Bioaccumulation of UV filters in *Mytilus galloprovincialis* mussel

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Abstract

In this study the bioaccumulation kinetics of organic UV filters, such as 4-MBC, BP-3, BP-4, OC and OD-PABA in wild *Mytilus galloprovincialis* mussels was investigated. The uptake and accumulation of waterborne 4-MBC, BP-4 and OC was very rapid, and after only 24 h of exposure to 1 µg L^{-1}, the tissular concentrations were 418, 263 and 327 µg Kg^{-1}d.w., respectively. The kinetics of bioaccumulation of BP-4 and OC significantly fitted to an asymptotic model with BCF values of 905 L Kg^{-1} and 2210 L Kg^{-1}, respectively. Measured bioaccumulation of the hydrophilic chemical BP-4 was much higher than predicted by K_{ow}-based bioconcentration models, which would lead to a marked underestimation of actual risk. On the other hand, the patterns of uptake found for BP-3 and OD-PABA suggest biotransformation ability of mussels for these two chemicals.

Keywords: *Mytilus galloprovincialis*, organic UV filters, bioaccumulation, 4-MBC, BP-4, OC.

Highlights

- The uptake of waterborne 4-MBC, BP-4 and OC was very rapid after only 24 h of exposure to 1 μ L^{-1}.
- Bioconcentration factor (BCF) was calculated for OC and BP-4.
- BP-3 showed a low accumulation in marine mussels.
- OD-PABA not tends to accumulate in biota due to their high capacity for elimination in mussels.

Introduction

Organic UV filters such as 4-methylbenzylidene-camphor (4-MBC), benzophenone-3 (BP-3), benzophenone-4 (BP-4), octocrylene (OC) and octyldimethyl p-aminobenzoic acid (OD-PABA) are aromatic compounds that adsorb UV radiation, used in sunscreens and other personal care products to protect the skin against sunlight, and as stabilizers in plastics and paints to enhance their durability and physical performance. These chemicals are considered emerging environmental pollutants, and they raise increasing concern due to the levels they reach in surface waters and their potential for endocrine disruption and developmental toxicity (Balmer et al., 2005; Richardson, 2010; Rodil et al., 2012).

Estrogenic activity for some UV filters, including 4-MBC (Inui et al., 2003), BP-3 (Kunz et al., 2006), BP-4 (Kunz et al., 2006), OC (Kunz and Fent, 2006) and OD-PABA (Kunz and Fent, 2006), has been reported in laboratory tests both *in vitro* using cell cultures or in vivo using fish and other aquatic organisms. These compounds recently became ubiquitous in the aquatic environment, reaching in marine coastal waters concentrations as high as 0.01 to 1 µg L^{-1} (Langford and Thomas, 2008; Bratkovics and Sapozhnikova, 2011). In addition to the indirect inputs common to other chemicals, particularly through wastewater effluents, sunscreen components are directly introduced in coastal waters by recreational activities that include sunbathing and swimming (Rodil et al., 2008; Bachelot et al., 2012).

Typically, combinations of three to eight separate UV filters are found in sunscreens and other cosmetics and can make up greater than 10% of products by mass (Brausch and Rand, 2011). In Europe Directive 76/768/CEE (EC, 1976) regulates the maximum content of the above compounds in commercial products at 4% for 4-MBC, 10% BP-3,

5% BP-4, 10% OC and 8% OD-PABA. In USA the composition of cosmetics is regulated by US Food and Drug Administration (2013), and it stipulates slightly different values for the maximum content of UV filters, namely: 6% BP-3, 10% BP-4, 10% OC and 8% OD-PABA, the use of 4-MBC is not allowed as a sunscreen component in USA.

The aim of this study is to assess the behaviour in seawater and the accumulation in the bivalve most commonly used in coastal pollution monitoring, the marine mussel, of a combination of five commonly used organic UV filters, 4-MBC, BP-3, BP-4, OC and OD-PABA. Due to their ubiquity, high water filtration rates and potential for bioaccumulation of chemicals the marine mussels of the *Mytilus* genus are the most common organisms used to monitor chemical pollution worldwide.

Materials and Methods

Experimental procedure

Mussels between 40 and 50 mm long were collected from a pristine area (Vidal-Liñán et al., 2010) from the outer part of Ria de Vigo (NW Iberian coast), and acclimated to incubation conditions in the laboratory for one week prior to experiments. Exposure was made in 30 L glass tanks with 6 mussels per tank, at constant temperature (16ºC), in darkness, using 1 µm filtered seawater (FSW) with oceanic characteristics from an uncontaminated area (salinity 34 ± 0.5 ‰, dissolved oxygen > 90% of saturation, DOC 11.5 ± 2.1 µM) located in the outer part of Ria de Vigo (42°12’11.55’’N; 8°48’21.12’’W). Exposure tanks were continuously aerated with 0.22 µm filtered air, and were allowed to equilibrate for 1 h, before introducing the mussels. Water was renewed every 48 h except on day one of exposure that was renewed at 24 h.

Mussels were fed before each water change for 1 h with a mixed diet of *Isochrysis galbana*, *Tetraselmis suecica* and *Chaetoceros gracilis*.

Experimental solutions

4-Methylbenzylidene-camphor (4-MBC; CAS 36861-47-9), Benzophenone-3 (BP-3; CAS 131-57-7), Benzophenona-4 (BP-4; CAS 611-99-4), octocrylene (OC; CAS 6197-30-4) and octyl dimethyl p-aminobenzoic acid (OD-PABA; CAS 21245-02-3) were obtained from Aldrich (Milwaukee, WI, USA) and Merck (Darmstadt, Germany). UV solution stocks were prepared in Dimethyl sulfoxide (DMSO) and stored in cold and darkness. DMSO concentration in the experiment was always under 0.1% (v/v).

Kinetics experiment

To evaluate the kinetics of accumulation of organic UV filters after different exposure times, over 450 mussels were exposed for 30 d to experimental solutions containing 1 µg L^{-1} of 4-MBC, BP-3, BP-4, OC and OD-PABA, followed by a 20 d depuration period. Samples of mussels were taken for chemical analyses after 0, 1, 2, 4, 8, 14, 22 and 30 d exposure, and after 2, 5, 9 and 20 d of depuration.

Chemical analyses

Matrix solid-phase dispersion

Matrix solid-phase dispersion (MSPD) extraction was used for the simultaneous extraction/cleanup of the mussel samples following a protocol developed previously for perfluorinated compounds (Villaverde-de Sáa et al., 2012). Briefly, 0.5 g of samples were dispersed with 0.2 g of diatomaceous earth using a glass mortar with a

pestle to achieve a complete homogenization. A 10 mL syringe barrel, containing a frit at the bottom, was filled with 1 g of anhydrous sodium sulfate and 4 g of silica followed by the homogenized sample and finally a second frit. Target analytes were eluted with 20 mL of acetonitrile. The eluate was concentrated to dryness under a nitrogen stream. Finally, the extract was reconstituted to a final volume of 500 µL in methanol, being ready for LC-MS/MS analysis.

Liquid chromatography – tandem mass spectrometry determination

UV filters determination in water samples was performed in a liquid chromatography-tandem mass spectrometry (LC–MS/MS) system from Varian (Walnut Creek, CA, USA). The system is equipped with two ProStar 212 high-pressure mixing pumps, a vacuum membrane degasser, an autosampler and a thermostated column compartment ProStar 410 module (Varian).A volume of 100 µL of water samples or 10 µL of MSPD mussel extracts was directly injected into an Ascentis Express C18 column (50 mm × 2.1 mm, 2.7 µm particle diameter) supplied by Supelco (Bellefonte, PA, USA) maintained at a constant temperature of 45 °C. The target compounds were separated at a flow rate of 0.2 mL min^{-1} using 5mM ammonium acetate in both, Milli-Q water (A) and MeOH (B) as eluents. The applied gradient was as follows: 0–1 min, 5% B; 1–2 min, linear gradient to 60% B; 2–9 min, linear gradient to 85% B; 9-9.5 min, linear gradient to 100%; 9.5-11.5 min, 100% B; 11.5-12 min linear gradient to 5% B and finally 12–16 min, 5% B. The system was interfaced to a Varian 320-MS triple quadrupole mass spectrometer equipped with an electrospray interface. Nitrogen was used as a nebulizing and drying gas and Argon was used as collision gas. The analytes were determined in the electrospray positive (4-MBC, BP-3, OC and OD-PABA) and negative (BP-4) and multiple-reaction monitoring (MRM) mode of acquisition. Two MRM transitions were used for each compoundas quantifier and qualifier respectively

(precursor > product ion, m/z values): 255 > 105 and 255 > 97 for 4-MBC, 229 > 151 and 229 > 105 for BP-3, 362 > 250 and 362 > 232 for OC, 278 > 166 and 278 > 151 for OD-PABA and 307 > 80 and 307 > 193 for BP-4.

Quality assurance

Procedural blanks were processed to check for possible contamination arising from laboratory materials, sorbent and solvents used during sample extraction.

The method for water samples (with an injection volume of 100 µL) provided quantification limits in the range 2 to 90 ng L^{-1}. Quantification was performed by injecting standards prepared in seawater in the 0.05 – 1 µg L^{-1} range.

In mussel samples the quantification limits ranged from 0.2 and 3 ng g^{-1} and analytical recoveries were between 90 and 110 %. The precision in terms of repeatability was below 15% (RSD). Quantification was performed by standard addition on non exposed mussel extracts in the 0.75 – 1000µg L^{-1}. This was carried out by dividing the 500 µL extract in four aliquots, spiked with increasing amounts of the analytes.

Bioaccumulation model

Bioaccumulation was modelled assuming first-order kinetics and constant BP-4 and OC concentrations in water according to the expression (Landrum et al. 1992):

$$C_a(t) = \frac{C_w K_u}{K_d}\left(1 - e^{-K_d t}\right) \quad (1)$$

Where $C_a(t)$ is the concentration (µg Kg^{-1}) accumulated in mussels at time t, C_w is the concentration in water (µg L^{-1}), K_u is the uptake rate coefficient (L Kg^{-1} d^{-1}), K_d

is the depuration rate coefficient (d^{-1}) and t is the time (days). K_u and K_d were estimated by least square fits of the accumulation data to equation (1) model.

The bioconcentration factor (BCF) is usually calculated as the ratio of the uptake rate coefficient to the depuration rate coefficient: BCF = K_u/K_d, with units L Kg^{-1}. The equation (1) can be rearranged to obtain directly the confidence intervals of BCF:

$$C_a(t) = C_w BCF\left(1 - e^{-K_d t}\right) \quad (2)$$

Results

Stability of the chemicals in solution

In a preliminary experiment, the stability of the tested chemicals dissolved in seawater was studied (Table 1). Aquaria with the same testing conditions that the exposure experiments but no mussels were spiked with fresh-made stocks of the chemicals to obtain a nominal concentration of 1 μg L^{-1} of each chemical, and samples were taken for chemical analyses after 30 min, 24 h and 48 h. In these conditions, 4-MBC, BP-3, BP-4 and OC showed remarkable stability, with a decrease after 48 h of 17.7% for 4-MBC, 18.1% for BP-3, 13.3% for BP-4 and 31.8% for OC. In contrast, OD-PABA was very unstable in solution, and its measured concentration showed a 86.8% reduction after 48 h.

During the mussel exposure experiments, water samples were also taken at the same time intervals. Measured concentrations (Table 2) revealed that initial 4-MBC, BP-3, BP-4, OC concentrations were 81-110% of the nominal concentrations, whereas that initial OD-PABA concentration was 11% of the nominal concentration, consistently with its unstable behaviour in seawater found in the preliminary experiment with no

mussels. A marked decrease in actual OC concentrations was observedalready after 24 h, consistent with the high bioaccumulation found for this chemical (see below).

Bioaccumulation in mussels

The results of UV-filters accumulation in mussels are given in Table 3. Concentrations in mussel tissues measured before exposure were similar to the background levels found in the NW Iberian coast and French coastal regions (Bachelot et al., 2012; Negreira et al., 2013). The uptake of waterborne 4-MBC, BP-4 and OC was very rapid, and after only 24 h of exposure to 1 μgL^{-1}, the tissular concentrations were 418, 263 and 327 $\mu g\ Kg^{-1}$d.w., respectively. In contrast, BP-3 and OD-PABA showed much lower accumulation, with 80 and 30 $\mu g\ Kg^{-1}$d.w., respectively. However, while BP-3 concentrations remained similar along the exposure period, mussels seemed capable to readily biotransform OD-PABA, which dropped to undetectable levels by the end of the exposure period. The kinetics of bioaccumulation of BP-4 and OC significantly fitted to the asymptotic model described by Eqs 1 and 2. For BP-4, the K_u was 169.8 ± 115.7 L Kg^{-1} day^{-1}, K_d was 0.19 ± 0.16 day^{-1}, and BCF was 905 ± 234 L Kg^{-1}, whilst for OC values were K_u = 281.7 ± 309.3 L Kg^{-1} day^{-1}, K_d = 0.13 ± 0.18 day^{-1} and BCF 2210 ± 1165 L Kg^{-1}. The 4-MBC bioaccumulation did not fit to that model due to the high variability of the data. Taking the peak concentration of 4-MBC in mussels recorded at day 14, a maximum BCF value of 801 L Kg^{-1} can be obtained.

Figs 1 and 2 show the actual concentrations of BP-4 and OC measured in the mussels, as well as the uptake and depuration kinetics predicted by the theoretical model of first order kinetics and a single compartment. For OC, and especially for BP-4, depuration rate was lower than predicted, and body burdens at the end of the depuration

phase were higher than predicted by the model. This may be due to accumulation in a second compartment, termed peripheral compartment, which functions as a storage compartment with virtually no elimination pathway. This pattern of accumulation was found in bivalves for chemicals that are preferentially stored in certain organs such as the digestive gland or the fat tissues (see Discussion).

Discussion

Coastal waters are subjected to direct inputs of UV filters, and reported maximum concentrations for most of these chemicals are within the same order of magnitude than those used in the present experiments (reviewed by Sánchez-Quiles and Tovar-Sánchez, 2015): 0.8 µg L^{-1} for 4-MBC, 3.3 µg L^{-1} for BP-3, 2.78 µg L^{-1} for OC and 0.39 µg L^{-1} for OD-PABA. Despite their presence is increasingly reported in surface waters very limited experimental information on their bioaccumulation potential is available. Balmer et al. (2005) detected 4-MBC, BP-3 and, to a lesser extent, OC in the fat tissues of fish from Swiss lakes.

Although the organic UV filters here studied are all aromatic compounds, their water and lipid solubility and biodegradability is very variable. The log K_{ow}, theoretically a proxy for bioaccumulation potential (MacKay, 1982), ranges from 0.37 for BP-4 to 7.53 for OC. However, both chemicals showed a similar experimental BCF in mussels. In other words, actual bioaccumulation of BP-4 recorded was much higher than that predicted from the common K_{ow} based bioaccumulation models frequently used in risk assessment. This finding highlights how much care must be taken when environmental risk is quantified on the basis of modelled rather than experimentally recorded parameters. In fact it is well known that bioaccumulation of proteinophilic

chemicals such as perfluoroalkyl compounds cannot be predicted on the basis of their partition between octanol or any other lipophilic surrogate and water (Kelly et al., 2009).

Moreover, the K_{ow} based bioaccumulation model assumes than lipophilic compounds passively partition among phases according to their chemical affinity, and does not take into account the metabolical biotransformation capability of the organisms. Adult zebrafish, for example, can biotransform BP-3 into BP-1 (2,4-dihydroxybenzophenone) and this limits BP-3 BCFs to values < 100 L Kg^{-1} (Blüthgen et al., 2012). Literature on the metabolism of emerging pollutants by bivalves or even fish, and UV filters in particular, is rather scarce, and this topic deserves further research. BP-3 could not be detected in *Dreissena polymorpha* mussels which did accumulate other UV filter (Fent et al., 2010). The patterns of bioaccumulation found for both BP-3 and OD-PABA, with maxima 1-2 days after exposure and decreasing levels afterwards, are in line with the expected response of biotransformable chemicals, where synthesis of biotransformation enzymes is induced shortly after exposure (Vidal-Liñán et al., 2015).

According to standard tests BP-3 is considered as readily biodegradable, whereas the remaining chemicals, BP-4, 4-MBC, OC and OD-PABA, are classified as not readily biodegradable (Brooke et al., 2008). This is in line with the lower bioaccumulation found for BP-3 compared to BP-4, 4-MBC and OC in the present study.

We have demonstrated here than a highly lipophilic compound such as OD-PABA (log K_{ow} = 6.15) does not accumulate in mussels whereas a water soluble organic compound such as BP-4 does attain BCF values of approximately 1000 L Kg^{-1}. In

mammals, PABA is metabolized by the Myeloperoxidase pathway (Sagone et al., 1993). According to Bachelot et al. (2012), OD-PABA was never detected in mussels from the French Atlantic and Mediterranean coasts, whereas OC was present in 55% of the samples, at concentrations ranging from 9 to 7112 ng g^{-1}d.w. Moreover, Bachelot et al. (2012) found in two of their sampling sites OC concentrations above 1 µg g^{-1}, higher than those obtained in the present laboratory study. Picot Groz et al. (2014) also found OC concentrations in mussels from South Portugal beaches above 1 µg g^{-1} d.w, associated to seasonal recreational activities, although they did detect OD-PABA also at concentrations up to 800 ng g^{-1}. These findings and the shape of the OC accumulation curve here reported (see Fig.2) suggest that actual OC BCF in wild mussels subjected to longer exposure periods may be even higher than the value here obtained using a first order kinetics model, which assumes saturation of the tissular concentration.

Depuration kinetics for BP-4 was slower and more incomplete than predicted by the first-order kinetic model, which considers the experimental animal as a single compartment. For modelling bioaccumulation in aquatic animals, more than one compartment must be considered when there is a widely different distribution between high perfusion (e.g. gills) and low perfusion (e.g. fat) tissues (Barron et al., 1990). Compared to the single compartment model, a more realistic and only slightly more complex approach is obtained when the organism is divided into two interconnected compartments: a central compartment that exchanges the chemical with the environment, and a peripheral compartment not connected with the outside with a higher affinity for the chemical. For hydrophobic chemicals such as OC (log K_{ow}= 6.88) this is the case of the fat tissue. BP-4 in contrast is a water-soluble chemical with a very low log K_{ow}= 0.37. Water soluble chemicals such as metals are preferentially accumulated in the digestive gland of bivalves (e.g. Walsh and O'Halloran 1997).

Unfortunately, the present study was not designed to investigate the internal compartmentalization of these chemicals, a subject that deserves future research.

Conclusions

The UV filters BP-4, OC and 4-MBC markedly accumulate in mussel tissues, with BCF values in the order of 1000 to 2000 L Kg^{-1}. The bioaccumulation for those three chemicals cannot be predicted on the basis of their K_{ow}, suggesting that other tissues apart from lipids are the final destination of the accumulated chemicals. This stresses the need to validate modeled values of bioaccumulation with experimental data in order to provide effective risk assessment.

In contrast, BP-3 and OD-PABA showed very limited bioconcentration, and the accumulation patterns found, with maxima 1-2 days after exposure and decreasing contents afterwards, support a biotransformation ability of mussels for those two chemicals. This study provides a preliminary assessment of the biotransformation ability of uv-filters by the mussels. More detailed mechanistic investigations are needed to identify the metabolic pathways involved, and field studies can assess their environmental relevance.

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Table 1. Actual concentrations measured in seawater without mussels 30 min, 24 h and 48 h after spiking with a nominal concentration of 1 μg L^{-1}.

	Measured concentration (μg L^{-1})		
	t_0	**t_{24}**	**t_{48}**
4-MBC	0,96	0,99	0,79
BP-3	1,16	1,17	0,95
BP-4	1,05	1,06	0,91
OC	1,57	1,21	1,07
OD-PABA	1,29	1,18	0,17

Table 2. Actual concentrations measured in seawater with mussels 30 min (t_0), 24 h and 48 h after spiking with a nominal concentration of 1 μg L^{-1}.

	Measured concentration (μg L^{-1})			
	t_0	**t_{24}**	**t_0**	**t_{48}**
4-MBC	0,87	0,61	0,86	0,42
BP-3	0,72	0,61	0,71	0,46
BP-4	1,10	0,65	1,08	0,79
OC	0,68	0	0,81	0
OD-PABA	0,11	0,11	0,37	0,11

Table 3. Organic UV filters concentrations measured in mussels (*Mytilus galloprovincialis*) exposed for 30 days to 1 µg L^{-1} and placed in clean seawater for 20 further days

Time exposure (days)	Measured concentrations (ng g^{-1} d.w)				
	4-MBC	BP-3	BP-4	OC	OD-PABA
0	10,5	<LOQ	6	21	<LOQ
1	418	80	263	327	30,5
2	528,5	66	270,5	451	46
4	437	51,5	328	144	11
8	<LOQ	56	429	469	1
14	801	67	520	559,5	13,5
22	411	62	739	712,5	11
30	9,5	59	615	833	<LOQ
32	<LOQ	13	90	141	<LOQ
35	<LOQ	<LOQ	6	<LOQ	<LOQ
39	<LOQ	<LOQ	91	41	<LOQ
50	5,5	<LOQ	186,5	32	<LOQ

Figure captions

Figure 1. Concentration of BP-4 in mussels (*Mytilus galloprovincialis*) exposed for 30 days to 1 µg L^{-1} and placed in clean seawater for 20 further days. The line describes the values predicted by a first-order kinetics model (see text).

Figure 2. Concentration of OC in mussels (*Mytilus galloprovincialis*) exposed for 30 days to 1 µg L^{-1} and placed in clean seawater for 20 further days. The line describes the values predicted by a first-order kinetics model (see text).

Figure 1.

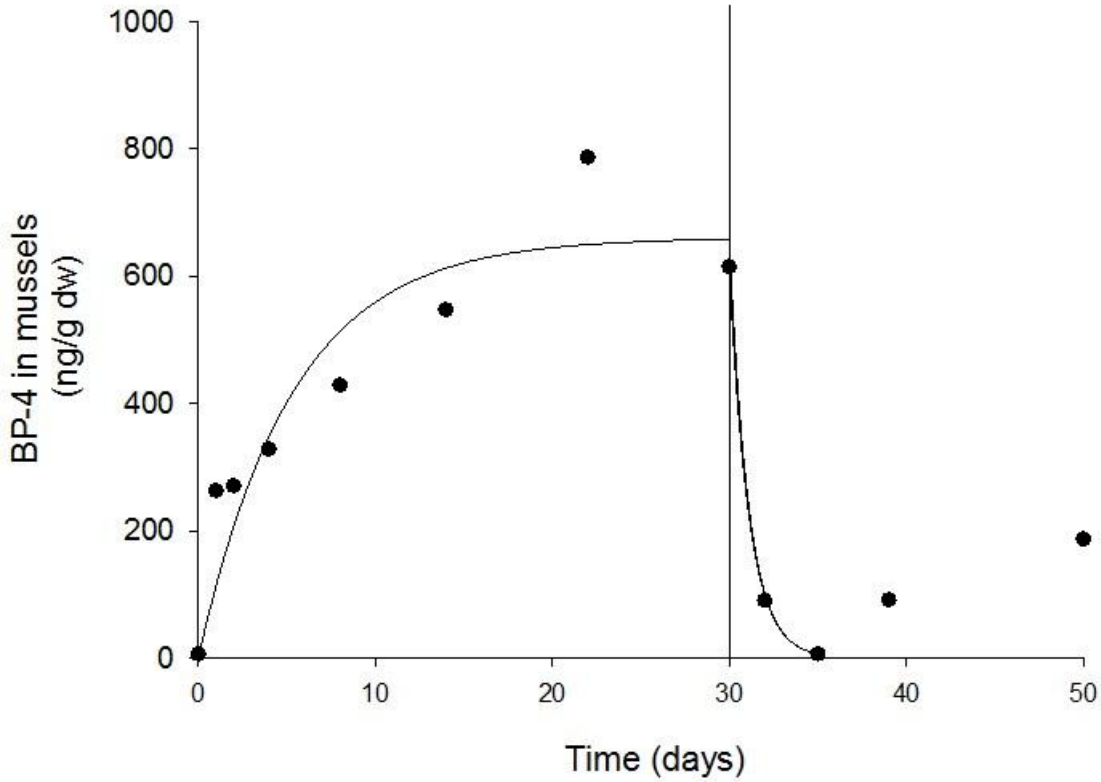
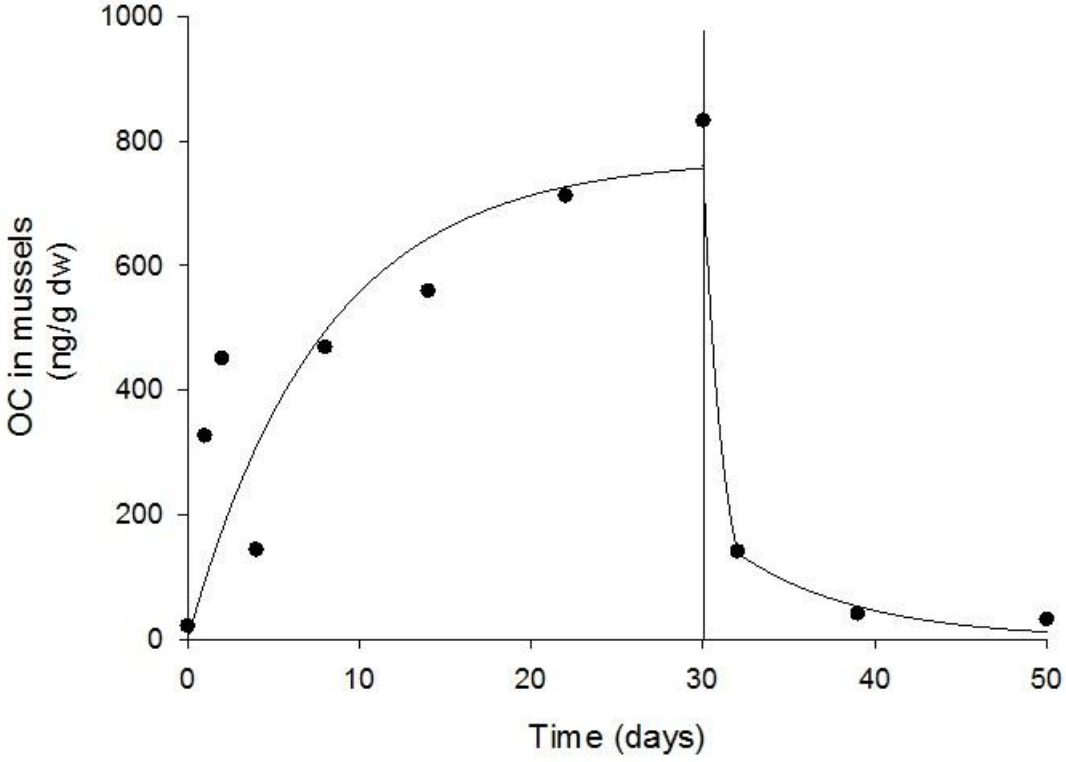

BP-4 in mussels
(ng/g dw)
1000
800
600
400
200
0
0
10
20
30
40
50
Time (days)

Figure 2.


1000
800
600
400
200
0
OC in mussels
(ng/g dw)
0
10
20
30
40
50
Time (days)