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Tissue distribution of organochlorine pesticides in largemouth bass (*Micropterus salmoides*) from laboratory exposure and a contaminated lake☆

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ABSTRACT

Tissue concentrations of persistent organochlorine pesticides in laboratory-exposed largemouth bass (*Micropterus salmoides*) and in bass collected from Lake Apopka, FL were determined by both total mass and lipid normalized mass to better understand the bioaccumulation pathways of contaminants. In the laboratory study, male bass were orally administered a single dose of a mixture of two pesticides (*p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) and dieldrin) and then fed uncontaminated food for 28 days. Gastrointestinal tract, liver, brain, gonad, kidney, spleen, and muscle were collected for chemical analysis. Different profiles were observed by total contaminant mass in tissues compared to lipid normalized mass. On a lipid normalized basis, *p,p′*-DDE was highest in the gastrointestinal tract followed by the liver, gonad, spleen, muscle, kidney and then brain. Dieldrin, on the other hand, was highest in the gastrointestinal tract and spleen and then followed by the gonad, muscle, liver, kidney, and brain. Distribution of the chemicals among the organs differed by their log K_{OW} values and generally followed the blood flow path after the gastrointestinal tract. The low contaminant levels found in kidney and brain suggest insufficient time for equilibration into these tissues, especially into the brain where the blood-brain barrier may be slow to traverse. In Lake Apopka fish, dichlorodiphenyltrichloroethanes (DDXs, sum of *p,p′*-DDE, *p,p′*-DDD, and *p,p′*-DDT), Drins (sum of aldrin, dieldrin, and endrin), and hexachlorocyclohexanes (HCHs) were found. For DDXs, the lipid normalized concentrations in each tissue were about the same, as predicted from theory. For Drins and HCHs, the lipid normalized concentrations were similar for kidney, spleen, brain, gonad and muscle, but much lower in the gastrointestinal tract and liver, probably because of metabolism occurring in those tissues.

1. Introduction

Organochlorine pesticides (OCPs) are chlorinated hydrocarbons that were widely used in agriculture and public health as insecticides and biocides from the 1940s through the 1960s (DHSS, 2010). OCPs that were used during that period include dichlorodiphenyltrichloroethane (DDT), methoxychlor, toxaphene, dieldrin, chlordanes, and lindane. Although their application has been banned or restricted in many countries, these pesticides, in particular DDT, continue to be used in some developing countries like Ghana and China (Hogarh et al., 2014; Li et al., 2016). OCPs are intractable in the environment and can act as endocrine disruptors, which interfere with reproduction and developmental processes in wildlife and humans (Johnson, 1999; Colborn et al., 1993; Kavlock et al., 1996).

In aquatic environments, OCPs are bioavailable to organisms and are transferred up the food chain to top predators including predatory fish, where they bioaccumulate in fatty tissues. Trophic transfer via food is often an important source of OCPs in fish since these contaminants have octanol-water partitioning coefficient (log K_{OW}) > 5 and are not freely dissolvable in water. Because most OCPs are not appreciably biotransformed in biological tissues, fish can be a suitable indicator for the contamination of their surrounding environments (Fisk et al., 1998; Lanfranchi et al., 2006). It is evident that consumption of contaminated fish is one of the most

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important pathways for transfer of pesticides into humans, encouraging many studies to focus on the magnitude of OCPs in edible tissues of fish species (Jiang et al., 2005; Yang et al., 2006; Li et al., 2008; Yohannes et al., 2014). In addition, understanding the distribution pattern of OCPs in other tissues (e.g., liver, kidney, gonad, brain, and spleen) provides insight into pathways of OCP bioaccumulation and helps define primary exposure in exposed animals and resultant human exposures from given levels of environmental contamination. OCP concentrations are normally found to be the lowest in muscle tissues but the highest in either hepatobiliary-related tissues such as liver, bile, and heart (Guo et al., 2008; Zhao et al., 2013, 2014) or brain (Sahagun et al., 1997; Zhou et al., 2007). These results have been primarily observed in studies where fish are chronically exposed to contaminants resulting in uptake of contaminants by different tissues in proportion to their lipid content at equilibrium. In addition, distribution of lipophilic contaminants among animal tissues can also depend on lipid content of that tissue, blood flow and time to reach equilibrium between blood and this compartment (Ondarza et al., 2011; Boon et al., 1994). Reaching to the brain, however, may take longer than other tissues since the contaminants need to transverse the blood-brain barrier.

The north shore area of Lake Apopka, FL has been extensively contaminated with OCPs from heavy agricultural activities from the 1940s to the 1970s. In 2011, a good 40 years after cessation of use, heavy levels of contaminants were still measured in the sediments (e.g., 1.0 ± 0.3, 9.5 ± 7.7, and 0.3 ± 0.1 μg/g dry wt for *p,p′*-DDE (the major metabolite of DDT), toxaphene, and dieldrin, respectively) (SJRWMD, 2011). Specifically, control largemouth bass (*Micropterus salmoides*, LMB) introduced into ¼ acre-ponds (mesocosms) from October 2007 to January 2008 in the highly OCP-contaminated north shore area of the lake contained a mean whole-body burden of 363.1, 185.1, and 11.5 μg/g lipid for *p,p′*-DDE, toxaphene, and dieldrin, respectively (Martyniuk et al., 2016). Although there have been extensive efforts by St. Johns River Water Management District (SJRWMD) to remediate the area since 2002 (SJRWMD, 2011), our previous study indicates that OCPs remain present in sediments of the north shore area and are bioavailable to blackworms (*Lumbriculus variegatus*) (Dang et al., 2016). The contaminated diets from benthic organisms and/or smaller fish could be a potential source of OCPs for LMB in mesocosms and possibly for LMB in the lake. To our knowledge, there is a paucity of information about tissue accumulation of OCPs in LMB given that LMB are top predators in most aquatic food chains as well as sought-after catch for anglers and subsistence fishermen (Soupir et al., 2000). Our main objective for this study was to better understand tissue-specific accumulation of pesticides in LMB exposed to 1) an acute dose of two OCPs (*p,p′*-DDE and dieldrin), and 2) a historical contamination of OCPs. We excluded toxaphene in this study due to complexity of its isomers that could be present in the environment. In addition, we administered individual LMB species with a single dose of a mixture of *p,p′*-DDE and dieldrin to minimize toxicity and variability of chemical doses among individuals as well as individual variations in tissue-specific concentrations of OCPs.

2. Materials and methods

2.1. Standards and solvents

Neat standards of *p,p′*-DDE (CAS# 72-55-9, 99% pure) and dieldrin (CAS# 60-57-1, 98.7% pure) were purchased from Aldrich (Milwaukee, WI). A stock solution of 18 OCPs containing α-, β-, γ-, δ-hexachlorocyclohexane (HCH) isomers, α- and β-chlordane isomers, *p,p′*-DDD, *p,p′*-DDE, *p,p′*-DDT, dieldrin, aldrin, endosulfan I, endosulfan II, endosulfan sulfate, endrin, heptachlor, heptachlor epoxide, and methoxychlor at a concentration of 2000 μg/ml in hexane:toluene (1:1 v/v) were purchased from Phenomenex (Torrance, CA). Isotope internal standards ($^{13}C_{12}$-*p,p′*-DDE and $^{13}C_{12}$-dieldrin) and a mixture of deuterated internal standards (acenaphthene-*d10*, chrysene-*d12*, and phenanthrene-*d10*) were obtained from Cambridge Isotope Laboratories (Tewksbury, MA) and Ultra Scientific (North Kingstown, RI), respectively. Organic solvents (hexane, acetone, and acetonitrile) were purchased from Fisher Scientific (Fair Lawn, NJ).

2.2. Oral dosing

Commercial salmon feed (sinking pellets, 4 mm in size) was purchased from Skretting USA (Tooele, UT) and ground into powder using a mortar and pestle. A mixture of *p,p′*-DDE and dieldrin (~10 mg of each) dissolved into 2 ml ethanol was mixed with 4 ml menhaden fish oil (Jandell Corp., Boyd, TX) to facilitate the coating of chemicals onto the feed. The mixture was then slowly added dropwise into 10 g of homogenized feed while mixing to obtain a nominal concentration of 1 mg chemical/g feed. The pesticide-laden feed was mixed thoroughly in a 60 ml amber glass vial using a clean stainless steel spatula and then placed inside a fume hood overnight to allow complete evaporation of ethanol. Unspiked feed also was prepared by adding 6 ml ethanol:oil (1:2 v/v), equal to the amount of vehicle added to the spiked feeds, into 10 g of the homogenized feed.

Male largemouth bass, 1–2 years of age, were purchased from American Sports Fish Hatchery (Montgomery, AL) in May 2013. Size of fish averaged 26.3 (±1.8) cm in length and 232.1 (±45.6) g in weight. Fish were maintained in a 120 gal, flow-through round tank supplied by filtered water (municipal water passed through granular activated carbon) and aerated on-site at the Center for Environmental and Human Toxicology (CEHT), University of Florida, FL. Fish were starved 24 h prior to oral dosing. On the day of dosing (early August 2013), fish ($n = 5$) were anesthetized in tricaine-methanesulfonate (MS 222, 150 mg/L) and orally introduced with a spiked feed pellet paste, which was prepared by compressing about 0.2 g of spiked feed (0.2 mg OCPs) into a 3 ml syringe with cut-off end to push the plug passed the esophagus. Total ingestion was verified visually in the tank for each fish. This method was used to obtain a nominal concentration of 1 μg chemical/g fish, which was 5 times lower than the concentration of *p,p′*-DDE and 2 times higher than the concentration of dieldrin, respectively, measured in bass introduced into the mesocosms on the north shore area of Lake Apopka, FL. Fish (5 per group) were also orally dosed with approximately 0.2 g (~0.1% of their body weight) of unspiked (control) pellet paste one time at the beginning of the experiment. Twenty-four hours following this one time exposure, fish were fed control commercial food (sinking pellets, 4 mm in size) for up to 30 days to ascertain that spiked feed containing OCPs was cleared out of the intestine and to give fish enough time to distribute the OCPs into tissues. All experiments were carried out at a water temperature of 24.8 (±1.7) °C with a photoperiod of 16 h:8 h (light:dark). Fish were starved for 24 h prior to sample collection to make sure that any food residues were flushed out of the intestine. After euthanasia with an overdose of MS 222, fish were measured for length and weight, and dissected to collect liver, gonad, muscle (with skin), gastrointestinal tract (GIT), kidney, spleen, and brain. Tissue samples were further stored frozen at −20 °C until analysis. All animals were treated as per the protocol by the University of Florida Institutional Animal Care and Use Committee.

2.3. Lake Apopka fish sampling

Lake Apopka LMB ($n = 10$, 5 males and 5 females) were collected

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