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High levels of polychlorinated biphenyls in tissues of Atlantic turtles stranded in the Canary Islands, Spain

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ABSTRACT

Polychlorinated biphenyls (PCBs 28, 31, 52, 101, 138, 153, 180, and 209) were measured in tissue samples (liver and fat) from 30 loggerhead turtles *Caretta caretta*, 1 green turtle *Chelonia mydas*, and 1 leatherback *Dermochelys coriacea* stranded on the coasts of the Canary Islands, trying to establish a possible relation between PCB concentrations and the lesions and causes of death. Tissues from these turtles contained higher levels of PCBs than those reported in turtles from other geographical regions. ∑PCB concentrations (1980 ± 5320 ng g^{-1} wet wt.) in the liver of loggerheads were higher than in the adipose tissue (450 ± 1700 ng g^{-1} wet wt.). Concentrations of PCB 209 in the liver (1200 ± 3120 ng g^{-1} wet wt.) of loggerheads and in the liver (530 ng g^{-1} wet wt.) and adipose tissue (500 ng g^{-1} wet wt.) of the leatherback were remarkable. Frequencies of detection of PCB 209 in the liver (15.5%) and adipose tissue (31%) were also remarkable. Cachexia was detected in 7 turtles (22%) and septicemia was diagnosed in 10 turtles (31%). Statistically, a positive correlation was detected between ∑PCBs concentration and cachexia. Poor physical condition, cachexia and/or septicaemia could explain the high levels of PCBs and tissue distribution. However, no histological lesions exclusively attributed to the acute effects of PCBs were described. The most prevalent histological lesions were ulcerative and purulent oesophagitis, purulent dermatitis, necrotizing enteritis, and granulomatous pneumonia. The bacteria most frequently isolated were *Escherichia coli*, *Staphylococcus* sp., and *Aeromonas* sp. Although immunosupression as a result of PCBs pollution has been described previously, other factors in this study, such as incidental fishing, nutritional status, and exposition to different micro-organisms, make it difficult to establish a clear association between PCB concentrations and causes of death.

1. Introduction

Two families and seven species of sea turtles are currently recognised (Pritchard, 1997) and included in the red list of the World Conservation Union (IUCN, 2007). The family Dermochelyidae includes only the leatherback (*Dermochelys coriacea*). The family Cheloniidae includes the green turtle (*Chelonia mydas*), loggerhead (*Caretta caretta*), hawksbill (*Eretmochelys imbricata*), Kemp's ridley (*Lepidochelys kempi*), olive ridley (*Lepidochelys olivacea*), and flatback turtles (*Natator depressa*). The most common species in the Canary Islands is the loggerhead turtle (Mateo et al., 1997). However, evidence of a decline in the population of turtles in the Canary Islands has been reported (López-Jurado and González, 1983; Blanco and González, 1992).

Diseases and causes of mortality among turtles stranded in the Canary Islands have been previously reported (Orós et al., 2004, 2005). However, data available for baseline levels of contaminants and effects on the turtle populations of the Canary Islands are scarce (Torrent et al., 2004).

Polychlorinated biphenyls (PCBs) have a particular significance because of their undesirable effects on environmental quality and animal health (Ahlborg et al., 1994). PCBs were manufactured from the 1930s to the 1970s for several industrial applications, such as liquid coolants for electrical transformers or as softeners in the production of plastics and as components of hydraulic fluids and lubricating oils. PCBs are able to bioaccumulate through the food chain and their effects have been reported on the immune, endocrine, and reproductive systems of different animal species (Fox, 2001). Although much was reported to date on PCBs concentrations in large predators, few studies have been dedicated to turtles. These studies have been focused on turtles from Long Island (Lake et al., 1994), Virginia (Rybitski et al., 1995), Scotland (Mckenzie et al., 1999), the Hawaiian Islands (Miao et al., 2001), the Baja California Peninsula (Gardner et al., 2003) and North Carolina (Keller et al., 2004, 2006). Studies focused on the Mediterranean Sea have a particular significance because of their number (Corsolini et al., 2000; Storelli and Marcotrigiano, 2000; Perugini et al., 2006; Storelli et al., 2007).

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The aim of this study was to evaluate the presence and patterns of eight PCB congeners (28, 31, 52, 101, 138, 153, 180, and 209) in tissue samples (liver and fat) from 32 turtles stranded on the coasts of the Canary Islands between August 2002 and November 2005. We also tried to determine a possible relation between the PCB concentrations and the lesions and causes of death using the Spearman's rho correlation method to calculate the correlation between ∑PCB concentrations in both tissues and physical conditions such as cachexia and septicaemia.

2. Materials and methods

2.1. Turtles

Between August 2002 and November 2005, 32 turtles that got stranded on the coasts of four islands belonging to the Canary Islands [Gran Canaria (n=25; 78.1%), Tenerife (n=3; 9.4%), Fuerteventura (n=2; 6.2%), and El Hierro (n=2; 6.2%)] were submitted for necropsy to the Veterinary Faculty, University of Las Palmas de Gran Canaria (ULPGC). Some of them had been previously submitted to the Tafira Wildlife Rehabilitation Center (TWRC) for health evaluation, medical management, and possible rehabilitation.

Species identifications were made according to Frick (1996). Turtles belonging to three different species were examined: 30 loggerheads, *C. caretta* (93.7%), 1 green turtle, *C. mydas* (3.1%), and 1 leatherback, *D. coriacea* (3.1%). Species, sex, age group, and biometrics data are shown in Table 1. Age group was determined on the basis of straight carapace length (SCL) (Bjorndal et al., 2001; Seminoff et al., 2004) and sexual maturity (estimated from the appearance of their gonads).

2.2. Pathological and microbiological studies

Necropsies were performed at the Veterinary Faculty (ULPGC) within the first 12 h after death. The gross postmortem examinations were carried out using the procedures previously described (Wolke and George, 1981; Orós and Torrent, 2001). Gross lesions were recorded and tissue samples from all major organs were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm for light microscopy and stained with haematoxylin and eosin. Special stains used on selected cases included Gram stain for bacteria, Ziehl-Neelsen stain for acid-fast organisms, periodic acid-Schiff stain for protozoa and fungal hyphae, Grocott's methenamine silver nitrate for fungi, and von Kossa stain for calcium (Bancroft and Stevens, 1996). For the microbiological studies, samples were taken from gross lesions and cultured on a variety of selective and non-selective media, including blood agar (Oxoid), MacConkey agar (Oxoid), Baird Parker agar (Oxoid) for staphylococci, and Sabouraud Dextrose agar (Oxoid) for fungi and yeasts. All cultures were incubated at 25 °C aerobically. Once a pure growth was obtained, bacteria were identified based on the biochemical profile (API 20 E, API 20 NE, and API 20 STAPH, BioMérieux).

2.3. Samples collection for PCB analysis

Tissues samples (liver and coelomic fat) were collected during necropsy. After collection, the samples were wrapped in an aluminium foil and stored at −20 °C until analysis. Prior to analysis, tissue samples were homogenized using a commercial blender.

2.4. PCBs analysis

Polychlorinated biphenyls (IUPAC Nos. 28, 31, 52, 101, 138, 153, 180, and 209) were analysed according to the method described by Tanabe et al. (1994). The validity of analytical methods was confirmed with Standard Reference Materials (CARP-2: ground whole carp, *Cyprinus carpio*) obtained from the National Research Council of Canada. Precision and accuracy are reported in Table 2.

Briefly, aliquots (4–7 g) of the homogenized samples were ground with anhydrous sodium sulphate in a mortar, and extracted using Soxhlet apparatus for 6 h with 300 mL of diethyl ether:hexane (3:1) solvent mixture. Extracts were concentrated in volume to 10 mL in Kuderna-Danish, and the aliquots (2 mL) were transferred to a glass column packed with 20 g of Florisil and dried by passing through nitrogen gas. Organochlorines adsorbed on Florisil were eluted with 150 mL of 20% hexane-washed water in acetonitrile and transferred to a separatory funnel containing 600 mL of hexane-washed water and 100 mL of hexane. After partitioning, the hexane layer was concentrated, cleaned up with sulphuric acid, and passed through a 12 g Florisil-packed glass column for separation.

Final determination of PCBs was carried out using a Varian 3600 gas chromatograph fitted with an electron capture detector (GC-ECD). In all the analyses a fused-silica capillary column Supelco (length = 30 m, inner diameter 0.53 mm and film thickness 0.50 μm) was used. The column oven temperature was programmed from 60 to 160 °C, held for 10 min, and then increased to 260 °C at a rate of 2 °C/min and held for 20 min. Injector and detector temperatures were set at 260 and 280 °C, respectively. Nitrogen was used as a carrier gas at 63.3 mL/min. The PCB patron used as an internal standard was the PCB-Mix 12, Iso-octane (Lab. Dr. Ehrenstorfer). Quantification interval used for PCBs was from 1 ng g^{-1} (instrumental detection limit) to 50 000 ng g^{-1} (optimum linear limit). Concentrations of PCBs, means of four measurements, are presented as ng g^{-1} on a wet weight basis.

2.5. Statistical analysis

Mann–Whitney U test was conducted to determine whether the difference in the levels of PCBs were related to the tissues. Spearman's rho correlation method was used to calculate the

Table 1
Species, sex, age group and biometrics data (mean ± standard deviation) of the turtles analysed

Species	Sex	Age group	Weight (kg)	SCL (cm)
Caretta caretta (n=30)	Female (n=27; 90%) Male (n=3; 10%)	Pelagic juvenile (n=12; 40%) Juvenile (n=18; 60%)	11.5 ± 8.3	41 ± 11.7
Chelonia mydas (n=1)	Female	Juvenile	21	52
Dermochelys coriacea (n=1)	Female	Adult	231.5	nm

SCL: straight carapace length.
n: number of turtles.
nm: not measured.

Table 2
Precision and accuracy of analytical methods obtained using a certified ground whole carp Cyprinus carpio (CARP-2)[a]

PCBs	CARP-2	
	Certified	Found[b]
PCB 28	34 ± 4.0	31 ± 3.1
PCB 52	138 ± 43	119.7 ± 13.9
PCB 101	145 ± 48	150.1 ± 24.1
PCB 138	103 ± 30	99.5 ± 18.7
PCB 153	105 ± 22	116 ± 16.6
PCB 180	53.3 ± 13.0	59.2 ± 10.7
PCB 209	4.6 ± 2.0	4.8 ± 3.6

[a] The concentrations are given in ng g^{-1} wet wt.
[b] Number de replicates is 4.