SOUTHERN HEMISPHERE CETACEANS: A STUDY OF THE POPs PCDDs/PCDFs AND DIOXIN-LIKE PCBs IN STRANDED ANIMALS FROM THE TASMANIAN COAST

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Persistent organic pollutants (POPs) have become an important group of organic pollutants that have attracted much attention internationally. Australia was a signatory to the Stockholm Convention in May 2001 and has set about investigating levels of these "dirty dozen" chemicals in its' environment[1]. Levels of POPs in environmental compartments in the Southern Hemisphere have not been studied as widely as their Northern Hemisphere counterparts but it is generally reported that levels are significantly lower[2]. This in particular is due to the much lower population densities, reduced amount of industrialisation and smaller continental masses which in turn has resulted in a lesser use and production of these POPs when compared with it's northern neighbours[3]. Over the past 25 years a concentration of research activities have been focused on the determination of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) a group of industrial byproducts and also the industrial compounds polychlorinated biphenyls (PCBs) in a variety of biota and environmental samples. Long range atmospheric transport (LRAT) has been used to describe the widespread distribution of a variety of POPs in the world's oceans that can also act as a sink for these compounds and their concentration from lower latitudes into the Polar regions particularly in the Arctic.[4,5,6,7,8] The meteorological phenomenon of air rising at the equator and moving towards the poles where the air subsides and then moves back to the equator is known as the Hadley Cells[9] and demonstrates little inter-hemispheric mixing.

In this paper, we examine levels of PCDDs/PCDFs and dioxin-like PCBs in a range of cetaceans stranded along the Tasmanian coast during 2002 and compare these levels with those reported for similar species in the Northern Hemisphere. Cetaceans, especially Sperm whales *(Physeter catodon)*, are open ocean dwellers and represent an important group of marine mammals being feeders at the higher trophic levels, usually long-lived and therefore levels of accumulated POPs can be a useful indicator of the amount of contamination in the oceans they inhabit.

Materials and methods

Blubber samples were taken after a mass stranding of Sperm Whales occurred in November 2002 in Waterhouse, Tasmania. All samples collected were of females with lengths ranging from 10.03m to 12.5m. It has been reported that females become sexually active at about 10m and grow to approximately 13m in length.[10] There was no evidence of any of these mammals lactating at the time of stranding. Further archived samples of a female Long-finned Pilot Whale *(Globicephala melas)* of 4.27m in length stranded on Sisters Beach in September 2002, an adult male Sperm Whale stranded on the West Coast in March 2002 and a male Grey's beaked whale *(Mesoplodon grayi)* of 5.12m in length stranded on Bruny Island in December 2002 were also examined.

PCDD/PCDF and dioxin-like PCB Analyses

Standards were all purchased from Wellington Laboratories (Ontario, Canada) and were used for calibration, quantification and determination of recovery of PCDD/PCDF and dioxin-like PCBs. Solvents were purchased as pesticide-quality standard and used as received. All chromatographic columns were purchased from Fluid Management Systems. (Waltham, MA, USA) and were used without any further treatment. They comprised multi-layer (basic/neutral/ acidic) silica, alumina and PX-21 carbon dispersed on celite.

Sample preparation

Accelerated solvent extraction was performed on thawed blubber samples that had been mixed with hydromatrix using a ASE 100 (Dionex, Utah, USA) with ethanol:toluene (68:32) as the extracting solvent and a temperature and pressure of 150^{o}C and 1500 psi, respectively. Approximately 1g of the extracted lipid was spiked with the respective PCDDs/PCDFs and dioxin-like PCB isotopically labeled $^{13}C_{12}$ surrogates. The extracts were then cleaned-up on the Power-Prep™ system using standard elution programs as supplied by the manufacturer.

Gas Chromatography High-Resolution Mass Spectrometric (GC-HRMS) Analysis

All experiments were conducted on a MAT95XL HRMS (ThermoFinnigan MAT GmbH, Bremen, Germany) coupled to an Agilent 6890 GC (Palo Alto, CA, USA) equipped with a CTC A200S autosampler. A DB-5 (J & W Scientific, Folsom, CA, USA) capillary column (60m x 0.25mm i.d., film thickness 0.25µm) was used as the primary analytical column. Resolution was maintained at 10,000 (10% valley definition) throughout the sample sequence. Multiple ion detection (MID) experiments were performed in the electron impact mode with monitoring of the exact masses of either M+ [M+2]+ or [M+4]+ ions for native and labeled compounds. Individual congeners are identified using the GC retention time and ion abundance ratios with reference to internal standards.

Results and Discussion.

This study is one of the first to determine PCDDs/PCDFs and dioxin-like PCBs in whales that have been stranded anywhere in Australia. The levels of PCDDs/PCDFs and dioxin-like PCBs have been calculated on a lipid weight basis using the most recent World Health Organisation toxic equivalency factors (TEFs)[11]. Recoveries of the $^{13}C_{12}$ PCDD/PCDF and $^{13}C_{12}$ PCB internal standards added prior to extraction and carried throughout the clean-up/fractionation steps averaged 45.8 ± 7.7% and 52.1 ± 6.0%, respectively. Concentrations of native analytes for PCDDs/PCDFs and PCBs were corrected for the recovery of these internal standards and middlebound concentrations were calculated assuming that all values of the different congeners less than the limit of determination are equal to one-half the limit of determination. The concentrations found are shown in Table 1.

PCDDs/PCDFs

Of the congeners detected, the major contributions to the PCDD/PCDF TEQ came from five congeners viz. 1,2,3,7,8-PeCDD ~2,3,4,7,8-PeCDF > 1,2,3,6,7,8-HxCDD >>1,2,3,4,6,7,8-HpCDD>> OCDD which were present in all Sperm whale samples. Interestingly, only 1,2,3,4,6,7,8-HpCDD and OCDD were detected in the other whales species analysed. Generally, the WHO-TEQ for all the whales examined were similar to those found in other Southern Hemisphere whale species[12,16] and lower than Northern Hemisphere whale data[14]. WHO-TEQ concentrations for all Southern Hemisphere whales were much less than for their dolphin counterparts albeit that from a geographical viewpoint their origins were quite distinct. The Humpback dolphin originated from the northern tropical Australian city of Darwin and the other study was from dolphins from the South Australia area.

Table 1. Concentrations of PCDDs/PCDFs and dioxin-like PCBs (pg WHO-TEQ/g lipid) in Cetacean Blubber from Southern & Northern Hemispheres

	PCDDs	PCDFs	Non-ortho PCBs	Mono-ortho PCBs	WHO-TEQ	Reference
Sperm Whales – Females (n=6)	2.51	1.49	23.6	4.86	32.5	This study
Beaked Whale – Male (n=1)	0.24	0.33	15.1	8.00	23.7	This study
Sperm Whales – Male (n=1)	1.43	1.25	14.3	2.66	19.6	This study
Long Finned Pilot Whale - Female (n=1)	0.33	0.32	11.4	10.2	22.2	This study
Humpback Dolphin (n=1)	12.7	2.22	20.0	155	190	Unpublished data
NZ Pilot Whales					1.82-43.7*	Schröder[12]
SA Dolphins	15.2-56.8		10.4-443		12.05-449.61	Ruchel[13]
North Sea and The Barents Sea Minke Whales	0.7-2.6		4.8-53.3	5.4-73.3	2.1-8.4	Skåre[14]
Minke Whales	0	0-0.75	1.62-6.97	0.2-2.47	2.1-8.4	WDCS[15]
Southern Hemisphere Minke Whales	0	0-0.75	1.62-6.97	0.2-2.47	2.1-8.4	WDCS[16]
North Pacific Minke Whales	0	0-12.6	11.2-95.9	9.7-29.7	15.6-127.2	WDCS[16]
Toothed Whale & Dolphin Products	0-14.6	1.61-71.8	18.8-452	4.9-148	27.1-691	WDCS[16]
Killer Whales	6.45-9.50	4.31-5.16	13.12-24.77	83.74-265.10	111.64-435.54	Ross[17]

*I-TEQ value is estimated from PCB data and extrapolation based upon a 9% contribution from PCDDs/PCDFs

dioxin-like PCBs

Concentrations of 17.0 – 35.9 pg WHO-TEQ/g lipid were determined across all whale species tested and contributed between 85.3 – 97.6% of the total WHO-TEQ. The female Sperm whales contained higher than the average WHO-TEQ. In all samples, the major coplanar PCB present was PCB#118 with the most important contributor to the WHO-TEQ in all cases was the non-ortho PCB#126. Non-ortho PCBs contribute on average greater than 70% of the WHO-TEQ. The PCB congener profiles between species were quite different with significantly higher contribution of the non-ortho PCB#77 and PCB#81 in the non-sperm whale samples.

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