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Substituted diphenylamine antioxidants and benzotriazole UV stabilizers in blood plasma of fish, turtles, birds and dolphins from North America

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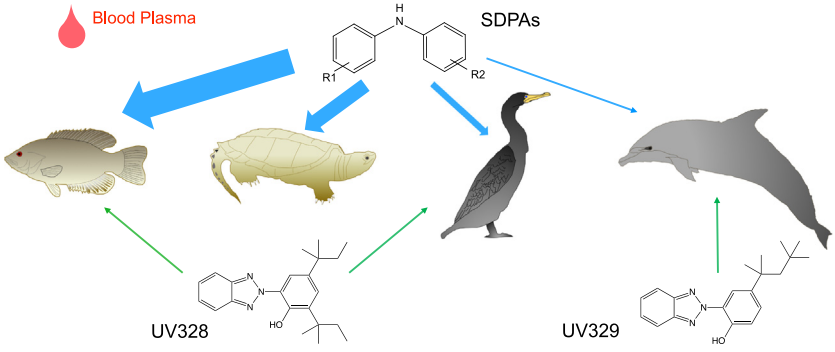
HIGHLIGHTS

- SDPAs and BZT-UVs detected in the blood of fish, turtles, birds and dolphins
- First report of SDPAs and BZT-UVs in reptiles
- Urban samples showed higher levels of SDPAs and BZT-UVs than rural areas.
- SDPAs levels followed the order of fish ≥ turtles > cormorants > dolphin.

GRAPHICAL ABSTRACT



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ABSTRACT

Substituted diphenylamine antioxidants (SDPAs) and benzotriazole UV stabilizers (BZT-UVs) are additives used in industrial and commercial applications to prevent degradation by oxidation and are contaminants of emerging environmental concern. Little is known about the fate of these contaminants in wildlife, particularly in reptiles, birds and marine mammals. Nine SDPAs and six BZT-UVs were measured in blood plasma of seven fish species, snapping turtles (*Chelydra serpentina*), double-crested cormorants (*Phalacrocorax auritus*), and bottlenose dolphins (*Tursiops truncatus*) from various locations in North America. Plasma SDPAs were more frequently (90–100%) detected and with higher concentrations (median: 25–270 pg g^{-1}, wet weight (ww)) in organisms from urban areas than rural locations (median: <method limits of quantification −136 pg g^{-1}). The concentrations of most SDPAs generally followed the order of fish ≥ snapping turtles > double-crested cormorants > bottlenose dolphins. Of the three quantifiable BZT-UVs, 2-(2H-benzotriazol-2-yl)-4,6-di-*tert*-pentylphenol (UV328) showed higher detection frequency in most species of fish, bird and turtle (range of 0–67%), indicating the widespread distribution of UV328 in the aquatic environment of lower Great Lakes region.

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1. Introduction

Exposure to anthropogenic contaminants is one crucial stressor affecting the health of wildlife and fish populations (Köhler and Triebskorn, 2013). However, little is known about the occurrence and distribution of many chemicals of emerging concern (CECs) in aquatic ecosystems, particularly in long-lived high trophic level vertebrates. Substituted diphenylamine antioxidants (SDPAs) and benzotriazole UV stabilizers (BZT-UVs) are CECs of environmental interest due to the high-volume production, multiple applications, and ecological risks (i.e., environmental persistence, bioaccumulation and chronic toxicity) (ECCC and HC, 2017; Lu et al., 2016a,b, 2017a,b, 2018; Nakata et al., 2009; Sühring et al., 2016). SDPAs and BZT-UVs are industrial additives typically used in automotive, lubricants and plastics to prevent the materials from degradation or color change (ECCC and HC, 2016, 2017). Most of SDPAs and BZT-UVs are high production chemicals in the USA and Europe (see production volumes summarized in Table S1).

Several studies have reported SDPAs (Lu et al., 2016a,b, 2017a, 2018; Sühring et al., 2016) and BZT-UVs (Kim et al., 2011; Lu et al., 2016a,b, 2017a, 2018; Nakata et al., 2009) in aquatic organisms worldwide. However, insufficient data of BZT-UVs and particularly SDPAs, in fish, birds and marine mammals are available, whereas no data are available for reptiles. The bioaccumulation of SDPAs and BZT-UVs is dependent on the habitat and the feeding ecology of the organisms, toxicokinetics of the chemicals, and biotransformation capacity as well as proximity to sources (Tierney et al., 2013). However, information on such factors affecting the bioaccumulation of these substances is still unknown. Snapping turtles (*Chelydra serpentina*) are a native reptile species to North America and have been previously demonstrated to be a suitable indicator species of local contamination because of their small terrestrial-aquatic interfacial habitats and longevity up to 100 years (de Solla et al., 2007; CSEWC, 2008). High levels of persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ether (PBDE) flame retardants, organochlorine pesticides (OCPs) and related metabolites have been detected in snapping turtles (Letcher et al., 2015). However, the concentration and distribution of SDPAs, BZT-UVs and many other CECs in snapping turtles are still unknown. In addition, BZT-UVs were suggested as potential endocrine disruptors (Fent et al., 2014; Giraudo et al., 2017; Hirata-Koizumi et al., 2007, 2008; Liang et al., 2017; Nagayoshi et al., 2014) which is of particularly important for snapping turtles as their reproductive (e.g., sex determination) and growth processes are susceptible to endocrine perturbation (de Solla et al., 1998).

Double-crested cormorants (*Phalacrocorax auritus*) are large colonial waterbirds that feed almost exclusively on fish and is widespread in North America with breeding colonies throughout the Great Lakes. Several studies have indicated the ecological sensitivity of this species to anthropogenic contaminants (Custer et al., 1999, 2001; Wires and Cuthbert, 2006). In the 1960s, cormorants in North American Great Lakes region were almost extirpated from the region due to the reproductive toxicity associated with exposure to POPs such as dichlorodiphenyltrichloroethane (DDT), on the breeding of this bird (i.e., including eggshell thinning) (Custer et al., 1999, 2001). Although cormorant populations have increased in the Great Lakes region after the ban of many POPs (including DDT) (Wires and Cuthbert, 2006), they continue to be monitored for POPs and select CECs under Canadian government initiatives (Braune et al., 2003).

SDPAs and BZT-UVs in the marine environment are still poorly understood with no data available for the bioaccumulation of SDPAs and BZT-UVs in any marine species from the Atlantic Ocean. Bottlenose dolphins (*Tursiops truncatus*) are apex predators in the marine environment and have been shown to accumulate contaminants (Houde et al., 2006; Kucklick et al., 2011). Investigation of the concentrations and distribution of SDPAs and BZT-UVs in bottlenose dolphin may provide insight into the bioaccumulation of CECs in marine mammals.

The objectives of this project were to address the significant knowledge gaps and determine the distributions of SDPAs and BZT-UVs in the freshwater and marine species from North America. It has been reported that BZT-UVs can bind with blood albumin via hydrogen bonds or electrostatic interactions (Zhuang et al., 2016), implying that blood is a possible reservoir for these contaminants. Blood plasma was therefore selected for SDPAs and BZT-UVs monitoring in this study considering that this approach is non-lethal and blood can be obtained with minimal harm to the animals (Keller et al., 2004). The central hypothesis was that biota from urban areas contains higher levels of target SDPAs and BZT-UVs than rural regions due to higher CEC emissions to aquatic environments near urban areas. To our knowledge, this is the first report of SDPAs and BZT-UVs in smallmouth bass (*Micropterus dolomieu*), common carp (*Cyprinus carpio*), brown bullhead (*Ameiurus nebulosus*), gizzard shad (*Dorosoma cepedianum*), rock bass (*Ambloplites rupestris*), largemouth bass (*M. salmoides*), double-crested cormorants and any reptile as well as North Atlantic marine mammal species.

2. Materials and methods

2.1. Sample collection

Fish, snapping turtles and double-crested cormorants were collected from two different freshwater food webs, namely Hamilton Harbour (HH) and Lake Joseph (LJ) (Ontario, Canada). HH is a historically contaminated aquatic ecosystem in the Great Lakes region and is a designated Area of Concern. The harbour receives wastewater effluents inputs from three wastewater treatment plants (WWTPs; serving about 700,000 people). Industrial activities, shipping and recreational boating, may also affect the water quality of HH. In contrast, LJ was selected given the absence of point sources of industrial contamination as well as the smaller population in this rural area. Anthropogenic activities in LJ are predominantly in the warmer months of June–September from recreational cottages. Blood from dolphins in the present study were collected from Charleston Harbor (CH) (South Carolina, USA). Previous work has shown that the dolphins in this area may be exposed to wastewater-related contaminants (e.g., triclosan) (Fair et al., 2009).

Sampling areas are shown in Fig. S1. Fish, snapping turtles and double-crested cormorants were collected in July 2016. Lake trout (*Salvelinus namaycush*) ($n = 4$) and smallmouth bass ($n = 3$) were caught from the LJ, while common carp ($n = 3$), brown bullhead ($n = 4$), gizzard shad ($n = 4$), rock bass ($n = 4$) and largemouth bass ($n = 4$) were collected from the HH. Fish in HH were collected using an electrofishing vessel (Smith-Root SR20H) and transported live to an on-site laboratory in an aerated tank with source water from individual sites. LJ lake trout were collected by gill net (4″ mesh) through the Ontario Ministry of Natural Resources and Forest (OMNRF) annual summer profundal index netting program (A. Ivany, OMNRF, pers. comm.). The length and weight of fish were measured, and the blood plasma was collected. Fish liver-somatic index is calculated as: (liver weight/body weight) × 100% and body condition is calculated as: (weight/length^3) × 100. Snapping turtles (LJ: $n = 1$; HH: $n = 9$) and large juvenile (~>1+ kg) double-crested cormorants (LJ: $n = 10$; HH: $n = 10$) were caught using hoop traps baited with fish and by hand, respectively. Whole blood was sampled from the ventral coccygeal vein for adult turtles and brachial vein of large cormorant chicks using syringes with 10 mL sodium heparin coated vacutainers. The blood samples were stored in a cooler on the ice and within 5 h were centrifuged for 5 min in a clinical centrifuge to separate the plasma. Then, the plasma was transferred to cryovials and stored ≤−80 °C. Blood samples were collected from the fluke vein of bottlenose dolphins ($n = 18$) from CH in 2013 as part of the Dolphin Health and Risk Assessment Project under National Marine Fisheries Science Permit No. 14352-02. Blood plasma was obtained by centrifugation of the blood within 10 min and stored in a liquid nitrogen cryogenic container. The samples were then stored