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Stereoselective bioaccumulation of *syn*- and *anti*-Dechlorane plus isomers in different tissues of common carp (*Cyprinus carpio*)

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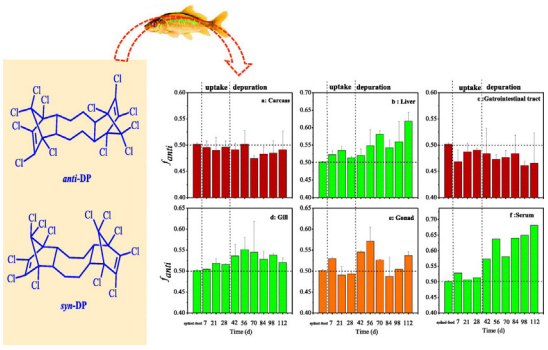
HIGHLIGHTS

- *Syn*- and *anti*-DP isomers were administered to common carps via diet.
- The BMFs of DP isomers were less than one in all tissues except for serum.
- A dynamic tissue distribution of DP isomers was observed in common carps.
- The isomer composition of the DPs also exhibited tissue specificity.

GRAPHICAL ABSTRACT



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ABSTRACT

Common carps (*Cyprinus carpio*) were exposed to *syn*- and *anti*-Dechlorane Plus (DP) isomers to investigate absorption, tissue distribution, and stereoselective bioaccumulation of DP isomers. The absorption efficiencies of *anti*-DP in the gastrointestinal system were higher than those of *syn*-DP. A linear accumulation was found for both isomers in all fish tissues except for serum; and the liver and gill exhibited the highest and lowest DP assimilation efficiency, respectively. The elimination of DP isomers in all tissues followed first-order kinetics, with the fastest depuration rate occurring in the liver and serum. The biomagnification factors (BMFs) of both isomers were less than one in all tissues, except for serum. *Anti*-DP was preferably accumulated in the liver, gill, and serum, whereas *syn*-DP was selectively accumulated in the carcass and gastrointestinal tract. As a whole, fish did not show selective accumulation of the *syn*- or *anti*-DP isomer in the uptake stage, whereas a selective accumulation of *syn*-DP in fish was observed during the depuration period, which could be due to a selective excretion of *anti*-DP. Metabolism cannot be ruled out as a possible reason considering the high f_{anti} values and the high elimination rate of DPs in the liver.

1. Introduction

Dechlorane plus (DP) is a highly chlorinated additive flame retardant, which has been widely used in computer monitors, cable coating, furniture, and other polymeric systems for approximately five decades (Peng et al. 2012; Sverko et al. 2011). Currently, DP is designated as a high production volume chemical by the United States Environmental Protection Agency (Gagne et al. 2017; Wang et al. 2016). Since the environmental occurrence of DP isomers was first reported by Hoh et al. (2006), DP has been consistently detected globally in ambient air, dust, sediment, water, tree bark, wildlife and humans (Peng et al.

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2015; Sühring et al. 2016; Wang et al. 2016). These studies indicated that DP is environmentally ubiquitous, persistent, potentially bioaccumulated in wildlife and humans, and biomagnified in food webs (Wang et al. 2016). Additionally, several studies have demonstrated that DP would exhibit toxic effects on both terrestrial and aquatic biota (Kang et al. 2016; Li et al. 2013b; Liang et al. 2014; Yang et al. 2016).

The commercial DP product primarily contains two stereoisomers, the *syn*-DP and *anti*-DP isomers, with a ratio of approximately 1:3 (Wu et al. 2010). Species-specific stereoselective enrichment of either *syn*-DP or *anti*-DP was observed in biota. Selective accumulation of *syn*-DP has been reported in zooplankton, mussels (Tomy et al. 2007), oysters (Jia et al. 2011), fish (Kang et al. 2010; Sun et al. 2016; Wu et al. 2010), and in waterbirds from southern China (Zhang et al. 2011a). Meanwhile, a preference for *anti*-DP was detected in walleye and goldeye fish species (Tomy et al. 2007), as well as peregrine falcon eggs from Spain (Guerra et al. 2011). Moreover, a negative correlation between the fraction of *anti*-DP and trophic level was observed in both bird and fish species (Kang et al. 2010; Sun et al. 2012; Wu et al. 2010; Zhang et al. 2011a), indicating that *syn*-DP is more bioaccumulative than *anti*-DP. However, the underlying cause of the stereoselective bioaccumulation is remain unclear.

A previous laboratory exposure study demonstrated that *syn*-DP exhibits higher accumulation potential than that of *anti*-DP in rainbow trout (*Oncorhynchus mykiss*), by exposing *syn*- and *anti*-DP separately, without consideration of gastrointestinal absorption (Tomy et al. 2008). Our previous study confirmed this observation, and also observed the potential for selective degradation of the *anti*-isomer, by exposing common carp (*Cyprinus carpio*) to a high dose of commercial DP mixture (DP-25) (Zeng et al. 2014). Moreover, the dose level that an organism is exposed to can also affect the accumulation potential for DP isomers. A stereoselective accumulation of *syn*-DP was observed in Sprague–Dawley rat (Li et al. 2013a) and quail (*Coturnix coturnix*) (Li et al. 2013b), only in the case of exposure to high doses of DP. The tissue-specific deposition of DP was also found in fish species (Peng et al. 2012; Zhang et al. 2011b), mainly determined by lipid partitioning. However, until now, there is little information on the tissue-specific and stereospecific bioaccumulation of DP isomers in fish.

In the present study, a dose of *syn*- and *anti*-DP isomers was administered to common carp specimens (*Cyprinus carpio*) through diet for 28 days, and then the carp were fed unfortified food for a further 84 days of depuration. The primary objectives of this study were to investigate the gastrointestinal absorption as well as the uptake and depuration behaviors of the DP isomers in different tissues, after exposure to relatively low doses of both isomers. On the basis of such results, we also investigated whether there is a dose-dependent accumulation and dechlorination metabolism of DP isomers in fish, by comparing the results of the present study to those of our previous exposure study (Zeng et al. 2014). Therefore, the results of this study will improve our understanding regarding the bioaccumulation process for DP in fish.

2. Materials and methods

2.1. Standards and reagents

Individual standard solutions for *syn*-DP, *anti*-DP, and brominated diphenyl ethers (BDE) 128 and 181 (50 mg/mL, in toluene, purity >95%) were purchased from AccuStandard (New Haven, CT, USA). Solutions of *anti*-Cl_{11}-DPand anti-Cl_{10}-DP (50 mg/mL, in toluene, purity > 95%) were supplied by Wellington Laboratories (Guelph, ON, Canada). Pesticide-grade acetone (Ace), dichloromethane (DCM), n-hexane (Hex) and methyl tert-butyl ether (MTBE) were purchased from CNW Technologies GmbH (Dusseldorf, Germany). Guaranteed reagent-grade concentrated sulfuric acid (H_2SO_4) and anhydrous sodium sulfate were acquired from Guangzhou Chemical Reagent Factory (Guangzhou City, China).

2.2. Food preparation

Approximately 100 μg of *syn*- and *anti*-DP isomers were dissolved in 10 g of cod liver oil (Peter Möller, Norway) in a stopper-sealed amber jars, and 140 g of commercial fish food pellets (Zhongshan President Enterprise Co. Ltd., P.R. China) was added into the oil solution, and then thoroughly blended by a shaking incubator (24 h, 20 °C). The resulting food was air-dried for 24 h and stored in the dark at −20 °C throughout their use. The designing concentration of each DP isomer in the food was 0.71 μg/g dry weight (dw). This concentration is comparable to the median concentration of *syn*-DP (720 ng/g dw) in sediment from the e-waste site (Zhang et al. 2011b), and approximately 1/10th of the *anti*-DP concentration of the spiked food in our previous exposure experiment (Zeng et al. 2014). Non-spiked food was prepared in the same manner minus the target compounds. Concentrations of the target compounds were determined in spiked and non-spiked food samples collected throughout the experiment using the same analytical procedures as for fish tissues described below.

2.3. Fish exposure and sampling

Sixty-four common carp with initial weights and lengths of 17.1 ± 2.7 g (mean ± SD, similarly hereinafter) and 10.3 ± 0.5 cm, respectively, were obtained from a local aquarium market in Guangzhou, China, and four of them were randomly collected as background samples. Twenty carp were randomly selected as the control group, and kept in one glass aquarium (150 cm × 45 cm × 100 cm), whilst the other forty carp were kept in another glass aquarium (150 cm × 45 cm × 100 cm) as the treated group. Each tank was filled with filtered dechlorinated tap water (maintained at 22 °C–23 °C, pH 6.5–7.5, and with 7.8–8.4 mg/L of dissolved oxygen), and a 12-h light: 12 h dark cycle was maintained. Fish were fed non-spiked for two weeks in the laboratory to acclimatize them to the new surroundings prior to exposure, and the daily feeding rate was 1% of mean weight of fish throughout the experiment.

After 28 days of exposure, the treated group was fed non-spiked food for a further 84 days to monitor deputation. Fish were sampled on days 0, 7, 21, 28, 42, 56, 70, 84, 98, and 112, respectively. On each sampling day, four fish were randomly chosen from the treated group, then the length and weight of each fish were recorded. Blood samples were taken from the dorsal aorta, centrifuged at 3000 rpm for 20 min, and serum was collected. Then, the fish were carefully dissected, separated into the gill, liver, gonad, gastrointestinal tract (GI), and carcass (whole fish minus liver, gonad, gill, and GI tract). The mass of each tissue was recorded, and then the gill, liver, gonad, and GI of fish collected on each sampling day were respectively pooled to form two composite samples, whereas the sera were pooled into one sample. Additionally, fish feces collected on days 1–7, 8–21, and 22–28, and on days 28–42, 43–56, 57–70, 71–84, 85–98, and 99–112 were respectively pooled into nine composite samples. All samples were freeze-dried, ground into powder, weighed, and stored at −20 °C prior to being analyzed. On each sampling day, two carp were randomly sampled from the control group, which was fed non-spiked food throughout the experiment, and treated in the same way as those in the exposure group. Meanwhile, 1 L of water was collected from the aquarium on each sampling day, and analyzed for the target compounds.

2.4. Sample cleanup and instrumental analysis

The analytical procedures for fish tissues (gill, liver, gonad, GI, and carcass), and food and composite feces samples were performed as those in the previous studies (Hoh et al. 2006; Zeng et al. 2014) with minor modification. In brief, freeze-dried samples were spiked with surrogate standard (BDE 128) prior to Soxhlet-extraction with 200 mL Hex/Ace (1:1, v/v) for 48 h. The extract was evaporated and redissolved to 10 mL hexane. An aliquot of the extract was used to determine the

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lipid content by gravimetric analysis. Lipids in the remaining extract was removed by treating with 3 mL of guaranteed reagent-grade concentrated H_2SO_4 twice. After removal of the residual H_2SO_4 with sodium sulfate (5%, w/w), the lipid-free extract was concentrated to 1 mL, further purified on a multilayer neutral/sulfuric acidified silica column. The DP isomers and their possible dechlorinated analogs were obtained by eluting with 30 mL Hex/DCM (v/v = 1:1). Finally, the extract was evaporated to near dryness and reconstituted in 300 μL isooctane for analysis. Recovery standard (BDE 181) was added before injection.

The water was filtered using glass fiber filter (Whatman 0.7 μm pore size, 47 mm diameter), and spiked with surrogate standard (BDE 128) prior to liquid-liquid extraction three times with 500 mL DCM. The combined extract was concentrated and purified as described above.

Serum pretreatment was performed as previously described with a minor modification (Malmberg et al. 2005; Zeng et al. 2014). Briefly, after spiking with the surrogate standard (BDE 128), approximately 0.5 g of serum was denatured by 1 mL of hydrochloric acid (6 mol/L) and 6 mL of 2-propanol, and then extracted thrice with 6 mL of MTBE/Hex (1:1, v/v). After washing with a potassium chloride solution (1%, w/w, 4 ml), the combined extracts were evaporated to near-dryness for determination of the lipid content by gravimetric analysis. The extracts were redissolved with hexane, then treated with H_2SO_4, and cleaned as described above.

Determination of DP and its possible dechlorinated analogs (*anti*-Cl_{11}-DP and *anti*-Cl_{10}-DP; and semi-quantitation will be achieved for *syn*-Cl_{11}-DP and *syn*-Cl_{10}-DP, if any, in reference to the response of *anti*-Cl_{11}-DP and *anti*-Cl_{10}-DP) was performed using gas chromatography/mass spectrometry analysis (Agilent 7890A GC/5975B MSD; Agilent Technology, Palo Alto, CA, USA) with an electron capture negative ionization in a selective ion mode, and a DB-XLB capillary column (30 m × 0.25-mm i.d. × 0.25-μm film thickness, J&W Scientific, Folsom, CA, USA) was used for separation. More details regarding the GC condition and oven temperature programs are given in the supporting information (SI).

2.5. Quality assurance and quality control

Quality assurance and control for quantification of *syn*- and *anti*-DP isomers was conducted by analyses of laboratory method blanks, blank spiking, surrogate spiking, and calibration-standard injections. The recoveries of *syn*-DP, *anti*-DP, *anti*-Cl_{11}-DP and *anti*-Cl_{10}-DP spiked in blank solvent and matrix ranged from 87% to 101%, from 85% to 116%, from 56% to 71%, and from 68% to 76%, respectively. No *syn*-DP and *anti*-DP,Cl_{11}-DP and Cl_{10}-DP was detected in the procedure blanks. The recovery for BDE 128 for all samples ($n = 161$) ranged from 88% to 115%.

The method detection limits (MDLs) were determined as the minimum amount of analyte which produced a peak with a signal to noise (S/N) ratio of 3. The MDLs for *syn*- and *anti*-DP, *anti*- and *syn*-Cl_{11}-DP, and *anti*- and *syn*-Cl_{10}-DP were ranged from 0.13 to 6.75 ng/g lw, 0.10 to 4.96 ng/g lw, and 0.17 to 5.18 ng/g lw in fish tissues, respectively; and ranged from 0.04 to 0.67 ng/g dw, 0.03 to 0.49 ng/g dw, and 0.03 to 0.51 ng/g dw in food and feces, respectively.

2.6. Data analysis

All concentrations in fish tissues and sera were lipid-based, unless otherwise specified. Equations for calculating the bioaccumulation parameters were similar to the previous study (Wong et al. 2002), and given in details in the SI. Statistical analyses were performed using the SPSS 21 software for Windows (SPSS), and the curve fitting was performed with Origin 8.5. One-way analysis of variance (ANOVA) was used to determine the possible statistical differences in the inter-tissue variability of DP levels, and paired-samples *t*-test was used to examine the difference between the weight and length of fish sampled on different days, and between the absorption/assimilation efficiencies of *syn*-DP and *anti*-DP. The relevance between wet-weight concentrations of DP isomers and the tissue lipid content was evaluated by Spearman's correlation analysis. The criterion for significance was set at $p < 0.05$.

3. Results and discussion

3.1. Background levels

After the duration of 112-d experiment, the weights and lengths of common carp were 17.8 ± 1.9 g and 10.5 ± 0.9 cm, respectively, which did not change significantly compared to the initial values (*t*-test, $p > 0.05$). The average concentrations of *syn*- and *anti*-DP ranged from 15.2 ± 10.1 pg/g lipid weight (lw) to 79.3 ± 26.5 pg/g lw, and from 14.8 ± 11.2 pg/g lw to 83.2 ± 23.5 pg/g lw in the control fish tissues, and was below the detection limit in the non-spiked food. These values were several orders of magnitude lower than those of the dosed fish and the spiked food. No possible dechlorinated products of DP isomers were observed in fish tissue and fecal samples, suggesting that common carp have a low DP dechlorination ability. The result indicated that metabolism of DP *in vivo* should not be the source of dechlorinated DP analogs in fish. Given the relatively lower recoveries of *anti*-Cl_{11}-DP and *anti*-Cl_{10}-DP spiked in blank solvent and matrix, the non-detection for the dechlorinated DP analogs in the present study could also result from the using of H_2SO_4 for lipid removal, because acid treatment for the samples might destroy the relevant analytes. However, dechlorinated DP metabolites were also undetected in rainbow trout liver extracts (Tomy et al. 2008), and *in vitro* study using liver microsomes of Chinese Sturgeon (Peng et al. 2012) and ring-billed gull (Chabot-Giguere et al. 2013). These three studies provide support to the present common carp *in vivo* study, suggesting that enzyme-mediated dechlorinated metabolism of DP isomers in fish might be very low, if it does occur.

3.2. Gastrointestinal absorption and fecal excretion of DP isomers

Syn-DP and *anti*-DP were detected in all the food and fecal samples. The concentrations of *syn*- and *anti*-DP isomers in the spiked food pellet homogenate were 715 ± 15.1 ng/g dw and 720 ± 22.3 ng/g dw, respectively, which were very close to the nominal concentration. The concentrations of *syn*- and *anti*-DP isomers in feces collected during the uptake period were respectively in the range of 1.24- to 2.28-fold and 1.20- to 2.25-fold of that in the administered food.

A ratio of the chemical concentration in the feces to the chemical concentration in the food is directly relevant to the chemical absorption and excretion in the gut, if the chemicals are not metabolized by endogenous enzymes in the gastrointestinal system (Zeng et al. 2014). The higher the ratio, the lower the absorption efficiency or the higher the excretion efficiency is. In addition, the losses of the target compounds from feces to water in this study could be neglected as the DP concentrations in water were two to three orders of magnitude lower than those in feces (Fig. 1). The ratios of feces to food for *anti*-DP (1.75, 1.20, 2.25) were always slightly lower than those of *syn*-DP (1.79, 1.24, 2.28) during the exposed period, providing a clue that the absorption efficiencies of the *anti*-isomer in the gastrointestinal system were higher than those of the *syn*-isomers, which was consistent with the results of the previous study (Zeng et al. 2014).

The concentrations of two isomers in feces collected in the depuration period sharply decreased significantly in comparison with those in the uptake phase. An exponentially decreasing trend in the concentration of target chemicals over the depuration period was observed (Fig. 1).

The fraction of *anti*-DP (f_{anti}, defined as the concentration of *anti*-DP divided by the total DP) in feces collected during the uptake period was very close to 0.50 (0.496), however, it increased linearly from 0.503 at day 7 of depuration to 0.718 at day 84 of depuration (Fig. 2), indicating that the *anti*-DPs are being selectively eliminated in common carp.

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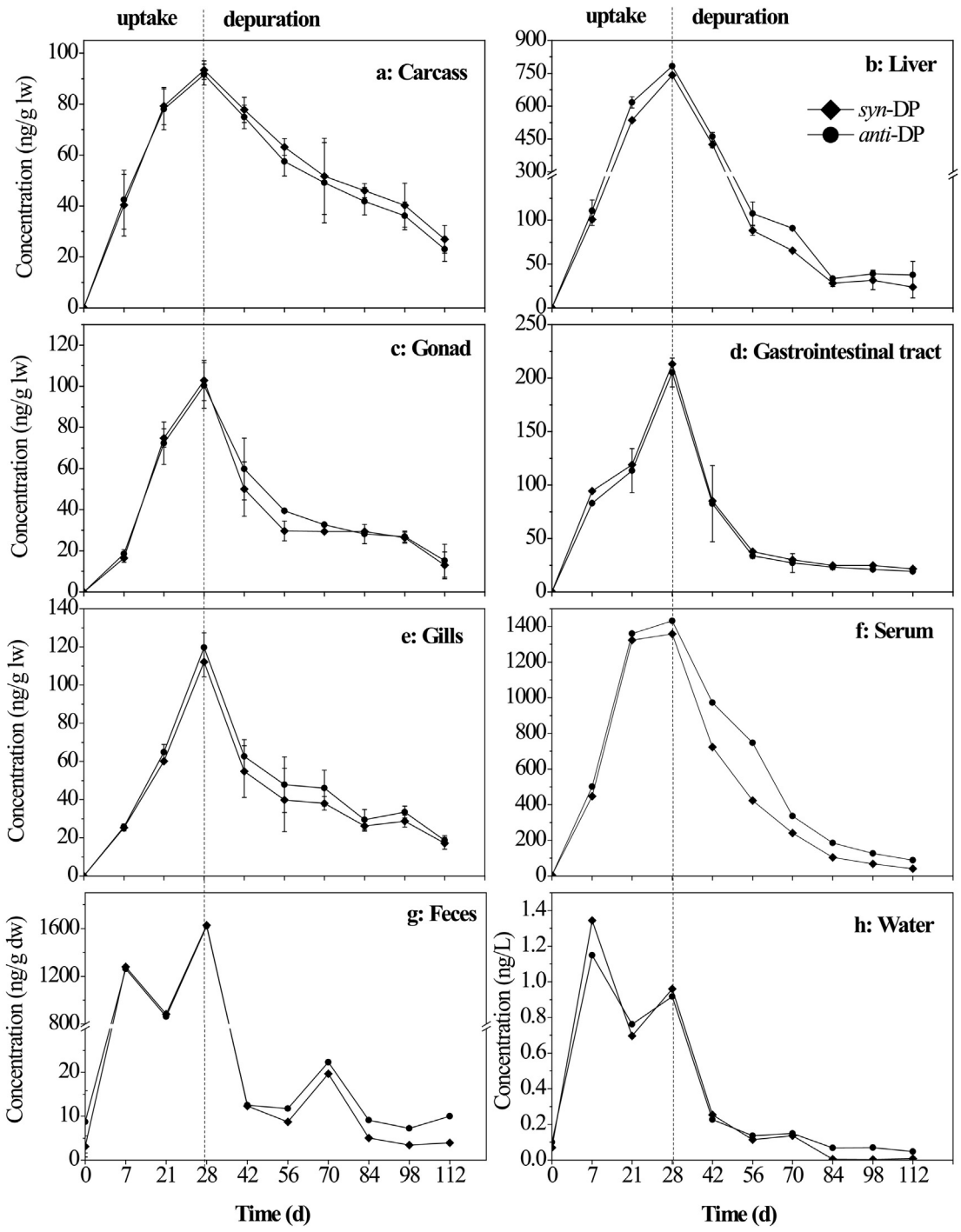

Fig. 1. Uptake and depuration curves of *syn*-DP and *anti*-DP throughout the experimental period in the tissues of common carp (*Cyprinus carpio*), and in feces and water. Error bar indicated ± standard deviation.

3.3. Bioaccumulation parameters of DP isomers

The uptake and depuration kinetic constants of DP in the carcass, liver, gonad, GI, gill, and serum were examined, and both isomers were detected in all the target tissues after seven days of exposure. The uptake curves were similar for both *syn*-DP and *anti*-DP. A linear accumulation was found in all tissues except for serum throughout the duration of the uptake phase (Fig. 1), both isomers reached their highest concentrations at the end of the uptake period (28 d) (Fig. 1), and no steady state was found during the 28-d exposure. However, both *syn*-DP and *anti*-DP has reached a steady state in serum at the end of the uptake period (Fig. 1), indicating an equilibrium for the concentrations of both isomers in the serum within 28-d exposure. Similarly, a steady state could be observed for *syn*-DP and *anti*-DP in GI tract when expressed the concentrations on a wet-weight basis (Fig. S1). The elimination of DP isomers in all the tissues showed an obvious decreasing trend during the depuration period, which followed first-order depuration kinetics. The uptake and depuration kinetics constants, including assimilation efficiencies (α), depuration rates (k_d), half-lives ($t_{1/2}$), and biomagnification factors (BMFs), of DP isomers in the carcass, liver, gonad, GI, gill, and serum were calculated (Table 1).

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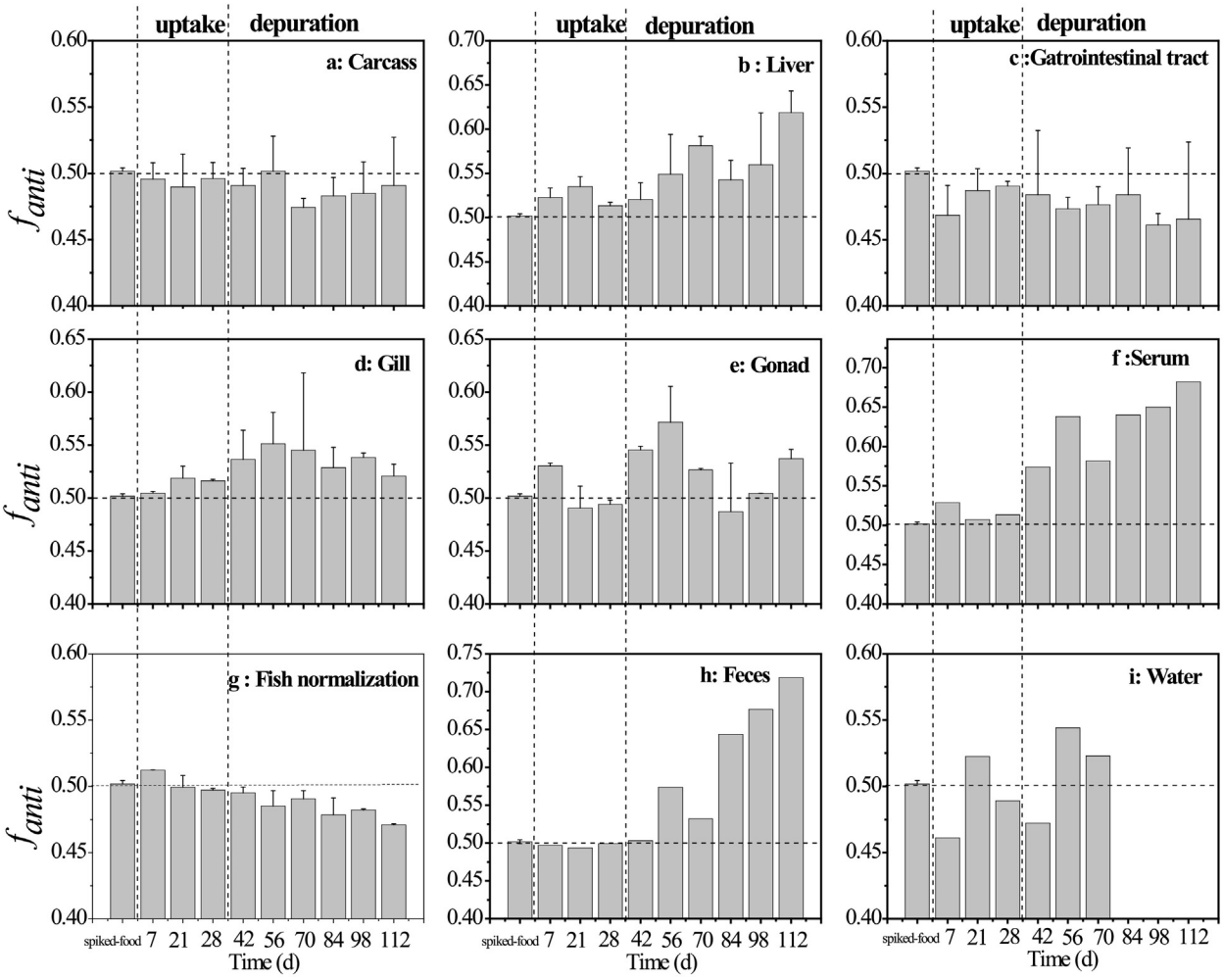

Fig. 2. f_{anti} values calculated in the fish tissues, and in feces and water throughout the experimental period. Error bar indicated ±standard deviation.

The highest assimilation efficiencies of DP isomers were in the liver (24.1 ± 0.8% and 26.4 ± 0.5% for *syn*- and *anti*-DP, respectively), followed by the GI (10.8 ± 0.5% and 9.9 ± 0.4%), serum (7.1 ± 0.1% and 7.0 ± 0.1%), carcass (4.8 ± 0.3% and 4.6 ± 0.4%) and gonad (2.8 ± 0.7% and 2.7 ± 0.5%), and the lowest was in the gill (2.2 ± 0.1% for both isomers). The assimilation efficiencies of DP isomers in the carcass were comparable to those determined in our previous study (3.8% and 3.2% for *syn*- and *anti*-DP, respectively) (Zeng et al. 2014), and also to those in juvenile rainbow trout (6.0% and 3.9% for *syn*- and *anti*-DP, respectively) (Tomy et al. 2008). However, no significant difference was found between the assimilation efficiency of the *syn*-DP isomer and *anti*-DP isomer in carcass (*t*-test, $p > 0.05$), which was different from the results in the previous studies (Tomy et al. 2008; Zeng et al. 2014).

Depuration rate constants (k_d) for *syn*-DP and *anti*-DP ranged from 0.0138 ± 0.0010 d^- in the carcass to 0.0425 ± 0.0068 d^- in the liver, and 0.0150 ± 0.0011 d^- in the carcass to 0.0388 ± 0.0069 d^- in the liver, respectively (Table 1). Based on the calculated k_d, half-lives ($t_{1/2}$) were derived for both *syn*-DP and *anti*-DP in all tissues, which ranged from 16.3 ± 2.7 d in the liver to 50.2 ± 3.7 d in the carcass, and 17.8 ± 3.3 d in the liver to 45.6 ± 3.5 d in the carcass, respectively (Table 1). Moreover, the depuration rates and half-lives calculated for the carcass in the present study were similar to those (k_d was 0.013 ± 0.003 d^- and 0.023 ± 0.004 d^-, and $t_{1/2}$ was 53. 3 ± 13.1 d and 30.7 ± 5.7 d for *syn*- and *anti*-DP, respectively) determined in rainbow trout (Tomy et al. 2008).

As shown in Table 1, the liver exhibited the highest assimilation efficiencies and the fastest depuration rates. While the serum showed comparable depuration rates to liver, the assimilation efficiencies in serum were much lower than those in the liver. Previous study demonstrated that the transport and equilibration of DP among lipid pools could affect the accumulation and distribution in biota (Li et al. 2013b). The liver is the first organ in which contaminants deposit after absorption from the GI. It is also a rich blood-perfused organ, and the main organ for the metabolism of xenobiotic chemicals (Zeng et al. 2014). These factors may contribute to the high assimilation efficiencies and fast depuration rates of DP isomers in the liver. On the other hand, the poor blood perfusion in muscle could be partly responsible for the low-elimination rate of chemicals in the carcass.

BMF is a comprehensive result of the different processes of uptake, depuration, and metabolism. The BMFs of *syn*-DP and *anti*-DP were less than one in all tissues except in the serum, and respectively ranged from 0.09 ± 0.02 in the gonad and the gill to 1.06 ± 0.12 in the serum, and from 0.09 ± 0.02 in the gonad to 1.23 ± 0.10 in the serum (Table 1), which were significantly lower than those for polychlorinated biphenyls (PCBs) (BMFs ranged from 1.0 to 2.1, except for those of the readily metabolized congeners) in our recent exposure study (Tang et al. 2017). The lower BMFs for DPs could be related to their high log K_{OW} values and large molecular size, which limits the ability to travel through cell membranes and thus, they could be sequestered by the tissue walls (Peng et al. 2012). Considering the fact that no possible dechlorinated DP analogs were observed in this study, the dechlorination metabolism

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Table 1
Bioaccumulation and depuration parameters of *syn*- and *anti*-DP isomers in fish tissues through dietary exposure to common carp.

	Depuration rate[a] (10^{-2}/day)	Half-life[b] (day)	Assimilation efficiency[c] (%)	BMF[d]
Carcass (lipid content[e]: 7.63 ± 2.18%)				
syn-DP	1.38 ± 0.10	50.2 ± 3.7	4.8 ± 0.3	0.21 ± 0.01
anti-DP	1.50 ± 0.11	45.6 ± 3.5	4.6 ± 0.4	0.18 ± 0.01
Liver (lipid content [e]: 3.86 ± 1.26%)				
syn-DP	4.25 ± 0.68	16.3 ± 2.7	24.1 ± 0.8	0.34 ± 0.05
anti-DP	3.88 ± 0.69	17.8 ± 3.3	26.4 ± 0.5	0.21 ± 0.06
Gonad (lipid content[e]: 8.24 ± 3.57%)				
syn-DP	1.91 ± 0.39	36.3 ± 5.8	2.8 ± 0.7	0.09 ± 0.02
anti-DP	1.94 ± 0.24	35.7 ± 4.4	2.7 ± 0.5	0.09 ± 0.02
Gastrointestinal tract (lipid content[e]: 8.75 ± 4.14%)				
syn-DP	2.49 ± 0.60	27.8 ± 7.2	10.8 ± 0.5	0.26 ± 0.11
anti-DP	2.64 ± 0.56	26.2 ± 5.9	9.9 ± 0.4	0.22 ± 0.10
Gill (lipid content[e]: 6.35 ± 2.35%)				
syn-DP	1.87 ± 0.29	37.1 ± 5.9	2.2 ± 0.1	0.09 ± 0.02
anti-DP	1.87 ± 0.27	37.1 ± 5.4	2.3 ± 0.1	0.11 ± 0.01
Serum (lipid content[e]: 0.75 ± 0.24%)				
syn-DP	4.23 ± 0.13	16.4 ± 0.5	7.1 ± 0.1	1.06 ± 0.12
anti-DP	3.52 ± 0.20	19.5 ± 1.1	7.0 ± 0.1	1.23 ± 0.10

[a] Depuration rate constants (k_d) (±standard error) were calculated using Eq. S2 with concentration on a lipid weight basis.
[b] Half-life (±standard error) calculated from Eq. S3.
[c] Assimilation efficiencies (±standard error) were calculated from Eq. S4 based upon the concentrations of DPs at days 7, 14, and 28 of the uptake phase.
[d] Biomagnification factors (BMFs) were calculated from Eq. S5.
[e] *Lipid content* (±standard deviation).

should not be held responsible for the low DP isomers in fish. However, hydroxylated metabolism or other phase II metabolism would occur (Tomy et al. 2008), but such metabolites cannot be detected by the method used in the present study.

To our knowledge, this study was the first of a comprehensive examination of bioaccumulation parameters of DP isomers in fish tissues, as Tomy et al. (2008) has assessed the bioaccumulation parameters of DPs merely in muscle and liver of rainbow trout. These parameters would be essential to provide a new insight to evaluate the ecotoxicology of DPs in aquatic organisms. The BMFs calculated for DP isomers were >1 in serum, implying that DPs will biomagnify in serum of common carp. Meanwhile, the concentrations of DP isomers reached a steady state only in serum within the 28-d exposure period. Therefore, there is reason to speculate that the BMFs (<1) calculated for DP isomers in other fish tissues did not allow underestimating the bioavailability of DP isomers in these tissues, because the concentrations of DP isomers did not reach steady state during the 28-d exposure period. And a longer time frame than 28 d for the exposure period could potentially have yield a biomagnification of DP isomers in these tissues, similar to which has been observed in the serum. Given that the DPs concentrations detected in the wild organisms could be accumulated over long periods of time (months, even years), significant biomagnification of DP isomers were generally observed in aquatic food webs (Tomy et al. 2007; Wu et al. 2010).

3.4. Dynamic tissue distribution and isomer composition

The fish serum exhibited the highest concentrations (lipid-based) of DP isomers throughout the experiment, followed by the liver (Fig. 1). The concentrations of DP isomers in these two tissues were significant higher than those in the carcass, gonad, GI, and gill (one-way ANOVA, $p < 0.05$, Fig. 1). Blood is in contact with all tissues and is in equilibrium with the organs and tissues where chemicals are deposited (Esteban and Castaño 2009). The significantly higher lipid-normalized concentrations of DP isomers in the serum than in other tissues might indicate that these tissues did not reach equilibrium with the serum throughout the experiment (Zeng et al. 2013).

The wet-weight concentrations of DP isomers in the liver were higher than those in the carcass, gonad, GI, gill, and serum (one-way ANOVA, $p < 0.05$, Fig. S1) in the uptake period. This result is consistent with that of our previous study on a high-dose exposure of commercial DP mixture (DP-25) to common carp (Zeng et al. 2014). At the late stage of the depuration period, the concentrations of DPs in the liver were slightly lower than those in the other tissues (Fig. S1), a finding different from that in our previous study (Zeng et al. 2014). In the previous study, DP levels in the liver were still higher than those in other tissues, although the ratio of the levels in the liver to those in the other tissues exhibited an exponential decline. This could be attributed to the higher depuration rates in the liver, and the relative lower concentrations accumulated in the liver resulted from the lower exposure dose compared to the previous study.

The wet-weight concentration of DP isomer was significantly correlated with the tissue lipid content in fish ($p < 0.0001$ for both isomers), excluding those in the liver of fish sampled on days 21 and 28 of the uptake period, and on day 14 of the depuration period, as well as those in the sera of fish sampled on day 28 of the uptake period (Fig. S2). This result revealing that DP isomers prefer to accumulate in lipid-rich tissues, which is in line with those observed in the previous studies (Sühring et al. 2015; Peng et al. 2012; Zheng et al. 2014). However, lipid content cannot explain the high DP deposits in the liver (Fig. S2). Previous study found that the liver of rats exhibited higher levels of DP than the muscle, by exposing rats to commercial DP mixtures (Li et al. 2013a). In Chinese sturgeon, the dechlorane concentrations in liver also found to be higher than those in the muscle and adipose tissue (Peng et al. 2012). Additionally, the livers of common carp in our previous study showed higher concentrations of DP and the analogs than other tissues, in spite of the higher lipid content of these tissues (Zeng et al. 2014). The result of the present study was in line with those in the previous studies, suggesting the sequestration of DP by hepatic proteins, although this was not identified (Li et al. 2013b).

A dynamic tissue distribution for DP among tissues was observed. During the exposure period, the lipid-normalized concentration ratios of the liver to other tissues generally exhibited an increasing trend (Fig. 3), whereas in the depuration period, the ratios exponentially decreased over time, to close to one at the start of the depuration period, and then slightly increased in the later stage of the experiment (Fig. 3). This trend is especially marked for the ratio of liver to serum, which was lower than one throughout the experiment (Fig. 3). The concentrations of DP in liver can be affected by the enterohepatic circulation and redistribution of lipophilic contaminants (Martin et al. 2006), and ultimately give rise to these ratios of concentration in the liver to other tissues. The results indicate that the liver is the first organ to which contaminants deposit after absorption from the GI tract in fish, and the DP concentrations in other tissues had not yet reach equilibrium with the sera throughout the experiment. This result was in line with previous studies on the tissue distribution of DP and its analogs in common carp (Zeng et al. 2014), and of PBDEs in two predator fish species (Zeng et al. 2013).

A tissue-specific isomer composition was observed in common carp (Fig. 2). The f_{anti} values of the liver, gill, and serum were higher than that of the spiked food (0.502 ± 0.002) throughout the experiment, indicating a preference of the *anti*-isomer in these tissues. An opposite trend was observed for the f_{anti} values in the carcass and gastrointestinal tract (Fig. 2). However, no regular trend was found for the f_{anti} values in the gonad during the whole experiment; this could be because gonads collected in different individuals may be in different stages of embryonic development. Sühring et al. (2015) found that *syn*-DP was preferably transferred into gonads in hormone treated eels with f_{anti} < 0.1 in gonads of mature eels while it was not dominant in gonads of non-mature eels (comparison group), which could be due to selective redistribution of *syn*-DP into gonads during maturation. The results

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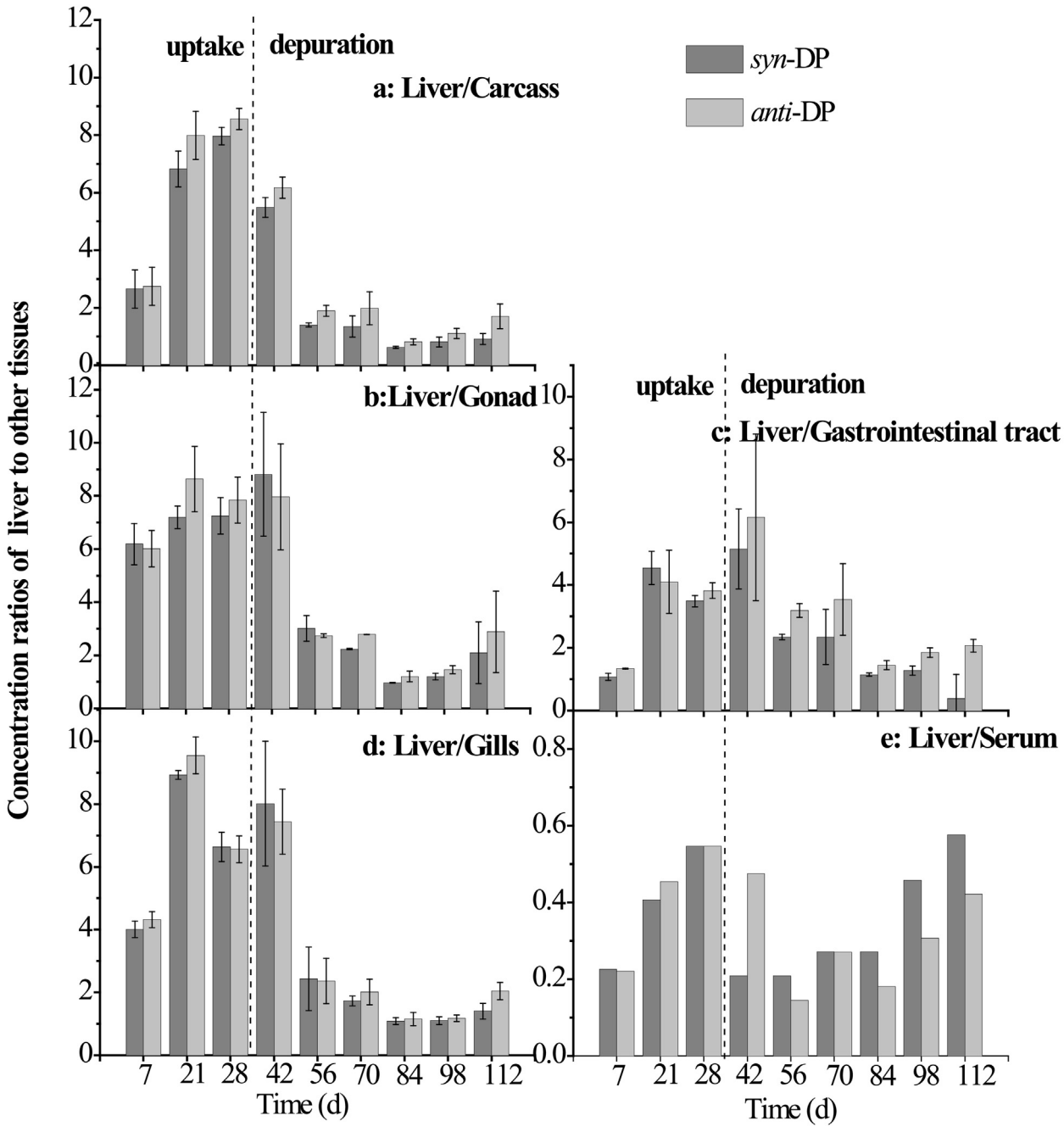

Fig. 3. Concentration ratios of *syn*-DP and *anti*-DP in the liver compared to other tissues of common carp throughout the experimental period. Error bar indicated ± standard deviation.

found in the present study, showing that the liver prefers *anti*-DP to *syn*-DP, were consistent with that of the experiment exposing rats to high doses of DP commercial mixture (Li et al. 2013a). Tissue-specific f_{anti} has also been reported in several previous studies. Zhang et al. (2011b) reported that the brain of the mud carp has a high affinity for *anti*-DP. Peng et al. (2012) found that f_{anti} in the heart, intestine, and gonad were lower than those in the muscle and liver.

As a whole, fish did not show selective accumulation of *syn*- or *anti*-DP isomers in the uptake stage in the present study (Fig. 2g). This was slightly different from the results in previous DP technical mixture exposure study (Zeng et al. 2014), in which the f_{anti} values in fish tissues were lower than those in the technical mixture in the early stage of the uptake experiment. Li et al. (2013a) had revealed that the isomer selective accumulation in rats was dose-dependent. A selective accumulation of *syn*-DP was only observed in the high-dose exposure group. Comparing the results of the present study to the previous high-dose exposure study (Zeng et al. 2014), a dose-dependence for the stereoselective accumulation of DP in the tissues of common carp also existed. However, it should be noted that the fish culture and growth stage of fish as well as the different experimental conditions could also affect the different observations between these two studies, since they were not conducted at same time.

During the depuration period, a selective accumulation of *syn*-DP in fish was observed (Fig. 2g). This result was consistent with those reported in most fish species sampled from the field, for which f_{anti} was generally lower than the technical mixtures (Tomy et al. 2007; Wu et al. 2010). As previously discussed, *anti*-DP being selectively eliminated by the feces of common carps could be one of the reasons. Considering

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the high f_{anti} and the highest elimination rate of DPs in the liver, metabolism cannot be ruled out as a possible reason for the isomer-specific accumulation of DPs in the fish.

4. Conclusion

In summary, the gastrointestinal absorption and the tissue-specific and stereoselective bioaccumulation of DP isomers in common carp were investigated in the present study. While the absorption efficiency of the *anti*-isomer could be higher than that of the *syn*-isomer in the GI system, the assimilation efficiency of *anti*-isomer in the GI was significantly lower than that of the *syn*-isomer, and conversely result was found in the liver, which exhibited the highest assimilation efficiencies and the fastest depuration rates for both *syn*- and *anti*-DP isomers. However, no significant difference was found between the assimilation efficiencies of *syn*-isomer and *anti*-isomer in the carcass, gonad, gill, and serum. The BMFs of both isomers were less than one in all tissues except for the serum, which could be related to the high log K_{OW} values and large molecular size of DP isomers. A dynamic tissue distribution of DP isomers was observed; liver is the first organ to which DP deposits after absorption from the GI tract in fish. The isomer composition of the DPs also exhibited tissue specificity; a preference for *anti*-DP was observed in the liver, gill, and serum, whereas *syn*-DP was selectively accumulated in the carcass and GI tract. As a whole, fish did not show selective accumulation of *syn*- or *anti*-DP isomers in the uptake stage, whereas during the depuration period a selective accumulation of *syn*-DP was observed.

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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.2017.10.183.

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