Environmental Toxicology and Pharmacology 122 (2026) 104935

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

# Limited bioconcentration of water-associated pharmaceutical active compounds through short-term exposure in signal crayfish (*Pacifastacus leniusculus*)

Anna Koubová [a,*], Worrayanee Thammatorn [a,b], Antonín Kouba [a], Vladimír Žlábek [a], Bent Speksnijder [c], Daniel Cerveny [d], Oksana Golovko [c]

[a] University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, Vodňany 389 01, Czech Republic
[b] Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand
[c] Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences (SLU), Uppsala 750 07, Sweden
[d] Department of Wildlife, Fish, and Environmental Studies, Swedish University of Agricultural Sciences, Umeå 901 83, Sweden

ARTICLE INFO

*Keywords:*
Pharmaceuticals
Exposure
Tissue distribution
Mixtures
Aquatic invertebrates

ABSTRACT

Pharmaceutical active compounds (PhACs) increasingly appear as complex mixtures in aquatic ecosystems, yet their bioconcentration in non-target organisms is poorly understood. This study examined tissue-specific distribution of five PhACs – bicalutamide, amitriptyline, furosemide, daidzein and sertraline – in signal crayfish (*Pacifastacus leniusculus*) after 96-hour exposure and an equal depuration period. Crayfish were subjected to environmentally relevant and 10-fold elevated mixture concentrations. Water and tissues (haemolymph, hepatopancreas, muscle) were analysed using ultra-high performance liquid chromatography–tandem mass spectrometry. Despite verified water concentrations and stable conditions, none of the compounds were quantifiable in tissues, except trace sertraline near the detection limit in some controls. The lack of detectable residues indicates minimal bioconcentration, likely due to physicochemical properties (low log $K_{ow}$), rapid metabolism and efficient excretion. Results underscore the need for longer exposures and metabolite-focused studies to better assess environmental fate, tissue kinetics and potential risks of PhAC mixtures in freshwater invertebrates.

## 1. Introduction

Since the European Commission's (EC) communication on 'The combination effects of chemicals – Chemical mixtures' (European Commission, 2012), concerns about mixture risk assessment (MRA) have grown substantially. However, the risk assessment and management of combined exposures to multiple chemicals is still challenging (Luijten et al., 2023). Although key aspects of MRA, such as occurrence and hazard screening of real-life mixtures in natural waters, have been explored (Malnes et al., 2022, 2023), insight into the dynamics, environmental fate, and bioconcentration of actual mixtures in exposed aquatic organisms remains limited. Although studies often focus on measurable bioaccumulation, documenting non-detectable or minimal accumulation is equally important for advancing environmental risk assessment. Such evidence provides valuable reference data for refining toxicokinetic models, evaluating the applicability of existing OECD bioaccumulation guidelines to invertebrates, and improving our understanding of how physicochemical properties constrain uptake under environmentally relevant exposure conditions. By presenting these findings, this study contributes critical insight into the limitations and boundary conditions of pharmaceutical mixture accumulation in aquatic organisms.

Contaminants of emerging concern, including pharmaceutical active compounds (PhACs), have been increasingly detected at low concentrations in aquatic environments (Osuoha et al., 2023). Although their composition can vary spatially and seasonally (Golovko et al., 2014, 2021; Malnes et al., 2022; Rehrl et al., 2020), their presence, even far from the original emission sources, raises concerns about their persistence, high mobility, and potential ecological and human health impacts. Many PhACs, especially compounds with n-octanol-water partition coefficient (log $K_{ow}$) values greater than 3, tend to accumulate and biomagnify in organisms along the aquatic food chain, potentially

* Corresponding author.
*E-mail address:* akoubova@frov.jcu.cz (A. Koubová).

https://doi.org/10.1016/j.etap.2026.104935
Received 31 August 2025; Received in revised form 29 December 2025; Accepted 8 January 2026
Available online 9 January 2026

leading to significant ecological consequences (OECD, 2005; Osuoha et al., 2023). For example, sertraline and amitriptyline, with log $K_{ow}$ values of 5.29 and 4.95, respectively (USEPA, 2007), could individually accumulate in aquatic biota after a 7-day exposure period (Rodrigues et al., 2015; Ziarrusta et al., 2017). However, even moderately water-soluble compounds, such as bicalutamide and daidzein, with log $K_{ow}$ values below 3 (USEPA, 2007), also showed some degree of bioaccumulation. Bicalutamide has been identified as potentially bioaccumulative in aquatic organisms (Li et al., 2021), and long-term exposure to daidzein in concentrations far above environmental relevance could lead to daidzein residue in gibel carp (*Carassius gibelio*) muscle (Li et al., 2016). Despite several reports of individual PhACs demonstrating persistence and bioaccumulation in aquatic organisms, even with the low to medium log $K_{ow}$ values, uncertainty remains regarding risk assessment and regulatory decision-making for multi-substance exposures (European Commission, 2012).

Therefore, in the present study, we aimed to address how actual mixtures of environmentally relevant chemicals accumulate and distribute among different tissues of freshwater crustaceans over short-term exposure. We selected signal crayfish (*Pacifastacus leniusculus*) due to its ecological significance, extensive accessibility, and sufficient body size (Kouba et al., 2010), and conducted a 4-day bioconcentration and 4-day depuration experiment in line with OECD Test Guideline 305 (OECD, 2019) under conditions assuming steady-state concentrations of the tested pharmaceuticals in the organism. Tissue distribution of PhAC mixtures was assessed at two experimental concentrations: i) an environmentally relevant concentration (54 ng/L amitriptyline, 190 ng/L bicalutamide, 16 ng/L daidzein, 160 ng/L furosemide, and 20 ng/L sertraline) and ii) a concentration 10-fold higher than levels detected in surface waters (540 ng/L amitriptyline, 1900 ng/L bicalutamide, 160 ng/L daidzein, 1600 ng/L furosemide, and 200 ng/L sertraline), to mimic typical and potentially increased contamination scenarios, respectively. The selection of PhACs was based primarily on risk quotient and detection frequency, using exposure levels reported in previous studies (Malnes et al., 2022, 2023). In Swedish rivers, these compounds (amitriptyline, bicalutamide, daidzein, furosemide, and sertraline) were detected with frequencies ranging from 26 % to 98 %, indicating consistent environmental presence despite regional or seasonal variability. Importantly, these pharmaceuticals span a spectrum of physicochemical properties (log $K_{ow}$ 2.3–5.29) and pharmacological classes (antidepressants, diuretics, antiandrogens, and phytoestrogens), which provides an opportunity to evaluate compound-specific bioaccumulation behaviour across a lipophilicity gradient. Our objective was to assess short-term tissue distribution patterns for pharmaceuticals with varying bioaccumulation potential, rather than to reproduce the global occurrence profile of all detected PhACs. We hypothesised that pharmaceutical mixtures would exhibit compound-specific tissue distribution patterns in signal crayfish and that even short-term exposures could lead to detectable bioaccumulation, particularly at elevated concentrations.

## 2. Materials and methods

### 2.1. Chemicals

All test PhACs, bicalutamide (CAS: 90357–06–5), sertraline hydrochloride (CAS: 79559–97–0), amitriptyline hydrochloride (CAS: 549–18–8), daidzein (CAS: 486–66–8), and furosemide (CAS: 54–31–9) were supplied by Tokyo Chemical Industry (Tokyo, Japan). Test water-based mixtures were prepared by separately dissolving powdered sertraline hydrochloride and amitriptyline hydrochloride in deionised water to obtain stock solutions, and the test mixtures of studied compounds were prepared by mixing and diluting the stock solutions with deionised water. Similarly, test ethanol-based mixtures were prepared using the same procedure as the water-based mixtures. Due to their limited water solubility, absolute ethanol (Penta, Czech Republic) was used as a solvent for bicalutamide, daidzein, and furosemide. The physicochemical properties and environmental risk assessment data of the compounds studied are presented in Table 1 and Table 2, respectively. Detailed procedures for chemical solution preparation are provided in the supplementary material (SM1–Text 1; SM1–Table S1-S3). Ultrapure water was generated by a Milli-Q Advantage Ultrapure water purification system and filtered through a 0.22 μm Millipak Express membrane and LC-Pak polishing unit (Merk Millipore, Billerica, MA). Methanol, acetonitrile, ammonium acetate, formic acid, ammonia, and ethyl acetate of high analytical grade were obtained from Sigma-Aldrich (USA).

### 2.2. Test organisms and experimental design

During the 9-day acclimation period, 200 signal crayfish were collected from Křesanovský brook (49.0604 N, 13.7583 E) and maintained at the University of South Bohemia České Budějovice, Vodňany, Czech Republic, as described by Van Nguyen et al. (2022). To suppress the invasive signal crayfish population at the source site, females were preferentially collected while males were largely left undisturbed, resulting in a male-to-female ratio of 1:7 for this experiment. Approximately 67 crayfish were randomly allocated into each of three aquaria (300 L, 64 × 97 × 53 cm) containing 50 L of dechlorinated, aerated tap water. Crayfish were fed with commercial fish pellets and shredded carrots until satiation once a day. After feeding, any residual feed was removed after 2 h, and aquarium water was renewed. Feeding was suspended for one day prior to the start of experimental period.

Following acclimation, 180 crayfish (carapace length, 25.7 ± 3.2 mm, mean ± SD; body weight 5.4 ± 1.8 g, mean ± SD; male-to-female ratio 1:7) were selected. For the treatment groups, 144 crayfish were divided into two groups with the same sex ratio of 72 crayfish each: one exposed to an environmentally relevant PhAC mixture concentration and the other to a concentration 10 times higher, as shown in Fig. 1. Each treatment group was further divided into three replicates of 24 crayfish, and each replicate was kept in an aquarium with 30 L of water. For the control groups, 36 crayfish were divided into two groups of 18 crayfish each (water and solvent control groups). The water control group consisted of 18 crayfish placed in the PhAC-free water without solvent. The solvent control group contained 18 crayfish in PhAC-free water with 0.01 % absolute ethanol (corresponding to the maximum ethanol volume used in the treatment groups to prepare spiking solutions; supplementary material SM1–Text 1). The experimental period consisted of a 96-hour exposure phase to PhACs followed by a 96-hour depuration phase in a static renewal system. Water (temperature: 17.5 ± 0.5°C, mean ± SD; pH: 7.7 ± 0.2, mean ± SD; dissolved oxygen: 8.0 ± 0.6 mg/L, mean ± SD) was completely exchanged daily to maintain stable PhAC levels during exposure and to avoid excess ammonia, nitrite, and nitrate accumulation in the aquaria. Two crayfish were sacrificed from each of the three independent aquaria for each treatment (6 crayfish per treatment) at the following time points: 1, 6, 12, 24, 48, 96, 97, 102, 108, 120, 144, and 192 h. Additionally, 6 crayfish from each control aquarium (6 crayfish per treatment) were sacrificed at time points of 0, 96, and 144 h to determine background-tested PhACs levels and evaluate any solvent effects. The crayfish were rinsed with dechlorinated tap water and anaesthetised by brief whole-body immersion in ice-cold water. Haemolymph, hepatopancreas, and muscle samples were collected and stored at –80°C as described by Thammatorn et al. (2024). In addition, experimental water (150 mL) was sampled from each aquarium at every sampling point and kept at –20°C until chemical analysis. Water samples collected at 144 and 192 h were not analysed further, as PhAC levels had already been confirmed to remain consistently low during the early depuration phase. Detailed information illustrating the experimental design and sampling can be found in the supplementary material SM2–Table 1. The preparation of water and biological samples for chemical analysis is described in supplementary material SM1–Text 2.

**Table 1**
Classification and physical-chemical properties of studied compounds.

| Compound | Category | Type | CAS number | Molecular formula | Monoisotopic mass (Da) | pKa | logKow[c] | LogD (pH 5.5)[d] | LogD (pH 7.4)[d] |
|---|---|---|---|---|---|---|---|---|---|
| Amitriptyline | Pharmaceutical | Antidepressant | 50–48–6 | $C_{20}H_{23}N$ | 277.1 | 9.4[a] | 4.95 | 1.73 | 2.96 |
| Bicalutamide | Pharmaceutical | Antineoplastic agent | 90357–06–5 | $C_{18}H_{14}F4N_2O_4S$ | 430.373 | pKa = 12.6 (est)[a] | 2.3 | | 2.71 |
| Daidzein | Isoflavone | | 486–66–8 | $C_{15}H_{10}O_4$ | 254.057909 | pKa1 = 8.96 (acid); pKa2 = -3 (base) (est) | 2.55 | | |
| Furosemide | Pharmaceutical | Diuretics | 54–31–9 | $C_{12}H_{11}ClN_2O_5S$ | 330 | 4.3[b] | 2.32 | -0.06 | -0.78 |
| Sertraline | Pharmaceutical | Antidepressant | 79617–96–2 | $C_{17}H_{17}Cl_2N$ | 305 | 9.5[a] | 5.29 | 2.13 | 3.14 |

[a] experimental pKa
[b] theoretical pKa
[c] KOWWIN
[d] ACD/Labs Percepta Platform – PhysChem Module
Modified from Malnes et al. (2023).

**Table 2**
Substances with at least one instance of high environmental risk in rivers. DF: detection frequency; RQ: risk quotient; RQ < 0.01: unlikely to pose an environmental risk; 0.01 < RQ < 1: low to moderate environmental risk; RQ > 1: high environmental risk. F: frequency of RQ > 1 exceedances.

| Substance | DF | RQ< 0.01 | 0.01 <RQ< 1 | RQ> 1 | Mean RQ | F | Concentrations in Swedish rivers, ng/L | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Mean | Median | Max |
| Amitriptyline | 49 % | NA | 19 | 4 | 0.053 | 9 % | 7.7 | 3.9 | 54 |
| Bicalutamide | 98 % | 14 | 30 | 2 | 0.0023 | 4 % | 19 | 5.6 | 190 |
| Daidzein[a] | 32 % | NA | 12 | 3 | 0.11 | 6 % | 5 | 4.4 | 16 |
| Furosemide | 40 % | NA | 4 | 15 | 0.07 | 32 % | 47 | 28 | 160 |
| Sertraline | 26 % | NA | 11 | 1 | 0.054 | 2 % | 4.8 | 4 | 20 |

[a]Daidzein has a partially natural origin; NA – not available

### 2.3. Determination of chemicals in water and biological extracts

Water samples and biological extracts (haemolymph, hepatopancreas, and muscle samples) were analysed using a DI-ONEX UltiMate 3000 ultra-high performance liquid chromatography (UPLC) system (Thermo Scientific, Waltham, MA, USA) coupled to a triple quadrupole (TSQ) mass spectrometer (TSQ QUANTIVA, Thermo Scientific, Waltham, MA, USA). For analysis of water samples, the system was configured for online solid-phase extraction (SPE) with automated extraction. An Acquity UPLC BEH-C18 column (2.1 ×50 mm, 1.7 μm particle size; Waters Corporation, Manchester, UK) was used as an analytical column for chromatographic separation. The temperature of the column oven was set at 40 ± 2°C. The system was equipped with a heated electrospray ion source with static spray voltage set at 3500 V in positive mode and 2500 V in negative mode. The temperatures of the ion transfer tube and the vaporiser were set at 325°C and 400°C, respectively. Xcalibur software (Thermo Fisher Scientific, San Jose, CA, USA) was used for the data acquisition, and the data were evaluated using TraceFinderTM 4.1. software (Thermo Fisher). For graphical representation of compound concentration in exposure water, values below or equal to the limit of quantification (LOQ) were divided by the square root of 2 according to Koubová et al. (2025) and Šauer et al. (2023).

### 2.4. Quality assurance and quality control

The performance of the method was assessed regarding its linearity in calibration, measurement of blank samples, LOQ, absolute recovery, precision, and matrix effects. The concentrations of target compounds measured in blanks were subtracted from the values measured in real samples. Detailed information can be found in the supplementary material (SM1–Table 4). Briefly, LOQs for the studied compounds in water ranged from 0.48 ng/L for amitriptyline to 17 ng/L for furosemide. In biological samples, the lowest LOQs were observed in haemolymph for most compounds (0.12 ng/mL for amitriptyline to 0.72 ng/mL for furosemide), followed by muscle (0.7 ng/g for bicalutamide to 2.1 ng/g for furosemide) and hepatopancreas (0.79 ng/g for bicalutamide to 23 ng/g for sertraline). Overall, amitriptyline exhibited the lowest LOQ values in water and haemolymph matrices, while the lowest LOQ values in muscle and hepatopancreas were observed in bicalutamide.

## 3. Results

### 3.1. Water concentrations

The measured concentrations of the studied PhACs confirmed that the exposure design successfully established three distinct treatment levels: control (non-detectable or trace), environmentally relevant (EC), and 10 × environmentally relevant (10 ×EC) concentrations. Overall, measured concentrations were within the same order of magnitude as the nominal values, indicating stable exposure conditions throughout the experiment (Fig. 1). Basic water parameters during the exposure test are provided in the supplementary material (SM1–Table 5; SM2–Table 2), while nominal and measured concentrations of the tested compounds in water and crayfish tissues are summarised in SM2–Table 3 and SM2–Table 4 for direct comparison.

Minor deviations were observed for some compounds: bicalutamide and furosemide were consistently measured below nominal levels, while sertraline was below the limit of quantification (LOQ) in the EC group and approximately one order of magnitude lower than nominal in the 10 ×EC group. Such deviations are commonly observed in complex mixture exposure experiments and are likely attributable to compound-specific solubility and adsorption properties.

Only amitriptyline was detected in control samples at very low levels (1 ng/L in the exposure control, 1.6 ng/L in the solvent control start, and 1.1 ng/L in the solvent control in the exposure phase), which likely reflects trace background contamination or analytical variability near LOQs. Despite these minor variations, the exposure gradient across treatments remained clear and consistent, confirming that crayfish in the control, EC, and 10 ×EC groups experienced the intended low-to-high concentration range of the pharmaceutical mixtures.

It should be noted that the exposure water was renewed daily to maintain stable conditions; however, the slight decreases observed in

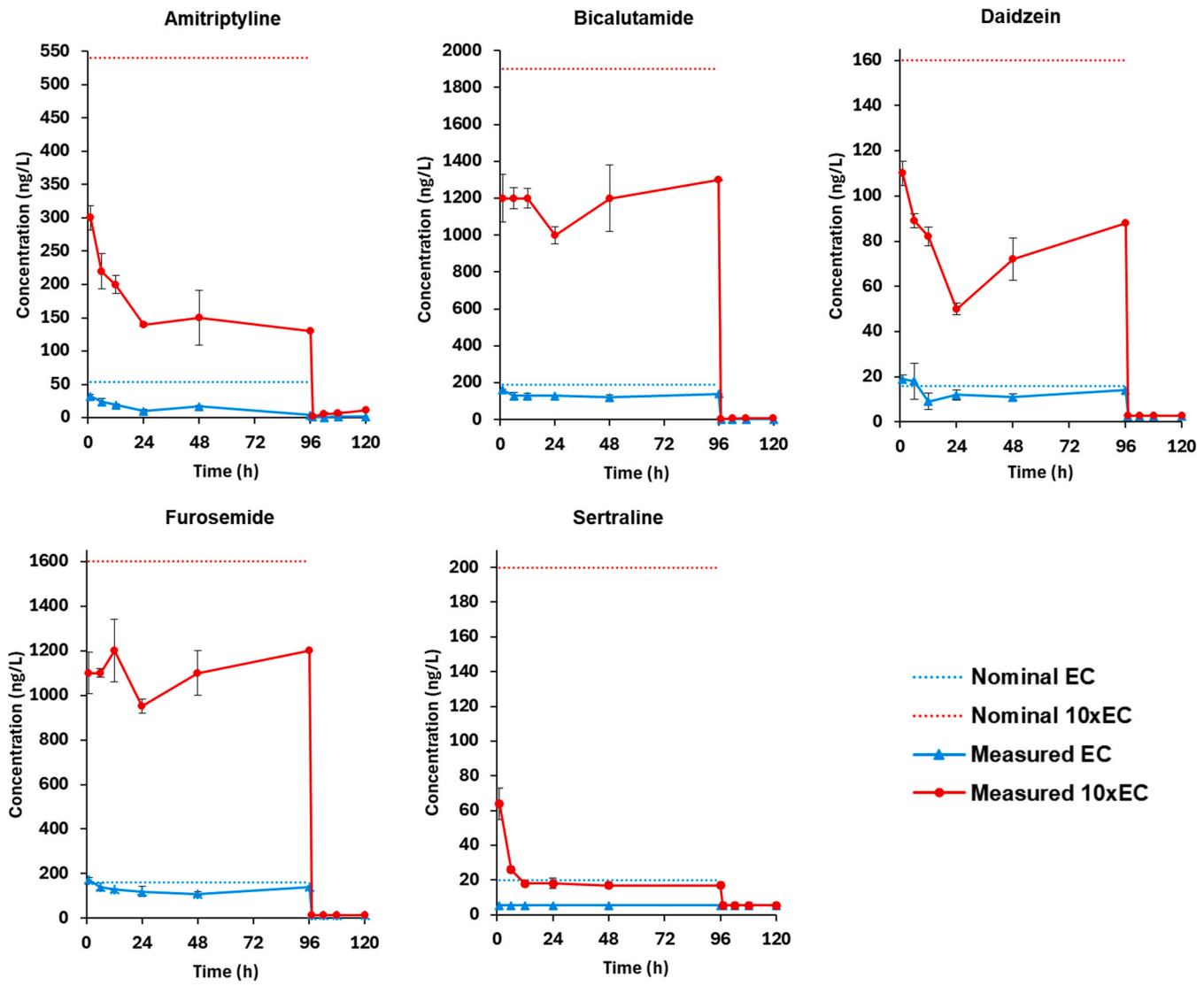


**Fig. 1.** Nominal and average measured concentrations (ng/L) of the studied compounds in exposure water. EC denotes the environmentally relevant concentration, and 10 ×EC represents 10 times the environmentally relevant concentration. Values at the levels below the limits of quantification were divided by the square root of 2. Error bars indicate the standard deviation (SD) of triplicate measurements (n = 3).

the measured concentrations of amitriptyline, daidzein, and furosemide over time likely reflect compound-specific properties, such as partial adsorption to aquarium surfaces or organic matter, as well as minor photolytic or microbial degradation between renewals.

### 3.2. *Concentrations in biological samples*

No mortality of crayfish was observed. None of the target compounds was detected in hepatopancreas or muscle samples. Similarly, no studied compounds were found in haemolymph, except for sertraline, which was detected in two control samples – solvent control (0.20 ng/mL) and exposure control (0.19 ng/mL) – values close to the limit of quantification (0.18 ng/mL). These findings likely reflect minor background contamination or analytical variability near the detection threshold. However, residual environmental exposure at the collection site cannot be entirely excluded, as trace levels of pharmaceuticals have been previously reported in similar freshwater systems within the region (Gregarová et al., 2024).

## 4. Discussion

With the increasing use of pharmaceuticals and personal care products, alongside improvements in detection sensitivity, efforts to protect and conserve aquatic biodiversity have intensified. Aquatic invertebrates, an integral component of freshwater ecosystems, are frequently exposed to pharmaceutical residues as non-target organisms. Although recent ecotoxicological research has largely focused on traditional model species, such as amphipods and *Daphnia* (De Lange et al., 2006; Nkoom et al., 2019), decapod crustaceans represent valuable model organisms for investigating the ecotoxicological effects of PhACs (Hossain et al., 2018; Vogt, 2008). Freshwater crayfish are large, long-lived invertebrates that often occur at high population densities (Haubrock et al., 2021). The signal crayfish (*P. leniusculus*), native to North America, is an invasive species in Europe and is known to be susceptible to diverse chemical pollutants. These characteristics make crayfish particularly suitable for ecotoxicological research and for the standardisations of methodologies aimed at evaluating the bioaccumulation dynamics of emerging contaminants (Kouba et al., 2010; Koutnik et al., 2014; Van Nguyen et al., 2023).

The results of this study indicate that the tested PhACs did not exhibit

significant bioconcentration in signal crayfish following 96-hour exposure, either at environmentally relevant concentrations or at levels ten times higher. This contrasts with several previous studies presenting the bioaccumulation of organisms following exposures to a single chemical with varying log $K_{ow}$. For the bioaccumulation potential of a single chemical exposure, sertraline and amitriptyline (log $K_{ow} > 3$; USEPA, 2007) could individually accumulate in aquatic biota after a 7-day exposure period (Rodrigues et al., 2015; Ziarrusta et al., 2017). Bicalutamide has been reported to exhibit potential for bioaccumulation in aquatic organisms (Li et al., 2021). Additionally, prolonged exposure to daidzein may result in the accumulation of daidzein residues in the muscle tissue of gibel carp (*Carassius gibelio*) (Li et al., 2016). This accentuates that exposure to a chemical mixture can lead to different bioaccumulation potentials in aquatic organisms compared to exposure to single chemicals, suggesting that complex chemical interactions within mixtures may influence bioaccumulation potentials from outside to inside the organism, possibly resulting in decreased bioconcentration as observed in our study. Furthermore, several physiological and biochemical factors may explain the absence or low detection of these PhACs in haemolymph, hepatopancreas, and muscle. One possible reason is that some of these compounds show limited bioaccumulation and are rapidly metabolised in crayfish. The study by Rønneberg et al. (2025), which analysed published research on other freshwater and marine planktonic crustaceans, reported that the sex ratio may play a role in enzyme activities in organisms exposed to pharmaceuticals. The meta-analysis there published showed that males are more vulnerable to pollutants, whereas females can better counteract the chemical load. However, little is known about sex-ratio-dependent biotransformation pathways in crayfish exposed to environmental residues of pharmaceuticals (Rodríguez et al., 2007). In addition, the physicochemical properties of the studied PhACs, such as high water solubility and low lipophilicity, likely reduce their potential to accumulate in tissues. Lipophilicity is a critical property influencing the absorption, distribution, metabolism, excretion, and toxicity of a compound within an organism (Arnott and Planey, 2012).

Absorption of PhACs from the water column is mainly passive in all aquatic organisms (Livingstone, 1998). Nonetheless, whether absorption rates are comparable between vertebrates and crustaceans is not well established, as they may depend on organism physiology and additional factors such as compound ionization, environmental pH, and chemical mixture composition. The ratio-based bioconcentration factors of pharmaceuticals in aquatic organisms vary significantly depending on experimental conditions, which underscores the need for robust studies to accurately assess the environmental impact of PhAC mixtures and their potential risks to ecosystems (del Carmen Gómez-Regalado et al., 2023).

The tissue-specific distribution also plays a crucial role in determining the presence of contaminants. The distribution differences are mainly derived from open and closed circulatory systems in crustaceans and fish, respectively (Reiber, 1992). Muscle tissue, primarily composed of proteins and water, generally has a low affinity for hydrophilic compounds, which may explain the absence of the studied PhACs. In contrast, hydrophobic compounds, such as organophosphate flame retardants, can accumulate in crayfish abdominal muscle via environmental exposure, highlighting the compound-specific nature of bioaccumulation processes (Peng et al., 2024). As the primary site for metabolism and detoxification, the hepatopancreas may have actively metabolised the compounds into metabolites before any significant accumulation occurred, resulting in observed <LOQ values of the test PhACs in hepatopancreas in our study. Nevertheless, pharmaceuticals would be expected to reach their highest concentrations in the hepatopancreas, as other polar compounds, such as PFAS, have been predominantly detected in this organ (Bian et al., 2024; Liu et al., 2025). This might imply the distinct bioconcentration between exposures to similarly acting chemical mixtures and the dissimilar ones on organisms.

In crustaceans, haemolymph functions as the primary transport medium for both nutrients and xenobiotics, analogous to blood in vertebrates. Furthermore, haemolymph may facilitate the rapid clearance of these substances, preventing their retention in tissues. For instance, excretory mechanisms for removing metabolites indicate different efficiencies in fish and crustaceans (Armitage et al., 2017; Livingstone, 1991). The distribution of chemicals between these compartments, often described by the apparent volume of distribution, influences their concentrations in circulatory fluids (Klaassen and Watkins, 2015). However, due to the semi-open circulatory system, chemical distribution in haemolymph may differ substantially from that in tissues, resulting in limited bioaccumulation of certain pharmaceuticals. Our measurements indicated that PhAC concentrations in haemolymph remained very low throughout the exposure period, supporting the idea that rapid circulation and excretory processes can prevent significant tissue retention. These findings are consistent with previous reports of low uptake of ionisable pharmaceutical diphenhydramine to crayfish haemolymph (Thammatorn et al., 2024, Van Nguyen et al., 2022) and highlight the importance of haemolymph-mediated transport in modulating the internal exposure of crayfish to waterborne pharmaceuticals.

Further research focusing on metabolite formation, prolonged exposure durations, and tissue-specific metabolic pathways could provide a more comprehensive understanding of the fate and behaviour of these compounds in aquatic invertebrates. However, these results suggest that the studied PhACs do not accumulate at significant levels in signal crayfish tissues under the given exposure conditions.

## 5. Conclusion

This study investigated the bioconcentration potential of selected PhACs in signal crayfish following short-term exposure under controlled laboratory conditions. Despite verified exposure concentrations and generally stable levels of tested compounds in the water phase, the target PhACs were not detected in crayfish haemolymph, hepatopancreas, or muscle tissues, except for trace sertraline levels near the limit of quantification in some control samples. These findings indicate limited bioconcentration of the tested compounds in crayfish and highlight the likely influence of physicochemical properties (such as low lipophilicity and high water solubility), rapid metabolic transformation and efficient excretory mechanisms in reducing tissue accumulation. Overall, the results underscore the need for extended exposure studies to better characterise the environmental fate and toxicokinetics of PhACs in non-target aquatic invertebrates.

## CRediT authorship contribution statement

**Anna Koubová:** Writing – original draft, Methodology, Investigation. **Worrayanee Thammatorn:** Writing – original draft, Methodology, Investigation. **Antonín Kouba:** Writing – review & editing, Investigation. **Vladimír Žlábek:** Writing – review & editing, Project administration, Conceptualization. **Bent Speksnijder:** Writing – review & editing, Methodology, Formal analysis. **Daniel Cerveny:** Writing – review & editing, Methodology, Formal analysis. **Oksana Golovko:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

## Declaration of generative AI and AI-assisted technologies in the writing process

OpenAI's ChatGPT was utilised exclusively to improve grammar, sentence structure, and overall readability of the manuscript. The scientific content, analyses, and interpretations remain solely the work of the authors. The use of this tool adhered to applicable ethical guidelines.

## Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgement

The study was financially supported by the Swedish Research Council FORMAS (grant numbers 2022–02084 and 2020–01052) and the Czech Science Foundation (23–07274S). This work was carried out with the support of LRI CENAKVA Research Infrastructure (ID 90238, MEYS CR, 2023–2026). The authors thank Tuyen Van Nguyen for his technical support. In addition, Worrayanee Thammatorn gratefully thanks the Anandamahidol Foundation for funding her doctoral scholarship.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.etap.2026.104935.

## Data availability

Data will be made available publicly and included in the paper.

## References

Armitage, J.M., Erickson, R.J., Luckenbach, T., Ng, C.A., Prosser, R.S., Arnot, J.A., Schirmer, K., Nichols, J.W., 2017. Assessing the bioaccumulation potential of ionizable organic compounds: Current knowledge and research priorities. Environ. Toxicol. Chem. 36, 882–897. https://doi.org/10.1002/etc.3680.

Arnott, J.A., Planey, S.L., 2012. The influence of lipophilicity in drug discovery and design. Expert Opin. Drug Discov. 7, 863–875. https://doi.org/10.1517/17460441.2012.714363.

Bian, J., Xu, J., Guo, Z., Li, X., Ge, Y., Tang, X., Lu, B., Chen, X., Lu, S., 2024. Per-and polyfluoroalkyl substances in Chinese commercially available red swamp crayfish (*Procambarus clarkii*): Implications for human exposure and health risk assessment. Environ. Pollut. 356, 124369. https://doi.org/10.1016/j.envpol.2024.124369.

del Carmen Gómez-Regalado, M., Martín, J., Santos, J.L., Aparicio, I., Alonso, E., Zafra-Gómez, A., 2023. Bioaccumulation/bioconcentration of pharmaceutical active compounds in aquatic organisms: Assessment and factors database. Sci. Total Environ. 861, 160638. https://doi.org/10.1016/j.scitotenv.2022.160638.

De Lange, H., Noordoven, W., Murk, A., Lürling, M., Peeters, E., 2006. Behavioural responses of *Gammarus pulex* (Crustacea, Amphipoda) to low concentrations of pharmaceuticals. Aquat. Toxicol. 78, 209–216. https://doi.org/10.1016/j.aquatox.2006.03.002.

European Commission, 2012. Communication from the Commission to the Council: The Combination Effects of Chemicals—Chemical Mixtures. In: COM, 252, pp. 1–10 (2012).

Golovko, O., Kumar, V., Fedorova, G., Randak, T., Grabic, R., 2014. Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. Chemosphere 111, 418–426. https://doi.org/10.1016/j.chemosphere.2014.03.132.

Golovko, O., Örn, S., Sörengård, M., Frieberg, K., Nassazzi, W., Lai, F.Y., Ahrens, L., 2021. Occurrence and removal of chemicals of emerging concern in wastewater treatment plants and their impact on receiving water systems. Sci. Total Environ. 754, 142122. https://doi.org/10.1016/j.scitotenv.2020.142122.

Gregarová, K., Zhao, S., Grabicová, K., Horký, P., Grabic, R., Slavík, O., Randák, T., Douda, K., 2024. Differential effects of antidepressant sertraline in glochidia-fish interactions involving drug transfer from parasite to host. Aquat. Toxicol. 273, 107012. https://doi.org/10.1016/j.aquatox.2024.107012.

Haubrock, P.J., Oficialdegui, F.J., Zeng, Y., Patoka, J., Yeo, D.C., Kouba, A., 2021. The redclaw crayfish: A prominent aquaculture species with invasive potential in tropical and subtropical biodiversity hotspots. Rev. Aquacult 13, 1488–1530. https://doi.org/10.1111/raq.12531.

Hossain, M.S., Patoka, J., Kouba, A., Buřič, M., 2018. Clonal crayfish as biological model: a review on marbled crayfish. Biologia 73, 841–855. https://doi.org/10.2478/s11756-018-0098-2.

Klaassen, C.D., Watkins, J.B. (Eds.), 2015. Casarett & Doull's Essentials of Toxicology, fourth edition. McGraw-Hill Medical, New York.

Kouba, A., Buřič, M., Kozák, P., 2010. Bioaccumulation and effects of heavy metals in crayfish: a review. Water Air Soil Poll. 211, 5–16. https://doi.org/10.1007/s11270-009-0273-8.

Koubová, A., Toušová, Z., Šauer, P., de Sales-Ribeiro, C., Vrana, B., Smutná, M., Kroupová, H.K., Grabicová, K., Schmidt-Posthaus, H., Randák, T., 2025. Biomarker responses in wild brown trout from a headwater stream and their causal link to water pollution assessed through chemical analysis and in vitro reporter gene bioassays. J. Hazard Mater., 138433 https://doi.org/10.1016/j.jhazmat.2025.138433.

Koutnik, D., Stara, A., Zuskova, E., Kouba, A., Velisek, J., 2014. The effect of subchronic metribuzin exposure to signal crayfish (*Pacifastacus leniusculus* Dana 1852). Neuroendocr. Lett. 35, 51–56.

Li, D., Chen, H., Liu, H., Schlenk, D., Mu, J., Lacorte, S., Ying, G.-G., Xie, L., 2021. Anticancer drugs in the aquatic ecosystem: Environmental occurrence, ecotoxicological effect and risk assessment. Environ. Int 153, 106543. https://doi.org/10.1016/j.envint.2021.106543.

Li, Y., Yu, H., Xue, M., Zhang, Y., Mai, K., Hu, H., Liu, J., 2016. A tolerance and safety assessment of daidzein in a female fish (*Carassius auratus gibelio*). Aquacult Res 47, 1191–1201. https://doi.org/10.1111/are.12575.

Liu, Y., Xia, Z., Wang, Q., Gong, Z., 2025. Per-and Polyfluoroalkyl Substances (PFASs) in crayfish (*Procambarus clarkii*) from Hubei Province, China: exposure, risk assessment and bioaccumulation. Emerg. Contam., 100579 https://doi.org/10.1016/j.emcon.2025.100579.

Livingstone, D., 1991. Organic xenobiotic metabolism in marine invertebrates. In: Livingstone, D. (Ed.), Advances in Comparative and Environmental Physiology, 7. Springer, Berlin, Heidelberg, pp. 45–185.

Livingstone, D., 1998. The fate of organic xenobiotics in aquatic ecosystems: quantitative and qualitative differences in biotransformation by invertebrates and fish. Comp. Biochem Physiol. A Mol. Integr. Physiol. 120, 43–49. https://doi.org/10.1016/S1095-6433(98)10008-9.

Luijten, M., Vlaanderen, J., Kortenkamp, A., Antignac, J.-P., Barouki, R., Bil, W., van den Brand, A., den Braver-Sewradj, S., van Klaveren, J., Mengelers, M., Ottenbros, I., Rantakokko, P., Kolossa-Gehring, M., Lebret, E., 2023. Mixture risk assessment and human biomonitoring: Lessons learnt from HBM4EU. Int J. Hyg. Environ. Health 249, 114135. https://doi.org/10.1016/j.ijheh.2023.114135.

Malnes, D., Ahrens, L., Köhler, S., Forsberg, M., Golovko, O., 2022. Occurrence and mass flows of contaminants of emerging concern (CECs) in Sweden's three largest lakes and associated rivers. Chemosphere 294, 133825. https://doi.org/10.1016/j.chemosphere.2022.133825.

Malnes, D., Waara, S., Figuière, R., Ahrens, L., Wiberg, K., Köhler, S.J., Golovko, O., 2023. Hazard screening of contaminants of emerging concern (CECs) in Sweden's three largest lakes and their associated rivers. J. Hazard Mater. 453, 131376. https://doi.org/10.1016/j.jhazmat.2023.131376.

Nkoom, M., Lu, G., Liu, J., Dong, H., Yang, H., 2019. Bioconcentration, behavioral, and biochemical effects of the non-steroidal anti-inflammatory drug diclofenac in *Daphnia magna*. Environ. Sci. Pollut. Res 26, 5704–5712. https://doi.org/10.1007/s11356-018-04072-3.

OECD, 2005. The assessment of persistency and bioaccumulation in the pesticide registration frameworks within the OECD region. In: OECD Series on Pesticides No, 25.

OECD, 2019. Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures. OECD Series on Testing and Assessment. OECD Publishing, Paris.

Osuoha, J.O., Anyanwu, B.O., Ejileugha, C., 2023. Pharmaceuticals and personal care products as emerging contaminants: Need for combined treatment strategy. J. Hazard Mater. Adv. 9, 100206. https://doi.org/10.1016/j.hazadv.2022.100206.

Peng, Q., Peng, L., Liu, J., Liu, Y., Liu, X., Yin, J., Duan, S., Liu, X., Li, Y., Gong, Z., 2024. Exposure, bioaccumulation, and risk assessment of organophosphate flame retardants in crayfish in the middle and lower reaches of the Yangtze River. Sci. Total Environ. 912, 168859. https://doi.org/10.1016/j.scitotenv.2023.168859.

Rehrl, A.-L., Golovko, O., Ahrens, L., Köhler, S., 2020. Spatial and seasonal trends of organic micropollutants in Sweden's most important drinking water reservoir. Chemosphere 249, 126168. https://doi.org/10.1016/j.chemosphere.2020.126168.

Reiber, C.L., 1992. The hemodynamics of the crustacean open circulatory system: Hemolymph flow in the crayfish (Procambarus clarkii) and the lobster (Homarus americanus). Univ. Mass. Amherst ProQuest Diss. Theses 1992, 9219486.

Rodrigues, A.P., Santos, L.H.M.L.M., Ramalhosa, M.J., Delerue-Matos, C., Guimarães, L., 2015. Sertraline accumulation and effects in the estuarine decapod *Carcinus maenas*: Importance of the history of exposure to chemical stress. J. Hazard Mater. 283, 350–358. https://doi.org/10.1016/j.jhazmat.2014.08.035.

Rodríguez, E.M., Medesani, D.A., Fingerman, M., 2007. Endocrine disruption in crustaceans due to pollutants: A review. Comp. Biochem Physiol. A Mol. Integr. Physiol. 146, 661–671. https://doi.org/10.1016/j.cbpa.2006.04.030.

Rønneberg, E., Dinh, K., Schneider, S.C., 2025. Sex-specific vulnerability of marine, freshwater and terrestrial animals to pollutants: A meta-analysis study. SETAC Europe 35th Annual Meeting. SETAC.

Šauer, P., Vrana, B., Escher, B.I., Grabic, R., Toušová, Z., Krauss, M., von der Ohe, P.C., König, M., Grabicová, K., Mikušová, P., Prokeš, R., Sobotka, J., Fialová, P., Novák, J., Brack, W., Hilscherová, K., 2023. Bioanalytical and chemical characterization of organic micropollutant mixtures in long-term exposed passive samplers from the Joint Danube Survey 4: Setting a baseline for water quality monitoring. Environ. Int 178, 107957. https://doi.org/10.1016/j.envint.2023.107957.

Thammatorn, W., Kouba, A., Nováková, P., Žlábek, V., Koubová, A., 2024. Effects of diphenhydramine on crayfish cytochrome P450 activity and antioxidant defence mechanisms: First evidence of CYP2C-and CYP3A-like activity in marbled crayfish. Ecotoxicol. Environ. Saf. 285, 117035. https://doi.org/10.1016/j.ecoenv.2024.117035.

USEPA, 2007. Estimation Program Interfaces Suites (EPI Suite) Version 3.2. US Environmental Protection Agency, Washington, DC.

Van Nguyen, T., Bořík, A., Velíšek, J., Kouba, A., Žlábek, V., Koubová, A., 2022. Integrated biomarker response in signal crayfish *Pacifastacus leniusculus* exposed to diphenhydramine. Chemosphere 308, 136382. https://doi.org/10.1016/j.chemosphere.2022.136382.

Van Nguyen, T., Bořík, A., Sims, J.L., Kouba, A., Žlábek, V., Koubová, A., 2023. Toxicological effects of diclofenac on signal crayfish (*Pacifastacus leniusculus*) as

related to weakly acidic and basic water pH. Aquat. Toxicol. 265, 106777. https://doi.org/10.1016/j.aquatox.2023.106777.

Vogt, G., 2008. The marbled crayfish: a new model organism for research on development, epigenetics and evolutionary biology. J. Zool. 276, 1–13. https://doi.org/10.1111/j.1469-7998.2008.00473.x.

Ziarrusta, H., Mijangos, L., Izagirre, U., Plassmann, M.M., Benskin, J.P., Anakabe, E., Olivares, M., Zuloaga, O., 2017. Bioconcentration and biotransformation of amitriptyline in gilt-head bream. Environ. Sci. Technol. 51, 2464–2471. https://doi.org/10.1021/acs.est.6b05831.