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The Science of the Total Environment 281 (2001) 247–253

the Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Man

www.elsevier.com/locate/scitotenv

Short communication

Congener-specific profile and toxicity assessment of PCBs in green turtles (*Chelonia mydas*) from the Hawaiian Islands

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Received 25 September 2000; accepted 10 March 2001

Abstract

Chemical pollution may play a role in the etiology of fibropapillomatosis in green turtles (*Chelonia mydas*). In this preliminary study, polychlorinated biphenyls (PCBs) were measured in the livers and adipose fats of green turtles collected after they were stranded on Oahu Island, Hawaii in 1992–1993. Average concentrations of total PCBs were 45–58 ng/g dry weight and 73–665 ng/g in the liver and adipose tissues, respectively. Hexachlorobiphenyls were predominant homologues, PCBs 153 and 138 were dominant congeners in all the turtle tissues. Among the most toxic coplanar congeners, in the order of abundance, were PCB 77 > 126 > 169. Estimated toxic equivalents (TEQs) of PCBs to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin were 8–15 pg/g in the livers and 13–48 pg/g in the adipose tissues. PCB 126 contributed 85–91% of the total TEQs.

Keywords: Polychlorinated biphenyls; PCBs; Green turtle; Marine pollution

1. Introduction

Hawaii's green turtles (*Chelonia mydas*) are listed as threatened under the US Endangered Species Act. A phenomenon prominently recorded in the populations of green turtles in Hawaii and Florida is a disease called fibropapillomatosis, the growing of large bullbous tumors predominantly on the animal's soft tissues. Surveys conducted in Kaneohe Bay on the island of Oahu during the 1990s indicated that more than one-half of the green turtles were infected and one-third of them off the island of Molokai (Balazs et al., 1998, 2000). Bergeron et al. (1994) observed male-to-female sex-reversal at male-producing temperatures when red-eared slider turtle eggs (*Trachemys scripta*) were exposed to some PCB congeners and hydroxyl metabolites. Several studies showed

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0048-9697/01/$ - see front matter
PII: S0048-9697(01)00835-X

elevated PCB levels in snapping turtles (*Chelydra serpentina*) (Olafsson et al., 1983; Bryan et al., 1987; Bishop et al., 1991; de Solla et al., 1998). Kannan et al. (2000a) reported that PCBs in yellow-blotched map turtles contributed 90–99% of the total estimated 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs). Several investigators measured concentrations of total PCBs or selected PCB congeners in green turtle tissues or eggs collected from Ascension Island, South Atlantic Ocean (Thompson et al., 1974), the east coast of Florida (McKim and Johnson, 1983) and Oahu Island in Hawaii (Aguirre et al., 1994; Rybitski et al., 1994). However, no Aroclors (PCBs) were detected in Hawaii's green turtles (Aguirre et al., 1994). In this study, all PCBs were determined to assess the congener profile and toxicity in Hawaii's green turtles.

2. Materials and methods

2.1. Sample collection and preparation

Liver and adipose tissue samples were collected during necropsies of three fresh-dead green turtles on the island of Oahu, Hawaii in 1992–1993. All samples were wrapped in aluminum foil and stored at −20°C until analyzed. Table 1 shows sampling locations and other information. Prior to analysis, liver (141–178 g) and adipose fat tissues (121–223 g) were homogenized with a blender, in an excess amount of dry ice, then freeze-dried.

2.2. Extraction and cleanup

PCBs in turtle tissues were analyzed in triplicates as previously described (Miao et al., 2000) with the following modifications. Basic alumina (60–325 mesh, 3 g) as an adsorbent for the lipids was placed on a 2-μm frit filter at the outlet end of a 20-ml supercritical fluid extraction cell. The freeze-dried turtle tissues (3.5 g liver or 1.6 g adipose fat) were then added. Decachlorobiphenyl (150 μl of 50 pg/μl) as a surrogate, which was absent in the non-spiked turtle samples, was added to the samples in the extraction cell and completely mixed (Stewart et al., 2000). The extracts were treated with concentrated sulfuric acid (3 × 3 ml), washed with 5% NaCl and were dried with anhydrous Na_2SO_4 (Murphy, 1972; Bernal et al., 1992). Sulfuric acid-treated extracts were subjected to further chromatographic cleanup as previously described (Miao et al., 2000).

Table 1
Specimen characteristics and PCB levels in the green turtle tissues collected from Oahu Island, Hawaii

Code	Sex	SCL[a]	Estimated age (year)	Weight (Kg)	Stranding zone	Stranding date	Tumor status[b]	Tissues	PCBs (ng/g)	Lipid content (%)
A	Female	96.0	> 25	~ 100	Makaha, S. Shore	09-28-93	0	Liver Adipose fat	52.1 664.7	14.1 55.0
B	Male (immature)	73.4	> 15	~ 50	Campbell Industrial Park, S. Shore	08-26-92	2	Liver Adipose fat	44.7 73.1	9.4 72.8
C	Male	88.1	> 25	~ 75	Laniakea, N. Shore	06-22-93	3	Liver Adipose fat	58.0 116.0	11.2 89.7

[a] SCL = Straight carapace length (cm).
[b] 0 = No tumors, 2 = moderate affliction, 3 = severe affliction.

2.3. High performance liquid chromatography (HPLC)

PCBs in the extracts were fractionated with normal phase HPLC (Dionex Corp., Sunnyvale, CA) with *n*-hexane at a flow rate of 0.5 ml/min. The column was a Cosmosil 5-PYE column [2-(1-pyrenyl)ethyldimethylsilylated silica gel, 250 × 4.6 mm i.d., particle size 5 μm, Nacalai Tesque Inc., Kyoto, Japan]. After the initial 3.5-ml fraction was discharged, subsequent 2.1-, 2.2- and 6-ml fractions were collected separately as the first, second and third fractions, respectively. The first fraction contained the bulk of the PCBs, the second the mono-*ortho* coplanar PCBs and the third the non-*ortho* coplanar PCBs (Ramos et al., 1999; Reich et al., 1999).

2.4. High resolution gas chromatography-electron capture detector (HRGC-ECD) and mass spectrometric detector (MSD)

After each fraction was concentrated to 50–200 μl in hexane, PCBs were analyzed with HRGC-ECD as previously described (Miao et al., 2000) with the following modifications. The GC column was DB-XLB, 30 m × 0.25 mm (i.d.) and 0.25-μm film thickness (J and W Scientific Inc., Folsom, CA). The oven temperature was programmed from 50 (held for 1 min) to 150°C at a rate of 10°C/min, and then to 300°C at 2.5°C/min with a final hold time of 5 min. Injector and detector temperatures were 280 and 320°C, respectively. The recoveries of PCBs from the samples ranged from 75 to 91% after the background PCB concentrations were corrected. Procedural blanks were carried out through the whole analytical procedure to check for interference and contamination. The limits of detection were in a range of 1–50 pg/g for various PCB congeners and approximately 5 pg/g for most individual congeners. The recoveries of the surrogate were 76–89%. Reported concentrations were not corrected for the recoveries of the surrogate. PCB congeners are represented by their IUPAC numbers throughout this article. A HP 5687MSD was used for chemical confirmation as previously reported (Miao et al., 2000).

The PCB standard mixtures C-CS-01 to C-CS-09 were purchased from AccuStandard, Inc. (New Haven, CT) and contained 209 PCB congeners. Lipid contents were determined by the Agricultural Diagnostic Service Center, University of Hawaii.

3. Results and discussion

Average concentrations of total PCBs were 45–58 and 73–665 ng/g dry weight in the turtle liver and adipose tissues, respectively (Table 1). A previous study showed that the total PCB levels in the green turtles were from below the limit of quantitation to 17.1 ng/g in livers and 58.2 ng/g in adipose tissues on wet weight although concentrations of specific PCBs were not reported (Rybitski et al., 1994). If the total PCB levels are calculated on a dry weight basis, the reported values are very close to those reported here. However, Aguirre et al. (1994) reported that no Aroclor residues were detected in the adipose fat, kidney, liver and brain tissues of the Hawaiian green turtles at the detection limit of 1.0 μg/g wet weight.

The PCB profiles in the turtle tissues were dominated by the higher chlorinated homologues. Hexachlorobiphenyls were predominant homologues, and accounted for 34–46% of total PCBs in the livers and 37–45% of total PCBs in the adipose fats (Fig. 1). These results were similar to those in our previous report, where hexachlorobiphenyls were also dominant, and averaged 43% of total PCBs in eels from French Frigate Shoals (Miao et al., 2000). PCB 153 was the most abundant congener in the turtle tissues followed by PCB 138. These two congeners collectively accounted for 21–35% of the total PCBs in livers and 23–35% of total PCBs in adipose tissues (Fig. 2). The other major congeners included PCBs 99, 105, 118, 128, 170 and 180. The PCB patterns were virtually identical between turtles as well as between liver and adipose tissues.

Non-*ortho* co-planar PCB congeners 77, 81, 126 and 169 and mono-*ortho* co-planar PCBs 105, 114, 118, 123, 156, 157, 167 and 189 were detected

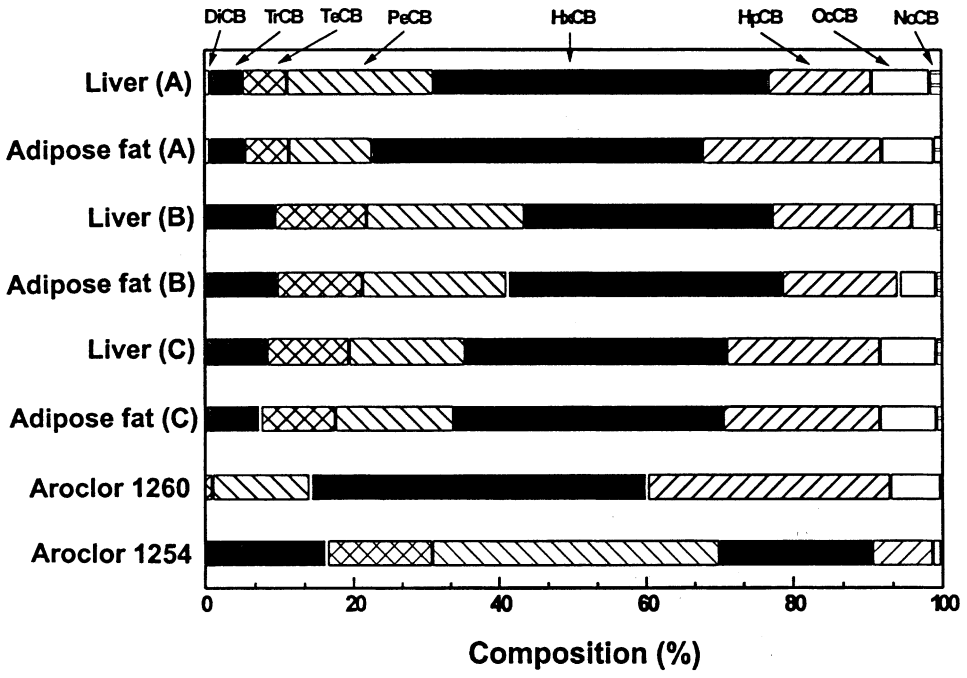

Fig. 1. Composition of PCB homologues in the green turtle livers and adipose fats from the island of Oahu, Hawaii and in Aroclors 1254 and 1260.

in the turtle tissues. The concentrations of the non-*ortho* co-planar PCBs were in the order of 77 and 81 > 126 > 169, a pattern also present in technical PCB preparations (Kannan et al., 1993) and in some marine species (Kannan et al., 2000b; Serrano et al., 2000). The levels of PCBs 77 and 81 were approximately equal. Non-*ortho*-substituted PCBs have a high affinity for the arylhydrocarbon receptor due to their tendency to assume a planar configuration, which is isosteric with 2,3,7,8-TCDD. These non-*ortho*-PCBs would be preferentially biomagnified because the *meta* and *para* substitutions increase stability and decrease excretion (Bright et al., 1995). Numerous studies have dealt with application of toxic equivalent factor (TEF) of PCBs to environmental and human specimens. The co-planar PCBs often contribute much more to TEQs than polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (Patterson et al., 1994; Bosveld et al., 1995). It was recently reported that PCBs contributed 90–99% of the total estimated TEQs when concentrations of PCDDs, PCDFs, PCBs and organochlorine pesticides were measured in yellow-blotched map turtles collected from the Pascagoula river drainage, Mississippi (Kannan et al., 2000a). PCB TEQ values can be representative when used to assess toxicity of contaminants.

Several different TEF schemes have been developed for co-planar PCBs. TEF values derived for co-planar PCBs varied by several fold depending upon the type of bioassays, end points and calculation methods of relative potencies (Giesy and Kannan, 1998; van den Berg et al., 1998). TEQs for mono- and non-*ortho* co-planar PCBs in the turtle tissues were estimated with 2,3,7,8-TCDD TEFs for humans/mammals which was suggested by van den Berg et al. (1998) for risk assessment (Table 2). The TEQs contributed by co-planar PCBs in the turtle liver and adipose tissues were 7.686 to 14.643 and 13.216 to 48.226 pg/g dry weight, respectively. A low TEQ contribution (< 0.8%) from the non-*ortho* congeners

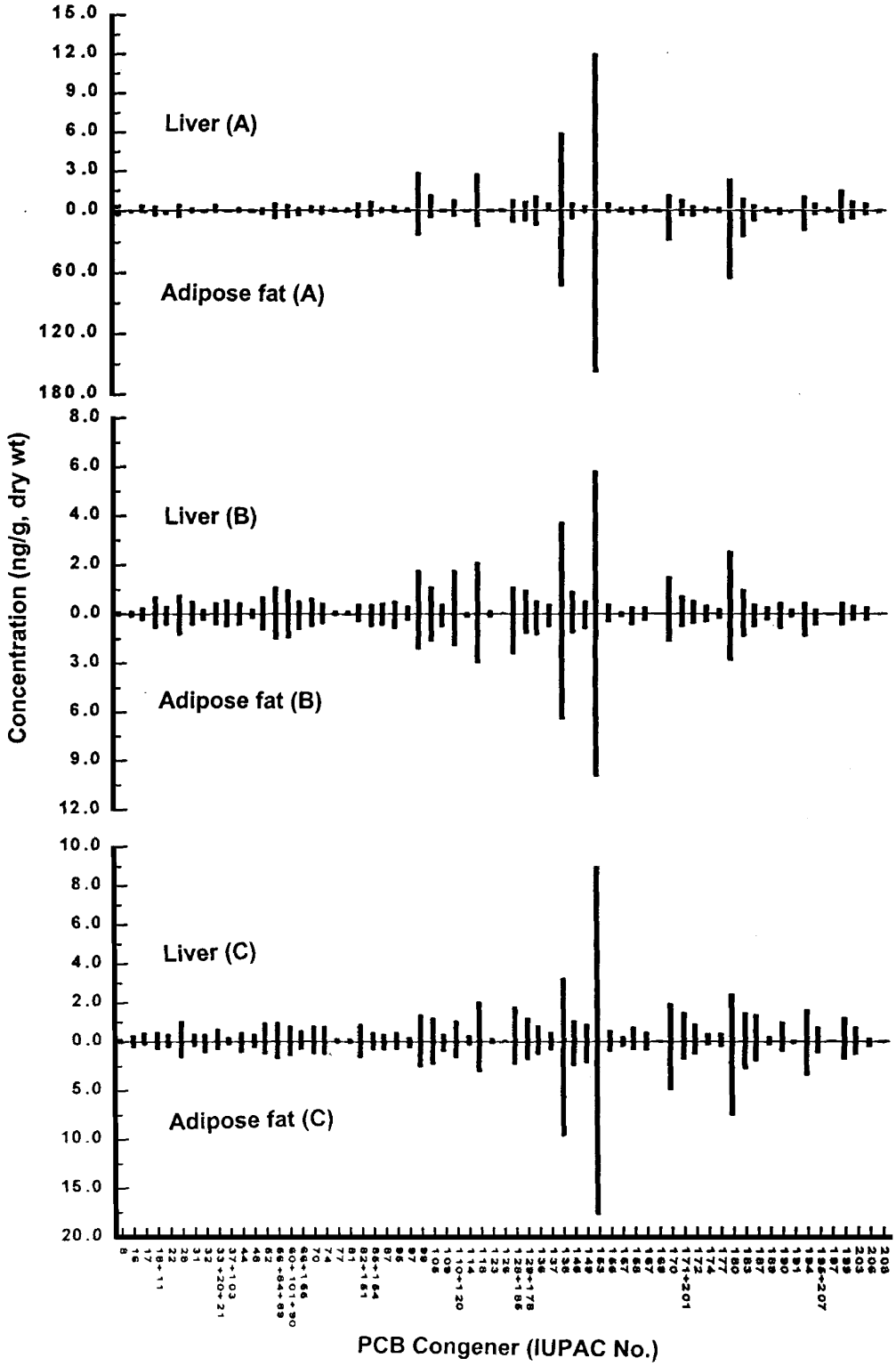

Fig. 2. Composition of PCB congeners in the turtle livers and adipose tissues.

Table 2
Toxic equivalents (TEQs) to 2,3,7,8-TCDD for coplanar PCBs in the turtle tissues

PCB Congener	TEF[a]	TEQs (pg/g dry weight)					
		A-liver	A-fat	B-liver	B-fat	C-liver	C-fat
81	0.0001	0.023	0.047	0.014	0.021	0.018	0.026
77	0.0001	0.024	0.062	0.016	0.025	0.020	0.028
126	0.1	13.000	41.000	7.000	12.000	10.000	18.000
169	0.01	0.700	1.800	–	–	0.500	0.800
105	0.0001	0.125	0.821	0.115	0.179	0.132	0.248
114	0.0005	0.055	0.215	0.055	0.140	0.100	0.275
118	0.0001	0.284	1.690	0.208	0.312	0.220	0.390
123	0.0001	0.013	0.051	0.015	0.032	0.015	0.040
156	0.0005	0.270	1.590	0.190	0.305	0.200	0.360
157	0.0005	0.125	0.770	0.045	0.150	0.120	0.205
167	0.00001	0.004	0.029	0.003	0.005	0.005	0.010
189	0.0001	0.020	0.151	0.025	0.047	0.023	0.054
Total		14.643	48.226	7.686	13.216	11.353	20.436

[a] TEFs for PCBs are from van den Berg et al., 1998.

77, 81 and 169 found in this study was similar to the reported results in shark and grouper tissues (Serrano et al., 2000). Non-*ortho* co-planar congener 126 was the dominant TEQ contributor, and accounted for, on average, 89% of the PCB-TEQs in the turtle tissues, which was followed by congeners 169, 118, 156 and 105 (Table 2). However, a larger set of specimens needs to be analyzed to establish possible relevance between green turtle fibropapillomatosis and pollutant exposure.

Acknowledgements

This work was supported in part by the US Fish and Wildlife Service. Authors are grateful to Karl Yanagihara, David Miyahira, Shandell Eames, Robert Morris (Makai Animal Clinic) and Denise Parker for their technical assistance, and Dr Lee Ann Woodward at the US Fish and Wildlife Service for helpful discussions.

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