TOXICITY OF HUMAN PHARMACEUTICALS AND PERSONAL CARE PRODUCTS TO BENTHIC INVERTEBRATES

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Abstract—Despite concerns about potential risks associated with the presence of pharmaceuticals and personal care products (PPCPs) in the environment, few toxicological data address the effects of these compounds. In aquatic systems, which often represent the final repository for PPCPs, increasing toxicological information regarding aquatic biota is improving our capacity to assess potential risks. However, responses of key biota, such as benthic invertebrates, have not been investigated as widely. In the present study, we examined the toxicity of four PPCPs—the lipid regulator atorvastatin (ATO), the antiepileptic drug carbamazepine (CBZ), the synthetic hormone 17α-ethinylestradiol (EE$_2$), and the antimicrobial triclosan (TCS)—to the midge *Chironomus tentans* and the freshwater amphipod *Hyalella azteca* in 10-d waterborne exposures. The toxicity of the four compounds varied between 0.20 and 47.3 mg/L (median lethal concentration), with a relative toxicity ranking of TCS > EE$_2$ > ATO > CBZ. *Hyalella azteca* was more sensitive than *C. tentans* to these compounds. The toxicity data were used in a hazard quotient approach to evaluate the risk posed by the four PPCPs to benthic invertebrates and other aquatic organisms. For each compound, a hazard quotient was calculated by dividing the lowest toxicity value by the highest exposure value found in the literature, to which an uncertainty factor was applied. With hazard quotients of 3.55 to 11.5, we conclude that potential risks exist toward benthic invertebrates for the toxicity of TCS and CBZ and that further investigations of these compounds are required to characterize more completely the risks to benthic organisms. In contrast, our data also indicate that considering the low concentrations currently detected in the environment, ATO and EE$_2$ pose negligible risks to benthic invertebrates.

Keywords—Benthic invertebrates   Pharmaceuticals   Personal care products   Toxicity

INTRODUCTION

In recent years, the environmental occurrence of pharmaceuticals and personal care products (PPCPs) has been a source of growing concern. The vast increase in production and usage of PPCPs has contributed to their prevalence in surface waters, a problem that has been exacerbated by existing water treatment facilities that are not designed to eliminate these compounds from waste streams [1,2].

Release of PPCPs into aquatic systems and groundwater mainly arises from the excretion of the drug or its metabolites from the target organism, inappropriate disposal of expired or unused medication, incomplete degradation during sewage treatment, and/or leaching from soils following land application of biosolids [1,3,4]. Increasingly, studies report detectable concentrations of PPCPs and their metabolites in surface water, sewage effluent, soils, sediments, groundwater, and drinking water [1–3,5]. Moreover, continuous consumption and release of PPCPs into aquatic systems contribute to their pervasiveness in the environment. Lastly, a unique aspect that also must be considered when assessing potential risks of PPCPs in the environment is that they are designed to elicit specific effects in target organisms and, hence, possess considerable biological activity [1].

Several studies have investigated acute effects of PPCPs on aquatic organisms [6–9]. The effects on benthic invertebrates, however, have not been widely investigated despite

their essential role in aquatic food webs and the potential for sediments to serve as a repository for anthropogenic contaminants [10,11]. Therefore, the objective of the present study was to evaluate the toxicity of four commonly used PPCPs on two benthic invertebrates, the midge *Chironomus tentans* and the freshwater invertebrate *Hyalella azteca*, using 10-d, water-only exposures. These data were then combined with environmental occurrence data to calculate a hazard quotient (HQ) and, thereby, provide a worst-case scenario assessment of the risks these compounds may pose to benthic organisms.

The selection of PPCPs for testing was based on Canadian usage data, chemical persistence, availability of analytical methods, and previous research within our group. This resulted in the selection of four chemicals.

Atorvastatin (ATO) is the most widely prescribed lipid regulator in Canada [12], and it has been detected in both sewage effluent and surface water [13,14]. Studies have revealed the phytotoxic effect of ATO to aquatic macrophytes at concentrations as low as 26 μg/L [15].

Carbamazepine (CBZ) is an antiepileptic drug, but is also used for treatment of psychological disorders and chronic pain [16]. Low degradation rates upon sewage treatment have been reported; thus, CBZ is commonly detected in sewage effluent and surface water, and was also detected in groundwater [3,17,18]. Its environmental persistence raises concerns about potential effects on nontarget organisms.

The synthetic hormone 17α-ethinylestradiol (EE$_2$), which is used for birth control and hormone replacement therapy, has been investigated extensively in vertebrates and has feminizing effects in fish at concentrations currently detected in

Table 1. Water-quality characteristics of test water monitored throughout the experiments[a]

	Chironomus tentans	*Hyalella azteca*
pH	8.4 ± 0.12 (254)	7.7 ± 0.30 (228)
Temperature (°C)	21.9 ± 0.73 (255)	22.4 ± 1.13 (228)
Dissolved oxygen (mg/L)	7.9 ± 0.39 (243)	6.5 ± 1.33 (228)
Conductivity (mS/cm)	606 ± 57 (132)	395 ± 20 (192)
Alkalinity (mEq)	0.35 ± 0.100 (23)	0.11 ± 0.008 (58)
Hardness (mmol)	0.25 ± 0.081 (26)	0.13 ± 0.004 (59)
Ammonia (mg/L)[b]	1.4 ± 0.69 (18)	0.3 ± 0.12 (23)

[a] Values are presented as the mean ± standard deviation. The number of samples is in parentheses. mS/cm = milli-Siemens per centimeter.
[b] Measurements performed on day-10 water residues.

the environment [19]. Until recently, comparatively few studies have examined its potential effects on nonvertebrate species.

Triclosan (TCS) has been used for more than 35 years as an antimicrobial and antifungal agent, and is a common constituent of household and personal care products, including soaps, shampoos, deodorants, and detergents. Its current widespread usage has raised concerns regarding possible effects on bacterial resistance, suggesting that potential effects of TCS on benthic invertebrates should be investigated.

MATERIALS AND METHODS

Chemicals

Acetone (distilled in glass), acetonitrile (MeCN; high-performance liquid chromatography grade), methanol (MeOH; high-performance liquid chromatography grade), dichloromethane (distilled in glass), toluene (distilled in glass), and silica gel (particle size, 100–200 μm) were purchased from Caledon (Georgetown, ON, Canada). Pentafluorobenzylbromide (purity, ≥99%) was obtained from Sigma-Aldrich (Oakville, ON, Canada).

Atorvastatin (purity, ≥99%) was obtained from Rugao Foreign Trade (Shanghai, Jiangsu, China). Carbamazepine (purity, ≥99%) was supplied by China Jiangsu Textiles (Nanjing, Jiangsu, China). 17α-Ethinylestradiol (purity, ≥98%) and TCS (purity, ≥97%) were acquired from Sigma-Aldrich.

Isotopically labelled $^2D_2$-17β-estradiol (atomic purity, >98%) was obtained from Sigma-Aldrich, and $^2D_3$-ibuprofen (atomic purity, ≥99%), used as a laboratory surrogate because isotopically labeled TCS was not available at that time, was purchased from CDN Isotopes (Pointe-Claire, QC, Canada).

Test organisms

A laboratory culture of *C. tentans* (now *Chironomus dilutus*), obtained from the U.S. Environmental Protection Agency (EPA; Duluth, MN, USA), was maintained in reconstituted water at the University of Guelph (Guelph, ON, Canada) following U.S. EPA guidelines [10]. New egg masses were seeded before each experiment, and 12-d-old animals were used in all toxicity tests. All experiments were performed in reconstituted water.

Hyalella azteca were obtained from Environment Canada (Burlington, ON, Canada). Culture maintenance and testing were performed in reconstituted standard artificial medium [20] at the University of Guelph following U.S. EPA guidelines [10]. Animals were selected for testing at 7 to 14 days of age.

Toxicity tests

The toxicity of each compound was investigated individually using 10-d assays following U.S. EPA guidelines [10].

All experiments were conducted in 300-ml tall-form beakers, to which 50 ml of sand (particle size, 250–500 μm) were added in the *C. tentans* experiments. A small piece of cotton gauze was added in the *H. azteca* experiments to serve as substrate [20]. For each toxicity test, either eight or nine concentrations were used (seven concentrations plus control; a 0.2% MeOH control was added for EE_2 and TCS tests to aid dissolution into the aqueous phase). The number of replicates (n = 4–5) and number of animals added to each beaker (n = 9–10 for *C. tentans* and 8–15 for *H. azteca*) depended on animal availability at the commencement of each test.

All tests were conducted under static-renewal conditions, with water renewals performed every 48 h. The test containers were aerated continuously during the *C. tentans* assay, and pH, temperature, dissolved oxygen, and conductivity were monitored throughout the experiments using a portable meter (Hach, Loveland, CO, USA) (Table 1). Water hardness, alkalinity and ammonia were measured by colorimetric assay (Hach) at the beginning and end of the experiments and are reported in Table 1. Water samples were collected for residue analysis. On experiment completion, surviving animals were counted, dried, and weighed for growth calculation using ash-free dry weight for *C. tentans* and dry weight for *H. azteca*.

Analytical chemistry

Atorvastatin and carbamazepine. Residue samples were collected before water renewal and frozen in glass vials for analysis. Quantification of ATO and CBZ was performed using an isocratic high-performance liquid chromatograph equipped with an ultraviolet detector at room temperature, using a Waters 600S pump (Waters, Milford, MA, USA) fitted with a Genesis C-18 reverse-phase column (length, 250 mm; inner diameter, 4.6 mm; film thickness, 4 μm; Jones Chromatography, Deerfield, IL, USA) and a Waters 996 photodiode-array detector. Target analytes were detected at wavelengths of 250 nm (ATO) and 290 nm (CBZ). A flow rate of 1 ml/min was used for all analyses. The mobile phase consisted of 60% MeCN:40% buffer (10 mM potassium phosphate monobasic, adjusted to pH 3 using *o*-phosphoric acid) for ATO analysis and 40% MeCN:60% buffer for CBZ analysis.

17α-Ethinylestradiol

Water residue samples were either frozen pending analyses or preconcentrated by solid-phase extraction (recovery, 87% ± 4.8% [mean ± standard error of the mean, n = 6]; Supelco ENVI-18; Sigma-Aldrich). Analyses were performed by liquid chromatography–tandem mass spectrometry (Waters 2695 separation module, Waters Quattro Ultima Micromass). Separation was achieved using a mobile phase consisting of a mixture of MeCN and H_2O operated at a flow rate of 200 μl/min. The

gradient characteristics were 0 to 5 min at 60% MeCN, 5 to 7.5 min at 80% MeCN, and 7.5 to 10 min at 60% MeCN. A postinjection base of 0.5% ammonium carbonate was added at a rate of 10 μl/min and facilitated detection of the target compound. The mass spectrometer was operated in negative-electrospray mode with a cone voltage of 20 kV, collision energy of 37 eV, parent ion 295.1 *m/z*, daughter ions 145.0 *m/z* (quantitation) and 159.0 *m/z* (confirmation), and retention time of 2.6 min. An internal standard of $^2D_2$-17β-estradiol served to correct for variations in ionization efficiency.

Triclosan

Samples were acidified to pH 2 with 50% sulfuric acid and preserved by addition of 50 to 100 ml of dichloromethane. Samples were refrigerated for less than two weeks pending extraction. After addition of a laboratory surrogate, water samples were extracted by liquid–liquid extraction in dichloromethane and esterified using 5% pentafluorobenzylbromide in acetone at 60°C. After cleanup through 5% deactivated silica gel, target analytes were isolated using 5% MeOH in toluene and analyzed by negative-ion chemical ionization gas chromatography–mass spectrometry using an Agilent 6890 gas chromatograph equipped with a HP5MS column (length, 30 m; inner diameter, 0.25 mm; film thickness, 25 μm) and a 5973 mass-selective detector (Agilent Technologies, Palo Alto, CA, USA). Helium was used as the carrier gas at a flow of 1.2 ml/min, and methane was the reagent gas providing negative-ion chemical ionization. Average method recovery was 100% ± 2.4% (mean ± standard error of the mean, *n* = 74).

Statistical analyses

Concentrations lethal to 50% (LC50), 25%, and 10% of organisms as well as concentrations producing effects on 50% (EC50), 25%, and 10% of organisms were estimated using four regression models (linear, exponential, Gompertz, and logistic). All estimates were based on measured concentrations. For each model, all data were tested to ensure that they conformed to the assumptions of normality and homoscedasticity using the Shapiro–Wilk statistic and by plotting predicted values versus residuals, respectively. The studentized residuals were compared to the critical values for approximate tests of outliers. Data that failed to meet the assumptions were transformed using either a log transformation (concentration data) or a square root or arcsine–square root transformation (survival and growth data). The model that best fit the data based on coefficient of determination and conformation to the assumptions of the analysis of variance was selected. All computations were performed using SAS® software (Ver 9.1; SAS institute, Cary, NC, USA).

Hazard quotient

Data from the present study were used to calculate a hazard quotient (HQ) for each chemical investigated using the specifications provided in the Environment Canada guidance document [21]. The calculation used was HQ = (exposure/effect)·UF, where exposure was the greatest concentration in surface water reported in the literature and UF was the uncertainty factor. The effect measure was the lowest measured LC50 or EC50 from the present study. Together, the exposure and effects data therefore represent worst-case scenarios. For all calculations, a UF of 1,000 was first selected for the conversion of our critical toxicity values to estimated no-effect values (two organisms), as suggested in the Environment Can-

ada guidelines [21]. A second HQ was also estimated by the incorporation of data from other studies, thus allowing us to reduce the UF to 100, because these HQ estimates were based on comparisons of acute toxicity data using three or more taxa [21].

RESULTS AND DISCUSSION

Toxicity

Of the four PPCPs tested, TCS was the most toxic compound, followed by EE_2, ATO, and CBZ, for both *C. tentans* and *H. azteca* (Tables 2 and 3 and Figs. 1 and 2). Concentrations required to cause a 50% effect (survival or growth) varied between 0.2 and 47.3 mg/L (Tables 2 and 3).

Hyalella azteca was significantly more sensitive than *C. tentans*, with the exception of the growth effects of CBZ, for which the EC50 of *C. tentans* was 9.5 mg/L, compared to 5.0 mg/L for *H. azteca*, with overlapping 95% confidence intervals, and with the exception of the toxicity of TCS, which was similar (Table 2 and Table 3).

For all four PPCPs, survival of *C. tentans* and *H. azteca* followed typical concentration–response relationships (Figs. 1 and 2). In *C. tentans*, growth was a more sensitive indicator of toxicity than survival (Table 2 and Fig. 1). In contrast, growth in *H. azteca* was not more sensitive than survival (Table 3 and Fig. 2). The smaller size and growth rate of *H. azteca* (average dry wt of 0.06 mg by the end of the experiments, compared with 0.95 mg for *C. tentans*) make differences more difficult to detect. For this reason, Borgmann [20] suggested that longer time intervals or a greater number of replicates may be more appropriate for detecting the more subtle cumulative effects of growth in *H. azteca*.

Atorvastatin

Atorvastatin was approximately 10-fold more toxic to *H. azteca* (LC50, 1.5 mg/L) than to *C. tentans* (LC50, 14.3 mg/L) (Tables 2 and 3). Few other studies have investigated the effects of this compound; among others, 7-d EC50s of 38 to 214 μg/L were reported for the duckweed *Lemna gibba* [15,22]. To the best of our knowledge, the present study is the first to report invertebrate toxicity.

Currently, few data are available regarding the fate and occurrence of ATO in aquatic systems. A monitoring study of statin drugs in a Canadian sewage treatment plant reported concentrations of 76 ng/L in raw sewage, 37 and 22.4 ng/L in sewage treatment plant effluents, and 1 ng/L in surface water [13,14].

The HQ, which was calculated using the exposure data of Miao and Metcalfe [13] and the effects data from the present study, indicates negligible risks of ATO to benthic invertebrates at concentrations currently detected in the environment (Table 4). Calculations using the lowest-effects data from the study by Brain et al. [22] did not change the HQ appreciably, confirming the low risks posed by the presence of this compound.

Carbamazepine

Carbamazepine was the least toxic of the four chemicals investigated, with LC50s and EC50s ranging from 9.9 to 47.3 mg/L (Figs. 1 and 2). Narcosis has been suggested as the main mechanism of action by CBZ in *Daphnia magna* [9]. The relatively lower toxicity of CBZ compared to the other compounds supports the likelihood that the toxic effect to *C. tentans* and *H. azteca* was also nonpolar narcosis.

Table 2. *Chironomus tentans* survival and growth estimates (mg/L) after 10-d exposures to four pharmaceutical and personal care products[a]

Survival (%)

	Atorvastatin	Carbamazepine	17α-Ethinylestradiol	Triclosan
LC50	14.3 (5.96–22.73)	47.3 (43.75–50.91)	4.1 (3.56–4.73)	0.4 (0.27–0.44)
LC25	10.1 (3.83–16.37)	23.7 (21.88–25.46)	2.7 (2.00–3.38)	0.1 (0.09–0.14)
LC10	8.2 (2.75–13.64)	9.5 (8.75–10.18)	1.6 (0.97–2.31)	0.02 (−0.011–0.055)
Model[b]	exp	lin	Gomp	exp[c]
r^2	0.78	0.91	0.96	0.92
Pr < W[d]	0.06	0.09	0.01[e]	0.05

Growth (% of control)

	Atorvastatin	Carbamazepine	17α-Ethinylestradiol	Triclosan
EC50	10.2 (3.94–16.42)	9.5 (7.91–11.37)	6.6 (2.06–11.19)	0.28 (0.208–0.362)
EC25	7.0 (2.45–11.52)	4.6 (3.61–5.85)	2.3 (0.44–4.11)	0.15 (0.088–0.212)
EC10	5.6 (1.69–9.41)	2.6 (1.84–3.43)	0.8 (−0.41–1.97)	0.08 (0.027–0.131)
Model	exp	log[c]	log	log
r^2	0.76	0.95	0.43	0.80
Pr < W	0.88	0.23	0.14	0.93

[a] LCx and ECx refer to the concentrations resulting in x% mortality and growth reduction, respectively. Values in parentheses represent the 95% confidence interval.
[b] exp = exponential; lin = linear; Gomp = Gompertz; log = logarithmic.
[c] Concentration data were log-transformed.
[d] Pr < W = Shapiro–Wilk statistic.
[e] None of the data transformations attempted resulted in a normal distribution of the residuals (log-transformation, arcsine, square root or arsine[square root]).

Other studies investigating the effects of CBZ have shown similar toxicity to species from various taxa. Measured toxicity values varied from a 7-d EC50 of 25.5 mg/L for the macrophyte *Lemna minor* [9] to a 24-h LC50 of 140 mg/L for the fairy shrimp *Thamnocephalus platyurus* [23]. The first published chronic CBZ toxicity study to date was a 28-d life-cycle assay with spiked sediments, which reported significant effects on *Chironomus riparius* emergence (EC50, ≈0.2 mg/kg dry wt) [24]. However, concentrations in overlying water were elevated (as high as 2.85 mg/L), making it difficult to conclude whether the toxicity resulted from exposure to sediment-bound CBZ, CBZ in the overlying water, or both. Indeed, the highest aqueous concentration measured in that study was 2.85 mg/L [24], which, when compared to those of the present study, would be sufficient to reduce the growth of *C. tentans* by 10%, as indicated by our 10% effect concentration of 2.6 mg/L in a water-only exposure (Table 2). Furthermore, studies in our laboratory have investigated the toxicity of CBZ in spiked sediments using Imhoff sedimentation cones and a water to sediment ratio of 39:1, which ensured minimal aqueous exposure and, thus, permitted evaluation of the specific effect of the sediment-bound compound. No significant toxic effects to either *C. tentans* or *H. azteca* were observed at concentrations as high as 56 mg/kg dry weight (È.B. Dussault, unpublished results).

Carbamazepine has been detected frequently in sewage effluents and surface water (82.5–100%) [3,25], has a long aqueous half-life (82 d) [26], and experiences poor removal during sewage treatment (5–29%) [3,25]. Reported CBZ concentrations in surface water range from 1.1 to 7,100 ng/L in Europe [3,27], and similar concentrations have been detected in North America [17,18].

The HQ calculated using the data from the present study (i.e., 0.72) revealed that there was no significant risk to benthic invertebrates (Table 4). We further assessed potential hazard using the chronic toxicity data of Oetken et al. [24], because we suspected that the observed toxicity may have been related to the concentration in the overlying water rather than to the concentration in the sediments. Given that their EC50 was calculated using the concentrations measured in the sediments, however, the former had to be converted from sediment concentration to overlying water concentration, both of which were reported in their study. We therefore used a linear regression predicting the concentration in the water from that in the sediments ($y = 0.9376x + 0.1975$, $r^2 = 0.9666$), which yielded an EC50 of 0.385 mg/L. Given the uncertainty associated with the assumption that toxicity was only related to the chemical in the aqueous phase, a UF of 100 was applied. The addition of this calculated value of 0.385 mg/L resulted in a HQ of 3.55, suggesting that further investigations should be conducted to assess the risk this compound may pose to benthic organisms.

17α-Ethinylestradiol

The synthetic hormone EE_2 was the second most toxic chemical to both species investigated, with 10-d LC50s of 4.1 mg/L (*C. tentans*) (Table 2 and Fig. 1) and 1.1 mg/L (*H. azteca*) (Table 3 and Fig. 2). Other studies investigating the toxicity of this compound to invertebrates reported a 96-h LC50 of 0.51 mg/L to the copepod *Nitocra spinipes* and a 24-h LC50 of 5.7 mg/L to the cladoceran *D. magna* [28,29]. Studies with the amphipod *Gammarus pulex* reported a 10-d

Table 3. *Hyalella azteca* survival and growth estimates (mg/L) after 10-d exposures to four pharmaceutical and personal care products[a]

	Survival (%)				Growth (% of control)			
	Atorvastatin	Carbamazepine	17α-Ethinylestradiol	Triclosan	Atorvastatin	Carbamazepine	17α-ethinylestradiol	Triclosan[b]
LC50	1.5 (1.10–1.97)	9.9 (6.53–14.82)	1.1 (0.98–1.26)	0.20 (0.144–0.257)	2.4 (1.30–3.56)	15.0 (10.37–19.64)	1.3 (0.98–1.71)	0.25 (0.09–0.40)
LC25	0.8 (0.49–1.12)	2.3 (1.74–2.98)	0.5 (0.41–0.50)	0.06 (0.039–0.084)	1.7 (0.40–2.97)	6.1 (2.84–9.29)	0.6 (0.40–0.80)	0.12 (0.05–0.20)
LC10	0.4 (0.17–0.67)	0.6 (0.50–0.74)	0.16 (0.15–0.18)	0.005 (−0.028–0.039)	1.4 (−0.09–2.80)	2.4 (0.14–4.76)	0.2 (0.09–0.39)	0.05 (0.02–0.08)
Model[c]	log	lin[d]	lin[d]	exp[d]	exp	log	Gomp	lin
r^2	0.89	0.72	0.88	0.88	0.69	0.76	0.73	0.25
Pr < W[e]	0.32	0.13	0.20	0.08	0.95	0.66	0.48	0.35

[a] LCx and ECx refer to the concentrations resulting in *x*% mortality or growth reduction, respectively. Values in parentheses represent the 95% confidence interval. A linear model was applied; however, the LC50 is based on an extrapolation. Data were not used for hazard quotient calculation and should be interpreted with caution.
[b] All animals died at the highest concentrations.
[c] log = logarithmic; lin = linear; exp = exponential; Gomp = Gompertz.
[d] Concentration data were log-transformed.
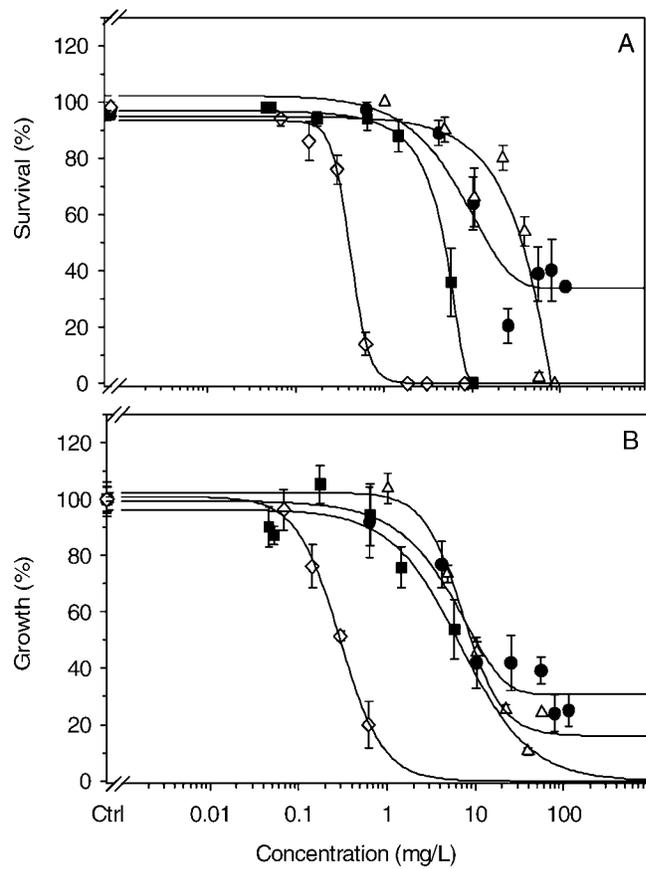[e] Pr < W = Shapiro–Wilk statistic.

Fig. 1. Survival (**A**) and growth (**B**; mean $\pm$ standard error of the mean) of *Chironomus tentans* following 10-d exposures to four pharmaceutical and personal care products. Trend lines are based on the regression models used for the estimation of median concentrations (see Table 2). ● = atorvastatin; △ = carbamazepine; ■ = 17α-ethinylestradiol; ◇ = triclosan.

LC50 of 0.84 mg/L [30], a value similar to those measured in the present study (Tables 2 and 3).

Although the mechanism of action and effects of EE_2 are well documented in vertebrates, the role played by sex steroids in invertebrate hormonal control is not as clear. Sex steroids are not known to play a role in the reproduction of insects or crustaceans [31,32]. The process involved with toxicity of EE_2 in the present study is not known, but is not likely to be related to its mechanism of action causing endocrine disruption in vertebrates [28].

In contrast with other widespread PPCPs, detection of EE_2 in aquatic systems is infrequent, and when detected, environmental concentrations generally are lower than those of other commonly found PPCPs. In 1999 to 2000, a survey of 139 U.S. streams revealed the sporadic presence of EE_2 in surface water at concentrations as high as 0.273 μg/L, but at a detection frequency of only 5.7% [33].

The HQ calculated for EE_2 yielded values of 0.25 and 0.05 (Table 4), revealing the low risk that this compound may represent for benthic and aquatic invertebrates.

Triclosan

With 10-d LC50s of 0.4 mg/L (*C. tentans*) (Table 2 and Fig. 1) and 0.2 mg/L (*H. azteca*) (Table 3 and Fig. 2), TCS was the most toxic of the four compounds tested. Other studies with the cladocerans *Ceriodaphnia dubia* and *D. magna* have reported similar sensitivity, whereas some algal species have
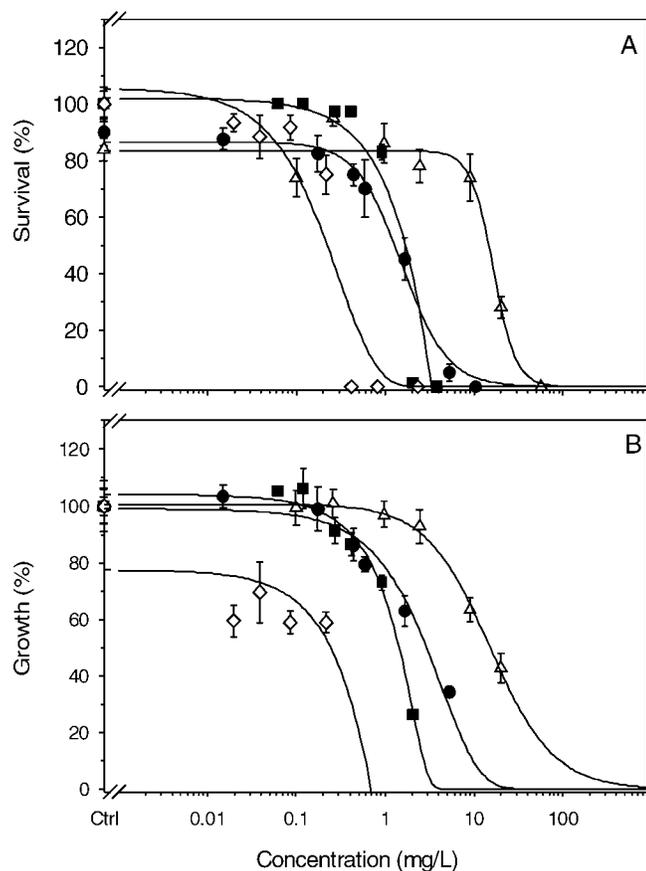
Fig. 2. Survival (**A**) and growth (**B**; mean $\pm$ standard error of the mean) of *Hyalella azteca* following a 10-d exposure to four pharmaceuticals and personal care products. Trend lines are based on the regression models used for the estimation of median concentrations (see Table 3). ● = atorvastatin; △ = carbamazepine; ■ = 17α-ethinylestradiol; ◇ = triclosan.

been reported to be more sensitive to TCS, with 72- to 96-h EC50s of 0.7 to 4.7 μg/L for *Scenedesmus subspicatus*, *Anabaena flos-aquae*, and *Pseudokirchneriella subcapitata* [6,7].

Despite its usage for more than 35 years, the fate and occurrence of TCS in aquatic environments has mostly been

investigated during the last decade. Its widespread addition to numerous household products results in significant release to sewage treatment plants. Although TCS does not appear to resist sewage treatment, with removal of as much as 95.5% from sewage treatment plants reported [34], the U.S. monitoring survey revealed that TCS was commonly detected in surface water at a frequency of 57.6% and at concentrations as high as 2.3 μg/L [2].

The HQ for TCS was 11.5, indicating that this compound could pose a risk to benthic invertebrates and that a more thorough risk assessment, including the effects of TCS in sediments, would be required to further elucidate potential risks. The addition of an EC50 from sensitive algae [6] resulted in a HQ of 329, confirming its potential risk and the necessity for undertaking a more complete assessment of the potential impacts this compound may have on aquatic systems.

CONCLUSION

Our investigations using 10-d survival and growth assays estimated the toxicity of four PPCPs (ATO, CBZ, EE$_2$, and TCS) to the benthic invertebrates *C. tentans* and *H. azteca*. All four PPCPs were found to be toxic only at concentrations at least 100-fold greater than those typically detected in aquatic environments. For ATO, EE$_2$, and CBZ, this was reflected in low HQs, ranging from 6.7×10^{-4} to 0.72. In most cases, the addition of data from other studies had little influence on the calculated HQ. For ATO and EE$_2$, the HQ remained below one. For CBZ, however, the use of the data from Oetken et al. [24] resulted in a HQ of 3.55, revealing a potential risk. Triclosan was the only compound for which a HQ greater than one was calculated using the data from the present study, indicating the need for further investigations to fully characterize the risks posed by its presence in aquatic systems. The bioavailability of sediment-bound PPCPs and their potential biological effects on benthic organisms remain largely undetermined and are the subject of current investigations within our laboratory.

Many investigations into the effects of PPCPs have focused on short-term toxicity (e.g., 24- to 96-h toxicity tests) with single substances [6–8,23]. Indeed, these studies are simpler and require less time and resources compared with longer chronic toxicity studies. Although acute toxicity testing pro-

Table 4. Hazard quotient for four pharmaceutical and personal care products using effects data from the present study and values reported in the literature (mg/L)[a]

	Atorvastatin	Carbamazepine	17α-Ethinylestradiol	Triclosan
Exposure (highest value, surface water)	0.001[b]	7.1[c]	0.273[d]	2.3[e]
Effects (lowest LC50 for *Hyalella azteca*)	1,500	9,900	1,100	200
Uncertainty factor, two species [21]	1,000	1,000	1,000	1,000
Hazard quotient	6.7×10^{-4}	0.72	0.25	11.5
Effects (lowest LC50 or EC50) from other studies	38[f]	385[g]	510[h]	0.7[i]
Uncertainty factor	100	100	100	100
Hazard quotient	2.6×10^{-3}	3.55	0.05	329

[a] LC50 and EC50 are the concentration resulting in 50% mortality or measured effect, respectively.
[b] Miao and Metcalfe [13].
[c] Wiegel et al. [27].
[d] Kolpin et al. [34].
[e] Kolpin et al. [2].
[f] Brain et al. [22].
[g] Value calculated from sediment data from Oetken et al. [24].
[h] Breitholtz and Bengtsson [28].
[i] Orvos et al. [6].

vides much needed answers for the assessment of potential risk, much debate exists regarding its usefulness and validity as an appropriate predictor of potential environmental impacts, because relying solely on this type of data may underestimate potential sublethal effects [10]. Furthermore, given the specific design and inherently high biological activity of PPCPs, one may wonder whether traditional testing methods possess the desired sensitivity to detect the effects of PPCPs. Clearly, further investigations should include chronic toxicity studies [24], which will contribute important information regarding the risks posed by these contaminants in the aquatic environment. Given the current widespread contamination of aquatic systems by numerous pollutants, organisms will most likely be exposed to mixtures of compounds, of which PPCPs represent only a fraction. A few recent studies have focused on evaluating the effects of PPCP mixtures [15,35,36]. In particular, Borgmann et al. [36] investigated the sublethal effects of a mixture of PPCPs on three generations of the amphipod *H. azteca*, revealing a slight but significant increase in the number of males. These studies raise questions concerning exposure to mixtures of compounds, and they highlight the need to address this issue in future research.

The present study indicates that among the PPCPs investigated, ATO and EE_2 pose low risk to benthic invertebrates but that further investigations are needed to provide a more complete assessment of the risk that CBZ and TCS may pose to aquatic invertebrates. Considerable uncertainty exists regarding the potential impacts of chronic exposure to mixtures of these biologically active contaminants. Further long-term studies are needed to fully address the risks and impacts of these compounds on aquatic organisms.

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