# Biokinetics and biotransformation of DDTs in the marine green mussels *Perna viridis*

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## ARTICLE INFO

## ABSTRACT

The biokinetics of *p,p′*-dichlorodiphenyltrichloroethane (DDT) and its metabolites, *p,p′*-dichlorodiphenydichloroethylene (DDE) and *p,p′*-dichlorodiphenyldichloroethane (DDD), in the green-lipped mussel *Perna viridis* were characterized in this study. We exposed the mussels to DDT in aqueous or dietary sources and then compared and evaluated the absorption, accumulation, distribution and elimination of DDT and its metabolites (DDD and DDE) in the mussels. In addition, a dynamic model was employed to quantify the depuration kinetics of each DDT compound in various organs of the mussels. The potential biotransformation pathway in the mussels after dietary exposure to DDT was also analyzed. Differing accumulation and elimination patterns of each DDT compound (DDT, DDD and DDE) in various organs were observed. Most of the DDT was confined to the hepatopancreas following either aqueous or dietary exposure, although the biological fate and biokinetics of DDT were differed significantly between routes of exposure. In addition, the elimination of dietary DDT was markedly slower than that following aqueous uptake. The biotransformation of DDT to DDE was rare in the mussels, suggesting that any DDE in the mussels came primarily from the ambient environment instead of through biotransformation process. Nevertheless, DDE may be retained in the mussels because of its exceptionally low elimination rate. In contrast, DDT was biotransformed to DDD in the mussels following dietary uptake, and this biotransformation may facilitate DDT elimination from the mussels.

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## 1. Introduction

The use of DDT has been officially banned in many countries, although recent studies have revealed that DDT pollution is still a concern in many coastal areas in Asian countries (Monirith et al., 2003). Contamination of seafood by DDT has been reported (Cheung et al., 2007; Meng et al., 2007), which raises the issue of the safety of seafood consumption, given that aquaculture and fishery products are the popular in the diets of many Asians and that a majority of the global aquaculture production is in Asia. Therefore, DDT is considered to be a continuing threat to human health as well as to the aquatic ecosystem.

The green-lipped mussel *Perna viridis* is commercially valuable seafood and is widely distributed in Asian coastal waters. Although mussels have long been used for environmental monitoring of DDT (Monirith et al., 2003), the biokinetics of DDT and its metabolites, DDD and DDE, in mussels is poorly understood. This is primarily because many complex biological processes may occur simultane-ously in mussels, such as differential absorption, bioaccumulation and elimination of each DDT compound (DDT, DDD and DDE), biotranslocation among organs, and potential biotransformation. These processes may alter the biological fate of DDT in the animals and they can have important toxicological implications, because the biokinetics and toxicity among different DDT compounds may be very different (Lotufo et al., 2000; Kwong et al., 2008). For example, conversion from DDT to DDE may increase the compound's retention in the animals, whereas conversion from DDT to DDD may facilitate its elimination (Kwong et al., 2008). Very recently, Binelli et al. (2008) demonstrated that DDE may cause genotoxicity in mussels at twice the potency of DDT and DDD. To evaluate the potential risk and the toxicological consequence of DDT in mussels, it is essential to understand the biotransformation process.

Many earlier studies have suggested that trophic transfer is a significant process for accumulating DDT in aquatic animals (Strandberg et al., 1998; Ruus et al., 1999), and the fate and biokinet-ics of dietary DDT may differ from that following aqueous exposure (Kwong et al., 2008). However, only a few recent studies have characterized the dietary uptake of DDT in aquatic animals (Wang and Wang, 2005; Kwong et al., 2008). Partitioning and the uptake kinetics of non-ionic organic compounds may be characterized

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by the octanol–water partition coefficient or the bioconcentration/bioaccumulation factor, but these models give little insight into the biotransformation pathway and may not be accurate in quantifying compounds that are susceptible to biotransformation (De Wolf et al., 1992). The accumulation and elimination of a compound may be modified upon biotransformation. Previously, a dynamic model was employed to evaluate the biokinetics of DDT in the marine fish *Acanthopagrus schlegeli* (Kwong et al., 2008). The dynamic model was shown to be a promising and a reliable tool to quantify the biokinetics of DDT and to provide valuable insight into the biotransformation pathway in the animals. In this study, we evaluated the absorption, bioaccumulation, distribution, and elimination of DDT and its metabolites (DDD and DDE) in a marine green mussel *P. viridis*. We compared the biokinetics of DDT from aqueous and dietary sources in the mussels. Following our experimental measurements, we used the dynamic model to examine the depuration kinetics of DDT in the mussels. We also attempted to evaluate the potential biotransformation processes that may occur in the mussels after dietary exposure.

## 2. Materials and methods

### 2.1. Organisms, chemicals and food preparation

Green-lipped mussels, *P. viridis* (shell length of approximately 5 cm; $2.6 \pm 0.5$ g wet wt, mean $\pm$ S.D., $N = 144$), were obtained from a fish farm at Yung Shue O, Sai Kung, Hong Kong. After the mussels were brought to the laboratory, the epibionts on their shells were removed immediately. The mussels were maintained in natural coastal seawater collected from Clear Water Bay, Hong Kong, at 23 °C and 30 psu, which received continuous aeration. The organisms were acclimated to laboratory conditions for 7 d in a flow-through system. During the acclimation and depuration period, the mussels were fed non-spiked diatoms, *Thalassiosira pseudonana* (clone 3H), at a ration of 2% of the mussel's tissue dry weight per day. *T. pseudonana* were cultured and maintained in f/2 medium (Guillard and Ryther, 1962) at 18 °C under a 14:10 h light:dark regime.

The DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane] was obtained from Sigma–Aldrich (St. Louis, MO, USA; product 386340). After serial dilution with acetone (high-performance liquid chromatography grade), the DDT was stored at 4 °C in the dark before use. In the dietary uptake experiment, the algae *T. pseudonana* were spiked with DDT prior to feeding to the mussels daily. The *T. pseudonana* were harvested during the exponential phase, then filtered and resuspended into 100 mL of 0.2 μm filtered seawater. Five micrograms of DDT was spiked into the culture for 2 h, then the algal suspension was filtered and resuspended again into filtered seawater.

### 2.2. Uptake and depuration of aqueous DDT in mussels

Thirty mussels were placed into each of three glass tanks containing 10 L of seawater and receiving continuous aeration. The mussels were first fed non-spiked *T. pseudonana* for one and a half hours every day. The water was renewed prior to aqueous DDT exposure to avoid adsorption of dosed DDT onto the algae. The exposure was first initiated by dosing DDT at a nominal concentration of 500 ng/L (actual measured average concentration over the exposure period was 504.5 ng/L, see Section 3 for DDT composition). The DDT was then continuously refreshed and added at a rate of 250 ng/L/h from a stock solution using a peristaltic pump. The solvent to seawater ratio was less than 0.1%. The seawater in each tank was changed daily. The mussels were exposed for 7 d, followed by three weeks of depuration in a flow-through system.

Two mussels were sampled from each tank for a composite sample (each tank represented one replicate, $n = 3$) on days 1, 2, 3, 5, 7 during the exposure period and on days 1, 3, 5, 7, 10, 14 and 21 during the depuration period. The mussels were dissected into gills, hepatopancreas and remaining tissue, weighed and then immediately stored at −80 °C until further analysis.

### 2.3. Uptake and depuration of dietary DDT in mussels

In the dietary uptake experiment, the mussels were exposed to DDT that had been spiked into *T. pseudonana* at a ration of 2% of the mussel's tissue dry weight per day. The measured DDT concentration in the algae was 2.58 ng/mg wet weight of the algae. No pseudo-feces was observed during the experimental period. The seawater in each tank was changed daily. During depuration, the mussels were kept in a flow-through system and fed non-spiked *T. pseudonana*. The mussels were exposed to the dietary DDT for 7 d in the static system, followed by three weeks of depuration in the flow-through system. Two mussels were sampled from each tank for a composite sample ($n = 3$) on days 1,2, 3, 5, 7 during the exposure period and on days 1, 3, 5, 7, 10, 14 and 21 during the depuration period. The mussels were dissected into gills, hepatopancreas and remaining tissue, weighed, and then immediately stored at −80 °C until further analysis.

### 2.4. Tissue extraction, sample clean-up and chemical analysis

The methods used for tissue extraction, sample clean-up, chemical analysis, and quality assurance/quality control have been described elsewhere (Kwong et al., 2008). Briefly, the mussel tissues were first freeze-dried and extracted three times with methylene chloride. Then, the mixture was centrifuged and the supernatant was collected. The supernatants were filtered via a glass fiber filter and the remaining extract was concentrated using a rotary evaporator. The solvent was exchanged by hexane and the volume was reduced to about 1 mL. Sample clean-up was facilitated by using the Florisil® cartridge (Waters; Milford, MA, USA). The identification and quantification of DDT, DDD and DDE were carried out by a Hewlett Packard 6890 series gas chromatograph equipped with both a microelectron capture detector (GC-μECD) installed with a DB-5MS capillary column (60 m length, 0.25 mm inner diameter, 0.25-μm film thickness, J&W Scientific, Folsom, CA, USA) and an auto injector and sampler (Hewlett-Packard 7683 series). Procedural blanks were included to check for potential contamination. Standard reference material (SRM-2978; National Institute of Standards and Technology, Gaithersburg, MD, USA) was used to test the recovery of DDT. The percentage recovery was over 80%, and no correction of data was made for the recovery.

### 2.5. Statistical analysis

One-way analysis of variance was used to test for differences in the accumulation of DDT among organs (gills, hepatopancreas, and remaining tissue), followed by a least significant difference test to identify the differences among groups. All statistical tests were performed using SPSS® for Windows® (Ver 10; Chicago, IL, USA). Data were reported as means $\pm$ standard deviation ($n = 3$), and statistical difference was accepted at $p < 0.05$.

### 2.6. Model development

The mathematical model employed in the present work was similar to that used in a previous study on the biokinetics of DDT and its metabolites in black sea breams (*A. schlegeli*) (Kwong et al., 2008). Generally, a two-level compartmental model was applied, meaning that the DDT from aqueous and dietary uptake was first
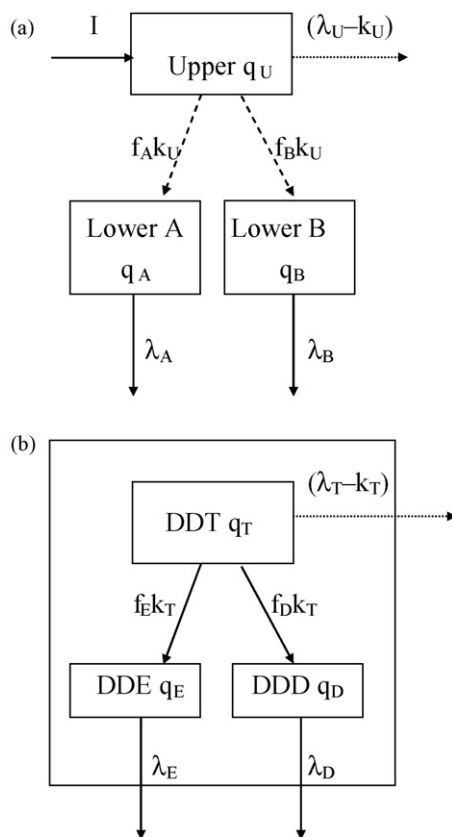
**Fig. 1.** The compartment model applied in the present study (a) a general two-level compartment model (i.e., with the dashed pathways) and a compartment model with biotranslocation neglected (i.e., without the dashed pathways, $k_U = 0$); (b) a two-level (sub-)compartment model for biotransformation from DDT to DDE and DDD in any one of the gill, hepatopancreas and remaining tissue compartments in the case of dietary uptake. The explanations of each parameter are shown in Table 1.

input into the first-level (or upper level) compartment(s), and the DDT in the first-level (or upper level) compartment(s) was then transferred to the second-level (or lower level) compartment(s). Regarding the clearance, the DDT from the first-level compartment(s) could be partially removed out of the compartment(s) directly into the surrounding environment and partially transferred to the second-level compartment(s); and the DDT from the second-level compartment(s) was only removed out of the compartment(s) directly into the surrounding environment. In the present work, a two-level compartment model with a single upper level (denoted as U) and two lower levels (denoted as A and B) was applied. The general compartment scheme is shown in Fig. 1a, with the parameters explained in Table 1.

As in the previous study (Kwong et al., 2008), the mathematical models in the present work focused on the depuration phase, i.e., $I = 0$. The differential equation governing the depuration for the upper compartment is:

$$\frac{dq_U}{dt} = -\lambda_U q_U. \qquad (1)$$

The differential equation governing the depuration for the lower compartments is ($j = A,B$):

$$\frac{dq_j}{dt} = \frac{m_U}{m_j} f_j k_U q_U - \lambda_j q_j. \qquad (2)$$

The time-dependent DDT concentrations in each of the three compartments during depuration with the initial conditions (on day 7), $q_U(0) = q_{Uo}$ and $q_j(0) = q_{jo}$, are:

$$q_U = q_{Uo} e^{-\lambda_U t}, \qquad (3)$$

$$q_j = q_{jo} e^{-\lambda_j t} + \frac{m_U}{m_j} f_j k_U q_{Uo} e^{-\lambda_U t} \left( \frac{e^{-\lambda_U t} - e^{-\lambda_j t}}{\lambda_j - \lambda_U} \right). \qquad (4)$$

The general two-level compartment model can be employed to describe both aqueous and dietary uptake. In modeling the aqueous uptake, we took the gill (G) as the upper compartment, while the hepatopancreas (H) and the remaining tissue (T) were the two lower compartments. In modeling the dietary uptake, the hepatopancreas was taken as the upper compartment, while the gill and the remaining tissue were the two lower compartments.

In both cases of aqueous and dietary uptake, the complete model was complicated by the possibility that DDT could be biotransformed and then biotranslocated among various compartments, or vice versa. Nevertheless, redistribution of DDT among tissue compartments was unlikely to occur during depuration (discussed later). Thus, we considered that the biotranslocation of DDT in the mussel was not significant during the depuration period, i.e., $k_U = 0$. The biotransformation process occurred within each compartment independently, and the compartment model was then modified to leave out the dashed pathways in Fig. 1a. In modeling without biotranslocation in the depuration phase, all compartments could be treated as first-level (or upper-level) compartments.

In considering dietary uptake, we also attempted to model the biotransformation among DDT, DDD, and DDE during the depuration period. With negligible biotranslocation among organs, DDT, DDD, and DDE were biotransformed only among themselves within the same compartment. Since DDT may be transformed to DDD via the reductive dechlorination process, or to DDE via the oxidative dehydrochlorination process, we employed the two-level compartment model as shown in Fig. 1a to model these processes in each organ. Each DDT compound (DDT, DDE and DDD) was treated as a sub-compartment within each organ compartment (gill, hepatopancreas and remaining tissue), with the DDT as the upper sub-compartment, and DDE and DDD as the lower sub-compartments. The compartment scheme for the biotransformation process is illustrated in Fig. 1b, and Eqs. (1) to (4) were used for the calculations. In the following, the term 'biotranslocation' refers to the transfer/distribution of DDT among organs, while the term 'biotransformation' refers to the conversion from DDT to DDE or DDD. In addition, the term 'DDTs' represents the sum of the DDT compounds (DDT + DDE + DDD), while DDT, DDE and DDD represents each compound alone.

## 3. Results

### 3.1. Composition of DDTs and growth of mussels

The aqueous DDTs mostly contained DDT (65.7%), with 32.7% as DDD, and only a trace amount of DDE (1.6%). Similarly, DDT was predominant in the algae *T. pseudonana*, contributing to 50.3% of the total DDT. Comparatively, a high proportion of DDD was also observed in the algae (40.0%) while DDE only comprised 9.7%.

**Table 1**
Parameters used in the compartment scheme shown in Fig. 1 ($i = $ U, A, B stands for the upper, lower A, and lower B compartments, respectively).

| Parameter | Description | Unit |
|---|---|---|
| $I$ | ng of DDT into the upper compartment per day | ng d$^{-1}$ |
| $f_A k_U$ | Transfer coefficient from upper to lower A | d$^{-1}$ |
| $f_B k_U$ | Transfer coefficient from upper to lower B | d$^{-1}$ |
| $q_i$ | ng g$^{-1}$ of toxin in the $i$th compartment | ng g$^{-1}$ |
| $m_i$ | Dry mass of the $i$th compartment | g |
| $\lambda_I$ | Removal rate from the $i$th compartment | d$^{-1}$ |
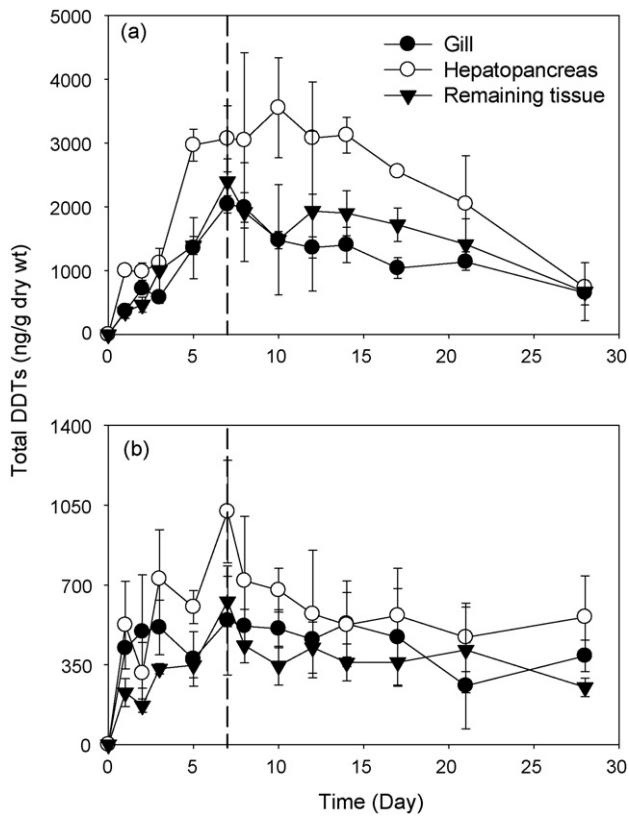
**Fig. 2.** Changes in total DDT concentrations (summation of DDT, DDD and DDE) of different compartments in the mussel *Perna viridis* through (a) aqueous DDT uptake and (b) dietary DDT uptake during the whole experimental period (day 1–7 exposure, day 8–28 depuration). Mean ± standard deviation (*n* = 3).

No significant differences in dry and lipid masses of the mussels between day 1 and day 28 (*p* > 0.05) were found, suggesting that neither positive nor negative growth occurred during the four-week experimental period.

### 3.2. Accumulation and depuration of aqueous DDTs

The concentration of total DDTs increased linearly along with exposure time (Fig. 2a). Maximum accumulation of DDTs occurred in the order of: hepatopancreas > remaining tissue > gills. On the last day on exposure (day 7), total DDTs in the hepatopancreas, remaining tissue, and gills reached 3067 ± 518, 2397 ± 354 and 2038 ± 134 ng/g dry wt, respectively. In addition, the concentrations of DDTs in the hepatopancreas was significantly greater than that in the gills and the remaining tissue (*p* < 0.05), while no statistical difference was recorded between the gills and the remaining tissue (*p* > 0.2). Elimination of total DDTs from the different organs was quite comparable; 68 ± 11%, 76 ± 3% and 72 ± 19% of total DDTs was eliminated from the gills, hepatopancreas and the remaining tissue, respectively. Overall, 73 ± 8% of total DDTs was eliminated from the whole bodies of the mussels by the end of the depuration period.

The DDT and its metabolites, DDD and DDE, increased with exposure time during the uptake period (Fig. 3). The accumulation profile of DDT, DDD and DDE in various organs was similar to the composition of the dosage (e.g., DDT > DDD > DDE). On day 7, the concentrations of DDT in the gills, hepatopancreas and remaining tissue reached 1335 ± 185, 1745 ± 120 and 1566 ± 380 ng/g, respectively, while the concentrations of DDD in these organs were 672 ± 78, 1204 ± 423 and 655 ± 129 ng/g, respectively. The concentrations of DDE remained fairly low over the course of the experiment. The highest DDE accumulation in the gills, hepatopan-
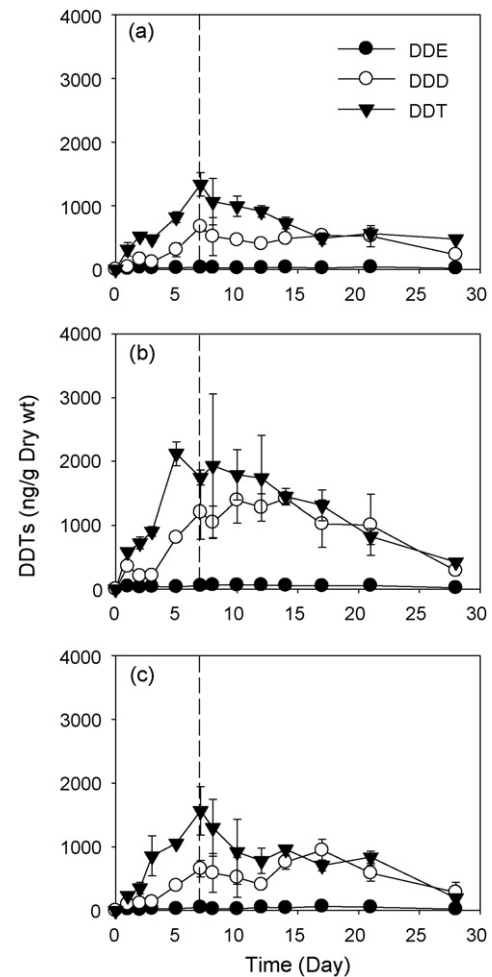


**Fig. 3.** Changes in concentrations of DDT, DDD and DDE in the (a) gills, (b) hepatopancreas, and (c) remaining tissue of the mussel *Perna viridis* exposed to aqueous DDT during the whole experimental period (day 1–7 exposure, day 8–28 depuration). Mean ± standard deviation (*n* = 3).

creas and remaining tissue over the whole experimental period ranged from 31 to 55 ng/g. Apparently, the higher concentrations of DDT and DDD in the water accounted for the higher levels of DDT in the mussels. During depuration, the elimination of DDE was least efficient than that of the other DDT compounds (DDT and DDD). Approximately 74 ± 11% of DDT, 70 ± 7% of DDD, and 61 ± 1% of DDE were lost from the whole bodies of the mussels.

The anatomical distributions indicated that total DDTs accumulation in each organ was relatively steady over the whole course of the experiment (Fig. 4a). The majority of total DDTs was confined to the hepatopancreas, accounting for 47% on average. Accumulation of DDTs in the gills and in the remaining tissue was comparable, accounting for 25% and 28%, respectively.

### 3.3. Accumulation and depuration of dietary DDTs

The concentration of total DDTs increased with the exposure time and reached the highest on the last day of exposure (day 7; Fig. 2b). On day 7, the concentrations of DDTs in the gills, hepatopancreas and remaining tissue were 544 ± 239, 1022 ± 225 and 627 ± 110 ng/g, respectively. The order of accumulation upon dietary exposure was similar to that following aqueous uptake (hepatopancreas > remaining tissue > gills). The hepatopancreas had significantly more DDTs when compared to the gills and the remaining tissue (*p* < 0.05), while no difference was recorded
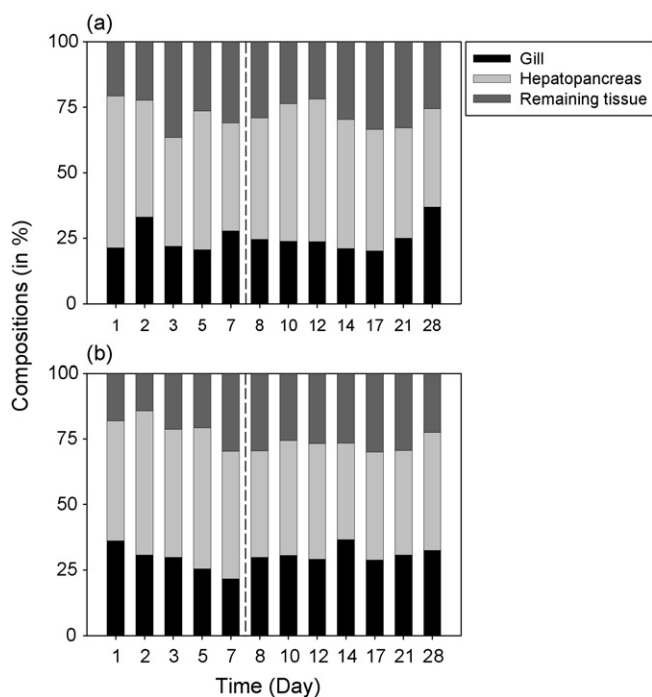
**Fig. 4.** Anatomical distribution of total DDT (summation of DDT, DDD and DDE) in the mussel *Perna viridis* through (a) aqueous DDT uptake and (b) dietary DDT uptake during the whole experimental period (day 1–7 exposure, day 8–28 depuration).

between the gills and the remaining tissue ($p > 0.5$). During depuration, only $29 \pm 6\%$ of DDTs was lost from the gills, while $45 \pm 7\%$ and $60 \pm 6\%$ of DDTs were lost from the hepatopancreas and the remaining tissue, respectively. Overall, $45 \pm 4\%$ of DDTs was eliminated from the whole bodies of the mussels after three weeks of depuration.

Similar to the experiment following aqueous uptake, the most dominant compound in all organs was DDT after dietary exposure (Fig. 5). However, when compared to the composition profile of DDTs in the food, the mussels appeared to preferentially absorb DDT over DDD. On day 7, the concentrations of DDT in the gills, hepatopancreas and remaining tissue reached $278 \pm 55$, $720 \pm 177$ and $402 \pm 71$ ng/g, respectively, while the concentrations of DDD in these organs were $158 \pm 68$, $272 \pm 45$ and $188 \pm 38$ ng/g, respectively. In contrast, the concentration of DDE was comparatively low during the experiment. The highest DDE concentrations reached in the gills, hepatopancreas and remaining tissue over the whole experimental period ranged from 19 to 31 ng/g. Over the three-week depuration period, $53 \pm 5\%$, $41 \pm 5\%$ and $34 \pm 3\%$ of DDT, DDD and DDE were eliminated, respectively, from the whole bodies of the mussels.

The anatomical distribution of total DDTs among different organs was steady over the whole course of the experiment (Fig. 4b). The majority of total DDTs was confined to the hepatopancreas, accounting for 45% on average. The proportion of DDTs in the gills and remaining tissue was similar, 30% and 25%, respectively.

### 3.4. Model fitting of aqueous uptake

The experimental data were fitted using user-defined expressions in the non-linear curve fit program, Microcal™ Origin™ (Version 6.0), with the parameters of interest as the user-defined parameters. Modeling the biokinetics of aqueous DDTs uptake in the mussels was comparatively straightforward since the model only involved single compartments and therefore Eqs. (1) and (3) were used. The best-fit plots are shown in Fig. 6 and the fitted parame-
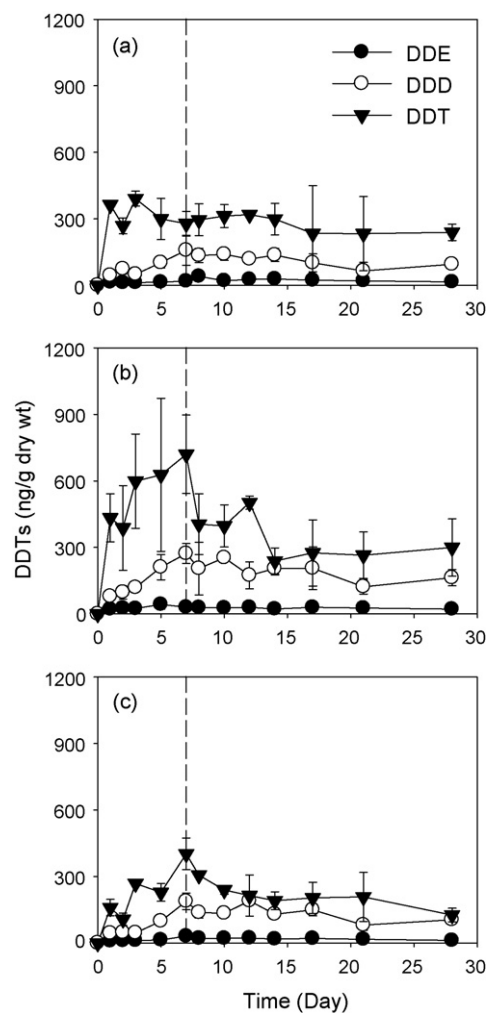


**Fig. 5.** Changes in concentrations of DDT, DDD and DDE in the (a) gills, (b) hepatopancreas, and (c) remaining tissue of the mussel *Perna viridis* exposed to dietary DDT during the whole experimental period (day 1–7 exposure, day 8–28 depuration). Mean ± standard deviation ($n = 3$).

ters are summarized in Table 2. $q_H$, $q_G$, and $q_T$ represent the DDTs concentrations in the hepatopancreas, gill, and remaining tissue, respectively, whereas $\lambda_T$, $\lambda_E$ and $\lambda_D$ represent the removal rates of DDT, DDE, and DDD from each compartment, respectively.

The $q_0$ values obtained from the fitted model were comparable to the actual measurements. It is worth noting that there were significant differences between the $\lambda$ values among DDT and its

**Table 2**
Summary of the results for aqueous uptake of DDT during depuration, including the concentrations at the end of the exposure and the removal rates from different compartments during the depuration period.[a]

|  | DDT | DDE | DDD |
|---|---|---|---|
| **Hepatopancreas** |  |  |  |
| $q_{Ho}$ (ng/g) | $2038 \pm 0.03$ | $84.16 \pm 2.78$ | $1285 \pm 0.03$ |
| $\lambda_T$, $\lambda_E$ or $\lambda_D$ (d$^{-1}$) | $0.0465 \pm 0.0000$ | $0.0616 \pm 0.0033$ | $0.0206 \pm 0.0000$ |
| **Gills** |  |  |  |
| $q_{Go}$ (ng/g) | $1221 \pm 0.05$ | N/A | $557.5 \pm 0.05$ |
| $\lambda_T$, $\lambda_E$ or $\lambda_D$ (d$^{-1}$) | $0.0712 \pm 0.0000$ | N/A | $0.0175 \pm 0.0000$ |
| **Remaining tissue** |  |  |  |
| $q_{To}$ (ng/g) | $1394 \pm 0.04$ | $48.49 \pm 1.58$ | $639.7 \pm 0.04$ |
| $\lambda_T$, $\lambda_E$ or $\lambda_D$ (d$^{-1}$) | $0.0786 \pm 0.0000$ | $0.0018 \pm 0.0067$ | $0.0107 \pm 0.0000$ |

N/A = Values could not be found using the current model.
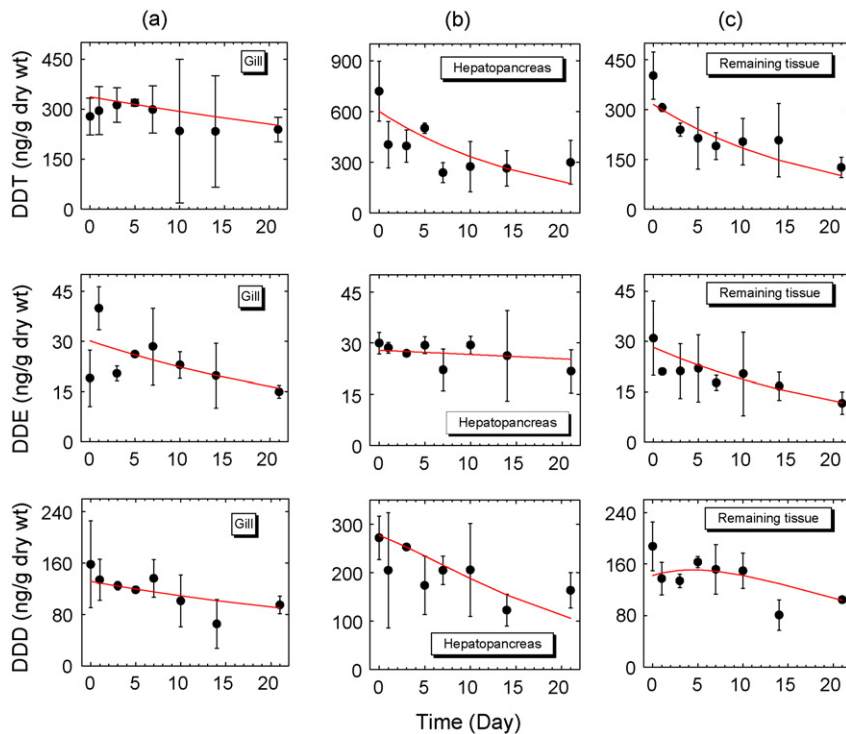
[a] Mean ± 95% confidence intervals.
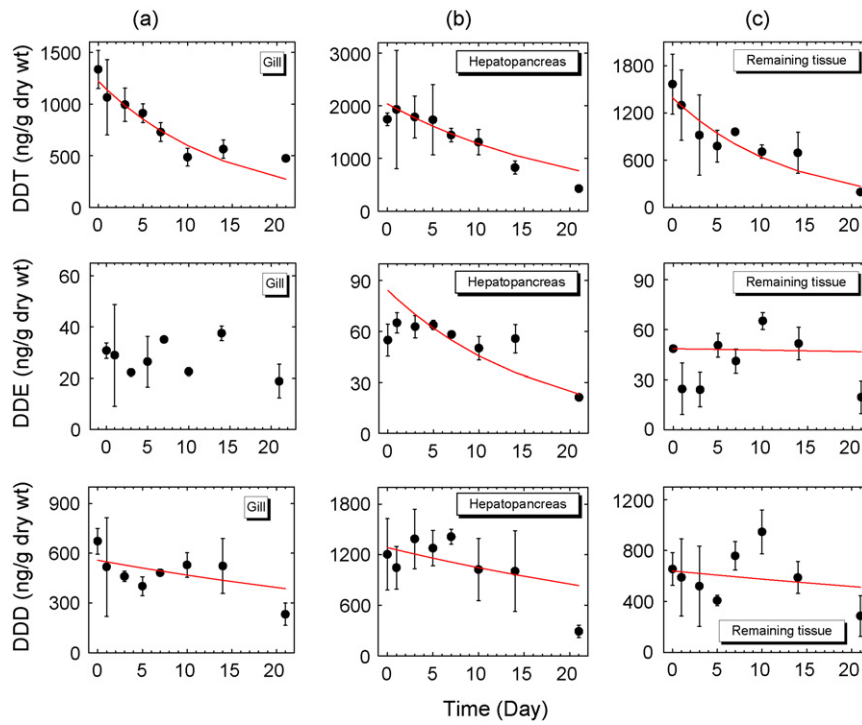
**Fig. 6.** Concentrations of DDT, DDE and DDD in the three compartments of *Perna viridis* – (a) gills, (b) hepatopancreas, and (c) remaining tissue, following aqueous uptake. The line indicates the best fit, starting from the last day of exposure (day 7). Mean ± standard deviation (*n* = 3). There is no fitting for DDE for the gill compartment.

metabolites in various compartments, suggesting differential elimination of each DDT compound from different organs. Generally, DDT had a comparatively higher elimination rate than DDD and DDE had in various tissue compartments, and the elimination rate of DDE in the remaining tissue was the lowest among different DDT compounds and tissues.

### 3.5. Model fitting of dietary uptake

The model fitting for dietary DDTs uptake was complicated because the model involved two-level sub-compartments (as shown in Fig. 1b), and therefore Eqs. (1) to (4) were used. The model was applied to evaluate the potential biotransformation



**Fig. 7.** Concentrations of DDT, DDE and DDD in the three compartments of *Perna viridis* – (a) gills, (b) hepatopancreas, and (c) remaining tissue, following dietary uptake. The line indicates the best fit, starting from the last day of exposure (day 7). Mean ± standard deviation (*n* = 3).

**Table 3**
Summary of the results for dietary uptake of DDT during depuration, including the concentrations at the end of exposure, the removal rates from different sub-compartments, and the transfer coefficients from the DDT sub-compartment to the DDE and DDD sub-compartments.[a]

|  | DDT | DDE | DDD |
| --- | --- | --- | --- |
| **Hepatopancreas** |  |  |  |
| $q_{Ho}$ (ng/g) | $600.8 \pm 76.4$ | $27.85 \pm 1.63$ | $276.8 \pm 19.2$ |
| $\lambda_T$, $\lambda_E$ or $\lambda_D$ (d⁻¹) | $0.0589 \pm 0.0227$ | $0.0055 \pm 0.0491$ | $0.1524 \pm 0.0191$ |
| $f_E$ or $f_D$ (d⁻¹) | – | $0.0010 \pm 0.0561$ | 1 |
| **Gills** |  |  |  |
| $q_{Go}$ (ng/g) | $336.9 \pm 18.7$ | $30.11 \pm 1.17$ | $131.5 \pm 8.6$ |
| $\lambda_T$, $\lambda_E$ or $\lambda_D$ (d⁻¹) | $0.0139 \pm 0.0086$ | $0.0302 \pm 0.0069$ | $0.0553 \pm 0.0109$ |
| $f_E$ or $f_D$ (d⁻¹) | – | 0 | 1 |
| **Remaining tissue** |  |  |  |
| $q_{To}$ (ng/g) | $315.8 \pm 13.1$ | $21.52 \pm 1.41$ | $142.3 \pm 10.0$ |
| $\lambda_T$, $\lambda_E$ or $\lambda_D$ (d⁻¹) | $0.0542 \pm 0.0126$ | $0.0447 \pm 0.1382$ | $0.0890 \pm 0.0048$ |
| $f_E$ or $f_D$ (d⁻¹) | – | $0.0291 \pm 0.221$ | 1 |

[a] Mean ± 95% confidence intervals.

process that might have occurred in the mussels. The DDT sub-compartment was the first-level compartment, while the DDE and DDD sub-compartments were the second-level compartments. The results are shown in Fig. 7, and the fitted parameters are summarized in Table 3. $f_E\lambda_T$ and $f_D\lambda_T$ signify transformation from DDT to DDE or DDD, respectively, where $\lambda_T$ is the total removal rate from the DDT compartment. Theoretically, due to the conservation of DDT, we should have $f_E\lambda_T + f_D\lambda_T \leq \lambda_T$ (or $f_E + f_D \leq 1$). This condition was imposed during the modeling.

The $q_0$ values determined from the fitted model were comparable to the actual measurements. There was an interesting observation during the model fitting of the DDD data. For the gill and remaining tissue compartments, the fitting procedures were pushed towards the limit of $f_D = 1$. For the hepatopancreas compartment, there was considerable inter-dependency among the parameters $q_{Ho}$, $\lambda_d$ and $f_D$, which might be partially due to the scattering of the experimental data. However, the data were also consistent with the fitting with the limit of $f_D = 1$. Therefore, it is likely that $f_D \sim 1$ is a general characteristic of DDT biotransformation in *P. viridis*, i.e., most DDT was biotransformed to DDD during depuration. However, if $f_D \sim 1$, $f_E$ should be close to 0 to satisfy the condition of $f_E + f_D \leq 1$, which has to be fulfilled for the model to be consistent. Interestingly, the model fitting for DDE data gave very small values of $f_E$ for the hepatopancreas and remaining tissue compartments (0.001 and 0.029, respectively). In addition, the fitting procedures were pushed towards the limit of $f_E = 0$ in the gills. Thus, it is likely that $f_E \sim 0$ is also a general characteristic of biotransformation in *P. viridis*, i.e., almost none of the DDT was biotransformed to DDE. The fulfillment of $f_E + f_D \leq 1$ indicates that our model is realistic and the results are reliable. Thus, it appeared that the majority of DDT was biotransformed to DDD in the mussels, whereas the biotransformation of DDT to DDE was unlikely.

## 4. Discussion

### 4.1. Uptake and biological fates of aqueous and dietary DDTs

The mussels rapidly accumulated DDTs from both sources, particularly in the hepatopancreas. The accumulation profile after aqueous or dietary exposure was similar (hepatopancreas > gill > remaining tissue), suggesting that the route of exposure did not affect the target organ where DDTs was stored. Apparently, the absorbed DDTs was preferentially distributed and stored in the hepatopancreas during exposure. On the other hand, the anatomical distribution of DDTs among organs was relatively steady, especially during the depuration period. This implies that

the redistribution of DDTs was unlikely to occur after DDTs was stored in the tissues. This scenario was also observed in a marine fish (Kwong et al., 2008). However, the biological fate of each DDT compound (DDT, DDE and DDD) differed significantly between routes of exposure. For example, the removal of DDT was more efficient than the removal of other DDT compounds after aqueous exposure, whereas the elimination of DDD was significantly greater than that of DDT and DDE after dietary exposure. On the other hand, DDE tended to be retained in the remaining tissue after aqueous exposure due to its apparently low removal rate. Conversely, DDE had the highest retention ability in the hepatopancreas after dietary exposure. The underlying mechanism of such a difference cannot be elucidated in this study, but it may be because DDTs was handled differently following different exposures.

In both aqueous and dietary uptake, the concentration of DDTs in the mussels increased linearly and did not reach a steady state by the end of the 7-d exposure period. Similarly, a previous study also reported that aqueous DDTs uptake was linear over a 20-d exposure period (Richardson et al., 2005). In addition, Richardson et al. (2005) found that elimination of DDTs from the mussels was inefficient over 10-d depuration. We observed that elimination of DDTs following aqueous uptake was particularly slow during the first week of depuration, although the removal of DDTs became more efficient starting in the second week of depuration, especially from the hepatopancreas. Approximately 74% of total DDT was removed from the whole body of the mussel after three weeks of depuration. Overall, elimination of total DDTs from the mussels following aqueous exposure was quite efficient.

The increase in DDTs concentrations in various tissue compartments following dietary DDTs exposure indicates that the mussels absorbed and assimilated DDTs from the algae. The elimination of dietary DDTs from the hepatopancreas occurred at a faster rate on the first day of depuration, followed by a very gradual decrease. The initial sharp decrease in the DDTs level was probably due to the evacuation of unassimilated algae from the digestive tract. In contrast to the findings on aqueous exposure, elimination of total DDTs following dietary exposure was markedly slower. Only 45% of total DDTs was eliminated from the whole bodies of the mussels after three weeks of depuration. The higher retention of dietborne DDTs than of the waterborne DDTs has also been reported in a marine fish (Kwong et al., 2008). However, we could not totally exclude the possibility that the different DDT concentrations used for dietary and aqueous exposure in the present study may affect the biokinetic parameters such as the elimination rate. Nevertheless, since ingestion of contaminated algae by mussels is ecologically relevant and important, the slower elimination of DDTs from dietary exposure may have important ecotoxicological implications. Many previous studies in mussels have primarily focused on aqueous exposure to DDTs and its toxicological effects (Siu et al., 2004; Binelli et al., 2008). Our finding suggests that the route of exposure significantly affected DDTs absorption, bioaccumulation and elimination; the route should thus be carefully considered when examining the potential toxicity of DDTs in mussels.

A recent study has demonstrated that a marine fish preferentially absorbs DDT over DDD from either aqueous or dietary sources (Kwong et al., 2008), suggesting that marine fish may not be ideal candidates for environmental monitors of DDT compound. In this study, the accumulation profile of DDTs in the mussels following aqueous exposure is consistent with the relative composition of DDTs in the water, suggesting that the concentration of each DDT compound in the exposure medium affects the bioaccumulation of DDTs in the mussels. Preferential or selective absorption of each DDT compound was unlikely in the mussels during aqueous exposure. This finding supports that the use of *P. viridis* for environmental DDTs monitoring (Phillips, 1985) is worthwhile since the body burden and the accumulation profile of DDTs in the mussels

may reflect the status of aqueous DDTs contamination in the ambient water. However, the differential absorption of dietary DDTs was observed in the present study. We found that the accumulation profile of dietary DDTs in the mussels was somewhat different from the relative composition in the food, and it appeared that the mussels preferentially absorbed DDT than DDD following dietary exposure, despite the concentration of DDD in the food was quite high. This is possibly because DDD has a lower lipophilicity than DDT, thereby lower absorption. This result indicated that the absorption ability of DDT and its metabolites by the mussels differed between routes of exposure. Thus, if the environment is historically contaminated by DDTs where the dietary DDTs is an important route of exposure, evaluating the relative composition of DDTs in the environment from the mussels may need careful interpretation.

### 4.2. Modeling of DDT biotransformation

It is known that DDT can be transformed to DDD by reductive dechlorination or to DDE by oxidative dehydrochlorination. However, the potential biotransformation pathway in mussels is not clearly understood. Cytochrome P450 (CYP450) or mixed-function oxidase (MFO) is believed to play a role in the oxidative dehydrochlorination of DDT to DDE (Murty, 1986). In bivalves, MFO activity is observed in blood cells, gills, and gonads, while the digestive gland (i.e., the hepatopancreas) has the highest MFO activity (Livingstone, 1991; Solé et al., 1994). To date, there is no evidence suggesting that the MFO activity in mussels can lead to the transformation of DDT to DDE. A recent study by Binelli et al. (2006) demonstrated that DDT in fact inhibits MFO activity. In this study, we observed that the concentrations of DDE remained fairly low during the whole course of the experiment, even in the hepatopancreas. The biotransformation of DDT to DDE appeared to be insignificant, possibly because MFO activity was not induced, and/or the functional capacity of MFO activity in DDT biotransformation in the mussels was low. Our modeling efforts also support the observation that the biotransformation of DDT to DDE was negligible in the mussels. In contrast, the biotransformation from DDT to DDD was likely a major activity in the mussels.

From the kinetic models developed in the present study, we observed that the biotransformation of DDT to DDE following dietary exposure was unlikely to occur in the mussels. This finding is somewhat surprising because it is quite commonly observed that among different DDT compounds, DDE is a predominant residue in mussels collected from contaminated waters. We suggest that mussels have a very limited capacity to undergo the dehydrochlorination process from DDT to DDE, and DDE in the mussels originates primarily from the ambient environment and not from biotransformation. However, due to its exceptionally low removal rates, DDE is preferentially retained in the mussels in comparison with other DDT compounds after exposure. On the other hand, there is evidence that the reductive dechlorination of DDT to DDD may occur in the gut and/or the liver of fish (Malone, 1970; Kitamura et al., 1999; Kwong et al., 2008). However, experiments indicating that the dechlorination process occurs in mussels are lacking. We found here that the dechlorination process from DDT to DDD may occur in the mussels. In fish, biotransformation from DDT to DDD may facilitate DDT elimination because of its higher removal rate (Kwong et al., 2008). Similarly, we observed that the removal rates of DDD in the mussels after dietary exposure were significantly greater than those of DDT and DDE. Thus, transformation from DDT to DDD in the mussels may be a plausible pathway for DDT elimination. The removal rate of DDT following aqueous exposure appeared to be greater than that of DDD. It is therefore possible that the mussels tended to eliminate the parent compound (DDT) after aqueous exposure, whereas DDT may be biotransformed to DDD after dietary exposure, leading to its elimination.

In conclusion, we demonstrated that the accumulation profile of DDTs after either aqueous or dietary exposure is similar (hepatopancreas > gill > remaining tissue). However, the biokinetics of DDTs differed significantly following different routes of exposure. On the other hand, we suggest that mussels have a very limited capacity to biotransform DDT to DDE. Although DDE is commonly reported to be the predominant residue among the DDT compounds in mussels collected from contaminated waters, our findings suggest that DDE in mussels primarily originates from the ambient water and/or food and not from biotransformation. However, since the elimination of DDE is very inefficient, the mussels may retain DDE in their bodies. Interestingly, the biotransformation of DDT to DDD was likely the major activity in the mussels following dietary exposure. This biotransformation pathway may facilitate the removal of DDT because of the markedly high removal rate of DDD. Our study suggests that the route of exposure may significantly affect the fate and biokinetics of DDTs, and it provides valuable insight about the biotransformation processes of DDT in mussels, which could have important ecotoxicological implications.

### Acknowledgements

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