HYDROXYLATED PBDE METABOLITES IN HUMAN BLOOD

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Introduction

Polybrominated diphenyl ethers (PBDEs) have been known as potential environmental contaminants for 30 years[1] and indeed as contaminants since the early 1980's[2]. Very much of the PBDE history is given in the review on brominated flame retardants (BFRs) presented at the 25th anniversary of the Dioxin Symposium series 2005[1] and is excluded herein. The large commercial production of PBDEs, their persistency and bioaccumulative properties have made these contaminants a ubiquitous environmental problem which is not solved by the recent legislative measures in the European Union and voluntary changes in production in the U.S.. It is noteworthy to mention the significant differences in human exposure to PBDEs around the world with one to two orders of magnitude higher concentrations in humans from U.S.A., Canada and Nicaragua[3-5].

Being well known contaminants for so long it is still little known about their metabolism and metabolites so far. Still, several studies have addressed individual PBDE metabolism as reviewed by Hakk and Letche[6]. Thereafter metabolites of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) have been identified[7], a study on a mixture of the major PBDE congeners in rats revealed a set of hitherto unknown metabolites in the rat blood[8] and preliminary data on hydroxylated metabolites of decaBDE (BDE-209) in humans has also been put forward[9]. The mechanisms for formation of hydroxylated PBDE metabolites (OH-PBDEs) are not different from what is known for the metabolism of other aromatic compounds, e.g. polychlorinated biphenyls (PCBs)[10]. However, reductive debromination seem to be an important transformation pathway[11,12]; possibly more pronounced than for PCBs. A major complicating factor in studies of OH-PBDEs is the occurrence of naturally formed poly-brominated phenoxyphenols as these compounds also may be called[13]. Some extensive work on identification of OH-PBDEs, in wildlife and alga particularly, have been pursued so far[14-17].

The aim of the present study is to determine if OH-PBDE metabolites are retained in human blood. This is a study pursued approximately ten years after the discovery of OH-PCB metabolites being retained in humans and wildlife[18]. The human subjects selected for analysis have previously been studied for their blood content of metals[19], several organochlorine compounds and their metabolites, including PCBs and OH-PCBs[20] and PBDEs[3].

Material and methods

Study groups: Blood from Nicaragua children, taken for organohalogen analysis, were analysed. These children were shown to be highly exposed to PBDEs in a previous study[3]. The children are between 12 and 16 years old. They are divided into five different pools[3,20]. The first pool comes from children that had worked for at least four years at the dump and they were also living at the dump. The second pool consists of samples from children that had worked at the dump for at least four years but they lived in Acahualinca. The third and fourth pool comes from children that did not work at the dump. These two pools are divided into two subgroups; pool three that eat fish from Lake Managua and pool four that do not eat fish from the lake. The fifth pool is a reference group from children living in another part of Managua. Also groups of women, exposed to PBDEs by eating fish from Lake Managua, were included in the study group. Four different pooled plasma samples were analyzed. The samples analyzed are presented in Table 1.

Chemical analysis: The chemicals used, extraction of serum, lipid determination, partitioning with an alkaline solution, procedure and analysis have been described in detail elsewhere[21] except that n-hexane was replaced with cyclohexane. The lipid removal step chosen was sulfuric acid treatment for both neutral and phenol-type

Table1. Composition of pools and subjects in each pool .

Pooled samples	*Pooled samples*	*n*
Pool A	Working and living at the dump	8
Pool B	Working at the dump, living in Acahualinca	21
Pool C	Eating fish from Lake Managua, living in Acahualinca	15
Pool D	Do not eat fish from Lake Managua, living in Acahualinca	7
Pool E	Reference group (living in another part of Managua)	8
Pool SF [a]	Young women from San Francisco libre, eating fish.	5
Pool Mat [a]	Young women from Mateares, eating fish	4
Young ref 1[a]	Young women from ref. area not eating fish	3
Old ref 2[a]	Older women from ref. area not eating fish	4
Pool 1	Working and living at the dump	11
Pool 2	Working at the dump, living in Acahualinca	23
Pool 3	Eating fish from Lake Managua, living in Acahualinca	16
Pool 4	Do not eat fish from Lake Managua, living in Acahualinca	10
Pool 5	Reference group (living in another part of Managua)	11

[a] The individuals including in the pool were analyzed individually for PCBs, OH-PCBs and pesticides[20] and PBDEs[3]

substances followed by an additional clean up step on sulfuric acid silica gel columns (1 g). Dichloromethane (10 ml) was applied as mobile phase for the column with any potential OH-PBDEs. Authentic reference standards were available through synthesis in house.

Identification and quantification were performed using a GC-MS Finnigan TSQ 700 (Thermoquest, Bremen, Germany) operating on electron capture chemical ionization (ECNI) mode tracing the bromine ions (*m/z* 79, 81). A DB-5HT column (15 m × 0.2 mm i.d. and 0.1 µm) from Supelco (Bellefonte, USA) was used with temperature program as follows; 80ºC (1min) – 15ºC/min – 300ºC – 2ºC/min – 320ºC (2 min). On-column injections were performed using a septum equipped programmable injector fitted with a high performance insert. The injector temperature was 60ºC and increased with 150ºC /min up to 300ºC for each injection. Helium was used as carrier gas. The transfer line temperature was 290ºC and the temperature in the ion-source was 200ºC.

Results and Discussion

A total of 18 OH-PBDE congeners were indicated in the human plasma analyzed. Two of the hydroxylated PBDEs were substituted with two hydroxyl groups. The chromatographic picture is visualized in Figure 1. Eight of the OH-PBDEs were tentatively identified and quantified by comparison to the authentic reference standards available to us as shown in Table 2. Several of the OH-PBDEs retained in the human plasma corresponded to OH-PBDE metabolites formed from the PBDE-mixture given to rats under experimental conditions[8].

This is, to the best of our knowledge, the first time it has been possible to show OH-PBDE metabolites being retained in the blood of a mammal. The OH-PBDEs previously reported in wildlife blood is related to the naturally occurring OH-PBDEs or possibly to PBDE metabolites to some extent. Our evidence for this conclusion are, *i:* the retention of OH-PBDEs with the same chromatographic properties as those metabolites being formed from major environmental PBDE congeners (BDE-47, 99, 100, 153, 154, 183 and 209)[8]; *ii:* The OH-PBDEs were dominated by *meta*- and *para*-substituted OH-PBDEs (14 out of 16 mono-OH-PBDEs) based on mass spectrometric fragmentation data and *iii:* The significantly lower concentration of OH-PBDEs than PBDEs in the blood (Table 2 and Fäldt et al, 2005[3]) and *iv:* the absence of detectable MeO-PBDEs in the human plasma.

The retention of OH-PBDEs in human blood is to be expected since numerous OH-PCBs have been shown to be retained and regarded to bind to transthyretin (TTR) the thyroxin (T_4) transporting protein[22] and the OH-PCBs have less of a structural resemblance to T_4 than the *meta*- and *para*-substituted OH-PBDEs. The question has more been a matter of having high enough PBDE concentrations in the subjects to be able to detect OH-PBDEs.

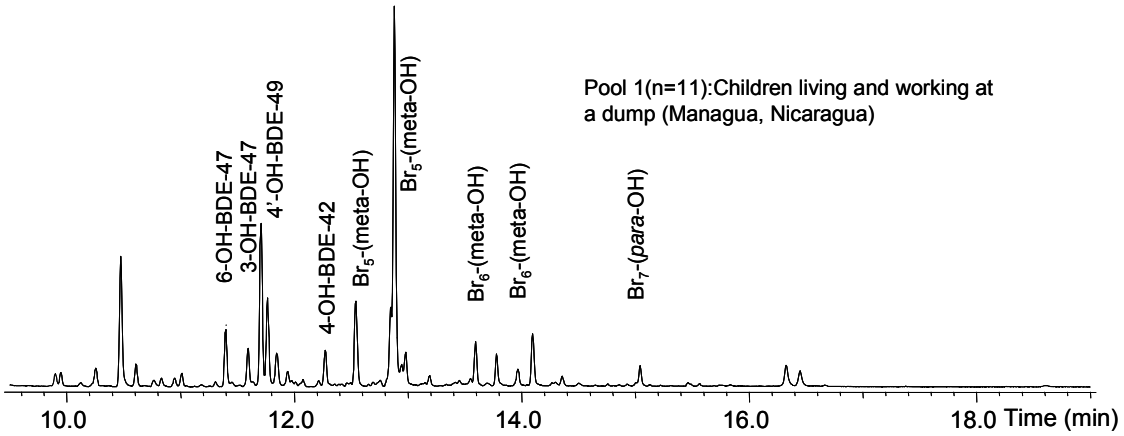

Figure 1. GC-MS chromatogram showing the presence of OH-PBDEs in the phenolic fraction of human blood

Table 2. Concentrations of OH-PBDEs (ng/g fat) in each sample from the city dump study and referents, and in women with varying fish consumption from Lake Managua.

	Fat %	4-OH-BDE17	6-OH-BDE 47	3-OH-BDE 47	4-OH-BDE 49	4-OH-BDE 42	6-OH-BDE 99	4-OH-BDE 90	6-OH-BDE 137
Pools from children working at the dump									
Work, live	0.41	7.2	6.1	3.3	9.4	4.9	0.16	32	3.1
Work, live	0.41	6.8	6.8	2.3	7.3	3.3	0.22	27	2.6
Work	0.38	0.57	2.4	0.43	0.28	0.28	0.14	0.28	1.3
Eat fish	0.39	0.33	1.0	0.16	0.49	0.16	0.08	0.41	0.25
No Fish	0.44	0.19	0.75	0.10	0.19	0	0.10	0.10	0.19
Ref area	0.41	0.24	0.72	0.08	0.24	0	0.08	0.16	0.24
Old pools from children working at the dump									
Pool 1	0.43	1.1	5.8	1.2	2.2	1.4	0.25	9.6	1.6
Pool 2	0.40	0.25	2.3	0.17	0.39	0.43	0.27	0.63	0.78
Pool 3	0.41	0.12	1.2	0.11	0.14	0.11	0.14	0.29	0.04
Pool 4	0.41	0.14	1.1	0.08	0.16	0.00	0.20	0.12	0.23
Pool 5	0.22	0.22	0.76	0.05	0.27	0.00	0.14	0.19	0.18
Pools from women eating fish from lake Managua									
Mateare	0.51	0.16	0.81	0.08	0.47	0.16	0	0.55	0.94
SF libre	0.35	0.06	1.3	0.06	0.11	0.34	0.13	0.51	0.51
Young ref	0.41	0.08	2.1	0.65	0.41	0.41	0.20	0.57	2.0
Adult ref. 1	0.71	0.42	2.0	0.23	0.65	0.17	0.01	0.90	0.25
Adult ref. 2	0.66	0.19	1.9	0.34	0.50	0.22	0	0.87	0.34

It was indeed surprising to find as high PBDE concentrations as reported in the children from Nicaragua[3] and these samples were accordingly screened for OH-PBDEs.

The results obtained are supported by data on competitive binding of some OH-PBDE congeners to TTR, showing higher affinity of those metabolites to the protein than affinity of the natural ligand[23]. Future work includes synthesis of an additional set of OH-PBDE standards with the hydroxyl group in the appropriate positions of the molecule and toxicological studies of the OH-PBDE metabolites.

Acknowledgements
We are grateful for the cooperation of Dr Danilo Hernandez Romero, and the staff from "Dos Generaciones", "Chateles project", and health centres in the study areas during the field work and Ioannis Athanassiadis for skilful mass spectrometry analyses. The study was performed within the framework of a multidisciplinary co-operation project between Lund University and the Autonomous University of Nicaragua (UNAN-Managua), funded by the Swedish International Agency for Research Cooperation with Developing countries, SAREC and faculty support from Stockholm University

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