POLYBROMINATED BIPHENYLS IN TASMANIAN DEVILS AS DETERMINED BY GC/ECNI-MSMS AND GC/EI-HRMS MEASUREMENTS

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Introduction

Tasmanian devils (*Sarcophilus harrisii*) are Australia's only nocturnal marsupial carnivore often feeding on carrion, lives for about six years and are about the size of a small dog. They were once widely distributed on the mainland but are now restricted to Australia's only island State of Tasmania whose pristine wilderness areas were inscribed in 1992 on the World Heritage List. Devil Facial Tumour Disease (DFTD) was first detected in 1996 and has spread widely throughout the Tasmanian devil population with major declines such that the devil is now considered 'a species at risk'. DFTD is a fatal infectious cancerous disease transmitted as allografts between fighting animals. In the course of investigating the reasons of DFTD, the contamination status of Tasmanian devils with anthropogenic pollutants was investigated[1]. In this study, we focused on the PBB contamination in Tasmanian Devil samples from DFTD diseased and non-diseased animals. PBBs have been used as flame-retardants in textile, electronic equipment, and plastics[2,3]. Mixtures with three different degrees of bromination have been marketed. Technical hexabromobiphenyl (THBB), which has a bromine content of 76% was mainly used in the USA. In Europe, technical octabromobiphenyl (TOBB, 81% bromine) and/or technical decabromobiphenyl (TDBB; 85%, bromine) played a larger role[4]. This is reflected by the PBB residue pattern in biota. While hexaBBs are dominating in a range of marine samples, the isomer patterns was varied if residues originated from THBB on one hand (e.g. marine mammals from North America[4]), or from TOBB and TDBB on the other hand (e.g. marine mammals and birds from Northern Europe[4,5]). This can be determined by the ratio of the abundance of PBB 153 (**Figure 1a**, major congener in THBB) versus PBB 154 or PBB 155 (**Figure 1b & 1c**, major metabolites of TOBB and TDBB[6]). It is noteworthy that PBB 153 is dealt as a new candidate for a global ban under the persistent organic pollutant (POP) mandate[7]. According to our knowledge PBBs were never manufactured or imported into Australia as industrial chemicals but their import contained in articles cannot be ruled out [8]. Samples were first analyzed for PBB 153 by GC/EI-HRMS-SIM using $^{13}C_{12}$-PBB 153 as internal standard. Thereafter, the PBBs in the extracts were also determined using GC/ECNI-MSMS-SRM. These comparisons were used for intercalibration of results obtained from both techniques followed by the determination of the residue pattern.

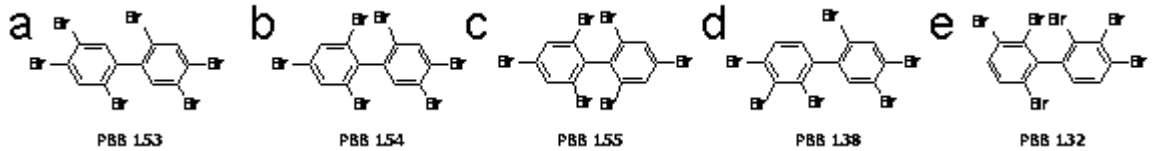

Figure 1: Structures and short terms of the hexabromobiphenyls determined in this study

Material and Methods

Chemicals and standards. THBB (lot number: NT01671) was obtained from ULTRA Scientific (North Kingstown, USA). Individual PBB standards were synthesized or isolated from TOBB or products of the photolysis of PBB 209[6,9]. $^{13}C_{12}$-PBB 153 was purchased from Wellington Laboratories (Guelph, Canada).

Samples and sample preparation. Tissue samples were collected between 2003 and 2007 from diseased (8) and non-diseased (8) animals, at post-mortem, frozen at -20 °C until they were sent, de-identified, to the National

Measurement Institute in May 2007. The animals selected were between two and five years of age of both sex and from different geographic locations throughout Tasmania.
All tissue samples were digested overnight with concentrated hydrochloric acid. Extraction of the lipid was then performed three times using dichloromethane:hexane (25:75) with end over end tumbling. Approximately 10 g of the extracted lipid was spiked with a known quantity of $^{13}C_{12}$ surrogates and analysed by isotope dilution HRMS for thirty-four PBDEs and 2,2',4,4',5,5'-hexabrominated biphenyl (PBB 153). The detailed analytical method have been previously described[10] and is based upon USEPA Method 1614.

GC/EI-HRMS-SIM analyses. Analyses were carried out with 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 DB5 column (10 m x 0.1 mm i.d. x 0.1 µm d_f) was used with the following GC oven program: after 2 min at 120 °C, the temperature was raised at 15 °C/min to 230 °C then 5 °C/min to 320 °C for 5 minutes. The total run time was 27 min. Injections of 1 µL were performed in splitless mode (split opened after 0.5 min). He (purity 99.9990%) was used as carrier gas with a constant flow of 0.4 mL/min. 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 for native and labelled compounds. For PBB 153 *m/z* 467.7006 and *m/z* 465.7026 were used as quantitation and confirmation ion with *m/z* 475.7449 and *m/z* 477.7429 used for $^{13}C_{12}$-PBB 153. To avoid any issues from potential contribution of BDE 154 *m/z* 483.6956 and *m/z* 481.6976 were chosen so that a resolution of 10,000 would be adequate to mass resolve the two co-eluting compounds.

GC/ECNI-MSMS-SRM analyses. Analyses were carried out with a 3800/1200 GC/MS system (Varian) according to von der Recke *et al.*[6]. A CP-Sil 8ms column (30 m x 0.25 mm i.d. x 0.25 µm d_f Factor Four®, Varian) was installed in the GC oven whose temperature was programmed as follows. The injection temperature of 50 °C was held for 2 min, then the temperature was raised at 10 °C/min to 300 °C which was maintained for 38 min. Injections were performed in splitless mode (split opened after 2 min). He (purity 99.9990%, Sauerstoffwerke, Friedrichshafen, Germany) was used as carrier gas with a constant flow of 1.2 mL/min. Methane (purity 99.995%, pressure ~8.5 Torr; Air Liquide, Bopfingen, Germany) and Ar (purity 99.995%, pressure 1.5 mTorr, Linde, Leuna, Germany) were used as reagent and collision gases, respectively. The electron energy was set at 70 eV, and the ion source temperature at 150 °C. The collision voltage was set at 12 V and the detector voltage at 1800 V. GC/ECNI-MSMS in the selected reaction monitoring mode was based on the fragmentation of the most abundant isotope peak of the respective molecular ion (*m/z* 233.0, *m/z* 310.9, *m/z* 390.8, *m/z* 469.7, *m/z* 548.6, *m/z* 627.5 (hexaBBs), *m/z* 706.5, *m/z* 785.4 for PBBs and *m/z* 515.7 for the internal standard 2´-MeO-BDE 68 (BC-2) with a peak width set to ± 3.0 u) as precursor ions. The bromide isotope ions (*m/z* 80 with a peak width of ± 1.5 u) were used as product ions.

Results and Discussion

Usually, PBBs are analyzed by GC/EI-MS-SIM using the most abundant isotope peaks of the molecular ion or by GC/ECNI-MS using the bromide ion isotopes[3]. Initial measurements in this study were carried out by isotope dilution analysis in the GC/EI-HRMS-SIM mode. The combination of high resolution analysis along with isotope labelled $^{13}C_{12}$-PBB 153 provided excellent S/N ratios paired with a high quality of the determination.
By contrast, it was reported that conventional GC/ECNI-MS-SIM using the bromide isotope ions is often not selective enough for the determination of PBBs because this method gives also response for PBDEs which are often more abundant than PBBs. GC/ECNI-MSMS-SRM was found to be suitable for a sensitive PBB determination[6]. The SRMs used for PBB determination are based on the transfer from M^- to Br^- [6]. In addition, this method allows for the use of ^{13}C labelled standards which is not possible when conventional GC/ECNI-MS is used[11]. As can be seen from **Figure 2**, co-eluting native and carbon-labelled PBB 153 did not interfere with each other. Thus, the isotope dilution technique is suitable for this technique. Impurities of PBB 153 in the blank sample were at least one order of magnitude lower than the lowest content of PBB 153 in any sample. In the first part of the study we compared the ratio of $^{13}C_{12}$-PBB 153 to native PBB 153 as determined by GC/EI-HRMS-SIM and GC/ECNI-MS-SRM.

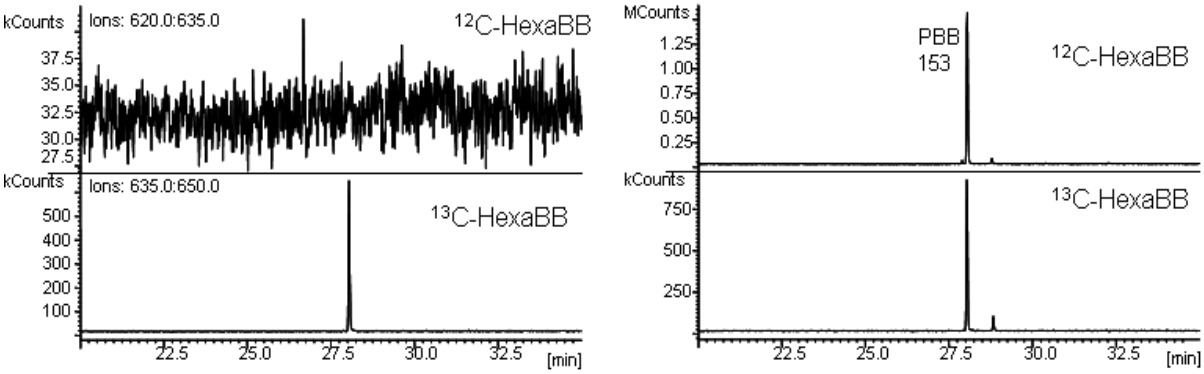

Figure 2: GC/ECNI-MSMS-SRM measurements of $^{13}C_{12}$-PBB 153 (left panels) and sample spiked with $^{13}C_{12}$-PBB 153 (right panels). The upper panels show the SRM typical of native, the lower those of labelled PBB 153.

The ratio of native PBB 153 to labelled $^{13}C_{12}$-PBB 153 determined by GC/ECNI-MSMS matched the results received by using GC/EI-HRMS (**Table 1**). Both methods are adequate for the determination of PBBs. In all samples, hexabromobiphenyls (hexaBBs) were dominating. Only traces of penta- and heptaBBs were detected but could not be quantified. The relative amounts of hexaBBs (**Table 1**) are corrected for different responses in GC/ECNI-MSMS [4]. Multiplication of the amount of PBB 153 results in quantitative data as listed in **Table 1**.

Table 1: Concentrations (pg/g lipids) of polybrominated biphenyls in samples from Tasmanian Devil Fat

Sample no.	Ratio EI-HRMS/ECNI-MSMS-SRM	GC/ECNI-MSMS-SRM	GC/EI-HRMS-SIM					
healthy animals		**153**	**153**	**138***	**132***	**155***	**154***	**sumPBBs**
N07/020715	1.00	1530	1530	28	21	1.5	1.5	1580
N07/020716	1.02	630	640	15	13	1.3	0.6	670
N07/020717	1.00	910	910	10	11			930
N07/020718	0.99	6630	6560	79	39	-	-	6680
N07/020719	1.06	310	330	4.6	3.3			340
N07/020720	0.99	1130	1120	12	16			1150
N07/020721	0.95	11000	10400	160	21	21	21	10600
N07/020722	-**	-**	330	4.6	4.0			340
Affected with DFTD								
N07/020723	1.00	1100	1100	25	21			1150
N07/020724	1.10	2925	3220	174	74	3.2	6.4	3580
N07/020725	1.03	3430	3520	155	42	3.5	3.5	3720
N07/020726	-**	-**	510	19	1.0			530
N07/020727	1.02	430	440	5.7	10			460
N07/020728	0.86	570	490	17	9.3			520
N07/020729	1.01	1790	1810	22	82	42	36	1990
N07/020730	1.06	1150	1220	43	3.7			1270

* ratios relative to PBB 153 determined with GC/ECNI-MS as previously shown [4]. Concentrations based on PBB 153 level as determined with GC/EI-HRMS

** not quantified

PBB 153 was the dominating PBB in all samples, and generally contributed with >90% to sum-PBBs. In addtion, PBB 138 (**Figure 1d**) and PBB 132 (**Figure 1e**) were the second and third most relevant congeners.
By contrast, PBB 154 and PBB 155 were only found at very low amounts in a few of the samples (**Table 1**). These two congeners are indicators for the previous use of technical octabromobiphenyl and technical decabromobiphenyl[6]. The virtual absence of PBB 154 and PBB 155 indicates that PBB residues in these samples almost exclusively originate from the previous use of technical hexabromobiphenyl. It is likely that this is valid for larger parts of Australia if not the whole continent. This result is supported by the detection of PBB 132 and PBB 138 which are minor congeners of technical hexabromobiphenyl. On the other hand, PBB 149 and PBB 167 which are both present in THBB were not detected in the Tasmanian devils (**Figure 3**).

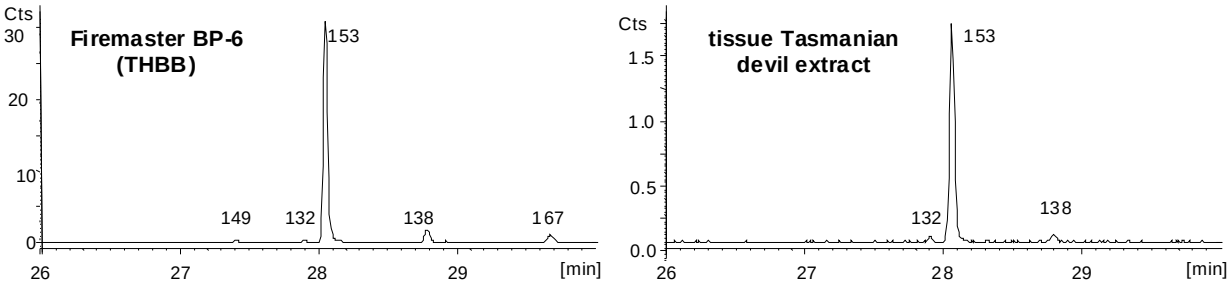

Figure 3: GC/ECNI-MSMS-SRM chromatograms of technical hexabromobiphenyl (left) and a purified extract of Tasmanian devil from Australia

Comparing the PBB burden of healthy and DFTD-affected animals led to no significant difference. While the mean value was higher in healthy animals (2790 vs. 1650 pg/g lipids), the median was lower in healthy animals (1040 versus 1210 pg/g lipids). Based on the samples number analyzed, there is no link between PBB concentrations and DFTD.
Supplementary GC/ECNI-MS analyses in the SIM and full scan modes clarified that all detected organobromine compounds originated from PBBs or polybrominated diphenyl ethers (PBDEs). For instance, the flame-retardant 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE) was not detected in the samples (< 1 pg/g lipids). Surprisingly, PBB 153 reached similar concentrations as were found for the dominating PBDE congeners. As stated above, no information exists on the previous use of PBBs in Australia. Our analyses confirm that environmental PBB residues need to be studied more in details. It became also clear that both GC/EI-HRMS-SIM and GC/ECNI-MSMS-SRM are complimentary methods for the determination of PBB residues in biota.

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