CONTAMINATION OF TRICLOSAN IN FISH TISSUES COLLECTED FROM SAVANNAH, GEORGIA, USA

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Abstract

Triclosan (TCS) is a common antibacterial chemical currently in widespread use in household, health care products in various consumer and personal care products. Earlier research has revealed considerable levels of these antibacterial agents in wastewater, river water, aquatic wildlife and humans. TCS are reported to be toxic and produce adverse health effects to wildlife and humans. Furthermore, TCS undergo photo-degradation and modified into lower chlorinated dioxins and phenols. During treatment process in wastewater treatment plants TCS also shown to convert into methyl triclosan (MeTCS) which is more stable. Consequently, in this study we measured TCS in selected fish species collected from various aquatic ecosystems in Savannah, Georgia USA. Concentrations of TCS in liver and muscle were in between 0.9-5.6 and 0.3-2.7 ng/g dry weight, respectively. Catfish collected from Lake Mayer contained maximum concentrations than other fish species collected from rivers, ponds, open oceans and estuarine ecosystem in Savannah. Observed concentrations were comparable to available literature despite meager study available so far.

Introduction

In past three to four decades the increase in the everyday use of antibacterial/antimicrobial products has raised concerns about the efficacy of these items and their effects in the rise of microbial resistance. Particularly, continued use of triclosan (TCS; [2,4,4'-trichloro-2'-hydroxydiphenyl ether]) a widespread antimicrobial agent which is used in consumer products, including personal care products, toothpaste, mouthwash, deodorants, soaps, textiles (socks, underwear), toys, liquid dishwashing soap, and plastic kitchenware creating a possibility of environmental contamination and resistant bacteria[1-3]. TCS was first introduced in 1965 and has been marketed as cloxifenol, and different forms of Irgasan. Its most common use is in antimicrobial hand soaps, but in the United States it can also be found in consumer products such as liquid dishwashing soaps, deodorants, and toothpastes. The concentrations used in products in the United States typically range from 0.15 to 0.3%. While allergy to triclosan-based products is uncommon, several cases of contact dermatitis secondary to triclosan have been reported[3].

Environmental occurrence of TCS has been reported in the aquatic ecosystem and human foodstuffs[4-6], and has special attention as an emerging environmental contaminant[7-8]. In amphibians, TCS can able to disrupt the thyroid hormone related genetic damage and can induce changes in the thyroid hormone-mediated metamorphosis[9]. TCS can also alter blood serum concentrations of total thyroxine in rats[10]. TCS is not acutely toxic to mammals, but it can interact with cytochrome P450-dependent enzymes, UDP-glucuronosyltransferases, and the human pregnane X receptor[11]. TCS have been found in sewage due to incomplete elimination during wastewater treatment processes[7]. Since TCS is hydrophobic in nature and thus these substances allows them to reside as sludge layers, contaminating wastewater streams. Due to incomplete elimination these compounds from effluent water and in sludge, these compounds are disposed to natural waters and consequently contaminated river ecosystem[12]. Due to enormous usage, disposal, persistency and lipophilicity TCS have shown to accumulate in aquatic wildlife[13]. There are not enough study focused on the TCS in fish tissues from the United States and therefore we monitored contamination status of TCS in various fish species collected from different aquatic ecosystems in Savannah, Georgia USA.

Materials and Methods

Sampling

Various aquatic organisms including sawtooth pen clam, white shrimp, fishes and shark (Atlantic sharp-nose shark and bonnethead shark) were collected in the Ogeechee River estuary at Richmond Hill, Skidaway Island, Lake Mayer, pond by Savannah Mall, St. Simons Island, Wassaw Sound, the Savannah River mouth and the Georgia Shelf from June 2006 to July 2007, because these sites receive wastewater from different origins (domestic, industrial and sewage treatment plants)[14]. Details of the aquatic wildlife samples analyzed in this study are illustrated in Senthil Kumar et al[14] and in Table 1. Immediately after collection, genus and species were determined and samples were wrapped in aluminum foil, packed in zip-lock bags containing ice and transported to the lab in a cooler filled with ice then stored at -20^{o}C until dissection. For each species, 1-9 individuals were selected and then dissected. The liver and muscle tissue were separated from large fish and shark species, while the soft tissue was separated from sawtooth pen clam and white shrimp. The liver from each species were pooled and homogenized in a stainless steel homogenizer and stored in a freezer until chemical analysis. Similarly, muscle tissue from each species was pooled and homogenized. However, individual liver and muscle tissue were analyzed for each Atlantic sharp-nose shark and bonnethead shark. Muscle and liver tissue were freeze-dried prior to chemical analysis.

Chemical analysis

Approximately 0.5-2g of freeze dried fish samples were loaded with Na_2SO_4 in a pressurized fluid extractor (ASE 200; Dionex, Sunnyvale, CA) and extracted after adding known amount of $^{13}C_6$-TCS. Extraction (3 cycles) was done with 95% acetone in 5% MeOH with pressure rate of 1500 psi at 100^{o}C for 5 min. All extracted samples were evaporated with TurboVap II (Caliper Life Science, Hopkinton, MA). Fractionation was conducted with 1-g 5% activated silicagel cartridges by fraction collector (Foxy 200; ISCO, Lincoln, NE). In fraction-1, 6-mL 20% dichloromethane in hexane was eluted which is discarded. The fraction-2, 1 mL of 100% dichloromethane was eluted and discarded. In fraction-3, 12-mL 50% dichloromethane in MeOH was eluted to collect TCS. Eluted samples were reduced to dryness and re-constituted with acetonitrile for LC-MS/MS analysis.

LC-MS/MS Analysis

Instrumental analysis was conducted using high-performance liquid chromatography (Shimadzu HPLC, USA)-interfaced with tandem mass spectrometer (Applied Bio Systems 3200 LC-MS/MS, USA). Acetonitrile and nano pure water were delivered at 0.2-μL/min with the Agilent HPLC. Aliquot (20 μL) of sample was injected onto Ultra IBD HPLC column with Trident integral inlet fitting; 2.1 mm x 150 mm; 5 μm (Restek, USA). The gradient method was adapted for acetonitrile and nano pure water mobile phase. The detector was an Applied Biosystems API-3200 tandem mass spectrometer operated in an electrospray interface in the negative ionization mode. The electron multiplier was set at 1.5 kV while the nebulizer gas was nitrogen. The recoveries of $^{13}C_6$-TCS spiked into liver samples were 82-86%. Recoveries of $^{13}C_6$-TCS spiked into muscle were 90-98%. Minimum five calibration points (0.1, 1, 5, 10, 50 and 100 ng/mL) of all TCS were freshly prepared for each batch and used to calculate the sample concentrations which gives the $r^2 = 0.999$. $^{13}C_6$-TCS and native TCS was detected with the m/z of 301.00 (daughter ion 35.00) and 289.00 (daughter ion 35.00), respectively. Blank sample were analyzed for each batch and we found no impurity of TCS in any of the samples. Concentrations of TCS in fish tissues were expressed as ng/g dry wt.

Quality Assurance and Quality Control

Quality assurance and quality control were maintained during entire research program. For example, we avoid using solid and liquid soap, antibacterial coated papers as maximum as possible. Soap washed glass wares other analytical materials were rinsed with acetone and methanol and dried using autoclave. The blank checks were run for fish liver and muscle. High purity internal as well as external (calibration standard) was used for the research. Calibration standards were freshly prepared for each set analysis in which relative response for each analysis was r^2=0.99.

Results and Discussion

TCS Concentrations

Concentrations of TCS in selected fish species have been illustrated in Table 1. Liver samples contained slightly higher concentrations than muscle tissue. Concentration of TCS in liver and muscle were in between 0.9-5.6 and 0.3-2.7 ng/g dry weight, respectively. Crustaceans like sawtooth pen clam and white shrimp soft tissues contained 0.4 to 1.2 ng/g dry weight which is also similar levels to those to shark muscle tissues. While fish tissues from different ecosystems showed almost similar concentrations of 0.3 to 1.8 in muscle and 0.9 to 5.6 ng/g dry weight. Catfish collected from Lake Mayer contained maximum concentrations (3.5-5.6 in liver and 1.2-2.7 in muscle) than other fish species collected from rivers, ponds, open oceans and estuarine ecosystem in Savannah. Concentrations of TCS in water and sediment from Savannah River (1.2-7.3 ng/L in water and 2.5-12 ng/g wet weight in sediment) and Ogeechee River was (2.1-9.3 ng/L in water and 4.8-16 ng/g wet weight in sediment). Concentrations of TCS in fish collected from both these rivers probably due to surrounding water and suspended sediment particles.

Comparisons

Observed concentrations were comparable to those to available literature[13]. For example, in Germany concentrations of TCS were limit of quantification to 3.4 ng/g wet weight. Another study revealed the presence of TCS in plasma of fish from a North American river at levels up to 10.4 ng/g[15]. During treatment process in wastewater treatment plants, TCS will be converted into methyl triclosan (MeTCS) by bacterial degradation. Therefore MeTCS found to ubiquitous and occurred more than TCS[13]. In aquatic biota, the presence of MeTCS was first reported for fish from the Tokyo Bay in Japan in 1984 where up to 38 ng/g MeTCS were detected (whole body basis)[16]. In Swiss lakes influenced by wastewater effluents the occurrence of MeTCS was observed[4] and levels of up to 35 ng/g MeTCS (wet weight basis) were detected in fish from these lakes[17]. Another study revealed the presence of TCS and MeTCS in plasma of fish from a North American river at levels of up to 10.4 ng/g and 0.0132 ng/g, respectively. Based on these findings it is assumed that MeTCS is more persistent than TCS[13]. Therefore study must be focused on e in fish tissues from various rivers and estuarine ecosystems in and around Savannah.

TCS Toxicity

There is evidence that TCS is acutely and chronically toxic to aquatic organisms. Research has shown that the presence of TCS may influence both the structure and the function of algal communities in stream[1 and 18]. TCS toxicity in different fish species also available in various literature that summarized in web pages[19]. Based on these studies concentrations of 3.0 to 1000 μg/L TCS in water may produce toxic effects depending upon species. In addition to aquatic toxicity, research suggests that TCS bio-accumulate in fish tissue. More briefly concentrations found in fish are thousands of times higher than what is found in the water column. Furthermore, at least one transformation product, MTCS, is relatively stable in the environment, making it also available for bioaccumulation. Thyroid hormones are critical for normal growth and development of humans. The developing brain of a child is particularly vulnerable to damage caused by disruption of the thyroid system. TCS may also disrupt other critical hormone systems. A recent lab study found the chemical to exert both estrogenic and androgenic effects on human breast cancer cells[20].

Table 1. Biometric details and concentration (ng/g dry wt.) of triclosan in fish samples analyed in this study.

Fish Species	Sampling Location	Scientific name	Weight (g)	Standard Length[e] (cm)	Moisture (%)[f] for L, M	Tissue Analyzed[c]	TCS in L	TCS in M
Sawtooth pen clam (n=2)	Sav-Wil Is Riv Mouth[b]	*Atrina serrata*	104.8	18.3	21	ST	0.6-0.9	
White shrimp (n=3)	Wassaw-Tybee Island	*Penaeus setiferus*	4.7-14.1	NM	29	ST	0.4-0.8	
White shrimp (n=4)	Sav-Wil Is Riv Mouth	*Penaeus setiferus*	4.4-31.9	8.9-17.3	23	ST	0.8-1.2	
Unidentified eel (n=1)	Pond Behind Savannah Mall		698	77.5	32, 27	L, M	3.3	1.2
Oyster toadfish (n=3)	St. Simons Island	*Opsanus tau*	103.5-334.9	18.3-25.2	40, 22	L, M	1.2-2.4	0.3-1.6
Oyster toadfish (n=1)	SKIO-Dock[d]	*Opsanus tau*	172.5	21.7	49, 23	L, M	1.7-4.4	0.5-1.8
Unidentified snapper (n=3)	Ogeechee River Richmond		62.6-79.5	16.1-18.1	30	M		0.6-0.7
Unidentified snapper (n=4)	Ogeechee River Richmond		59.2-62.6	14.8-15.3	26	M		0.4-0.8
Unidentified catfish (n=3)	Lake Mayer	family lutaluridae	916.3-965.2	39.5-42.7	63, 21	L, M	3.5-5.6	1.2-2.7
Unidentified catfish (n=8)	Ogeechee River Richmond	family lutaluridae	130.7-274.6	23.6-34.2	47, 23	L, M	1.1-2.6	0.7-1.4
Atlantic croaker (n=4)	St. Simons Island	*Micropogonias undulatus*	82.9-125.8	19.4-22.5	22	M		1.0-1.4
Southern kingfish (n=5)	St. Simons Island	*Mentichrrhus americanus*	118.7-169.6	21.4-28.9	21	L, M	2.7-3.4	0.3-1.6
Southern stingray (n=2)	SKIO-Dock	*Dasyatis americana*	777.87-819.8	69.7-74.1	24	L, M	2.1-2.8	0.8-1.4
Atlantic croacker (n=5)	SKIO-Dock	*Micropogonias undulatus*	91.1-96.8	19.5-21.1	23	M		0.4-1.7
Silver perch (n=9)	SKIO-Dock	*Bairdiells chrysoura*	65.1-72.5	17.4-18.6	30	M		1.0-1.7
Southern kingfish (n=3)	Wassaw-Tybee Island	*Mentichrrhus americanus*	52.1-86.3	17.3-20.8	21	M		0.3-0.7
Spot (n=3)	Wassaw-Tybee Island	*Leistomus xanthurus*	75.5-111.0	17.5-19.9	29	M		0.3-1.1
Snshore lizardfish (n=1)	Atlantic Ocean	*Synodus foetens*	390.8	41.4	41, 27	L, M	1.4-2.7	0.4-1.0
Tomtate (n=4)	Atlantic Ocean	*Haemulon aurolineatum*	79.2-220.1	19.2-26.5	37, 23	L, M	1.0-1.7	0.3-0.6
Sea robin (n=4)	Sav-Wil Is Riv Mouth	*Prionotus sp.*	127.6-133.3	20.1-22.4	23	M		0.3-1.1
Black sea bass (n=3)	SKIO-Dock	*Centroprinstis striata*	69.9-71.5	16.2-17.3	30	M		0.7-1.2
Large mouth bass (n=1)	Pond Behind Savannah Mall	*Micropterus salmoides*	352.7	31.3	22	M		1.5
ASNS[a] (n=5)	Wassaw-Tybee Island	*Rhizoprionodon terranovae*	NM	69.0-90.1	27-28	L, M	0.9-2.3	0.3-0.7
Bonnethead shark (n=3)	Wassaw-Tybee Island	*Sphyrna tiburo*	NM	82.3-97.1	27-29	L, M	1.2-1.9	0.3-0.5

[a]Atlantic sharp-nose shark, [b]Savannah Wilmington Island River Mouth, [c]ST=soft tissue, L=liver, M=muscle; [d]Skidaway Institute of Oceanography Dock, [e]length from head to tail, [f]moisture for pooled samples except sharks that analyzed individuals; NM=not measured

Studies of fish suggest that TCS may have weak androgenic[21] or anti-estrogenic effects[22], while a metabolite of TCS may have estrogenic effects[23].

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