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Occurrence of triclosan in plasma of wild Atlantic bottlenose dolphins (*Tursiops truncatus*) and in their environment

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Triclosan in bottlenose dolphin plasma and their environment.

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ABSTRACT

The presence of triclosan, a widely-used antibacterial chemical, is currently unknown in higher trophic-level species such as marine mammals. Blood plasma collected from wild bottlenose dolphins (*Tursiops truncatus*) in Charleston, SC (CHS) ($n = 13$) and Indian River Lagoon, FL (IRL) ($n = 13$) in 2005 was analyzed for triclosan. Plasma concentrations in CHS dolphins ranged from 0.12 to 0.27 ng/g wet weight (mean 0.18 ng/g), with 31% of the sampled individuals having detectable triclosan. The mean IRL dolphin plasma concentrations were 0.072 ng/g wet weight (range 0.025–0.11 ng/g); 23% of the samples having detectable triclosan. In the CHS area, triclosan effluent values from two WWTP were both 190 ng/L and primary influents were 2800 ng/L and 3400 ng/L. Triclosan values in CHS estuarine surface water samples averaged 7.5 ng/L ($n = 18$) ranging from 4.9 to 14 ng/L. This is the first study to report bioaccumulation of anthropogenic triclosan in a marine mammal highlighting the need for further monitoring and assessment.

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1. Introduction

The accumulation of anthropogenic chemical agents in the aquatic environment and their potentially deleterious effects on wildlife and humans is an increasing concern. Pollution monitoring of the aquatic environment has focused primarily on conventional priority pollutants, especially agricultural and industrial persistent organic pollutants (POPs). It has been well-established that marine mammals, especially fish-eating species and those from coastal regions with dense human populations and greater industrial and agricultural activities, harbor high concentrations of POPs (Aguilar et al., 2002; Houde et al., 2005a; O'Shea, 1999; O'Shea and Tanabe, 2003).

POPs are only one group of chemicals that may pose a risk to aquatic mammals. Another group of bioactive chemicals that pose a potential risk include a wide diversity of active ingredients in pharmaceuticals and personal care products (PPCPs). PPCPs represent a diverse group of compounds including human and veterinary drugs, hormones, antibiotics, antiseptics, and biocides. They are ubiquitous and persistent in urban receiving waters (Ellis, 2006) and a source of prevalent anthropogenic contaminants in the aquatic environment (Kolpin et al., 2002). A marked increase has been observed in the number of studies investigating their occurrence, fate and risk assessment (Heberer, 2002; Jones et al., 2001; Kummerer, 2001). One of the more recently studied chemicals of concern used in a number of PPCPs (e.g., deodorants, toothpastes, and cosmetics) is triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether), a synthetic, broad-spectrum antibacterial agent. In addition to its use in PCPS, triclosan has also been incorporated into polymers and textile fibers used in a variety of other consumer products (e.g., toys, undergarments, and cutting boards) to provide antibacterial properties (Schweizer, 2001). Over 95% of triclosan's uses are in consumer products that are disposed of in residential drains (Reiss et al., 2002). Since wastewater treatment plants (WWTPs) do not completely remove this compound and, with its widespread use and mostly 'down-the-drain' disposal, triclosan has been found in waterways that receive discharge at concentrations ranging from 10 ng/L to 98 ng/L (Boyd et al., 2003; Singer et al., 2002). A recent

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review of chemical fate during wastewater treatment found that triclosan was among those with the highest influent concentrations (Heidler and Halden, 2008).

A U.S. Geological Survey study of 95 contaminants in United States (US) streams across 30 states found triclosan to be one of the most frequently detected and highly concentrated compounds (Kolpin et al., 2002). Because triclosan has a high hydrophobicity with a 4.8 log k_{ow} value, it can accumulate in fatty tissues. It has been detected in the bile of fish placed in cages downstream from wastewater processing plants and high levels have been measured in human milk samples (Adolfsson-Erici et al., 2002; Allmyr et al., 2006). A major environmental concern is the tendency for triclosan to be transformed into other, potentially more toxic substances such as chlorinated dibenzo-*p*-dioxins. Triclosan may be biotransformed into methyl-triclosan, a more persistent compound, by biological methylation (Lindstrom et al., 2002); chlorinated to form toxic chlorophenols, such as 2,4-dichlorophenol (Canosa et al., 2005; Rule et al., 2005) and phototransformed into lower chlorinated dioxins, in particular 2,7/2,8-dibenzodichloro-*p*-dioxin (Aranami and Readman, 2007; Mezcua et al., 2004). Triclosan is listed in EPAs draft Dioxin Reassessment as "could be" and "suspected to be" contaminated with dioxins (USEPA, 1994).

Triclosan accumulation and toxicity has been described primarily for freshwater aquatic organisms. As repositories for point and nonpoint-source contaminants, estuarine and near coastal ecosystems are particularly vulnerable to pollution. Thus far, studies in the aquatic environment have only measured triclosan levels in lower trophic-level organisms such as algae, crustacean and fish. High levels of triclosan (0.24–4.4 mg/kg) were reported in the bile of fish living downstream of WWTP discharges in Sweden (Adolfsson-Erici et al., 2002) and in blood plasma of fish in the Detroit River of North America (Valters et al., 2005).

The toxicity of triclosan among several aquatic organisms has been described (Capdevielle et al., 2008; DeLorenzo et al., 2008; Ishibashi et al., 2004; Orvos et al., 2002) and toxic effects have been observed when rats and mice were exposed to triclosan (Bhargava and Leonard, 1996). Reports have indicated that triclosan causes endocrine disruption (Foran et al., 2000; Gee et al., 2008; Ishibashi et al., 2004) and interferes with thyroid hormone metabolism (Crofton et al., 2007; Veldhoen et al., 2006). The extent that PPCP chemicals, such as triclosan, may accumulate in marine mammals is unknown. As a widely-distributed top-level predator in near coastal waters, bottlenose dolphins are affected by local anthropogenic activities. As such, they represent an important sentinel species for biomonitoring spatial and temporal trends in contaminants and health of ecosystems (Bossart, 2006; Fair and Becker, 2000; Reddy et al., 2001; Wells et al., 2004).

Our objective was to determine the presence and concentration of triclosan in blood plasma of bottlenose dolphins sampled in two southeast U.S. estuarine sites, Charleston, South Carolina (CHS) and the Indian River Lagoon, Florida (IRL). The dolphins in these two locations exhibit high site-fidelity, as indicated by long-term photo-identification research (Mazzoil et al., 2005; Speakman et al., 2006; Zolman, 2002). For the CHS site, we further analyzed ambient water samples to determine relevant environmental concentrations and WWTP samples to investigate potential sources.

2. Materials and methods

2.1. Study sites

Samples were collected during dolphin capture-release studies conducted under NMFS Permit 998-1678 at two study sites: Charleston, SC (CHS) and the Indian River Lagoon, FL (IRL). For the CHS study site, capture–release surveys were conducted out of Charleston, SC (32°46′35″N, 79°55′51″W), and included the Charleston Harbor, portions of the main channels and creeks of the Ashley River, Cooper River, Wando River, and the Stono River Estuary. For the IRL study site, capture–release surveys were conducted out of Titusville, FL (28°36′43″N, 80°48′27″W) and Stuart, FL (27°11′51″N, 80°15′10″W) and included portions of the Mosquito Lagoon, Indian River, Banana River, north and south forks of the St. Lucie River, and the St. Lucie Inlet. This study was part of the Bottlenose Dolphin Health and Risk Assessment (HERA) Project, aimed at assessing the health status of dolphins in these two areas and investigating associations between health status and environmental stressors (Fair et al., 2006).

2.2. Sample collections

Techniques used for the capture, sampling, and release of dolphins have been previously described (Fair et al., 2006). Once restrained, dolphins were placed on a processing vessel for veterinarian health examinations and collection of samples. Blood samples were drawn from the periarterial venous rete in the flukes within the first 10 min of capture with a 19-gauge, 1.9 cm, butterfly catheter into heparin vacutainers (Becton Dickinson, Franklin Lakes, New Jersey, USA). Samples were centrifuged and plasma separated and stored at −80 °C until analyzed. A total of 26 plasma samples were collected during summer of 2005 (13 from CHS and 13 from IRL). Age was determined by examining the post-natal dentine layers of an extracted tooth (Hohn et al., 1989). All animal capture and sampling protocols were conducted under National Marine Fisheries Permit No. 998-1678 and approved by the Harbor Branch Oceanographic Institution Institutional Animal Care and Use Committee.

A single primary influent 24 h composite and effluent water samples were collected from two WWTPs with discharges into Charleston Harbor, SC in September 2008. Within 24 h of the collection of the WWTP samples, a total of 18 ambient water samples were collected from nine different locations within the CHS study site. Each of the nine sites was sampled during a low and high tidal stage. Water samples were collected in 1 L amber glass containers and shipped overnight on ice packs to the National Water Research Institute, Burlington ON Canada. Measures were taken to minimize photo degradation including the use of amber bottles and shielding from light from the time the sample was taken, during shipment and in the laboratory. Samples were received by the analytical laboratory within 24 h and all samples were then immediately acidified to pH 3 with 1 N HCl and kept at 4 °C in the dark to reduce triclosan degradation. The WWTP samples were extracted within 36 h while the surface water samples were extracted within seven days.

2.3. Chemical analysis

Triclosan was isolated and quantified in the same bottlenose dolphin plasma samples that were previously analyzed for hydroxylated polychlorinated biphenyls (OH-PCBs) (Houde et al., 2006). Method details are provided in Supporting information. In brief: Samples were spiked with $^{13}C_{12}$-triclosan, two surrogate recovery standards for PCBs: chlorobiphenyl (CB)30 (2,4,6-trichlorobiphenyl) and CB204 (2,2′,3,4,4′,5,6,6′-octachlorobiphenyl)], and five $^{13}C_{12}$-labeled OH-PCBs (4-OH-CB12, 4-OH-CB29, 4-OH-CB61, 4-OH-CB120, and 4-OH-CB187; 50 ng/ml). Following extraction with MTBE, extracts were fractionated into neutral and phenolic fractions. The phenolic extracts were derivatized with diazomethane to yield methyl-triclosan while the neutral fraction was analyzed directly. Samples were analyzed by high resolution gas chromatography high resolution mass spectrometry (HRGC/HRMS) in electron ionization (EI) mode. Source temperature was 280 °C and the resolving power of the analyzer was 10,000.

Analysis of lab blanks for plasma analysis indicated no detectable triclosan. The GC–HRMS instrument detection limit (IDL) defined as the lowest quantifiable response (S/N = 3) was 0.005 ng/g while the Method Detection Limit (MDL), defined as 3 standard deviation of the lowest calibration standards in the absence of background levels (Gomez-Taylor et al., 2003), was calculated at 0.033 ng/g. The recovery for $^{13}C_{12}$-triclosan was 51 ± 23%.

Sewage (250 mL) and surface water (500 mL) samples were extracted using previously developed solid-phase extraction (SPE) procedure (Lee et al., 2005). Further details are provided in the Supporting information. In brief: Samples were filtered (1.2 μm) and spiked with $^{13}C_{12}$-triclosan. The SPE cartridge was washed with methanol/water (washing discarded) before triclosan was eluted with 5 mL of methanol. The methanol fraction was evaporated and derivatized with pentafluoropropionic acid anhydride (PFPA) to yield the pentafluoropropionyl (PFP) derivative of triclosan. Sample extracts were analyzed for PFP-triclosan by electron-impact ionization (EI) GC-low resolution MS in selected ion mode. Recovery for $^{13}C_{12}$-triclosan in the surface water and WWT samples averaged 76% and 107%, respectively. Based on a concentration factor of 1000, the MDL for triclosan were 3 ng/L and 10 ng/L for surface water and wastewater, respectively.

3. Results

3.1. Triclosan in dolphins

In CHS dolphins, plasma triclosan concentrations ranged from 0.12 to 0.27 ng/g wet weight, with 31% of the sampled individuals having detectable triclosan (Table 1). For the CHS study site, triclosan levels as well as dolphin identification (ID) codes are shown in Fig. 1 for the dolphins sampled and the collected water