INTRODUCTION

Persistent organochlorine pollutants (POPs) were associated with adverse effects on aquatic organisms as early as the 1940s [1]. Characterized by hydrophobicity and low volatility, most POPs are halogenated hydrocarbons that bioaccumulate and biomagnify, particularly at higher trophic levels. These widely distributed contaminants were first described in marine mammals during the 1960s, provoking a global assessment of burdens and toxicity. Although POP concentrations in marine mammal populations have decreased in some regions of the world [2,3], extremely recalcitrant POPs, such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs; including DDTs, chlordane, and mirex), and chlorinated monoterpenes (toxaphene) were measured using gas chromatography with electron-capture detection and gas chromatography with electron-capture negative ion mass spectrometry (GC-ECNI-MS) in blubber of free-ranging and stranded bottlenose dolphins (Tursiops truncatus). Mean total PCBs (78.6 ± 32.4 μg/g lipid) and toxaphene (11.7 ± 9.3 μg/g lipid) were significantly higher in dolphins sampled in the TBRE than in dolphins stranded near Savannah (GA, USA) 80 to 100 km to the north. Levels of OCPs were several-fold lower than levels of PCBs; moreover, PCBs comprised 81 and 67% of the total POP burden in TBRE and non-TBRE dolphins, respectively. Analyses with GC-ECNI-MS revealed that 2,2,5-endos,endo-endo,8,8,9,10-octachloroborneane (P-42a), a major component in technical toxaphene and a major residue congener in local estuarine fish species, was the most abundant chloroborneale in both sets of blubber samples. Mean total POP concentrations (sum of PCBs, OCPs, and toxaphene) approached 100 μg/g lipid for the TBRE animals, well above published total PCB thresholds at which immunosuppression and/or reproductive anomalies are thought to occur. These results indicate extended utilization of the highly contaminated TBRE as habitat for a group of coastal estuarine dolphins, and they further suggest that these animals may be at risk because of elevated POP concentrations.

Keywords—Tursiops truncatus Toxaphene Persistent organochlorine pollutants Polychlorinated biphenyls Bottlenose dolphins

ERIN L. PULSTER,†‡ KELLY L. SMALLING,‡ ERIC ZOLMAN,§ LORI SCHWACKE,|| and KEITH A. MARUYA*#
†Marine Sciences Department, Savannah State University, Savannah, Georgia 31404, USA
‡Skidaway Institute of Oceanography, 10 Ocean Science Circle, Savannah, Georgia 31411, USA
§National Oceanic and Atmospheric Administration, National Ocean Service, Center for Coastal Environmental Health and Biomolecular Research, 219 Fort Johnson Road, Charleston, South Carolina 29412-9110, USA
||National Oceanic and Atmospheric Administration, National Ocean Service, Hollings Marine Laboratory, 331 Fort Johnson Road, Charleston, South Carolina 29412, USA
*#Southern California Coastal Water Research Project, 3535 Harbor Boulevard Suite 110, Costa Mesa, California, 92626, USA

PERSISTENT ORGANOCHLORINE POLLUTANTS AND TOXAPHENE CONGENER PROFILES IN BOTTLENOSE DOLPHINS (TURSIOPS TRUNCATUS) FREQUENTING THE TURTLE/BRUNSWICK RIVER ESTUARY, GEORGIA, USA

Abstract—Although the Turtle/Brunswick River Estuary (TBRE) in coastal Georgia (USA) is severely contaminated by persistent organochlorine pollutants (POPs), little information regarding POPs in higher-trophic-level biota in this system is available. In the present study, polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs; including DDTs, chlordane, and mirex), and chlorinated monoterpenes (toxaphene) were measured using gas chromatography with electron-capture detection and gas chromatography with electron-capture negative ion mass spectrometry (GC-ECNI-MS) in blubber of free-ranging and stranded bottlenose dolphins (Tursiops truncatus). Mean total PCBs (78.6 ± 32.4 μg/g lipid) and toxaphene (11.7 ± 9.3 μg/g lipid) were significantly higher in dolphins sampled in the TBRE than in dolphins stranded near Savannah (GA, USA) 80 to 100 km to the north. Levels of OCPs were several-fold lower than levels of PCBs; moreover, PCBs comprised 81 and 67% of the total POP burden in TBRE and non-TBRE dolphins, respectively. Analyses with GC-ECNI-MS revealed that 2,2,5-endos,endo-endo,8,8,9,10-octachloroborneane (P-42a), a major component in technical toxaphene and a major residue congener in local estuarine fish species, was the most abundant chloroborneale in both sets of blubber samples. Mean total POP concentrations (sum of PCBs, OCPs, and toxaphene) approached 100 μg/g lipid for the TBRE animals, well above published total PCB thresholds at which immunosuppression and/or reproductive anomalies are thought to occur. These results indicate extended utilization of the highly contaminated TBRE as habitat for a group of coastal estuarine dolphins, and they further suggest that these animals may be at risk because of elevated POP concentrations.

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INTRODUCTION

Persistent organochlorine pollutants (POPs) were associated with adverse effects on aquatic organisms as early as the 1940s [1]. Characterized by hydrophobicity and low volatility, most POPs are halogenated hydrocarbons that bioaccumulate and biomagnify, particularly at higher trophic levels. These widely distributed contaminants were first described in marine mammals during the 1960s, provoking a global assessment of burdens and toxicity. Although POP concentrations in marine mammal populations have decreased in some regions of the world [2,3], extremely recalcitrant POPs, such as polychlorinated biphenyls (PCBs), DDT, or chlorinated monoterpenes (toxaphene), remain elevated in some areas [4].

Because of their association with densely populated areas, elevated levels of POPs are common in estuaries and coastal marine environments [5], which are essential habitats for resident populations of odontocetes like bottlenose dolphins (Tursiops truncatus) [6]. The severity of exposure and potential for deleterious effects from POPs in these environments depends on several factors, including the physicochemical properties of parent compounds and their transformation products, total mass loading and input duration, hydrodynamic considerations (e.g., flushing time), predator–prey relationships, and sensitivity of target species [7]. For year-round residents of the mid-Atlantic U.S. coast, the biomagnification potential of POPs may be increased by a diet comprised primarily of locally abundant fish species [6,8], limited POP biotransformation capacity [9], and in particular, long-term fidelity to specific, highly contaminated tidal estuaries [6,10]. If high enough, the biomagnification potential of POPs for such animals may lead to exceedence of threshold concentrations associated with toxic effects.

Historically, Brunswick (GA, USA) is one of the most contaminated coastal areas in the United States, with several Superfund sites and numerous other hazardous waste sites. Most notable among these are former chloralkali and toxaphene manufacturing facilities, the discharges of which have impacted the surrounding Turtle/Brunswick River estuary (TBRE; GA, USA). Unique within the region and, possibly, across the globe, PCB contamination in the TBRE is largely attributable to Aroclor 1268, a highly chlorinated formulation used at the LCP Chemicals plant (Brunswick, GA, USA) between 1955 and 1994 [11]. Unlike Cl2–Cl10 PCB congeners that are ubiquitous in the environment, the highly chlorinated Cl2–Cl10 homologues in Aroclor 1268 dominate PCB profiles in sediment and biota of the TBRE [11–13]. Nearby, the Hercules
Fig. 1. Locations of free-ranging bottlenose dolphins (Tursiops truncatus) biopsied in the Turtle/Brunswick River estuary (St. Simons Sound, GA, USA) and stranded T. truncatus in estuaries near Savannah (GA, USA).

The objective of the present study was to measure and compare concentrations of legacy POPs, including PCB congeners; organochlorine pesticides (OCPs), such as DDTs, chlordanes, and mirex; and chlorinated monoterpenes (toxaphene) in the blubber of free-ranging and stranded T. truncatus frequenting the highly contaminated TBRE and other nearby coastal Georgia estuaries. Our second goal was to compare and contrast congener profiles by POP class, particularly for toxaphene. Finally, we compared levels both to those reported from around the globe and to estimated threshold levels associated with adverse effects to evaluate toxicological implications associated with our observations.

MATERIALS AND METHODS

Sample collection

In December 2004, blubber samples from seven free-ranging bottlenose dolphins (T. truncatus) in the TBRE (Fig. 1 and Table 1) were collected via dart biopsy following the methods described by Barrett-Lennard et al. [17]. Briefly, a mod-
Blubber samples from biopsies (~0.5–1.0 g) and strandings (1.0–1.1 g) were macerated and ground with approximately 30 g of kiln-fired (~500°C for 13 h) Na₂SO₄ and packed into 33-ml, stainless-steel extraction cells. Samples were extracted with CH₂Cl₂ using a Dionex 200 ASE system programmed for three sequential extraction cycles at 100°C and 2,000 psi (5 min of equilibration and static periods per cycle), followed by a 60-s, ultrahigh purity (>99.999%) nitrogen purge. Ten percent by volume of each raw extract was allowed to evaporate to a constant weight in a fume hood for gravimetric lipid determination to the nearest 0.0001 g using a microbalance.

Sample extracts were recombined and reconstituted in approximately 5 ml of hexane before application onto a glass column (length, 600 mm; inner diameter, 25 mm) packed with approximately 100 g of BioBeads SX-3 (Bio-Rad). The first fraction containing lipids and other high-molecular-weight interferences was eluted with 100 ml of a 50% (v/v) mixture of CH₂Cl₂ and hexane. Compounds of greater polarity were targeted in a third fraction, which was eluted with 100 ml of a 20% (v/v) mixture of CH₂Cl₂ and hexane. Florisil fractions were reduced to approximately 5 ml of hexane before application onto a glass column (length, 600 mm; inner diameter, 25 mm) packed with approximately 100 g of BioBeads SX-3 (Bio-Rad). The first fraction containing lipids and other high-molecular-weight interferences was eluted with 100 ml of a 50% (v/v) mixture of CH₂Cl₂ and hexane. Compounds of greater polarity were targeted in a third fraction, which was eluted with 100 ml of a 20% (v/v) mixture of CH₂Cl₂ and hexane. Florisil fractions were reduced to approximately 1 ml and exchanged to hexane (if necessary) using the TurboVap II. Final extracts were transferred to solvent-rinsed, 2-ml, amber-glass gas chromatography (GC) vials, which were then sealed with a Teflon-lined septum and stored at −20°C until analysis.

Extracts (injection volume, 1 μl) were analyzed on a Varian 3400CX GC with electron-capture detection (ECD) and a Model 8200 autosampler. Analyte separation was achieved using...
a DB-XLB fused silica column (length, 30 m; inner diameter, 0.25 mm; film thickness, 0.25 μm; J&W Scientific) with ultrahigh-purity helium as the carrier gas. The split/splitless injector and detector temperatures were 250°C and 300°C, respectively. The initial GC oven temperature of 120°C (1-min hold) was followed by an increase to 200°C at a rate of 10°C/min (1-min hold) and a final increase to 280°C at rate of 2°C/min (10-min hold). Total GC run time was 60 min. Extracts also were analyzed using a Hewlett-Packard 6890 Plus Series GC coupled to a 5973 mass-selective detector operating in the electron-capture negative ionization (ECNI) mode for toxaphene congener analysis and to confirm the identity of other target analytes. Ultrahigh-purity methane, at a cavity pressure of approximately 2.0 × 10⁻⁴ torr, was used as the moderating gas. A column similar to that used for GC-ECD analyses (i.e., DB-XLB) and helium at a linear velocity of 30 cm/s were used to separate target analytes. The 60-min GC oven program was as follows: 60°C (1-min hold), increase to 150°C at a rate of 10°C/min, and increase to 300°C at a rate of 4°C/min (11.5-min hold). The detector was operated in scanning (200–500 amu, ~1 Hz) and selected-ion-monitoring modes.

Total toxaphene concentration (ΣTOX) was estimated using GC-ECNI with mass spectrometry (MS) by summing the peaks with m/z 275, 309, 343, 379, 413, and 445 (representing Cl₁₀–Cl₁₀ PCB congeners) and applying the corresponding mean response factor for a 0.111 to 55.4 μg/ml dilution series of a technical toxaphene product standard provided by Hercules [19]. A 22-component mixture containing 17 chlorobornanes and five chlorocamphenes, including 2-end,3-exo,5-end,6-exo,8,8, 10,10-octachlorobornane (P-26), 2-end,3-exo,5-end,6-exo, 8,9,10-octachlorobornane (P-40), 2-end,3-exo,5-end,8,9,9,10-octachlorobornane (P-41), and 2-end,3-exo,5-end,6-exo,8,9,9,10-nonachlorobornane (P-50) (Dr. Ehrenstorfer), was used for congener identification and quantification. In addition, 2-exo,3-exo,6-exo,8,9,10-hexachlorobornane (Hx-Sed), 2-end,3-exo,5-end,6-exo,8,8,10-heptachlorobornane (B7-1000), and 2-end,3-exo,5-end,6-exo,8,9,10-heptachlorobornane (Hp-Sed) isolated/purified from environmental samples were quantified using a mean response factor for all Cl₁₀–Cl₁₀ components in the 22-component standard [20].

Of the 65 Cl₂–Cl₁₀ PCB congeners, identified by their International Union of Pure and Applied Chemistry (IUPAC) number, targeted for analysis using the external calibration method in the dart biopsies, 24 congeners were not detected in any sample. As a result, total PCB concentrations for these blubber samples were represented as the sum of 41 detected congeners (Σ₁⁰PCBs). Forty-nine individual Cl₁₀–Cl₁₀ PCB congeners were targeted in stranded (Savannah, GA, USA, area) blubber tissue; however, 13 of these were not detected. Thus, total PCB concentrations in stranded blubber were represented as the sum of 36 detected congeners (Σ₁⁰PCBs). Of the 15 congeners (IUPAC 3, 71, 95, 92, 85, 59, 149, 151, 156, 186, 178, 175, 185, 172, and 189) analyzed in the dart biopsies but not in the stranded samples, 10 were not detected in any sample. On average, the remaining five additional congeners that were detected (IUPAC 71, 95, 92, 178, and 172) in the dart biopsies accounted for 1.3% or less of Σ₁⁰PCBs. Thus, we conclude that the difference between Σ₁⁰PCBs (for the free-ranging TBRE animals) and Σ₁⁰PCBs (for the stranded Savannah area animals) was minimal. For both free-ranging and stranded samples, 28 OCP compounds were targeted for analysis with 12 analytes confirmed by GC-ECNI-MS in the dart biopsies (Σ₁⁰OCPs) and 14 analytes confirmed in the strandings (Σ₁⁰OCPs). The two OCPs not detected in the dart biopsies (dieldrin and HCB) accounted for less than 2.8% of Σ₁⁰OCPs in the stranded samples, resulting in a minimal difference between Σ₁⁰OCPs (for the free-ranging TBRE animals) and Σ₁⁰OCPs (for the stranded Savannah area animals). Nominal method detection limits (MDLs) for ΣPCBs, ΣOCPs, and ΣTOX were approximately 0.001, 0.001, and 0.1 μg/g lipid weight, respectively. Nominal congener-specific MDLs were approximately 0.05 ng/g lipid for PCBs and OCPs and approximately 5 ng/g lipid for chlorinated monoterpenes (toxaphene).

Quality control

All sample glassware was washed exhaustively by hand and rinsed with tap water, followed by acetone and hexane, before use. Solvents (Optima grade) and reagents (Na₂SO₄) were purchased from Fisher Scientific. Individual and standard mixtures of PCBs were purchased from the National Institute of Standards and Materials (NIST), Ultra Scientific, or AccuStandard. A performance-based quality assurance and quality control program, which included the parallel analysis of procedural blanks, matrix spikes, and Standard Reference Materials as well as participation in the 2004 NIST Marine Mammal Interlaboratory Calibration Exercise, was implemented to ensure data of the highest quality. Instrument response was monitored every 10 to 12 samples with PCB and technical toxaphene product check standards. Target analytes in sample extracts were confirmed by retention time (±0.05 min) and mass spectral matching (minimum, 70%) compared to standards and were not detected in procedural blanks consisting of 40 to 50 g of kiln fired Na₂SO₄. Recoveries (mean ± standard deviation [SD]) of dibromooctafluorobiphenyl and ε-hexachlorocyclohexane added before sample extraction as recovery surrogates for the PCB and OCP fractions, respectively, were 102% ± 19% and 116% ± 25%, respectively. Recoveries of PCBs and OCPs in Standard Reference Material 1945, a pilot whale homogenate from NIST, were 98% ± 16% and 111% ± 17%, respectively.

Statistical analysis

Individual toxaphene congener concentrations, ΣTOX, ΣPCBs, and ΣOCPs were expressed on a lipid-weight basis (μg/g lipid wt or ng/g lipid wt) to account for variations in blubber lipid content. Total POP concentration (ΣPOPs) was estimated by summing ΣPCBs, ΣOCPs, and ΣTOX. Log-transformed, lipid-normalized ΣPCBs, ΣOCPs, and ΣTOX were evaluated for differences by gender and location using a t test for independent samples (α = 0.05; Statistica, Ver 6; StatSoft). Detectable concentrations that were less than the reported MDL were included (as is) in all statistical analyses; undetectable values were replaced with one-half of the reported MDL. Toxaphene congener relative abundance was computed by dividing congener-specific concentrations by the sum of all congeners detected.

RESULTS AND DISCUSSION

POPs in bottlenose dolphins along Georgia (USA) coast

Lipid content, ΣPCBs, ΣOCPs, ΣTOX, and ΣPOPs for all blubber samples are summarized in Table 1. The most abundant POP class was PCBs, contributing 81 and 67% on average to ΣPOPs for the TBRE and Savannah area animals, respectively. For strandings (n = 6), ΣPCBs ranged from 0.725 to 17.8 μg/g.
than stranded beluga whales (Delphinapterus leucas) from the St. Lawrence River Estuary [4]. As with PCB levels, ΣOCPs (0.013–4.13 μg/g lipid wt) in our stranded samples were similar to total DDT concentrations (ΣDDTs) reported for the Gulf of Mexico mortality event (0.82–2.3 μg/g lipid wt, n = 5) [21]. For the lone stranded adult female in the present study, ΣOCPs (0.315 μg/g lipid wt) (Table 1) was up to several-fold lower than in two stranded adult female (0.68–1.9 μg/g lipid wt) animals in the aforementioned Australian study [22].

In contrast, ΣPCBs in free-ranging TBRE dolphins (35.4–125 μg/g lipid wt) were somewhat higher compared with levels in T. truncatus sampled (surgically as well as remotely) in Florida, North Carolina, and South Carolina (2.02–110 μg/g lipid wt; all USA) [25] and common dolphins (Delphinus delphis) sampled in the Mediterranean (5.26–124 μg/g lipid wt) [26]. In the TBRE animals, PCB levels also were up to an order of magnitude higher than reported in free-ranging D. delphis off northwestern Spain (0.55–22.9 μg/g lipid wt) [27]. Mean ΣPCBs for the TBRE animals (78.6 ± 32.4 μg/g lipid wt) was almost fourfold lower than the median ΣPCBs (282 μg/g lipid wt) for free-ranging Mediterranean striped dolphins (Stenella coeruleoalba) [28], which is among the highest levels ever reported for dolphins. Mean ΣOTX was up to twice as high as that reported for Alaskan beluga whales (5.18 ± 4.77 μg/g lipid wt) [29] but was similar in magnitude to those in L. acutus and S. bredanensis mentioned previously [24]. Total persistent organochlorine pollutants (0.34–17.6 μg/g lipid wt) generally were lower for TBRE animals compared with other free-ranging T. truncatus from the southeastern United States (1.15–87.3 μg/g lipid wt) [25] as well as compared with D. delphis in the Mediterranean (2.88–165 μg/g lipid wt) [26]. These comparisons, however, do not necessarily take into account the variability of POPs associated with gender (see Spatial and gender differences).

Spatial and gender differences

In addition to health status and condition, contaminant concentrations in marine mammals can be influenced by gender,
age, reproductive status, diet, and season. Thus, it is important to recognize that stranded animals may have been compromised by disease or anthropomorphic factors, leading to abnormal rates of pollutant metabolism or excretion, increased or abnormal rates of lipid mobilization, and extended or uncertain time periods stranded under variable environmental conditions [30]. Conversely, POP levels in free-ranging specimens are considered to represent the average state of the population. From a gender perspective, data comparison among adult males is preferable because of highly variable concentrations associated with adult females. Mean ΣPCBs in male TBRE dolphins (84.9 ± 27.6 μg/g lipid wt) was similar to that reported for free-ranging male *D. delphis* (88.3 ± 37.7 μg/g lipid wt) in the Mediterranean [26], which remains at the high end of the global contamination spectrum documented for odontocetes (Fig. 3). This comparison includes top-level predators, such as resident Pacific killer whales (*Orcinus Orca*) [31]; however, mean ΣPCBs for male TBRE dolphins was threefold lower than those of migratory *O. orca* versus the resident populations [31]. The particularly high contaminant burdens reported in this latter study may be attributable to a diet rich in marine mammals [31], in contrast to a diet consisting primarily of lower-trophic-level fish (up to 88%) for resident *O. orca* [31] and coastal estuarine populations of *T. truncatus* in the southeastern United States [6,8].

Given the small sample size and aforementioned issues, the statistical differences in POP levels between the TBRE and stranded dolphins in the Savannah area should be interpreted with caution. Considering only males, however, free-ranging TBRE dolphins still had significantly higher ΣPCBs (84.9 ± 27.6 μg/g lipid wt) than the Savannah area dolphins (10.2 ± 10.8 μg/g lipid wt, n = 2, p = 0.0004). This difference could be attributed in part to differences in animal condition or the depth of blubber sampled using the two different methods, as indicated by the twofold higher (on average) lipid content for stranded animals (Table 1). A recent study concluded that lipid content was two- to threefold higher in blubber layers beneath the outer surface layer, but POP concentrations did not vary with depth after normalizing to lipid (J. Kucklick, NIST, personal communication). Another possible confounding factor was age; only dolphins judged to be older than two years were targeted for dart biopsy sampling, compared with five subadults out of six stranded Savannah area animals (Table 1) (see also Pulster and Maruya [19]). Despite these possible contributing factors, however, the observed differences were consistent with spatial trends for PCBs and toxaphene in preferred prey of *T. truncatus* taken from the TBRE compared with those collected from the Savannah and Jacksonville (FL, USA) coastal areas [13], clearly indicating the importance of the extent of local POP contamination. Additional supporting evidence for this geospatial distribution of POPs is given below (see PCB and OCP congener profiles).

Because female odontocetes transfer POPs to their offspring during gestation and lactation, body concentrations increase with age (like in males) until the animal reaches sexual maturity, at which time levels stabilize or decrease as a result of reproductive off-loading [9]. The extent of the reproductive offloading is variable, however, depending on species and compound [32]. In the present study, ΣPCBs for the presumed mother and calf pair stranded in Wassaw Sound were nearly identical (0.82 vs 0.73 μg/g lipid wt) (Table 1), yet ΣOCPs in the calf (0.64 μg/g lipid wt) was roughly twice that measured in the mother (0.32 μg/g lipid wt). In the calf, the OCPs were comprised of 75% ΣDDTs, which was 2.5-fold higher (0.477 μg/g lipid wt) than in the mother (0.191 μg/g lipid wt); the ratio was only 1.1 for ΣPCBs. This trend was similar to that observed by Borrell and Aguilar [32], who reported that ΣDDTs and ΣPCBs were 3.4- and 1.9-times higher, respectively, in the calf than in the mother. Thus, DDTs may be more easily transferred to offspring than PCBs are, perhaps because of their molecular structure (i.e., fewer chlorines). Unfortunately, the small number of adult females and female/calf pairings sampled in the present study precluded further analysis of the effect of gender and reproductive state on POP levels.

**PCB and OCP congener profiles**

The use of PCB congener profiles to distinguish among dolphin populations frequenting the TBRE has been discussed in detail elsewhere [19]. Briefly, the congener distribution associated with TBRE dolphins was dominated by Cl₂-Cl₁₀ homologues that are uniquely characteristic of Aroclor 1268, a rare technical mixture that was used extensively at the LCP Chemicals chloralkali facility. In contrast, the dominant congeners in blubber collected from stranded dolphins were IUPAC 153, 101, 138, 187, and 118/188, representing a more common signature found in marine mammals worldwide [19,25,26]. Moreover, the Aroclor 1268 fingerprint found in dolphins using the TBRE was distinguishable from those in dolphins sampled near Charleston (SC, USA) and in the Indian River Lagoon (FL, USA) [25]. This unique PCB signature may prove to be useful in assessing habitat use and health impacts resulting from POPs for resident estuarine marine mammals in the northwestern Atlantic [19].

In the present study, the most abundant (nontoxaphene) OCP in all of the free-ranging dolphins and in four of the six stranded dolphins was 4,4′-dichlorodiphenyldichloroethylene (DDE). No significant differences (p = 0.07) were found in 4,4′-DDE levels between the free-ranging (4.59 ± 3.44 μg/g lipid wt) and the Savannah area (0.821 ± 0.16 μg/g lipid wt) dolphins. This highly persistent and bioaccumulative metabolite of 4,4′-DDT, the major component in technical DDT, contributed 66 and 52% of the OCP body burden for TBRE and Savannah area animals, respectively (data not shown). Much lower contributions from 4,4′-nonachlor were the next most abundant OCPs detected, comprising 9 to 25%, respectively, of ΣOCPs. Lesser amounts of oxychlordane, heptachlor epoxide, cis- and trans-chlordane and cis-nonachlor, as well as dieldrin also were detected. Similar patterns have been observed in other marine mammals globally [3,23,25,34].

Total DDT concentrations in male free-ranging dolphins (5.87 ± 2.29 μg/g lipid wt) from the present study were up to an order of magnitude lower compared with those in male free-ranging bottlenose dolphins of other regions along the southwestern U.S. Atlantic coast (12.6–55.2 μg/g lipid wt) [25] and in free-ranging striped dolphins in the Mediterranean (55.1 ± 15.3 μg/g lipid wt) [3]. The ΣDDTs in male stranded dolphins in the present study (1.13 ± 1.31 μg/g lipid wt) was similar to that in male stranded bottlenose dolphins in the Gulf
detected in more than 50% of the TBRE samples, whereas only three congeners were detected in 50% of the strandings. The most abundant congener in both sample sets was 2,2,5-endo,6-exo,8,8,9,10-octachlorobornane (P-42a), with concentrations (mean ± SD) of 85.1 ± 110 and 1,170 ± 1,350 ng/g lipid weight for Savannah and TBRE animals, respectively (Table 2). Other prominent congeners, ranging in concentration between 100 and 1,000 ng/g lipid weight, were P-26, P-40, P-50, Hx-Sed, and Hp-Sed. Congeners detected with relatively low abundance (e.g., <5–100 ng/g lipid wt) were P-41, 2-endo,5,5,6,8,8,9,10,10-octachlorobornane (P-44), Tursiops truncatus from coastal Georgia (USA).
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Fig. 4. Mean relative abundance of toxaphene congeners in blubber of stranded and free-ranging *Tursiops truncatus* from coastal Georgia (USA).

and 2-exo,3-endo,5-exo,6-exo,8,8,9,10,10-octachlorobornane (P-63). Trace amounts and/or infrequent detections of 5,6-exo,8,8,9,10,10-octaCC-octachlorobornane (P-31), 2,2,5-endo,6-exo,8,8,9,10,10-octachlorobornane (P-56), 2,2,3-exo,5,5,8,9,10,10-octaCC-octachlorobornane (P-58), and 2,2,5-endo,6-exo,8,9,9,10,10-octachlorobornane (P-59) were observed in the TBRE samples. Mean congener concentrations were an order of magnitude or more greater for the TBRE samples (Table 2), further validating the difference noted in estimates of ΣTOX (Fig. 2). Substantial variability for a given congener, however, also was observed, indicating a wide range of concentrations among the animals even within the same geographical grouping.

Overall, toxaphene congener profiles for the TBRE and Savannah area samples were not strikingly different. Unlike previous reports on marine mammals [24,35], the dominant toxaphene congener in both TBRE and Savannah area blubber samples was identified as P-42a (Fig. 4). Only two previous papers report high abundance of this congener, one on Arctic ringed seal blubber [36] and the second in livers of adult swallows (*Petrochelidon* spp.) of the Rio Grande River valley in Texas (USA) [20]. As one of the most abundant components of technical toxaphene, it is not surprising that P-42a also was highly abundant in TBRE fish based on proximity to the former toxaphene plant [13,16]. This elevated abundance also provides a direct link for animals sampled in the present study with their preferred prey. Interestingly, another study also detected P-42a in fish but not in harp seals (*Pagophilus groenlandicus*) and penguins (family *Spheniscidae*) [37].

Several more subtle differences between the toxaphene congener profiles also were apparent. First, TBRE animals contained on average more Hx-Sed and Hp-Sed, reductive dechlorination metabolites of higher-chlorinated toxaphene congeners (including P-42a) that are abundant in aquatic sediments [38] and fish in systems originally contaminated with technical toxaphene, such as the TBRE [15]. The relative persistence of Hx-Sed and Hp-Sed in higher biota, however, is expected to be low, as suggested by their rapid elimination in fish [39] and as further supported by the decreased abundance of Hx-Sed and Hp-Sed in bowhead whales (*Balaena mysticetus*) compared to zooplankton [40]. Second, the abundance of the globally persistent congeners P-26 and P-50 is highly variable in our samples (Table 2). This could reflect differences in the level and timing of exposure of dolphins frequenting the present study area to toxaphene-contaminated media (i.e., prey fish), as discussed by Pulster and Maruya [19]. Also, differences in physicochemical properties [41] and vulnerability to transformation based on chlorine substitution of toxaphene structures [42] may help to explain the lower persistence and bioaccumulative potential of selected congeners, such as those with geminal dichloro substituents like P-42a, P-31, P-56, P-58, and P-59 (Table 2). In fact, one would expect a decrease in the relative abundance of these labile congeners as the level of exposure to toxaphene-contaminated media decreased. A concomitant increase in the relative abundance of 2,3,5,6-substituted (persistent) chlorobornanes like P-26, P-40, and P-50 (Table 2) also would be expected.

Although we report the most abundant toxaphene peak in our samples as P-42a, a study of toxaphene congeners in seabird eggs [43] using high resolution gas chromatography (HRGC) and an HT-8 stationary phase revealed a prominent, but unidentified, octachlorobornane that eluted very close to P-42a. Unfortunately, no discussion of differences in ECNI-MS mass spectra was included, thus leading readers to conclude that the identification reported by Witte et al. [43] was based solely on retention time matching (the unidentified compound eluted 0.05 min after the expected retention time for P-42a). In our samples, the ECNI-MS mass spectrum corre-
sponding to the most abundant peak was indistinguishable from the spectrum recorded for the P-42a standard peak. It remains a possibility, however, that another interfering octachlorobornane is present, rendering our P-42a identification somewhat tentative.

Another interesting observation by Witte et al. [43] was that the relative abundances of P-41, P-50, and P-63 appeared to decrease, whereas those of P-26 and P-40 remained stable, over the time period in which samples were collected (1981–1997). If one assumes that the TBRE animals in the present study were continually exposed to toxaphene residues via the local habitat and food web, as recently indicated [19], then the lower relative abundance of labile compounds, such as Hx-Sed and Hp-Sed, and the higher abundance of the more persistent congeners, such as P-26 and P-40, in stranded Savannah animals (Fig. 4) is consistent with a lower overall exposure to toxaphene-contaminated media associated with the TBRE for the latter grouping. Moreover, a report on toxaphene congener residues in birds [44] supports our results and subsequent hypothesis that suggests a high degree of recalcitrance for P-40, particularly with respect to P-41 (Fig. 4). This is important in that these isomers are not resolvable on DB-5–like columns, necessitating the combined reporting of data for these congeners, such as P-40/P-41. If P-40 is universally more persistent than P-41 (in marine mammals as a surrogate for other higher species), it follows that concentrations of P-40/P-41 based on DB-5 analyses in samples from remote areas such as the Arctic are composed largely of P-40.

Toxicological implications

Kannan et al. [45] derived a threshold concentration of 17 µg/g lipid weight for PCBs in marine mammal blubber from published results of semifield or field toxicity studies conducted with a combination of marine and terrestrial species. The toxicity endpoints in the form of no-observable-adverse-effect and lowest-observed-adverse-effect levels for this risk assessment were hepatic vitamin A, thyroid hormone concentrations, suppression of natural killer cell activity, and proliferative response of lymphocytes to mitogens. Schwacke et al. [46] independently derived a threshold concentration of 1.4 µg/g lipid weight for southeastern U.S. coastal T. truncatus by combining PCB tissue residue data collected from three populations with a dose–response curve based on experimental assessment of lymphocyte response to mitogens. Schwacke et al. [46] more critically evaluated PCB and toxaphene residues available to higher marine predators in the Arctic, as well as measures to reduce the amount of PCBs and toxaphene residues available to higher marine predators appear to be warranted. In addition, more studies are needed to assess the relative impact and/or toxicological response of individual toxaphene residue congeners, such as locally abundant labile congeners (e.g., Hx-Sed, Hp-Sed, and P-42a) versus globally persistent structures (e.g., P-26, P-40, and P-50).

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