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ACCUMULATION AND EFFECTS OF 90-DAY ORAL EXPOSURE TO DECHLORANE PLUS IN QUAIL (*COTURNIX COTURNIX*)

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Abstract: While a number of studies have addressed bioaccumulation of the flame retardant Dechlorane Plus (DP), little information is available regarding the adverse effects of DP on animals, especially on bird species. In the present study, male common quails (*Coturnix coturnix*) were consecutively exposed to commercial DP-25 by gavage for 90 d at 1-mg/kg/d, 10-mg/kg/d, and 100-mg/kg/d dosages. Concentrations of DP isomers in liver, muscle, and serum were determined after exposure. Liver enzyme activity involved in xenobiotic biotransformation processes and oxidative stress was measured, as well as glutathione and maleic dialdehyde content. The results showed that DP was more prone to accumulate in the liver than in muscle and serum in all exposed groups. In tested tissues, syn-DP dominated in the high-exposure groups (10 and 100 mg/kg/d), whereas anti-DP tended to accumulate in the low-exposure group (1 mg/kg/d). The concentration ratios of anti-DP to total DP (f_{anti} values) in the tissues examined were close to commercial DP in the low-exposure group; however, the f_{anti} values were significantly decreased in the high-exposure groups. Enzyme activity of 7-pentoxyresorufin-O-demethylase (PROD) decreased significantly in all exposed groups compared with the control group, whereas activity of erythromycin N-demethylase (ERND) and the antioxidant enzyme catalase significantly increased in high-exposure groups. The results implied that DP exposure levels influenced isomeric compositions in organs and that DP exposure altered hepatic alkoxyresorufin O-dealkylase (AROD) activity and contributed to the biological effects of DP. *Environ Toxicol Chem* 2013;32:1649–1654. © 2013 SETAC

Keywords: Dechlorane Plus Birds Accumulation pattern Biological effects Flame retardant

INTRODUCTION

Flame retardants have received considerable attention in recent years due to their global distribution, persistence, bioaccumulative properties, and potential toxicities. With contamination and high detection in the environment, penta- and octa-diphenyl ethers and brominated flame retardants (BFRs) face worldwide regulation and possible prohibition [1]. The chlorinated flame retardant Dechlorane Plus (DP) has been used since the 1960s in electrical wiring and cable coatings, plastic roofing material, hard connectors in computers, and automotive lubricants [2,3]. The use of DP has been unregulated, and production volumes have ranged from 1 000 000 pounds to as high as 10 000 000 pounds annually [4,5].

Dechlorane Plus has been consistently and globally detected in ambient air, dust, sediment, wildlife, and human beings [6–10], suggesting that DP is ubiquitous in the environment. Commercial DP is a mixture of syn-DP and anti-DP stereoisomers, at a ratio of approximately 1:3 [2]. Isomer-selective uptake accumulation has been observed in wildlife. The content of anti-DP was approximately 2 times greater than syn-DP in the plasma of bald eagles from the Great Lakes region (USA and Canada) [11] and was the predominant isomer in herring gull eggs from the Great Lakes and white stork eggs from Spain [12,13], while preferential accumulation of syn-DP was found in aquatic organisms from an e-waste recycling site reservoir in China [14]. The anti-DP fraction (f_{anti}), defined as the concentration of anti-DP divided by total DP, has been used to characterize isomer-specific accumulation in biota. Although species-specific bioaccumulation exists [14], field studies have observed negative associations between f_{anti} values and exposed DP levels in the same species—that is, smaller f_{anti} values observed in individuals with higher DP residues [3]. Li et al. [15] found that f_{anti} values in Sprague–Dawley rats were greater in the lower exposure groups than in the higher exposure groups. More recently, Mo et al. [16] observed a significant negative correlation between f_{anti} values and DP concentrations in kingfishers (*Alcedo atthis*) from an electronic waste recycling site and a reference site. These findings raise concerns about the influence of DP exposure levels on isomeric compositions in biota.

Limited data are available on the toxicity of DP [17,18]. A recent study found that 5000-mg/kg DP doses had no effect on in vivo parameters or clinical and anatomic pathology during a 28-d repeat dose experiment on rats, and no effects were observed on reproductive and fertility indices or fetal growth in the developmental and reproductive toxicity phase [17]. Using in vitro and in ovo approaches, Crump et al. [18] observed no overt toxic effects (up to the highest nominal dose group of 500 ng/g/egg) in *Gallus domesticus* (hepatocytes and pipping success) and no changes in the transcript levels of 11 target genes, identified as responses to BFRs, after DP treatment. Although these studies indicate that DP had no obvious acute toxicity on the organisms tested, information on subchronic toxicities of DP remains very limited.

In the present study, male common quails (*Coturnix coturnix*) were orally administered commercial DP-25 in corn oil at different dosages for 90 d. The concentrations of DP in muscle, liver, and serum were measured, as was the dechlorinated products Cl_{11}-DP and other possible DP metabolites. Cytochrome P450s (CYPs) have critical roles in the metabolism of endogenous and xenobiotic chemicals, and the antioxidant defense system acts on

All Supplemental Data may be found in the online version of this article.

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reactive oxygen species through reduction reactions to render them harmless. To assess the influence of DP on quail, we measured the activity of several hepatic enzymes, including CYPs, catalase (CAT), and superoxide dismutase (SOD), as well as glutathione (GSH) and maleic dialdehyde (MDA) content, an indicator of lipid peroxidation.

MATERIALS AND METHODS

Chemicals and standards

Commercial DP-25 (≥99% purity) was obtained from Jiangsu Anpon. Corn oil was purchased from Sigma-Aldrich. The anti-DP, syn-DP, and anti-Cl_{11}-DP standards were obtained from Wellington Laboratories. Brominated diphenyl ethers (BDEs) BDE-128 and BDE-181 were purchased from AccuStandard. Acetone, dichloromethane, hexane, and other solvents and reagents were of analytical grade.

Animals

A total of 60 male quails (6–8 wk old, with an average weight of 125 g) were obtained from a farm in Beijing, China. The animals were raised individually in suspended, unattached cages in a mass air-displacement room at 20 °C to 26 °C. After 14 d of acclimation, they were randomly separated into 4 groups and orally administered corn oil with commercial DP-25 dosages of 0, 1, 10, and 100 mg/kg/d for 90 d, respectively. All quails were euthanized by decapitation after the experiment. Livers were quickly perfused in situ with ice-cold 0.9% w/v sodium chloride until the entire organ became pale. Livers were removed from the bodies and rinsed with ice-cold 0.9% w/v sodium chloride, and were then weighed, cut into pieces, frozen in liquid nitrogen, and stored at −80 °C until chemical and enzyme activity analyses. Pectoral muscle and serum were also immediately collected, frozen in liquid nitrogen, and stored at −80 °C for chemical analyses. All experiments and procedures were approved by the Animal Ethics Committee of the Institute of Zoology, Chinese Academy of Sciences.

Gas chromatography/mass spectrometry analysis

The DP isomers and their dechlorinated analogs in the samples were identified by an Agilent 6890 gas chromatograph (GC) system coupled with a 5975C mass spectrometer (MS) using electron capture negative ionization in the selective ion-monitoring mode. Target chemicals, along with surrogate and internal standards, were separated using a 30-m DB-XLB capillary column (0.25 mm inner diameter, 0.25 μm thickness; Agilent Technologies). Detailed GC/MS procedures for DP analysis have been reported elsewhere [19]. Ions were scanned 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, *m/z* 584 and 586 for anti-Cl_{10}-DP, and *m/z* 79 and 81 for the polybrominated diphenyl ether congeners. All GC/MS peaks were calculated by their peak area. Semiquantitative determination was achieved for syn-Cl_{11}-DP by reference to the response of anti-Cl_{11}-DP on GC/MS.

Sample preparation

For liver and muscle, 1 g of tissue was homogenized with ashed anhydrous sodium sulfate, spiked with recovery standard BDE-181, and extracted using the Soxhlet extraction technique with 50% acetone in hexane for 48 h. A portion of the extract was used for gravimetric lipid determination. The remainder was subjected to gel permeation chromatography and eluted for lipid removal with dichloromethane:hexane (1:1, v/v). Fractions from 90 mL to 280 mL containing target compounds were collected and concentrated to 1 mL under a gentle stream of nitrogen. The extract was further purified on a silica column (1 cm inner diameter) packed with 8 cm neutral silica and 8 cm acidified silica and eluted with 40 mL of hexane/dichloromethane (1:1 v/v). The elute was concentrated and redissolved in 270 μL of isooctane, and then spiked with an internal standard (BDE-128) before instrumental analysis.

Serum extraction and purification were performed as described previously [20], with some modifications. In brief, 2 mL 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. Both DP and its metabolites were twice extracted with 6 mL of hexane:methyl tert-butyl ether (1:1 v/v) mixture. The combined organic extracts were washed with potassium chloride solution (1%; 3 mL) and concentrated to dryness under N_2 for gravimetric lipid weight determination. Extracts were redissolved with 6 mL of hexane. 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 with hexane (6 mL) for complete extraction. The neutral phase was treated with 2 mL of concentrated sulfuric acid to remove lipids, and subsequently purified by silica column as described above. The elutes were concentrated to near dryness under N_2, redissolved in 270 μL of isooctane, and spiked with a known amount of internal standard (BDE-128) before instrumental analysis.

Quality assurance and quality control

Quality assurance was conducted by analyses of procedural blanks, blank spikes, and sample duplicates. Procedural blanks were used in parallel with the treated samples for every extraction, with a procedural blank performed for each batch of 11 samples. Recoveries of surrogates for all tissue and serum samples ranged from 70.4% to 90.6%, with an average of 80.9%. The relative standard deviation of duplicates among triplicate samples was less than 20%. The method detection limit (MDL) was set as the average value plus 3-fold standard deviation for analytes, which were detected in the procedural blanks ($n = 6$). For analytes not detected in the blanks, a signal-to-noise ratio of 10 was set as the MDL. The MDLs of syn-DP, anti-DP, and anti-Cl_{11}-DP were 27.85 ng/g, 46.12 ng/g, and 0.68 ng/g lipid weight in muscle and liver, and 0.20 ng/mL, 0.054 ng/mL, and 0.042 ng/mL in serum, respectively.

Enzyme activity analyses

Liver microsomal alkoxyresorufin O-dealkylase assays (resorufin assays) were performed, whereby alkoxyresorufins were metabolized by O-dealkylases to a resorufin product. The alkoxyresorufins used (and names of their assays) were 7-ethoxyresorufin (EROD), 7-methoxyresorufin (MROD), and 7-pentoxyresorufin (PROD). Hepatic microsomal erythromycin N-demethylase (ERND) and 7-benzyloxy-4-trifluoromethylcoumarin debenzylase (BFCD) assays were also performed. Detailed information on microsomal preparation and enzyme activity assays are shown in the Supplemental Data. Enzyme activity of CAT and SOD, as well as GSH and MDA content, were detected using commercial kits. Detailed information on sample preparation and methods is included in the Supplemental Data.

Statistical analysis

All statistical analyses were conducted using SPSS 16.0 for Windows. The statistical significance of concentrations and

other experimental statistics were determined using one-way analysis of variance followed by Fisher's least significant difference test. Normal distribution and homogeneity of variance were confirmed before analysis of variance. Significant differences were set at $p < 0.05$.

RESULTS AND DISCUSSION

DP accumulation patterns

Quail mortality during the 90-d exposure experiment was zero. At the end of the trial, body and liver weights were not significantly altered between the control and exposure groups (Supplemental Data, Figure 1). Concentrations of syn-DP, anti-DP, syn-Cl_{11}-DP, and anti-Cl_{11}-DP were detected in all samples examined (Table 1), as well as in the control group. For example, average syn-DP and anti-DP values in muscle of the control group were 5800 ± 2000 ng/g and 1000 ± 330 ng/g lipid weight, respectively, which may relate in part to the widespread distribution of DP in the environment, including the exposure room and feedstuff [21].

Accumulation of DP isomers was observed in the exposed quails. The highest concentrations of syn-DP occurred in liver of the 10-mg/kg/d group, being nearly 10 times greater than observed in the 1-mg/kg/d group and 3 times greater than observed in the 100-mg/kg/d group. For anti-DP, however, the highest concentrations occurred in the 1-mg/kg/d group. In addition, anti-DP concentrations decreased with increasing DP exposure dosage. These findings indicate that isomer-specific and concentration-related pharmacokinetics of DP existed in the exposed quails. However, the mechanism for the observed dose-dependent accumulation of DP isomers is not understood based on the present data.

The monodechlorination products of DP, syn-Cl_{11}-DP, and anti-Cl_{11}-DP were also detected in the quails. The highest concentrations of syn-Cl_{11}-DP were found in the 10-mg/kg/d group among all tested samples (Table 1). In muscle, for example, the mean concentration of syn-Cl_{11}-DP was 80 ± 16.7 ng/g lipid weight in the 1 mg/kg/d group, which increased significantly in the 10-mg/kg/d group (309 ± 59 ng/g lipid wt; $p = 0.001$). Similar trends were also observed in liver and serum. The anti-Cl_{11}-DP content in the various tissues showed a decreasing trend from the 1-mg/kg/d to the 100-mg/kg/d group. In serum, however, the content of anti-Cl_{11}-DP decreased significantly from 417 ± 28.6 ng/g lipid weight in the 1-mg/kg/d group to 180 ± 37.8 ng/g lipid weight in the 10-mg/kg/d group ($p = 6 \times 10^{-5}$) and 195 ± 39.3 ng/g lipid weight in the 100-mg/kg/d group ($p = 0.0001$) (Table 1). The syn-DP, anti-DP, syn-Cl_{11}-DP, and anti-Cl_{11}-DP contents in test tissues in the 100-mg/kg/d group were less than those in the 10-mg/kg/d group.

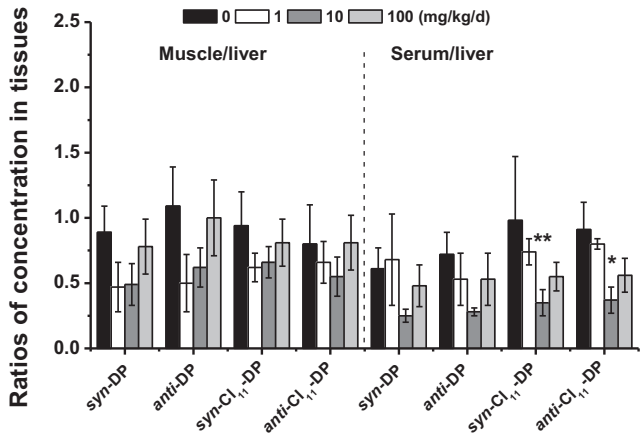

Figure 1. Concentration ratios of muscle:liver and serum:liver for syn-Dechlorane Plus (DP), anti-DP, syn-Cl_{11}-DP, and anti-Cl_{11}-DP measured in the 0-mg/kg/d, 1-mg/kg/d, 10-mg/kg/d, and 100-mg/kg/d groups after 90 d of exposure. Error bars represent ± 1 standard error. Significant differences from control are indicated as $^{*}p < 0.05$ and $^{**}p < 0.01$.

In addition, at least 2 other DP dechlorinated analogs were detected in the quail samples and in the commercial DP (data not shown). Unfortunately, no standard was available to make an accurate determination. Because syn-Cl_{11}-DP, anti-Cl_{11}-DP, and the 2 unknown dechlorinated analogs were also found in commercial DP and sediment samples [22], we could not exclude the possibility that they originated from the commercial product, and were not biotransformation products.

The muscle:liver and serum:liver concentration ratios for syn-DP, anti-DP, syn-Cl_{11}-DP, and anti-Cl_{11}-DP in the 3 exposed groups and the control group are shown in Figure 1. The results suggest that DP tended to accumulate in the liver. This agrees with previous findings in wild fish species [23,24]. Like other persistent pollutants, not only may DP accumulation and distribution in the body be controlled by transport and equilibration among lipid pools, but sequestration of DP isomers by hepatic proteins may also account for their disposition in the liver, although the hepatic proteins that bind DP were not identified. For example, exposure to xenobiotic pollutants such as polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, and coplanar PCBs may induce hepatic binding proteins, leading to hepatic sequestration of the compounds [25,26].

DP isomeric composition

In the 1-mg/kg/d group, f_{anti} values were 0.70, 0.68, and 0.68 in the muscle, liver, and serum, respectively (Figure 2). In the 10-mg/kg/d and 100-mg/kg/d groups, however, f_{anti} values were significantly lower (0.21 and 0.24 in muscle, 0.16 and 0.19 in liver, and 0.18 and 0.21 in serum, respectively; $p < 10^{-10}$). The f_{anti} values for tissues in the low-exposure group (1 mg/kg/d) were close to those in commercial DP. In the high-exposure groups (10 and 100 mg/kg/d), however, the f_{anti} values were lower than commercial DP. Similar results have been found in hair from occupationally exposed workers (f_{anti} 0.55) and nonoccupationally exposed residents (f_{anti} 0.62) from an e-waste area in China [9]. Ren et al. [10] also reported f_{anti} values of 0.58 ± 0.11 in serum from dismantling workers in another e-waste area in China. Lower values of f_{anti} than commercial DP have also been observed in environmental samples. For example, f_{anti} values ranged from 0.35 to 0.47 in Atlantic seawater in the East Greenland Sea [27] and Atlantic transect, and to 0.3 in aquatic organisms from Lake Winnipeg (Manitoba, Canada) [2]. Furthermore, the low f_{anti} value in all measured tissues in the current 10-mg/kg/d and 100-mg/kg/d DP study may be due to increasing syn-DP in the high-exposure groups, or more efficiently removed or less stable anti-DP at high DP dosages in animal tissues. We speculate that stereoselective enrichment in biota might occur under high DP exposed concentrations. Stereoselection of s*yn*-DP and anti-DP did not occur in the 1-mg/kg/d group in the present study or in some environment samples of previous research [13,14], so the f_{anti} values were close to the commercial DP. However, the higher concentration of DP isomers in the 10-mg/kg/d and 100-mg/kg/d groups and high contamination area samples may promote stereoselection between syn-DP and anti-DP, accompanying the low value of f_{anti}. This phenomenon might be explained by the following 3 factors. First, structural differences between syn-DP and anti-DP, whereby the pendant chlorocyclopentene

Table 1. Concentrations (ng/g lipid wt) of syn-Dechlorane Plus (DP), anti-DP, syn-Cl_{11}-DP, and anti-Cl_{11}-DP measured in the muscle, liver, and serum of quails exposed to 0-mg/kg/d, 1-mg/kg/d, 10-mg/kg/d, and 100-mg/kg/d dosages for 90 d[a]

		Exposed groups			
Chemical	Tissue	0 mg/kg/d	1 mg/kg/d	10 mg/kg/d	100 mg/kg/d
Syn-DP	Muscle	5800 ± 2000 C,D	61 000 ± 18 000 C	560 000 ± 120 000 A,B,D	240 000 ± 58 000 A,C
	Liver	10 000 ± 5900 C	150 000 ± 69 000 C	1 500 000 ± 400 000 A,B,D	460 000 ± 180 000 C
	Serum	3900 ± 1200 C,D	42 000 ± 5600 C,D	310 000 ± 39 000 A,B,D	160 000 ± 53 000 A,B,C
Anti-DP	Muscle	1000 ± 330 B,C	140 000 ± 38 000 A	160 000 ± 40 000 A	77 000 ± 17 000
	Liver	15 000 ± 8100 B,C	300 000 ± 120 000 A	260 000 ± 39 000 A	100 000 ± 34 000
	Serum	6500 ± 1600 B,C,D	87 000 ± 7600 A,D	68 000 ± 7400 A	43 000 ± 13 000 A,B
Syn-Cl_{11}-DP	Muscle	25 ± 3.4 C,D	80 ± 16.7 C,D	309 ± 59 A,B	278 ± 57.2 A,B
	Liver	48 ± 14.4 C,D	127 ± 8.1 C,D	655 ± 125.4 A,B	424 ± 90.3 A,B
	Serum	53 ± 8.7 C,D	101 ± 8.5 C	233 ± 69.8 B	203 ± 39.1 A
Anti-Cl_{11}-DP	Muscle	55 ± 11.4 B,C,D	371 ± 75.1 A	364 ± 76.1 A	266 ± 56.6 A
	Liver	83 ± 37.1 B,C,D	611 ± 88.0 A	570 ± 100.0 A	401 ± 92.0 A
	Serum	75 ± 10.5 B,C,D	417 ± 28.6 A,C,D	180 ± 37.8 A,B	195 ± 39.3 A,B

[a]Values represent means ± standard error. The letters A, B, C, and D denote significant differences compared with 0-mg/kg/d, 1-mg/kg/d, 10-mg/kg/d, and 100-mg/kg/d groups, respectively ($p < 0.05$).

moieties structure of anti-DP would not block metabolism more effectively than syn-DP, thus increasing susceptibility to a biological attack on DP double bonds. Second, depuration rate differences between syn-DP and anti-DP, as observed in studies on the elimination half-lives of syn-DP (53.3 ± 13.1 d) and anti-DP (30.4 ± 5.7 d) in whole fish (minus the liver) [28]. Third, uptake rate differences between the isomers, as found in juvenile rainbow trout, in which syn-DP had significantly greater uptakes than anti-DP (0.045 nmol/d and 0.018 nmol/d, respectively) [28]. In addition, the biomagnification factor of syn-DP (5.2) was greater than that of anti-DP (1.9) in exposed rainbow trout (whole fish minus the liver), which suggested that syn-DP was more bioavailable [28].

Hepatic enzyme activities

Research has shown that DP isomers are widespread in birds, such as in herring gull eggs, peregrine falcon eggs, and bald eagle plasma [3]. However, little information exists on the adverse effects of DP isomers on bird species [18]. Cytochrome P450s have critical roles in the metabolism of endogenous chemicals and process the burden of drugs and other xenobiotics [29]. In the present study, decreasing trend of the enzyme activity of EROD, MROD, PROD, and BFCD were observed in the 1-mg/kg/d, 10-mg/kg/d, and 100-mg/kg/d groups compared with those of in the control group (Figure 3A). The PROD activity significantly decreased in the exposed groups compared with the control. This may have adverse effects on quails because PROD activity is an indicator of CYP2B in mammals, which is, in turn, involved in the metabolism or inactivation of several endogenous chemicals such as steroid and gonadal hormones [30]. For ERND, the 10-mg/kg/d and 100-mg/kg/d groups showed higher enzyme activities than the control group (Figure 3A). Activity of ERND is mainly CYP3A-dependent in mammals [31]. Forms of CYP3A are among the most abundant and important xenobiotic-metabolizing CYP enzymes, mediating the metabolism of numerous xenobiotics—including pollutants, pesticides, and food contaminants—as well as endogenous compounds such as steroid hormones and bile acids [32,33]. In the present study, the increased ERND activity in the 10-mg/kg/d and 100-mg/kg/d groups might alter the detoxification and metabolism activities of CYP3A to endogenous and exogenous compounds, and partly contribute to the biological effects of DP.

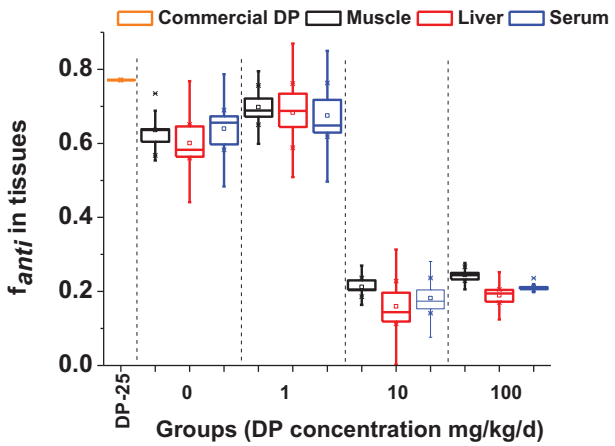

Figure 2. Concentration ratios of anti-Dechlorane Plus (DP) to total DP (f_{anti}) values in commercial DP and muscle, liver and serum of quails exposed to 0-mg/kg/d, 1-mg/kg/d, 10-mg/kg/d, and 100-mg/kg/d DP dosages. Values represent means ± standard error.

Defense system antioxidants are composed of enzymatic or nonenzymatic molecules that exist in extra- and intracellular compartments. These molecules act on reactive oxygen species through reduction reactions to render them harmless [34]. In the present study, the activity or content of GSH and the antioxidants CAT, SOD, and MDA were determined. An increasing trend of CAT and SOD was observed among the exposed groups compared with the control group. A significant increase occurred in the 10-mg/kg/d and 100-mg/kg/d groups for CAT ($p = 0.009$ and 0.004, respectively) and in the 1-mg/kg/d group for SOD ($p = 0.006$; Figure 3B), suggesting that DP exposure may induce oxidative stress. With H_2O_2 as its substrate, CAT is involved in protection against oxidative reaction, and the level of SOD reflects the body's ability to scavenge oxygen free radicals. The increase in SOD levels in the exposed groups may be a manifestation of the body's stress reaction.

Our results demonstrated dose-dependent and tissue-specific accumulation of DP isomers and potential subchronic toxicities in exposed quails. Both syn-DP and anti-DP were prone to accumulate in the liver. In the tested tissues, syn-DP tended to accumulate in the high-exposure groups, whereas anti-DP was dominant in the low-exposure group. The f_{anti} values decreased in the high-exposure groups, which differed from commercial DP. Exposure to DP decreased PROD enzyme activity but

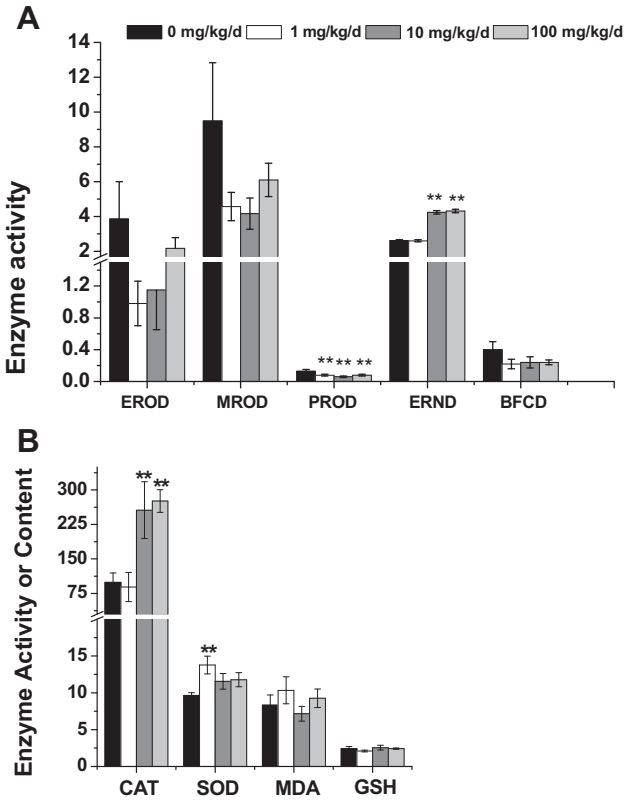

Figure 3. (**A**) Activity (mean ± standard error) of 7-ethoxyresorufin (EROD), 7-methoxyresorufin (MROD), 7-pentoxyresorufin-O-demethylase (PROD), erythromycin N-demethylase (ERND), and 7-benzyloxy-4-trifluoromethylcoumarin debenzylase (BFCD), and (**B**) activity or contents of catalase (CAT), superoxide dismutase (SOD), maleic dialdehyde (MDA), and glutathione (GSH) in the liver of quail exposed to 0-mg/kg/d, 1-mg/kg/d, 10-mg/kg/d, and 100-mg/kg/d Dechlorane Plus dosages. Activity is expressed as product produced per minute per milligram of protein in the assays. The unit of measurement is pmol/mg/min for EROD, MROD, and PROD; nmol/mg/min for ERND, BFCD, and GSH; μmol/mg/min for MDA; and U/mg for CAT and SOD. Significant differences between control and treatments are indicated as $^{*}\ p < 0.05$ and $^{**}\ p < 0.01$.

increased ERND activity. Results of CAT and SOD activity indicated induction of oxidative stress in quails exposed to certain DP doses. The mechanisms of the dose-dependent bioaccumulation of DP isomers as well as their long-term effects on wildlife should be investigated further.

SUPPLEMENTAL DATA

Supplementary Methods.
Figure S1. (168 KB PDF).

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REFERENCES

1. Alaee M, Arias P, Sjödin A, Bergman Å. 2003. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environ Int* 29:683–689.
2. Tomy GT, Pleskach K, Ismail N, Whittle DM, Helm PA, Sverko E, Zaruk D, Marvin CH. 2007. Isomers of Dechlorane Plus in Lake Winnipeg and Lake Ontario food webs. *Environ Sci Technol* 41: 2249–2254.
3. Xian Q, Siddique S, Li T, Feng YL, Takser L, Zhu J. 2011. Sources and environmental behavior of Dechlorane Plus—A review. *Environ Int* 37:1273–1284.
4. Betts KS. 2006. A new flame retardant in the air. *Environ Sci Technol* 40:1090–1091.
5. Ren NQ, Sverko E, Li YF, Zhang Z, Harner T, Wang DG, Wan XN, McCarry BE. 2008. Levels and isomer profiles of Dechlorane Plus in Chinese air. *Environ Sci Technol* 42:6476–6480.
6. Hoh E, Zhu LY, Hites RA. 2006. Dechlorane Plus, a chlorinated flame retardant, in the Great Lakes. *Environ Sci Technol* 40:1184–1189.
7. Qiu X, Hites RA. 2008. Dechlorane Plus and other flame retardants in tree bark from the Northeastern United States. *Environ Sci Technol* 42:31–36.
8. Zhu J, Feng YL, Shoeib M. 2007. Detection of Dechlorane Plus in residential indoor dust in the city of Ottawa, Canada. *Environ Sci Technol* 41:7694–7698.
9. Zheng J, Wang J, Luo XJ, Tian M, He LY, Yuan JG, Mai BX, Yang ZY. 2010. Dechlorane Plus in human hair from an e-waste recycling area in South China: Comparison with dust. *Environ Sci Technol* 44: 9298–9303.
10. Ren GF, Yu ZQ, Ma ST, Li HR, Peng PG, Sheng GY, Fu JM. 2009. Determination of Dechlorane Plus in serum from electronics dismantling workers in South China. *Environ Sci Technol* 43:9453–9457.
11. Venier M, Wierda M, Bowerman WW, Hites RA. 2010. Flame retardants and organochlorine pollutants in bald eagle plasma from the Great Lakes region. *Chemosphere* 80:1234–1240.
12. Gauthier LT, Letcher RJ. 2009. Isomers of Dechlorane Plus flame retardant in the eggs of herring gulls (*Larus argentatus*) from the Laurentian Great Lakes of North America: Temporal changes and spatial distribution. *Chemosphere* 75:115–120.
13. Muñoz-Arnanz J, Sáez M, Hiraldo F, Baos R, Pacepavicius G, Alaee M, Jiménez B. 2011. Dechlorane Plus and possible degradation products in white stork eggs from Spain. *Environ Int* 37:1164–1168.
14. Wu JP, Zhang Y, Luo XJ, Wang J, Chen SJ, Guan YT, Mai BX. 2010. Isomer-specific bioaccumulation and trophic transfer of Dechlorane Plus in the freshwater food web from a highly contaminated site, South China. *Environ Sci Technol* 44:606–611.
15. Li Y, Yu L, Wang J, Wu J, Mai B, Dai J. 2013. Accumulation pattern of Dechlorane Plus and associated biological effects on rats after 90 days of exposure. *Chemosphere* 90:2149–2156.
16. Mo L, Wu JP, Luo XJ, Sun YX, Zheng XB, Zhang Q, Zou FS, Mai BX. 2012. Dechlorane Plus flame retardant in kingfishers (*Alcedo atthis*) from an electronic waste recycling site and a reference site in South China: Influence of residue levels on the isomeric compositions. *Environ Pollut* 174C:57–62.
17. Brock WJ, Schroeder RE, McKnight CA, VanSteenhouse JL, Nyberg JM. 2010. Oral repeat dose and reproductive toxicity of the chlorinated flame retardant Dechlorane Plus. *Int J Toxicol* 29:582–593.
18. Crump D, Chiu S, Gauthier LT, Hickey NJ, Letcher RJ, Kennedy SW. 2011. The effects of Dechlorane Plus on toxicity and mRNA expression in chicken embryos: A comparison of in vitro and in ovo approaches. *Comp Biochem Phys C* 154:129–134.
19. Wang J, Tian M, Chen SJ, Zheng J, Luo XJ, An TC, Mai BX. 2011. Dechlorane Plus in house dust from e-waste recycling and urban areas in South China: Sources, degradation, and human exposure. *Environ Toxicol Chem* 30:1965–1972.
20. Malmberg T, Athanasiadou M, Marsh G, Brandt I, Bergman A. 2005. Identification of hydroxylated polybrominated diphenyl ether metabolites in blood plasma from polybrominated diphenyl ether exposed rats. *Environ Sci Technol* 39:5342–5348.
21. Venier M, Hites RA. 2011. Flame retardants in the serum of pet dogs and in their food. *Environ Sci Technol* 45:4602–4608.
22. Sverko E, Reiner EJ, Tomy GT, McCrindle R, Shen L, Arsenault G, Zaruk D, MacPherson KA, Marvin CH, Helm PA, McCarry BE. 2010. Compounds structurally related to Dechlorane Plus in sediment and biota from Lake Ontario (Canada). *Environ Sci Technol* 44:574–579.
23. Zhang Y, Wu JP, Luo XJ, Wang J, Chen SJ, Mai BX. 2011. Tissue distribution of Dechlorane Plus and its dechlorinated analogs in contaminated bottom fish: High affinity to the brain for anti-DP. *Environ Pollut* 159:3647–3652.
24. Peng H, Zhang K, Wan Y, Hu JY. 2012. Tissue distribution, maternal transfer, and age-related accumulatiton of Dechloranes in Chinese sturgeon. *Environ Sci Technol* 46:9907–9913.
25. Kubota A, Iwata H, Tanabe S, Yoneda K, Tobata S. 2004. Levels and toxicokinetic behaviors of PCDD, PCDF, and coplannar PCB congeners in common commorants from Lake Biwa, Japan. *Environ Sci Technol* 38:3853–3859.

26. Chen D, Zhang X, Mai B, Sun Q, Song J, Luo X, Zeng EY, Hale RC. 2009. Polychlorianted biphenyls and organochlorine pesticides in various bird species from northern China. *Environ Pollut* 157:2023–2029.
27. Moller A, Xie Z, Sturm R, Ebinghaus R. 2010. Large-scale distribution of dechlorane plus in air and seawater from the Arctic to Antarctica. *Environ Sci Technol* 44:8977–8982.
28. Tomy GT, Thomas CR, Zidane TM, Murison KE, Pleskach K, Hare J, Arsenault G, Marvin CH, Sverko E. 2008. Examination of isomer specific bioaccumulation parameters and potential *in vivo* hepatic metabolites of syn- and anti-Dechlorane Plus isomers in juvenile rainbow trout (*Oncorhynchus mykiss*). *Environ Sci Technol* 42:5562–5567.
29. Guengerich FP. 1991. Reactions and significant of cytochrome P-450 enzymes. *J Biol Chem* 266:10019–10022.
30. Rosenbrock H, Hagemeyer CE, Singec I, Knoth R, Volk B. 1999. Testosterone metabolism in rat brain is differentially enhanced by phenytoin-inducible cytochrome P450 isoforms. *J Neuroendocrinol* 11: 597–604.
31. Bornheim LM, Correia MA. 1990. Selective inactivation of mouse liver cytochrome P-450IIIA by cannabidiol. *Mol Pharmacol* 38:319–326.
32. Lamda JK, Lin YS, Schuetz EG, Thummel KE. 2002. Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliv Rev* 54:1271–1294.
33. Guengerich FP. 1999. Cytochrome P-450 3 A4: Regulation and role in drug metabolism. *Annu Rev Pharmacol Toxicol* 39:1–17.
34. Halliwell B, Gutteridge JMC. 2007. *Free Radicals in Biology and Medicine*. Oxford University Press, Oxford, UK.