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Effects of dechlorane plus on the hepatic proteome of juvenile Chinese sturgeon (*Acipenser sinensis*)

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ABSTRACT

Dechlorane Plus (DP), an alternative to decabromodiphenyl ether (BDE-209), is a widely used polychlorinated flame retardant that is frequently detected in aquatic ecosystems. While the mechanisms of toxicity of BDE-209 have been well documented, less is known about the toxicity of DP. In this study, juvenile Chinese sturgeon (*Acipenser sinensis*) were treated with DP at doses of 1, 10, and 100 mg/kg wet weight for 14 days via a single intraperitoneal injection (i.p.). After 14 days, liver proteomes of juvenile Chinese sturgeon were analyzed using two-dimensional electrophoresis (2-DE) coupled matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry (MALDI–TOF/TOF–MS). A total of 39 protein spots were significantly altered in abundance (>2-fold) and of these proteins, 27 were successfully identified. Proteins related to the stress response that included heat shock cognate protein 70 and T-complex protein 1 were significantly increased and decreased in abundance, respectively. Moreover, Ras-related protein Rab-6B and GDP dissociation inhibitor 2, proteins that are involved in small G-protein signal cascades, were decreased in abundance 2- to 5-fold. Annexin A4, which is associated with Ca^{2+} signaling pathways, was also markedly decreased by 2-fold in the liver. Pathway analysis of differentially regulated proteins revealed that DP interfered with metabolism and was associated with proteins related to apoptosis and cell differentiation. Based upon protein responses, we suggest that DP has effects on the generalized stress response, small G-protein signal cascades, Ca^{2+} signaling pathway, and metabolic process, and may induce apoptosis in the liver. This study offers novel mechanistic insight into the protein responses induced in the liver with DP, an increasingly used and understudied flame retardant.

1. Introduction

Dechlorane Plus (DP) is used as a replacement of Mirex, penta- and octa-BDE products which were banned from widespread use due to their toxicity, persistence, and bioaccumulation (Hoh et al., 2006). DP and its analogs are high production volume chlorinated flame retardants that are used in coating electrical wires and cables, computer connectors, and plastic roofing material (Betts, 2006). Relatively high concentrations of DP in environmental media and biota, as well as their persistence, bioaccumulation, and long-range transportation, suggests that DP and its analogs might be persistent organic pollutants (POPs) (Sverko et al., 2011). After the chemical was first identified in wildlife in the North American Great Lakes in 2006, DP has been detected on a more global scale (Möller et al., 2010, 2012; Qi et al., 2010). For example, concentrations of DP in surface water ranged from 0.0013 to 2.4 ng/L, while in suspended sediment from an E-waste recycling site in South China, concentrations of DP were as high as 78.8 μg/g dry weight (Xian et al., 2011; Zhao et al., 2011). It has been reported in other studies that the levels of DP and its isomers in aquatic and terrestrial biota such as zooplankton, shellfish, fish and birds can vary from 0.02 to 2200 ng/g lipid (Feo et al., 2012). Additionally, DP has been detected in human hair (Zheng et al., 2010), serum (Ren et al., 2009) and breast milk samples (Siddique et al., 2012). Thus it appears as though DP can be relatively ubiquitous in aquatic systems.

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However, despite growing evidence that DP is detectable across multiple taxa and tissues, the health risks of DP exposure to aquatic organism and humans are not fully characterized.

The bioaccumulation of DP in tissues has been reported in aquatic organisms including zooplankton, shellfish and fish (Feo et al., 2012). For example, in teleost fishes such as juvenile rainbow trout (*Oncorhynchus mykiss*), the biomagnification factor (BMF) for isoforms of DP is 5.2 and 1.9 for syn-DP and anti-DP, respectively; thus it appears as though the syn form of DP is more readily bioavailable (Tomy et al., 2008). In a freshwater food web from a reservoir in the vicinity of electronic waste recycling workshops in South China, there were trophic magnification factors (TMFs) of 11.3 and 6.6 for syn-DP and anti-DP, respectively (Wu et al., 2010). In addition to bio-magnification, there is evidence that DP can preferentially accumulate in tissues in aquatic organisms. For example, higher concentrations of DP were observed in liver and brain of Northern snakehead and Mud carp compared to muscle (Zhang et al., 2011). A recent study in Chinese sturgeon indicated high bioaccumulation potential of DP in heart, liver and eggs as well as high maternal transfer efficiencies of DP based on its tissue distribution (Peng et al., 2012). Therefore, the data support the hypothesis that DP can bioaccumulate readily in aquatic food webs and bioconcentrate in fish tissues.

The uptake and bioconcentration of pollutants can be associated with adverse biological effects in aquatic organisms. Studies have demonstrated that exposure to polybrominated diphenyl ethers (PBDEs) are associated with reproductive and developmental effects, neurobehavioral toxicity, thyroid hormone disruption, immunotoxicity and that DP exposures are potentially related to cancers (Siddiqi et al., 2003). While there have been a number of studies reporting on the toxicology of PBDEs, few data are available for DP. Studies on the toxicity of DP that measured higher level biological endpoints, as well as clinical or anatomical pathology, have demonstrated that the toxic effects of DP may be relatively low (Brock et al., 2010; Crump et al., 2011). However, research on DP at the molecular level suggests that DP can induce adverse effects and there may be subtle endpoints that can be used to assess biological impacts in organisms (Li et al., 2013; Wu et al., 2012). For example, sub-chronic exposures to DP in rats (90 days period, 1–100 mg/kg/d) showed no adverse effect based upon histopathology and survival; however mRNA levels of *sulfotransferase* (*SULT*) *1A1*, *1C2*, and *2A1* in the low dosage group (1 mg/kg/d) were significantly decreased and enzyme activity of CYP 2B1 was increased with DP exposure (Li et al., 2013). Recently, hepatic oxidative damage, perturbations in metabolism, and signal transduction in mice were reported to be induced by DP at the dose of 500–5000 mg/kg by daily gavage for 10 days (Wu et al., 2012). These studies indicate that DP may induce toxic effects in organisms at the level of the transcriptome and metabolome. However, additional research on the toxicity of DP at the protein level is required to better assess the overall impact of DP exposure and to more fully characterize the molecular responses underlying DP exposure.

To address this knowledge gap, the proteomic response in the liver of Chinese sturgeon (*Acipenser sinensis*) was measured after animals were treated with DP to learn more about the pathways affected by DP in this detoxifying tissue. Quantitative proteomics, a powerful tool for the global evaluation of protein expression, provides an effective method for characterizing toxicity pathways of chemical pollutants (Monsinjon and Knigge, 2007). To date, proteomics approaches have been successfully employed in several studies on the toxic effects of PBDEs in aquatic organisms (Chiu et al., 2012; Kling and Förlin, 2009; Kling et al., 2008). The model chosen for this study was the Chinese sturgeon because (1) it is listed as a grade I protected animal in China (since 1988) and because (2) this species is experiencing dramatic declines in population number due to overfishing, loss of natural habitat for reproduction, and anthropogenic activities (Qiao et al., 2006). Moreover, the Chinese sturgeon is an excellent sentinel species for monitoring environmental organic contaminants because it is a long-lived predatory fish (Wei et al., 2002).

2. Materials and methods

2.1. Chemicals

Dechloranes Plus (CAS no. 13560-89-9; M.W. 653.7; purity > 95%) was purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada). Due to its extremely lipophilic character, DP was dissolved in corn-oil for intraperitoneal injections according to Wu et al. (2012).

2.2. Exposure experiment

Juvenile Chinese sturgeon individuals were obtained from Fisheries College of Huazhong Agricultural University. These juveniles are offspring of artificially propagated individuals, and the parents are released into the Yangtze River under the supervision of the local government, as described previously (Wan et al., 2006). Fish (about 1.0 kg in weight) were randomly stocked in 1200 L glass tanks that included replicated controls and three treatment groups. The fish were maintained in aerated de-chlorinated tap water (using an activated carbon filter) at a constant temperature ($15 \pm 2\,^{\circ}C$) with a photoperiod of 16 h:8 h (light:dark) in order to mimic their optimal temperature range in the natural environment. Fish were acclimated for one week prior to experimental injections. DP was dissolved in corn oil in order to prepare the stock solution. The control group was injected with corn oil only. Individuals in the treatment groups were injected intraperitoneally once with 1, 10, 100 mg/kg fresh wet weight DP. Over the experimental period of 14 days, fish were fed with tubificid worms twice a day. The DP exposure doses employed were chosen on the basis of a report of Wu et al. (2012) and toxicity data provided by OxyChem and U.S. EPA.

2.3. Sampling

Three fish were sampled at each dose after the 14-day exposure. Deep anesthesia was induced by a 0.05% solution of MS-222 (Sigma, USA). The liver samples from juvenile Chinese sturgeon were collected within 30 min of exsanguinations by tailing and immediately dipped into liquid nitrogen and stored at $-80\,^{\circ}C$. The experimental procedures were based on the standards of the Chinese Council on Animal Care.

2.4. Proteomic analysis

2-DE coupled MALDI–TOF–TOF was performed to quantify the proteomic response of juvenile Chinese sturgeon after injection of DP in order to better elucidate toxicity responses of DP at the protein level.

2.4.1. Protein extraction and separation

Protein extraction and 2-DE were performed according to Fang et al. (2010). Please refer to the Supporting Information for more specific details.

2.4.2. Protein identification and data analysis

After in-gel tryptic protein digestion, the resulting peptide mixtures were subjected to MALDI–TOF/TOF–MS analysis according to Meng et al. (2009) using a Bruker UltraFlex MALDI–TOF/TOF instrument. The mass signals generated from the MS mode