the presence of phenol groups in their structures can give them some mobility in the aquatic environment (Negreira et al. 2009; Richardson 2009).

UV filters are classified as inorganic (physical) or organic (chemical) according to their chemical nature. The inorganic UV filters (e.g., titanium dioxide and zinc oxide), basically act

by reflecting, scattering, and absorbing UV light, whereas the organic UV filters (e.g., benzophenone, octocrylene) act mainly by absorbing UV light (Cuderman and Heath 2007).

Some of these UV filters protect from UVB radiation, others offer protection against wavelengths in the UVA range, and few are efficient against both UVA and UVB. Thus, to ensure an efficient sun protection factor (SPF), high concentrations of UV filters have been used in personal care products (PCPs) and, in general, a combination of UV filters is used. Consequently, consumers can be exposed to high concentrations of several UV filters simultaneously (Fent et al. 2010; Manová et al. 2013). To protect the public health, the UV filters used in PCPs are regulated in some countries, mainly in the European Union and North America.

However, no worldwide environmental regulatory rules exist. In Brazil, this input to the environment is probably excessive. Considering that Brazil is a tropical country and has a high incidence of solar radiation, there is high consumption of products that contain UV filters. According to the Brazilian Association of Toiletries, Perfumes & Cosmetics, Brazil is the third largest consumer of cosmetics in the world, trailing only the USA and Japan (ABIHPEC 2014), and it still has an unreliable sanitary sewer system. According to the Brazilian Institute of Geography and Statistics, only 28.5 % of Brazilian cities collect and treat sewage, which leads to the hypothesis that the environmental levels of these compounds in Brazil can be higher than those found in other countries.

Although several papers in the literature report on the estrogenic activity of UV filters (Fent et al. 2008; Kunz and Fent 2006; Schlumpf et al. 2004), as well as on their occurrence and environmental fate (especially regarding water resources; Liu et al. 2011; Magi et al. 2012; Zhang and Lee 2012) and tap water (Basaglia et al. 2011; Díaz-Cruz et al. 2012; Zhang and Lee 2013), papers about Brazil could not be found. Only, an extraction method development for the determination of PCPs from drinking-water treatment sludge including benzophenone-3 (BP-3; Cerqueira et al. 2014). Therefore, this study aims to provide information about the occurrence and fate of the UV filters BP-3, ES, EHMC, and OC in Brazil, which are widely used in cosmetics. The study is focused on the occurrence and concentrations of these UV filters in aqueous samples from water treatment plants (WTP). The WTPs selected for study are located in the central region of São Paulo. Sao Paulo State has the better basic sanitary conditions in Brazil, collecting 87 % and treating 53 % of the sewage (SNIS 2013).

Materials and methods

Chemicals and reagents

The compounds selected for this study were four UV filters used with high frequency in sun creams largely sold in Brazil. In Brazil, there is not available information about retail marketing for cosmetics, including sunscreens. Thus, we conducted an informal survey based on the more traditional cosmetics brands sold in Brazil, by multinational and Brazilian companies. This survey included several types of sunscreen formulation and protection factors (n=52). Among the substances found most frequently in the formulation, as described in the products labels, those with the greatest estrogenic potential, according to the literature, were selected to be included in this study (BP-3, ES, EHMC, and OC).

The UV filters (BP-3, ES, EHMC, and OC), the surrogate benzophenone-d10 (BP-d10) and the internal standard, and benzyl cinnamate (BC), all of high purity (>97 %), were obtained from Sigma-Aldrich (St. Louis, MO, USA). Names, abbreviations, chemical structures, and some physicochemical data for the UV filters are shown in Table 1. High-purity solvents (methanol [MeOH] and ethyl acetate [AcOEt] were of HPLC grade) were obtained from Mallinckrodt Baker Inc. (Paris, KY, USA). Hydrochloric acid (HCl, 37 % m/v) was purchased from the JT Baker Chemical Co. (Phillipsburg, NJ, USA) to adjust the pH of the water samples.

Standard solutions of the individual UV filters were prepared at a concentration of 1,000 mg L^{-1} in AcOEt and then diluted (with the same solvent) to achieve a stock solution mixture at 5 mg L^{-1} for each compound. All the standard solutions were stored at −20 °C. Working solutions of different concentrations were prepared daily by appropriate dilution (v/v) of the stock solution in AcOEt.

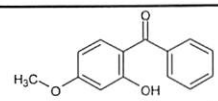
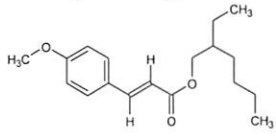
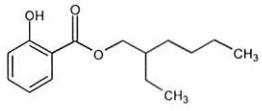
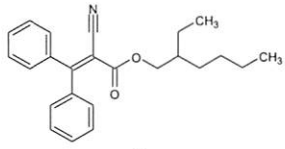
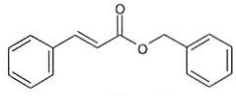
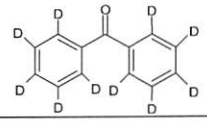
Cleaning of glassware

Considering that UV filters are ingredients of PCPs (shampoo, lipstick, soap, and sunscreen), laboratory contamination appeared to be imminent. To avoid contamination from this source, nitrile gloves were worn during all procedures during the breakup of the glassware and materials to be used, sampling, sample pre-treatment, and analysis. Glassware cleaning consisted of the following steps: a running tap water rinse, immersion in neutral Extran 5 % detergent (Merck, Darmstadt, Germany) overnight, a tap water rinse followed by a distilled water rinse and finally, an acetone rinse. The clean glassware was air dried for a minute or two, then transferred to an oven at >100 °C. Volumetric glassware was air dried. All glassware was rinsed with AcOEt immediately prior to use.

Sampling sites description

The area selected for study was the Unit of Water Resources Management number 13 (UWRM-13), located in southeastern Brazil, in the central region of São Paulo State (Fig. 1). São Paulo is divided into 22 Units of Water Resources

Table 1 Analytes, abbreviations, chemical structures, molecular weights, Log K_{ow} and pK_a values for the UV filters included in this work

Analyte (abbreviations)	Chemical structure	Molecular weight (g mol^{-1})	Log K_{ow}	pKa
Benzophenone-3 (BP-3)		228.24	3.79	7.56
Ethylhexyl methoxycinnamate (EHMC)		290.40	5.80	-
Ethylhexyl salicylate (ES)		250.33	5.97	8.13
Octocrylene (OC)		361.49	6.88	-
Benzyl cinnamate[a] (BC)		238.28	4.06	-
Benzophenone -d_{10}[b] (BP-d_{10})		192.28	3.18	-

[a]internal standard; [b]surrogate

Management (UWRM). UWRM-13, also called Hydrographic Basin Tietê-Jacaré, is composed of 34 municipalities that contain approximately 3.5 % of the São Paulo State population, about 1.5 million inhabitants (CETESB 2012) and was selected as the study area. The municipalities included in this study were Araraquara, Bueno de Andrada (Araraquara district), Bauru, Jau, São Carlos, and Trabiju. In the municipalities of Araraquara, Bauru, Jau, and São Carlos, the water treatment steps are coagulation, flocculation, sedimentation, filtration, chlorination, and fluoridation. In Bueno de Andrada and Trabiju, the water source is groundwater and the treatment consists only of disinfection by chlorination and fluorination (which occurs directly in the piping).

Sampling and sample preparation

Water samples were collected from October 2012 to February 2014, in the Water Treatment Plants (WTP)

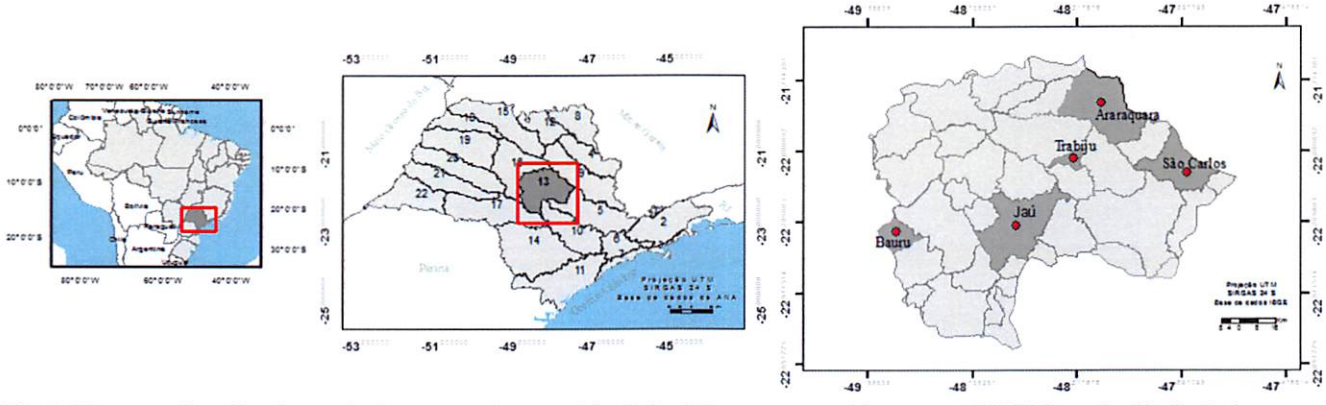


Fig. 1 Water sampling cities. São Paulo State at Brazil map and the Unit of Water Resources Management (UWRM), on the São Paulo State map, showing the cities where water samples were collected

Table 2 Concentrations of UV filters in natural, treated, and chlorinated water (ng L^{-1})

	Analyte	River water	Treated water	Chlorinated water	River water	Treated water	Chlorinated water	River water	Treated water	Chlorinated water
Araraquara		March/2013			April/2013			May/2013		
	BP-10[a]	102(5)	100(2)	110(10)	76(9)	83(3)	84(8)	89(5)	86(2)	98(11)
	ES	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	19(10)	18(9)	18(9)	23(3)	25(4)	21(8)	n.d.	n.d.	n.d.
	EHMC	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		June/2013			July/2013			August/2013		
	BP-10[a]	59(6)	63(5)	71(2)	80(5)	92(1)	85(4)	89(9)	90(11)	97(10)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	EHMC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	59(67)	<LOQ
		September/2013			October/2013			November/2013		
	BP-10[a]	111(6)	109(11)	109(12)	68(11)	82(7)	85(4)	77(8)	73(8)	86(5)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	22(1)	19(2)	20(4)	33(2)	28(15)	25(7)	38(2)	29(9)	31(4)
	EHMC	n.d.	n.d.	75(11)	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		December/2013			January/2014			February/2014		
	BP-10[a]	58(8)	61(12)	69(8)	59(3)	63(15)	65(5)	106(9)	106(16)	141(5)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	26(4)	23(3)	23(10)	36(5)	32(4)	28(3)	30(6)	25(13)	23(7)
	EHMC	<LOQ	<LOQ	57(64)	65(19)	55(11)	70(58)	55(10)	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Bauru		April/2013			May/2013			June/2013		
	BP-10[a]	107(15)	105(5)	131(3)	48(6)	81(17)	115(23)	59(6)	63(5)	71(2)
	ES	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	28(15)	33(14)	115(1)	20(36)	26(27)	72(54)	n.d.	n.d.	n.d.
	EHMC	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	OC	<LOQ	<LOQ	87(57)	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.
		July/2013			August/2013			September/2013		
	BP-10[a]	45(12)	57(3)	73(8)	93(6)	100(18)	111(9)	123(16)	106(9)	105(3)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	EHMC	n.d.	n.d.	n.d.	669(5)[b]	707(6)[b]	309(6)[b]	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	188(2)[b]	211(4)[b]	199(4)[b]	n.d.	n.d.	n.d.
São Carlos		April/2013			May/2013			June/2013		
	BP-10[a]	110(8)	121(7)	93(3)	93(9)	85(5)	81(11)	80(2)	82(10)	82(4)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	n.d.	n.d.	n.d.	<LOQ	<LOQ	n.d.	44(10)	60(31)	n.d.
	EHMC	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	101(10)	<LOQ	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		July/2013			August/2013			September/2013		
	BP-10[a]	86(11)	84(5)	80(8)	94(10)	94(15)	92(40)	105(6)	104(9)	114(6)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	<LOQ	n.d.
	BP-3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	EHMC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 2 (continued)

	Analyte	River water	Treated water	Chlorinated water	River water	Treated water	Chlorinated water	River water	Treated water	Chlorinated water
Jau		March/2013			April/2013			May/2013		
	BP-10[a]	96(5)	100(3)	105(6)	79(5)	79(11)	82(5)	65(23)	73(10)	74(17)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	18(8)	18(1)	n.d.	24(8)	26(6)	28(15)	n.d.	n.d.	n.d.
	EHMC	<LOQ	n.d.	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		June/2013			July/2013			August/2013		
	BP-10[a]	60(1)	59(7)	66(4)	83(6)	84(1)	92(6)	115(6)	123(6)	135(13)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	21(20)	<LOQ	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
	EHMC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	755(8)[b]	629(16)[b]	292(3)[b]
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	208(12)[b]	176(10)[b]	183(24)[b]

BP-10 was used as a surrogate. Average recoveries and relative standard deviations (RDS) for $n=3$

n.d. not detected, *<LOQ* below the limit of quantification

[a] BP-10 used as surrogate at 200 ng L^{-1}

[b] Quantification disregarded (contamination was suspected)

at the municipalities included in this study at three points: the entrance of the WTP, before any treatment (*river water*), after treatment without chlorination (*treated water*), and after chlorination (*chlorinated water*). Sampling in Bauru, Bueno de Andrada, Jau, São Carlos, and Trabiju was performed over a period of 6 months, while in Araraquara, the sampling was performed over a period of 12 months to evaluate the influence of seasonality on the occurrence of the UV filters.

All samples were collected inside the water treatment plant, except in Bauru, where there was no collection point for one of the sample types (river water or raw water) inside the station, so it had to be directly collected from the river. In Trabiju and Bueno de Andrada, the water is collected underground and is disinfected directly in the pipe, so only two types of water samples were analyzed: groundwater and chlorinated water.

The samples were collected in the morning between 07:00 and 10:30 in amber Pyrex glass bottles with screw caps of 4 L capacity. After collection, the samples were transported to the laboratory under refrigeration at 4 °C (ice packs) and protected from light. In the laboratory prior to extraction, the samples were filtered through a glass fibre filter with a pore size of 0.6 μm (Macherey-Nagel GF3), acidified to pH 3, spiked to 200 ng L^{-1} with a surrogate (BP-d10) and immediately extracted, and analyzed according to the previously developed and validated solid-phase extraction (SPE) method and gas chromatography coupled to tandem mass spectrometry (GC-MS/MS; Silva et al. 2013; Silva et al. 2015).

SPE extraction and GC-MS/MS analysis

The SPE method employed was a previously published method by Silva et al. (2013). The extraction of the sample analytes was performed using 200 mg/6 mL, Strata X SPE cartridges obtained from Phenomenex (Torrance, USA). The SPE cartridges were first conditioned with 5 mL of AcOEt, 5 mL of MeOH and 5 mL of deionized water. Sample volumes of 500 mL at pH 3 were passed through the cartridge. The cartridge was then dried under total vacuum for 5 min and 500 μL of MeOH was added. The analytes were eluted with 3×2 mL of AcOEt. Then, 500 μL of an internal standard solution of 100 μg L^{-1} BC was added to the eluate. The eluate was evaporated to 1 mL under a gentle flow of nitrogen gas prior to analysis by GC-MS/MS.

GC-MS/MS analysis was performed on a Varian CP-3800 gas chromatograph (Varian, Walnut Creek, CA, USA) that was equipped with a Saturn 2000 ion trap mass spectrometer . The chromatography was performed under the following conditions: helium was the carrier gas (maintained at a constant flow rate of 1.2 mL min^{-1}); an injector temperature of 300 °C, operating in splitless injection mode with a sampling time of 1 min. The separation of the analytes was performed with a fused-silica ZB-5MSi capillary column (30 m length × 0.25 mm i.d. and 0.25 mm film thickness; Phenomenex, Torrance, CA, USA). The temperature program was as follows: 60 °C for 1 min; ramped at 25 °C min^{-1} to 160 °C, which was maintained for 1 min; and ramped at 10 °C min^{-1} to 300 °C, which was maintained for 2 min. The total run time was 22.0 min. The transfer line, trap, and manifold temperatures were maintained at 300, 50, and 220 °C, respectively.

Results and discussion

Quality parameters of the method

The analytical method used to determine UV filters in water, in this work, was based on SPE and GC-MS/MS. The detailed study of multivariate optimization procedure for this method was published previously (Silva et al. 2013), as well as, the validation parameters, including matrix effect (Silva et al. 2015).

For all samples (6 months to 1 year) for all cities (Araraquara, Bauru, Jau, São Carlos, Trabiju and Bueno de Andrada), the recovery of the surrogate (BP-d10) ranged 70–120 %, and the relative standard deviations (RSDs) was below 20 %, as shown in Tables 2 and 3. Therefore, the method satisfied the requirements for precision and accuracy for quantification.

Sampling aspects

The most favorable Brazilian scenario (south-eastern Brazil) regarding basic sanitation, where the sewage collection far exceeds the national average, was used as the study area. However, note that the treatment is performed in only 48 % of collector municipalities. This absence of sewage treatment in more than half of the collector municipalities in the region of the country that has the best sanitary conditions clearly shows the seriousness of the national environmental situation. The poor collection and treatment of sewage in Brazil implies serious negative consequences to public health and the environment. In this context, the quantitative determination of UV filters in the raw water and the drinking water under the "best" conditions (south-east) is of importance.

The state of São Paulo in the Southeast Brazil is divided into 22 UWRM. UWRM-13 was selected as the study area

Table 3 Concentrations of UV filters in ground and chlorinated water (ng L^{-1})

	Analyte	Ground water	Chlorinated water	Ground water	Chlorinated water	Ground water	Chlorinated water
Bueno de Andrada		March/2013		April/2013		May/2013	
	BP-10[a]	133(7)	143(11)	107(10)	99(9)	108(5)	112(10)
	ES	n.d.	<LOQ	n.d.	n.d.	<LOQ	n.d.
	BP-3	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
	EHMC	<LOQ	n.d.	<LOQ	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		June/2013		July/2013		August/2013	
	BP-10[a]	71(8)	72(23)	105(13)	122(3)	130(40)	80(30)
	ES	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.
	BP-3	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.
	EHMC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Trabiju		March/2013		April/2013		May/2013	
	BP-10[a]	105(21)	113(3)	88(8)	88(9)	59(14)	66(14)
	ES	n.d.	105(258)	n.d.	n.d.	n.d.	n.d.
	BP-3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	EHMC	137(148)	<LOQ	n.d.	n.d.	n.d.	n.d.
	OC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		June/2013		July/2013		August/2013	
	BP-10[a]	60(5)	61(6)	93(3)	85(2)	115(15)	112(6)
	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	BP-3	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
	EHMC	n.d.	n.d.	n.d.	n.d.	724(4)[b]	273(2)[b]
	OC	n.d.	n.d.	n.d.	n.d.	175(22)[b]	174(19)[b]

BP-10 was used as a surrogate. Average recoveries and relative standard deviations (RDS) for $n=3$

n.d. not detected, *<LOQ* below the limit of quantification

[a] BP-10 used as surrogate at 200 ng L^{-1}

[b] Quantification disregarded (contamination was suspected)

because it is a populous region in which the sewage collection is nearly 100 %, although in some municipalities, the sewage collected is not treated. For example, Bauru, the most populous municipality in this basin, treats only 10 % of the sewage collected (CETESB 2012).

Study of the occurrence of UV filters in environmental samples

The concentrations of the UV filters in samples from the six WTPs in the UWRM-13 are presented in Tables 2 and 3. All UV filters were detected in the samples from the WTPs, however, quantifiable concentrations were found only for BP-3 and EHMC. The concentrations of BP-3 and EHMC ranged 18–115 and 55–101 ng L^{-1}, respectively. Although the other analytes were present in some samples, they were not present at levels that could be reliably quantified.

Both BP-3 and EHMC, among other UV filters, have been found throughout the world. They have been detected in water, soil, sediment, sludge, and biota. BP-3 and EHMC have been detected in river and tap water worldwide (Table 4). The maximum level of BP-3 detected in river water was 114 ng L^{-1} in Slovenia (Cuderman and Heath 2007), according to a review by Kim and Choi (2014). EHMC has been found in slightly higher concentrations; Kameda and collaborators found concentrations up to 1,040 ng L^{-1} in highly polluted rivers in Japan (Kameda et al. 2011). It was reported by Díaz-Cruz et al. (2012) that concentration of BP-3 and EHMC were up to 295 and 256 ng L^{-1}, respectively, in the public water supply. These literature values are comparable to the values obtained for river water and drinking water in this study.

BP-3, EHMC, and OC are the UV filters most used in PCPs. BP-3 is the most polar, which is possibly why it occurs with the highest frequency in the samples studied. BP-3 was quantified in Araraquara during almost every sample period (March 2013 to February 2014) except only May, June, July, and August 2013. BP-3 was quantified in Bauru during April and May 2013; in São Carlos, only in June 2013; and in Jau during March, April, and June 2013. EHMC was quantified in Araraquara only in September 2013, January 2014, and February 2014; and it was quantified in São Carlos in June 2013. The other two UV filters (ES and OC) were below quantifiable levels, although they were detected. Figure 2 shows (1) a chromatogram of the SPE products and the GC-MS/MS analysis for the UV filters at the limit of quantification and (2) a chromatogram of the SPE products and the GC-MS/MS analysis for the UV filters in river water from the Araraquara WTP in January 2014.

The high concentrations of EHMC and OC in August in Bauru, Jau, and Trabiju might be explained by contamination during the collection and/or extraction of the sample. Although these compounds were detected (below the LOQ) in previous months, these values appear to be discordant with others from the same sampling sites Even if their coefficients of variation are excellent, the values are suspect because these analytes were detected in Bauru, Jau, and Trabiju at similar concentrations. Because the samples from the three municipalities were collected on the same day and also processed together, the possibility of contamination cannot be discarded. Contamination was also not confirmed because an entire cleaning procedure of glassware and materials was conducted,

Table 4 Summary of the measured concentrations of UV filters in river water and tap water (ng L^{-1})

Sample	Maximum concentration of BP-3 (ng L^{-1})	Maximum concentration of EHMC (ng L^{-1})	Country	Reference
River water	44	101	Brazil	This article
Drinking water	105	75	Brazil	This article
River water	114	88	Slovenia	Cuderman and Heath (2007)
River water	30	21	Spain	Rodil and Moeder (2008)
River water	52	–	Spain	Negreira et al. (2009)
River water	<LOQ	<LOQ	Australia	Liu et al. (2011)
River water	12	1040	Japan	Kameda et al. (2011)
River water	n.d.	n.d.	Singapore	Zhang and Lee (2012)
Tap water	295	256	Spain	Diaz-Cruz et al. (2012)
River water	69	10	Italy	Magi et al. (2012)
River water and groundwater	38	–	Spain	Gago-Ferrero et al. (2013)
River water	15	–	Taiwan	Wu et al. (2013)
Tap water	n.d.	n.d.	Singapore	Zhang and Lee (2013)
Groundwater	19	–	Spain	Jurado et al. (2014)

<LOQ below the limit of quantification, *n.d.* not detected, – not studied

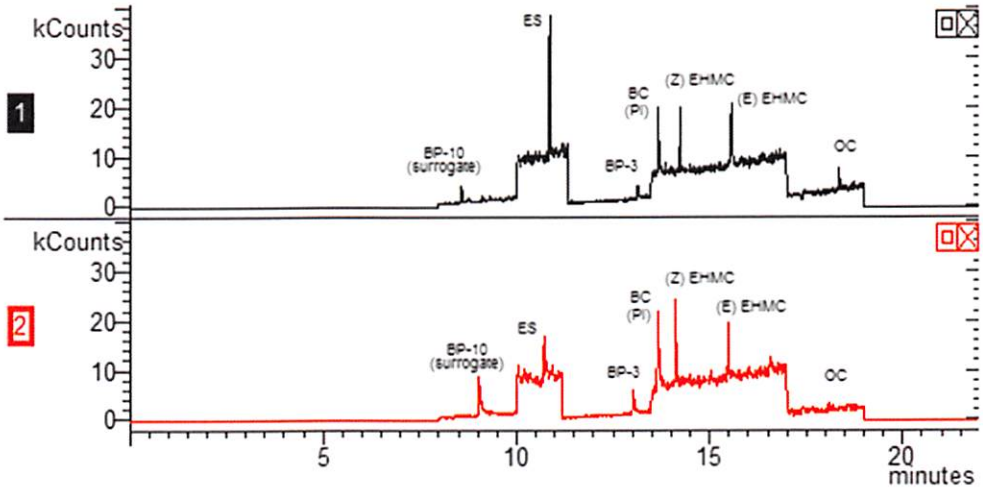


Fig. 2 SPE and GC-MS/MS chromatogram in MRM mode of the analysis of *1* WTP (treated water—Araraquara) spiked at 10, 100, 10, 50, and 50 ng L^{-1} to BP10, ES, BP3, EHMC, and OC, respectively. The internal standard BC was spiked at 50 μg L^{-1}. *2* WTP (river water—Araraquara—January) the surrogate BP-10 and the internal standard BC was spiked at 100 μg L^{-1}. EHMCs are present as both (*E*) and (*Z*) isomer

in addition to care with the use of gloves during the procedure for separation of the glassware for the collection until the moment of injections.

In Araraquara, the sampling was performed over one year period (March 2013 to February 2014), so in that municipality, the seasonal occurrence of BP-3 was evident, as shown in Fig. 3. It should be noted that during winter time (May–August) UV filters were not quantifiable probably because of the lower consumption of sunscreens in the winter season.

Figure 3 indicates a lower concentration of BP-3 in chlorinated water samples compared to treated and river samples, probably because of the chlorination of the aromatic ring of the BP-3 (Negreira et al. 2008). However, the concentration in chlorinated water is only slightly lower than in treated and river water. It is suggested that the water treatment procedure used was not sufficient to eliminate these substances from the drinking water. This finding is disturbing because BP-3 has often been reported as an endocrine disruptor (Fent et al. 2008), inducing hormonal activity in vitro and in vivo. Benzophenones have been associated with the induction of vitellogenin, alterations in the gonads, a reduction in fertility and reproduction, and feminization of sexual characteristics in male fish (Díaz-Cruz and Barcelo 2009).

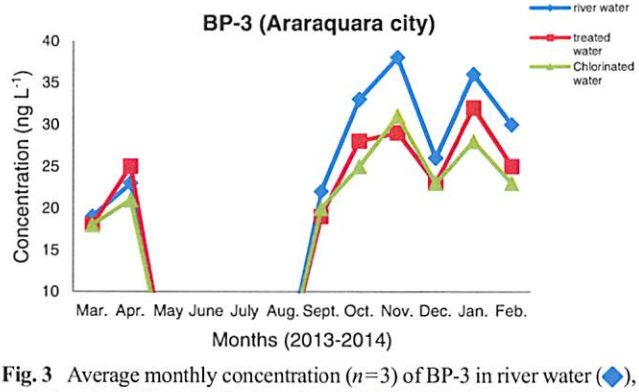


Fig. 3 Average monthly concentration ($n=3$) of BP-3 in river water (◆), treated water (●), and chlorinated water (▲) in the Araraquara WTP

Despite the low concentration found in this work for natural water analysis, to achieve a more complete environmental risk analysis discussion is mandatory to include UV filters determination in other environmental compartments as sediment and water particulate matter. On the other hand, in the literature, conclusive studies regarding the temporal effects of environmentally relevant concentrations are absent. However, even at very low concentrations, long-term exposure can present deleterious effects (Díaz-Cruz and Barcelo 2009). Additionally, the propensity for rapid accumulation of these substances and their potential combined effects should not be neglected because they are introduced into the environment daily in a mixture with other compounds. All these issues clearly indicate the need for further studies to evaluate the environmental occurrence and fate of UV filters, as well as toxicological and ecotoxicological studies (which are currently lacking) that can support the worldwide environmental regulation of these compounds.

Conclusions

UV filters were found in low concentrations in raw water and in the public water supply from treatment plants in the UWRM-13. All of UV filters studied were detected at some point during the study, both in the raw water and the drinking water, but only BP-3 (18–115 ng L^{-1}) and EHMC (55–101 ng L^{-1}) were found in quantifiable concentrations.

The concentrations of BP-3 increased during the summer, most likely due to direct and indirect environmental input. Although the concentrations found were low (in nanograms per liter level), it does not indicate that these substances are not liable to cause environmental or human damage because safe environmental or human exposure levels for these compounds have not yet been determined. Additionally, this is the first report of these compounds in Brazil, but only water samples were analyzed. The physicochemical properties of some of

these compounds indicate a high potential for accumulation in particulate matter, sediments and even in the biota.

The occurrence and quantification of UV filters in drinking water raises the question about the safe levels of these substances, indicating the need for improved water treatment processes for their removal. Thus, our results contribute to a discussion about the improvement of the water and sewage treatment in Brazil, as well as the development of global environmental legislation regarding emerging contaminants.

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