TISSUE DISTRIBUTION AFTER DEVELOPMENTAL EXPOSURE TO LOW DOSE PBDE-99

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Introduction

Human tissue concentrations of persistent organochlorine compounds (POCs), such as polychlorinated biphenyls (PCBs) and 1,1,1-trichloro-2-2-bis (p-chlorophenyls) ethane (*p,p´*- DDT) peaked in 1970s before their use was banned resulting in decreasing levels[1,2]. In contrast, human tissue levels of PBDEs have been increasing throughout the last decades[1-6]. Experimental investigations in rodents indicate that polybrominated diphenyl ethers (PBDEs) posess a wide-range of dose related effects, mainly through thyroid hormone disruption and neurotoxicity[7-14]. Recently, we demonstrated that developmental exposure to low doses of the 2,2'4,4'5-pentabromodiphenyl ether (PBDE-99) congener (60 µg or 300 µg PBDE-99/kg BW) caused permanent effects in rat offspring as indicated by changes in the male and female reproductive systems and locomotor activity[8,14]. In this paper, we present the tissue concentration levels of PBDE-99 which were associated with the effects published in the above mentioned studies. Our data indicate that the tissue concentrations achieved in the animals studies were not much higher than reported human body burdens .

Methods and Materials

Animals and treatment: Wistar dams were treated by gavage on gestation day six with a single dose of 60 or 300 µg PBDE-99/kg body weight or peanut oil (control). **Tissue concentration:** Tissue concentrations of PBDE-99 were determined in liver and adipose tissue samples from dams and offspring. During lactation, dams and offspring were sacrificed at different time points (PNDs 1, 14 and 22) and tissue (adipose tissue and liver) was pooled based on experimental group. Thus, PBDE-99 concentration was determined in one pool / treatment group / day of sacrifice. The analytical method employed for the quantification of PBDE- 99 and its validation has been described in detail[15]. A brief explanation of the method follows: ***Extraction:*** Prior to extraction, adipose tissue was mixed with a surplus of water-free sodium sulphate (Na_2SO_4) and pulverized in a mortar, and liver was freeze-dried. Then, dried samples were extracted by accelerated solvent extraction (ASE) with toluene 10 (140 bar, 175 °C, 5 static cycles of 5 min.). Internal $^{13}C_{12}$-labelled PBDE-99 standard (Wellington, Berlin, Germany) was added before extraction. The extraction cells (33 ml) were filled up with silica 60 (ICN). A blank sample, consisiting of an extraction cell filled with silica, or in the case of adipose tissue with silica and Na_2SO_4, was added to each series of extractions. Extracts were concentrated to dryness to determine lipid content. ***Clean up:*** Extracts were first purified on a column with silica / 44% concentrated sulphuric acid and eluted with n-heptane. In the second clean up, extracts were purified on a column containing 2.5 g alumina B super I, eluted with 25 ml n-hexane : dichloromethane (DCM) (98:2) and 25 ml n-hexane : DCM (1:1), where the PBDEs are found. ***Separation and detection:*** Before GC/MS measurement, recovery standard was added. Separation was performed by GC (gas chromatography) on a DB-5MS capillary column (15 m, 0.25 mm inner diameter, 0.1 µm film thickness) and detection was performed with EI-MS (electron impact ionization – mass spectrometry) in the selected ion monitoring (SIM) mode with the following masses: 404/564 u for native and 416/576 u for $^{13}C_{12}$-labelled PBDE-99. The maximum variation of the intensity of the ratio masses (406/566 u and 418/578 u, respectively) was allowed to be 15% of the theoretical value. For quantification, actual response factors were determined by three point calibration prior to each sequence of samples.

Table 1: PBDE-99 concentration in lactating dams after a single gestational (day 6) exposure to low dose PBDE-99.

PND		Adipose Tissue				Liver			
		Control	PBDE 60	PBDE 300	Lipid content (%)	Control	PBDE 60	PBDE 300	Lipid content (%)
1	Lipid weight	3.63	306	1994	76	6.03	230	1170	1.7
22	Lipid weight	4.23	421	2310	60	2.02	18.6	431	7.6
1	Wet tissue weight	2.84	221	1540	-	0.10	4.14	20.4	-
22	Wet tissue weight	2.29	259	1490	-	0.03	0.27	6.80	-

Values are expressed as ng/g.

Results and Discussion

Tissue distribution data are important for the interpretation of toxicological experiments as they are particularly useful for the extrapolation of animal experimental data to humans (risk assessment) and for investigation of species differences[16]. This issue is critical when evaluating the effects of environmental contaminants and often the tissue concentrations of test substances are not determined in reproductive and developmental toxicity studies due to the prohibitive costs of such analyses. The data on tissue concentrations of PBDE-99 presented in this study provide a relevant contribution to human risk assessment since a reliable comparison between animal experiments to human exposure can be performed. The doses of 60 µg or 300 µg PBDE-99/kg BW are pertinent to human exposure levels because a study by Schecter *et al.* found a mean level of 14.0 µg PBDE-99 /kg fat in human breast adipose tissue with a range from 0.7 to 111.0 µg PBDE-99 /kg fat[6]. Significant amounts of the parent compound were found in offspring tissue at the end of lactation, clearly indicating that body burdens of PBDE-99 continued after weaning (Table 2). These data confirmed our hypothesis that gestational administration to a single dose of PBDE-99 leads to a long term exposure. *Geyer et al* (2004) reported that the terminal total body elimination half-life for PBDE-99 in humans is 2.9 years and in adult rat adipose tissue, the half-life for the same compound was calculated to be 41.6 days in females[17]. In our study, the high tissue concentration of PBDE-99 found in dams and offspring at the end of lactation (approximately 37 days after single exposure) (Table 1 and 2) corroborates the data from *Geyer* et al (2004), indicating that PBDE-99 possesses a long half-life. A general overview of tissue distribution in adipose tissue and liver can be obtained by calculating the ratio between PBDE-99 levels in both compartments. Through this calculation, we observed that over lactation, the ratios vary according to the dose administered. On PND 1, the ratio of PBDE-99 concentration (adipose tissue : liver) was 55 for the 60µg/kg group and 77 for the 300 µg/kg group. However, a dose specific tissue distribution was observed on PND 22 with the adipose tissue : liver ratio being higher in the PBDE 60µg/kg group (863) than the PBDE 300µg/kg group (212). In other words, over lactation, PBDE-99 elimination from hepatic tissues is faster in the 60µg PBDE-99/kg group than in animals exposed to 300µg PBDE-99/kg. This also indicates that PBDE-99 accumulation in adipose tissue is much higher than in liver. This type of distribution exhibited by PBDE-99 is contrary to what has been observed for other persistent organohalogen compounds, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and other 2,3,7,8-chlorinated dibenzo-p-dioxins and dibenzofurans and 2,3,7,8-tetrabromodibenzo-pdioxin (TBDD) in rodents where accumulation in liver was much higher than in adipose tissue[18-20]. Investigations of persistent environmental compounds which administer high doses to animal models may not be suitable for human risk assessment because the tissue distribution/elimination varies according to the dose as has been demonstrated by other authors[17] and in this study. Therefore, the human exposure (to very low doses) scenario differs immensely from experimental animal studies employing repeated high dose protocols. We compared the tissue concentration of PBDE-99 in rat dams with human breast milk levels reported in U.S. mothers[6]. For this purpose, the tissue concentration of PBDE-

99/ng lipid found in adipose tissue from dams on PND 1 (Table 1) was plotted together with the mean and maximum PBDE-99/ng lipid reported in human milk by Schecter *et al*., 2003 (Figure 1). The tissue concentrations achieved in the present study are about 22- fold and 142-fold higher than the average concentration of PBDE-99 found in human milk (Figure 1). If we take into account the highest reported concentration of this PBDE congener in human breast milk, the tissue concentrations in this experimental study are only about 3-fold and 18-fold higher than levels found in humans. The effects on the male and female reproductive systems and locomotor activity which we described in previous studies [7,14] occurred, therefore, at tissue level concentrations very close to those described for humans.

Table 2: PBDE-99 concentration in offspring after a single gestational (day 6) exposure to low dose PBDE-99.

PND		Adipose Tissue				Liver			
		Control	PBDE 60	PBDE 300	Lipid content (%)	Control	PBDE 60	PBDE 300	Lipid content (%)
1	Lipid weight	-	-	-	-	9.68	571	2960	5.5
14		6.36	323	3590	45	6.35	291	1890	2.2
22		8.93	170	1580	64	22.1	169	1260	2.4
1	Wet tissue weight	-	-	-	-	0.54	32.3	162	-
14		2.57	169	1550	-	0.13	5.86	46.5	-
22		5.77	99.4	1070	-	0.50	4.45	29.8	-

Values are expressed as ng/g.

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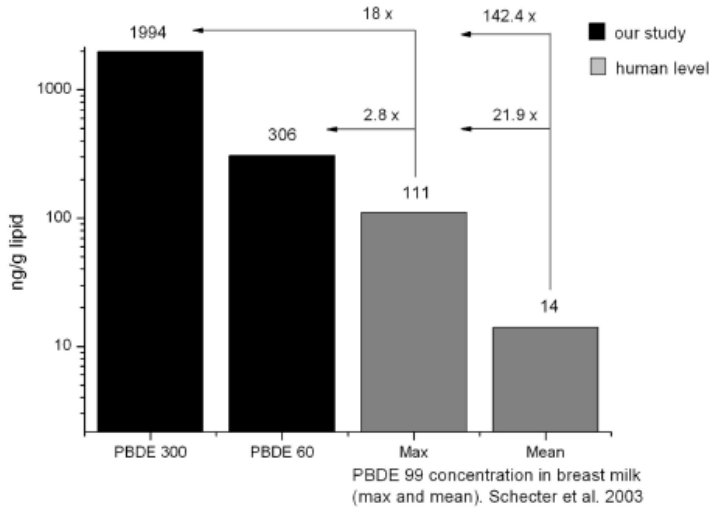
Figure 1: Tissue concentration of PBDE-99 in rat adipose tissue compared to human milk fat levels reported in U.S. women. human milk values (mean and maximum) were taken from from Schecter *et al*. 2003.



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