Journal of Hazardous Materials 360 (2018) 420–427

Contents lists available at ScienceDirect

Journal of Hazardous Materials

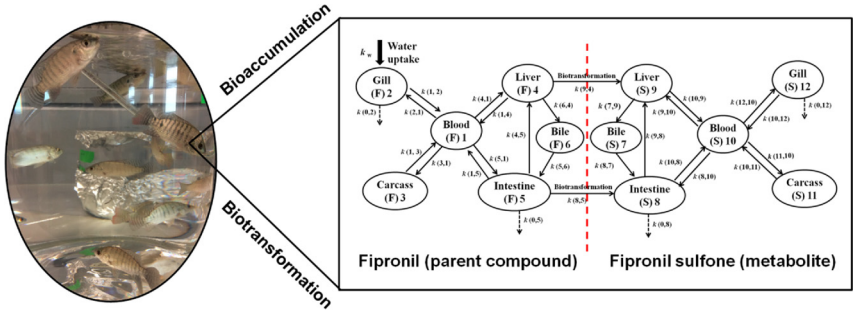
journal homepage: www.elsevier.com/locate/jhazmat

# Multi-compartmental toxicokinetic modeling of fipronil in tilapia: Accumulation, biotransformation and elimination

Huizhen Li[a,b], Jing You[b,*], Wen-Xiong Wang[a,*]

[a] Department of Ocean Science, The Hong Kong University of Science and Technology (HKUST), Clear Water Bay, Kowloon, Hong Kong
[b] School of Environment and Guangdong Key Laboratory of Environmental Pollution and Health, Jinan University, Guangzhou 510632, China

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Keywords:*
Multi-compartmental toxicokinetic modeling
Fipronil
Tissue-specific accumulation
Biotransformation
Inter-compartmental transport

## ABSTRACT

Bioaccumulation and biotransformation are critical processes modifying toxicity of easily metabolizable chemicals to aquatic organisms. In this study, tissue-specific accumulation, biotransformation and elimination of a current-use pesticide fipronil in tilapia (*Oreochromis niloticus*) were quantified by combining *in vivo* measurements and a newly developed multi-compartmental toxicokinetic model. Waterborne fipronil was taken up via gills and metabolized rapidly and solely to fipronil sulfone. Significant decrease of fipronil residues in liver and intestine during exposure period strongly suggested the induction of metabolism in these two organs. Significant transport of fipronil and fipronil sulfone in the liver-bile-intestine system implied that hepatobiliary excretion and enterohepatic re-absorption played important roles in fipronil metabolism and system circulation of the parent compound and the metabolite. The multi-compartmental model quantitatively described the highly dynamic inter-compartmental transport and rapid branchial clearance of fipronil in fish. Modeling results also suggested that uptake and biotransformation were the stronger driving forces for the inter-compartmental transport of fipronil in fish than the inherent partitioning capacity. Overall, our findings highlight the importance of biotransformation on internal disposition of fipronil in fish, which helps to improve aquatic toxicity assessment of this pesticide.

## 1. Introduction

Tracking ecological impacts of pesticides is of global significance especially considering that pesticides are increasing in demand, use and release into the environment [1]. Evidence suggested that systemic insecticides such as neonicotinoids and fipronil were likely responsible, at least partially, for the observed decline of biodiversity of aquatic microinvertebrates [2,3]. As a broad spectrum phenylpyrazole

* Corresponding authors.
*E-mail addresses:* youjing@jnu.edu.cn (J. You), wwang@ust.hk (W.-X. Wang).

https://doi.org/10.1016/j.jhazmat.2018.07.085
Received 11 May 2018; Received in revised form 5 July 2018; Accepted 23 July 2018
Available online 03 August 2018
0304-3894/ 

insecticide, fipronil targets the γ-amino butyric acid (GABA) receptors, resulting in continuous central nervous system activity [4]. This specific mode of action makes fipronil extremely toxic to non-target invertebrates [5]. Fipronil is much less toxic to mammals compared to invertebrates, but it exhibits high lethal toxicity to fish (e.g. 4-d LC50 was 94.2 μg $L^{-1}$ for medaka [6]) and also can cause sublethal effects to fish (e.g. growth inhibition at 30 μg $L^{-1}$ and possible endocrine disruption at 10 μg $L^{-1}$ to madaka [7]). A statewide investigation in the U.S. found that fipronil residues in 70% and 20% of the urban and agricultural waterways, respectively, exceeded the aquatic life benchmark for this chemical [8], calling for concerns on the risk of this insecticide to aquatic species.

Biotransformation significantly influences the bioaccumulation process and further modifies the toxicity of a compound to an organism [9]. Understanding the accumulation and biotransformation processes is essential to assess the impact of pesticides on aquatic organisms. Although studies have focused on evaluating fipronil effects on fish [10], its accumulation and biotransformation processes in fish are still less known [11]. While fipronil is easily degraded and metabolized, its degradates/metabolites are equal or even more toxic to many aquatic species than the parent compound [12–14], and are also more stable in the environment [3]. Different compositions of fipronil and its metabolites in water and wild European eel was observed, indicating significant biotransformation of fipronil in wild fish [15]. The transformation process of xenobiotics in organism is tissue-specific [16], however, little information is available on the time-dependent and tissue-specific accumulation and biotransformation of metabolizable pesticides in fish. Multi-compartmental model, which considers multiple tissues, is particularly suitable for studying the fate of xenobiotics in fish and recognizing the importance of individual tissues in processing the xenobiotics [16].

The main objective of the present study was to investigate the tissue-specific accumulation, biotransformation and elimination of waterborne fipronil in tilapia (*Oreochromis niloticus*). A multi-compartmental model was constructed to simulate the dynamic transport of the parent compound and metabolites among six compartments (blood, gill, carcass, liver, bile and intestine). In addition, the commonly used one-compartmental model was applied to support the results of the multi-compartmental model. Specifically, the current study was conducted to better understand (1) the disposition and transport of fipronil and its metabolites in fish and the importance of different compartments to this process; (2) the significance of the liver-bile-intestine system in the specific biotransformation, excretion and circulation of fipronil in fish; (3) the influence of biotransformation on the accumulation and disposition of fipronil in fish. Tilapia is an important commercial fish, which is cultured worldwide with production increasing rapidly in recent years [17]. With high consumption and the associated human health concerns, tilapia has been used as a model species to study the accumulation of metals in aquatic organisms [18,19], while the tissue-specific accumulation and biotransformation of pesticides in this species is unknown. Concerning the impacts of pesticides on fish in paddy fields of rice-fish cultivation, there is a great need to understand the toxicokinetics of pesticides in this cultured fish [20].

## 2. Materials and methods

### 2.1. Uptake, elimination and sampling

The bioaccumulation testing (96-h uptake and 96-h elimination regimes) was performed in 20-L glass beakers using acclimated tilapias (fresh weight of approximately 12 g) at 21 ± 1 °C. The elimination half-life for fipronil in rainbow trout was reported to be 0.6 d [11], thus the respective 4-d accumulation and elimination experiments in the current study was deemed appropriate. The exposure concentration of fipronil in water was set at 1 μg $L^{-1}$, which was environmentally relevant and was monitored throughout the testing. The system was static with about 80% of water being renewed every 12 h. A laboratory control was conducted simultaneously. The fish were not fed during the whole bioassay. After 96-h exposure, the fish were taken out and transferred to non-dosed water to initiate the elimination. More details on fish acclimation and bioaccumulation testing are presented in the Supporting Information.

At pre-determined time points during the uptake regime (4, 10, 24, 48, 72 and 96 h) and elimination regime (99, 102, 106, 120, 144, 168 and 192 h), four fish were randomly sampled (one fish from each tank) and narcotized in cold water to collect blood from caudal fin by capillary pipette and then dissected into five compartments (gill, liver, bile, intestine and carcass). The intestinal tract including the upper and lower intestine was taken as the intestine sample and intestinal contents in the intestinal tract were extruded gently using tweezers. The tissue samples were stored at −80 °C prior to analysis after being weighed for fresh weight. Meanwhile, fipronil-dosed water were collected for analysis at the time points of 0 (before adding the fish), 4, 10, 48 (before water change and thereafter) and 96 h.

### 2.2. Sample preparation and instrumental analysis

Freeze-dried gill, liver, intestine and carcass were grounded and extracted with acetonitrile, while fresh blood and bile samples were directly extracted with respective acetonitrile and diethyl ether using vortex-assisted sonication. Lipid was removed from the extracts by repeated freezing and centrifugation. Water samples were extracted by liquid-liquid extraction with dichloromethane. Fipronil and its metabolites in the samples were quantified using high performance liquid chromatography and mass spectrometer system (HPLC/MSMS). A batch of quality assurance and quality control (QA/QC) procedures, including internal calibration, recoveries of surrogate and target compounds in the blanks, matrix spikes and samples, and instrumental calibration standard check, were performed to assess the effectiveness of sample preparation and quantification. Details of the sample preparation, instrumental analysis and QA/QC are presented in the Supporting Information.

### 2.3. Statistical analysis

One-way ANOVA with Tukey's multiple comparison was performed using SPSS 17.0 to evaluate the statistical significance in the levels of fipronil and its metabolites among the time points. Significant difference was set at $p < 0.05$.

### 2.4. Model development

A multi-compartmental model based on mass balance was constructed to quantitatively assess the uptake, transport, biotransformation and elimination kinetics of fipronil and its metabolite in tilapia. Six compartments were included in the model with blood connecting the individual organs (Fig. 1). Fipronil in water was taken up through gill along with the movement of water and blood in the gill and then transferred to blood [21]. The blood served as a carrier that distributed fipronil to the other organs, which drove the systemic circulation. The liver was recognized as the primary organ to metabolize xenobiotics in fish [22]. The bile was produced by liver and served as a transient compartment for excreting fipronil and its metabolites to the small intestine. The compounds excreted to the small intestine may be re-absorbed by the intestine and transferred back to the liver, which was known as enterohepatic cycling. The carcass, including muscle, bones and other organs, accounted for more than 90% of the whole fish weight and therefore was considered as the largest pool for fipronil and its metabolites. Considering the possible biotransformation in the gastrointestinal tract [23], biotransformation was set in both liver and intestine. After biotransformation, the metabolites entered systemic circulation which was driven by the blood flow and the enterohepatic

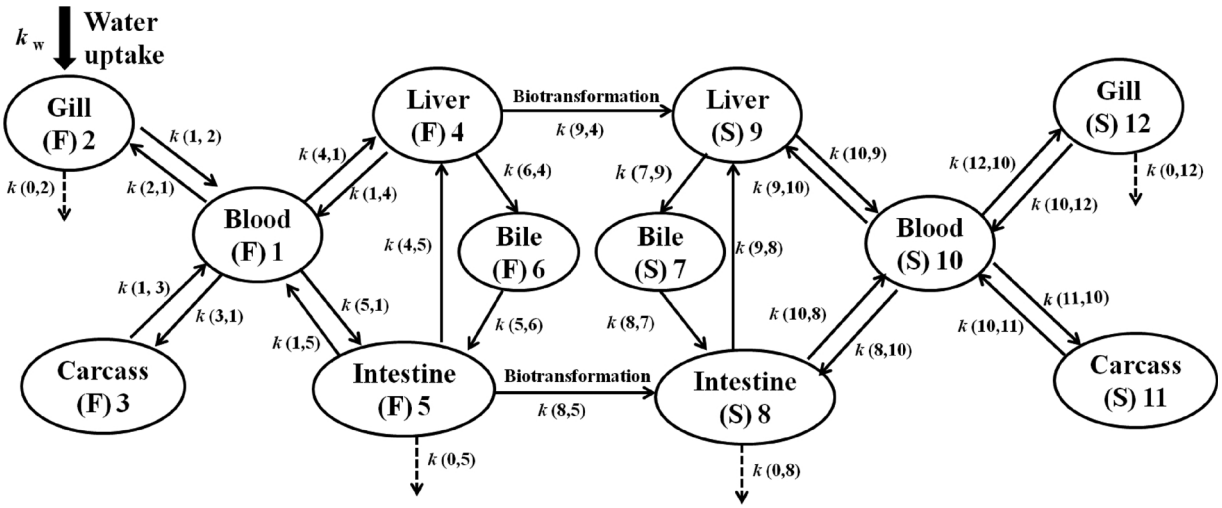



**Fig. 1.** The schematic multi-compartmental transport of fipronil and its metabolite, fipronil sulfone in tilapia. The arrows show transfer direction and $k_{(i,j)}$ represents the inter-compartmental rate coefficients ($h^{-1}$) from *j*th compartment to *i*th compartment, except for the uptake rate coefficient from water ($k_w$, $h^{-1}$) and the transformation rate coefficients ($k_{(9,\ 4)}$ and $k_{(8,\ 5)}$, $h^{-1}$). The capital letters "F" and "S" in parentheses refer to fipronil and its metabolite fipronil sulfone, respectively, in the specific compartment. The numbers are symbols for individual compartments which are used for easier explanation of the rate coefficients.

cycling, and then excreted to outside environment (i.e., water) via gill ventilation and intestine.

Assuming that inter-compartmental transfer of fipronil and its metabolites followed first-order kinetics, the flux from the *j*th to *i*th compartment ($F_{(i,j)}$, ng $h^{-1}$) was calculated using Eq. (1).

$$F_{(i,j)} = k_{(i,j)} \times Q_j \quad (1)$$

Where $k_{(i,j)}$ ($h^{-1}$) is the rate coefficient between the two compartments, $Q_j$ (ng) is the amount of chemical in the *j*th compartment at time *t* and it is calculated by multiplying chemical concentration (ng $g^{-1}$) in the compartment and its fresh weight (g). Total blood volume of the fish was taken into account to calculate the amount of fipronil and its metabolite in the blood, assuming 40 mL blood for 1 kg tissue in teleosts and 1 mL of blood equaled 1 g of fresh weight [24]. The fresh weights, water contents and lipid contents of individual tissues are presented in Table S1. The following differential equations (Eqs. 2–13) were applied to model the dynamic flux of fipronil (F) and its metabolite, fipronil sulfone (S), in the various compartments of the fish.

Blood:

$$dQ_1/dt = k_{(1,2)} \times Q_2 + k_{(1,4)} \times Q_4 + k_{(1,5)} \times Q_5 + k_{(1,3)} \times Q_3 - (k_{(2,1)} + k_{(4,1)} + k_{(5,1)} + k_{(3,1)}) \times Q_1 \quad (2)$$

$$dQ_{10}/dt = k_{(10,9)} \times Q_9 + k_{(10,12)} \times Q_{12} + k_{(10,8)} \times Q_8 + k_{(10,11)} \times Q_{11} - (k_{(9,10)} + k_{(12,10)} + k_{(8,10)} + k_{(11,10)}) \times Q_{10} \quad (3)$$

Gill:

$$dQ_2/dt = k_w \times Q_w + k_{(2,1)} \times Q_1 - (k_{(1,2)} + k_{(0,2)}) \times Q_2 \quad (4)$$

$$dQ_{12}/dt = k_{(12,10)} \times Q_{10} - (k_{(10,12)} + k_{(0,12)}) \times Q_{12} \quad (5)$$

Carcass:

$$dQ_3/dt = k_{(3,1)} \times Q_1 - k_{(1,3)} \times Q_3 \quad (6)$$

$$dQ_{11}/dt = k_{(11,10)} \times Q_{10} - k_{(10,11)} \times Q_{11} \quad (7)$$

Liver:

$$dQ_4/dt = k_{(4,1)} \times Q_1 + k_{(4,5)} \times Q_5 - (k_{(1,4)} + k_{(9,4)} + k_{(6,4)}) \times Q_4 \quad (8)$$

$$dQ_9/dt = k_{(9,4)} \times Q_4 + k_{(9,10)} \times Q_{10} + k_{(9,8)} \times Q_8 - (k_{(10,9)} + k_{(7,9)}) \times Q_9 \quad (9)$$

Bile:

$$dQ_6/dt = k_{(6,4)} \times Q_4 - k_{(5,6)} \times Q_6 \quad (10)$$

$$dQ_7/dt = k_{(7,9)} \times Q_9 - k_{(8,7)} \times Q_7 \quad (11)$$

Intestine:

$$dQ_5/dt = k_{(5,1)} \times Q_1 + k_{(5,6)} \times Q_6 - (k_{(1,5)} + k_{(4,5)} + k_{(8,5)} + k_{(0,5)}) \times Q_5 \quad (12)$$

$$dQ_8/dt = k_{(8,5)} \times Q_5 + k_{(8,10)} \times Q_{10} + k_{(8,7)} \times Q_7 - (k_{(10,8)} + k_{(9,8)} + k_{(0,8)}) \times Q_8 \quad (13)$$

Where $k_{(0,2)}$ and $k_{(0,12)}$ are elimination rate coefficients of fipronil and its metabolite through gill, respectively, while $k_{(0,5)}$ and $k_{(0,8)}$ are their respective elimination rate coefficients through feces. The parameter $Q_w$ is the amount of fipronil in water effectively passing through the gill per hour during respiration, and $k_w$ is rate coefficient of fipronil transferring from water to gill.

$$Q_w = C_w \times V \quad (14)$$

Where $C_w$ (μg $L^{-1}$) is concentration of fipronil in water, and $V$ (L $h^{-1}$) is ventilation rate, which equals to 0.079 L $h^{-1}$ for 12.3 g tilapias based on measurement that ventilation rate was 6.6 L $h^{-1}$ $kg^{-1}$ for tilapia at 21 °C [25].

Biotransformation of fipronil in the liver and intestine was recognized as a rate-limiting step controlled by enzymatic activity, therefore a modified Michaelis–Menten type model was applied to simulate the biotransformation rate coefficients (Eqs. 15 and 16) [26]:

$$k_{(9,4)} = V_{max\text{-}L}/(K_{d\text{-}L} + Q_{L\text{-}F}) \quad (15)$$

$$k_{(8,5)} = V_{max\text{-}I}/(K_{d\text{-}I} + Q_{I\text{-}F}) \quad (16)$$

Where $k_{(9,4)}$ and $k_{(8,5)}$ ($h^{-1}$) are the respective biotransformation rate coefficients of fipronil in the liver and intestine, and $V_{max\text{-}L}$ and $V_{max\text{-}I}$ (ng $h^{-1}$) are the respective maximum biotransformation rate of fipronil in the liver and intestine, $K_{d\text{-}L}$ and $K_{d\text{-}I}$ (ng) are the respective amount of fipronil in the liver and intestine at which the biotransformation rate equals to half of the $V_{max}$, and $Q_{L\text{-}F}$ and $Q_{I\text{-}F}$ (ng) are the amount of fipronil in the liver and intestine, respectively.

The SAAM II modeling software (version 2.3.1, SAAM Institute, University of Washington, Seattle, WA, USA) was applied to simulate the kinetics, which has been successfully used to model the accumulation, distribution and elimination of metals in fish [16]. A process of trials was performed until reaching the best fit. The quality of the fitted parameters was evaluated based on fractional standard deviation (FSD) of the parameters (< 0.5), the parameter correlation matrix (< 0.9) and the appearance of the fitted data-model plots. More details in data

fitting are presented in the Supporting Information. Accumulation, biotransformation and elimination of waterborne fipronil in whole fish were simulated using a one-compartmental model (Fig. S1) (Scientist 3.0, MicroMath Inc., St. Louis, MO, USA). Detailed descriptions on the one-compartmental model and the calculation of bioconcentration factor (BCF) are presented in the Supporting Information. Concentrations of fipronil and fipornil sulfone on a weight basis were used for modeling.

## 3. Results and discussion

### 3.1. Accumulation, biotransformation and elimination in whole fish

Water concentration of fipronil was relatively constant ($0.81 \pm 0.18\,\mu g\ L^{-1}$) over the exposure period (Fig. S2). The three degradates of fipronil were detected in water with concentrations lower than their reporting limits (RLs), suggesting that the 12-h interval of water changes guaranteed the constant exposure scenario. As shown in Fig. S3, fipronil rapidly reached steady-state in tilapia after 24 h of uptake, and then remained constant until the end of exposure, followed by a sharp decrease to near zero at 144 h during the elimination regime. Fipronil was rapidly transformed to fipronil sulfone in tilapia by oxidation at the sulfinyl moiety, while the other two metabolites (fipronil sulfide and fipronil desulfinyl) were both lower than the RLs, which was consistent with that in other fish species [11,15]. Consequently, we focused on the parent compound and its primary metabolite fipronil sulfone in this study. The concentrations of fipronil sulfone in tilapia increased linearly and exceeded the parent compound at 35 h, and were about three times higher than fipronil concentrations at the end of uptake period (96 h). Unlike the rapid elimination of the parent compound, the concentrations of fipronil sulfone only slightly decreased during the elimination period (96 h).

The uptake and elimination profiles of fipronil and fipronil sulfone in the whole body of tilapia were well simulated by the one-compartmental model (Fig. S1 and S3). The simulated biotransformation rate of fipronil to fipronil sulfone in tilapia was $0.03 \pm 0.003\,h^{-1}$, and their $t_{1/2}$ values were $6.5 \pm 1.9$ and $204 \pm 36$ h, respectively, suggesting that the metabolite was more retainable in fish than fipronil (Table S2). Fipronil was transformed to fipronil sulfone at a rate of $0.042\ h^{-1}$ and their $t_{1/2}$ values were $15 \pm 0.7$ and $57 \pm 1.7$ h, respectively, in rainbow trout under dietary exposure [11]. The authors estimated the biotransformation rate based on an empirical relationship between $t_{1/2}$ and log $K_{ow}$, and demonstrated that biotransformation contributed to the most of elimination of fipronil in rainbow trout (i.e., 88%) [11]. In contrast, biotransformation only accounted for 31% of the total elimination of fipronil in tilapia in this study. In addition, elimination of fipronil sulfone from tilapia in this study was about four times slower than that from rainbow trout [11]. Different modeling methods, species sensitivity and exposure routes (waterborne *vs*. dietary) may result in inconsistent kinetics [27,28].

The bioaccumulation potential of waterborne fipronil in tilapia was evaluated by BCF values. The equilibrium-based (Eq. S7) and kinetic-based (Eq. S8) BCFs (1016 and $1047\ L\ kg^{-1}$ lipid, respectively, Table S2) were similar, which provided evidence that the one-compartmental model adequately described the whole body accumulation of fipronil in fish. Regarding the essential role of inter-organ transport in processing xenobiotics [28–30], tissue-specific disposition, biotransformation and inter-compartmental dynamic changes were analyzed to better understand the fate of fipronil in tilapia.

### 3.2. Tissue-specific disposition

Accumulation profiles of fipronil and fipronil sulfone in different compartments of tilapia are shown in Fig. S4. Overall, concentrations of fipronil and fipronil sulfone in individual compartments during the exposure period followed the same trend: liver > intestine ≈ gill > bile ≈ carcass > blood. Higher concentrations of fipronil and fipronil sulfone was also found in the liver than the muscle of wild European eel [15], suggesting that the liver was an important organ for storage and transformation of fipronil likely due to its higher lipid content than other tissues [13,22]. The uptake and elimination trends of fipronil and fipronil sulfone in the individual compartments were similar to those in the whole fish except for fipronil in the liver and intestine. Fipronil levels in the liver and intestine could not achieve a steady-state and started to decrease after reaching the highest point during the exposure period. The decrease suggested possible biotransformation in these two organs. A similar decreasing trend of triphenyl phosphate, a metabolizable organophosphate flame retardant, in the liver of zebrafish was observed during the waterborne exposure period [9].

Based on the physiological-biochemical processes and the tissue-specific accumulation profiles, a multi-compartmental model with two metabolic sites (liver and intestine) was constructed. The simulated

**Table 1**
Simulated rate coefficients of fipronil and its metabolite, fipronil sulfone, between every two compartments in tilapia exposed to waterborne fipronil.

| Transfer direction/ coefficients | Fipronil | | Fipronil sulfone | |
|---|---|---|---|---|
| | Rate coefficients ($h^{-1}$) | Value (mean ± SD)[a] | Rate coefficients ($h^{-1}$) | Value (mean ± SD) |
| Gill to blood | $k_{(1,\ 2)}$ | 1.3 ± 0.09 | $k_{(10,\ 12)}$ | 0.40 ± 0.16 |
| Blood to gill | $k_{(2,\ 1)}$ | 6.9 ± 0.52 | $k_{(12,\ 10)}$ | 1.8 ± 0.68 |
| Blood to carcass | $k_{(3,\ 1)}$ | 13 ± 0.54 | $k_{(11,\ 10)}$ | 2.3 ± 0.20 |
| Carcass to blood | $k_{(1,\ 3)}$ | 0.26 ± 0.04 | $k_{(10,\ 11)}$ | 0.05 ± 0.007 |
| Blood to liver | $k_{(4,\ 1)}$ | 4.6 ± 0.51 | $k_{(9,\ 10)}$ | 3.5 ± 0.22 |
| Liver to blood | $k_{(1,\ 4)}$ | 0.60 ± 0.19 | $k_{(10,\ 9)}$ | 1.5 ± 0.19 |
| Blood to intestine | $k_{(5,\ 1)}$ | 3.1 ± 0.87 | $k_{(8,\ 10)}$ | 0.96 ± 0.47 |
| Intestine to blood | $k_{(1,\ 5)}$ | 0.69 ± 0.20 | $k_{(10,\ 8)}$ | 1.1 ± 0.30 |
| Liver to bile | $k_{(6,\ 4)}$ | 0.55 ± 0.16 | $k_{(7,\ 9)}$ | 0.51 ± 0.10 |
| Bile to intestine | $k_{(5,\ 6)}$ | 1.9 ± 0.47 | $k_{(8,\ 7)}$ | 4.0 ± 0.90 |
| Intestine to liver | $k_{(4,\ 5)}$ | 0.39 ± 0.0.2 | $k_{(9,\ 8)}$ | 0.20 ± 0.11 |
| Gill to water clearance | $k_{(0,\ 2)}$ | 3.8 ± 0.27 | $k_{(0,\ 12)}$ | 0.04 ± 0.01 |
| Intestine to water excretion | $k_{(0,\ 5)}$ | 0.01 ± 0.01 | $k_{(0,\ 8)}$ | 0.01 ± 0.005 |
| Water to gill | $k_w$ | 0.14 ± 0.015 | | |
| Maximum biotransformation rate in the liver (ng $h^{-1}$) | $V_{max\text{-}L}$ | 2.5 ± 0.72 | | |
| Maximum biotransformation rate in the intestine (ng $h^{-1}$) | $V_{max\text{-}I}$ | 2.5 ± 0.87 | | |
| Amount of fipronil in the liver at which the biotransformation rate equals to half of the $V_{max}$ (ng) | $K_{d\text{-}L}$ | 0.66 ± 0.52 | | |
| Amount of fipronil in the intestine at which the biotransformation rate equals to half of the $V_{max}$ (ng) | $K_{d\text{-}I}$ | 0.75 ± 0.11 | | |

[a] Standard deviation (SD).

parameters and modeled curves of fipronil and fipronil sulfone are presented in Table 1 and Fig. 2, respectively. Most of the simulated parameters (90%) had FSDs lower than 0.5, thus the statistical analysis was sufficiently accurate. In addition, the simulated parameters were all functioning independently and the model was not over-parameterized with all correlation coefficients between two parameters lower than 0.9. Overall, the accumulation and elimination profiles of fipronil and its metabolite were well fitted by the constructed model, which validated the application of the model. Uncertainty in the modeling was from the inputs of the model, including fipronil concentration in water, the ventilation rate for tilapia and the fipronil/fipronil sulfone concentrations on weight basis in the six compartments. Overall, the modeled kinetics shed a light on the transport and biotransformation of fipronil inside the fish body.

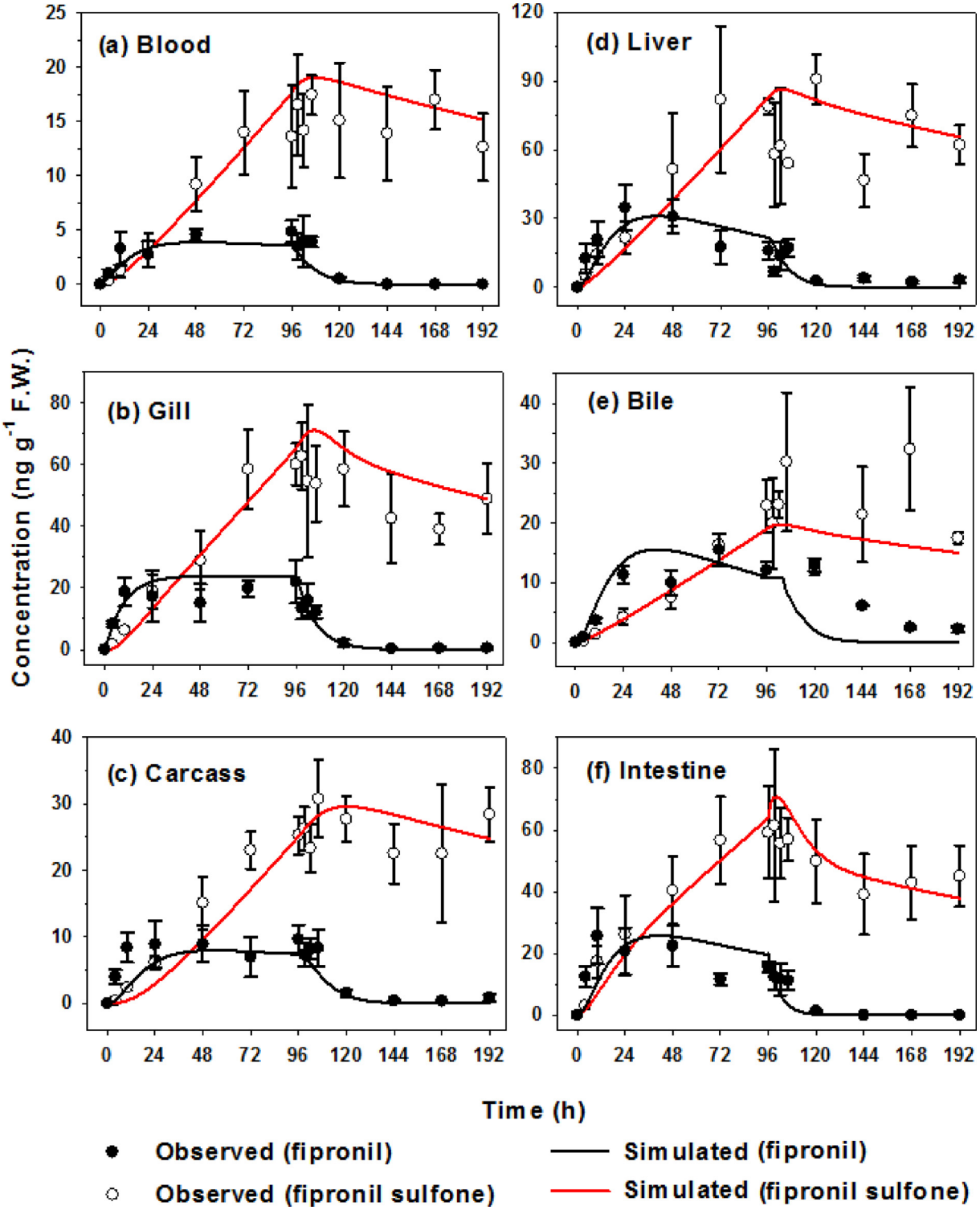



**Fig. 2.** The observed and simulated accumulation (96 h) and elimination (96 h) profiles of fipronil and its metabolite, fipronil sulfone, in different compartments of the tilapia, including blood (a), gill (b), carcass (c), liver (d), bile (e) and intestine (f). The observed data are presented as the mean ± standard deviation, $n = 4$.

### 3.3. Inter-compartmental transport kinetics

In water-only exposure scenario, freely dissolved fipronil is taken up by gills. It was estimated that 64 ng of fipronil was taken up per hour, which was rapidly partitioned to the blood with a rate coefficient of 1.3 ± 0.09 $h^{-1}$, and then subsequently distributed to other parts of the body. As shown in Fig. 3, the largest proportion of fipronil in the blood was transported to the carcass due the dominant mass (more than 90% of the whole body weight) of carcass compared to other tissues, followed by the gill, liver and intestine. A similar flux from the carcass was transported back to the blood, suggesting that the carcass (mainly muscle) was not a storage compartment for fipronil. The flux of fipronil from the gill to blood was 1.7 times higher than that from the blood to gill on average. Gill was the organ taking up waterborne fipronil, and a large proportion of fipronil in the gill was transported to the blood for systemic circulation. On the contrary, the fluxes of fipronil from the blood to the liver and intestine were 2.2 and 1.5 times higher than those transported back to blood on average, respectively, corresponding to the role that these two organs served in transforming and/or storing fipronil.

Fipronil was transformed to fipronil sulfone mainly in the liver and intestine, and then distributed to different parts of the fish via blood circulation. The trends for inter-compartmental transport of fipronil sulfone was different from that of the parent compound (Fig. 3). In comparison to fipronil, the blood-to-intestine flux of fipronil sulfone only accounted for half of that from the intestine to blood. The liver and gill had higher affinity for the hydrophobic fipronil sulfone (log $K_{ow}$ = 3.7) [15] than the intestine and carcass due to their higher lipid contents (Table S1), which may result in the higher transport of fipronil sulfone from the blood to these two compartments. As a richly perfused tissue, the intestine receives high level of volume-normalized blood flow [21,31], which may drive the export of fipronil sulfone from the intestine. Fipronil and fipronil sulfone had different inter-compartmental transport behavior, although they were expected to behave similarly due to their similar hydrophobicity [13,15] (Fig. 3). The opposite blood-intestine net transport fluxes for fipronil (from the blood to intestine) and fipronil sulfone (from the intestine to blood) indicated biotransformation of fipronil in the intestine. Moreover, their opposite net transport fluxes between the blood and intestine/gill suggested that accumulation processes, e.g. uptake and biotransformation, might be stronger driving forces for the transport behavior of xenobiotics in fish than the inherent partitioning capacity of fipronil [32].

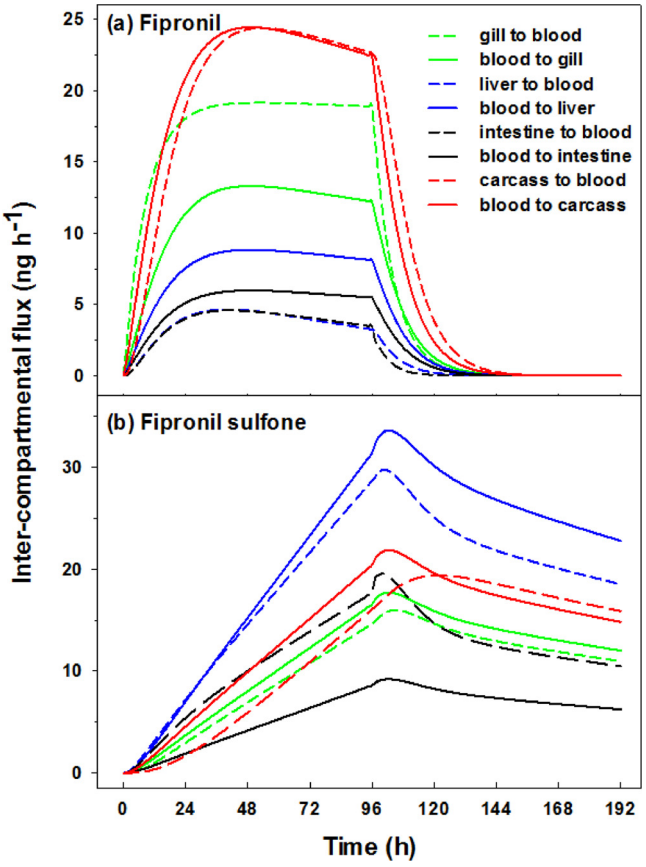



**Fig. 3.** The inter-compartmental flux (ng $h^{-1}$) of fipronil and its metabolite, fipronil sulfone, in tilapia during the accumulation (96 h) and elimination (96 h) regime.

During systemic circulation, elimination of the compounds from the fish occurs through branchial clearance and intestine excretion [28]. The mean estimated uptake (water to gill) and elimination (gill to water) fluxes of fipronil via gill during exposure period were 58 and 52 ng $h^{-1}$, respectively (Fig. S5). Branchial uptake and elimination rates of organic contaminants are controlled by the effective respiration volume and the partitioning coefficient between blood and water, which correlates to chemical hydrophobicity [32]. The uptake and elimination rates based on whole fish in this study (Table S2) were used to calculate the corresponding fluxes. Assuming $k_{ep}$ (Eq. S2) was the elimination rate from branchial clearance, the calculated flux of branchial elimination accounted for 66 ± 24% of the branchial uptake flux, which supported the high proportion of branchial elimination in the multi-compartmental modeling (88% on average). In addition, 58% of fipronil from the gill to blood was transported back to the gill, suggesting that a large proportion of branchial eliminated fipronil involved in systemic circulation instead of being eliminated directly (Fig. 3). Compared to branchial elimination, elimination of fipronil through intestine excretion was negligible with a mean flux of 0.06 ng $h^{-1}$ during exposure. It is reasonable that branchial elimination dominates the elimination of organic contaminants from fish body in waterborne exposure scenario without feeding [28,33].

The limited intestine excretion was from biliary excretion, among which could either be re-absorbed by the intestine and further involved in the systemic circulation, or could be excreted out of the fish body [13,34]. Fipronil sulfone was much more retainable than fipronil in the fish, with smaller mean fluxes of branchial clearance and intestine excretion (i.e., 1.0 and 0.13 ng $h^{-1}$, respectively). The fact that fipronil sulfone persisted longer in organism than the parent compound has been previously reported, yet the mechanism is unknown [13]. From the perspective of multi-compartmental elimination and transport, the slower branchial elimination of fipronil sulfone than fipronil may be the direct reason for the longer half-life of the metabolite in tilapia. In addition, the net transport of fipronil sulfone from the blood to the liver, gill and carcass (mainly muscle) for storage may also prolong its retainability in fish.

### 3.4. Hepatobiliary-intestinal biotransformation

Recognizing metabolic site(s) and quantifying biotransformation rate are essential to evaluating the bioaccumulation and toxicity of xenobiotics in fish. Given the consensus that the liver is the primary organ metabolizing pesticides in fish [22] and the observation that fipronil concentrations in tilapia liver significantly decreased during exposure (Fig. 2d), the liver was set as the site for biotransformation. Regarding the significant decrease of fipronil concentrations in the intestine during exposure (Fig. 2f), there were two possible reasons: (1) fipronil was transformed in the intestine, or (2) fipronil was transformed in the liver and then transferred to the intestine via the rapid hepatoenteral and/or blood circulation. If the latter happened, fipronil concentrations in the bile and/or blood should follow the same decreasing trend as the liver and intestine. However, fipronil in the bile and blood reached steady-state and then kept constant till the end of exposure period (Fig. 2). Thus, biotransformation occurring in the

intestine was the more plausible explanation for the observed decrease of fipronil in this organ. Biotransformation of xenobiotics in the intestinal tract of fish has been commonly observed in dietary exposure scenario due to resident bacteria, digestive and intestinal mucosal enzymes [35]. A recent study found that intestinal biotransformation was the dominant metabolic pathway for most of the tested chemicals via dietary exposure, and somatic (including the liver) biotransformation was the dominant pathway via waterborne exposure [23]. However, there were some exceptions, for example, *trans*-decalin was mostly transformed in the intestinal tract under the waterborne exposure [23].

Considerable efforts have been undertaken to obtain whole body and tissue-specific biotransformation rates [23,26,36]. Constant biotransformation rates were conventionally used in modeling, however, the metabolic process is rate limited by the activities of metabolic enzymes, which follows a dose-responsive change with the concentrations of xenobiotics [13]. In this study, constant biotransformation rates failed to model the decreasing trend of fipronil in the liver and intestine during exposure, so a Michaelis–Menten type model was introduced in the simulation. The sum biotransformation flux of fipronil in the liver and intestine (5.02 ng $h^{-1}$ on average during exposure) was consistent with its biotransformation flux (4.55 ng $h^{-1}$) estimated from the one-compartmental whole fish model, confirming the applicability of the developed multi-compartmental model. The biotransformation fluxes of fipronil in the liver and intestine were comparable (Fig. S5), suggesting that the two organs equally contributed to the biotransformation of fipronil to fipronil sulfone in tilapia. However, the two organs metabolized fipronil differently. The biotransformation flux of fipronil in the liver continued to increase till the end of exposure, yet it increased until it reached steady-state and then remained constant in the intestine for the remaining exposure time (Fig. S5). There is limited information on the mechanisms of hepatic and intestinal biotransformation of fipronil. Comparable metabolic capacity of organophosphate pesticides in liver microsomes and intestinal enterocytes of rats were observed, but the metabolic profiles were different for the two pesticides [37]. On the contrary, liver was found to have higher capacity to metabolize a drug than small intestine based on the total enzyme activity and inherent clearance [38]. It appears that metabolic function in regards to processing xenobiotics is species-, tissue- and compound-specific. The present study quantitatively evaluated the biotransformation of fipronil in the liver and intestine of tilapia, but more studies are required to mechanistically understand the tissue-specific biotransformation of fipronil in fish.

After being metabolized in the liver, the formed metabolite is excreted to the intestine via bile along with the parent compound [34,39]. The compounds excrete to the small intestine may be re-absorbed and transferred back to the liver, namely enterohepatic cycling, which may prolong the half-life of the compounds in the fish body. Therefore, the dynamic transport of fipronil and fipronil suflone in the liver-bile-intestine system is an important part of systemic circulation. The transport flux of fipronil from the liver to the bile resembled that from the liver to the blood, suggesting that hepatobiliary excretion accounted for half of the systemic circulation in the liver (Fig. S6). The liver-to-bile and bile-to-intestine fluxes of fipronil were exactly the same, supporting the notion that the bile was a transient compartment between the liver and intestine [34]. A mean flux of 2.0 ng $h^{-1}$ from the intestine to the liver was observed and accounted for half of the flux from the intestine to the blood, suggesting significant enterohepatic cycling of fipronil in tilapia (Fig. S6a). Compared to the parent compound, the hepatobiliary (34% on average) and enterhepatic (18% on average) circulations of fipronil sulfone accounted for a smaller proportion of the corresponding circulation via blood (Fig. S6b). Therefore, the liver-bile-intestine circulation was an important part of the systemic circulation and not only influenced the *in vivo* biotransformation of fipronil, but also the inter-compartmental transport kinetics of the parent compound and the metabolite in fish.

## 4. Conclusions and perspectives

Overall, the developed multi-compartmental model for the first time quantified the uptake, biotransformation, inter-compartmental transport and elimination of fipronil and its metabolite in tilapia. This model helped to interpret the *in vivo* disposition and biotransformation of fipronil and recognize the importance of individual compartments within the fish. Based on the observed and simulated results, fipronil was transformed to fipronil sulfone in the liver and intestine under waterborne exposure. Hepatobiliary excretion and enterohepatic re-absorption in the liver-bile-intestine system played an important role in fipronil biotransformation and systemic circulation of the parent compound and the present metabolite. The modeled kinetics revealed that fipronil was highly dynamic in tilapia experiencing rapid biotransformation, inter-compartmental transport and branchial clearance, which significantly influenced the body residues, disposition and toxicity of fipronil. The metabolite, fipronil sulfone, was equally or even more toxic to fish [13,40] and was much more retainable than fipronil in fish, implying that *in vivo* biotransformation would largely increase the toxicity of fipronil to fish. As such, the incorporation of biotransformation into bioaccumulation modeling surely improves the toxicity evaluation and prediction of easily metabolizable toxicants. Fipronil is known to induce neurotoxicity, while tissue-specific toxicity has also been observed for the liver [41] and kidney [42]. The modeled metabolism and inter-compartmental transport kinetics provided by the present study gives quantitative information on the tissue-specific disposition of fipronil and its metabolite in fish. It is expected that this information will assist in quantify their toxicity to specific organs in future investigations.

## Acknowledgements

We thank Jie Zhang for help in sample preparation. This research was supported by the National Natural Science Foundation of China (Grant No. 41473106) and the Natural Science Foundation of Guangdong Province, China (Grant No. 2017A030313065).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jhazmat.2018.07.085.

## References

[1] H.-R. Köhler, R. Triebskorn, Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond? Science 341 (2013) 759–765.
[2] S. Stehle, R. Schulz, Agricultural insecticides threaten surface waters at the global scale, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 5750–5755.
[3] J.M. Bonmatin, C. Giorio, V. Girolami, D. Goulson, D.P. Kreutzweiser, C. Krupke, M. Liess, E. Long, M. Marzaro, E.A.D. Mitchell, D.A. Noome, N. Simon-Delso, A. Tapparo, Environmental fate and exposure: neonicotinoids and fipronil, Environ. Sci. Pollut. Res. 22 (2015) 35–67.
[4] C.C.D. Tingle, J.A. Rother, C.F. Dewhurst, S. Lauer, W.J. King, Fipronil: environmental fate, ecotoxicology, and human health concerns, Rev. Environ. Contam. Toxicol. 176 (2003) 1–66.
[5] D.P. Weston, M.J. Lydy, Toxicity of the insecticide fipronil and its degradates to benthic macroinvertebrates of urban streams, Environ. Sci. Technol. 48 (2014) 1290–1297.
[6] M.G. Nillos, K. Lin, J. Gan, S. Bondarenko, D. Schlenk, Enantioselectivity in fipronil aquatic toxicity and degradation, Environ. Toxicol. Chem. 28 (2009) 1825–1833.
[7] L.W. Sun, R. Jin, Z.H. Peng, Q.W. Zhou, H.F. Qian, Z.W. Fu, Effects of trilostane and fipronil on the reproductive axis in an early life stage of the Japanese medaka (*Oryzias latipes*), Ecotoxicology 23 (2014) 1044–1054.
[8] W.W. Stone, R.J. Gilliom, K.R. Ryberg, Pesticides in U.S. streams and rivers: occurrence and trends during 1992–2011, Environ. Sci. Technol. Lett. 48 (2014) 11025–11030.
[9] G. Wang, Z. Du, H. Chen, Y. Su, S. Gao, L. Mao, Tissue-specific accumulation, depuration, and transformation of triphenyl phosphate (TPHP) in adult zebrafish (*Danio rerio*), Environ. Sci. Technol. 50 (2016) 13555–13564.
[10] S. Beggel, I. Werner, R.E. Connon, J.P. Geist, Impacts of the phenylpyrazole insecticide fipronil on larval fish: time-series gene transcription responses in fathead minnow (*Pimephales promelas*) following short-term exposure, Sci. Total Environ.

426 (2012) 160–165.
[11] B.J. Konwick, A.W. Garrison, M.C. Black, J.K. Avants, A.T. Fisk, Bioaccumulation, biotransformation, and metabolite formation of fipronil and chiral legacy pesticides in rainbow trout, Environ. Sci. Technol. 40 (2006) 2930–2936.
[12] O.G. Júnior, W.B. Neto, A.E.H. Machado, D. Daniel, A.G. Trovó, Optimization of fipronil degradation by heterogeneous photocatalysis: identification of transformation products and toxicity assessment, Water Res. 110 (2017) 133–140.
[13] X. Wang, M.A. Martinez, Q.H. Wu, I. Ares, M.R. Martinez-Larranaga, A. Anadon, Z.H. Yuan, Fipronil insecticide toxicology: oxidative stress and metabolism, Crit. Rev. Toxicol. 46 (2016) 876–899.
[14] H. Qu, R.-x. Ma, D.-h. Liu, J. Gao, F. Wang, Z.-q. Zhou, P. Wang, Environmental behavior of the chiral insecticide fipronil: enantioselective toxicity, distribution and transformation in aquatic ecosystem, Water Res. 105 (2016) 138–146.
[15] N. Michel, M. Freese, M. Brinkmann, J.-D. Pohlmann, H. Hollert, U. Kammann, M. Haarich, N. Theobald, W. Gerwinski, W. Rotardd, R. Hanel, Fipronil and two of its transformation products in water and European eel from the river Elbe, Sci. Total Environ. 568 (2016) 171–179.
[16] X. Wang, F. Wu, W.-X. Wang, *In vivo* mercury demethylation in a marine fish (*Acanthopagrus schlegeli*), Environ. Sci. Technol. 51 (2017) 6441–6451.
[17] M.P. Matlala, M.D. Kpundeh, Y. Yuan, Production and marketing systems of farmed tilapia in China, Int. J. Fish. Aquac. 5 (2013) 12–18.
[18] R. Wang, W.-X. Wang, Importance of speciation in understanding mercury bioaccumulation in tilapia controlled by salinity and dissolved organic matter, Environ. Sci. Technol. 44 (2010) 7964–7969.
[19] C.M. Liao, H.M. Liang, B.C. Chen, S. Singh, J.W. Tsai, Y.H. Chou, W.T. Lin, Dynamical coupling of PBPK/PD and AUC-based toxicity models for arsenic in tilapia *Oreochromis mossambicus* from blackfoot disease area in Taiwan, Environ. Pollut. 135 (2005) 221–233.
[20] B. Clasen, V.L. Loro, R. Cattaneo, B. Moraes, T. Lopes, L.A. de Avila, R. Zanella, G.B. Reimche, B. Baldisserotto, Effects of the commercial formulation containing fipronil on the non-target organism *Cyprinus carpio*: implications for rice – fish cultivation, Ecotoxicol. Environ. Saf. 77 (2012) 45–51.
[21] J.W. Nichols, J.M. McKim, M.E. Andersen, M.L. Gargas, H.J. Clewell, R.J. Erickson, A physiologically based toxicokinetic model for the uptake and disposition of waterborne organic chemicals in fish, Toxicol. Appl. Pharmacol. 106 (1990) 433–447.
[22] D. Schlenk, Pesticide biotransformation in fish, Biochem. Molecul. Biol. Fishes 6 (2005) 171–190.
[23] J.C. Lo, D.J. Letinski, T.F. Parkerton, D.A. Campbell, F.A.P.C. Gobas, In vivo biotransformation rates of organic chemicals in fish: relationship with bioconcentration and biomagnification factors, Environ. Sci. Technol. 50 (2016) 13299–13308.
[24] M.H. Horn, Feeding and digestion, in: D.H. Evans (Ed.), The Physiology of Fishes, CRC, Boca Raton, FL, USA, 1998, pp. 65–97.
[25] M. Maricondi-Massaria, A.L. Kalinina, M.L. Glassb, F.T. Rantina, The effects of temperature on oxygen uptake, gill ventilation and ecg waveforms in the nile tilapia, *Oreochromis niloticus*, J. Therm. Biol. 23 (1998) 283–290.
[26] J.W. Nichols, P.N. Fitzsimmons, L.P. Burkhard, In vitro-in vivo extrapolation of quantitative hepatic biotransformation data for fish. II. Modeled effects on chemical bioaccumulation, Environ. Toxicol. Chem. 26 (2007) 1304–1319.
[27] F. Dang, W.-X. Wang, P.S. Rainbow, Unifying prolonged copper exposure, accumulation, and toxicity from food and water in a marine fish, Environ. Sci. Technol. 46 (2012) 3465–3471.
[28] B. Streit, Bioaccumulation of contaminants in fish, Fish Ecotoxicol. 86 (1998) 353–387.
[29] A. Grech, C. Brochot, J.L. Dorne, N. Quignot, F.Y. Bois, R. Beaudouin, Toxicokinetic models and related tools in environmental risk assessment of chemicals, Sci. Total Environ. 578 (2017) 1–15.
[30] M. Brinkmann, C. Schlechtriem, M. Reininghaus, K. Eichbaum, S. Buchinger, G. Reifferscheid, H. Hollert, T.G. Preuss, Cross-species extrapolation of uptake and disposition of neutral organic chemicals in fish using a multispecies physiologically-based toxicokinetic model framework, Environ. Sci. Technol. 50 (2016) 1914–1923.
[31] J. Stadnicka, K. Schirmer, R. Ashauer, Predicting concentrations of organic chemicals in fish by using toxicokinetic models, Environ. Sci. Technol. 46 (2012) 3273–3280.
[32] D. Mackay, A. Fraser, Bioaccumulation of persistent organic chemicals: mechanisms and models, Environ. Pollut. 110 (2000) 375–391.
[33] P.N. Fitzsimmons, J.D. Fernandez, A.D. Hoffman, B.C. Butterworth, J.W. Nichols, Branchial elimination of superhydrophobic organic compounds by rainbow trout (*Oncorhynchus mykiss*), Aquat. Toxicol. 55 (2001) 23–34.
[34] M. Lahti, J.-M. Brozinski, A. Jylhä, L. Kronberg, A. Oikari, Uptake from water, biotransformation, and biliary excretion of pharmaceuticals by rainbow trout, Environ. Toxicol. Chem. 30 (2011) 1403–1411.
[35] K.M. Kleinow, M.O. James, Z. Tong, C.S. Venugopalan, Bioavailability and biotransformation of benzo(a)pyrene in an isolated perfused in situ catfish intestinal preparation, Environ. Health Persp. 106 (1998) 155–166.
[36] D.T.F. Kuo, D.M.D. Toro, Biotransformation model of neutral and weakly polar organic compounds in fish incorporating internal partitioning, Environ. Toxicol. Chem. 32 (2013) 1873–1881.
[37] T.S. Poet, H. Wu, A.A. Kousba, C. Timchalk, In vitro rat hepatic and intestinal metabolism of the organophosphate pesticides chlorpyrifos and diazinon, Toxicol. Sci. 72 (2003) 193–200.
[38] M.F. Paine, M. Khalighi, J.M. Fisher, D.D. Shen, K.L. Kunze, C.L. Marsh, J.D. Perkins, K.E. Thummel, Characterization of inter- and intra-intestinal differences in human CYP3A dependent metabolism, J. Pharmacol. Exp. Ther. 283 (1997) 1552–1562.
[39] J.-M. Kallio, M. Lahti, A. Oikari, L. Kronberg, Metabolites of the aquatic pollutant diclofenac in fish bile, Environ. Sci. Technol. 44 (2010) 7213–7219.
[40] D. Hainzl, L.M. Cole, J.E. Casida, Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct, Chem. Res. Toxicol. 11 (1998) 1529–1535.
[41] P.R.D. Oliveira, G.H. Bechara, S.E. Denardi, R.J. Oliveira, M.I.C. Mathias, Cytotoxicity of fipronil on mice liver cells, Microsc. Res. Tech. 75 (2012) 28–35.
[42] P.C. Badgujar, N.N. Pawar, G.A. Chandratre, A.G. Telang, A.K. Sharma, Fipronil induced oxidative stress in kidney and brain of mice: Protective effect of vitamin E and vitamin C, Pest. Biochem. Physiol. 118 (2015) 10–18.