Environmental Toxicology and Chemistry—Volume 43, Number 8—pp. 1767–1777, 2024
Received: 5 March 2024 | Revised: 29 March 2024 | Accepted: 17 April 2024

Special Series

The Importance of Including Variable Exposure Concentrations When Assessing Toxicity of Sediment-Associated Pharmaceuticals to an Amphipod

Sara Nicoline Grønlund,[a,b,*] Casper D. Læssøe,[a] Nina Cedergreen,[c] and Henriette Selck[a]

[a]Department of Science and Environment, Roskilde University, Roskilde, Denmark
[b]Department of Wildlife, Fish and Environmental Studies, Swedish University of Agricultural Sciences, Umeå, Sweden
[c]Department of Plant and Environment Sciences, University of Copenhagen, Copenhagen, Denmark

Abstract: Pharmaceuticals have been classified as an environmental concern due to their increasing consumption globally and potential environmental impact. We examined the toxicity of sediment-associated diclofenac and citalopram administered as both single compounds and in a mixture to the sediment-living amphipod *Corophium volutator*. This laboratory-based study addressed the following research questions: (1) What is the toxicity of sediment-associated diclofenac and citalopram to *C. volutator*? (2) Can the mixture effect be described with either of the two mixture models: concentration addition (CA) or independent action (IA)? (3) What is the importance of the choice of (i) exposure measure (start concentration, time-weighted average [TWA], full exposure profile) and (ii) effect model (concentration–response vs. the toxicokinetic–toxicodynamic model general unified threshold model for survival in its reduced form [GUTS-RED]) for the derived effect concentration values? Diclofenac was more toxic than citalopram to *C. volutator* as a single compound (10-day exposure). Diclofenac exposure to *C. volutator* provided median lethal concentrations (LC50s) within the same range (11 $\mu g\ g^{-1}$ dry wt sediment) using concentration–response based on TWA and both GUTS-RED models. However, concentration–response based on measured start concentrations provided an approximately 90% higher LC50 ($21.6 \pm 2.0\ \mu g\ g^{-1}$ dry wt sediment). For citalopram, concentration–response parameters were similar regardless of model or concentration used (LC50 85–97 $\mu g\ g^{-1}$ dry wt sediment), however, GUTS-RED with the assumption of individual tolerance resulted in a lower LC50 (64.9 [55.3–74.8] $\mu g\ g^{-1}$ dry wt sediment). The mixture of diclofenac and citalopram followed the CA quite closely, whereas the result was synergistic when using the IA prediction. In summary, concentration–response based on TWA and GUTS-RED provided similar and reasonably good fits compared to the data set. The implications are that GUTS-RED will provide a more flexible model, which, in principle, can extend beyond the experimental period and make predictions based on variable exposure profiles (toxicity at different time frames and at different variable exposure scenarios) compared to concentration–response, which provides contaminant toxicity at one point in time. *Environ Toxicol Chem* 2024;43:1767–1777. © 2024 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: *Corophium volutator*; Citalopram; Diclofenac; Ecotoxicology; Environmental modeling; Mixture toxicology; Pharmaceuticals; Sediment toxicity

INTRODUCTION

Wastewater treatment plants (WWTPs) are handling increasing amounts of pharmaceutical compounds (PCs) because of increased use and consumption globally (Organisation for Economic Co-operation and Development, 2017). However, most WWTPs are not designed to handle PCs, which generally results in low removing efficiencies (Jelic et al., 2011; Kasprzyk-Hordern, 2010; Kasprzyk-Hordern et al., 2009; Thiebault et al., 2017) and frequent occurrence in the aquatic environment

This article includes online-only Supporting Information.

* Address correspondence to sanigr@ruc.dk
Published online 28 May 2024 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.5894

(Alygizakis et al., 2016; Andreozzi et al., 2003; Kostich et al., 2014; Wilkinson et al., 2022). The European Environment Agency (2010) has classified PCs as an environmental concern and has specified the need for enhanced monitoring of compounds such as antibiotics, antiparasitics, hormones, analgesics, and psychotropic medicines, especially in water and, to some extent, in sediments. However, despite an increasing experimental focus on the impact of pharmaceuticals in the aquatic environment, the primary focus on water compared to sediment could potentially result in an underestimation of the environmental risks of some PCs that, because of their hydrophobicity, are prone to accumulate in the sediment (Patel et al., 2019).

We examined the effect of diclofenac, a nonsteroidal anti-inflammatory drug, and citalopram, a selective serotonin reuptake inhibitor antidepressant, on the tube-dwelling amphipod *Corophium volutator*. Toxicity (mortality) was assessed for diclofenac and citalopram both as single compounds and as a mixture because chemicals in the environment never occur in isolation but always exist as multicomponent mixtures (Backhaus, 2014; Inostroza et al., 2023). In addition, because diclofenac and citalopram have different modes of action, we wanted to examine if there was any indication of a mixture interaction.

The choice of assessing the toxicity of diclofenac and citalopram was based on their high annual consumption (Gan, 2010; Marasine et al., 2021; Stahl, 1998; Zhang et al., 2008) and frequent detection in both fresh- and marine waters (Alygizakis et al., 2016; Han et al., 2006; Weber et al., 2014; Wilkinson et al., 2022). Diclofenac is reported to have a log octanol–water partition coefficient (K_{OW}) of approximately 3.9 to 4.5 (in its neutral form) and a negative base-10 logarithm of the acid dissociation constant (pK_a) of 4.16, causing K_{OW} to decrease with increasing pH. Diclofenac (in its neutral form) has a partitioning coefficient between organic carbon and water (log K_{OC}) of 2.39 (Ferrari Beno et al., 2004; Han et al., 2006; Scheytt et al., 2005). Citalopram is reported to have a log K_{OW} of 3.7, a *p*Ka of 9.78, and a log K_{OC} of 5.63, making it significantly more hydrophobic compared to diclofenac. Further, diclofenac was included because it is on the first European Union's Water Framework Directive Watch List, and, therefore, has been frequently monitored and detected in the aquatic environment in 50 countries, with surface water concentrations reaching >1 μg L^{-1} (Han et al., 2006; Weber et al., 2014).

The test organism *C. volutator* is not commonly used in ecotoxicological studies but is considered a good candidate for testing marine sediments due to its global occurrence and ecological importance. *Corophium volutator* is a cosmopolitan amphipod with high ecological relevance, serving as prey for a variety of fish and birds (McLusky, 1968; Møller & Riisgård, 2006; Peer et al., 1986). The intertidal infaunal amphipod has an endobenthic lifestyle, burrowing in the upper part (up to 6 cm depth) of the sediment, where *C. volutator* can switch between several ways of feeding (deposit feeding, suspension feeding, and epipsammic browsing) depending on food availability (Gerdol & Hughes, 1994; McLusky, 1968; Møller & Riisgård, 2006; Nielsen & Kofoed, 1982; Siebeneicher et al., 2013; Wilson & Parker, 1996). Because of its habitat choice and feeding strategy, *C. volutator* may be exposed to both sediment-associated and dissolved chemicals (Droge et al., 2008; Siebeneicher et al., 2013).

The present study was based on laboratory experiments and addressed the following research questions: (1) What is the toxicity of sediment-associated diclofenac and citalopram to *C. volutator*? (2) Can the mixture of the two compounds be described with either of the two mixture models: concentration addition (CA) or independent action (IA)? (3) What is the importance of the choice of (i) exposure measure (start concentration, time-weighted average [TWA], full exposure profile) and (ii) effect model (concentration–response vs. the toxicokinetic–toxicodynamic [TKTD] model general unified threshold model for survival in its reduced form [GUTS-RED]) for the derived effect concentration (EC) values? Results are presented first as single compounds, then as the mixture (CA and IA), and lastly the different modeling approaches (start concentrations, TWA, and GUTS-RED).

MATERIALS AND METHODS

Collection and preparation of C. volutator

Corophium volutator was collected from Herslev Beach (N: 55.4039; E: 11.5681), Denmark, in June 2018, and Lyndby Harbor (N: 55.4007; E: 11.5909), Denmark, between September and November 2018, when water temperatures and salinities ranged between 9°C and 23°C and 15‰ and 18‰, respectively. The amphipods were collected by wet-sieving the top 5 to 10 cm of the sediment through a 500-μm-mesh sieve and transferred using soft tweezers to a plastic bucket containing water from the location. In the laboratory, *C. volutator* were acclimated to experimental conditions, 18 ± 1°C and a salinity of 31‰, over a 24 to 48-h period. Subsequently, the amphipods were transferred using a small mesh sieve, to two separate aerated culture tanks (6 L), containing 1 L ≤500 μm (wet) sediment and 2 L 31‰ S natural 0.2 μm filtered seawater at pH 7.8. Both cultures were fed sediment (<125 μm) as needed. For experimental use, *C. volutator* with a body length >0.5 cm was selected from the two tanks and randomly distributed between treatments. The lower size limit was set to make sure only adult specimens were included in the test, and the upper represents the maximum length of *C. volutator* (~1.2 mm).

Sensitivity of C. volutator

Based on the literature, there are speculations over whether field-collected amphipods should be used within 15 days of collection because cultivation might affect their sensitivity (Bat & Raffaelli, 1996). Because *C. volutator* were collected at different times and seasons, their sensitivity was tested in a 72-h water-only cadmium chloride ($CdCl_2$) setup modified from Ciarelli (1994) and Ré et al. (2009), assessing mortality. The test was performed in 250-mL beakers at 21°C to nominal concentrations of 0, 1, 7, and 14 mg/L $CdCl_2$. The test was

conducted using *C. volutator* kept in culture for 3 to 5 months and newly collected (<15 days) specimens, at two collection times (~2 months apart), and on two batches from different locations because more specimens were needed during the experiments. The first sensitivity test compared *C. volutator* collected at Herslev beach (Batch A) to a batch collected at Lyndby Harbor (Batch B), whereas the second sensitivity test compared a combination of the two first batches (AB) to another batch form Lyndby Harbor (Batch C).

Because the sensitivity tests indicated equal sensitivity among batches (Supporting Information), *C. volutator* used throughout experiments were a combination of all three batches.

Collection and preparation of sediment

Sediment for all experiments was collected from Herslev Beach, Denmark, between June and October 2018, when water temperature and salinity ranged between 18°C and 23°C and 15‰ and 18‰, respectively. Sediment treatment followed descriptions in Grønlund et al. (2023). Briefly, the top layer of the sediment (~10 cm depth) was scraped off using a shovel and sieved to ≤500 μm in situ. The sediment was subsequently frozen (−18°C, minimum 24 h), thawed, and washed with salt water corresponding to the test system (31‰ S, pH 7.8). After washing, the sediment was left to settle for 48 h before removing the overlying water. The sediment water content (105°C, 24 h: ~25%) and total organic content (550°C, 2 h: ~1%) were determined. The sediment was spiked with diclofenac sodium and/or citalopram hydrobromide dissolved in methanol (MeOH), supplied by Acros Organics. Both chemicals were acquired at the highest possible purity (≥97.5%). The chemical extracts were added to clean glass beakers, and MeOH was evaporated (2–4 h) before adding wet homogenized sediment. Two control sediments were included for each experiment: one solvent control (an equal amount of MeOH was added) and one without solvent addition (i.e., natural sieved sediment). All sediments were homogenized (30 min) on a shaking table (200 rpm) before being placed in the refrigerator (24 h). Subsequently, all sediments were mixed thoroughly with a spoon prior to experimental use. Screening tests were conducted to establish the sensitivity of *C. volutator* to sediment-associated diclofenac and citalopram. Based on the screening tests, two different setups were used: a single-compound experiment (conducted twice) with sediment spiked to nominal concentrations of 0.1 to 100 μg diclofenac g^{-1} dry weight sediment (nine concentrations) or 0.1 to 200 μg citalopram g^{-1} dry weight sediment (10 concentrations) and a mixture experiment with sediment spiked to eight nominal concentrations of 0.1 to 100 μg diclofenac g^{-1} dry weight sediment together with a constant concentration of citalopram corresponding to the citalopram median lethal concentration (LC50) obtained in the single-compound experiment based on nominal concentrations.

Experimental setup

The experiments were conducted in 20-mL scintillation vials covered with lids, with three boreholes: one for aeration (one to two bubbles per second through a needle connected to a tube) and two as air elimination paths. Experimental duration was 10 days. The sediment organic matter content was sufficient to allow food ad libitum, and it was not necessary to supply additional food during the exposure period.

One day prior to experimental start, 15 g wet weight (~11.3 g dry wt) sediment and 8 mL salt water were added to each scintillation vial, and vials were left to settle overnight with aeration. Before transferring *C. volutator* to the test vials, 4 mL salt water was replaced with 4 mL aerated salt water because a lack of water exchange has been observed to impact organisms negatively, possibly because of release of impurities from the sediment during spiking (Grønlund et al., 2023). *Corophium volutator* were removed from the culture tanks using a small mesh sieve and distributed randomly among the experimental treatments. Four replicates were included per treatment containing five *C. volutator* per replicate in the first setups and four individuals per replicate in the last setup because of a lack of individuals living up to the quality criteria. The remaining sediment from each concentration was saved in 50-mL Falcon tubes and used when determining sediment concentration to Day 0. All samples were frozen (−18°C) until further chemical analysis.

All replicates were examined daily to check potential water evaporation, mortality, and burrowing behavior/activity. Mortality was distinguished from molting: Dead individuals were nontransparent, and molted exoskeletons were semi-transparent. Deionized water was added in case of evaporation. Salinity (31‰) and pH (7.8) of the water were measured at experimental start and end (randomly selected concentrations) and did not change significantly over the duration of the experiment.

Experimental termination and sampling

At experimental termination, *C. volutator* were removed from the sediment using soft tweezers and transferred to clean salt water, where mortality was registered. If a specimen was missing at Day 10, it was considered dead.

At the end of the experiment, water (~4 mL) and sediment (~4 mL, wet homogenized) from each replicate per treatment were pooled in 50-mL plastic Falcon tubes, resulting in one sediment and one water sample per concentration. All samples were frozen (−18°C) until further chemical analysis.

Extraction

Sediment extraction. Diclofenac and citalopram were extracted from the sediment using Bond Elut, Sample Prep Solutions (Agilent Technologies). Extractions were made using 6 to 7 g wet weight (~4.5–5.3 g dry wt) homogenized (with a spoon) sediment. Each sediment sample had MeOH added (10 mL) and was manually shaken (1 min). Then, a Bond Elut QuEChERS extraction pouch (59820650) and a ceramic homogenizer were added, and the sediment was manually shaken (1 min) and centrifuged (5 min, 4000 rpm). Subsequently, 6 mL of the aliquot was transferred to a Bond Elut dSPE 15-mL tube,

which was manually shaken (1 min) and centrifuged (5 min, 4000 rpm).

Water extraction. Water samples (5 mL) were concentrated by evaporating the sample to complete dryness (~6 h) under a constant stream of nitrogen while placed on a heating block (50°C). The samples were redissolved in MeOH (1 mL).

All extractions then had 50 µL internal standard (imipramine hydrochlorine) added to determine compound recovery. Extraction products were transferred to gas chromatography vials (sediment 1.5 mL, water 0.2 mL) and stored in the freezer (−18°C) until further analysis.

The measured concentrations of diclofenac and citalopram in the sediment (Day 0 and Day 10) and overlying water (Day 10) were determined using high-performance liquid chromatography with florescence detection (FLD; Thermo-Scientific Ultimate^{TM} 3000), with an excitation wavelength of 246.0 nm and an emission wavelength of 365.0 nm. The limit of detection and limit of quantification for the FLD analysis were determined based on a standard curve of concentrations, 0.5 to 50,000 µg L^{-1} of diclofenac and citalopram, and were 8 and 24 µg L^{-1} for both compounds (diclofenac and citalopram), respectively (Shrivastava & Gupta, 2011).

Data analysis

Contaminant budget. The contaminant budget for the system was determined by relating the measured concentration in each compartment (i.e., water and sediment) with the quantity of sediment (15–11.3 g dry wt) and water (8 mL) in each replicate for each exposure concentration. Recovery at the start of the experiment was determined relating the measured actual start concentration to the nominal start concentrations of the sediment, whereas recovery for Day 10 was determined by relating the concentrations from Day 10 to the start concentrations.

Concentration–response. The toxicity of diclofenac and citalopram to *C. volutator*, in terms of mortality, after a 10-day period was assessed by a log-logistic concentration–response curve.

Concentration–response relationships were determined in R Ver. 4.3.2 (R Core Team, 2023) using the packages "tidyverse" and "drc". A three-parameter log-logistic model assuming binary distribution of data (Ritz & Streibig, 2005) was used, to allow for estimation of control mortality:

$$y = \frac{d}{1 + (\frac{x}{e})^b} \quad (1)$$

In Equation (1), y is the surviving fraction of organisms, d is the surviving fraction of the control treatment, e is the concentration killing 50% of the organisms (LC50), and b is the slope around the LC50. The concentration, x, was fitted to the measured sediment concentration (micrograms per gram dry wt sediment) at experimental start or the TWA concentration (see below). A significance level of 0.05 was used throughout.

TWA. As chemicals degrade during an experiment, using start concentrations will overestimate the true exposure of the organisms during the experiment, consequently leading to higher effect concentrations than the "true" one. Using TWA is a way to estimate exposures more precisely, and that will be more comparable to those changing concentrations measured in the environment; TWA was calculated using the measured concentration (micrograms per gram dry wt sediment) at Days 0 and 10, assuming an exponential decay process:

$$\mathrm{TWA} = \frac{\sum_{i=1}^{n} A_i}{\sum_{i=1}^{n} (t_{i+1} - t_i)} \quad (2)$$

In Equation (2), $t_{i+1} - t_i$ is the time (days) between concentration measurements, and A_i is the area under the decreasing exponential curve between t_i and t_{i+1}, which is defined in Equation (3):

$$A_i = \frac{(c_i - c_{i+1})(t_{i+1} - t_i)}{\ln c_i - \ln c_{i+1}} \quad (3)$$

Mixture effects. Based on the single-substance curves, CA (Loewe & Muischnek, 1926) and IA (Bliss, 1939) predictions for the mixture effect were calculated and compared to the observed concentration–response curve of the mixture. When categorizing the combined effect of toxicants in a mixture, these can be described as additive (described by either of the two reference models), synergistic (higher effect than predicted by the reference model), or antagonistic (lower effect than predicted by the reference model Cedergreen et al., 2013).

We used two models to predict the mixture effects: CA, which assumes that chemicals with similar mode of action in a mixture could be considered as dilutions of each other, each having a different chemical efficiency (Backhaus, 2014; Cedergreen, 2014; Loewe & Muischnek, 1926):

$$\sum_{i=1}^{n} \frac{C_i}{\mathrm{EC}x_i} = 1 \quad (4)$$

In Equation (4), c_i gives the individual concentrations of the substances i, which are present in a mixture that create the definite effect x; $\mathrm{EC}x_i$ is the concentration of compound i which provokes an x% effect if applied alone, for example, EC50. The ratio $c_i/\mathrm{EC}x_i$ is a toxicant concentration expressed as a fraction of the concentration of the pure compounds that yields a predefined effect, also known as a toxic unit (TU; Backhaus, 2014; Cedergreen et al., 2013; Faust et al., 2003). To calculate the CA-predicted effect of a mixture at all effect levels, the complete concentration–response functions for all the chemicals in the mixture need to be available, and Equation (2) must be solved iteratively for x (Cedergreen et al., 2013; Faust et al., 2003).

On the other hand, IA assumes that the probability of surviving exposure to two chemicals with dissimilar modes of action would be equal to the probability of surviving the first chemical multiplied by the probability of surviving the second chemical (Backhaus, 2014; Bliss, 1939; Cedergreen, 2014). Considering that the probability of surviving is 1 minus the

probability of dying from concentration i of a chemical ($E[c_i]$), the probability of dying from a mixture ($E[c_{mix}]$) can be written as follows:

$$E(c_{mix}) = E(c_1 + I + c_n) = 1 - \prod_{i=1}^{n}[1 - E(c_i)] \quad (5)$$

In Equation (5), $E(c_{mix})$ is the probability of dying from a mixture composed of n chemicals at a total concentration, c_{mix}, and $E(c_i)$ is the probability of dying from chemical i when applied alone in concentration c. To calculate the effect concentration (e.g., EC50) of a mixture for all concentrations, Equation (5) should be solved iteratively (Cedergreen et al., 2013; Faust et al., 2003).

Toxicokinetic–toxicodynamic model. In addition to concentration–response relations to both start and TWA concentrations, we applied the TKTD model GUTS (Jager et al., 2011) in its reduced form (GUTS-RED). The reduced model was applied to account for the changes in sediment concentration and mortality over time. The GUTS-RED model was estimated both under the assumption of stochastic death (SD) and under that of individual tolerance (IT). Stochastic death assumes that all individuals are equally sensitive; however, death is considered a stochastic process on an individual level where the probability of dying increases with concentration. Hence, the ones dying are not necessarily less tolerant but rather the unlucky ones (Jager et al., 2011). On the other hand, IT assumes that individuals have different sensitivity; hence, they will show effects at different internal concentrations (Jager et al., 2011). The OpenGUTS Ver. 1.1 software was used (Jager, 2021). An array of parameters were estimated, with the following being relevant for each model: for SD, k_d, the dominant rate constant; m_w, the median distribution; h_b, the background hazard (control mortality); and b_w, the mortality rate; for IT, k_d, m_w, and β, the shape parameter of the sensitivity distribution (Jager & Ashauer, 2018). We applied the measured sediment concentration at the start (Day 0) and end (Day 10) of the experiment in GUTS-RED, assuming a first-order decay model.

When assessing the effect of the mixture experiment, we used the assumption that 1 TU of both compounds would yield the same effect; that is, they are interchangeable and can be assumed to be and used as a common concentration measure.

Statistics

Recovery data are presented as mean ± standard deviation, concentration–response parameters are presented ± standard error (SE), and GUTS-RED predictions and parameters are presented as best fits ±95% confidence intervals (CIs).

RESULTS AND DISCUSSION

Diclofenac

At experimental initiation (prior to the addition of overlying salt water) the measured concentration of diclofenac constituted 63.3 ± 12.3% ($n = 16$) of the nominal concentration (Supporting Information, Table S2). After 10 days of exposure, 19.5 ± 2.7% ($n = 10$) and 32.0 ± 13.0% ($n = 16$) of the measured mass of diclofenac were recovered from the sediment and overlying water, respectively. Thus, on average 55.8 ± 16.3% ($n = 16$) of the incubated diclofenac had disappeared from the system after 10 days. Our choice of water extraction method might have affected the recovery at Day 10, particularly for diclofenac, because heating may have increased compound degradation. However, the water concentration was exclusively used to account for the mass balance of the compounds, and the disappearance rate constants will, therefore, remain the same even with a poor recovery in the water analysis. For citalopram the mass balance at the end of the experiment corresponded well to the nominal concentrations with approximately 81% of the total amount of citalopram recovered at Day 10, while for diclofenac recovery at Day 10 was approximately 46%, which could be due to a faulty quality control of the method. We speculate that filtering the water samples (see Koba et al., 2018) would probably have been a more preferable preparation method. When assuming first-order disappearance rate constants, sediment-associated diclofenac had a rate constant of 0.12 day^{-1}, corresponding to a disappearance half-time (DT50) from the sediment of 5.9 days ($n = 10$–16), whereas diclofenac in the total system (sediment and overlying water) had a DT50 of 33.7 days ($n = 10$–16). Similar recovery and disappearance rates were found for sediment-associated diclofenac in a freshwater system: 76.7% of the nominal concentration was recovered at experimental start; further, over a 21-day period, the DT50 of diclofenac was 5.5 days (solely based on the highest concentration reported; Nieto et al., 2017).

At experimental conditions, diclofenac, being a weak acid (pK_a <4.5), is mainly in its negatively charged form, resulting in a log K_{OW} <2 (1.9 at pH 7.2), hence being poorly retained by the sediment (Patel et al., 2019). This is in line with the chemical analyses revealing a larger proportion of the diclofenac concentration having partitioned to the overlying water at Day 10. The disappearance of diclofenac after 10 days may indicate metabolization or degradation, likely to a hydroxylated metabolite (e.g., its primary metabolite 4'-hydroxy diclofenac; Altman et al., 2015; Stülten et al., 2008). However, we cannot confirm if this is the case.

After 10-day diclofenac exposure of *C. volutator*, the concentration response based on TWA and both GUTS-RED models resulted in LC50 values within the same range (i.e., 11 μg g^{-1} dry wt sediment; Table 1). However, the concentration response based on measured start concentrations resulted in an LC50 approximately 90% higher (i.e., 21.6 ± 2.0 μg g^{-1} dry wt sediment). This difference was expected due to both TWA and GUTS-RED take the disappearance of diclofenac over time into account, but it also shows that basing EC values on starting concentrations can underestimate toxicity twofold in the case of a compound with a half-life counted in days. The observed decrease in survival occurred over a very narrow concentration span, resulting in a steep slope of the concentration–response curve (Figure 1 and Table 2).

TABLE 1: The estimated median lethal concentration values (micrograms per gram dry wt sediment) for single-compound exposures after 10 days

	Measured start concentrations	Time-weighted average	GUTS-RED-SD	GUTS-RED-IT
DCF	21.6 ± 2.0	10.7 ± 1.0	11.1 (8.28–15.3)	11.4 (9.37–14.1)
CP	97.3 ± 8.4	86.3 ± 8.0	85.2 (81.6–89.6)	64.9 (55.3–74.8)

Measured start concentrations and time-weighted average are displayed as mean ± standard error, and GUTS-RED-SD and GUTS-RED-IT are displayed as mean with 95% confidence interval in parentheses.
CP = citalopram; DCF = diclofenac; GUTS-RED-IT = GUTS-RED with the assumption of individual tolerance; GUTS-RED-SD = general unified threshold model for survival in its reduced form with the assumption of stochastic death.

Both GUTS-RED models seemed to describe the interactions between sediment-associated diclofenac and survival of *C. volutator* over a 10-day exposure period equally well, with IT being marginally better (based on log-likelihood; Figure 2 and Table 3).

Comparing the toxicity of sediment-associated diclofenac across sediment-dwelling invertebrates suggests that *C. volutator* is more sensitive to diclofenac exposure than the commonly used insect larvae *Chironomus riparius*, which showed no significant decrease in survival at concentrations up to 34.0 μg g^{-1} dry weight sediment (10 days; Nieto et al., 2017), but less sensitive than the sentinel amphipod *Hyalella azteca*, where the LC50 was 0.467 μg g^{-1} dry weight sediment (72 h; Oviedo-Gómez et al., 2010).

Based on the rapid diclofenac partitioning from sediment to overlying water, we find it unlikely that diclofenac would pose a risk to the sediment community because the chemical properties of diclofenac make it more likely to primarily stay in the surface water under environmental conditions, thus making water monitoring likely to be sufficient when assessing the environmental impact of diclofenac. In addition, the measured lethal concentrations are very high and above environmentally realistic concentrations, with measured water concentrations being at the low micrograms per liter range, probably corresponding to

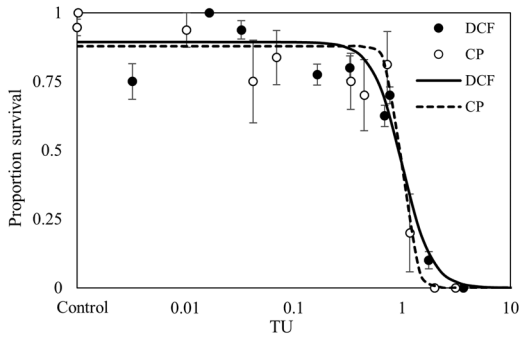

FIGURE 1: Proportion survival after 10-day exposure to sediment-associated diclofenac or citalopram presented as their individual toxic unit). One toxic unit corresponds to a median lethal concentration of the compound. The symbols represent the mean survival within each concentration (n = 4–16, 4–5 individuals per replicate) ± standard error, and the lines represent the concentration–response relationship; full circles and full line for diclofenac and open circles and dotted line for citalopram. DCF = diclofenac; CP = citalopram; TU = toxic unit.

TABLE 2: The estimated values of the three-parameter log-logistic fit (mean ± standard error) for *Corophium volutator* after 10-day exposure to diclofenac or citalopram as single compounds using either measured start concentrations or time-weighted average

	b	*d*
Measured start concentrations		
DCF	3.2 ± 0.8	0.9 ± 0.02
CP	7.4 ± 3.6	0.9 ± 0.03
Time-weighted average		
DCF	3.2 ± 0.8	0.9 ± 0.02
CP	6.6 ± 3.3	0.8 ± 0.03

CP = citalopram; DCF = diclofenac.

even lower sediment concentrations. However, sublethal endpoints (i.e., reproduction) might still be relevant at environmentally realistic concentrations over longer exposure durations because diclofenac is designed to affect prostaglandins, which are believed to be involved in the regulation of reproduction in several marine invertebrates (Varvas et al., 2009).

Citalopram

The measured concentration of citalopram constituted 104.9 ± 22.2% (n = 17) of the nominal concentration at experimental start (Supporting Information, Table S2), and 75.5 ± 8.4% (n = 15) and 5.4 ± 2.8% (n = 15) of the starting citalopram amounts were present in the sediment and overlying water, respectively, after 10 days of exposure. Thus, on average, 19.1 ± 9.0% (n = 17) of the starting concentration of citalopram had disappeared from the system after 10 days. Assuming first-order disappearance rate constants, sediment-associated citalopram had a rate constant of 0.03 day^{-1}, corresponding to a DT50 from the sediment of 21.0 days (n = 15–17), whereas citalopram in the total system (sediment and overlying water) had a DT50 of 26.6 days (n = 15–17). Compared to diclofenac, citalopram is relatively uninvestigated, hence, it has not been possible to retrieve comparable data on environmental disappearance. However, the high partitioning of citalopram to the sediment fraction was expected due to its hydrophobicity (log K_{OC} of 5.63, meaning that it will bind strongly to the organic fraction of the sediment (Christensen et al., 2007; Minguez et al., 2014; Yang et al., 2017).

After 10 days of exposure, concentration–response parameters were similar disregarding the model or concentration used, giving LC50 values within the same range (Table 1). The GUTS-RED-IT model resulted in the lowest LC50 (64.9 [55.3–74.8] μg g^{-1} dry wt sediment) compared to the others (85–97 μg g^{-1} dry wt sediment). We have no immediate explanation as to why GUTS-RED-IT was more conservative compared to the other models, but considering the prediction intervals of the GUTS model and the SE of the fitted parameters, differences are only marginally lower. Considering the slow dissipation rate constant of citalopram, it makes sense that EC values are more similar than those for diclofenac. As observed for diclofenac, mortality of *C. volutator* exposed to citalopram increased drastically within a narrow range of

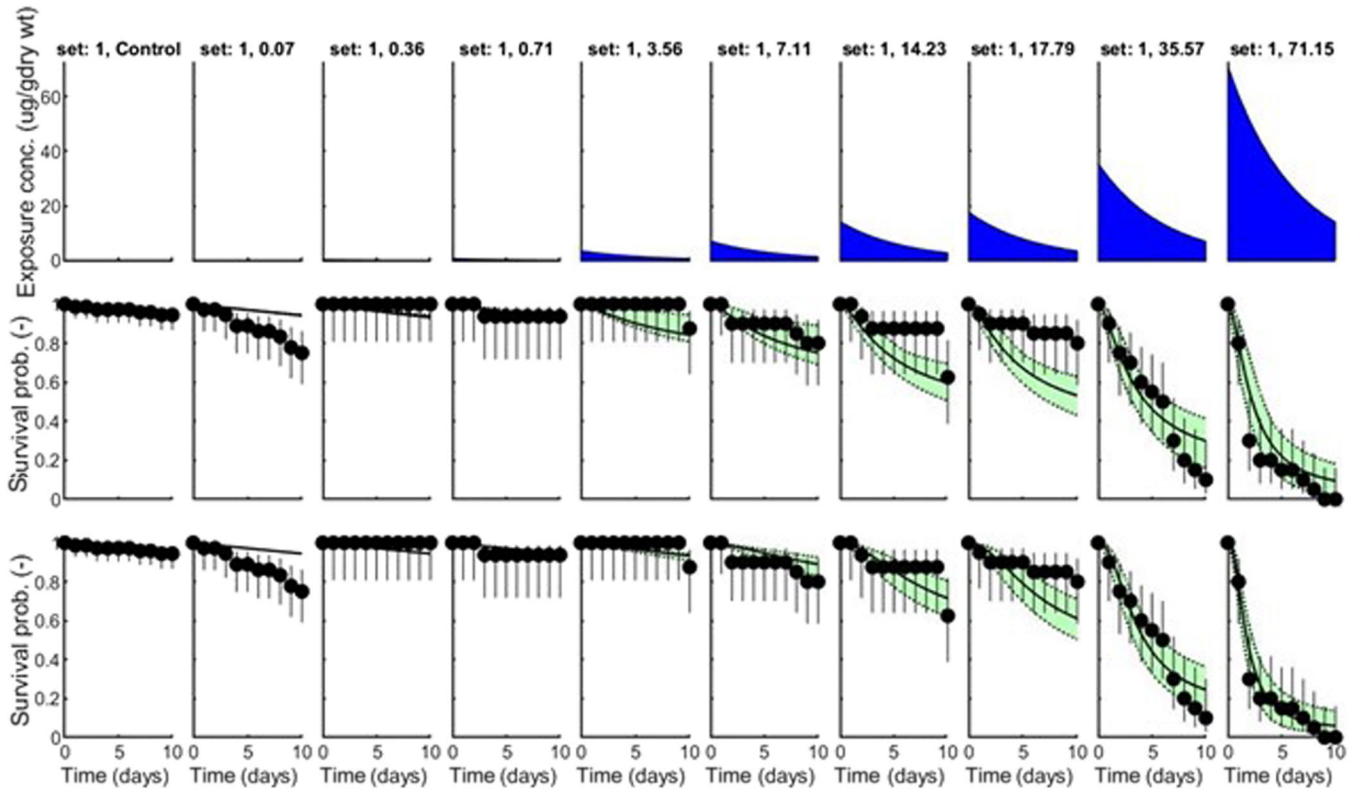

FIGURE 2: General unified threshold model for survival in its reduced form with the assumption of stochastic death (SD) or the assumption of individual tolerance (IT) predictions based on single toxicity concentrations of diclofenac over time (Day 0–10). Top row: Sediment exposure concentration over time as a first-order decay model based on measured start and end sediment concentrations. Middle row: Survival as SD. Bottom row: Survival as IT, with the actual survival (circles = mean ± 95% confidence interval [CI]) and the survival probability (lines = mean ± 95% CI; green area). prob. = probability; conc. = concentration; dw = dry weight.

concentrations, having an even steeper mortality slope compared to diclofenac (Figure 1 and Table 2). As with diclofenac, both GUTS-RED models seemed to describe the interactions between sediment-associated citalopram and *C. volutator* survival equally well; however, SD provided a slightly better fit based on log-likelihood (Figure 3 and Table 3). Though both models overestimated survival at lower concentrations, IT largely underestimated survival approaching the LC50 values and slightly overestimated survival at the two highest concentrations.

To our knowledge, there are no published studies exposing invertebrates to sediment-associated citalopram. However, citalopram has been detected in the nanograms per liter range in influent and effluent water from WWTPs and in fresh- and marine waters (Alygizakis et al., 2016; Wilkinson et al., 2022).

Our findings on sediment partitioning suggest that relying on water monitoring and ignoring the sediment likely underestimates the environmental concentration of citalopram, and thus also the potential impact in the sediment compartment. Based on monitored water citalopram concentrations and the sediment/water partitioning observed in the present study (~15/1), citalopram is unlikely to directly pose a lethal risk to the *C. volutator* community. However, as with diclofenac,

TABLE 3: The estimated parameter of GUTS-RED-SD and GUTS-RED-IT presented as best fit (±95% confidence interval) and the corresponding log-likelihood of the modeled fractions of survival in *Corophium volutator* exposed (10 days) to diclofenac and citalopram as single compounds

	SD					IT			
	LL	k_d (day^{-1})	m_w (μg g^{-1} dry wt)	b_w (μg g^{-1} dry wt day^{-1})	h_b (day^{-1})	LL	k_d (day^{-1})	m_w (μg g^{-1} dw)	β
DCF	−251	1.53 (0.305–7.761)	9.6×10^{-6} (9.6×10^{-6}–2.989)[a]	0.007 (0.005–0.013)	0.006	−248	0.002 (0.002–0.020)[a]	0.19 (0.154–2.11)	2.4 (1.78–3.22)
CP	−253	1.43 (1.041–2.307)	77.97 (74.05–83.07)	0.013 (0.009–0.020)	0.006	−262	0.18 (0.112–0.246)	54.9 (38.84–66.70)	3.4 (2.35–4.63)

[a]Lower limit of 95% parameter confidence interval has run into a boundary.
b_w = killing rate; CP = citalopram; DCF = diclofenac; GUTS-RED-IT = GUTS-RED with the assumption of individual tolerance; GUTS-RED-SD = general unified threshold model for survival in its reduced form with the assumption of stochastic death; h_b = background hazard (control mortality); k_d = dominant rate constant; LL = log-likelihood; m_w = median distribution; β = shape parameter of the sensitivity distribution.

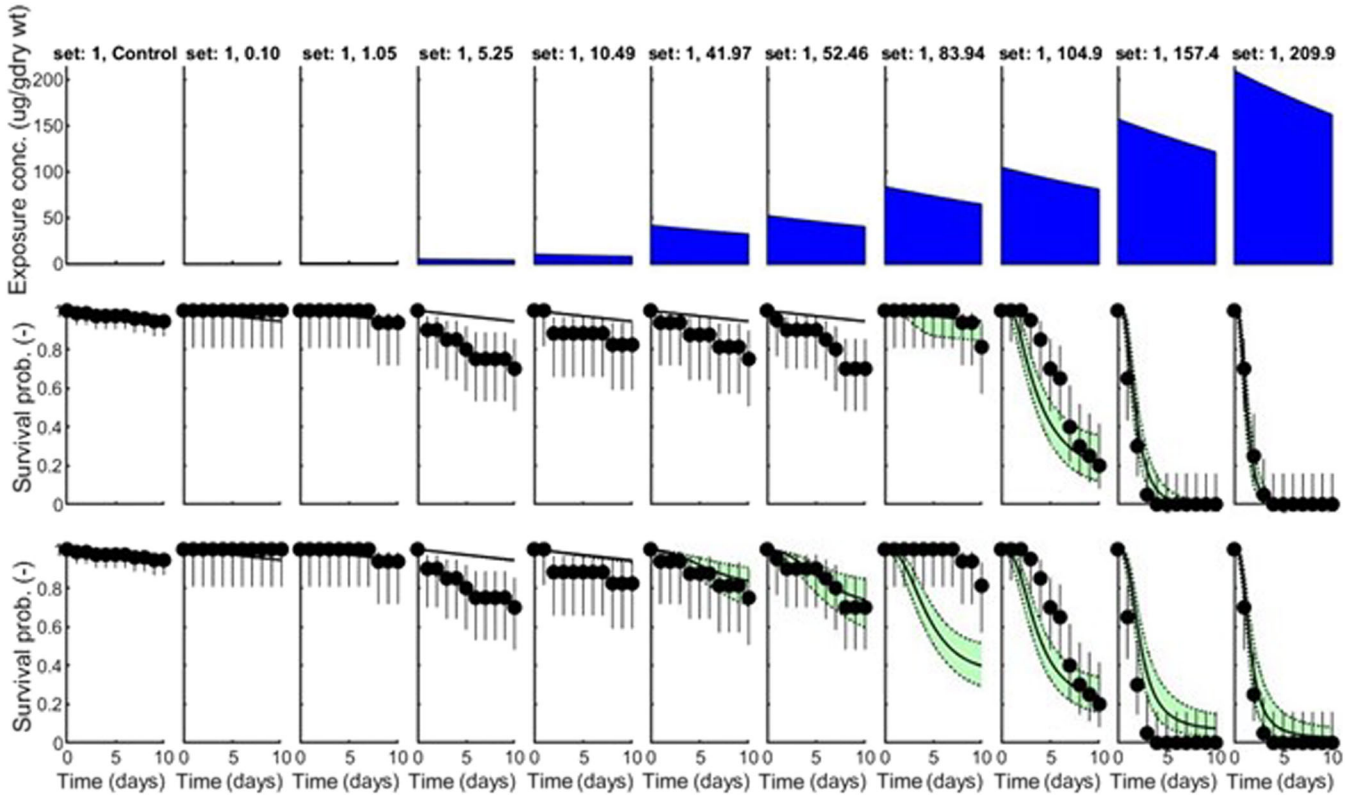

FIGURE 3: General unified threshold model for survival in its reduced form with the assumption of stochastic death (SD) or the assumption of individual tolerance (IT) predictions based on single toxicity concentrations of citalopram over time (Day 0–10). Top row: Sediment exposure concentration over time as a first-order decay model based on measured start and end sediment concentration measurements. Middle row: Survival as SD. Bottom row: Survival as IT, with the actual survival (circles = mean ± 95% confidence interval [CI]) and the survival probability (lines = mean ± 95% CI; green area). prob. = probability; conc. = concentration; dw = dry weight.

citalopram may also cause sublethal effects because of its impact on serotonin levels, as illustrated for crustaceans where serotonin is suggested to play a key role in regulating behavior, that is, dominance, aggression, and reaction to light (Huber et al., 1997; Kravitz, 2000; Perrot-Minnot et al., 2013). As an example, Bose et al. (2022) found that water-associated citalopram concentrations approaching 1 μg^{-1} L reduced the predation efficiency of dragonfly nymphs.

Mixture

The mixture study followed the model of CA quite closely, however, the results were synergistic when comparing with the IA prediction (Figure 4). Though there are studies assessing mixture effects of contaminants in sediments to invertebrates (see Schmitt et al., 2012; Verrhiest et al., 2001), there are to our knowledge no reports comparing mixture toxicity and predictions by mixture reference models of sediment-associated pharmaceuticals to invertebrates. Based on toxicity assessment of pharmaceuticals in aquatic mixtures, CA generally predicts mixture toxicity of pharmaceuticals to crustaceans more accurately than IA, which generally indicates synergy as observed in the present study (Cleuvers, 2005; Drzymała & Kalka, 2020; Henry & Black, 2007). It is a common observation that as a reference model CA leads to predictions within a twofold error for the majority of tested mixtures, irrespective of the individual component's mode of action, if the mixtures do not include potential synergists (Altenburger et al., 1996; Cedergreen, 2014; Cedergreen et al., 2008). In more complex systems, that is, assessing effects of mixtures

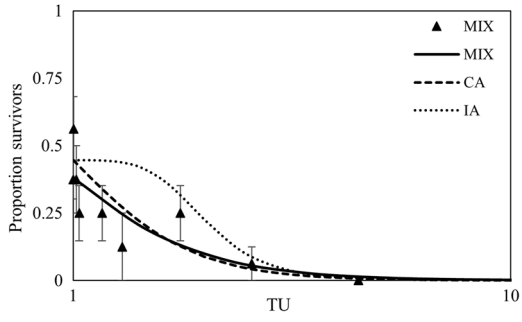

FIGURE 4: Proportion survival after 10-day exposure to sediment-associated diclofenac and citalopram as a mixture (full line) together with concentration addition (dotted line) and independent action (small-dotted line) predictions of the mixture presented as toxic units (TUs) on a logarithmic *x*-axis. The mixture and mixture prediction are assessed with the assumption that 1 TU citalopram corresponds to 1 TU diclofenac (calculated using their respective median lethal concentration). Triangles represent mean survival within each concentration (n = 4–8, 4 individuals per replicate) ± standard error. The graphs start at 1 because 1 TU was used as the baseline for the mixture experiment; no predictions were made before this point. CA = concentration addition; IA = independent action.

on a multispecies or ecosystem level, IA has been suggested as the superior model (Cedergreen, 2014).

Concentration response versus GUTS modeling

When comparing the output of log-logistic concentration response and GUTS-RED, the concentration response provides information on the toxicity at one point in time, whereas GUTS-RED includes changes in the exposure concentration over time and in mortality over time. Further, we found that neither GUTS-RED-SD nor GUTS-RED-IT fit the data better than the other, which corresponds well with the general assumption that an organism's sensitivity to contaminants is based on both individual differences as well as stochastic processes (Newman & McCloskey, 2000); and other studies comparing the fit of the two models have reached the same conclusion (Brock et al., 2021; Dalhoff et al., 2020).

In the present study, concentration response based on TWA concentrations and GUTS-RED provided similar and reasonably good fits compared to the data set. Though GUTS in general requires more input (e.g., exposure concentrations over time, mortality over time) than both the conventional concentration–response approach (e.g., requires a single input for exposure concentration, mortality) and TWA (e.g., requires concentrations over time), GUTS, contrary to both concentration–response approaches, can be used predictively to forecast toxicity over different time frames and at variable exposure scenarios (e.g., higher/lower exposure, fluctuating seasonal exposure). For regulatory purposes LC-values can be calculated based on the GUTS parameters. Hence, the relatively small additional effort of monitoring lethality and/or concentration over time makes it possible to use a much more flexible model, which can be used for multiple purposes. Consequently, toxicity predictions using GUTS-RED can, in principle, be extended beyond the experimental period; and, more importantly, predictions may include a variable exposure profile.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5894.

Acknowledgments—Henriette Selck was supported by the European Union's Horizon 2020 Marie Sklodowska-Curie Action (956009). Additional funding was received from Roskilde University, Swedish University of Agricultural Sciences, and University of Copenhagen.

Conflict of Interest—The authors declare no conflict of interest.

Author Contribution Statement—**Sara Nicoline Grønlund**: Conceptualization; Formal analysis; Investigation; Methodology; Visualization; Writing–original draft. **Casper D. Læssøe**: Conceptualization; Investigation; Methodology. **Nina Cedergreen**: Formal analysis; Methodology; Visualization; Writing–review & editing. **Henriette Selck**: Conceptualization; Formal analysis; Methodology; Writing–review & editing.

Data Availability Statement—Data and associated metadata are available in the Supporting Information and from the corresponding author (sanigr@ruc.dk) upon reasonable request.

REFERENCES

Altenburger, R., Boedekers, W., Faust, M., & Grimmes, L. H. (1996). Regulations for combined effects of poillutants: Consequences from risk assessment in aquatic toxicology. *Food and Chemical Toxicology*, *34*, 1155–1157.

Altman, R., Bosch, B., Brune, K., Patrignani, P., & Young, C. (2015). Advances in NSAID development: Evolution of diclofenac products using pharmaceutical technology. *Drugs*, *75*(8), 859–877. https://doi.org/10.1007/s40265-015-0392-z

Alygizakis, N. A., Gago-Ferrero, P., Borova, V. L., Pavlidou, A., Hatzianestis, I., & Thomaidis, N. S. (2016). Occurrence and spatial distribution of 158 pharmaceuticals, drugs of abuse and related metabolites in offshore seawater. *Science of the Total Environment*, *541*, 1097–1105. https://doi.org/10.1016/j.scitotenv.2015.09.145

Andreozzi, R., Raffaele, M., & Nicklas, P. (2003). Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. *Chemosphere*, *50*(10), 1319–1330.

Backhaus, T. (2014). Medicines, shaken and stirred: A critical review on the ecotoxicology of pharmaceutical mixtures. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1656), Article 20130585. https://doi.org/10.1098/rstb.2013.0585

Bat, L., & Raffaelli, D. (1996). The *Corophium volutator* (Pallas) sediment toxicity test: An inter-laboratory comparison. *Journal of Fisheries and Aquatic Sciences*, *13*, 433–440.

Bliss, C. I. (1939). The toxicity of poisons applied jointly. *Annals of Applied Biology*, *26*, 585–615.

Bose, A. P. H., McCallum, E. S., Avramović, M., Bertram, M. G., Blom, E.-L., Cerveny, D., Grønlund, S. N., Leander, J., Lundberg, P., Martin, J. M., Michelangeli, M., Persson, L., & Brodin, T. (2022). Pharmaceutical pollution disrupts the behavior and predator–prey interactions of two widespread aquatic insects. *iScience*, *25*(12), Article 105672. https://doi.org/10.1016/j.isci.2022.105672

Brock, T., Arena, M., Cedergreen, N., Charles, S., Duquesne, S., Ippolito, A., Klein, M., Reed, M., Teodorovic, I., van den Brink, P. J., & Focks, A. (2021). Application of general unified threshold models of survival models for regulatory aquatic pesticide risk assessment illustrated with an example for the insecticide chlorpyrifos. *Integrated Environmental Assessment and Management*, *17*(1), 243–258. https://doi.org/10.1002/ieam.4327

Cedergreen, N. (2014). Quantifying synergy: A systematic review of mixture toxicity studies within environmental toxicology. *PLOS ONE*, *9*(5), Article e96580. https://doi.org/10.1371/journal.pone.0096580

Cedergreen, N., Christensen, A. M., Kamper, A., Kudsk, P., Mathiassen, S. K., Streibig, J. C., & Sørensen, H. (2008). A review of independent action compared to concentration addition as reference models for mixtures of compounds with different molecular target sites. *Environmental Toxicology and Chemistry*, *27*(7), 1621–1632.

Cedergreen, N., Svendsen, C., & Backhaus, T. (2013). Toxicity prediction of chemical mixtures. *Encyclopedia of Environmental Management*, 2572–2581.

Christensen, A. M., Faaborg-Andersen, S., Ingerslev, F., & Baun, A. (2007). Mixtures and single-substance toxicity of selective serotonin reuptake inhibitors toward algae and crustaceans. *Environmental Toxicology and Chemistry*, *26*(1), 85–91.

Ciarelli, S. (1994). *Guideline for conducting 10-day static sediment toxicity tests using marine or estuarine amphipods*. National Institute for Coastal and Marine Management (RIKZ).

Cleuvers, M. (2005). Initial risk assessment for three β-blockers found in the aquatic environment. *Chemosphere*, *59*(2), 199–205. https://doi.org/10.1016/j.chemosphere.2004.11.090

Dalhoff, K., Hansen, A. M. B., Rasmussen, J. J., Focks, A., Strobel, B. W., & Cedergreen, N. (2020). Linking morphology, toxicokinetic, and toxicodynamic traits of aquatic invertebrates to pyrethroid sensitivity. *Environmental Science & Technology*, *54*(9), 5687–5699. https://doi.org/10.1021/acs.est.0c00189

Droge, S. T. J., Postma, J. F., & Hermens, J. L. M. (2008). Sediment toxicity of a rapidly biodegrading nonionic surfactant: Comparing the equilibrium partitioning approach with measurements in pore water. *Environmental Science & Technology*, *42*(11), 4215–4221. https://doi.org/10.1021/es702802p

Drzymała, J., & Kalka, J. (2020). Ecotoxic interactions between pharmaceuticals in mixtures: Diclofenac and sulfamethoxazole. *Chemosphere*, *259*, Article 127407. https://doi.org/10.1016/j.chemosphere.2020.127407

European Environment Agency. (2010). *Pharmaceuticals in the environment: Results of an EEA workshop*. Office for Official Publications of the European Communities.

Faust, M., Altenburger, R., Backhaus, T., Blanck, H., Boedeker, W., Gramatica, P., Hamer, V., Scholze, M., Vighi, M., & Grimme, L. H. (2003). Joint algal toxicity of 16 dissimilarly acting chemicals is predictable by the concept of independent action. *Aquatic Toxicology*, *63*, 43–63.

Ferrari Beno, M. R., Vollat, B., Fraysse, B., Paxé, N. U., Giudice, R. L., Pollio, A., & Garric, J. (2004). Environmenatl risk assessment of six human pharmaceuticals: Are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment? *Environmental Toxicology and Chemistry*, *23*(5), 1344–1354.

Gan, T. J. (2010). Diclofenac: An update on its mechanism of action and safety profile. *Current Medical Research and Opinion*, *26*(7), 1715–1731. https://doi.org/10.1185/03007995.2010.486301

Gerdol, V., & Hughes, R. G. (1994). Effect of *Corophium volutator* on the abundance of benthic diatoms, bacteria and sediment stability in two estuaries in southeastern England. *Marine Ecology Progress Series*, *114*, 109–115.

Grønlund, S. N., Chan, W. S., D'Amico, E., Flodgaard, M., Lyngsie, G., McCallum, E. S., Palmqvist, A., Sandgaard, M. H., Santobuono, M., Thit, A., & Selck, H. (2024). When and how to conduct ecotoxicological tests using natural field-collected sediment. *Environmental Toxicology and Chemistry*, *43*, 1757–1766. Advance online publication. https://doi.org/10.1002/etc.5792

Han, G. H., Hur, H. G., & Kim, S. D. (2006). Ecotoxicological risk of pharmaceuticals from wastewater treatment plants in Korea: Occurrence and toxicity to *Daphnia magna*. *Environmental Toxicology and Chemistry*, *25*(1), 265–271.

Henry, T. B., & Black, M. C. (2007). Mixtures and single-substance acute toxicity of selective serotonin reuptake inhibitors in *Ceriodaphnia dubia*. *Environmental Toxicology and Chemistry*, *26*(8), 1751–1755.

Huber, R., Smith, K., Delago, A., Isaksson, K., & Kravitz, E. A. (1997). Serotonin and aggressive motivation in crustaceans: Altering the decision to retreat. *Proceedings of the National Academy of Sciences of the United States of America*, *94*, 5939–5942.

Inostroza, P. A., Elgueta, S., Krauss, M., Brack, W., & Backhaus, T. (2023). A multi-scenario risk assessment strategy applied to mixtures of chemicals of emerging concern in the River Aconcagua basin in central Chile. *Science of the Total Environment921*, Article 171054. https://doi.org/10.1101/2023.08.23.554257

Jager, T. (2021). OpenGUTS. Supporting mechanistic modelling for survival. https://openguts.info/

Jager, T., Albert, C., Preuss, T. G., & Ashauer, R. (2011). General unified threshold model of survival—A toxicokinetic–toxicodynamic framework for ecotoxicology. *Environmental Science & Technology*, *45*(7), 2529–2540. https://doi.org/10.1021/es103092a

Jager, T., & Ashauer, R. (2018). *Modelling survival under chemical stress: A comprehensive guide to the GUTS framework*. Leanpub.

Jelic, A., Gros, M., Ginebreda, A., Cespedes-Sánchez, R., Ventura, F., Petrovic, M., & Barcelo, D. (2011). Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. *Water Research*, *45*(3), 1165–1176. https://doi.org/10.1016/j.watres.2010.11.010

Kasprzyk-Hordern, B. (2010). Pharmacologically active compounds in the environment and their chirality. *Chemical Society Reviews*, *39*(11), 4466–4503. https://doi.org/10.1039/c000408c

Kasprzyk-Hordern, B., Dinsdale, R. M., & Guwy, A. J. (2009). The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. *Water Research*, *43*(2), 363–380. https://doi.org/10.1016/j.watres.2008.10.047

Koba, O., Grabicova, K., Cerveny, D., Turek, J., Kolarova, J., Randak, T., Zlabek, V., & Grabic, R. (2018). Transport of pharmaceuticals and their metabolites between water and sediments as a further potential exposure for aquatic organisms. *Journal of Hazardous Materials*, *342*, 401–407. https://doi.org/10.1016/j.jhazmat.2017.08.039

Kostich, M. S., Batt, A. L., & Lazorchak, J. M. (2014). Concentrations of prioritized pharmaceuticals in effluents from 50 large wastewater treatment plants in the US and implications for risk estimation. *Environmental Pollution*, *184*, 354–359. https://doi.org/10.1016/j.envpol.2013.09.013

Kravitz, E. A. (2000). Serotonin and aggression: Insights gained from a lobster model system and speculations on the role of amine neurons in a complex behavior. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology*, *186*(3), 221–238. https://doi.org/10.1007/s003590050423

Loewe, S., & Muischnek, H. (1926). Effect of combinations: Mathematical basis of problem. *Archives of Experimental Pathalogy and Pharmacology*, *114*, 313–326.

Marasine, N. R., Sankhi, S., Lamichhane, R., Marasini, N. R., & Dangi, N. B. (2021). Use of antidepressants among patients diagnosed with depression: A scoping review. *BioMed Research International2021*, Article 6699028. https://doi.org/10.1155/2021/6699028

McLusky, D. S. (1968). Some effects of salinity on the distribution and abundance of *Corophium volutator* in the Ythan estuary. *Journal of Marine Biological Association of the United Kingdom*, *48*, 443–454.

Minguez, L., Farcy, E., Ballandonne, C., Lepailleur, A., Serpentini, A., Lebel, J. M., Bureau, R., & Halm-Lemeille, M. P. (2014). Acute toxicity of 8 antidepressants: What are their modes of action? *Chemosphere*, *108*, 314–319. https://doi.org/10.1016/j.chemosphere.2014.01.057

Møller, L. F., & Riisgård, H. U. (2006). Filter feeding in the burrowing amphipod *Corophium volutator*. *Marine Ecology Progress Series*, *322*, 213–224. https://doi.org/10.3354/meps322213

Newman, M. C., & McCloskey, J. T. (2000). The individual tolerance concept is not the sole explanation for the probit dose-effect model. *Environmental Toxicology and Chemistry*, *19*(2), 520–526.

Nielsen, M. V., & Kofoed, L. H. (1982). Selective feeding and epipsammic browsing by the deposit-feeding amphipod *Corophium volutator*. *Marine Ecology Progress Series*, *10*, 81–88. https://doi.org/10.3354/MEPS010081

Nieto, E., Corada-Fernández, C., Hampel, M., Lara-Martín, P. A., Sánchez-Argüello, P., & Blasco, J. (2017). Effects of exposure to pharmaceuticals (diclofenac and carbamazepine) spiked sediments in the midge, *Chironomus riparius* (Diptera, Chironomidae). *Science of the Total Environment*, *609*, 715–723. https://doi.org/10.1016/j.scitotenv.2017.07.171

Organisation for Economic Co-operation and Development. (2017). *Health at a glance 2017: OECD indicators*. https://www.oecd-ilibrary.org/social-issues-migration-health/health-at-a-glance-2017_health_glance-2017-en

Oviedo-Gómez, D. G. C., Galar-Martínez, M., García-Medina, S., Razo-Estrada, C., & Gómez-Oliván, L. M. (2010). Diclofenac-enriched artificial sediment induces oxidative stress in *Hyalella azteca*. *Environmental Toxicology and Pharmacology*, *29*(1), 39–43. https://doi.org/10.1016/j.etap.2009.09.004

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman, C. U., & Mohan, D. (2019). Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods. *Chemical Reviews*, *119*(6), 3510–3673. https://doi.org/10.1021/acs.chemrev.8b00299

Peer, D. L., Linkletter, L. E., & Hicklin, P. W. (1986). Life history and reproductive biology of *Corophium volutator* (Crustacea: Amphipoda) and the influence of shorebird predation on population structure in Chignecto Bay, Bay of Fundy, Canada. *Netherlands Journal of Sea Research*, *20*(4), 359–373.

Perrot-Minnot, M. J., Dion, E., & Cézilly, F. (2013). Modulatory effects of the serotonergic and histaminergic systems on reaction to light in the crustacean *Gammarus pulex*. *Neuropharmacology*, *75*, 31–37. https://doi.org/10.1016/j.neuropharm.2013.06.028

R Core Team. (2023). *R: A language and environment for statistical computing*. R Foundation for Statistical Compution. https://www.R-projects.org/

Ré, A., Freitas, R., Sampaio, L., Rodrigues, A. M., & Quintino, V. (2009). Estuarine sediment acute toxicity testing with the European amphipod *Corophium multisetosum* Stock, 1952. *Chemosphere*, *76*(10), 1323–1333. https://doi.org/10.1016/j.chemosphere.2009.06.041

Ritz, C., & Streibig, J. (2005). Bioassay analysis using R. *Journal of Statistical Software12*, (5), 1–22.

Scheytt, T., Mersmann, P., Lindstädt, R., & Heberer, T. (2005). 1-Octanol/water partition coefficients of 5 pharmaceuticals from human medical care: Carbamazepine, clofibric acid, diclofenac, ibuprofen, and propyphenazone. *Water, Air, and Soil Pollution*, *165*, 3–11.

Schmitt, S., Reifferscheid, G., Claus, E., Schlüsener, M., & Buchinger, S. (2012). Effect directed analysis and mixture effects of estrogenic compounds in a sediment of the river Elbe. *Environmental Science and Pollution Research*, *19*(8), 3350–3361. https://doi.org/10.1007/s11356-012-0852-x

Shrivastava, A., & Gupta, V. (2011). Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of Young Scientists*, *2*(1), 21–25. https://doi.org/10.4103/2229-5186.79345

Siebeneicher, S., Wahrendorf, D. S., Wetzel, M. A., & Jungmann, D. (2013). Analysis of burrowing ability as a sublethal endpoint in a marine sediment bioassay with *Corophium volutator* (Pallas). *Journal of Soils and Sediments*, *13*(1), 197–206. https://doi.org/10.1007/s11368-012-0619-5

Stahl, S. M. (1998). Mechanism of action of serotonin selective reuptake inhibitors serotonin receptors and pathways mediate therapeutic effects and side effects. *Journal of Affective Disorders*, *51*, 215–235.

Stülten, D., Zühlke, S., Lamshöft, M., & Spiteller, M. (2008). Occurrence of diclofenac and selected metabolites in sewage effluents. *Science of the Total Environment*, *405*(1–3), 310–316. https://doi.org/10.1016/j.scitotenv.2008.05.036

Thiebault, T., Boussafir, M., & Le Milbeau, C. (2017). Occurrence and removal efficiency of pharmaceuticals in an urban wastewater treatment plant: Mass balance, fate and consumption assessment. *Journal of Environmental Chemical Engineering*, *5*(3), 2894–2902. https://doi.org/10.1016/j.jece.2017.05.039

Varvas, K., Kurg, R., Hansen, K., Järving, R., Järving, I., Valmsen, K., Lõhelaid, H., & Samel, N. (2009). Direct evidence of the cyclooxygenase pathway of prostaglandin synthesis in arthropods: Genetic and biochemical characterization of two crustacean cyclooxygenases. *Insect Biochemistry and Molecular Biology*, *39*(12), 851–860. https://doi.org/10.1016/j.ibmb.2009.10.002

Verrhiest, G., Ment, B. C., & Blake, G. (2001). Single and combined effects of sediment-associated PAHs on three species of freshwater macroinvertebrates. *Ecotoxicology*, *10*, 363–372.

Weber, F.-A., Aus Der Beek, T., Carius, A., Grüttner, G., Hickmann, S., Ebert, I., Hein, A., Küster, A., Rose, J., & Koch-Jugl, J. (2014). *Pharmaceuticals in the environment—The global perspective: Occurrence, effects, and potential cooperative action under SAICM*. German Environment Agency. https://www.umweltbundesamt.de/sites/default/files/medien/378/publikationen/pharmaceuticals_in_the_environment_0.pdf

Wilkinson, J., Boxall, A., Kolpin, D., Leung, K., Lai, R., Galbán-Malagón, C., Adell, A. D., Mondon, J., Metian, M., Marchant, R. A., Bouzas-Monroy, A., Cuni-Sanchez, A., Coors, A., Carriquiriborde, P., Rojo, M., Gordon, C., Cara, M., Moermond, M., Luarte, T., … , Teta, C. (2022). Pharmaceutical pollution of the world's rivers. *Proceedings of the National Academy of Sciences*, 119(8), https://doi.org/10.1073/pnas.2113947119

Wilson, Jr., W. H., & Parker, K. (1996). The life history of the amphipod, *Corophium volutator*: The effects of temperature and shorebird predation. *Journal of Experimental Marine Biology and Ecology*, *196*, 239–250.

Yang, H., Lu, G., Yan, Z., Liu, J., Ma, B., & Dong, H. (2017). Biological effects of citalopram in a suspended sediment–water system on *Daphnia magna*. *Environmental Science and Pollution Research*, *24*(26), 21180–21190. https://doi.org/10.1007/s11356-017-9763-1

Zhang, Y., Geißen, S. U., & Gal, C. (2008). Carbamazepine and diclofenac: Removal in wastewater treatment plants and occurrence in water bodies. *Chemosphere*, *73*(8), 1151–1161. https://doi.org/10.1016/j.chemosphere.2008.07.086