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Toxicokinetic/toxicodynamic-based risk assessment of freshwater fish health posed by microplastics at environmentally relevant concentrations

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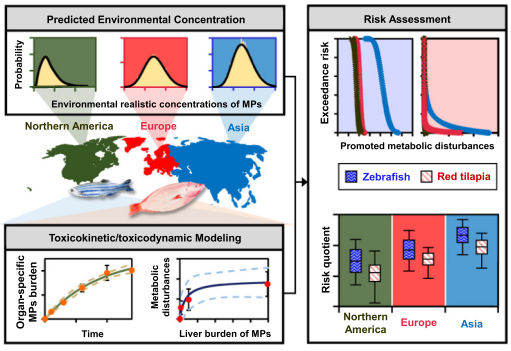
HIGHLIGHTS

- A TK/TD risk assessment framework for freshwater fish-MPs system is established.
- An alarming is implicated for MPs pollution in major freshwaters over Asia.
- MPs pollution is likely to enhance fish health risk due to metabolic disturbances.

GRAPHICAL ABSTRACT



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ABSTRACT

The pervasive contamination of microplastics (MPs) in freshwater ecosystems is of emerging concern. Mechanistic link between exposure and effect on assessing health risk of freshwater fish posed by environmental MPs, however, is more limited. Our study filled this gap by developing a toxicokinetic/toxicodynamic (TK/TD)-based risk assessment framework to examine health effects of zebrafish and red tilapia responses to environmental concentrations of MPs appraised with a variety of valuable published data on a global scale. We assessed organ-specific TK parameters and mean residence times for polystyrene (PS)-MPs-exposed freshwater fish in size- and concentration-dependent manners. We estimated the relatively sensitive benchmark concentrations (BMCs) of PS-MPs for oxidative stress in zebrafish and detoxification in red tilapia to be ~1.0 and ~119 μg g^{-1}, respectively. Based on continental scale MPs trends, the high MPs concentrations were over Asia, with a mean value of 36 mg L^{-1}. Given metabolic disturbances in zebrafish and red tilapia as bioindicators, we found that MPs pollution was highly likely to enhance fish health risks and that this factor must therefore be considered in evaluations of MPs susceptibility of freshwater fish. Our TK/TD-based risk scheme could help inform intensified efforts to mitigate environmental MPs pollution in order to benefit freshwater fish species and people who depend on healthy stocks of different fish.

1. Introduction

Microplastics (MPs) (plastic particle <5 mm) have become pervasive in all environmental compartments including aquatic, terrestrial, and atmospheric environments (Dris et al., 2018; Mai et al., 2018). Since the ubiquity and massive number of MPs in oceans have been

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revealed, more attention has given to the contribution of freshwater systems as main emitting sources of MPs. A recent report indicated that a total of 106 studies on plastic pollution in freshwater environments worldwide were recorded (Blettler et al., 2018). Two studies carried out in Africa showed that 35% and 76% of the sampled Nile tilapia from Lake Victoria and Nile River, respectively, had MP residues in their digestive tracts (Biginagwa et al., 2016; Khan et al., 2020).

Given the broad evidence of freshwater fish prone to MPs ingestion, the MPs-fish interactions including accumulation, excretion, and a wide range of sublethal endpoints such as tissue inflammation have been largely explored under the laboratory conditions (Huang et al., 2020; Khan et al., 2018; Qiao et al., 2019; Yang et al., 2020). Based on a Google Scholar searching, 27 and 9 publications have studied the interactions between MPs and zebrafish and tilapia, respectively. Available data suggested that significant alterations of metabolic disturbances such as oxidative stress and enzymatic detoxification in fish have been used in many toxicological studies as prominent indicators for environmental toxicant-induced stresses (Batel et al., 2020; Hamed et al., 2020; Qiao et al., 2019; Zhang et al., 2019). However, whether the extent of the MPs-induced potential health impact on freshwater fish in real habitats is often not clearly examined. A prior need for appraising bioaccumulation kinetics and dose-response functions of MPs in fish bodies could efficiently address such knowledge gap.

While elements for characterizing the risks of MPs are still limited, the ecological risks of MPs to organisms are an emerging issue proposed in several studies (Adam et al., 2019; Besseling et al., 2019; Burns and Boxall, 2018). As anthropogenic MPs pollution in freshwater ecosystems continues the risks to biota will increase over time, with future predictions indicating that a potentially catastrophic impact is imminent (Adam et al., 2019). Yet to date, our ability to develop environmental MPs mitigation measures are insufficient to sustain MPs-exposed fish in a healthy status. Mainly because of the fragmented toxicological information and scarcity of field and experimental data, limiting our understanding of the linkage between environmental MPs exposure and its effect on assessing the health risk of freshwater fish in a mechanistic point of view.

The purpose of this study was to provide a risk-based predictive model to assess the potential impact of MPs on freshwater fish health risk by combining field and experimental data to generate the parsimonious risk metrics on the global scale. A toxicity-based toxicokinetic/toxicodynamic (TK/TD) modeling was developed for mechanistically describing the organ-specific bioaccumulation trends and MPs-induced metabolic disturbances in zebrafish and red tilapia. Based on the continent-specific predicted environmentally relevant concentrations of MPs in freshwater environments, we performed a reliable prediction for exposure risk assessment of zebrafish and red tilapia in response to MPs.

2. Materials and methods

2.1. Study data

To investigate and to quantify uptake, distributions, and induced adverse effects of MPs in freshwater fish, our study adopted valuable datasets of bioaccumulation and toxicity assays related to zebrafish *Danio rerio* and red tilapia *Oreochromis niloticus* from Lu et al. (2016) and Ding et al. (2018), respectively. They used polystyrene (PS)-MPs to surrogate MPs, a most common polymer type MP debris. Briefly, Lu et al. (2016) selected two sizes of 5 and 20 μm PS-MP beads together with zebrafish as a model organism. After 2 weeks of acclimation and a 24-h starvation, six adult zebrafish (5-mon-old with 0.29 ± 0.022 g (mean ± SD) in wet wt) were placed into 30 glass tanks each with 20 mg L^{-1} fluorescent PS-MPs. Three replicates of tanks were sampled on the 4th, 12th h, 1st, 2nd, and 7th day. Gills, liver, and gut were then removed to determine the amounts of PS-MPs in two particle sizes (Table S1). Due to no 20 μm PS-MP beads were found in liver, the time-course of 20 μm PS-MPs in liver was not available (Table S1).

The other exposure experiment was conducted by Ding et al. (2018) by using red tilapia (21 ± 3.9 g in wet wt, and 9.5 ± 1.7 cm in body length). Three exposure concentrations of 0.1 μm fluorescent PS-MP beads prepared in each three replicate tanks were 0.001, 0.01, and 0.1 mg L^{-1}. One fish from each tank was sampled for accumulation tests for PS-MPs in brain, gills, liver, and gut after the 0th, 1st, 3rd, 6th, 10th, and 14th day (Table S2).

The MPs-induced toxicity, oxidative stress and detoxification activity in liver of zebrafish and red tilapia, respectively, were measured (Ding et al., 2018; Lu et al., 2016). Toxic exposure of 5 μm PS-MPs was adopted in this study since no accumulation was observed in liver posed by 20 μm PS-MPs (Table S1). In brief, 60 zebrafish were divided into 12 tanks. The exposure concentrations of 5 μm PS-MPs in three tanks were 0, 0.02, 0.2, and 2 mg L^{-1}, respectively. After 3 weeks of exposure, fish livers were sampled to identify activities of catalase (CAT) and superoxide dismutase (SOD) proteins. The normalized data of oxidative stress in fish liver at different exposure concentrations to the control were then reanalyzed (Table S3).

The other toxicity test used four MPs exposure concentrations (0, 0.001, 0.01, and 0.1 mg L^{-1}) to measure the activities of 7-benzyloxy-4-trifluoromethyl-coumarin *O*-dibenzyloxylase (BFCOD) and 7-ethoxyresorufin O-deethylase (EROD) related to cytochrome P450 (CYP) enzymes in liver of red tilapia during 14 days of exposure (Ding et al., 2018). We presented the raw response data instead of normalization to exhibit the significant decreases followed by increases compared to the control (Table S3). We then reanalyzed experimental data for both fish species to mechanistically estimate TK/TD behaviors of MPs exposure.

2.2. Predicting environmentally relevant concentration of MPs

A systematic review was conducted for data collection of environmental concentrations of MPs at a global scale to comprehend the severity of MPs pollution that freshwater fish have faced with. Search terms like MPs, freshwater, river, lake, and wastewater were entered into Google Scholar to retrieve as many publications or reports as possible. A total of 73 articles were initially considered for inclusion in the systematic review as of October 2019. Data extracted from studies included sampling location, depth, and period, as well as MPs abundance. The tow depth was determined based on one-half mouth height of nets if authors did not provide directly in articles.

We developed an algorithm for data compilation and treatment of environmental concentrations of MPs constrained to the following criteria and instructions (Fig. S1). First, given the typical habitats of zebrafish and red tilapia, concentration data sampled from surface water were considered to perform realistic exposure conditions for pelagic fish (CABI, 2019; Engeszer et al., 2007). The selected freshwater systems were mainly categorized into river, lake, and wastewater. Due to highly temporal variation, the second tier of screening criteria was that literature needed to be published in recent years from 2015 to 2019.

Concentration data of MPs from 44 remained researches were reported in various units. Those expressed as a mass unit were ideal for predicting internal burden. Whereas data in numerical concentrations needed to be converted into mass concentrations by using a log-log linear-square regression derived from Cózar et al. (2014),

$$\log m = 1.21 \times \log a - 3.99 \quad (1)$$

where m and a are the MPs concentration in mass unit (g km^{-2}) and amount unit (items km^{-2}), respectively. Each tow depth would then

be applied to convert all data in mass unit into "mg mL^{-1}". During this process, 9 papers were excluded due to insufficient information, such as unclear mouth dimension of net and concentration data only presented in a single value.

Finally, we compiled the selected concentration data and found out that all sampled sites were distributed in Northern Hemisphere. Geographically, 13 studies were performed in Asia (China, South Korea, and Vietnam), 10 in Europe (Spain, Portugal, Italy, Germany, France, Swiss, UK, and Finland), and 12 in Northern America (USA and Canada) (Table S7). Data in the same continent were pooled into a predicted environmental concentration (*PEC*) based on the assumption of lognormal (LN) distribution performed by Monte Carlo (MC) simulation.

2.3. Toxicokinetic (TK) modeling of PS-MPs in freshwater fish

A one-compartment TK model was used to perform the time course of internal PS-MPs accumulations in target organs of freshwater fish. The differential equation could be written as

$$\frac{dC_{f,i}(t)}{dt} = k_u C_w - k_e C_{f,i}(t) \tag{2}$$

with a solution of

$$C_{f,i}(t) = C_{f,i}(t=0)e^{-k_e t} + \frac{k_u}{k_e} C_w \left(1 - e^{-k_e t}\right) \tag{3}$$

where $C_{f,i}(t)$ is the PS-MPs concentration in specific organ i of fish (mg g^{-1} bw) at the exposure time t (d), k_u is the uptake rate constant of organ from the culture water with PS-MPs by zebrafish or red tilapia (mL g^{-1} d^{-1}), k_e is the elimination rate constant of PS-MPs in organ by zebrafish or red tilapia (d^{-1}), and C_w is the constant PS-MPs concentration of culture water in tank (mg mL^{-1}).

When $C_{f,i}(t)$ no longer varies with exposure duration, the dynamic PS-MPs accumulations of specific organ i in zebrafish nearly approached a steady-state process. Whereas the PS-MPs accumulations in red tilapia did not reach equilibrium at any scenarios owing to insufficient exposure period. Therefore, only the TK models of zebrafish can be simplified to obtain the steady-state bioconcentration factor (BCF_{ss}),

$$C_{f,i}(t=\infty) = \frac{k_u}{k_e} C_w = BCF_{ss} \times C_w \tag{4}$$

The BCF_{ss} could be calculated as the ratio of uptake and elimination rate constants. It potentially reflects the equilibrium of PS-MPs accumulation in organs from continuous exposure.

2.4. Organ-specific mean residence time (MRT) estimates

To fathom the difference of size-specific or concentration-dependent PS-MPs accumulations in organs, the mean residence times (MRTs) were also estimated. The organ-specific MRT could be calculated based on the data from accumulation bioassays (Tables S1 and S2),

$$\overline{t_i} = \frac{\int_0^t t C_{f,i}(t)dt}{\int_0^t C_{f,i}(t)dt} \tag{5}$$

where $\overline{t_i}$ is the MRT of PS-MPs in a specific organ i (d).

2.5. Toxicodynamic (TD)-based concentration-response profiles

The concentration-response relationship between internal concentrations of PS-MPs and biological effects in freshwater fish can be described by a non-linear Hill-based TD model. The Hill equation firstly formulates a reversible reaction to the variable concentration of a substance and is widely used in pharmacology and toxicology (Hill, 1910). In red tilapia system, exposure PS-MPs concentrations were firstly converted into internal effect concentrations in liver based on the developed TK model. The detoxification function of liver posed by PS-MPs can then be described by a four-parameter Hill-based TD model as,

$$E(C_L) = E_{min} + \frac{(E_{max} - E_{min}) \times {C_L}^n}{EC50^n + {C_L}^n} \tag{6}$$

where C_L is the PS-MPs burden in liver (mg g^{-1}), $E(C_L)$ represents the activity of BFCOD or EROD enzymes (pmol mg^{-1} pro min^{-1}) to a specific liver burden of PS-MPs (C_L) in red tilapia, E_{min} is the minimum biomarker expression in the absence of exposure to PS-MPs (pmol mg^{-1} pro min^{-1}), E_{max} is the maximal activities of enzymes (pmol mg^{-1} pro min^{-1}) for an infinite C_L, $EC50$ is the effective concentration of PS-MPs (μg g^{-1}) evoking 50% maximal enzyme activity ($E_{max} - E_{min}$), and n is the Hill coefficient characterizing the slope of the concentration-response curve at the midpoint. If $n > 1$, there is a positive interaction between PS-MPs and the biomarker (Gesztelyi et al., 2012; Yifrach, 2004).

In zebrafish system, internal PS-MPs concentrations in fish liver were obtained from multiplications of exposure concentrations by the estimated BCF_{ss} values. Owing to the normalized process to biomarkers responses resulting in $E_{min} = 0$ in zebrafish system, the TD model can be simplified to a three-parameter Hill equation as,

$$E(C_L) = \frac{E_{max} \times {C_L}^n}{EC50^n + {C_L}^n} \tag{7}$$

where $E(C_L)$ represents the activity of CAT or SOD to a specific burden of PS-MPs (C_L) and E_{max} is the maximum change of CAT and SOD activities (fold increase from control).

To further determine the threshold level of PS-MPs burden, a biomarker-based benchmark approach was employed based on the present constructed TD model. The benchmark response (BMR) was set to a conservative percentage of 10% promoted effect (BMR_{10}) in the concentration-response relationship. Correspondingly, the effective concentration that induced BMR_{10} would be referred to as benchmark concentration (BMC_{10}) for being the threshold values in fish liver.

2.6. Exposure risk profiling

Exceedance risk (ER) and risk quotient (*RQ*) as two risk metrics were used to perform the probabilistic risk assessment. *PEC*s of MPs in freshwater systems were transformed into C_L via the constructed TK model. Based on the developed TD model, we could understand the continent-based concentration-response relationship of the metabolic disturbances occurring at internal concentration levels. Therefore, given the probabilistic distribution of C_L as prior probability ($P(C_L)$) and the conditional probability of $P(E|C_L)$, the joint probability of $\Phi(R)$ could be obtained as:

$$\Phi(R) = P(C_L) \times P(E|C_L) \tag{8}$$

where $\Phi(R)$ indicates the risk of MPs burden-induced effect in freshwater fish. The ER can then be expressed as $1 - \Phi(R)$.

On the other hand, the continent-based *RQ* can be calculated by utilizing BMC_{10} as:

$$RQ = \frac{C_L}{BMC_{10}} \tag{9}$$

where C_L and BMC_{10} were treated probabilistically. $RQ > 1$ indicates that there is a potential risk for the freshwater fish health

posed by the likely concentrations of MPs in liver, whereas $RQ < 1$ implies no significant risk of environmentally relevant waterborne MPs.

2.7. Uncertainty analysis and simulation schemes

The overall schematic framework delineates the mechanistic approach and key elements for global impact assessment of MPs (Fig. S2). We employed the TableCurve 2D (Version 5.01, AISN Software Inc., Mapleton, OR, USA) to simulate all model fittings to the published data. Optimal model fittings were based on least squared criterion provided by TableCurve 2D, and a value of $p < 0.05$ was judged significant. Ordinary differential equations in the TK modeling were solved by the Mathematica® (Version 11.2, Wolfram Research Inc., Champaign, IL, USA). Berkeley Madonna 8.0.1 (Berkeley Madonna was developed by Robert Macey and George Oster of the University of California at Berkeley) was used to calculate internal PS-MPs concentrations based on the TK models. For statistical data analysis, the Oracle® Crystal Ball software (Version 11.1, Oracle Corporation, Redwood Shores, CA, USA) was employed to implement the MC simulation. Each simulation was performed with 10,000 iterations to quantify probabilistic results with uncertainty.

3. Results

3.1. TK analysis of PS-MPs in freshwater fish

The TK modeling revealed that a rapidly increasing trend of bioaccumulation dynamics of PS-MPs was found among organs of freshwater fish (Figs. 1 and 2). Physiological parameters including k_u and k_e were obtained (Tables S4 and S5). For zebrafish, the bioaccumulation of PS-MPs in 5 μm size showed better-fit ($r^2 = 0.63$–0.83, $p < 0.001$) than that in 20 μm ($r^2 = 0.48$–0.51, $p < 0.01$) (Fig. 1). Among all k_us of organs, liver posed by 5 μm PS-MPs had a highest value with 78.48 ± 13.24 (mean ± SE) mL g^{-1} d^{-1} (Tables S4). Results also revealed that k_us of both gills and gut exposed to 5 μm PS-MPs were greater than those of 20 μm PS-MPs (Tables S4). However, there is little difference among all k_es in specific organs of zebrafish (Table S4). Overall, k_us had greater

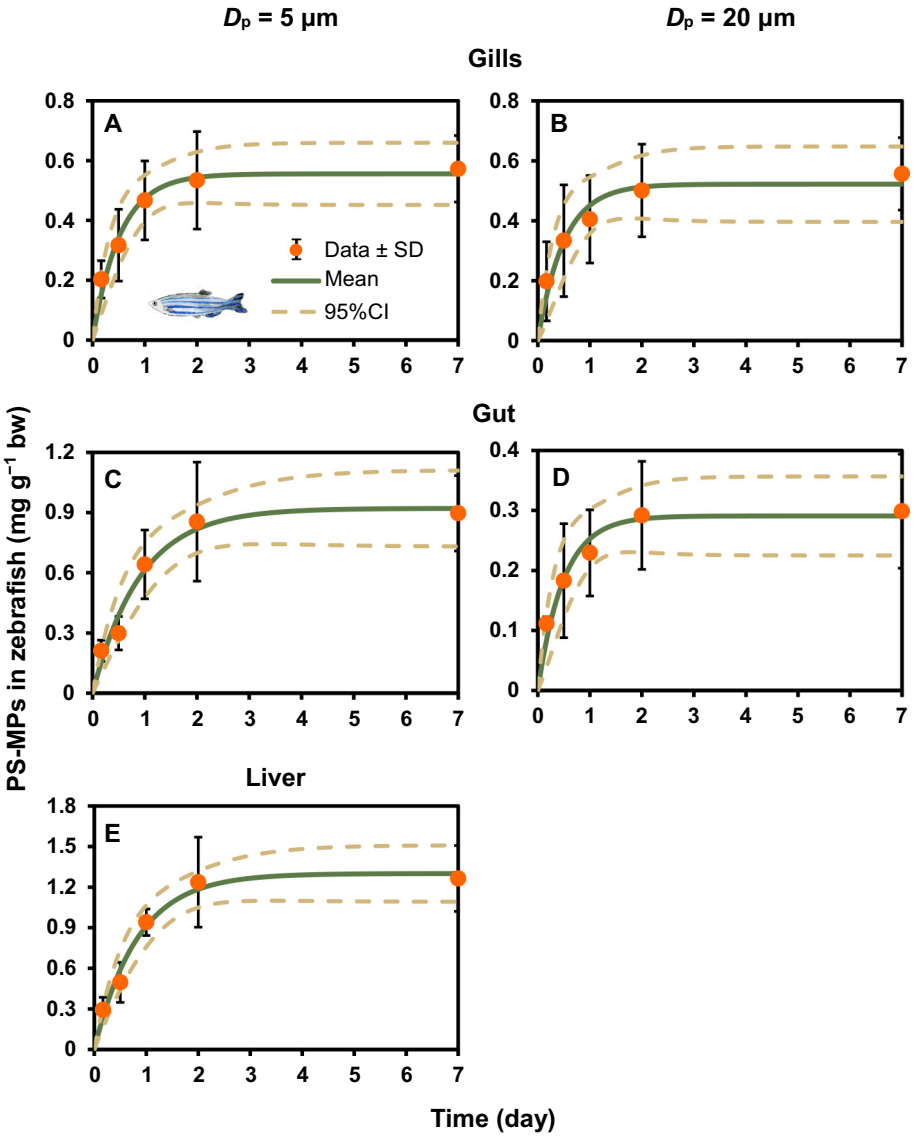

Fig. 1. Fitting the toxicokinetic model to particle diameter (D_p) = 5 and 20 μm PS-MPs burdens in (A, B) gills, and (C, D) gut, and D_p = 5 μm PS-MPs in (E) liver of zebrafish during a 7-day exposure.

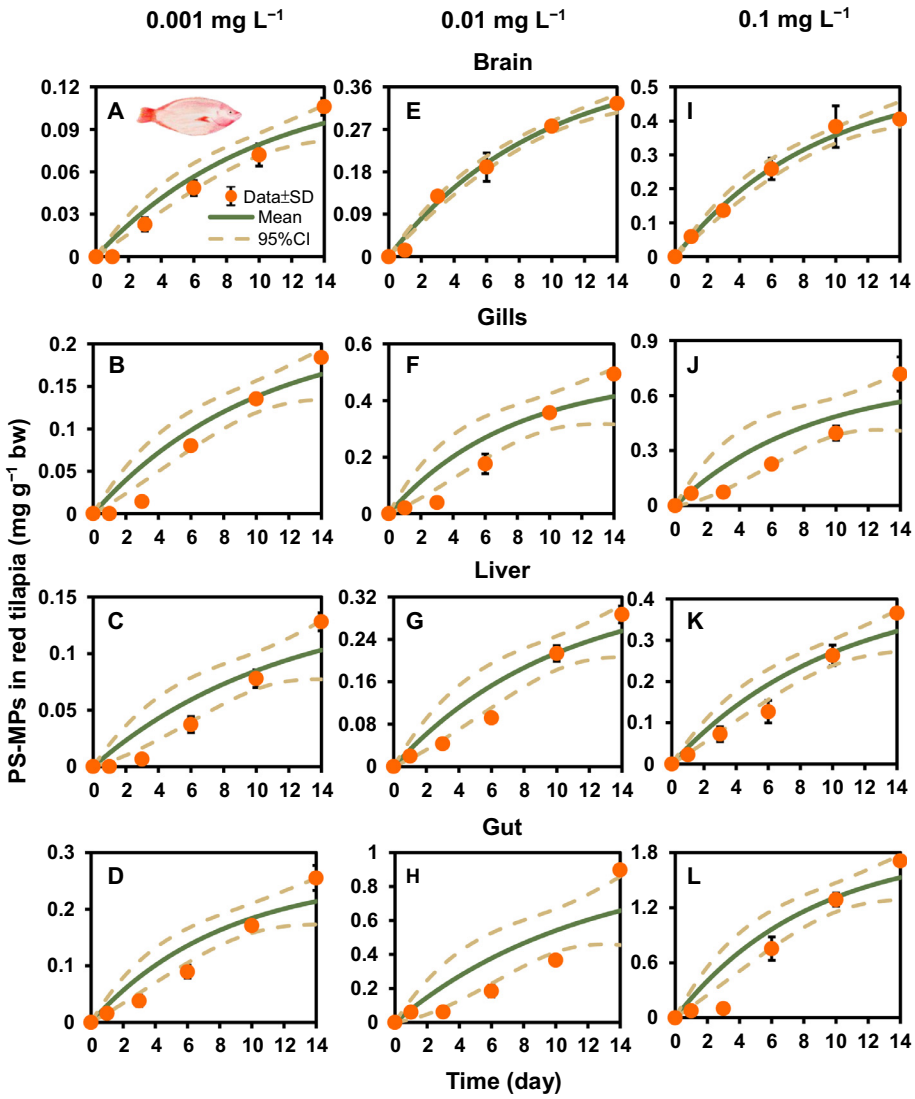

Fig. 2. Fitting the toxicokinetic model to PS-MPs burdens in brain, gills, liver, and gut of red tilapia posed by (A–D) 0.001, (E–H) 0.01, and (I–L) 0.1 mg L^{-1} PS-MPs treatments during a 14-day exposure.

values than those of k_es, resulting in all BCF_{ss}s greater than 1. The BCF_{ss}s of 5 μm PS-MPs were higher than those of 20 μm PS-MPs with the highest to lowest organ-specific BCF_{ss}s in zebrafish posed by 5 μm PS-MPs in the order of 67 ± 19 in liver, 50 ± 18 in gut, and 31 ± 12 in gills (Table S4).

Given red tilapia treated with lower exposure concentration, smaller particle size of MPs, and longer exposure period, the overall k_us in red tilapia were higher than those in zebrafish (Fig. 2; Table S5). Results showed that the higher the PS-MPs exposure concentrations, the lower the k_us (Table S5). The mean k_es had much smaller magnitude within a narrow range (10^{-1}) compared to k_us (10^2–10^4) among organs under any PS-MPs exposure levels, implying that k_e had environmental concentration-independent characteristics (Table S5). Gut had the highest k_us with mean values of 31,864, 8031, and 2255 mL g^{-1} d^{-1}, exposed to 0.001, 0.01, and 0.1 mg L^{-1} PS-MPs exposures, respectively (Table S5). Under 0.001 mg L^{-1} PS-MPs exposure, brain had the lowest k_u of 12,537 ± 2131 mL g^{-1} d^{-1}, whereas liver had the lowest k_u of 3376 ± 915, and 428 ± 84 mL g^{-1} d^{-1} under 0.01 and 0.1 mg L^{-1} PS-MPs exposures, respectively (Table S5). Overall, results of red tilapia posed by 0.001 (r^2 = 0.81–0.92, $p < 0.001$), 0.01 (r^2 = 0.71–0.98, $p < 0.001$), and 0.1 mg L^{-1} (r^2 = 0.76–0.95, $p < 0.001$) PS-MPs treatments indicated good fitting of TK data.

3.2. MRT of PS-MPs in freshwater fish

During a 7-day exposure treatment of zebrafish, the median MRTs of 20 μm PS-MPs were 3.89 d in gills and 4.17 d in gut, whereas 3.78 d in liver, 3.8 d in gills, and 4.12 d in gut were found for 5 μm PS-MPs (Fig. 3A). To explore the size influence, overall size-specific probability density functions (pdfs) of MRT were obtained (Fig. 3B). Results showed that the pdfs of MRT with LN distributions of 5 and 20 μm PS-MPs were LN(4.06 d, 1.2) and LN(4.22 d, 1.4), respectively (Fig. 3B).

In the red tilapia system, owing to large uncertainties of the k_e estimates of MPs, only the mean values of TK parameters were applied in MC simulation for MRT estimates (Table S5). We found that the orders of organ-specific MRTs were all different among concentrations (Fig. 3C). PS-MPs in the brain had longer residence time than those in the gut at any exposure concentrations (Fig. 3C). In addition, MRTs of PS-MPs in liver slightly prolonged with increasing of exposure

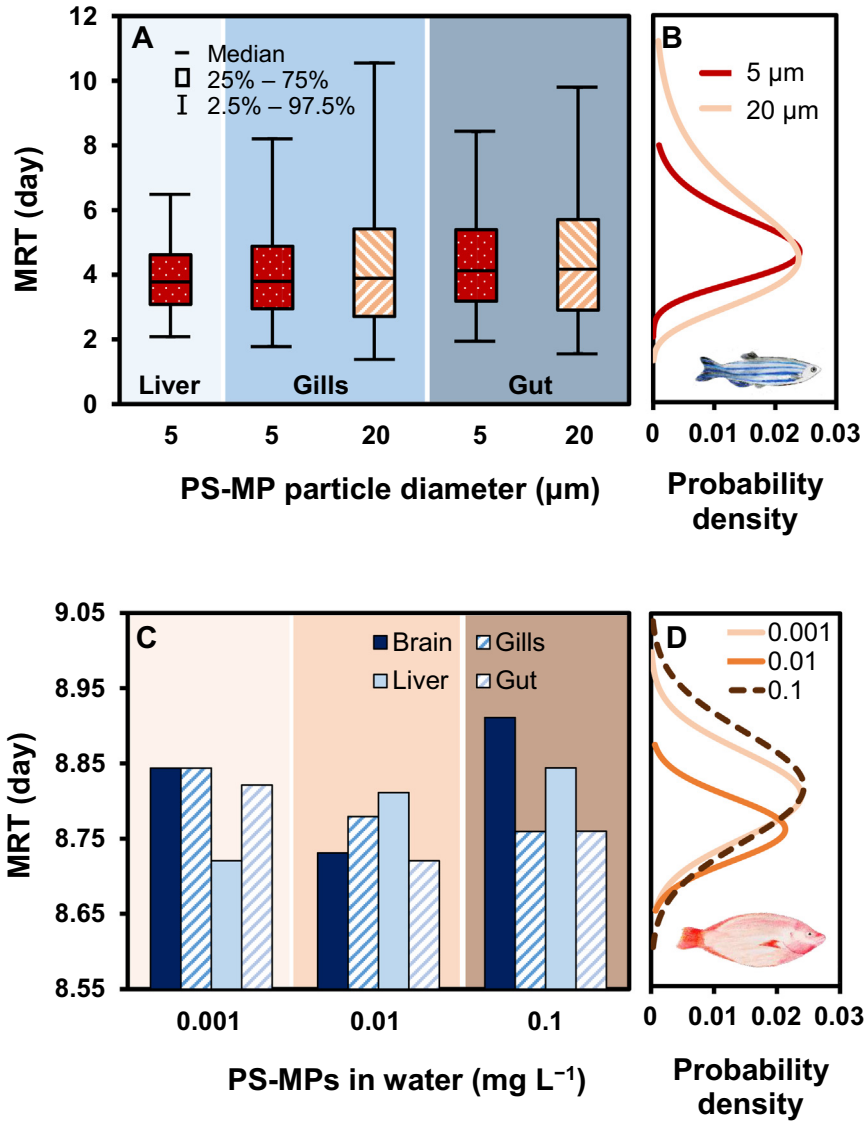

Fig. 3. (A) PS-MPs particle size-specific mean residence times (MRTs) in liver, gills, and gut of zebrafish with (B) their probability distribution. (C) Exposure concentration-dependent MRTs in brain, gills, liver, and gut of red tilapia with (D) their probability distribution.

concentrations, whereas a declining MRT was found in gills (Fig. 3C). Moreover, to investigate the effect of PS-MPs exposure concentrations on MRTs in red tilapia, MRT values among four organs under the same exposure concentration were grouped into a pdf presented in LN distributions (Fig. 3D). The averaged MRTs were in the order of LN(8.82 d, 1.01), LN(8.81 d, 1.01), and LN(8.76 d, 1) at 0.1, 0.001, and 0.01 mg L^{-1} PS-MPs exposure concentrations, respectively (Fig. 3D).

3.3. TD analysis of PS-MPs for metabolic disturbances

To investigate the responses in liver of freshwater fish posed by PS-MPs burden, exposure concentrations of 5 μm PS-MPs in zebrafish experiment were firstly converted into internal concentrations via the estimated liver BCF_{ss} of 67.4 ± 18.7 (mean ± SE). The relationships between 5 μm PS-MPs burdens and biomarkers of CAT and SOD activities related to oxidative stress in zebrafish liver were best-fitted by a three-parameter Hill-based TD model ($r^2 \geq 0.6$, $p < 0.04$) (Fig. 4A, B). Whereas the mean PS-MPs concentrations in red tilapia liver of 128, 287, and 366 μg g^{-1} at 14th day adopted from Ding et al. (2018) were applied instead of exposure concentrations. The average E_{max}s of CAT and SOD activities were 1.1- and 1.9-fold increase from control, respectively (Table S6). *EC*50s of promoted CAT and SOD activities were 15.0 ± 12.2 and 8.3 ± 7.9 μg g^{-1} with *n* of 1.3 and 1.0, respectively (Table S6).

The relationships between CYP enzymes activities and PS-MPs burden in liver were shown by fitting the four-parameter Hill-based TD model to available data ($r^2 \geq 0.7$, $p < 0.02$) (Fig. 4C, D). The fitted E_{max} and E_{min} for BFCOD activity were 4.0 ± 1.9 and 0.7 ± 0.3 pmol mg^{-1} pro min^{-1}, respectively, for EROD activity, whereas the fitted E_{max} and E_{min} were 3.7 ± 1.5 and 1.1 ± 0.5 pmol mg^{-1} pro min^{-1}, respectively (Table S6). Comparing *EC*50s between the two CYP enzymes activities, BFCOD activity with estimates of 261.3 ± 72.4 μg g^{-1} was higher than EROD activity with an estimate of 237.0 ± 96.8 μg g^{-1} (Table S6). All fitted *n* were greater than 1 (Table S6).

Additionally, based on the benchmark concept, BMC_{10} of CAT, SOD, BFCOD, and EROD activities were 2.2 (95% CI: 0.09–11.38), 1.0 (0.03–5.15), 119.4 (24.62–327.92), and 126.3 (32.44–354.87) μg g^{-1}, respectively (Fig. 4; Table S6). Undoubtedly, promoted SOD

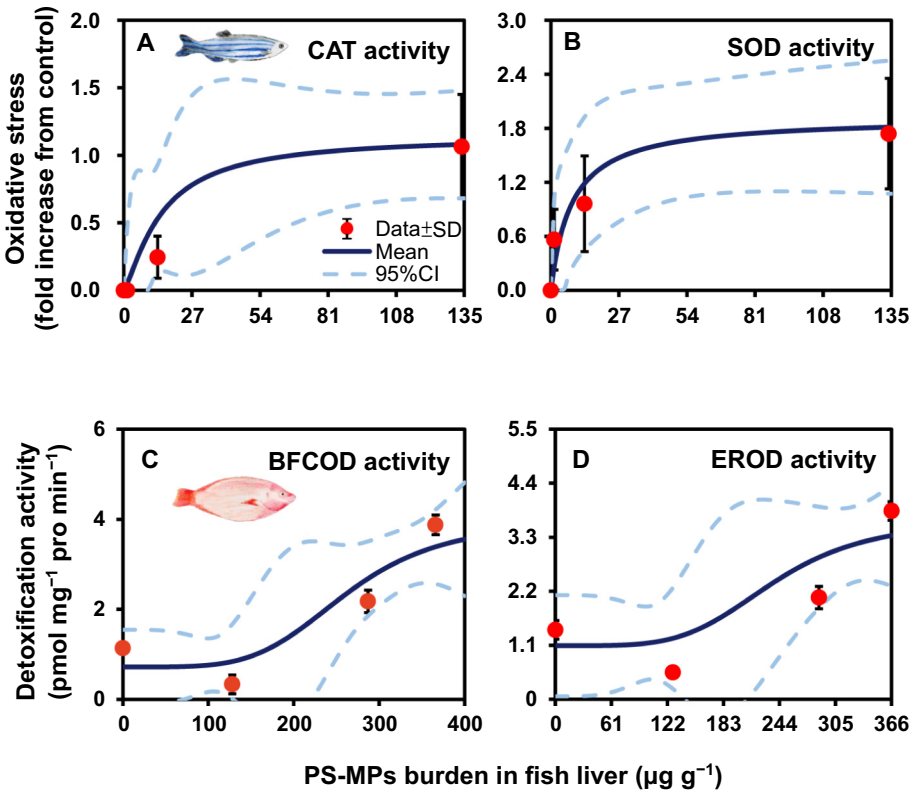

Fig. 4. Optimal fit of the Hill model to PS-MPs burden in liver-response profiles for oxidative stress: (A) catalase (CAT) and (B) superoxide dismutase (SOD) activities in zebrafish; as well as for detoxification activity: (C) 7-benzyloxy-4-trifluoromethyl-coumarin *O*-dibenzyloxylase (BFCOD) and (D) 7-ethoxyresorufin O-deethylase (EROD) activities in red tilapia.

activity in liver was much more sensitive to accumulated PS-MPs than CAT activity, no matter which threshold concentration was used (Table S6). However, the BFCOD activity showed higher sensitivity than EROD activity at strict threshold of BMC_{10} value, in contrast to the results under *EC*50 comparison (Table S6). To assess the exposure risk for MPs-induced metabolic disturbances, SOD and BFCOD activities were chosen to be the representative biomarkers of oxidative stress in zebrafish and detoxification capability in red tilapia, respectively.

3.4. Exposure risk prediction in freshwater fish

Based on the established algorithm, concentration data from 35 studies of MPs in a variety of freshwater systems were listed in Table S7 (Fig. S1). Freshwater systems included river, reservoir, lake, wastewater treatment plant (WWTP), wetland, and channel (Table S7). Our BMC_{10} estimates had μg MPs per g body weight as measurement unit, and we thus used environmental data with the same unit. By utilizing the developed TK modeling, continent-based *PEC*s were firstly converted into internal concentrations in fish liver based on estimated TK parameters.

Specially for red tilapia, concentration-dependent TK parameters were used to form a simple exponential regression between k_u of liver and exposure concentrations (C_w) as $k_u = A_0 \exp(-A_1 C_w) + A_2$ with A_0, A_1, and A_2 of 14,233 ± 2227 (mean ± SE), 157 ± 67, and 427 (95% CI: 5.74–2606.14), respectively, prior to conversion between exposure concentration and internal burden (Fig. S3). On the other hand, due to similar values among three exposure treatments, k_es of liver were averaged out to 0.10 ± 0.03 d^{-1} (Table S5).

Internal concentrations in liver were simulated at the 21st and 14th day in zebrafish and red tilapia, respectively, corresponding to the end of exposure durations in the adopted toxicity tests. The MC simulation was then employed and all MPs burdens in fish at different sites were described by LN distributions. Results showed that liver MPs burdens in zebrafish were LN(2.33 mg g^{-1}, 1.16), LN (0.99 mg g^{-1}, 1.09), and LN(0.84 mg g^{-1}, 1.10) in Asia, Europe, and Northern America respectively, whereas those in red tilapia were LN(104.20 mg g^{-1}, 1.73), LN(46.75 mg g^{-1}, 1.60), and LN (28.05 mg g^{-1}, 2.93), respectively (Fig. 5A). We found that the highest gsd values of both fish species were at Northern America with a right-skewed distribution (Fig. 5A).

The ER curves for promoted metabolic disturbances subject to predicted MPs burden of freshwater fish in Asia, Europe, and Northern America were assessed (Fig. 5B, C). The promoted oxidative stress in zebrafish response to continent-based MPs exposure concentration with probabilities exceeding 50% ranged from 0.18–0.42-fold increase from control (Table S8). Percentages of promoted detoxification activity in red tilapia were calculated based on the difference between effect and E_{min} then normalized to ($E_{max} - E_{min}$) from the TD model. ER of red tilapia in Asia was the highest, showing 1.32% MPs-promoted detoxification with probabilities exceeding 50% (Fig. 5C; Table S8). Overall, the risks among selected regions ranked from highest to lowest were Asia, Europe, and Northern America (Fig. 5B, C).

The *RQ*s for oxidative stress in zebrafish and detoxification capability in red tilapia were calculated based on the BMC_{10} values of SOD and BFCOD activities, respectively (Fig. 6). All *RQ* estimates were much larger than 1, indicating that MPs-polluted freshwater sites in Asia, Europe, and Northern America were highly likely to pose significant risks to freshwater fish (Fig. 6; Table S9). Among these three continents, Asia had the greatest potential risks to zebrafish and red tilapia health with median *RQ* of 5678.5 and 1087.9, whereas the lowest risks were

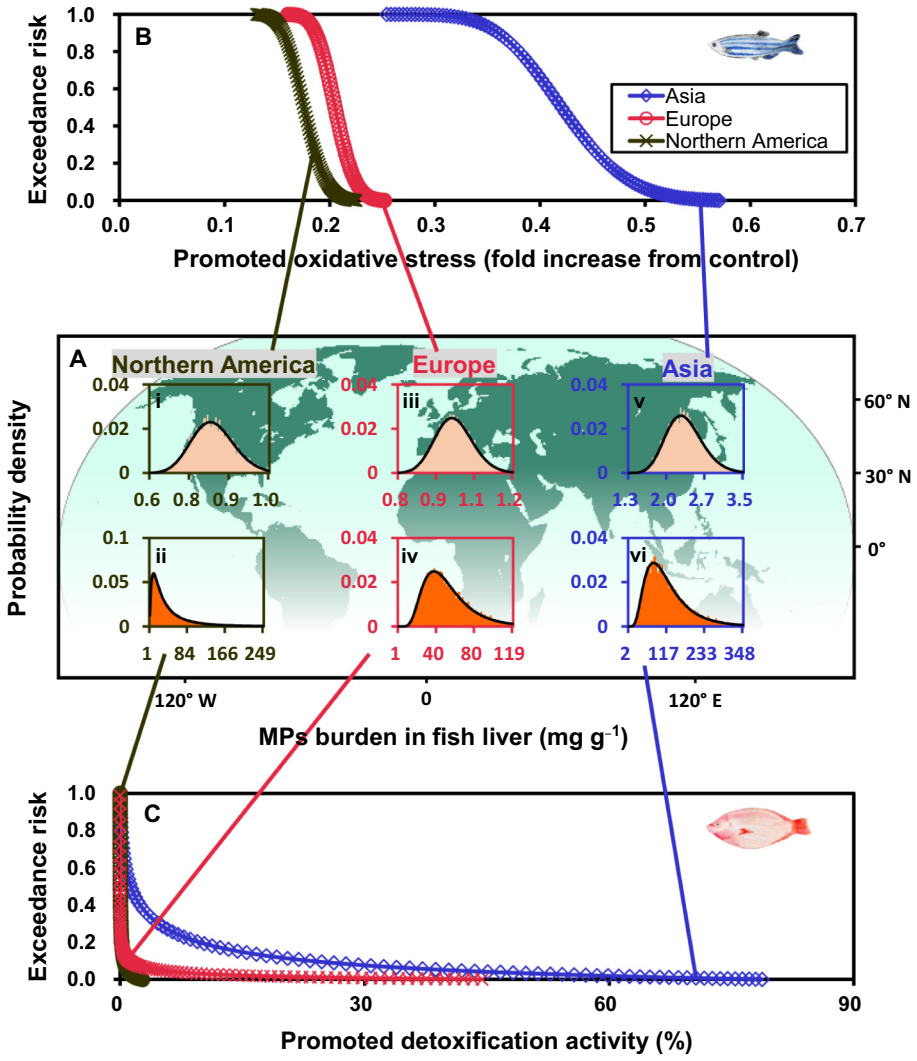

Fig. 5. (A) Probabilistic distributions of MPs burden in liver of zebrafish and red tilapia posed by predicted environmental concentrations of MPs in freshwater regions over (i, ii) Northern America, (iii, iv) Europe, and (v, vi) Asia, respectively. (B, C) Exceedance risk estimates for promoted oxidative stress and detoxification activity of zebrafish and red tilapia, respectively, in Northern American, Europe, and Asia. Map generated using Natural Earth (http://naturalearthdata.com).

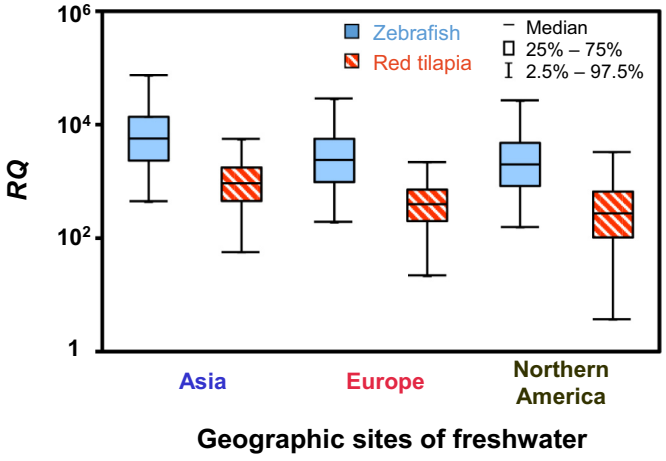

Fig. 6. Box and whisker plots of promoted oxidative activity- and detoxification activity-associated risk quotients (*RQs*) in zebrafish and red tilapia, respectively, exposed to MPs in freshwaters over Asia, Europe, and Northern American.

occurred at Northern America with median *RQ* of 1986.7 and 292.5, respectively (Table S9).

4. Discussion

4.1. Comparison of TK parameters and bioaccumulation in freshwater fish

Due to the high potential to accumulate persistent pollutants, fish are excellent bioindicators revealing the relative health of freshwater ecosystems (Lasheen et al., 2012). Tilapia being one of the most abundant, widely introduced, and commercially valued species is known for withstanding environmental stressors (Abdel-Tawwab et al., 2017; CABI, 2019). Whereas information of zebrafish in the wild is mostly lacking, its particular traits and being sensitive to environmental pollutants make zebrafish the most effectively used model vertebrate (Engeszer et al., 2007; US Fish and Wildlife Service, 2018).

A field study investigated MPs pollution in sea and freshwater fish from China, indicating that the size of sampled MPs ranging from 0.04 to 5 mm accounted for 36.8–92.3% of the total plastics number in each specific species (Jabeen et al., 2017). MPs have been found in different fish tissues such as the brain, gills, muscle, stomach, gut, and liver

(Jabeen et al., 2017; Su et al., 2019a, 2019b). Our findings showed that the organ distribution pattern of MPs in red tilapia was in the order of gut > gills > liver ≈ brain, whereas MPs accumulation in liver of zebrafish was the highest.

The size-specific TK analysis for zebrafish offered an opportunity to determine the role of MPs particle size to each organ. In general, MPs size smaller than 20 μm would enter bloodstream (Lusher et al., 2017a). The particle size in 5 μm is able to be transferred to liver in zebrafish, and 0.1 μm size is capable of penetrating the blood-brain barrier and eventually reaches brain in red tilapia (Ding et al., 2018; Lu et al., 2016). A MPs biomonitoring within the Greater Melbourne Area revealed that MP sizes ranged from 0.09 to 4.86 mm in bodies and 0.22–2.01 mm in heads were found in *Gambusia holbrooki* (Su et al., 2019a). Another study found that particle sizes of MPs larger than 20 μm were absent in liver and muscle of Asian seabass caught from China coast (Su et al., 2019b). They also showed that MPs in gills had smaller sizes than those found in guts.

Our BCF_{ss} estimates for PS-MPs were greater than 1 for both smaller and larger sizes, evidencing the bioaccumulation potential of PS-MPs in specific organ of zebrafish. High BCF in zebrafish is likely due to the absence of food during constant exposure (Lu et al., 2016). In gills, BCF_{ss} showed no significant difference to the selectivity of PS-MPs size. Whereas BCF_{ss} of PS-MPs in smaller size was nearly three folds in the gut than that in larger size. The laboratory-derived BCF is only a conservative estimate of the residues likely to occur in the environment. MPs burden from various routes of exposure and food supply during exposure were not considered in this study. Therefore, BCFs need to be more accurately determined.

Many laboratory studies regarding MPs exposure rely solely on preproduced plastics that are easily purchased; however, these are not representative of the diverse forms currently present in the environment (Lusher et al., 2017b). In natural environments, fragmented forms and weathered particles were found more ubiquitous, and fibers and fragments were thus predominantly ingested by organisms. (Meng et al., 2020; Zheng et al., 2019). It was consistent with the type of observed MPs in wild tilapia which 65% were fibers (Khan et al., 2020). In addition, the presence of biofilms on the plastic surface increased the likelihood of MPs ingestion by triggering sensory responses in organisms (Carbery et al., 2018).

Compared to MPs in homogeneous size distribution and spherical shape, irregular form might result in different TK behaviors with lower k_e and higher BCF, furtherly increasing the probability of toxic reaction. The ingested MPs in irregular shape are likely to entangle within the intestinal tract, resulting in the gut-blockage and longer gut retention times (GRTs) (Cole et al., 2013; Wright et al., 2013). The entanglement and other physical effects would also potentially occur in other tissues. Longer retention of MPs could aggravate its impact on the ingesting wildlife which may impair tissue function (Cole et al., 2013).

Previous studies mainly focused on MPs accumulation and retention in gut (Biginagwa et al., 2016; Jabeen et al., 2017; Zheng et al., 2019). GI tract dynamics encompasses the uptake-depuration interaction and the resulting load of MPs retain. Yet, there is limited information on the dynamics of MPs in other organs. Our findings provided better understanding of the organ-specific retention of MPs in two different freshwater fish. In zebrafish system, MRTs of PS-MPs in gills and gut were probable to exceed the exposure duration (7 d). One study found that 90% GRT for MPs in goldfish post 1 h consumption was 33.4 h (Grigorakis et al., 2017). However, an expectation of smaller particles being removed from tissues more easily was not remarkably observed in the present study. Further research is needed to determine the influence of particle characteristics of MPs on retention time in fish organs.

4.2. Metabolic disturbances in freshwater fish exposed to MPs

The effects at the cellular level of biological organization generally occur rapidly, which act as a good early warning to assess the potential toxicological effects at higher levels of biological organization (Clements, 2000). The antioxidative system is involved in detoxification and elimination processes of toxic substances from body (Xia et al., 2020). Cellular defense mechanisms against MP-induced oxidative stress were triggered by activating antioxidant enzymes (Huang et al., 2020). SOD is at the first defense line to prevent cellular oxidative damage by injuries of MPs in tissues (Prokić et al., 2019). It is a plausible explanation for the *EC*50 of SOD activity nearly half that of CAT activity. Several experimental studies demonstrated that MPs led to promoted CAT and SOD activities in tilapia and other freshwater fish, such as guppy, carp, and discus fish (Hamed et al., 2020; Huang et al., 2020; Wen et al., 2018; Xia et al., 2020).

BFCOD and EROD are common biomarkers for assessing CYP enzyme activity responsible for detoxification and metabolism. A significant correlation was found between quantity of MPs in larval fish and gene expression of CYP1A (Mazurais et al., 2015; Xia et al., 2020). The upregulation of CYP is known to serve as protective response to eliminate xenobiotics (Xia et al., 2020). However, there are currently limited data and knowledge of mechanisms on CYP enzyme activity subject to MPs. Apart from CYP proteins, a slight increase of glutathione S-transferase enzyme involved in phase II detoxification process in liver of mullet fish reflected an induction of the detoxification system (Alomar et al., 2017). Our results showed that both endpoints had similar *EC*50 value.

Despite the distribution and habitat preferences of zebrafish and red tilapia, this study regarded them as representative indicators of sensitive and resilient species to emerging pollutants, respectively (CABI, 2019; Engeszer et al., 2007). We found that zebrafish had lower *EC*50s than red tilapia though their metabolic biomarkers function differently. The use of different biomarkers at different levels of biological organization in different species and other experimental factors such as dosing approaches and level of exposure are new challenges for toxicity identification and evaluation. Moreover, the Microplastics Expert Workshop advocated the prior need for the TK/TD assessment of organisms to make better quantitative predictions of the detection of biomarkers and the heterogeneity in adverse outcomes of MPs exposures in individuals (Murphy, 2017).

4.3. Modeled threshold levels and model limitations

Previous studies used predicted-no-effect concentration (PNEC) as threshold in risk assessment (Adam et al., 2019; Burns and Boxall, 2018). The mean PNEC of environmental MPs was 8×10^{-2} μg L^{-1} (Adam et al., 2019). However, the PNEC calculation relied on no-observed-effect concentrations (NOECs) or lowest-observed-effect concentrations (LOECs) which were all determined by a traditional approach selecting a single outcome from a set of toxicity tests. Our work provided a first insight into estimating toxicity burdens to cellular damage in fish by considering the physiological distribution of MPs. Up to date, though a battery of functional tests has been rapidly screened in laboratory studies, no internal threshold of MPs burden for freshwater fish is available. Only few studies have used the dose-response model in data analysis to obtain the critical thresholds.

The impact of particle size was only evaluated in TKs. However, it has been evidenced in many toxicological assays showing significant difference (Enfrin et al., 2020; Yang et al., 2020). A broad overview of ecotoxicity endpoints in terms of NOECs and LOECs was classified by particle size of MPs (Burns and Boxall, 2018). Therefore, the particle behavior might be a matter of concern in threshold estimates. To systematically improve the ecological risk assessment and provide rational policy measures, a standard approach is needed to set allowable MP levels in environment and safe thresholds for biota.

Recent studies have shown increasing focus on selecting environmentally relevant concentrations in treatment, whereas polymer size and shape are far different from those observed in environments where targeted species live. To bridge the gaps, we suggested that laboratory studies should explain reasons for using the model organism and

MPs type when describing experimental design and procedure. Reasons ought to have connections to ecologically realistic conditions to avoid overreaction or misinterpretation of MPs toxicity (Lenz et al., 2016).

In this study, we developed a parsimonious one-compartment model to describe TK processes of MPs in fish organs. The model reduced variables related to fish characteristics and physical properties of MPs which were complex and might result in a poor goodness-of-fit. A limitation of this approach was that results between species were hardly comparable. As such, applicability of the present TK parameters to new species or other different treatments was limited without a thorough validation process.

Due to few experimental groups of different exposure concentrations, the validity in fitting the inflection of TD curve was limited and thus reflected in the uncertainties and significance of *EC*50 and *n*. The other limitation was the ability of TK/TD model describing biological interactions between MPs and target sites in fish. More work needs to be done to enhance understanding of mechanisms of the TK/TD processes leading to toxicity of MPs.

4.4. Implications for risk assessment of MPs

Our results revealed that MPs pollution is highly likely to threaten freshwater fish regardless its variation within species and their endurance to withstand pollutants. Due to the high *PEC*s of pelagic MPs pervading most freshwater sites in Western China, the most populous and rapidly developing area, we estimated that freshwater fish in Asia were most at risk among three continents. A global river plastic inputs model showed that 15 out of the top 20 MPs polluting rivers were located in Asia which is responsible for half of global plastic production (Lebreton et al., 2017; PlasticsEurope, 2019). Although a preliminary ecotoxicological risk assessment indicated no immediate MPs pollution risk to global freshwater environments, an alarming was implicated from which there were 0.4% of the *RQ* values greater than 1 in Asia (Adam et al., 2019).

Notably, our *RQ* estimates are greater than 1, in contrast to previous studies whose risk estimates were far below 1 (Adam et al., 2019; Burns and Boxall, 2018). The divergence could be ascribed to updated exposure concentrations in our dataset in which several data showed severe MPs pollution, resulting in higher risks to freshwaters. On the other hand, our work improved the threshold concentration estimating process via TK/TD modeling without directly determined from ecotoxicity experiments that were constrained by limited and discrete doses to responses.

MPs concentrations and their characteristics were varied with the hydrodynamic and meteorological conditions of freshwater systems, even sampled multiple times from the same system within different studies like the Pearl River (Lin et al., 2018; Mai et al., 2019; Yan et al., 2019) and the Three Gorges Reservoir (Di and Wang, 2018; Zhang et al., 2015, 2017). Uncertainty of each *PEC* value was also attributed to spatiotemporal distribution. A study might have multiple subsampling sites and sampling frequencies by month or season, whereas we only derived an averaged concentration value to represent this overall field investigation. The other uncertainty came from unit conversion based on a regression equation in the algorithm for *PEC* calculation. The real process of conversion might be governed by factors like polymer density and shape, leading to the difference between measured and calculated value. We thus strongly suggested researchers to provide both number and mass concentrations which need to be considered in risk assessment.

MPs pollution levels at selected sampling sites also reflect different degrees of development and land-use characteristics influencing the release of MPs in the freshwater environment (Baldwin et al., 2016; Sighicelli et al., 2018). Based on the site description in the present adopted studies, most regions are located within populous urban areas and communities, increasing the likelihood of MPs appearance. Thus, we implicated that manufacturing industries, sewage treatment plants, civic facilities and fishing industries provide an avenue for MPs released. Wang et al. (2017) indicated that MPs abundance negatively correlated with the distance from city center, which identified the crucial role of anthropogenic factors in MP distribution. A sampling strategy is thus suggested that monitoring of MP pollution in freshwaters is necessary with the prioritization of locations with dense urban populations (Khan et al., 2018).

In our datasets, there were 18 and 8 studies, respectively, correlated to MPs pollutions in rivers and lakes, the most studied freshwater systems. Both ecosystems serve as local sinks for MPs; moreover, river acts as a MPs carrier and drains into other systems. Field studies found that small-sized MPs in lakes or ponds were more abundant than those in inflowing rivers (Ma et al., 2020; Xiong et al., 2018). Though there are no standard methods for the monitoring and identification of MPs in freshwaters, among 35 papers adopted in the present dataset, we found various sampling methods such as pumping with filter device or using a trawl net with a mesh size of 112–330 μm (Faure et al., 2015; Lin et al., 2018; Talvitie et al., 2017; Zhang et al., 2017).

A study reported that MPs concentrations in Swedish waters were up to 10^5 times larger when sampled with an 80 μm rather than a 450 μm mesh (Norén, 2007). Obviously, mesh size is an important factor for sampling water surface MPs, leading to heterogeneity of environmental concentrations of MPs among sampling sites. The smallest size of MPs samples was 5 μm, detected in Taihu Lake where another study found 95.7% of freshwater fish containing MPs (Jabeen et al., 2017; Su et al., 2016). Therefore, the estimated health risks for freshwater fish are likely to be underestimated due to limited mesh size of current sampling tools and observation limit for identification.

GESAMP (2015) reported that the risk assessment of MPs in environments is still in the hazard characterization phase due to limited information on exposure levels and established effect levels. In view of 5 of the top 10 largest rivers in the world located in South America and the exceptional biodiversity in Africa and Oceania, an unjustified lack of attention to these continents in Southern Hemisphere hampers the global impact assessment of MPs pollution. Investigations of MP residues in wild tilapia all carried out in African freshwaters, indicating the average abundance of 0.5–7.5 particles per fish (Khan et al., 2020; Naidoo et al., 2020). Reliable data on concentrations, sizes, shapes, and polymer types of MPs in environments are still needed, particularly as freshwater ecosystems are less well understood. Proposing standardized protocols for sampling and identification of MPs in environments and organisms is recommended, allowing for comparisons across researches. On the other hand, field investigations need more focus on possible presence of smaller MPs in size of 20 to 0.1 μm which are highly likely to cause significant toxicity at various levels of biological organization (Lusher et al., 2017a). For future researches, elucidating sources and pathways of MPs in freshwater ecosystems would be a major challenge as this information plays a crucial role on management strategies and risk reduction measures.

It was noticed that many field investigations focused on freshwaters in China and the United States. China has been the world's largest producer of tilapia yet with great concern of MPs pollution (Dai et al., 2020). In view of the evidence of uptake and effects of MPs in fish, of most concern to tilapia aquaculture or other species would be water quality whether MPs concentrations in ponds beyond the threshold level. A field investigation demonstrated that MP concentration in fish pond inlets originating from natural streams was always higher than that in the outlets (Bordós et al., 2019). MPs in water sources should be effectively managed and controlled, such as processed with additional filtration device (Ma et al., 2020). Our study first provided threshold concentrations of MPs for metabolic disturbances in red tilapia. The values could be used as a health assessment index for improving fish health status. Nonetheless, further studies still need to give a comprehensive assessment on all potential impacts of MPs on tilapia health in farming systems for sustainable management.

5. Conclusions

A TK/TD-based quantitative risk assessment was performed based on the extent of MPs bioaccumulation and on the metabolic disturbances at cellular level in zebrafish and red tilapia, underscoring the importance to investigate the relationships between environmental pollution of MPs and freshwater fish health. Metrics like BCF, MRT, BMC_{10}, and *RQ* provided a mechanistic perspective on determining the biological fate of MPs in zebrafish and red tilapia and associated risks. We found that MPs pollution was likely to enhance fish health risks due to promoted metabolic disturbances and that this factor must therefore be considered in evaluations of MPs-related susceptibility. By integrating environmental exposure assessment and laboratory-based effect assessment, the significant risk estimates among Asia, Europe, and Northern America revealed the undesirable situation of freshwater fish against MPs pollution in their living environments worldwide.

Our finding also highlights the urgency of global conservation actions and policy initiatives (e.g., coordinating fragmented monitoring, and acquiring more available data on toxicological potentials for most species), if MPs pollution-induced harmful effects on freshwater fish around the world are to be moderated in the future. Given a sufficient database involving serious consideration on all potential impacts, the extension of our TK/TD-based risk scheme would be readily for those vulnerable or high-value species posed by MPs in living habitats to support decision-making processes under scientific basis. Over the long term, such approach could help inform intensified efforts to mitigate MPs pollution that could benefit many freshwater fish species and people who depend on health of fish stocks.

CRediT authorship contribution statement

Chi-Yun Chen: Investigation, Formal analysis, Data curation, Visualization, Writing - original draft, Validation. **Tien-Hsuan Lu:** Formal analysis, Data curation, Investigation, Validation. **Ying-Fei Yang:** Project administration, Methodology, Validation, Supervision. **Chung-Min Liao:** Conceptualization, Supervision, Writing - original draft, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.144013.

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