A Study of the Presence of Brominated Flame Retardants in Australian Fauna

Robert Symons[1], Debbie Burniston[1], Nino Piro[1], Gavin Stevenson[1], Alan Yates[1]

[1]Australian Government Analytical Laboratories, Sydney

Brominated flame retardants, in particular polybrominated diphenyl ethers (PBDEs) gained prominence in the late nineties when Norén et al.[i,ii,iii] reported an exponential increase in PBDE levels found in Swedish mothers milk over a quarter of a century period with an associated decrease in levels of dioxin-like compounds. PBDEs have since become exceptionally widely studied being detected in most environmental compartments[iv] and food[v] as well as human tissues[vi]. Only limited information on the distribution if PBDEs is available for the Southern Hemisphere, however, elevated levels of PBDEs in pork fat were detected during the routine screening for organochlorine pesticide residues. More recently an investigation of breast milk for PBDE levels[vii] also demonstrated that levels were comparable with those in the Northern Hemisphere. BFRs are not manufactured in Australia but it has been estimated that over 500 tonnes are imported yearly of which 340 tonnes are PBDEs.[viii] In addition, the amount of PBDEs that are contained in imported articles used both in domestic and industrial applications is unknown. In this paper, we report levels of PBDEs in a range of different Australian fauna that show that these POPs have indeed become widely distributed both in terms of the types of the fauna but also the levels determined.

Materials and methods

Fauna, except fish, were collected opportunistically from recently deceased animals in locations throughout Australia. Appropriate wildlife permits allowing scientific research were obtained prior to collection. Personnel from the National Parks and Wildlife Service, relevant State Government employees in natural resource management positions, wildlife rescue and recovery organisations and individuals from educational institutions were among the network used to collect the respective samples. No live animals were collected. The overall project manager was David Ellis from the Commonwealth Scientific and Industrial Research Organisation (CSIRO).

Marine Mammals

Whale species including Sperm whales *(Physeter catodon)*, long-finned Pilot Whale *(Globicephala melas)* and a Gray's beaked whale *(Mesoplodon grayi)* stranded along the Tasmanian coast had previously been analysed for dioxins and dioxin-like PCBs and were low compared to levels reported in the Northern Hemisphere.[ix].

Terrestrial Mammals

Tasmanian devils (*Sarcophilus harrisii*) are one of the world's largest surviving carnivorous marsupials. While they were once distributed throughout Australia they are now only found in Tasmania. The devil is mainly a scavenger and feeds on whatever is available. Powerful jaws and teeth enable it to completely devour its prey - bones, fur and all.

Avian

A black shouldered kite (*Elanus axillaris)*, peregrine falcons (*Falco peregrinus)*, Kestral (*Falco cenchriodes)* were collected from South Australia with a sparrow hawk (*Accipiter cirrhocephalus*) being collected from Western Australia. These species are all small birds of prey feeding mainly on other birds, small mammals and reptiles.

Fish

Atlantic salmon (*Salmo salar*) and Ocean Trout (*Oncorhynchus mykiss*) cutlets were purchased from the fresh fish section of a local supermarket.

PBDE Analyses

Standards were all purchased from Wellington Laboratories (Ontario, Canada) and were used for calibration, quantification and determination of recovery of PBDEs. 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 and basic alumina.

Sample preparation

Acid digestion was used for birds (breast meat), fish and Tasmanian devil samples to extract the lipid prior to clean-up. Briefly, this involves the addition of concentrated hydrochloric acid and tumbling overnight with a dichloromethane/hexane (25/75) solvent mixture. After centrifugation the organic phase was removed and then the extraction process repeated with two more portions of solvent. The solvent extracts were combined into a tared vessel and then the solvent removed under vacuum until constant weight is achieved.

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.

Between 1-5g of the extracted lipid was spiked with the respective PBDE isotopically labeled $^{13}C_{12}$ surrogates (MBDE-MXE) and dissolved in hexanes. The extracts were first cleaned up using multiple extractions with concentrated sulfuric acid until the hexanes remained clear. The hexanes extracts were washed several times with water and dried through clean anhydrous sodium sulfate. The extracts were then concentrated prior to clean-up on the Power-Prep™ system. The elution through the different columns is computer controlled and requires applying the hexanes extract first onto the multi-layer silica and using hexanes at a flow rate of 10 mL/min onto the second column consisting of alumina. Dichloromethane:hexanes (2:98) at 10 mL/min was used initially and then the solvent strength was modified to dichloromethane:hexanes (50:50). This fraction contains the PBDEs and is concentrated under vacuum with the respective recovery standard (BDE-CVS-EISS BDE139L) added and then further concentrated using clean dry nitrogen to a final volume of 50 µL prior to HRGC/HRMS analysis.

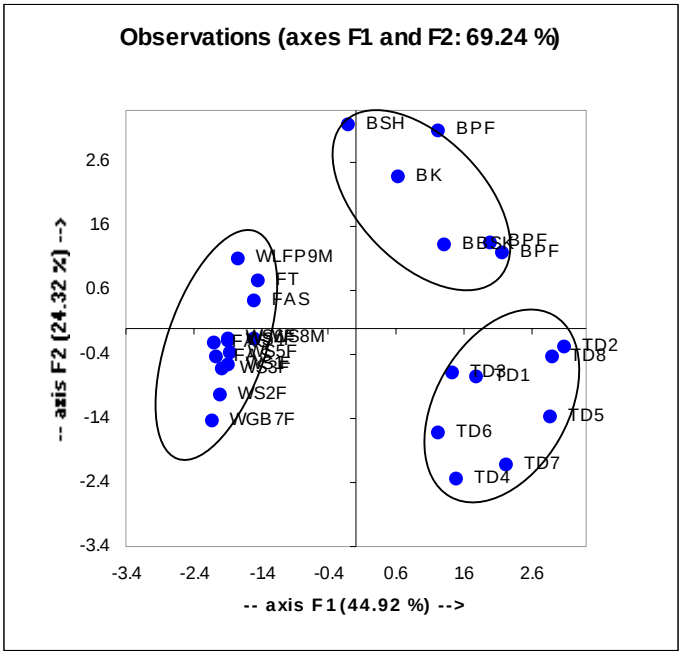
Gas Chromatography High-Resolution Mass Spectrometric (GC-HRMS) Analysis

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 with ultra-high purity Helium as the carrier gas. A flow rate of 1.0 mL/min was maintained throughout the chromatographic run. The temperature program for the PBDE analysis was: 110°C (isothermal for 1 min.) then ramp 1 to 200°C at 15oC/min, ramp 2 to 300°C at 3°C/min and then ramp 3 to 310°C (isothermal 8 min) at 15°C/min. A 1µL splitless injection with an injector temperature of 280°C for PBDE analysis was employed for all standards and sample extracts. The mass spectrometer operating conditions were: ion source and transfer line temperatures, 240°C and 280°C, respectively; ionisation energy 45eV, filament current 0.7mA and electron multiplier voltage set to produce a gain of 10^6. 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 appropriate ions for native and labelled compounds. Individual congeners are identified using the GC retention time and ion abundance ratios with reference to internal standards.

Results and Discussion.

The following PBDE congeners were tested BDE17, BDE28, BDE47, BDE49, BDE66, BDE71, BDE77, BDE85, BDE99, BDE100, BDE119, BDE126, BDE138, BDE153, BDE154, BDE183. Those congeners detected at levels greater than three times the level found in the blank and that passed retention time and ion ratio quality assurance criteria were reported. Levels determined for the major congeners in the various samples are shown in Table 1. Generally levels are low but the distribution of congeners between the different classes of animals was different and therefore principal component analysis was used to endeavour to further elucidate this observation. The data was normalised and analysed using XLSTAT (Addinsoft, Paris, France). Figure 1 shows a distinct clustering of the bird data (B..) from the Tasmanian Devil (TD.) samples with the Whale (W..) and the fish data (F.) co-existing. This may suggest different sources of the PBDEs or in fact discrete bioaccumulation by the different species studied.

Future research will be conducted to determine the levels of the PBDE in other biotic compartments as well as extend the nature of the PBDEs to include BDE209.



	BDE28	BDE47	BDE49	BDE99	BDE100	BDE153	BDE154	BDE183	ΣBDE
Sperm Whale Female	0.22	2.31	1.37	0.68	0.36	0.14	0.59	<0.05	5.67
Sperm Whale Female	0.22	2.29	1.62	0.52	0.30	0.11	0.46	<0.05	5.52
Sperm Whale Female	0.16	1.75	1.57	0.50	0.30	0.13	0.53	<0.05	4.93
Sperm Whale Female	0.14	1.77	1.20	0.53	0.34	0.14	0.57	<0.05	4.70
Sperm Whale Female	0.19	1.76	0.86	0.57	0.35	0.10	0.42	<0.05	4.26
Sperm Whale Female	0.21	2.23	1.28	0.73	0.46	0.15	0.72	<0.05	5.78
Beaked Whale Male	0.40	3.15	2.95	0.70	0.46	0.15	0.56	<0.05	8.36
Sperm Whale Male	0.20	1.89	0.85	0.95	0.30	0.11	0.29	<0.05	4.58
Long Finned Pilot Whale Female	1.19	19.0	2.88	6.15	4.71	3.09	11.3	<0.05	50.3*
Atlantic Salmon	1.33	15.6	4.12	2.08	5.92	0.52	0.92	<0.05	30.5
Ocean Trout	0.22	3.63	0.80	1.43	1.16	0.24	0.34	<0.05	7.8
Atlantic Salmon	0.35	5.55	1.23	1.94	1.56	0.32	0.41	<0.05	11.4
Atlantic Salmon	1.29	18.1	4.63	1.90	5.39	0.58	1.19	<0.05	33.9
Peregrine Falcon	0.33	3.04	1.00	17.9	4.81	51.5	10.6	8.3	97.5
Sparrow Hawk	0.17	4.77	2.58	11.4	7.04	5.91	2.80	0.90	35.6
Kestral	0.09	0.94	0.06	2.01	1.09	2.00	0.35	0.21	6.7
Peregrine Falcon	0.16	15.7	3.11	82.3	27.1	65.9	15.7	11.1	221
Black Shouldered Kite	0.10	0.07	<0.05	0.11	0.03	0.11	0.01	0.03	0.38
Tasmanian Devil	<0.05	0.20	0.01	0.17	0.05	0.61	0.02	0.08	1.16
Tasmanian Devil	<0.05	1.95	0.11	0.90	0.33	5.90	0.07	1.85	11.1
Tasmanian Devil	<0.05	2.14	0.11	0.50	0.17	4.16	0.21	0.20	7.5
Tasmanian Devil	<0.05	0.11	0.01	0.06	0.01	0.31	0.01	0.04	0.56
Tasmanian Devil	<0.05	0.15	<0.01	0.13	0.02	3.83	0.01	0.06	1.02
Tasmanian Devil	<0.05	0.22	0.04	0.08	0.03	0.59	0.01	0.02	1.02
Tasmanian Devil	<0.05	0.01	0.01	0.05	0.02	0.60	<0.01	0.02	0.74
Tasmanian Devil	<0.05	0.87	0.05	0.40	0.14	2.53	0.03	0.74	4.79

* ΣBDE also contains contributions from BDE66 1.38 ng/g, BDE119 0.31 ng/g and BDE126 0.19 ng/g.

Table 1.
Concentrations (ng/g lipid) of PBDEs in Cetacean Blubber, Salmon, Birds of Prey and Tasmanian Devils from Australia

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