Polybrominated diphenylethers in human adipose tissue and relation with watching television - a case study

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Introduction

Polybrominated diphenylethers (PBDEs) are used as flame retardants. Due to more stringent fire regulations in many countries and the increased use of plastic materials, the use of flame retardants in general has increased over the last decade. In 1992, 600,000 tonnes of flame retardants of which 150,000 tonnes were brominated compounds, were used world-wide. The total consumption of PBDEs was 40,000 tonnes, where it was less than 20,000 tonnes in 1984 (1). PBDEs have been found in considerable concentrations in many sediments and aquatic organisms (2).

In 1990 the International Agency for Research on Cancer (IARC) concluded that there was limited evidence for carcinogenicity, indicating that DeBDE, at present exposure levels, does not present a carcinogenic risk for humans (3). The acute toxicity of these PBDEs for laboratory animals is low (LD_{50}>1 g/kg body weight). The dermal toxicity of PeBDE in rabbits is also low. DeBDE and OcBDE are not skin irritants (3). PBDEs are listed as compounds that can affect the regulation of thyroid and steroid hormones (3). The results for mutagenicity of PeBDE, OcBDE and DeBDE were negative (3). PBDEs induced two enzymes which catalyse the conjugation in phase II metabolism (*O*-ethyl-*O*-*p*-nitrophenyl phenylphosphothionate and uridine diphosphate-glucuronosyltransferase). The inducers were not only potent, but the induction was long lasting (4). The DeBDE congener did not cause any enzyme induction (4). Although PBDEs seem to have affinity to the same biological receptors as the polychlorinated biphenyls (PCBs), they did not inhibit the metabolism of CB congeners used as positive controls (5). The US EPA found hyperplasia of the thyroid gland in rats (6). The half-life of PBDEs in humans is unknown, but, because of the presence of the oxygen atom, it is assumed to be less than that of the polybrominated biphenyls (PBBs). Half-lives of ca. four years were found for higher brominated PBBs in rhesus monkeys (7).

In this paper a case study is described in which PBDEs were determined in a biopsy and a blood sample from a young man. In 1982, at age 13, this person watched television and played computer games, using a newly purchased television set in a small non-ventilated, insulated room (27 m^3), for several hours per day during a period of 8 consecutive months. A strong smell was observed during the first weeks of this period, which disappeared later. One month after the start of this period the person studied visited a local physician, complaining of headache, painful lesions on the soles of his feet, dizziness and other symptoms. Normal scalp hair and facial hair was lost and replaced by abnormal hair of a metallic texture and darker colour. The patient associated the altered hair color and texture with the onset of chronic craniofacial pains. Two and a half years later, by mid 1985, the skin of his face became severely affected by acne-like lesions. Later physical examination resulted in the diagnosis of acne vulgaris, severe papulocystic acne of the entire back with hyperpigmentic scarring and slightly enlarged paratoid glands. In 1986 "marfanoid features" were diagnosed, which refers to an abnormality in skeletal proportions. Four years later, a fine postural tremor of hands was diagnosed by a neurologist. In the same year a blood specimen was sent to the French Commissariat of Atomic Energy, where 200 white blood cells were examined for chromosomal abnormalities. Four chromosomal fragments were found, which, according to the report, is consistent with chemical exposure. A karyotype chromosomal test, together with the highly unusual nature of the disorders ruled out a hereditary cause. Among other health effects observed were an enlarged liver and a shrunken gallbladder. Because a relationship between the health effects and the exposure to the vapours from the TV set was supposed, an adipose tissue specimen was surgically removed from the person studied in 1991. It was analysed, together with a blood sample, for PBDEs at the DLO-Netherlands Institute for Fisheries Research. Later, after 13 years of daily use, parts of the TV set were also analysed for PBDEs and other flame retardants at the Institute of Ecological Chemistry and Waste Analysis, Braunschweig, Germany.

Methods and materials

Analyses of human samples. PBDEs were determined in a human adipose tissue sample which was surgically removed from a male Israeli from Jerusalem, aged 21 years.
Details of the analytical method can be found in de Boer (1988) (8). A Bromkal 70-5 DE was used as a standard. This solution contained 2,4,2',4'-tetra BDE, 2,4,5,2',4'-penta BDE and an unknown (x,y') penta BDE in concentrations of 67, 73 and 13 ng/ml, respectively. The final analysis was carried out by gas chromatography/mass spectrometry (HP 5988A, quadrupole) using electron capture negative ionization (GC/ECNI-MS).
Analysis of TV set. Wipe samples were taken with ethanol soaked Kleenex tissues from different inner parts of the TV set (Sharp color TV 2001G, serial no. 511117). It was first planned to take air samples from the inside of the TV housing under operating conditions. Since the TV set was out of order and repair seemed to be coupled with unforeseeable problems, it was decided to simulate working conditions by heating inside of the TV housing with two infrared bulbs to steady state temperatures of 42 -110 °C. Air samples were taken from air inside and outside the TV set. No brominated substances were present in the blank control of outside laboratory air. The elution from the adsorption material of the sampling tubes was done with ethyl acetate. Parts of the side wall were cut off the television set and parts of the back wall (5 g) were taken by a drill. Chips and shavings were collected and extracted in a Soxhlet extractor with toluene for 16 hours. Certain part of the main printed circuit board was separated from electronic devices and cut. The circuit board material was homogenised by a centrifugal mill (ZM1, Retsch, Haan) using a 0.5 mm sieve. 5 g were extracted using n-heptane (8 h) and n-heptane/acetone (2:1, 8 h) in the Soxhlet extractor (9).
Prior to the PBB/PBDE analyses the extracts were cleaned up by passing through chromatography columns (20 g aluminium oxide neutral, deactivated with 2 % water; 5 g Na_2SO_4), eluted with 60 ml n-heptane/ethyl acetate (19:1) and concentrated to 2 ml. PBDE and PBB determination was conducted by two different GC/MS systems (1: GC HP 5890A/MSD HP 5970B; 2: Varian GC 3400/MS Finnigan SSQ 700). Permanent clean up control and quality assurance was done by corresponding examinations using PBDE and PBB standards (PBDE: Bromkal 70-5DE, 79-8DE, 82-0DE, Promochem, Wesel; PBB: Dow FR 250, Amchro, Sulzbach, Germany).
Polar brominated phenols, tetrabromobisphenol-A (TBBP-A) and polybrominated benzenes were determined with GC/MSD after liquid/liquid partition with 40 ml 0.1 M K_2CO_3 and heptane,

and derivatization of the phenols. Polybrominated dibenzo-p-dioxins (PBBDs) and dibenzofurans (PBDFs) were determined by GC/MSD after clean-up using alumina and silica/ sulfuric acid/sodium hydroxide columns.

Results and Discussion

The PBDE concentrations found in the blood and the adipose tissue are given in Table 1. In addition, a cow's milk and a poultry fat sample from Israel were analysed for PBDEs, to determine the possible presence of PBDEs in the local environment (Table 1). The results of the analysis of the TV set are given in Table 2.

Table 1. PBDE concentrations in human adipose tissue and blood, cow's milk and poultry fat from Israel, in µg/kg wet weight

Sample	2,4,2',4'-TBDE	2,4,5,2',4'PeBDE	x,y'-PeBDE	Fat content (g/kg)
Adipose tissue	2	4	1	716
Blood	<0.02	<0.01	<0.02	1.5
Cow milk	<0.02	<0.01	<0.02	19
Poultry fat	*<0.8*	*<0.1*	*<0.5*	*930*

Table 2. PBDE concentrations in various samples taken from the TV set.

PBDE	Back wall wipe (µg/m²)	Side wall wipe (µg/m2)	Circuit board wipe (µg/m2)	Air inside (ng/m3)	Circuit board (µg/kg)
TeBDE				39	46
PeBDE I				10	<0.02
PeBDE II				26	<0.4
PeBDEIII				51	
HxBDE I			<0.4	52	3.1
HxBDE II			0.3		14
HpBDE		3			
NoBDE I	15	<1			
NoBDE II	43	<10			
DeBDE II	40	<10			

I, II, III: different single isomers

The presence of PBDEs in human adipose tissue of the person studied has unambiguously been determined (Table 1). PBDEs could not be detected in the blood sample of the person, nor in the cow's milk and poultry fat samples. The PBDE levels found in the adipose tissue correspond with PBDE levels found in German human breast milk, 0.6-11 µg/kg lipid weight (10). However, since there was a period of almost 10 years between exposure and sampling, the original PBDE concentration in the adipose tissue may have been much higher. Based on 4-24-fold differences in half-lives of PBBs and PBDEs, a worst-case calculation would lead to a much higher original PBDE level, in the order of mg/kg -g/kg wet weight.

It is known that a delayed ossification of normally developed bones in rats only occurs at high dose levels of octa BDE (25-50 mg/kg body weight) (3). Dietary levels of 100 mg/kg octa DBE led to increased liver weights in rats (3). All doses mentioned seem to be relatively high. This would mean that the effects found could have been caused by the PBDEs themselves only if we would assume that the original PBDE level shortly after exposure would have been in the order of mg/kg to g/kg. The confirmation of the presence of the PBDEs in the adipose tissue, possibly originally in relatively high concentrations, the type of health effects, and the similarities with

health effects observed in Yusho victims are strong arguments in favour of the hypothesis that exposure to vapours from the television set has caused the observed dramatic health effects. The work of Zelinski *et al.* (11) confirms the risks of the use of PBDEs as flame retardants in television sets. Because of the person studied was an adolescent, and fasted for considerable periods, effects may have been intensified.
On the other hand, the PBDE concentrations found in the TV set and inside-air samples are not extremely high and a cause-effect relationship between these concentrations and the effects observed is difficult to prove. It is possible that certain parts in the electronic circuit board would contain higher concentrations of PBDEs, but it would be a tremendous work to analyse all these parts. In addition to the PBDE concentrations in the TV set, other brominated compounds were found in the printed circuit board of the TV set such as several mono-tribrominated phenols at mg/kg levels as well as tetrabromobisphenol-A (TBBP-A) in a concentration of 266 mg/kg. However, due to its polar character TBBP-A was not emitted to the inside and outside air of the TV set. PBBDs and PBDFs were not found at a level of 3-8 ng/m^2 in wipe samples.

Conclusions

The presence of PBDEs in the adipose tissue of this individual, the presence of these compounds in the TV set, the result of the study on chromosomal abnormalities indicating chemical exposure, and the similarities between the observed health effects and effects observed with Yusho patients point to a possible relationship between the exposure to vapours from the TV set and the observed health effects. However, the time lag between exposure and analysis, and the PBDE concentrations in the TV set, which were not extremely high, hinder a proof of such a relationship. Possibly, parts of the electronic circuit board which have not been analysed until now, have played a certain role in the process. Other brominated flame retardants such as TBBP-A present in the TV set may also have played a role. The similarities in the toxicity and behaviour of PBDEs with PCBs, which have been banned for many years should trigger a re-evaluation of the production and use of these compounds. The same is true for other brominated flame retardants such as TBBP-A.

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