Polychlorinated dibenzothiophenes: toxicological evaluation in mice.

Mäntylä, E.[A], Ahotupa, M.[B], Nieminen, L.[A], Paasivirta, J.[C], Sinkkonen, S.[C]

[A]Orion Corporation Farmos, Toxicological Laboratory, SF-20101 Turku, Finland
[B]Department of Physiology, University of Turku, SF-20520 Turku, Finland
[C]Department of Chemistry, University of Jyväskylä, SF-40351 Jyväskylä, Finland

Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are highly toxic environmental pollutants that are formed e.g. during waste combustion in incinerators. The most toxic of these compounds is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Of the PCDFs the structural stereoisomers of TCDD, e.g. 2,3,4,7,8-pentachlorodibenzofuran and 2,3,7,8-tetrachlorodibenzofuran (TCDF) are the most toxic congeners. The toxicity pattern of these compounds is quite complex: wasting syndrome, hepatotoxicity, immunotoxicity, teratogenicity etc[1,2,3]. Recently, the presence of polychlorinated dibenzothiophenes (PCDTs), sulfur analogues of PCDFs, was confirmed in incinerator gas[4,5] or fly ash [6,7] samples. As nothing was known about the toxicity of these new environmental contaminats the present study was initiated to elucidate this aspect.

Of the PCDTs 3,4,6,7- and 2,3,7,8-tetrachlorodibenzothiophenes (3,4,6,7-TCDT and 2,3,7,8-TCDT) and 1,3,4-trichlorodibenzothiophene (1,3,4-TriCDT) were synthetized and characterized as described[4]. Ah-responsive male C57BL/6J mice were used in the experiments. The compounds were dissolved in corn oil and administered as a single i.p. dose into the mice: 3,4,6,7-TCDT and 2,3,7,8-TCDT 10, 100 or 300 µg/kg (2 days) (31, 310 or 930 nmol/kg) or 300 µg/kg (8 days); 1,3,4-TriCDT 100 or 500 µg/kg (2 days) or 300 µg/kg (8 days) (350, 1040 or 1740 nmol/kg). After 2 or 8 days the mice were sacrificed. A liver sample, thymus, spleen and a mesenteric lymph node were studied histologically. In addition bone marrow smear (femur) was studied. Livers were prepared for biochemistry as described[8]. Hepatic reduced glutathione (GSH) content was evaluated in homogenates and hepatic microsomal preparation was used to determine cytochrome P450-mediated monooxygenase activities: ethoxyresorufin O-deethylase (EROD)[9] and ethoxycoumarin O-deethylase (ECOD)[10].

The compounds studied did not change thymus weights and the changes in the weights of other organs were small: a slight increase in liver and spleen size was observed with 2,3,7,8-TCDT (300 µg/kg; 8 days). In the bone marrow smears there could be seen an elevated myeloid/erythroid cell ratio with all the studied compounds (0.6 in controls and 0.8-1.6 in PCDT-animals) at most doses. Histologically a diffuse hepatic necrosis was found in one of the 3,4,6,7-TCDT-treated animals (10 µg/kg) and in one of the 1,3,4-TriCDT-treated animals (500 µg/kg). The latter animal was the only

showing thymic atrophy (minimal) in histological examination. In the spleen moderate extramedullary haematopoesis was noticed with 3,4,6,7-TCDT and 2,3,7,8-TCDT at 8 days (300 µg/kg); pigmentation was noticed with the same dose at 2 days. Also 1,3,4-TriCDT caused splenic pigmentation at the two highest doses. After 2 days a slight increase (up to 1.5-fold) could be seen in the hepatic monooxygenase activities with the TCDTs (300 µg/kg) and the TriCDT (500 µg/kg).

PCDTs were shown to be considerably less toxic than TCDD or the most toxic PCDFs. Only with the highest PCDT doses studied some marginal toxic effects could be seen. These effects, liver and thymus atrophy, spleen changes, bone marrow changes, are typical ones caused by toxic PCDDs and related compounds.[1,2,3] At the doses studied minimal thymic atrophy was detected only in one of the 1,3,4-TriCDT-treated (1740 nmol/kg) mice. The ED_{50}-value for TCDD-induced thymic atrophy is about 10 nmol/kg[1]. The induction of microsomal EROD-activity (cytochromes P448) is a very sensitive measure for the toxic potency of PCDDs and related compounds: a linear correlation has been noticed between toxicity and inducing potency[2]. In the present study this activity increased only less than 1.5-fold with 2,3,7,8-TCDT or 1,3,4-TriCDT (doses 930 or 1740 nmol/kg). The ED_{50}-value for EROD induction is about 2 nmol/kg with TCDD and about 10 nmol/kg with TCDF (about 10-fold induction maximally).[1,2] The three most toxic nonortho coplanar PCBs are 3,4,5,3',4'-penta-, 3,4,5,3',4',5'-hexa- and 3,4,3',4'-tetrachlorobiphenyl (in decreasing toxicity and EROD inducing potency order).[1,3] The 3,4,3',4'-tetrachlorobiphenyl (TCB) induces hepatic EROD activity 1.4- or 7-fold in 2 days with a single dose of 680 or 3400 nmol/kg, respectively, (maximal induction over 70-fold at 34 µmol/kg). The TCB dose 3400 nmol/kg causes moderate thymic atrophy.[11] According to this the enzyme inducing and toxic potency of the studied PCDTs is at least not higher than that encountered with TCB, but more studies at higher doses are needed. The concentration of PCDTs in fly ash samples is equal or about 10-times lower than the concentration of PCDDs or PCDFs[7], so it may be that these new environmental contaminats are toxicologically of minor importance. However, other environmental sources are possible and more studies are needed to elucidate the distribution and toxicity of these and other PCDT congeners (e.g. the penta- and hexachlorinated ones) and that of the methylated dibenzothiophenes. Further, nothing is known about the long-term toxicity (e.g. carcinogenicity) of these compounds. In **conclusion** PCDTs were shown to have minor toxic effects at the highest doses studied (900-1700 nmol/kg). The toxicity of these compouns is clearly less than that with the most toxic coplanar PCBs

1 *Health effects of halogenated aromatic hydrocarbons*, Nicholson, WJ, Moore, JA, eds. *Ann NY Acad Sci* 1979;320:1-730.

2 Safe, S. Comparative toxicology and mechanism of action of polychlorinated dibenzo-p-dioxins and dibenzofurans. *Ann Rev Pharmacol Toxicol* 1986;26:371-99.

3 Safe, S. Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology, and mechanism of action. *CRC Crit Rev Toxicol* 1984;13:319-95.

4 Sinkkonen, S, Koistinen, J. Chlorinated and methylated dibenzothiophenes: preparation of the model compounds and their analysis from some environmental samples. *Chemosphere* 1990;21:1161-71.
5 Sinkkonen, S, Paasivirta, J, Koistinen, J, Tarhanen, J. Tetra- and pentachlorodibenzothiophenes are formed in waste combustion. *Chemosphere* 1991;23:583-7.
6 Buser, H-R, Rappe, C. Determination of polychlorodibenzothiophenes, the sulphur analogues of polychlorodibenzofurans, using various gas chromatographic/mass spectrometric techniques. *Anal Chem* 1991;63:1210-17.
7 Buser, H-R, Dolezal, IS, Wolfenberger, M, Rappe, C. Polychlorodibenzothiophenes, the sulfur analogues of the polychlorodibenzofurans identified in incineration samoles. *Environ Sci Technol* 1991;25:1637-43.
8 Ahotupa, M, Mäntylä, E. Adipose tissue content as a modifier of the tissue distribution, biological effects, and excretion of a hexachlorobiphenyl in C57BL and DBA mice. *Mol Pharmacol* 1983;24:464-70.
9 Prough, RA, Burke, MD, Mayer, RT. Direct fluorometric methods for measuring mixed-function oxidase activity. *Methods Enzymol* 1978;52:372-75.
10 Aitio, A. A simple and sensitive asay of 7-ethoxycoumarin deethylation. *Anal Biochem* 1978;85:488-91.
11 Mäntylä, E. Unpublished results.

Acknowledgements: The expert technical assistance provided by Ms Kirsti Nieminen, Ms Leena Söderholm and Ms Kerttu Tammi is greatly appreciated.