Bioconcentration of pharmaceuticals in benthic marine organisms (*Holothuria tubulosa, Anemonia sulcata and Actinia equina*) exposed to environmental contamination by atenolol and carbamazepine.

María del Carmen Gómez-Regalado[a], Julia Martín[b*], Felix Hidalgo[c], Juan Luis Santos[b], Irene Aparicio[b], Esteban Alonso[b], Alberto Zafra-Gómez[a,d,e*]

[a] *Department of Analytical Chemistry, Sciences Faculty, University of Granada, E-18071 Granada, Spain*

[b] *Department of Analytical Chemistry, Escuela Politécnica Superior, University of Seville, C/ Virgen de África 7, E-41011 Seville, Spain*

[c] *Department of Zoology, Sciences Faculty, University of Granada, E-18071 Granada, Spain*

[d] *Instituto de Investigación Biosanitaria, Ibs.Granada, E-18016 Granada, Spain*

[e] *Institute of Nutrition and Food Technology, INYTA, University of Granada, Spain*

[*]Corresponding authors: jbueno@us.es (Julia Martín) and azafra@ugr.es (Alberto Zafra-Gómez)

ABSTRACT The extensive use of pharmaceuticals leads to their ubiquitous occurrence in coastal aquatic environments. Their accumulation in aquatic organisms is an issue that has received increasing attention in recent years since previous attempts to estimate this phenomenon have been ineffective. Invertebrates such as benthic organisms are key in the functioning of marine ecosystems and can be used as sentinel species of water quality status. The aim of the present work is to assess the bioconcentration kinetics of atenolol (ATN) and carbamazepine (CBZ) in three common marine organisms including cotton spinner sea cucumber (*Holothuria tubulosa*), snakelocks anemone (*Anemonia sulcata)* and beadlet anemone (*Actinia equina*) under controlled laboratory conditions. The type of organism, the tissue and structure of the compound have a significant impact upon their toxicokinetic processes. CBZ exhibited higher uptake and excretion rates resulting higher bioconcentration factor (BCF) (41-537 L/kg for CBZ vs 7-50 L/kg for ATN) although both are far below these threshold limits established by the European Union. The measured BCF using kinetic data showed some slightly differences with those predicted using the concentrations measured at the steady-state, probably explained because the steady state was not ready reached. The animal-

specific BCF generally followed the order of cotton spinner sea cucumber > beadlet anemone > snakelocks anemone for ATN while was the opposite for CBZ. The study also highlighted between-tissues differences in the digestive tract and the body wall of the sea cucumber. The work presented here was the first to model bioconcentration of ATN and CBZ in holothurian and anemone animal models.

Keywords: Atenolol; Carbamazepine; Up-take/Depuration; Animal model; Benthic organisms

1. Introduction

In recent years, the consumption of a number of pharmaceutically active compounds (PhACs) has increased considerably due to their high medical and veterinary prescription and uses (Duarte et al. 2023; Xu et al., 2019). Some of these PhACs have been recognized as an important group of emerging pollutants (EPs) (Cerveny et al., 2021). The release of these pollutants into the environment can occur through a variety of pathways, including municipal wastewater treatment plants (WWTPs), as these compounds are not completely removed by traditional treatments, meaning that many of them are found in surface waters around the world (McCallum et al., 2019). The emissions into the environment is continuous (Xu et al., 2019), which has led them to be considered pseudo-persistent pollutants, despite the fact that many of them degrade (Cravo et al., 2022). Controlled laboratory studies on exposure to these compounds have shown that they affect the development, physiology and behaviour of aquatic species. Even so, it is very complex to demonstrate that these observed effects will be the same in nature (Duarte et al., 2023).

Atenolol (ATN) is a β-blocker used for heart rhythm-related diseases, but also used in combination with other drugs to treat hypertension (Mastrángelo et al., 2022). Picó et al. (2019) detected it in treated wastewater at concentrations ranging 0.1 to 0.9 μg L^{-1} and Biel-Maeso et al. (2018) demonstrated its persistence even in treated water, which makes it an emerging pollutant of global concern. On the other hand, carbamazepine (CBZ) is a widely used anti-epileptic drug in clinical practice, in fact, worldwide consumption of CBZ was estimated at 1014t per year (Yang et al., 2023). It is considered one of the most important and relevant compounds due to its high toxicity as well as its persistence in the environment, and its toxic effects on aquatic organisms have been demonstrated. Almeida et al. (2014) studied its effects

in clams and Dumas and colleagues (2022) focused on molecular alterations in mussels. In recent years, much research has been devoted to determining the effects and risks produced by pharmaceuticals once they reach the environment, and it is one of the most quantifiable pharmaceuticals in urban waters, it has been widely used as a marker of anthropogenic pollution (Vitale et al., 2020).

According to the Marine Strategy Framework Directive (European Union, MSFD 2008), research on the accumulation of emerging contaminants in water, sediments and aquatic organisms is essential. For this purpose, the quantification of pollutants takes place by means of some factors such as the bioconcentration factor (BCF) (Martín et al., 2020). Valdés et al. (2014), Meylan et al. (1999) and Fu et al. (2009) studied the bioconcentration of ATN in the whole organism of different fish species, obtaining BCFs between 0.008 and 3.16, while Steinbach et al. (2014) calculated bioconcentration by differentiating fish parts and found that ATN accumulation varied according to the body compartment of the fish. Moreover, some studies have focused on the bioaccumulation of CBZ in different aquatic organisms due to its toxicity. Boillot et al. (2015), Serra-Compte et al. (2018), Daniele et al. (2017) and Contardo-Jara et al. (2011) focused their studies in mussels, in which they obtained BCFs for CBZ in the range of 3.9 to 90, while García et al. (2012) and Valdés et al. (2014, 2016) studied the CBZ bioconcentration in fish, with BCFs between 0.7-9, differentiating in most cases the body compartments.

Bioindicators are important tools for detecting changes in the environment. They allow the health of ecosystems to be assessed through the biological responses they provide (Bonanno et al., 2018). The essential characteristics that a good bioindicator should have, are abundance, ease of sampling, moderate tolerance to disturbance and a wide distribution throughout the ecosystem, which allows for a true assessment of exposure (Urban et al., 2012). Holothurians have proven to be useful bioindicators of marine pollution as, on the one hand, they are widely distributed in coastal areas and on the other hand, their feeding mode consists of ingesting sediments from which they capture organic matter (Bulleri et al., 2021; Chahrour et al., 2021). Sea anemones also exhibit characteristics that make them potential biomonitors of pollution in the sea due to their wide distribution, generalist feeding behaviour, ease of sampling and sessile habit (Morais et al., 2020).

In this context, the main objective of the present study was to assess the bioaccumulation of ATN and CBZ, in a laboratory model, using cotton spinner sea cucumber (*Holothuria tubulosa*), snakelocks anemone (*Anemonia sulcata)* and beadlet anemone (*Actinia equina*) as bioindicators. The duration of the experiment was 80 days to determine the relationship between

compound concentrations in specimens, water and sediment. BCFs were calculated by steady-state and a mass balance models, using the different body compartments of the sea cucumber as well as the two types of anemones.

2. Material and methods

2.1. Chemical and reagents

Ultrapure water (18.2 MΩ cm) was obtained using a Milli-Q Plus® system (Millipore, Madrid, Spain). Analytical grade formic acid (≥ 98%) was used as mobile phase additive. LC-MS grade methanol (MeOH) was acquired from VWR Prolabo CHEMICALS (Barcelona, Spain). ATN (≥ 98.0%) and CBZ (≥ 98.0%) were purchased from Sigma Aldrich (Madrid, Spain). Cinchophen (CIN, surrogate) was supplied by Alfa Aesar (Massachusetts, MA, USA). The chemical structure and physicochemical properties of the analytes are shown in Table S1. Nitrate, nitrite and ammonium analyses were essayed with Hanna instruments® test kits (HI 3874, 3873 and 3826, respectively). Stock solutions were prepared in MeOH and stored at -20 °C until use.

2.2. Laboratory test

2.2.1. Acclimation period

The specimens under study were captured in February 2022 by divers at a depth of 10-15 m at random points along the coast of Granada (Spain). The animals were transported, in refrigerated containers at 4 ºC, to the facilities where the laboratory test was carried out. Once in the laboratory, they were introduced into the experimental tanks containing natural sea water and sediments and allowed to acclimatize and purify for a period of 15 days. In this process, 50 cotton spinner sea cucumbers were captured, 45 of them were used as samples (mean 193.7 ± 10.7 g). Fifty snakelocks anemones (*Anemonia sulcata*) and beadlet anemones (*Actinia equina*) were also captured.

2.2.2. Up-take and depuration test

The research was carried out at the Zoology Department of the University of Granada (Spain), where a 300 L glass aquarium containing recirculating natural seawater was installed with artificial illumination by a 6000ºK metal halide lamp. The system was filtered with a biological filter with foam rubber and bioballs, and the aeration was carried out with compressed air. Photoperiods of 10 hours of light and 14 hours of darkness were carried out. Throughout the experiment, the physicochemical characteristics of the water were checked twice a week, with mean values of temperature 19.7 ± 0.2 ºC, pH 8.2 ± 0.0, salinity 37.5 ± 0.2 PSU, dissolved O_2 6.3 ± 0.1 mg/L, ammonium 0.20 ± 0.03 mg/L, nitrites 0.23 ± 0.01 mg/L, nitrates 51 ± 3 mg/L. Twice a week a scoop containing a mixture of dehydrated fish meal and spirulina (1:1) was added to the aquarium to feed the holothurians.

The experimental design was as follow. During the 28 days of the assay (uptake), after acclimatization time, 3 mg of each compound were added daily to the tank. As the tank capacity was 300 L, the final concentration of each compound was 10 µg/L per day. The exposure concentration used is in agreement with the OECD 305 method taking into account the LC_{50} reported values in aquatic organisms (OECD, 2012; Kim et al., 2009; Heye et al., 2016). During exposure period three specimens of each animal were sampled every four days. For sea cucumbers, body wall and digestive tract were separated. After day 28, all the remaining specimens were transferred to a new tank with the same characteristics as the previous one, initiating the depuration period, which took place for another 52 days, being the total duration of the experiment 80 days. Two specimens of each animal were sampled every four days during the depuration period. The remainder, up to the 50 specimens of each animal in the tank, were captured to allow a margin for mortality and evisceration of the studied animals. Immediately after sample collection, a simple pre-treatment of each specimen, consisting of freeze-drying and pulverization, was carried out. All samples were stored at -20 ºC until analysis.

2.3. Analytical methods

Aliquots of pulverized material from cotton spinner sea cucumber, snakelocks anemone and beadlet anemone, as well as sediment (0.2 g dry weight (dw)) were taken and weighed into 10 mL glass tubes together with a methanolic solution containing the surrogate standard (CIN) (200 ng/g dw). The samples were left in the dark for 24 hours to ensure contact between the matrix and the surrogate. Then, 2.2 mL of MeOH was added, vortexed for 30 seconds and an ultrasound-assisted extraction (UAE) was done for 12 minutes at 40% amplitude. The samples were then centrifuged for 5 minutes at 4000 rpm and the supernatant was collected in another

tube. This procedure was repeated, mixing the collected supernatants. The samples were then completely dried under a stream of N_2. Finally, the residue was dissolved in an 80/20 (v/v) H_2O/MeOH mixture (composition of the initial mobile phase) to a final volume of 0.2 mL. Finally, 2 µL were injected into the UHPLC-MS/MS equipment.

Water samples were treated as follows. They were collected in 100 mL plastic bottles. Aliquots of 10 mL of sample were separated and 50 µL of a methanolic solution of CIN were added to achieve a final sample concentration of 200 ng/mL. The solution was frozen and then freeze-dried for 48 hours at -109 ºC. After drying, 1 mL of MeOH was added and vortexed for 1 min. Afterwards the sample was centrifuged for 5 min at 4000 rpm and the supernatant was separated. The procedure was repeated once more and the two supernatants collected were pooled and evaporated under a stream of N_2. The dry extract was dissolved in the initial chromatographic mobile phase as for the solid samples.

The analysis by ultrahigh performance liquid chromatography-tandem mass spectrometry was performed using an ACQUITY HSS T3 column (100 mm x 2.1 mm internal diameter, 1.8 µm particle size). Compounds were separated by using a mobile phase gradient which consisted of an aqueous buffer with 0.05% formic acid (v/v) as phase A and MeOH as phase B. Thus, the method was performed in 8 min with the subsequent gradient program: 0-1.5 min, isocratic gradient at 80% A. 1.5-3 min, linear gradient from 80% to 60% A, 3-4 min, linear gradient from 60 to 15% A, and 4-6 min, isocratic gradient at 15% A. 6-6.1 min, return to initial conditions for 2 min in order to prepare for the next injection. For each analyte, two multiple reaction monitoring (MRM) transitions were selected, the first for quantification, and the second for confirmation. Transitions and MS parameters are shown in Table 1.

Table 1

Mass spectrometer operated in positive ESI mode. The method was successfully validated according to the ICH guideline (ICH, 2005) in terms of linearity, range, sensitivity and accuracy (trueness and precision). The validation procedure is described in supplementary material section. Table S2 summarizes the limits of detection and quantification as well as the linearity and linear dynamic range. Recovery assays showed recoveries close to 100%, with standard deviations below 15%, for all compounds and matrices.

2.4. Toxicokinetic and bioconcentration calculation

The accumulation (k_1) and depuration (k_2) rate constants in each animal model were studied

as a first-order process (Mackay and Fraser, 2000) described mathematically as:

$$\frac{dC_b}{dt} = k_1 C_w - k_2 C_b \quad (1)$$

C_b is the concentration in biota (ng/g dw), C_w the concentration in water (ng/mL), k_1 the first-order uptake constant (mL/g·d), and k_2 the first-order elimination rate constant (1/d). With the integration of Eq. 1:

$$C_b = \frac{k_1}{k_2} C_w (1 - e^{-k2t}) + C_{b,0}(e^{-k2t}) \quad (2)$$

$C_{b,0}$ is the concentration when the depuration phase begins (ng/g dw), t the time of exposure (days).

For the depuration phase, C_w is assumed to be zero and the equation 2 may then be reduced to:

$$C_b(\text{depuration}) = C_{b,0} e^{-k_2 t} \quad (3)$$

Performing a linear regression of ln(concentration) versus time, we can obtain k_2 from the slope of the regression line. And, the 50 percent depuration will then be reached at the time (t1/2):

$$t_{1/2} = \frac{\ln 2}{k_2} \quad (4)$$

Then, k_1 could be calculated as follows:

$$k_1 = \frac{C_b k_2}{C_w (1 - e^{-k2})} \quad (5)$$

The BCF through the kinetic parameters was calculated as:

$$BCF_{kin} = \frac{k_1}{k_2} \quad (6)$$

In addition, a second way to estimate the BCF_{ss} of each compound was used suing the average concentration in each animal and the corresponding concentration in the aqueous phase at steady state by the equation 7:

$$BCF_{SS} = \frac{C_b}{C_w} \quad (7)$$

C_b is the concentration in the organism (ng/g dw) and C_w is the concentration in the water (ng/mL), both measured at the same time and at the steady-state. The steady stated was estimated at 28 days. Moreover, the C_w was kept within 20% of the measured mean (n = 3).

2.5. Quality and exposure control

Before the experiment, pharmaceuticals were measured in the water and sediment of the aquaria as well as in control organisms (N = 5) of each class. No adverse behaviour was observed in either the control or treated groups during the entire assay period. Mortalities of each group were lower than 1%. The condition index (control and contaminated) remained stable, with low variability, under these conditions being thus optimal for the cotton spinner sea cucumber and the anemones during the experiment. Unfortunately, there are currently no standard exposure tests for this type of animals. Different publications put forward a cut-off level of 10% mortality in the blank controls, as quality measure (Vellinger et al., 2012; Vellinger et al., 2013).

In order to guarantee the quality assurance of the results, a protocol involving the use of control samples including fortified organisms samples with the target compounds (100 ng/g dw), a mixture of the standards in pure solvent (100 ng/mL), solvent (MeOH:water, 50:50 v/v) and procedural blanks injections were included in each analytical batch. For quantification purposes, eight-point calibration in matrix curves were prepared containing ATN and CBZ in the range from method quantitation limit to 500 ng/g dw.

3. Results and discussion

3.1. Pharmaceutical concentrations in exposure water and sediment compartments

Data for concentrations of the target analytes in the aqueous and the surrounding sediments throughout the experiment are displayed in Table 2.

Table 2

PhACs were not detected in control aquaria, thus indicating the absence of contamination. During the exposure stage (spiking with a nominal concentration of 10 ng/mL of each of them) the values measured showed a minimal variation between 3.60 and 8.90 ng/mL for ATN and between 27.1 to 92.2 ng/mL for CBZ. ATN was less stable in water samples and only was detected in few samples after 20 days of the exposure experiment. The concentration levels measured during the exposure period were maintained relatively stable and meet the 20% maximum variation commitment of OECD 305 (OECD, 2012).

ATN was not detected in water from the depuration tank. CBZ present a decrease in the concentrations up to 5.8 ng/mL after 4 days of depuration and not detected after 28 days. CBZ was however detected at higher concentration in the sediment compartment than in water samples from 0.22 ng/g dw in control sediment to 751 ng/g on the last day (28) of the exposure experiment, which suggest that sediments may be an important source of selected compounds for benthic biota like is the case of cotton spinner sea cucumber.

3.2. Uptake and depuration kinetics of carbamazepine and atenolol

Concentrations of ATN and CBZ in control animals were below detection limits. The uptake and depuration pattern in the sea cucumber and the anemones were determined and the results for both compounds are presented in Figures 1 and 2, respectively.

Figure 1 and Figure 2

Throughout the exposure phase, it was observed that the concentrations of the compounds under study increased significantly, with rapid absorption being observed in all groups of animals and tissues. As for the levels of contaminants found after the exposure treatment, all compounds showed bioconcentration, although to different degrees. CBZ was detected at the highest concentration after 20 days of exposure (5803 ng/g dw) in the digestive tract of cotton spinner sea cucumber and 28 days (15387 ng/g dw) in snakelocks anemone. These concentrations were 100-fold higher than those found in the water phase. A concentration up to 215 and 204 ng/g dw was reached for ATN in the digestive tract of cotton spinner sea cucumber and in beadlet anemone, respectively. CBZ visually appeared to reach a maximum level after approximately 20 days of exposure for the digestive tract of the sea cucumber and snakelocks anemone, however ATN did not level off even within 28 days uptake phase. According to OECD 305E testing guidelines (OECD, 2012), the estimated times to 95% of steady state (i.e., $t_{95} = 3.0/k_2$) in the uptake phase were not less than 45 d for CBZ and 93 d for ATN. Thus, we speculated that ATN visually appearing to level off should be a pseudo- steady state which might be attributed to lack of longer exposure time. The capacity of stress tolerance of selected organisms together with their low mobility, allows this group of animals to be suggested as potential sentinel organisms for the monitoring of environmental pollution.

During the depuration phase, a significant decrease in the concentrations of both drugs was observed with a high initial elimination rate of over 83 % for ATN and 91 % for CBZ (except

for beadlet anemone, 46 %) on day 32 (i.e. 4 days of depuration). From this day on, the fall curves slowed down markedly. On the other hand, it was observed that the concentrations of the two PhACs in cotton spinner sea cucumber were significantly dependent on the selected tissue. Higher concentrations were measured in the digestive tract, almost twice than in body wall. For example, the concentration of ATN in digestive tract on last exposure day was of 214 ng/g dw to 96 ng/g dw in the body wall. In the anemone group, concentration levels of CBZ tend to be higher in snakelocks anemone than those to beadlet anemone (5 fold) after 28 days of exposure.

The uptake and depuration kinetics constants, bioconcentration factor, and biological half-lives ($t_{1/2}$) are summarized in Table3.

Table 3

The bioconcentration data fit the toxicokinetic model well with high correlation coefficients ($r^2 \geq 0.748$) for all animals. The uptake rate constants of ATN were between 0.206 L/kg·d in snakelocks anemone and 1.202 L/kg·d in the digestive tract of sea cucumber. The uptake rate constants of CBZ were between 1.729 L/kg·d in the body wall of sea cucumber and 6.204 L/kg·d in snakelocks anemone. This could be because the difference in absorption rates may be associated with the lower permeability of ATN across biological membranes.

The depuration rate constants were between 0.011-0.032 and 0.012-0.066 1/d for ATN and CBZ, respectively. CBZ (log K_{ow} = 2.45) were less hydrophilic than ATN (log K_{ow} = 0.16) and thus excreted faster which was agreement with several previous publications in which hydrophilic compounds had been proved to have low excretion rates (Hendriks et al., 2001). Moreover, it was noted that the uptake and depuration rates of two PhACs in the digestive tract were greater than those estimated in body wall, which could indicate that digestive metabolism might be a main transformation pathway for them. Biological half-lives were 21–64 d for ATN and 11-60 d for CBZ. The longest half-lives were in body wall.

3.3. Bioconcentration factors

It is important to note that, in the literature, BCF values are usually calculated as the ratio of the instantaneous concentrations measured in biota to those in water. In a regulatory context, BCF is a kinetic measure established using a standardized test protocol (e.g., OECD 305) in which the ratio of the rate of absorption to the rate of elimination is determined. Thus, it is *in*

vivo laboratory exposure studies that can elucidate toxicokinetic properties to identify hazards associated with exposure and help prioritize compounds of concern for specific analytical approaches in environmental monitoring campaigns. In the present work, the two different approaches were used to estimate the BCF in the different animal models and similar values were get in case of CBZ while significant differences were observed in some cases probably explained as a consequence that the steady state was not reached (Table 3). Animal-specific BCF were in the ranges of 41-537 L/kg for CBZ and 7-50 L/kg for ATN. Following the indications from REACH legislation, those compounds with a BCF > 2000 are considered bioaccumulative, and very bioaccumulative if the corresponding BCF > 5000. Although the BCFs are higher for CBZ than for ATN, both are well below these European Union (EU) thresholds.

Table 4 summarizes the research found in the literature dedicated to the evaluation of the bioaccumulation potential of these two PhACs in aquatic organisms under laboratory studies.

Table 4

Comparatively, there are many efforts made for CBZ regarding ATN. Although the list of antiepileptic drugs is broad, CBZ is mainly used in the context of environmental studies due to its high consumption and low degradation rate which in turn results in its presence in the environment. Therefore, there are many studies confirming its adverse effects on aquatic organisms (Ferrari et al., 2003). However, bibliographic research demonstrates that uptake data available for aquatic organisms is mostly devoted to fish and mussels. In addition, most of the published works have estimated the BCF taking into account the concentration measured at steady-state, but have not fully addressed their bioconcentration kinetics.

The highest laboratory BCF reported was for CBZ that reached 25.8-35.3 L/kg in the mussel (*Mytilus galloprovincialis*) (Serra-Compte et al., 2018). These values are similar to those of cotton spinner sea cucumbers, but lower than those of anemones. However, other estimates of accumulation were found to be 5 times lower (Table 4), although for different animals and under different experimental conditions. There are inherent difficulties in interpreting and comparing BCF. Recently, Vitale et al. (2020) evaluated the ability of anemones to accumulate CBZ at two level exposure concentrations in a short period experiment (1 and 100 ng/mL). It seems that at low concentrations anemones do not exhibit CBZ bioconcentration but at high concentrations, some mechanisms such as biotransformation processes or multi-resistance systems get saturated or are not efficient enough. Therefore, CBZ

is not eliminated at the same rate as it is taken up, so bioconcentration occurs (BCF = 29 and 24 in *A. equina* and *A. sulcate*, respectively). In the present work, slightly higher values were observed when anemones were exposed for 28 days at intermediate concentration levels (10 ng/mL). These differences could be observed between the two ways of calculation, probably explained because the steady state was not ready reached. The 305 guidelines in 2012 suggests to use toxicokinetic models as the first-order two-compartment model to estimate the BCF if the steady state is not clearly reached. However, kinetic model is not commonly applied in the previous reported studies (OECD, 2012; Molina-Fernández et al., 2021). Moreover, we observed a different pattern in the anemone group, BCF of CBZ tend to be higher in *A. sulcata* anemone than those to *A. equina*, which might be related with the different capacities to accumulate and/or metabolize compounds of the different type of anemones. *A. equina* has the ability to absorb dissolved organic matter through ectodermal cells (Schlichter, 1978) and *A. sulcata* has a larger relative body surface area than *A. equina* due to its larger and thinner tentacles. In fact, unlike *A. equina*, *A. sulcata* is an endosymbiotic species that exchanges nutrients and metabolites with its endosymbiotic microalgae (Stambler and Dubinsky, 1987; Vitale et al., 2020). BCF values are species-specific, and it is hazardous to compare BCF between different species and different genera as their physiology are so different.

As for ATN, the BCF reported in fish are significantly lower than those found in the lower invertebrate organisms studied in the present work. However, it is largely unknown whether these compounds can bioaccumulate in echinoderms. Previously published studies have shown that the log BCF values of hydrophobic compounds are usually closely related to their octanol-water partition coefficient (log K_{ow}) (Barron, 1990). However, estimating the BCF for ionized compounds is highly complicated based on the difference in lipophilicity of neutral and ionized species. Meylan et al. (1999) developed an improved method of estimating BCF at the screening-level and proposed BCF values of 3.16 for ionizable compounds with log $K_{ow} < 5$.

Fu et al. (2009) designed a model for calculating the BCF of ionizing organic compounds and separately established BCF regression models for acids and bases based on pK_a and log K_{ow}. This model performed better than some previously established ones. In the present work, the BCF values of ATN and CBZ were predicted according to the methods of Meylan and Fu under similar experimental conditions (0.34 for ATN and 1.57 for CBZ), and then the data obtained have been compared with the BCF determined by the predictions of the two models. The results showed that our measured BCF values were not consistent with the predicted values. However, the predicted models have been developed considering data reported in fish species, thus the importance of measured data in other animal models. Sea cucumber and anemones

have resulted interesting models for studying bioconcentration since they can reflect changes in the concentration of the contaminant from the surrounding environment, accumulate contaminants without being seriously affected by the concentrations as well as are considered representative and abundant in the marine environment (OSPAR commission, 2012). In addition, anemones are sessile organisms (like the mussel) and we can associate them with a specific place as opposed to a fish. They could be considered secondary or tertiary consumers (they feed on animals that can be herbivores -primary consumers- or carnivores -secondary consumers), thus can have a higher bioconcentration capacity from each trophic level. Sea cucumber although is not sessile, it is sedentary with very low mobility and can also be associated with a specific place because its locomotion capacity is much reduced.

Furthermore, aquatic sediment might also be important sources of contamination of aquatic food webs (Martín et al., 2017). Sea cucumbers are detritivorous animals that feed by ingesting organic matter and food particles from the surface layer of marine sediment, during this process they can concentrate higher amounts of toxic chemicals (Martín et al., 2019; Jiang et al., 2015; Sugni et al., 2007). In our work, sediments were also collected in the same sampling point as the water samples and the BCF_{sed} was calculated as the ratio between the concentration in the organism and the sediment, both measured at the same time and at the steady-state. A BCF_{sed} up to 53 in the digestive tract of *H. tubulosa* and to 20 in *A. sulcate* were noted for ATN and CBZ. Similar levels or even doubled to those estimated from the water compartment BCF_{ss} were calculated for ATN and the contrary effect, slightly lower, were observed in case of CBZ. However, it is important to note that most of the accumulation studies did not examine suspended solids or sediments and the role of the sediment compartment in biota accumulation is still poorly understood.

4. Conclusions

The present study is centered in the determination of the bioconcentration (uptake/depuration) kinetic of ATN and CBZ in cotton spinner sea cucumber and anemones as animal model. The research demonstrates the highest capacity of non-target but common marine organisms to accumulate CBZ than other pharmaceuticals such as ATN. The results also show that the type of organism, the tissue and physico-chemical properties of the compound have a significant impact upon their toxicokinetic processes and could significantly affect the

uptake and depuration rates and BCF. CBZ exhibited higher uptake and excretion rates resulting BCF of 41-537 L/kg vs 7-50 L/kg for ATN, although both are far below these threshold limits established by the EU. The animal-specific BCF generally followed the order of snakelocks anemone > beadlet anemone > sea cucumber for CBZ while was the opposite for ATN. The uptake and depuration rates of two compounds in the digestive tract were both much greater than those in body wall, which indicated digestive metabolism might be a main transformation pathway for them. Therefore, a full understanding of this phenomenon also requires an understanding of metabolic processes. Therefore, in the next step, the data must be complemented by experiments to determine whether and to what extent certain compounds undergo biotransformation.

An important gap within aquatic ecosystems is that, despite the above, the effects of PhACs have been studied only in a few of the many species that exist. Moreover, in our opinion, a credible estimation of the problem of bioconcentration remains a major challenge. Although numerous models exist, their accuracy is still a questionable issue, especially for ionic compounds. In this work, significant differences were found with those predicted BCF values of ATN and CBZ according to Meylan's and Fu's methods under similar experiment conditions, highlighting that experimental practices are crucial for reliable results and accurately calculated field-derived BCF values (Meylan et al., 1999, Fu et al., 2009).

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CRediT authorship contribution statement

María del Carmen Gómez Regalado: Methodology, resources and conceptualization, writing; Julia Martín: Methodology, resources, conceptualization, writing, review & editing; Felix Hidalgo: Methodology, resources and conceptualization, writing; Juan Luis Santos:

Conceptualization, Supervision, review & editing; Irene Aparicio: Conceptualization, Supervision, writing, review & editing; Esteban Alonso: Conceptualization, Supervision, Funding acquisition and Project administration; Alberto Zafra-Gómez: Conceptualization, writing, review & editing, supervision, funding acquisition and project administration.

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.

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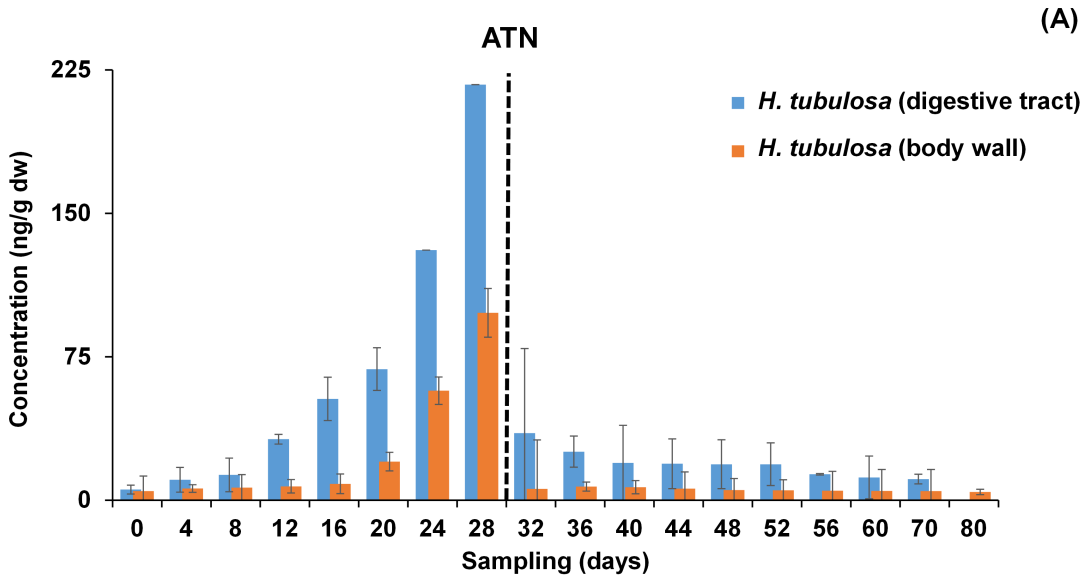
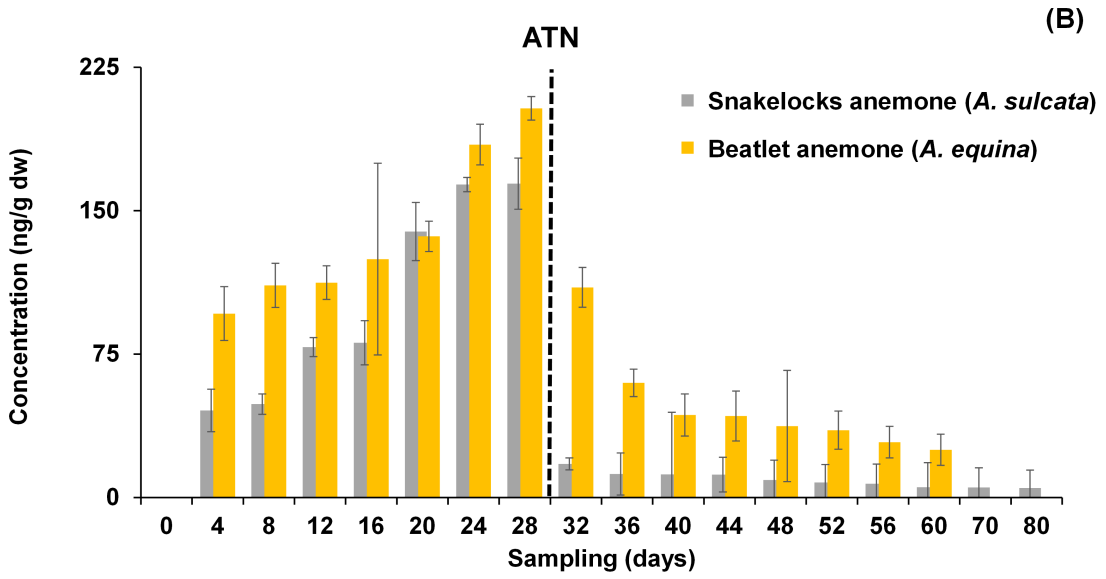
Figure Captions

Figure 1. Kinetics of accumulation (days 0-28) and depuration (days 32-80) of ATN in sea cucumber (*Holothuria tubulosa*) (A) and snakelocks anemone (*Anemonia sulcata)* and beadlet anemone (*Actinia equina*) (B).

Figure 2. Kinetics of accumulation (days 0-28) and depuration (days 32-80) of CBZ in sea cucumber (*Holothuria tubulosa*) (A) and snakelocks anemone (*Anemonia sulcata)* and beadlet anemone (*Actinia equina*) (B).


(A)
ATN
225
150
75
0
Concentration (ng/g dw)
H. tubulosa (digestive tract)
H. tubulosa (body wall)
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 70 80
Sampling (days)


(B)
ATN
225
150
75
0
Concentration (ng/g dw)
Snakelocks anemone (A. sulcata)
Beatlet anemone (A. equina)
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 70 80
Sampling (days)

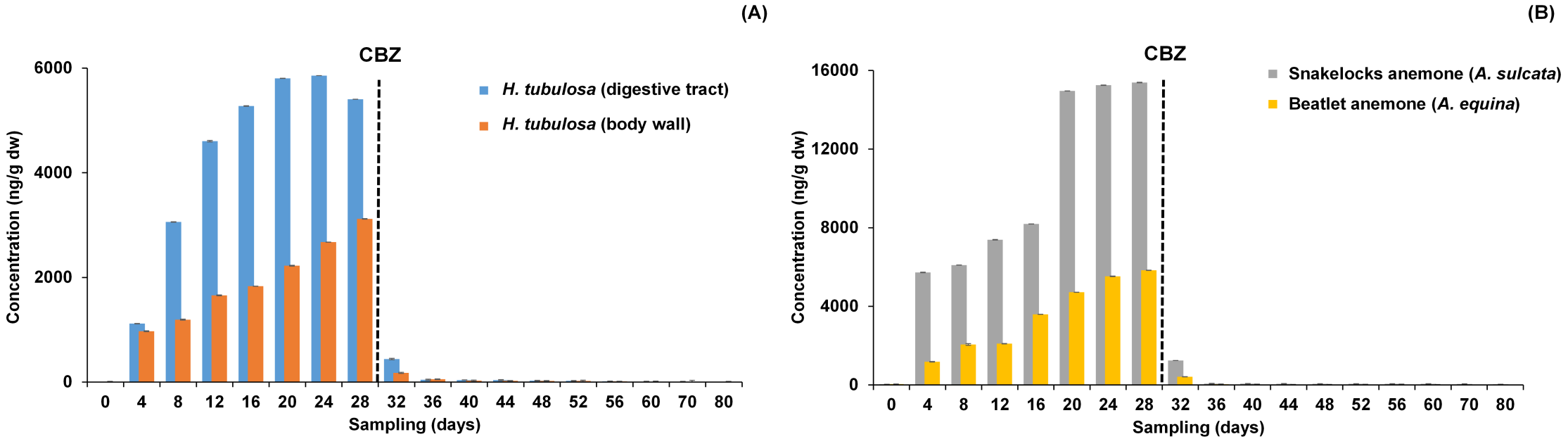

(A)
CBZ
6000
4000
2000
0
Concentration (ng/g dw)
H. tubulosa (digestive tract)
H. tubulosa (body wall)
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 70 80
Sampling (days)
(B)
CBZ
16000
12000
8000
4000
0
Concentration (ng/g dw)
Snakelocks anemone (A. sulcata)
Beatlet anemone (A. equina)
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 70 80
Sampling (days)

Table 1. UHPLC-MS/MS conditions for the determination of ATN and CBZ.

Compound	t_R (min)	Transitions	CV (V)	CE (eV)	Ratio (a/b)
ATN	0.96	267.1 > 145.1[a]	20	24	2.1
		267.1 > 190.0[b]	20	14	
CBZ	4.91	237.1 > 194.0[a]	38	18	8.6
		237.1 > 178.9[b]	38	34	
Spectrometric conditions					
Impactor voltage		1 kV	Nebulizer gas pressure		7.0 bar
Source temperature		150 ºC	Cone/desolvation gas		N_2 (≥ 99.995 %)
Desolvation temperature		600 ºC	Collision gas		Ar (99.999 %)
Cone gas flow		150 L h^{-1}	Dwell time		25 ms
Desolvation gas flow		500 L h^{-1}	Inter-scan delay		3 ms

[a] *Transition used for quantification;* [b] *transition used for confirmation; CV: cone voltage; CE: collision energy*

Table 2. Mean concentration and relative standard deviation of ATN and CBZ in the aqueous phase and in the sediments throughout the experiment.

Sampling (days)	Water phase				Sediments			
	ATN		CBZ		ATN		CBZ	
	Mean (ng/mL)	RSD (%)	Mean (ng/mL)	RSD (%)	Mean (ng/g)	RSD (%)	Mean (ng/g)	RSD (%)
0	ND	-	ND	-	ND	-	0.22	23.0
4	ND	-	27.1	5.3	12.0	10.8	101	9.7
8	ND	-	58.6	4.8	5.30	2.6	149	10.6
12	ND	-	56.9	6.3	4.57	22.0	207	5.6
16	ND	-	81.8	3.6	5.00	15.2	407	14.0
20	3.60	4.1	92.2	2.6	5.14	0.7	624	3.1
24	5.98	2.9	85.7	5.1	11.3	5.0	578	14.3
28	8.90	0.7	74.6	2.2	6.00	6.3	751	7.4
32	ND	-	5.77	5.6	3.60	1.2	23.3	10.0
36	ND	-	0.18	5.3	3.64	2.2	3.00	19.5
40	ND	-	0.18	8.7	3.77	2.4	2.00	10.2
44	ND	-	0.20	-	3.59	2.3	2.26	15.8
48	ND	-	0.21	10.7	3.58	1.2	1.72	12.2
52	ND	-	0.49	0.7	3.72	4.3	1.60	14.5
56	ND	-	0.20	76.8	3.65	2.0	2.00	4.8
60	ND	-	ND	-	3.62	3.3	1.02	12.4
70	ND	-	ND	-	3.51	1.8	2.11	3.8
80	ND	-	ND	-	4.00	15.6	1.26	10.2

ND: not detected

Table 3. Kinetic data of ATN and CBZ in sea cucumber and anemones.

Compound	k_2 (1/d)	R^2	$t_{1/2}$ (d)	k_1 (L/kg·d)	R^2	BCF_{kin} (L/kg)	BCF_{ss} (L/kg)	BCF_{sed} Sed
ATN								
H. tubulosa (digestive tract)	0.024	0.890	29	1.202	1.000	50	50	53
H. tubulosa (body wall)	0.011	0.846	64	0.504	0.994	47	11	24
A. sulcata	0.030	0.884	23	0.206	0.685	7	18	41
A. equina	0.032	0.949	21	0.585	0.892	18	23	50
CBZ								
H. tubulosa (digestive tract)	0.066	0.928	11	4.851	0.885	74	69	7
H. tubulosa (body wall)	0.037	0.877	19	1.729	0.748	41	42	4
A. sulcata	0.012	0.938	60	6.204	0.884	537	206	20
A. equina	0.024	0.760	29	5.195	0.888	127	78	8

k_1: first-order uptake constant; k_2: first-order elimination rate constant; R^2: Determination coefficient of linear representations; BCF_{kin}: bioconcentration factor based in kinetic parameter; BCF_{ss}: bioconcentration factor based in concentration measured at the steady state; BCF_{sed}: bioconcentration factor in the sediment compartment.

Table 4. BCF observations for ATN and CBZ in aquatic organisms.

	Organism	Type of study	Nominal exposure concentration (ng/mL)	Exposure/ depuration period	Tissue; dry weight (dw) or wet weight (ww)	BCF	Reference
ATN	Fish (*Gambusia affinis*)	steady-state	10-1000	96 h/0	whole organism (ww)	0.08-0.13	Valdés et al., 2014
	Juvenile rainbow trout *(O. mykiss)*	steady-state	1-1000	42/0	liver	0.21	Steinbach et al., 2014
					kidney	0.10	
					muscle	0.002	
	Sea cucumber (*Holothuria tubulosa*)	kinetic	10	28/52	digestive tract (dw)	50	The present work
	Sea cucumber (*Holothuria tubulosa*)				body wall (dw)	47	
	Sea anemone (*Anemonia sulcata*)				whole organism (dw)	7	
	Beadlet anemone (*Actinia equina*)				whole organism (dw)	18	
	Estimated					3.16	Meylan et al., 1999
	Estimated					0.34	Fu et al., 2009
CBZ	Mussel (*M. Galloprovincialis*)	kinetic and steady-state	10	7 / 7	whole organism (dw)	3.9	Boillot et al., 2015
	Shrimps (*Gammarus pulex*)	kinetic	100	48 h / 48 h	whole organism (dw)	5.47-8.93	Meredith-Williams et al., 2012
	Water boatman (*Notonecta glauca*)					0.17-0.33	
	Mussel (*M. Galloprovincialis*)	steady-state	<15.7	20 / 20	whole organism (dw)	25.8-35.3	Serra-Compte et al., 2018
	Fish (*Pimephales notatus*)	steady-state	1	14 / 0	muscle (ww)	1.9	García et al., 2012
					liver (ww)	4.6	
	Fish (*Ictalurus punctatus*)	steady-state	1	14/0	muscle (ww)	1.8	
					liver (ww)	1.5	
					brain (ww)	1.6	
					plasma (ww)	7.1	
	Fish (*Gambusia affinis*)	steady-state	10-1000	96 h/0	whole organism (ww)	0.7-0.9	Valdés et al., 2014
	Zebra mussel *(Dreissena polymorpha)*		0.05-5	6 months	whole organism (dw)	3.4-14.8	Daniele et al., 2017
	Zebra mussel *(Dreissena polymorpha)*	steady-state	0.236-236	7/0	whole organism (dw)	90	Contardo-Jara et al., 2011
	Fish *(Jenynsia multidentata)*	steady-state	0.5-100	48h/0	gill (ww)	5	Valdés et al., 2016
					intestine (ww)	5	
					liver (ww)	9	
					brain (ww)	9	
					muscle (ww)	6	

Sea anemone *(A. sulcata)*	steady-state	0-100	8/0	whole body	0.65-29	Vitale et al., 2020
Beadlet anemone *(A. equina)*	steady-state	0-100	8/0	whole body	0.62-24	Vitale et al., 2020
Clam (*Ruditapes sp.*)	steady-state	0.3-9	96 h/0	soft tissue	0.1-1.2	Almeida et al. (2014)
Clam (*Ruditapes decussatus)*	steady-state	30-50	14	gills	20-32	Abdelhafidh et al. (2018)
Plantonic crustacean (*Daphnia magna*)	steady-state	5-100	48 h/0	whole body	202-20	Nkoom et al. (2019)
Mussel (*Mytilus edulis)*	kinetic	1-100	7/7	whole body	2.15-2.17	Boillot et al., 2018
Clam (*Ruditapes philippinarum*)	steady-state	1	28/0	gills	1.7	Almeida et al. (2018)
Sea cucumber (*Holothuria tubulosa*)	kinetic	10	28/52	digestive tract (dw)	74	The present work
Sea cucumber (*Holothuria tubulosa*)	kinetic	10	28/52	body wall (dw)	41	
Sea anemone (*Anemonia sulcata*)	kinetic	10	28/52	whole organism (dw)	537	
Beadlet anemone (*Actinia equina*)	kinetic	10	28/52	whole organism (dw)	127	
Estimated					3.16	Meylan et al., 1999
Estimated					1.57	Fu et al., 2009