Accumulation pattern of Dechlorane Plus and associated biological effects on rats after 90 d of exposure

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HIGHLIGHTS

► We exposed DP on rats in different dose levels to detect the accumulation pattern and biological effects.
► DP preferentially accumulated in the liver rather than in muscle at all exposure levels.
► Syn-DP was prone to accumulate in all tissues tested at high exposure concentration (10 and 100 mg kg g^{-1}).
► The value of f_{anti} is different in various exposure concentration.
► No observable-effect in histopathology and death during the whole experiment.

GRAPHICAL ABSTRACT
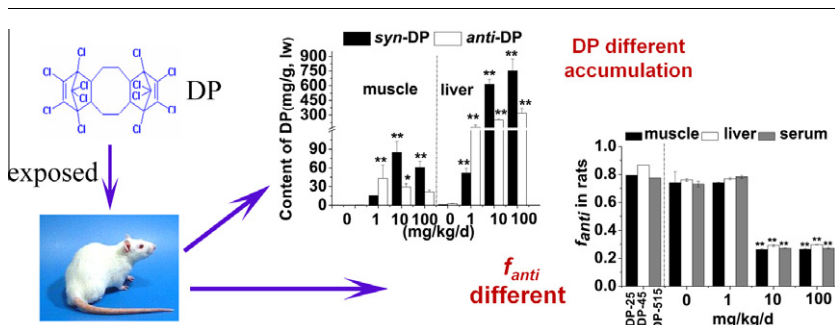
ABSTRACT

Recent studies have indicated that Dechlorane Plus (DP) is widespread in the environments. However, different isomer-specific enrichment pattern of syn-DP and anti-DP was reported in biological samples from the field. In this study, Sprague–Dawley rats were consecutively exposed to commercial DP 25 by gavage for 90 d at different doses (0, 1, 10, and 100 mg kg^{-1} d^{-1}) to investigate the accumulation pattern of syn-DP and anti-DP in liver, muscle, and serum of rats. The possible biological effects of DP on rats were also examined. Results showed that DP preferentially accumulated in the liver rather than in muscle at all exposure levels. No significant stereoselectivity of anti-DP or syn-DP in tissues was observed in the low DP exposure groups (0 and 1 mg kg^{-1} d^{-1}) with f_{anti} values (defined as the concentration of the anti-DP divided by the sum of concentrations of anti- and syn-DP) ranging from 0.74 to 0.78. However, f_{anti} values reduced (f_{anti} ranged from 0.26 to 0.30) significantly in the high DP exposure groups (10 and 100 mg kg^{-1} d^{-1}) and syn-DP was predominant in all tissues. Biochemical parameters in serum, the mRNA expression levels of certain enzymes and their activities in liver were detected. There was no observable-effect in histopathology and death during the experiment, although the mRNA expression levels of some genes in the low dosage group decreased significantly and enzyme activity of CYP 2B2 increased.

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1. Introduction

Dechlorane Plus (DP) is an unregulated chlorinated flame retardant used as a replacement of toxic Dechlorane and Mirex, which was first introduced into the market in the 1960s. Commercial

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DP has two isomers (*syn*-DP and *anti*-DP), with the ratio of 1:3 (Tomy et al., 2007), which has been widely employed in products such as electrical wires, cable coatings, plastic roofing materials, and hard connectors in computers and televisions (Weil and Levchik, 2004). Three types of DP products are currently available (DP-25, DP-35, and DP-515), which are identical in chemical composition but differ in particle size (Zhu et al., 2007). Annual production of DP is estimated at ∼5000 tons in the European Union and is marketed worldwide (Ren et al., 2008), while current production in China is estimated at ∼300 tons (http://info.china.alibaba.com/news/detail/v4-d1001424159.html, in China). Recently, DP was listed by the European Commission as a possible replacement for the newly restricted decabromodiphenyl ether (Deca-BDE) for several applications such electrical products (Sverko et al., 2011), which might lead to increasing usage in newly industrializing countries in Asia where the majority of these products are manufactured.

Previous studies have investigated the occurrence and behavior of DP in the atmosphere, sediment, soil, water, and biota including wildlife and humans, demonstrating that DP is a persistent, bioaccumulative environmental contaminant, and susceptible to long-range atmospheric transport (Sverko et al., 2011; Xian et al., 2011). One of the most discussed issues among researchers regarding the environmental behavior of DP is the stereoisomeric fractionation of DP in various environmental matrices. While selective depletions of *anti*-DP during long-range transport were observed in the atmosphere from the Great Lakes region and along the northern Atlantic Ocean (Moller et al., 2010; Sverko et al., 2011), no significant stereoisomeric fractionations of DP were found in air samples across China (Ren et al., 2008). The DP isomer profiles measured in biota samples are more complicated. Wu et al. (2010) described net *syn*-isomer enrichment in the aquatic food web from a reservoir in an e-waste recycling site in China. Tomy et al. (2007) reported *anti*-isomer biomagnifications in the food web at Lake Winnipeg but an absence of a stereoisomer biomagnifications in the food web at Lake Ontario. In bald eagle plasma from the Great Lakes region, the content of *anti*-DP was about two times than *syn*-DP (Venier et al., 2010). No significant stereoselective enrichments were detected within herring gull eggs from the Laurentian Great Lakes of North America or white stork eggs from Spain (Gauthier and Letcher, 2009; Munoz-Arnanz et al., 2011), but preferential accumulation of *syn*-DP was observed in water birds from the above-mentioned e-waste recycling site (Zhang et al., 2011). From a few studies of DP in mammals, a preferential depletion of *anti*-DP was measured in serum samples of residents from an e-waste dismantling region in China, with little stereoselectivity of DP found in serum samples of people living in a nearby region (Ren et al., 2009). Only slight depletion of *anti*-DP was reported in a small number of pet dog serum samples from the United States (Venier and Hites, 2011). These available data on DP stereoselectivity give a picture that biota samples from e-waste sites generally have more stereoselectivity than other samples in the environment, leading to the speculation that stereoselectivity of DP in organisms may depend on exposure concentration.

Very little data exists on the toxicity of DP (Brock et al., 2010; Crump et al., 2011). A recent study examined the reproductive toxicity of DP in rats and found that DP doses of 5000 mg kg^{-1} had no effect on in-life parameters or clinical and anatomic pathology during the 28-d repeat dose toxicity phase, and no effects were observed on reproductive or fertility indices or on fetal growth in the developmental and reproductive toxicity phase (Brock et al., 2010). Additionally, Crump et al. (2011) determined the effects of DP exposure on cytotoxicity, embryo toxicity and mRNA expression levels in the domestic chicken using *in vitro* and *in ovo* approaches; no overt toxic effects (hepatocytes and pipping success) were observed up to the highest nominal dose group of 500 ng g^{-1} egg, and none of the eleven gene targets identified as responsive to the brominated flame retardant, hexabromocyclododecane, changed after DP treatment. Although the few available toxicity data indicate that DP has no significantly overt toxic effects on test organisms, additional molecular markers should be analyzed to identify the potential targets that might be responsive to DP, and subchronic/chronic toxicity studies on DP are needed to evaluate the risk of DP on the environment and humans before it is used as an alternative for Deca-BDE.

In this study, male Sprague Dawley (SD) rats were orally administrated commercial DP-25 mixed with corn oil (existing service is used) for 90 d at different doses. Another two groups were exposed to a known amount of DP-25 for 45 d followed by 45 d depuration. The DP levels in liver, muscle, and serum were measured after exposure to investigate the tissue distribution of DP and its stereoisomeric fractionations following different doses (in the range of 0–100 mg kg^{-1} d^{-1}). The dechlorinated DP products and other possible DP metabolites in various tissues were also screened using GC/ECNI-MS and GC/EI-MS. We also assessed possible toxicity of DP by monitoring a suite of biochemical parameters, including thyroid hormone levels, thirteen clinical chemistry parameters, the response to environmental pollutants of mRNA expression levels, and the activity of certain enzymes.

2. Materials and methods

2.1. Animals

Forty-two Male SD rats (35-d-old) with average weight of 110 g were obtained from the Weitong Lihua Experimental Animal Central (Beijing, China) and were housed individually in a mass air-displacement room with a 12-h light–dark cycle at 20–26 °C and a relative humidity of 50–70%. After 1 week acclimation, they were separated into six groups: the rats in four groups were exposed to consecutive dosages of 0, 1, 10, and 100 mg kg^{-1} d^{-1} for 90 d, while the other two groups were exposed to 0 and 100 mg kg^{-1} d^{-1} for 45 d followed by 45 d of depuration during which the rats were fed unfortified food. All rats were euthanized after their exposure period. Liver, muscle, and serum were immediately collected from each rat and were frozen in liquid nitrogen and stored at −20 or −80 °C until the chemical analysis and toxic experiments, respectively.

2.2. Chemicals and chemical analysis

Commercial DP 25 (⩾99% purity) was obtained from Jiangsu Anpon Co., Ltd and corn oil was purchased from Sigma–Aldrich (Oakville, ON, Canada). The *anti*-DP, *syn*-DP, *anti*-Cl$_{11}$-DP, and *anti*-Cl$_{10}$-DP standards were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). All solvents and reagents used in these experiments were of analytical grade, and organic solvents were redistilled using the glass system. The BDE-77, BDE-181, BDE-128, and BDE-118 were purchased from AccuStandard (New Haven, US).

The DP was scanned by an Agilent 6890 gas chromatograph coupled with an Agilent 5975C mass spectrometer (GC/MS) using electron capture negative ionization (ECNI) in the selective ion-monitoring (SIM) mode and separated by a DB-XLB (30 m × 0.25 mm × 0.25 μm, J&W Scientific) capillary column. The initial oven temperature was set as 110 °C (held for 1 min), ramped at 8 °C min^{-1} to 180 °C (held for 1 min), and then 2 °C min^{-1} to 240 °C (held for 5 min), 2 °C min^{-1} to 280 °C (held for 15 min), and finally 10 °C min^{-1} to 310 °C (held for 10 min). We manually injected 1 μL of sample in the pulsed splitless mode. The temperature of the injection port was set at 280 °C. The

monitored and quantitative ions were as follows: m/z 653.8 and 651.8 for DP isomers (syn-DP and anti-DP), m/z 618 and 620 for anti-Cl_{11}-DP and syn-Cl_{11}-DP, and m/z 584 and 586 for anti-Cl_{10}-DP. The syn-Cl_{11}-DP was previously identified as a dechlorinated product of syn-DP by Wang et al. (2011). Semi-quantitation was achieved to syn-Cl_{11}-DP by reference to the response of anti-Cl_{11}-DP on GC/MS. We also attempted to screen some possible methoxy- and/or methylsulfone-DP in rat serum and liver. Such DP metabolites, if present, would ionize similar to OH-CB and/or $MeSO_2$-CB by scavenging an electron to show a dominant M^- in ENCI ion source. Corresponding m/z values of potential oxidative metabolites were demonstrated by Tomy et al. (2008).

2.3. Sample preparation

Details of the analytical procedures for liver and muscle are described in the supplementary data. Pretreatment for serum was performed as Malmberq (Malmberg et al., 2005) with minor modification. Two milliliter of serum was spiked with surrogate (BDE-181). The protein was denatured with 1 mL of hydrochloric acid (6 M) and 6 mL of 2–propanol, and the mixtures were shaken vigorously. The DP and its metabolites were twice extracted with 6 mL of 1:1 hexane/methyl tert-butyl ether (MTBE) (V/V = 1:1) mixture. The combined organic extracts were washed with a potassium chloride solution (1%, 3 mL), and the solvent was concentrated to dryness under N_2 for gravimetric lipid weight determination. The extract was redissolved with 6 mL of hexane, and the aqueous phase compounds were separated from neutrals by partitioning with potassium hydroxide (0.5 M in 50% ethanol). The aqueous phase was re-extracted again with hexane (6 mL) for complete extraction. Neutral phase was treated with 2 mL of concentrated sulfuric acid to remove lipids, and subsequent processing was eluted with 40 mL hexane/dichloromethane (V/V = 1:1) in multi-layer silica column (i.d. = 1 cm) packed with 8 cm neutral silica and 8 cm acidified silica. Elutes were concentrated to near dryness under N_2 and redissolved in 270 μL of isooctane and spiked with a known amount of internal standard (30 μL BDE-128) before instrumental analysis.

2.4. QA/QC and data analysis

Procedural blanks covering the whole procedure were performed in parallel with the samples on each batch of extraction. The average relative standard deviations (RSDs) among triplicates samples ranged from 3% to 9.6% for all targets. The recoveries for surrogates ranged from 71.7% to 112.9% in all detected samples. The method detection limit (MDL) was defined as the mean value plus 3-fold standard deviation for analytes, which were detected in the procedural blanks ($n = 6$) and for others which were not detected in blanks, a signal-to-noise ratio of ten was set as MDL. The MDLs for syn-DP, anti-DP, and anti-Cl_{11}-DP were 108.31, 70.44, and 0.98 ng g^{-1} lw in muscle and liver, and were 0.20, 0.054, and 0.042 ng ml^{-1} for syn-DP, anti-DP and anti-Cl_{11}-DP in serum, respectively. Statistical analysis was performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL) for comparisons of means between and within treatment groups. The f_{anti} was delegated to ratio of anti-DP/(anti-DP + syn-DP), which was used to evaluate the profiles of DP in the tissue of experiment animals. One-way analysis of variance (ANOVA) was used to determine differences in concentrations and other experimental statistics. Significance was set at $p < 0.05$.

2.5. Serum biochemical parameter and thyroid hormone analysis

Standard spectrophotometric methods for the HITAC 7170A automatic analyzer were applied to measure serum parameters including alanine amino transferase (ALT), aspartate aminotransferase (AST), albumin (ALB), alkaline phosphatase (ALP), total bile acids (TBA), urea nitrogen (BUN), creatinine (CRE), cholesterol (CHO), triglycerides (TG), high density lipid–cholesterol (HDL-C), low density lipid–cholesterol (LDL-C), creatine kinase (CK), and glucose. The levels of thyroid hormones, total triiodothyronine (T3) and thyroxine (T4), free T3 and T4 in serum were detected by chemiluminescence. To avoid fluctuation in thyroid hormone levels, serum was collected from the control and exposed groups at the same time during 1 d.

2.6. Gene expression and enzyme activities analysis

Gene response to environmental pollutants was detected, including Nat2 and SULT isomers, and the expression levels of selected genes and their PCR primers are given in supplementary data Table A1. Total RNA from the 90-d exposed rat livers were isolated by TRizol reagent and measured by absorbance at 260 nm performed in a UV1240 spectrophotometer (Shimadzu, Japan) and 260/280 nm absorbance ratio for estimating its purity. Every sample was analyzed in triplicate. β-actin was chosen for the housekeeping gene and was used as an internal control. Cycle threshold (Ct) data were normalized to β-actin, and the fold changes in target genes were calculated using the 2$-\Delta\Delta$Ct (20). Enzyme activities were measured according to CYP1A2, CYP2B1, CYP2B2, CYP3A, and GST kit, respectively (Genmed Scientifics Inc. USA).

3. Results and discussion

3.1. DP distribution in rat tissues

To investigate the distribution profile of DP in the rat, syn-DP, anti-DP, syn-Cl_{11}-DP, and anti-Cl_{11}-DP were detected in liver, muscle, and serum. The details of the concentration in tissues are given in supplementary data (Table A2). In the control group, detection frequencies of syn-DP and anti-DP were 100% in all tissues measured. The average concentrations of anti-DP and syn-DP were found in liver up to 2800 ± 1200 and 890 ± 400 ng g^{-1} lw, respectively, which indicate that syn-DP and anti-DP accumulated in organs from background exposure such as feed or air-borne dust, which may be due to the fact that DP was widespread in the environment including the food (Venier and Hites, 2011). Neither syn-Cl_{11}-DP nor anti-Cl_{11}-DP was found in the tissues of the control group.

In the exposure groups, the highest concentrations of syn-DP and anti-DP were both detected in liver from the 100 mg kg^{-1} d^{-1} dose group, which were nearly 12–15-folds than that in muscle and 4.8–5.4-folds that found in serum from the same group, respectively (Fig. 1A). In liver, the concentration of syn-DP and anti-DP increased with DP exposure dosage. In muscle, the highest content of syn-DP occurred in the 10 mg kg^{-1} d^{-1} group (84 ± 39 μg g^{-1} lw). However, the highest concentration of anti-DP was detected in the 1 mg kg^{-1} d^{-1} group in muscle, which reduced with increasing DP exposure dosage (Fig. 1A). In serum, the concentration of syn-DP increased with DP exposure dosage and anti-DP was also prone to accumulate in the 1 mg kg^{-1} d^{-1} group (Fig. 1B).

We next analyzed the Cl_{11}-DP in rat tissues. The highest average concentration of syn-Cl_{11}-DP and anti-Cl_{11}-DP occurred in liver in the 100 mg kg^{-1} d^{-1} group, reaching 140 ± 51 and 480 ± 170 ng g^{-1} lw, respectively. The average concentrations of syn-Cl_{11}-DP and anti-Cl_{11}-DP in liver in the 100 mg kg^{-1} d^{-1} group were 4.7- and 8.3-fold greater than that found in muscle in the same group. In liver, syn-Cl_{11}-DP and anti-Cl_{11}-DP increased with DP exposure
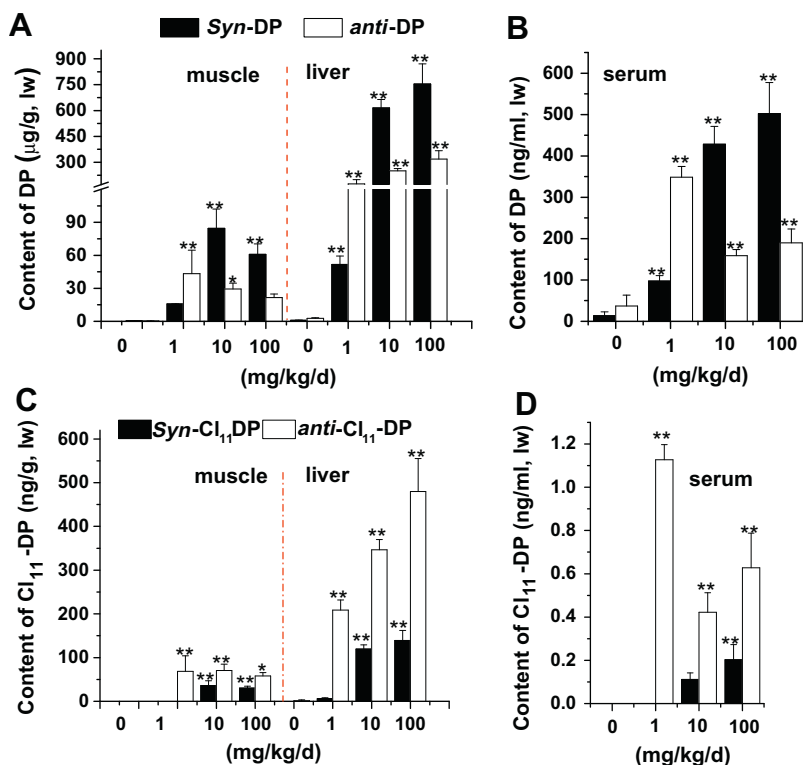
Fig. 1. Levels of syn-DP ($\mu g\,g^{-1}$ lw), $anti$-DP ($\mu g\,g^{-1}$ lw), syn-Cl_{11}-DP ($ng\,g^{-1}$ lw), and $anti$-Cl_{11}-DP ($ng\,g^{-1}$ lw) measured in the liver, muscle, and serum of rats exposed to 0, 1, 10, and 100 $mg\,kg^{-1}\,d^{-1}$ DP dosages. Values represent the mean and standard error of five rats in each group. Statistically significant differences between controls and treatments are indicated by *for $p < 0.05$. (A) Content of DP in muscle and liver; (B) content of DP in serum; (C) content of syn-Cl_{11}-DP and $anti$-Cl_{11}-DP in muscle and liver; (D) content of syn-Cl_{11}-DP and $anti$-Cl_{11}-DP in serum.

dosage. In muscle, syn-Cl_{11}-DP and $anti$-Cl_{11}-DP were accumulated; however, no significant change was observed among the DP exposure groups (Fig. 1C). In serum, the highest concentration of $anti$-Cl_{11}-DP was detected in the 1 $mg\,kg^{-1}\,d^{-1}$ group (Fig. 1D). It is worth noting that the contents of $anti$-Cl_{11}-DP in the three tissues were higher than those of syn-Cl_{11}-DP in corresponding groups, indicating that $anti$-Cl_{11}-DP was more accumulated than syn-Cl_{11}-DP in the tissues measured. Since syn-Cl_{11}-DP and $anti$-Cl_{11}-DP are also detected in commercial DP-25, we could not exclude the possibility that these chemicals originated from commercial products.

To further explore possible DP degradation in tissue samples, we conducted three photolytic degradation experiments by exposing $anti$-DP, syn-DP, and commercial DP solutions to UV light, the degradations were mainly [$-Cl + H$] and [$-2Cl + 2H$] processes. Compared to rat liver samples, syn-DP, $anti$-DP, syn-Cl_{11}-DP, and $anti$-Cl_{11}-DP were mapped to the syn-DP (UV), $anti$-DP (UV), and commercial product (Fig. 2). In addition to syn-DP, $anti$-DP, syn-Cl_{11}-DP, and $anti$-Cl_{11}-DP, at least two unknown products (U1 and U2) were identified in all samples and in commercial DP. Although it was difficult to make an accurate identification for U1 and U2, our results showed that U1 and U2 were possibly prone to accumulate in liver. However, we could not exclude that U1 and U2 were biotransformed from DP or its metabolites or originated from the commercial product. This result was consistent with reports that found unidentified peaks in sediment samples (Sverko et al., 2010).

To investigate the elimination of DP in tissues, rats were exposed to 100 $mg\,kg^{-1}\,d^{-1}$ DP for 45 d followed by 45 d depuration. The amounts of syn-DP and $anti$-DP in muscle and liver showed no significant change after depuration, although the amounts of syn-DP and $anti$-DP in serum significantly decreased after depuration

(Fig. 3A and B). However, the content ratio of syn-DP and $anti$-DP in liver to those in liver plus muscle significantly increased after depuration compared with exposed group in 45 d (Fig. 3D). In addition, the content ratio of syn-DP and $anti$-DP in liver to those in liver plus muscle demonstrated no significant change in any group after 90 d exposure (Fig. 3C). These data suggest that DP was more prone to accumulate in liver or that the eliminating rate in liver was lower than that in muscle. The content of both syn-Cl_{11}-DP and $anti$-Cl_{11}-DP in the liver decreased significantly after depuration and neither was detected in serum after depuration (Fig. 3E and F). Our result showed that the contents of $anti$-Cl_{11}-DP were more than syn-Cl_{11}-DP in liver and muscle, indicating that $anti$-Cl_{11}-DP might be more accumulative than syn-Cl_{11}-DP in liver and muscle. However, since the content of $anti$-Cl_{11}-DP is much higher than syn-Cl_{11}-DP in the commercial product (Fig. 2) as well as both $anti$- and syn-Cl_{11}-DP could be biotransformed from DP in different rate, we could not exclude this coming from above reasons.

In addition, elimination half-life of syn-DP was about 179 d in liver, 44 d in muscle, 24 d in serum, 54 d of $anti$-DP in muscle, and 25 d of $anti$-DP in serum. These results suggest that $anti$-DP and syn-DP in liver were more difficult to eliminate than in muscle and serum; in other words, DP isomers were more likely to accumulate in the liver. We did not calculate the elimination half-life of $anti$-DP as it increased, albeit not significantly, after depuration. These results implied that $anti$-DP in the liver was more difficult to eliminate than syn-DP or $anti$-DP possibly transfer to liver from other organs. Our results for elimination half-life of syn-DP are similar to previous reports that demonstrated biological half-lives in the whole body of lake trout (minus the liver) of 53 d for the syn-DP isomers dosed with syn-DP in food for 49 d followed by 112 d of depuration (Tomy et al., 2008). For $anti$-DP, our results may differ
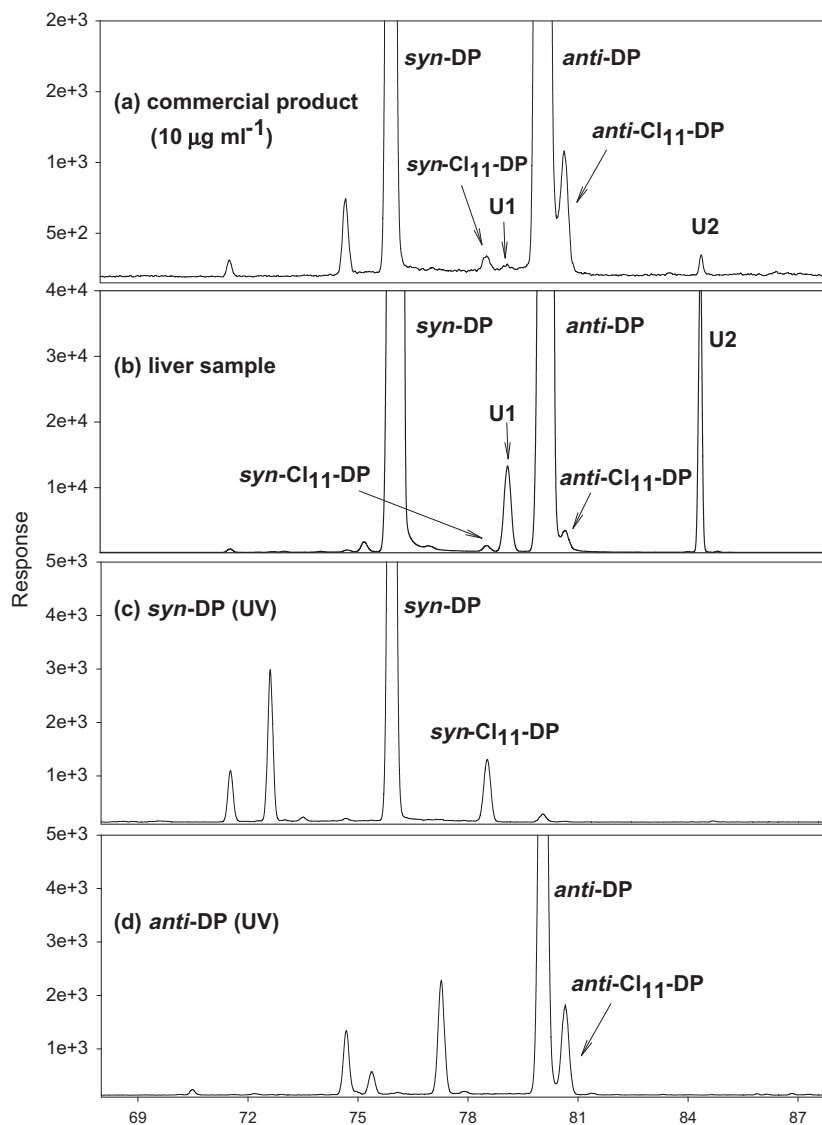
Fig. 2. Gas chromatograph-electron capture negative ionization-mass spectrometer chromatogram (m/z 653.8/651.8, 618.0/620.0) of Dechlorane Plus (DP) isomers and Cl_{11} dechlorinated products in (a) commercial DP (10 μg ml^{-1}), (b) degradation products of liver samples; (c) degradation products of *syn*-DP under ultraviolet (UV) light; and (d) degradation products of anti-DP under ultraviolet (UV) light. The identified degradation products were numbered consecutively by their retention time; superscripts of 1 signify [−Cl + H] dechlorinated DP products. The separation was performed on a DB-XLB column (30 m length × 0.25 mm inner diameter, 0.25 μm film thickness).

from Tomy's et al. report due to the different species (rat *versus* rainbow trout) or the different tissue (liver *versus* whole body). Our results are consistent with previous research which showed that *anti*-DP was more persistent than *syn*-DP in walleye and goldeye and accounted for more than 90% of the total body burden of DP (Tomy et al., 2007). The mechanism of the different DP isomers accumulation dependent with its concentration is needed to be elucidated in the further.

Like most persistent halogenated compounds, DP showed higher accumulation in the liver than in the muscle tissue. Distribution of DP in tissues may not only be controlled by transport and equilibration among lipid pools, but other mechanisms such as specific protein binding in the liver may also exist for DP isomers during their partitioning within the body. Hepatic protein association has also been observed for other persistent chlorinated and brominated contaminants, for example, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and polybrominated diphenyl ethers in organisms and humans (Guvenius et al., 2002; Iwata et al., 2004).

3.2. f_{anti} in DP

Interestingly, the fractions of the *anti*-isomer (ratio of the *anti*-DP concentration to total DP concentration, or f_{anti}) were 0.75, 0.77, and 0.80 for muscle, liver, and serum, respectively, in the lower exposed group (1 mg kg^{-1} d^{-1}), which were close to ratio in commercial DP-25 (0.8) (Fig. 4A). However, the f_{anti} significantly decreased in the 10 mg kg^{-1} d^{-1} and 100 mg kg^{-1} d^{-1} exposed groups (0.26 and 0.26 in muscle, 0.29 and 0.30 in liver, 0.27 and 0.27 in serum, respectively). The DP trend of f_{anti} after depuration was similar to the higher DP exposure group, near 0.3 (Fig. 4B). The f_{anti} for all tissues in the low DP exposure group was similar to the findings in most wild animals from Northern America and Europe, where no significant stereoselective enrichment were detected in wildlife such as birds relative to the commercial mixture (Gauthier and Letcher, 2009; Guerra et al., 2011; Munoz-Arnanz et al., 2011). However, in our study, the f_{anti} values decreased with the increase in DP exposure dose. Similarly, greater amounts of *syn*-DP than *anti*-DP have been reported in the serum of workers and birds in
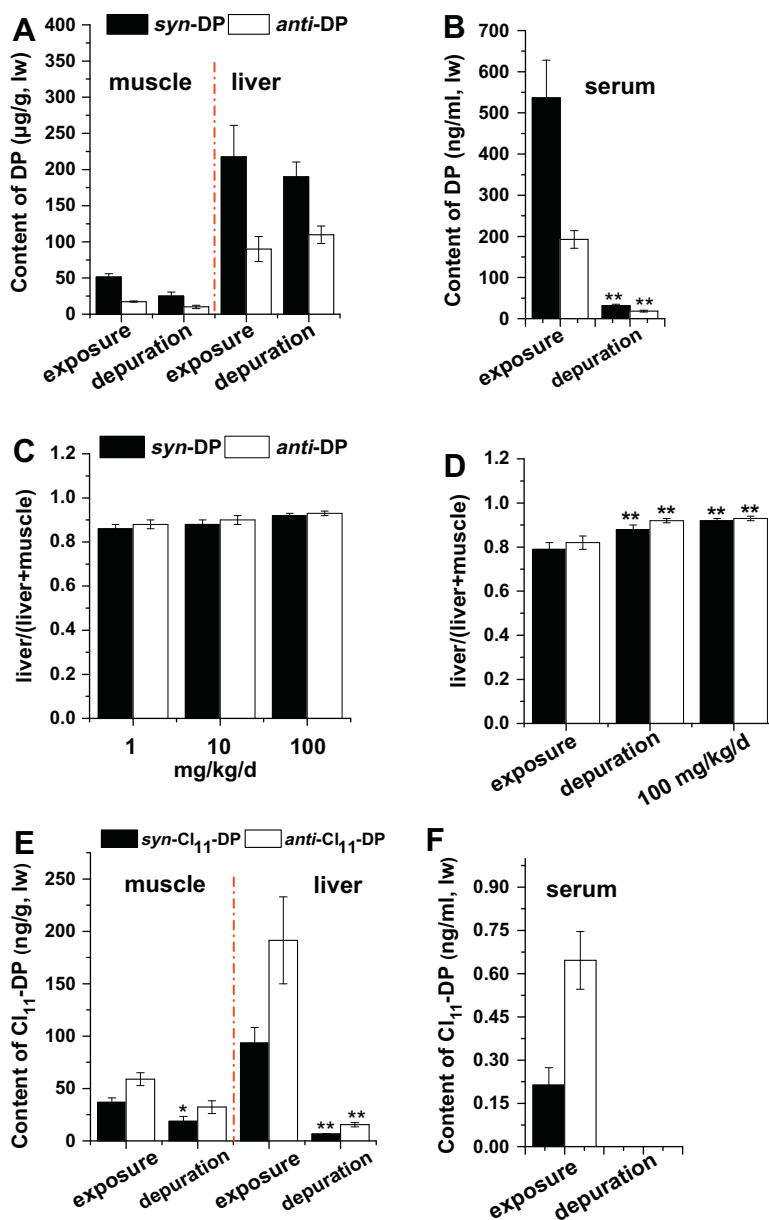
Fig. 3. Levels of *syn*-DP (μg g^{-1}, lw), *anti*-DP (μg g^{-1}, lw), *syn*- Cl$_{11}$-DP (ng g^{-1}, lw), and *anti*-Cl$_{11}$-DP (ng g^{-1}, lw) measured in the liver, muscle, and serum of rats exposed to 100 mg kg^{-1} d^{-1} DP for 45 d, followed by 45 d depuration. Values represent the mean and standard error of five rats per group. Statistically significant differences between exposure and depuration are indicated by ∗ for $p < 0.05$. (A) Liver and muscle; (B) serum; (C) ratio of liver/(liver + muscle) for 90 d exposure; (D) ratio of liver/(liver + muscle) for 90 d exposure; (E) content of *syn*-Cl$_{11}$-DP and *anti*-Cl$_{11}$-DP in muscle and liver; and (F) content of *syn*-Cl$_{11}$-DP and *anti*-Cl$_{11}$-DP in serum.

e-waste dismantling regions (Ren et al., 2009; Zhang et al., 2011). An enrichment of *syn*-DP could be explained by the following factors: (1) isomer-specific structural configuration and (2) different binding affinity to protein. The structure of *syn*-DP could block effectively metabolism than *anti*-DP since the configuration of the pendant chlorocyclopentene moieties of *anti*-DP render it more susceptible to biological attack than the less sterically hindered *syn*-DP (Hoh et al., 2006; Tomy et al., 2008). It could be that *anti*- and *syn*-DP have different binding affinity to protein in liver, and that of *syn*- is much higher than *anti*-DP due to its steric hindrance. At lower dosage, there are enough binding sites for both *syn*- and *anti*-DP; at higher dosage, the binding sites are mostly available for *syn*-, resulting the discriminating of *anti*-DP. In addition, Tomy et al. (2008) had found that the biomagnification factor of *syn*-DP (5.2) was greater than *anti*-DP (1.9) in exposed rainbow trout, which suggested that *syn*-DP was more bioavailable. They also

reported that the uptake rate of *syn*-DP (0.065 nmol d^{-1} in liver) was significantly faster than *anti*-DP (0.024 nmol d^{-1} in liver) in chicken (2008).

3.3. Clinical chemistry parameters

To evaluate whether or not DP have the adverse effects of rats, we observed the weight change and clinical biochemistry parameters, such as alanine amino transferase (ALT), aspartate aminotransferase (AST), albumin (ALB), alkaline phosphatase (ALP), total bile acids (TBA), urea nitrogen (BUN), creatinine (CRE), cholesterol (CHO), triglycerides (TG), high density lipid–cholesterol (HDL-C), low density lipid–cholesterol (LDL-C), creatine kinase (CK), and glucose. No significant changes in absolute body and liver weight or in relative liver weight were observed in the exposure and depuration groups compared to the controls (data not shown).
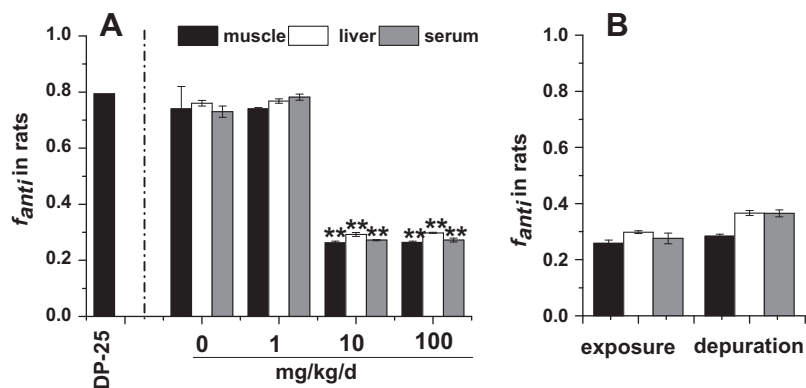
Fig. 4. The f_{anti} values of DP in commercial DP products and rat tissues in different treatments. The f_{anti} defined as *anti*-isomer divided by *anti*-isomer plus *syn*-isomer. (A) The f_{anti} of DP in the liver, muscle, and serum exposed 0, 1, 10, and 100 mg kg^{-1} d^{-1} DP, as well as the f_{anti} in commercial DP. (B) The isomer of f_{anti} in DP was measured in the liver, muscle, and serum in exposure and depuration groups. Values represent the mean and standard error of five rats per group. Statistically significant differences between controls and treatments are indicated by ∗ for $p < 0.05$.

This may indicate that the dosage levels used did not cause overt toxicity. The activities of ALT, ALP, TBA, and level of glucose were significantly decreased ($p < 0.05$) (Table A3). We did not observe histopathological liver damage in our experiment. To investigate the potential influence of DP and its metabolites on endocrine-related processes, thyroid hormone levels (FRT4, FRT3, TSH, TT3, and TT4) in serum were also measured. Results showed no significant influence or related dose diversification (Table A3). No systemic toxicity was observed in rats gavaged with the high concentration of 1500 mg kg^{-1} d^{-1} following repeated oral dosing over a 28-d period (Brock et al., 2010). Taken together, we inferred that DP did not cause adverse effects on the liver and its lipid metabolism or on endocrine levels.

3.4. The mRNA expression levels of certain enzymes

N-acetyltransferase 2 (Nat2) plays an important role in metabolism of methylsulfone-PCBs and the sulfide acid metabolic pathway (Saneto et al., 1982). The sulfotransferase (SULT) family is comprised of important phase II conjugation enzymes for the detoxification of xenobiotics and activity modulation of physiologically important endobiotics such as thyroid hormones, steroids, and neurotransmitters. Sulfotransferase activity may be inhibited in humans when exposed to certain xenobiotics including certain pharmaceuticals and environmental chemicals (hydroxylated polychlorinated biphenyls, hydroxylated polyhalogenated aromatic hydrocarbons, pentachlorophenol, triclosan, and bisphenol A) (Wang and James, 2006). Accordingly, we further investigated the transcriptional effect of DP on the liver in relation to mRNA expressions of Nat2, SULT1A1, 1C2, 1C3, 1E1, and 2A1 in rats. The expression of Nat2 decreased markedly in the 10 mg kg^{-1} d^{-1} group (Fig. 5A) ($p < 0.05$), and a significant reduction was also observed in the expression of SULT1A1, SULT1C2 and SULT2A1 in the 1 mg kg^{-1} d^{-1} group ($p < 0.05$) (Fig. 5A). These changes may relate to biological responses when the body is stimulated by DP exposure. Crump et al. also detected CYP1A4, CYP1A5, CYP2H1, CYP3A37, UGT1A9, DI2, THRSP14-α, L-FABP, and IGF-1 in chicken embryonic hepatocytes and chicken embryos following injection of DP into the air cell of eggs prior to incubation at 24 or 36 h, which showed no adverse effect of DP on embryonic viability or pathways associated with the genes (Crump et al., 2011).

3.5. Determination of enzyme activities

Cytochrome (CYP) monooxygenases are a group of proteins responsible for the oxidation of pharmaceuticals, environmental pollutants, and endogenous compounds (Guengerich, 1991). Because mRNA expression increases or decreases cannot fully explain changes in enzyme activity, we measured the activities of CYP1A2, CYP2B1, CYP2B2, CYP3A, and glutathione S-transferase (GST). Significant changes in CYP2B1 were observed in the 1 mg kg^{-1} d^{-1} group compared with the control group (Fig. 5B) ($p < 0.05$), but no significant variation was observed in the activity of the other enzymes (Fig. 5C). Taken together, no adverse effect on enzyme activity was observed after subchronic exposure to DP.
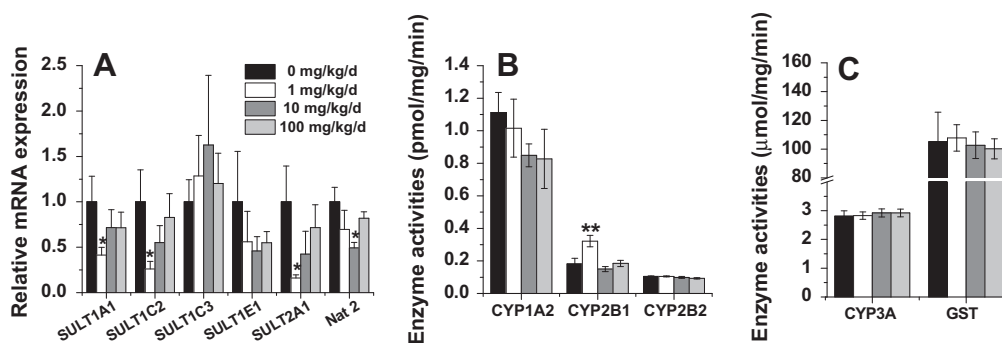
Fig. 5. (A): Relative liver mRNA expression of Nat2, SULT1A1, 1C2, 1C3, 1E1, and 2A1 from 0, 1, 10, and 100 mg kg^{-1} d^{-1} DP in exposed rats (mean ± SE; n = 6). (B and C): enzyme activities of CYP1A2, CYP2B1, CYP2B2, CYP3A, and GST measured among 0, 1, 10, and 100 mg kg^{-1} d^{-1} DP exposed rats (mean ± SE, n = 6). Statistically significant differences between controls and treatments are indicated by ∗ for $p < 0.05$, ∗∗ for $p < 0.01$.

4. Conclusion

In summary, our results showed that DP preferentially accumulated in the liver rather than in muscle at all exposure levels. No significant stereoselectivity of anti-DP or syn-DP in tissues was observed relative to the commercial mixture in the low DP exposure groups with f_{anti} values ranging from 0.74 to 0.78. However, f_{anti} values shifted significantly from 0.78 in 1 mg kg^{-1} d^{-1} to 0.26 in 10 and 100 mg kg^{-1} d^{-1} and syn-DP was predominant in all tissues in the high DP exposure groups (10 and 100 mg kg^{-1} d^{-1}). No overt toxicity was observed after subchronic exposure to DP, although the mRNA expression levels of SULT 1A1, 1C2, and 2A1 in the low dosage group decreased significantly and enzyme activity of CYP 2B2 increased.

Appendix A. Supplementary material

Detailed information for sequences of primers (Table A1), concentration of the three tissues in exposure and depuration groups (Table A2), effect of DP on selected clinical chemistry parameters and the thyroid hormone levels (Table A3) are all given in supplementary data. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.10.106.

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