METABOLISM OF DECABROMODIPHENYL ETHER (BDE-209) IN THE RAT

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Introduction

Polybrominated diphenyl ethers (PBDE) belong to the group of brominated flame-retardants used as additive in polymers and textiles. The use has resulted in PBDEs are found in the environment both in biotic and abiotic samples[1,2]. Decabromodiphenyl ether (decaBDE) is the most abundant commercially produced PBDE today, with a world-wide production of approximately 54.000 tons[3].

The absorption, metabolism and excretion of pollutants are important factors for the estimation of potential risk. DecaBDE has low solubility in both water and organic solvents[4] and the absorption has been reported to be low[5, 6]. The molecular weight of decaBDE is M_w 959g/mol and according to proposed limits for absorption (M_w<700 g/mol)[7] decaBDE should not be bioavailable. Still, decaBDE is present in human plasma[8] and after an oral dose of radiolabelled decaBDE to rats, radiolabelled material was found in tissues[5]. The excretion and metabolism of decaBDE has previously been studied, and the results showed that the major part of an oral dose was excreted in faeces (99% of the dose) and only traces in urine, <0.5%[5, 6]. Although the occurrence of metabolites in faeces and bile has been reported, the information regarding characterisation of the metabolites is limited[5].

The present study was primarily aimed to identify metabolites formed by the rat after an oral dose of radiolabelled decaBDE.

Material and methods

Animals: Two groups of male Sprague-Dawley rats, conventional (n=4) and bile duct cannulated (n=2) were dosed orally with ^{14}C-labelled BDE-209 (3µmol/kg, 15Ci/mol). DecaBDE was dispersed in a suspending vehicle, consisting of Lutrol F127, soya phospholipid and water. The animals were kept in metabolism cages and excreta were collected in 24h intervals; 0-24h, 24-48 and 48-72h. Bile from the bile duct cannulated rats was collected at 0-4 h, 4-12 h, 12-24 h, 24-48 h and 48-72 h after dose. The radioactivity content was determined in the faeces, urine and bile. From the conventional rats liver, adipose tissue, lung, kidney, adrenals, skin, muscle, spleen, testis, thymus, heart, plasma, red blood cells (RBC), colon wall and content, jejunum content and jejunum wall were collected and radioactivity content measured.

Extraction and clean up: The extraction and clean up steps used have been described previously[9] but a brief outline of the method is given.

Faeces: Freeze-dried and ground faecal samples were extraction with chloroform/methanol (2/1, 200 ml) in a Soxhlet-apparatus[10]. The extract was redissolved in hexane washed with H_3PO_4 (0.1M in 0.9% NaCl)[11] and the lipid weight determined. The radioactivity content in extracts, water phases and residues were determined. The extracted compounds were fractionated by gel

permeation chromatography (GPC) and one fraction containing lipids (GPC-LF, 0-130 ml) and one essentially lipid-free fraction containing BDE-209 and non-conjugated metabolites (GPC-MF 130-260 ml) were collected. The radioactivity content in GPC-LF and GPC-MF were determined. The GPC-MFs was pooled by day for each group and partitioned into neutral and phenolic compounds[8]. The phenolic fraction was derivatised with acetic acid anhydride, and both the neutral and phenolic fractions were analysed by GC/MS (NICI (negative ion chemical ionisation))[12], in the mass range m/z 50-1000.

Tissues: Liver, adipose tissue, lung, kidney and intestinal tissues were pooled for each group and homogenised in hexane/acetone (1/3.5, 45ml) followed by extraction with hexane/MTBE (9/1, 25ml)[13]. The solvent was evaporated and the lipid weight determined. The radioactivity content in extracts, water phases and residues were determined. The extracts, pooled for each group, were dissolved in hexane/dichloromethane (1/1) separated by GPC, into GPC-LF, 0-130ml and GPC-MF, 130-260ml.

Results

The major route of excretion for decaBDE was faeces, approximately 90% of the dose was excreted within three days and only traces were excreted in urine, <0.05% of the dose. Excretion via the bile from bile duct cannulated rats corresponded to approximately 9.5% of the dose within three days. Approximately 3% of the dose was retained in the tissues, 0.9% of the dose, 0.4% and 0.7% in liver, skin and muscle, respectively. The tissue distribution of radioactivity, as concentration mol/mg lipid weight, is presented in Figure 1.

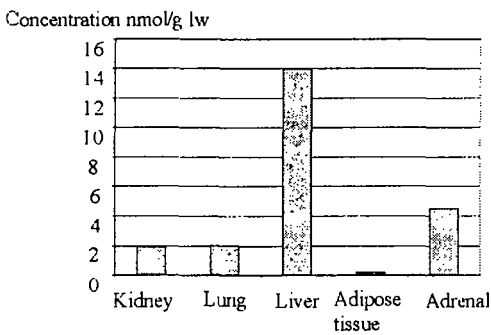

Figure 1, Distribution of ^{14}C, as concentration nmol/g lipid weight (lw), in kidney, lung, liver, adipose tissue and adrenal from rats dosed orally with radiolabelled decaBDE

The radiolabelled material in faeces and tissues was characterised by the distribution in various fractions obtained in the sample work-up. The relative distribution of non-extracted, water soluble, lipid bound and decaBDE/non-conjugated metabolites are presented in Figure 2. Notable is the high relative proportion of non-extracted radioactivity in the jejunum wall (60%) and in the liver (30%). The distribution of radioactivity in different metabolite types for the faecal samples both from different rats and time points were very similar and therefore the mean value is presented in Figure 2.

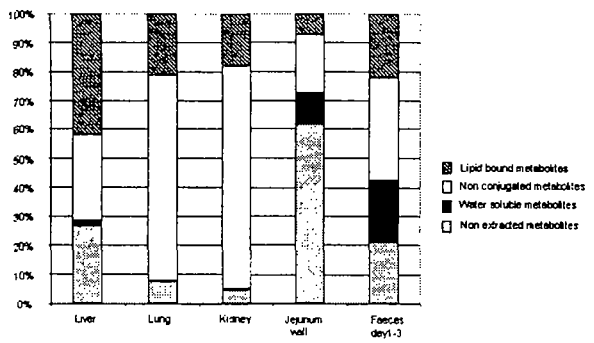

Figure 2, Relative distribution of lipid bound, non conjugated, water soluble and non extractable metabolites in liver, lung, kidney, jejunum wall and faeces (day 1-3) from rats dosed orally with decaBDE.

22%, 42% and 45% of the radioactivity in faecal GPC-MF corresponded to phenolic metabolites in samples from day 1, day 2 and day 3, respectively. GC/MS (NICI), as penta- to heptabrominated diphenyl ethers with guaiacol structure. The phenolic metabolites were identified as their acetyl derivatives. The neutral substances corresponded mainly to decaBDE, but trace of amounts of three nonabrominated diphenyl ethers was also observed. Analysis of the tissue samples is on going.

Discussion

In the present study, decaBDE was shown to be absorbed from the GI tract and 9.5% of the oral dose was excreted in bile within three days. The highest concentration of decaBDE was found retained in liver, followed by skin and muscle. Also kidney, lung and adrenals have higher concentrations than the adipose tissue (Figure 1). Thus, decaBDE does not seem to be readily distributed to adipose tissue. This may be due to slow distribution kinetics or due to high binding to proteins in tissues. The distribution is different from the distribution of many other halogenated aromatic pollutants, e.g. PCBs, and requires further studies.

DecaBDE is metabolised via oxidative debromination, as deduced from the presence of debrominated hydroxylated and metoxylated-hydroxylated metabolites that were observed in faeces and certain tissues. The presence of both a hydroxy- and a metoxy-group in the same phenyl ring, according to mass fragmentation, indicates that the structure is a guaiacol structure, thus the methoxy- and hydroxy-group on adjacent carbon atoms. Such structures are formed by methylation (often by catechol-O methyl transferase) of one of the carbons of an ortho-cathecol.. Oxidation to an epoxide and further to a diol that one hydroxy group were methylated could explain the formed metabolites. However, arene oxide formation is difficult when bromine atoms occupy all carbons. Only traces of debrominated diphenyl ethers were observed three nonaBDEs. Hexabromobenzene

have shown to be metabolised to debrominated-, hydroxylated- and sulphur-containing metabolites[14].

Dihydroxylated metabolites of polychlorinated biphenyls (PCBs) may form reactive quinone intermediates and further react with both sulphur and nitrogen nucleophiles in the cell[15]. Transformation of decaBDE to similar intermediates, dihydroxylated PBDE forming quinone intermediates, could explain the non-extractable radioactivity and lipid bound metabolites. Lipid bound metabolites have previously been shown for PCBs[16]. The potential reactive intermediate metabolites require further studies.

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References

1) Sellström, U., Nylund, K., de Wit, C. And Jansson, B. *Environ. Toxicol. Chem.* **1998**, 17, 1065-1072.
2) Sellström, U., Jansson, B., Kierkegaard, A., de Wit, C., Odsjö, T. and Olsson, M. *Chemosphere* **1993**, 26, 1703-1718.
3) According to BSEF (Bromine Science and Environmental Forum, Brussels, Belgium, website; www.bsef.com
4) Brominated diphenylethers, *IPCS, World Health Criteria*, 162., **1994.**
5) El Dareer, SM., Kalin, JR., Tillery, KF. and Hill, DL. *J. Toxicol. Environ. Health.*, **1987**, 22, 405-415.
6) Norris, JM., Kociba, RJ., Schwertz, BA., Rose, JQ., Humiston, CG., Jewett, GL., Gehring, PJ. and Mailhes, JB. *Environ. Health Perspec.*, **1975**, 11, 158-161.
7) Absorption. Chapter 3. *In Clinical Pharmacokinetics Concepts an Application. 3.* M. Rowland and T. N. Tozer. LEA, FEBIGER, Philadelphia, **1980**.
8) Sjödin, A. Occupational and Dietary Exposure to Organohalogen Substances, with Special Emphasis on Polybrominated Diphenyl Ethers. [Ph.D thesis]. Stockholm, Sweden: Institution of Environmental Chemistry, Stockholm University, **2000**.
9) Klasson Wehler, E., Hovander, L. and Lund, B.-O. *Chem. Res. Tox.*, **1996**, 9, 1340-1349.
10) Klasson Wehler, E., Bergman, Å., Brandt, I., Darnerud, P.O. and Wachtmeister, C.A. *Drug Metab. Dispos.*, **1989**, 17, 441-448.
11) Örn, U. and KlassonWehler, E. *Xenobiotica.*, **1998**, 28, 199-211.
12) Sjödin, A., Carlsson, H., Thuresson, K., Sjölin, S., Bergman, Å. and Östman, C. *Environ. Sci. Technol.*, **2001**, 35, 448-454.
13) Bergman, Å., Athanasiadou, M., Bergek, S., Haraguchi, K., Jensen, S. and Klasson Wehler, E. *Ambio.*, **1992**, 21, 570-576.
14) Yamaguchi, Y., Kawano, M. and Tatsukawa, R. *Arch. Environ. Contam. Toxicol.*, **1988**, 17, 807-812.
15) Amaro, A.R., Oakley, G.G., Bauer, U., Spielmann, H.P. and Robertson, L.W. *Chem. Res. Toxicol.*, **1996**, 9, 623-629.
16) Mörck, A. Lipidbindning as a metabolic pathway, studies on 3,3',4,4'-tetrachlorobiphenyl metabolites. [Ph. lic. thesis]. Stockholm, Sweden. Institution of Environmental Chemistry, Stockholm University, **1999.**