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PERFLUOROALKYL CONTAMINANTS IN PLASMA OF FIVE SEA TURTLE SPECIES: COMPARISONS IN CONCENTRATION AND POTENTIAL HEALTH RISKS

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Abstract—The authors compared blood plasma concentrations of 13 perfluoroalkyl contaminants (PFCs) in five sea turtle species with differing trophic levels. Wild sea turtles were blood sampled from the southeastern region of the United States, and plasma was analyzed using liquid chromatography tandem mass spectrometry. Mean concentrations of perfluorooctane sulfonate (PFOS), the predominant PFC, increased with trophic level from herbivorous greens (2.41 ng/g), jellyfish-eating leatherbacks (3.95 ng/g), omnivorous loggerheads (6.47 ng/g), to crab-eating Kemp's ridleys (15.7 ng/g). However, spongivorous hawksbills had surprisingly high concentrations of PFOS (11.9 ng/g) and other PFCs based on their trophic level. These baseline concentrations of biomagnifying PFCs demonstrate interesting species and geographical differences. The measured PFOS concentrations were compared with concentrations known to cause toxic effects in laboratory animals, and estimated margins of safety (EMOS) were calculated. Small EMOS (<100), suggestive of potential risk of adverse health effects, were observed for all five sea turtle species for immunosuppression. Estimated margins of safety less than 100 were also observed for liver, thyroid, and neurobehavorial effects for the more highly exposed species. These baseline concentrations and the preliminary EMOS exercise provide a better understanding of the potential health risks of PFCs for conservation managers to protect these threatened and endangered species. Environ. Toxicol. Chem. 2012;31:1223–1230. © 2012 SETAC

Keywords—Marine turtles Reptiles Environmental pollutants Trophic magnification Persistent organic pollutants

INTRODUCTION

Perfluoroalkyl compounds (PFCs) have been used for many purposes, including stain-resistant coatings, fire-fighting foams, and emulsifiers to make fluoropolymer plastics [1,2]. Despite their usefulness, PFCs have become global pollutants, and exposure to them can produce lethal and sublethal toxic effects [1,2]. The most studied and commonly detected PFCs in the environment are perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). These substances have been detected in human and wildlife samples worldwide, including several species of seabirds and marine mammals [3,4] and have been shown to biomagnify in various food webs [4–6]. Two studies have previously reported PFCs in sea turtle samples, with interesting contrasts between species, as well as geographical and temporal differences in PFC concentrations [7,8]. Perfluorooctane sulfonate, the predominant PFC, was shown to be the organic pollutant at the highest concentration measured to date in sea turtle blood, even higher than total polychlorinated biphenyls [7]. Perfluorooctanoic acid was below detection limits in loggerhead sea turtles (*Caretta caretta*) captured at four sites along the Atlantic coast of the United States, but was detected in loggerhead turtles from Florida Bay, Florida [8]. Kemp's ridley sea turtles (*Lepidochelys kempii*) had higher PFC concentrations than loggerheads, suggesting that they select slightly different prey items found higher on the food web than other marine turtles studied [7]. A robust spatial and temporal trend analysis using loggerhead plasma found that PFOS increased in concentration with capture latitude from Cape Canaveral, Florida, in the south to the Chesapeake Bay (Virginia and Maryland) in the north. However, Florida Bay, which is south of Cape Canaveral, did not follow this pattern. Florida Bay loggerhead turtles generally had the highest or second highest PFC concentrations [8]. On a temporal scale, PFOS and perfluorononanoic acid (PFNA) have significantly declined in concentration in loggerhead turtles sampled annually near Charleston, South Carolina, from 2000 to 2008 [8].

Although sea turtles are highly migratory throughout their lives, older juveniles and adults appear to exhibit foraging site fidelity, returning to the same general site annually [9,10]. Therefore, sea turtles of these age classes may be good bioindicators of regional-scale contamination of their foraging grounds, thus reflecting the geographical differences seen by O'Connell et al. [8]. By comparing contaminant concentrations among different species of sea turtles, the biomagnification property of contaminants may be assessed because sea turtle species vary in trophic status [11]. Musick and Limpus [11] reviewed what is known about the dietary habits of these species. Herbivorous green turtles (*Chelonia mydas*) feed mostly on sea grasses and algae. Hawksbill sea turtles (*Eretmochelys imbreicata*) feed primarily on coral reef sponges, and leatherbacks (*Dermochelys coriacea*) eat mostly gelatinous zooplankton. Loggerheads are opportunistic carnivores feeding on an assortment of crabs, mollusks, and gelatinous zooplankton, and the Kemp's ridleys forage primarily on various crabs and, to a lesser degree, mollusks.

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Baseline concentrations of PFCs are vastly unknown for most sea turtle species throughout their large ranges, making understanding potential health risks from this threat difficult. As our primary objective, the present study is the first to report PFC concentrations in green, leatherback, and hawksbill sea turtles and to compare biomagnifying PFC concentrations across five sea turtle species. Based on their known prey items and assumed trophic status, we hypothesized that PFC concentrations would be lowest in green turtles, followed by increasingly higher levels in hawksbill, leatherback, loggerhead, and Kemp's ridley turtles. Our hypothesis is further justified by previous studies that have compared concentrations of other biomagnifying persistent organic contaminants, such as polychlorinated biphenyls, in adipose tissue among four sea turtle species [12]. Results of these comparisons coincided with trophic status, revealing that green turtles indeed had the lowest concentrations, followed by leatherbacks, loggerheads, and Kemp's ridleys.

A second objective of the present study was to compare the measured PFC concentrations in the sea turtles with concentrations that are known to cause toxicity in laboratory-exposed animals. Estimated margins of safety (EMOS) were calculated to better understand the potential health risks of PFCs to help resource managers protect these threatened and endangered species.

MATERIALS AND METHODS

Sampling

Juvenile Kemp's ridley ($n = 10$), loggerhead ($n = 15$), and green ($n = 10$) sea turtles were captured incidentally in a pound net fishery located in Core Sound, NC, from June to October, 2006. Juvenile hawksbills ($n = 5$) were hand-captured on a reef offshore of Juno Beach (Florida, USA) during June to August 2006. Adult female leatherbacks ($n = 7$) were sampled during nesting in April and May 2007 on Juno Beach. Turtles were tagged, measured for carapace length, and sampled for blood. Heparinized blood was centrifuged on the day of collection, and plasma was removed and stored at −80°C. All juvenile turtles had straight carapace lengths ranging from 25 to 70 cm, and all samples were selected from warmer months to reduce the chance of including transient, seasonally migrating turtles.

Extraction and PFC quantification

Samples were analyzed for perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), PFOA, PFNA, perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnA), perfluorododecanoate (PFDoA), perfluorotridecanoate (PFTriA), perfluorotetradecanoate (PFTA), perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), PFOS, and perfluorooctane sulfonamide (PFOSA), according to the methods in O'Connell et al. [8] and Keller et al. [13]. Briefly, an internal standard solution (consisting of $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFDoA, $^{18}O_2$-PFBS, $^{13}C_4$-PFOS, and $^{18}O_2$-PFOSA) was added gravimetrically to 0.15 ml or 1.2 ml plasma, followed by the addition of formic acid in water (50% volume fraction). Samples were loaded onto 3-cm^3, 60-mg Oasis WAX solid-phase extraction columns (Waters Corp.) using a RapidTrace solid-phase extraction module (Caliper Life Sciences), and after column washing steps, PFCs were eluted with 1% (volume fraction) ammonium hydroxide in methanol. Extracts were filtered, evaporated, and amended with $^{13}C_2$-PFOA as a recovery standard.

Procedural blanks were tested in triplicate. These blanks consisted of Millipore water that had been processed through unused blood collection needles and tubes of the same or similar lots as used during sample collection. National Institute of Standards and Technology, Standard Reference Material 1957 Organic Contaminants in Non-Fortified Human Serum [13], as well as an in-house reference material of pooled loggerhead turtle plasma ("Cc pool"), were also analyzed alongside the samples for method quality assurance. A six-point calibration dilution series was used for quantification, and these calibration solutions were processed through all extraction steps alongside samples of each batch.

Samples were injected (20 μl) onto a Thermo Betasil C8 column (100 mm × 2.1 mm × 5 μm) and quantified using an Agilent 1100 high-performance liquid chromatography interfaced to an Applied Biosystem negative electrospray ionization tandem mass spectrometer. The solvent gradient consisted of methanol and 20 mmol/L ammonium acetate in water. Perfluoroalkyl compounds were quantified using a relative response ratio to an internal standard compound that most closely matched the compound. Linear and branched isomers of each compound were integrated together as a single peak. The reporting limit was determined as the maximum of either: (1) the lowest detectable calibration solution (ng) per gram of extracted sample or (2) the average nanograms measured in three blanks plus three times the standard deviation of the blanks, all divided by the mass (g) of the extracted sample.

Statistical methods

Statistical tests were performed as suggested by Helsel [14], using the R NADA package for handling data with nondetects without needing to substitute nondetects with an arbitrary value. Kaplan-Meier or regression on order models were chosen to estimate central tendency and variability based on recommendations by Helsel [14]. A censored Kruskal-Wallis test followed by pairwise censored Wilcoxon tests were used to determine species differences in PFC concentrations after Bonferroni adjustment to the alpha value ($p < 0.005$ was considered significant).

Estimated margins of safety

Margin of safety is routinely calculated as the ratio of the no observed adverse effect level in laboratory-exposed animals (such as mice and rats) to the exposure level of the animal of concern (usually humans; in this case, sea turtles), and a value under 100 suggests potential risk of toxic effects in the animal of concern [15]. Because no observed adverse effect levels were rarely available from PFOS toxicity studies, we calculated an EMOS as the ratio of the average plasma or serum PFOS concentration in laboratory-exposed animals in the lowest observed adverse effect level (LOAEL) dose group to either the average or maximum sea turtle plasma PFOS concentration. From our literature review, we selected PFOS toxicity studies that resulted in the two lowest of the LOAELs for effects on the liver, the primary target of PFOS toxicity, as well as more sensitive physiological systems of the thyroid, neurobehavior, and immune functions.

RESULTS

The PFC concentrations were in the low nanogram per gram wet mass range in the plasma, and species differences were demonstrated for PFOS and other compounds (Table 1). Perfluorooctane sulfonate was detected in all samples and was the

Table 1. Perfluoroalkyl contaminant concentrations (pg/g wet mass) in plasma of five sea turtle species[a]

Species statistic	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTriA	PFBS	PFHxS	PFOS	PFOSA
Green											
Mean	<RL	<RL	<RL	196	342	151	586	92.1	60.0	2,410	17.6
(SD)				(117)	(203)	(71)	(561)	(267)	(43.7)	(1120)	(3.4)
Median	<RL AB	<RL A	<RL A	160 A	293 A	140 AC	390 A	0.0547	30.0 AB	2,370 A	17.0 B
Range	<10–170	<70–<9,060	<70–<182	<90–377	156–845	<90–262	<140–2,080	<20–846	18.0–111	871–3,870	<10–21.3
% >RL	10	0	10	40	50	40	50	20	90	100	50
Leatherback											
Mean	<RL	<RL	273	320	447	106	849	<RL	17.1	3,950	157
(SD)			(204)	(206)	(294)	(63)	(334)		(4.9)	(2,510)	(152)
Median	<RL A	<RL A	184 A	190 A	470 A	110 A	890 AB	<RL	20.0 A	4,420 AB	92.0 C
Range	<20–70	<80–110	<170–648	<90–670	<60–843	<40–208	<170–1,200	<20–<175	14.5–22.6	884–7,830	15.5–435
% >RL	14	0	29	57	86	71	86	0	100	100	100
Loggerhead[b]											
Mean	<RL	<RL	1,340	501	674	203	715	20.3	136	6,470	10.4
(SD)			(3,680)	(602)	(754)	(452)	(508)	(42.9)	(106)	(7,520)	(5.6)
Median	<RL A	<RL A	240 A	220 A	340 A	70.0 A	630 A	1.9	110 B	3,130 AB	9.18 A
Range	<20–<690	<90–<9,250	<180–1,440	<90–2,300	<60–2,800	<40–1,760	<280–2,170	<10–126	16.1–317	305–26,500	<10–25.5
% >RL	0	0	66	73	80	53	73	20	100	100	20
Kemp's ridley											
Mean	<RL	<RL	2,470	2,070	2,240	509	1,860	22.5	597	15,700	15.4
(SD)			(1,600)	(965)	(1,260)	(338)	(909)	(16.6)	(378)	(9,860)	(5.5)
Median	<RL A	<RL A	2,180 B	1,910 B	1,740 B	380 BC	1,720 B	17.7	505 C	10,800 C	17.0 AB
Range	<20–<690	<540–188	539–5,370	1,010–3,860	981–4,930	230–1,090	538–3,600	<10–55.8	146–1,310	6,850–35,000	9.89-23.3
% >RL	0	10	100	100	100	50	100	30	100	100	50
Hawksbill											
Mean	120	3,780	1,730	4,400	4,790	400	620	40.9	724	11,900	11.2
(SD)	(91)	(3,630)	(1,210)	(789)	(2,180)	(82)	(352)	(58.8)	(486)	(6,270)	(3.3)
Median	100 B	1,840 B	17,000 C	4,130 C	3,810 C	390 B	370 A	7.87	550 C	11,900 BC	11.0 ABC
Range	30–220	1,070–9,730	3,870–30,800	3,560–5,380	3,570–8,660	300–522	358–1,030	<20–139	258–1,460	5,450–21,200	6.96–15.5
% >RL	100	100	100	100	100	100	100	40	100	100	100

[a] Different letters after the median values indicate significant differences among species for that compound.

[b] Loggerhead samples reported here were included in samples reported in O'Connell et al. [8].

PFHpA = perfluoroheptanoate; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFDA = perfluorodecanoate; PFUnA = perfluoroundecanoate; PFDoA = perfluorododecanoate; PFTriA = perfluorotridecanoate; PFBS = perfluorobutane sulfonate; PFHxS = perfluorohexane sulfonate; PFOS = perfluorooctane sulfonate; PFOSA = perfluorooctane sulfonamide; SD = standard deviation; % >RL = percent of samples above the reporting limit.

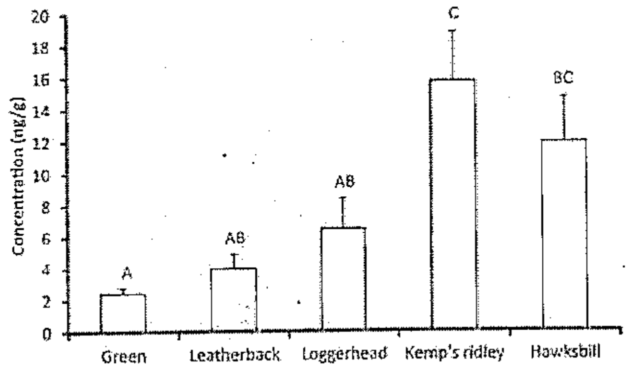

Fig. 1. Perfluorooctane sulfonate (PFOS) plasma concentrations (mean with standard error) in plasma of five sea turtle species. Different letters above bars indicate statistically significant differences among species ($p < 0.005$).

predominant PFC measured in all species except hawksbills. For PFOS, Kemp's ridleys had the highest average concentrations, followed by hawksbills, loggerheads, leatherbacks, and greens (Fig. 1). Of the perfluorocarboxylic acids, PFNA, PFDA, PFUnA, and PFTriA were the most abundant. For PFNA, PFDA, and PFUnA, the species rank from highest to lowest was hawksbill, Kemp's ridley, loggerhead, leatherback, and green. The concentration of PFTriA was highest in Kemp's ridleys followed by loggerhead, leatherback, hawksbill, and then green turtles. Perfluorohexanoate and PFTA were not detected in any samples (data not shown). Perfluorooctanoic acid was detected in all hawksbill samples and only 10% of the Kemp's ridleys; it was not detected in the other species. The PFOA concentrations in the hawksbills and Kemp's ridleys were three- to fivefold lower than PFOS concentrations. Perfluorobutane sulfonate was detected in only a few samples from each species, with the exception of leatherbacks, for which it was nondetectable. Most of the detectable values of PFBS (0.02–0.14 ng/g) ranged from just above the reporting limit to three times the reporting limit, except for one green turtle with a PFBS concentration of 0.85 ng/g. This outlier resulted in green turtles having the highest average PFBS concentration compared with the other species; however, the median was lower than that of the Kemp's ridley as expected. Perfluorooctane sulfonamide was significantly higher in the leatherbacks compared with all other species examined ($p < 0.005$).

The PFC patterns differed between species (Fig. 2). Perfluorooctane sulfonate was predominant in all species except for the hawksbills. Hawksbills were distinguished from the other species because of their higher proportions of many perfluorocarboxylic acids, such as PFOA, PFNA, and PFDA.

To calculate estimated margins of safety for sea turtles, we first reviewed the literature for studies that assessed the toxicity of PFOS on the liver, thyroid, neurobehavioral, and immune systems in laboratory-exposed animals. Studies demonstrating the two lowest of the lowest observed adverse effect levels for each biological system were compiled and reviewed in Table 2. Studies that included serum/plasma PFOS concentrations measured in the animals of the LOAEL test group were selected. From this literature comparison, it was apparent that immunosuppression was the most sensitive toxic effect of PFOS compared to liver, thyroid, and neurobehavioral effects.

Estimated margins of safety for the five sea turtle species (Table 3) provide a preliminarily assessment of potential health risks of their exposure to PFOS in the wild. Smaller EMOS values (<100) indicate that sea turtle exposure to PFOS is similar to concentrations that are known to cause a toxic effect in laboratory animals. Estimated margins of safety values less than 1 indicate that sea turtles are exposed to concentrations above the threshold known to cause toxic effects. The EMOS for the sea turtle exposed to average PFOS in the current study ranged from 35,270 for green sea turtle risk of liver toxicity to

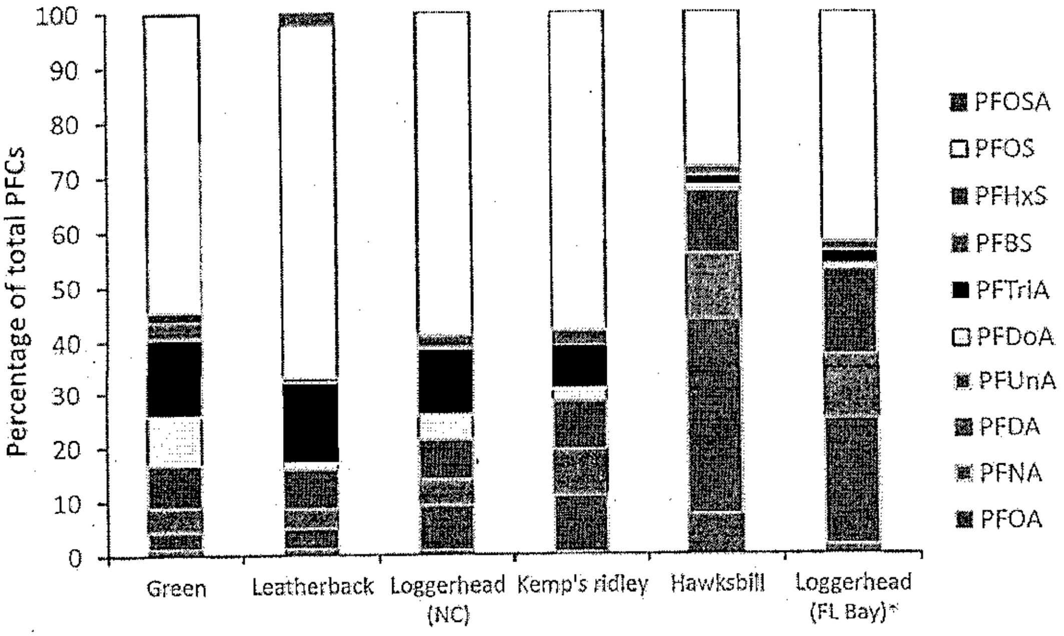

Fig. 2. Perfluoroalkyl contaminant patterns in plasma of five sea turtle species. PFOSA = perfluorooctane sulfonamide; PFOS = perfluorooctane sulfonate; PFHxS = perfluorohexane sulfonate; PFBS = perfluorobutane sulfonate; PFTriA = perfluorotridecanoate; PFDoA = perfluorododecanoate; PFUnA = perfluoroundecanoate; PFDA = perfluorodecanoate; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; NC = North Carolina; FL = Florida. * Data from loggerhead sea turtles captured in Florida Bay, shown for comparison with the Juno Beach hawksbills, were taken from O'Connell et al. [8].

Table 2. Review of perfluorooctane sulfonate toxicity studies resulting in the lowest of the lowest observed adverse effect levels

System	Test species/strain	Age/sex of dosed subjects	Route (duration)	LOAEL dose	Age/sex of LOAEL test subject	Adverse effect	Mean serum PFOS at LOAEL (ng/g or ng/mL)	Reference
Liver	Sprague-Dawley rat	Adult F	Gavage (21 d)	3 mg/kg bw/d	Perinatal fetus M and F	↑ neonate mortality, ↑ hepatocellular hypertrophy; altered hepatic gene expression (for peroxisome proliferation, fatty acid activation and biosynthesis, bile acid synthesis, etc.)	85,000^{a}	[24,25]
Liver	Sprague-Dawley rat	Adult M and F	Dietary (28 d)	2 mg/kg diet	Adult F	↑ liver:body weight	1,500	[26]
Thyroid	Sprague-Dawley rat	M	Drinking water (91 d)	1.7 mg/L water	M	↓Total T4; ↑ Total T3	5,000	[27]
Thyroid	Wistar rat	Adult F	Dietary (GD1-PND14)	3.2 mg/kg diet	Adult F at PND1	↓Total T4	2,290	[28]
Neurobehavioral	Sprague-Dawley rat	Adult F	Gavage (GD0-PND20)	0.3 mg/kg bw/d	M pups at PND17	↑ Motor activity.	5,048^{b}	[29,30]
Neurobehavioral	Zebrafish	Embryos	Immersion in water (6–120 hpf)	1.0 mg/L water	Embryos at 120 hpf	Altered development of motor neurons	1,000^{c}	[31]
Immune	B6C3F1 mouse	F	Gavage (21 d)	0.025 mg/kg bw/d	F	↓ Survival to influenza A virus	670^{d}	[32]
Immune	B6C3F1 mouse	Adult M and F	Gavage (28 d)	0.00166 mg/kg bw/d	Adult M	↓Plaque-forming cell response (T-cell dependent IgM antibody response)	91.5	[33]

[a] Fetal serum PFOS concentration was determined by Lau et al. [25] using a different set of female rats that were exposed to the same dose and duration as in Bjork et al. [24].

[b] Male pup serum PFOS concentration was determined in Chang et al. [30] at PND21, similar to the timing of the LOAEL at PND17.

[c] This is not the internal serum concentration, but rather the embryos' water exposure concentration.

[d] Mean PFOS concentration in plasma.

LOAEL = lowest observed adverse effect level; PFOS = perfluorooctane sulfonate; F = female; M = male; GD = gestational day; PND = postnatal day; hpf = hours post-fertilization; bw = body weight; T4 = thyroxine; T3 = triiodothyronine.

5.8 for Kemp's ridley sea turtle risk of immunotoxicity. For the maximally exposed sea turtles, EMOS ranged from 21,964 for green sea turtle risk of liver toxicity to 2.6 for Kemp's ridley sea turtle risk of immunotoxicity. The EMOS for Kemp's ridley and loggerhead sea turtles sampled in 2006 of the present study were higher than the EMOS for turtles sampled in 2003 (when PFOS concentrations were higher [7]), indicating a declining risk of toxicity as the concentrations decline in these populations. Margins of safety under 100 are considered by regulatory agencies to be suggestive of potential risk to toxic effects [15]. Using these criteria, maximally exposed hawksbill, Kemp's ridley, and loggerhead sea turtles are at risk of liver and neurobehavioral toxicity from PFOS exposure at the 2006 level. Likewise, Kemp's ridley and loggerhead sea turtles are at risk of thyroid disruption, and all sea turtle species, even herbivorous greens, at average PFOS exposure are at risk of

Table 3. Estimated margin of safety for average/maximum sea turtle exposure to perfluorooctane sulfonate based on effects previously seen in lab-exposed animals^{a}

Adverse effect in lab-exposed animals [previous study]	Hawksbill	Kemp's ridley (2006)	Kemp's ridley (2003)^{b}	Loggerhead (2006)	Loggerhead (2003)^{b}	Leatherback	Green
Neonate mortality, altered liver histology and gene expression [24,25]	7,142/4,009	5,414/2,429	2,157/1,412	27,157/3,208	7,727/878	21,519/10,856	35,270/2,1964
Increased liver weight [26]	126/71^{c}	96^{c}/43^{c}	38^{c}/25^{c}	479/57^{c}	136/16^{c}	380/192	622/388
Altered thyroid hormones [27]	420/236	319/143	127/83^{c}	1,597/189	455/52^{c}	1,266/639	2,075/1,292
Altered thyroid hormones [28]	192/108	146/65^{c}	58^{c}/38^{c}	732/86^{c}	208/24^{c}	580/292	950/592
Increased motor activity [29,30]	424/238	322/144	128/84^{c}	1612/190	459/52^{c}	1,278/645	2,095/1,304
Altered development of motor neurons [31]	84^{c}/47^{c}	64^{c}/29^{c}	25^{c}/17^{c}	320/38^{c}	91^{c}/10^{c}	253/128	415/258
Decreased survival to influenza A virus (immunosuppression) [32]	56^{c}/31^{c}	43^{c}/19^{c}	17^{c}/11^{c}	214/25^{c}	61^{c}/6.9^{c}	169/86^{c}	278/173
Decreased T-cell dependent IgM antibody response (immunosuppression) [33]	7.7^{c}/4.3^{c}	5.8^{c}/2.6^{c}	2.3^{c}/1.5^{c}	29^{c}/3.5^{c}	8.3^{c}/0.95^{c}	23^{c}/12^{c}	38^{c}/24^{c}

[a] Estimated margins of safety were calculated as the ratio of the serum/plasma PFOS concentration at the LOAEL of test subjects to either the average or the maximum plasma PFOS concentration measured in sea turtles.

[b] Estimated margins of safety were calculated for Kemp's ridley and loggerhead sea turtles sampled in 2003 based on mean and maximum PFOS concentrations reported in Keller et al. [7].

[c] Estimated margins of safety <100, which is suggestive of potential health risks.

immunotoxicity. In fact, the maximally exposed loggerhead turtle sampled in 2003 had an EMOS of 0.95, which means that it had accumulated a PFOS concentration higher than the laboratory-exposed mice showing immunosuppression.

DISCUSSION

We hypothesized that PFC concentrations would be highest in Kemp's ridley turtles, followed by sequentially lower levels in loggerhead, leatherback, hawksbill, and green turtles. As expected, Kemp's ridley turtles had the highest average PFOS concentration, followed unexpectedly by hawksbills, and then loggerheads, leatherbacks, and greens. This order follows the putative trophic status of each species except for the hawksbill. Hawksbill turtles, based on their trophic level of eating filter-feeding sponges, were expected to have lower PFOS levels than loggerheads and similar or lower levels than leatherbacks. Although sponges are known to contain unique natural chemicals, many of which are halogenated [16], PFOS and the other PFCs are anthropogenic [1]. One possible explanation for the unexpected species differences may have to do with a different capture location for the hawksbills. Our goal was to eliminate all confounding factors, but a perfect sampling design that includes five endangered and threatened species all from one location and one age class was not possible. Few to no hawksbills inhabit North Carolina waters, where loggerheads, Kemp's ridleys, and greens are routinely sampled. Likewise, we wanted to sample juvenile leatherbacks; however, no congregating areas where juveniles of this species may be easily sampled are known, so nesting adult female leatherbacks were the only available samples. Juno Beach, where the hawksbills were captured and observed foraging, may have different contamination levels than other regions. Geographical differences in PFC concentrations along the Atlantic coastline of the United States have previously been observed in loggerhead sea turtles [8]. Interestingly, Juno Beach is approximately half the distance between Cape Canaveral (the site with the lowest loggerhead PFC concentrations) and Florida Bay (one of the sites with the highest loggerhead PFC concentrations). The hawksbills at Juno Beach had a PFC pattern more similar to that of the loggerheads in Florida Bay [8] than any other sea turtle species or location (Fig. 2). This could be attributable to a different source of PFC contamination in southern Florida than the other locations. Correspondingly, just south of Cape Canaveral is the delineation between two marine ecoregions [17], which groups Juno Beach and Florida Bay into the more tropical South Florida/Bahamian Atlantic ecoregion. Core Sound in North Carolina is in the temperate Virginian Atlantic marine ecoregion. Differences in the biogeography and currents within these ecoregions may play a role in different fate, transport, and accumulation of PFCs.

Differences in hawksbill PFC levels compared with the other species along with their unexpectedly high concentrations may relate with their foraging location, but there may be other undetermined explanations. Few hawksbill samples have ever been measured for other persistent organic pollutants; however, two studies suggest that hawksbill turtles bioaccumulate higher persistent organic pollutant levels than other sea turtle species in the same location. One hawksbill blood sample from Australia had higher polybrominated diphenylether concentrations than green turtle blood [18], and three hawksbill liver samples from Japan had higher concentrations of polychlorinated biphenyls and organochlorine pesticides than samples from greens and loggerheads [19]. Even more interesting is that four hawksbills from the eastern Pacific Ocean had higher trophic stable isotope signatures ($\delta^{15}N$) than four other species from that region (J. Seminoff, National Oceanic and Atmospheric Administration, La Jolla, California, personal communication). Both the recent contaminants and stable isotope results are unexpected and difficult to explain based on the relatively low expected trophic level of hawksbills and highlight the need for future studies to focus on food webs that include marine sponges.

As mentioned, the leatherback samples were from adult turtles, whereas the other species were juveniles. Additionally, leatherbacks were sampled a year later than all other species. Recently, Stewart et al. [20] showed that nesting leatherback turtles transfer a portion of their accumulated persistent organic pollutant burden into eggs. This finding, in conjunction with previous studies showing transfer of PFOS specifically into bird eggs [21], suggests that the leatherback females used in this study may have levels lower than a juvenile population because of reproductive offloading. Despite the age difference, the leatherback turtles ranked as expected within the species trophic levels for PFC concentrations. Regardless of potential confounding factors, this is the first study to measure PFC contaminants in leatherback, hawksbill, and green turtles.

In this study, we can confidently compare the Kemp's ridley, loggerhead, and green sea turtles with each other because they were all juveniles and all captured at the same location and time. Our results demonstrate that the concentrations of the five most abundant PFCs (PFOS, PFNA, PFDA, PFUnA, and PFTriA) in these turtles do indeed rank as expected to their different trophic levels (Table 1). Our findings also support previous studies that showed PFC concentrations to be significantly higher in Kemp's ridleys than in loggerheads ($p < 0.05$) [7] and that persistent organic pollutants are observed in decreasing concentrations beginning with Kemp's ridley, loggerhead, leatherback, and then green turtles [12]. Thus, we confirm that these five PFCs biomagnify up marine food webs, and by comparing PFCs in sea turtle species at different trophic levels, we may have a useful tool for examining biomagnification of environmental contaminants.

Additional interesting results were seen when comparing the species for PFC patterns. Perfluorooctanoic acid was not detected in most sea turtle species from most locations, however, it is a common PFC measured in human plasma [3] and is frequently detected in fish and marine mammals [3], but most often at concentrations lower than PFOS. The lack of PFOA in most turtles suggests that they are either less exposed to this compound or they have an efficient pathway to eliminate this compound from their blood. The only sea turtle samples with consistently detected concentrations of PFOA were the hawksbills from Juno Beach in the present study and the loggerheads from Florida Bay that were measured previously [8]. The high proportion of PFOA (and other perfluorocarboxylic acids) compared with PFOS in turtles from these sites (Fig. 2) suggests that the contamination profile in southern Florida is different from that of the rest of the Atlantic coast of the United States. However, the leatherbacks sampled at Juno Beach showed a PFC profile that was more similar to that of the turtles from North Carolina (Fig. 2). This finding may be explained because adult leatherbacks accumulate PFCs from their foraging grounds, which are located far from the nesting grounds. Satellite telemetry data from leatherbacks nesting in southeastern Florida show that most of them forage in coastal areas along northeast Florida to New Jersey, or return to these areas after an oceanic voyage [22]. Thus, adult leatherbacks are exposed to a PFC profile more similar to that of the turtle species sampled in North Carolina and different from that of the hawksbills that were foraging directly off of Juno Beach.

The green turtles had concentrations of PFBS many times higher than concentrations in the other species, but this result was driven by one elevated outlier. Detection of this compound in this turtle is particularly surprising because not only does the green turtle have the lowest trophic status, but PFBS is also known to have a very low bioaccumulation factor compared with longer-chain PFCs [6]. Although this one detection is unusual, PFBS has been detected in human plasma, particularly after occupational exposure [23]. Perfluorobutane sulfonate detection in wildlife may become more common as environmental concentrations of this more recently manufactured compound increase. Concentrations of PFBS might be expected to increase because of the replacement of PFOS-related chemistry with PFBS-related chemistry around 2002.

Toxicity testing of PFCs has focused largely on only two compounds (PFOS and PFOA) and on mammals rather than reptiles. Because of these limitations, the EMOS calculated in this study should be considered a cursory exercise to only begin understanding the potential health risks of PFCs to sea turtle populations. Aside from these limitations, the comparison suggests that individual turtles with average or higher PFOS exposure of all sea turtle species sampled have a potential risk of immunosuppressive effects of PFOS (EMOS < 100). In addition, species that accumulate higher concentrations, such as the hawksbill, Kemp's ridley, and loggerhead sea turtles, have potential risks of enlarged livers, thyroid disruption, and neurobehavioral changes because of exposure to PFOS. Because PFOS concentrations are declining in loggerhead sea turtles from the southeastern region of the United States [8], their risk of health effects may also be declining, as shown by the increasing EMOS from 2003 to 2006 for both loggerhead and Kemp's ridley sea turtles. The risks posed by PFCs other than PFOS or those in combination with other environmental pollutants are still unknown, as are the risks to sea turtle populations from other regions, species, or age classes that remain to be analyzed.

In conclusion, this study provides the first baseline concentrations of PFCs for three sea turtle species (leatherback, hawksbill, and green sea turtle) and provides additional measurements for two other sea turtle species (Kemp's ridley and loggerhead). Although different capture locations and age classes may impact the accumulation of PFC, we observed an increasing trend in PFC concentrations in relation to the trophic status of each turtle species, apart from hawksbills. The estimated margins of safety indicated that some sea turtles inhabiting the southeastern United States are exposed to PFOS at levels that could have the potential to cause health effects; however, one must consider this exercise preliminary and realize that the comparisons are being extrapolated from mammals to reptiles. With more research, we may discover that reptiles are less or more sensitive than mammals to PFC toxic effects. This work provides direction for future research into chemical biomagnification in sea turtles as well as highlights the need for a better understanding of chemical fate, metabolic pathways, and toxicological consequences of these compounds in these endangered and threatened species.

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