Toxicology Letters 179 (2008) 6–14

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

A 28-day oral dose toxicity study in Wistar rats enhanced to detect endocrine effects of decabromodiphenyl ether (decaBDE)

Leo T.M. Van der Ven[a,*], Ton van de Kuil[a], Pim E.G. Leonards[b], Wout Slob[c], Rocío F. Cantón[d], Silke Germer[e], Theo J. Visser[f], Sabina Litens[g], Helen Håkansson[g], Dieter Schrenk[e], Martin van den Berg[d], Aldert H. Piersma[a], Josephus G. Vos[a,1], Antoon Opperhuizen[a]

[a] Laboratory for Health Protection Research, National Institute of Public Health and the Environment (RIVM), P.O. Box 1, 3720 BA Bilthoven, The Netherlands
[b] Institute for Environmental Studies, Vrije Universiteit Amsterdam, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands
[c] Centre for Substances and Integrated Risk Assessment, RIVM, P.O. Box 1, 3720 BA Bilthoven, The Netherlands
[d] Institute for Risk Assessment Sciences (IRAS), Utrecht University, P.O. Box 80.176, 3508 TD, Utrecht, The Netherlands
[e] Food Chemistry and Environmental Toxicology, University of Kaiserslautern, D-67663 Kaiserslautern, Germany
[f] Department of Internal Medicine, Erasmus Medical Centre, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands
[g] The Institute of Environmental Medicine, Karolinska Institute, Box 210, 171 77 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 10 September 2007
Received in revised form 3 March 2008
Accepted 4 March 2008
Available online 12 March 2008

Keywords:
Decabromodiphenyl ether
Brominated flame retardants
Endocrine disruption
Reproductive health
Hazard identification
Risk assessment

ABSTRACT

Decabromodiphenyl ether (decaBDE) is a widely used brominated flame retardant, considered to be of low toxicity. However, previous toxicity studies applied exposure methods with low bioavailability of this compound, and the actual hazard of decaBDE for humans, which are environmentally exposed to decaBDE, may thus be underestimated in current risk assessments. The present 28 days oral toxicity study in Wistar rats was designed to facilitate detection of endocrine and immune modulating effects of decaBDE using an exposure protocol with improved bioavailability. A technical preparation of high purity decaBDE was thus tested by daily exposure through gavage with an emulsion of soy phospholipon/lutrol as a carrier. Most sensitive effect in males were increased weight of seminal vesicle/coagulation gland with BMDL of 0.2 mg/kg bw/day and increased expression of hepatic CYP1A and CYP2B (BMDLs 0.5–0.7 mg/kg bw/day). In females the most sensitive effect was decreased activity of P450c17 (CYP17), which is a key enzyme in the androgen synthesis pathway, in adrenals (BMDL 0.18 mg/kg bw/day). These results suggest that decaBDE may represent an as yet unreported hazard for reproductive health.

1. Introduction

Decabromodiphenyl ether (decaBDE) is a brominated flame retardant (BFR) which has its main application in plastics (television cabinets, other electronic and electrical devices) and textile (drapery, upholstery, carpets) industry (Hansen et al., 2002). Current technical mixtures are of high purity, containing 97–98% decaBDE (BDE209), with nonabromodiphenyl ether as a major impurity. With an estimated annual production of about 30,000 tonnes worldwide, of which 7500–11,000 tonnes per year are used in the EU, this is the most widely used polybrominated diphenyl ether. In recent decades, decaBDE is appearing as a contaminant in various environmental compartments (Law et al., 2006), even in higher trophic levels in marine food chains (Sørmo et al., 2006). Measurable levels of decaBDE are also observed in humans, and both exposure through the food chain and direct contact are probable sources (Sjödin et al., 1999). DecaBDE has a relatively short half-life in humans (15 days), and while it is commonly present in humans in general, the metabolism and excretion must be counterbalanced by continued exposure to this flame retardant (Thuresson et al., 2006).

Regarding human health, the current EU RAR concludes that there is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already (Hansen et al., 2002). Similar conclusions were reached for environmental health, although some uncertainties were identified, mainly regarding long-term effects in view of persistence of the compound, secondary poisoning, and toxicity of break down products, particularly as a result of debromination.

These uncertainties led to regulatory proposals and decisions regarding the use of decaBDE. In the EU, decaBDE is approved for use in new electrical and electronic equipment only, and based on the conclusion that continued use of decaBDE would lead to further increases in environmental concentrations in the Baltic region (Palm et al., 2004), several Nordic countries proposed a ban or further restrictions in the

* Corresponding author. Tel.: +31 30 274 2681; fax: +31 30 274 4446.
E-mail address: Leo.van.der.ven@rivm.nl (L.T.M. Van der Ven).
[1] Deceased author.

0378-4274/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.toxlet.2008.03.003

use of decaBDE. Similar considerations led Canada to develop a strategy to minimize releases of decaBDE to the environment (http://www.ec.gc.ca/toxics/docs/substances/pbde/consult-09-06/en/pbde.pdf).

The oral dose toxicity studies in mammals, on which the EU RAR conclusions on human health are based, were all performed by dietary exposure (Hansen et al., 2002). However, bioavailability through this route is known to be very low, and enhanced uptake is achieved when the compound is administered in an emulsion (Mörck et al., 2003; Sandholm et al., 2003). The current study was part of an integrated program to address uncertainties regarding hazards and risks of brominated flame retardants (see Acknowledgement), and therefore designed to improve the toxicokinetic and toxicological database of decaBDE. It was anticipated that improved bioavailability would facilitate this aim. The relevance for humans of testing with enhanced bioavailability is further underlined by recent cases of higher body burdens than previously reported, particularly in children, who may experience additional exposure through dust, compared to dietary exposure in adults (Watson, 2005; Fischer et al., 2006).

A major focus in the analyses was on endocrine disruption, and particularly of the thyroid hormone (TH) system, which has been identified as a target for potential endocrine disruptive hazard of BFRs in general (Legler and Brouwer, 2003). Bone parameters were analyzed because bone physiology is subject to endocrine regulation, and immunologic and haematological endpoints were included because these are also commonly affected by persistent organic pollutants (Andrews et al., 2001).

2. Animals, materials and methods

2.1. Exposure protocol

Forty Wistar rats (HdsCpb:WU) of both sexes, were purchased from Harlan (Horst, NL) and housed individually to allow recording of individual feed consumption, and to avoid bias from hierarchical stress. Housing was in plastic Macrolon cages with stainless steel wire cover and chopped wood bedding. Light/dark regime was 12/12 h. Standard pelleted rat feed without soy (RMH-GS, Hope Farms/ABDiets, Woerden, NL) and drinking water were supplied ad libitum. Decabromodiphenyl oxide (decaBDE) was obtained as a composite of equal portions of three manufacturer's commercial products (Albermarle Corporation, Great Lakes Chemical Coorporation, and Bromine Compounds Limited) through BSEF (with kind cooperation of Dr. Klaus Rothenbacher). The composition of the commercial product is more than 97% decaBDE with remainder nonabromodiphenyl oxide; trace amounts of octabromodiphenyl oxide may be present (Hardy, 2002).

The formulation for administration of decaBDE was based on a protocol by Dr. Anna Sandholm-Mörck (Mörck et al., 2003), who kindly advised with development of a large scale preparation. Thus, a smooth (lump-free) emulsion of phospholipon 90NG (Phospholipid, Cologne, Germany) was prepared by adding 175 mL water to 10.24 g phospholipon and stirring for 30 min. Lutrol F127 (21.76 g; BASF, Burgbernheim, Germany) was then slowly added (5 min) under stirring, which was then continued for another 25 min. At this point, the net weight of the emulsion was about 200 g.

DecaBDE (1200 mg) was dissolved in 200 mL toluene by sonification (30 min at 35 °C). This solution (or 200 mL toluene without decaBDE for carrier-only exposure) was then slowly added to the emulsion, under continued stirring, and the toluene subsequently evaporated under a flow of nitrogen, at 35 °C, while stirring was continued. Approximately 140 mL of water was added after 3.5 h, and the evaporation procedure continued to allow optimal removal of toluene (at least another 16 h), while infusing 9 mL water per hour to compensate for evaporated volume. The final volume was adjusted to the original 200 g by further evaporation or by adding water. The emulsion was stored in the dark at 4 °C, in bottles which were churned daily.

Appropriate concentrations of decaBDE in the emulsion were based on daily gavage of 5 mL/kg bw. Target exposure doses were 0–0/0–1.87–3.75–7.5–15–30–30/30 mg/kg bw/day, where the controls (0 and 0/0) represent carrier-only exposure. Due to limited solubility, the highest dose was administered as a duplicate dose of the 30 mg/kg bw/day dose, given with an interval of 4 h. The exposure range was therefore completed with a duplicate vehicle exposure (0/0). Intermediate doses were prepared by dilution of the top dose with carrier-only.

The experimental protocol was based on the OECD407 28 day subacute toxicity guideline, which was enhanced for endocrine and immunological endpoints (Andrews et al., 2001). The animals were distributed among a larger number of dose groups than advised in the guideline each with ten animals, i.e. five rats per sex per dose group, for improved assessment of dose–response relationships (Kavlock et al., 1996; Slob, 2002).

Exposure started at age 11 weeks. In life observations during the exposure period, and necropsy at the end of the exposure period were performed as described previously (Van der Ven et al., 2006), including analysis of epididymal sperm, collection of spleen, blood and bone marrow for immunological analysis, and dissection of organs for fresh weighing (except thyroid and pituitary glands, which were weighed after fixation), and for histological analysis. Targeted necropsy of females in the first day of diestrous was abandoned because staging of the estrous cycle on the basis of vaginal smears was not consistent with further histological staging of uterus and vagina epithelium. Organ weights are reported as wet weight, to avoid erroneous interpretation from weights relative to body or brain weight (Van der Ven et al., 2006). Observed organ weight changes were interpreted in the context of concomitant other changes, particularly of body weight. Defined parts of the liver, intestines, brain, one of each pair of adrenals, testes and ovaries, and samples of muscle and fat were snap frozen in liquid nitrogen, stored at −80 °C and distributed for analysis of metabolic activity, retinoid analysis, and analysis of decaBDE and its debromination products (liver only). Plasma aliquots were stored at −20 °C for analysis of thyroid hormones and further clinical chemistry. A distal body preparation including lumbar vertebrae, pelvic bones and one intact hind limb was frozen at −20 °C for analysis of bone parameters. All remaining dissected organs/tissues were fixed in standard formalin for further histological processing.

Experiments were approved by the institutional Committee on Animal Experimentation, according to Dutch legislation.

2.2. Compound analysis

Body burdens of decaBDE in liver of five male and five female animals per dose group were determined. About 0.1–0.6 g of liver was weighed, dried with sodium sulphate (Merck, Darmstadt, Germany) and stored for 2 h. Pressurized liquid extraction was performed with an ASE300 equipment (Dionex, Sunnyvale, CA, USA) using a 34 mL cell. The cell was filled from bottom to top with two paper filters, 33% H_2SO_4 deactivated silica gel (10 g), the dried liver sample, and sodium sulphate. One milliliter of ^{13}C-labeled decaBDE (25 ng/mL, Accustandard) was added to the cells as internal standard. The ASE cells were extracted with hexane using two cycles (pressure was 1500 psi). After addition of 1 mL of toluene and evaporation with nitrogen to 1 mL, the extract was analyzed with gas chromatography (GC) coupled to mass spectrometer (MS). The instrument was run in ECNI mode using methane. A DB-5 GC column 15 m, 0.25-mm internal diameter, 0.1-μm film thickness (J&W Scientific, Folsom, CA) was used. Helium was used as carrier gas. Peak identification was based on the retention time of the ^{13}C-labelled compound and the bromine clusters of m/z 79, 81 and 486.7, 488.7. For more details on the GC–MS instrumentation see De Boer et al. (2003). Additionally, nona-BDEs (BDE206, 207 and 208) were quantified in all samples. Chromatograms were also screened for other brominated BDEs (in total 126 different BDE congeners), including octa- (BDE198, 203, 204) and heptaBDEs (BDE173, 181, 182, 183, 184, 195, 190, 191, 192).

The technical decaBDE was analyzed with GC–HRMS for contaminants by Dr. Wim Traag, RIKILT, Wageningen, The Netherlands. For this purpose, the compound was dissolved in toluene in a concentration of 4.5 mg per mL. At least two masses, at a resolution of 10,000, per brominated dioxin and furan were measured. A standard solution of EDF-2046 (CIL, Andover, MA, USA) was used for comparison.

2.3. Histology

After fixation, organs sampled for histology were dehydrated and paraffinized and embedded according to standard sampling and trimming procedures. Sections of 4 μm were stained with haematoxylin and eosin in an automated way. Microscopic observations were done by initial unblinded comparison of all control and top dose samples. Blind and/or semi-quantitative scoring was applied when changes were suggested by the initial inspection.

2.4. Hepatic drug metabolism

Analysis of mRNA, protein, and enzyme activity of hepatic cytochrome P450 enzymes CYP1A(1/2) and CYP2B(1/2) were performed as described previously (Germer et al., 2006). In brief, for mRNA analysis, total RNA was isolated, dissolved in pure water and quantified with a spectrophotometer. Diluted samples were analyzed to control RNA integrity. RNA was then transcribed using quantitative RT-PCR, and the produced cDNA was assayed using the same primers as in the reference. Ct-values of the target gene were normalized to the housekeeping gene GAPDH, and treated groups were related to the untreated control. CYP enzyme protein was analyzed by Western blotting of CYP enzymes using vertical SDS gel electrophoresis and semi-dry membrane blotting, and visualization of immunoreactive bands. The catalytic activities of CYP 1A (1/2) and CYP2B (1/2), 7-ethoxyresorufin O-deethylase (EROD) and 7-pentoxyresorufin O-dealkylase (PROD), respectively, were measured in isolated hepatic microsomes with adjustment for total protein.

2.5. Thyroid hormone analysis

Total concentrations of circulating thyroid hormones thyroxin T4 and T3 were determined in plasma by in-house radioimmunoassays (Internal Medicine Labora-