Metabolism of polychlorinated naphthalenes and a tetrabrominated diphenyl ether

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Introduction.
Persistent organic pollutants are generally considered to be an environmental problem because of their persistence in the physical environment. Biotransformations are however often efficient and most organohalogens are biotransformed to some extent, even 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153)[1].

Most organohalogens such as polychlorinated naphthalenes, biphenyls and diphenyl ethers lack functional groups and therefore the first step is oxidation, either by direct insertion of a hydroxy-group or via an arene oxide. The formation of 1,2-shifted hydroxylated metabolites are generally considered to be an evidence for metabolism via arene oxide and was first reported by Jerina and Daly[2]. For PCB, 1,2-shifted metabolites of several congeners have also been reported - e.g. CB-77 [3,4] and CB-105 [5]. Another indication of arene oxides is the formation of mercapturic acid pathway metabolites (MAP) [6] and the formation of aryl methyl sulphone PCB metabolites. Metabolism via direct insertion occurs in the *meta*-position of, at least, 2,2',5,5'-tetrachlorobiphenyl, as shown by Preston and coworkers [7]. Naphthalenes lacking, or with only few chlorine atoms, have been reported to be metabolised via arene oxides and to form both hydroxylated and MAP metabolites[8-10]. Chlorinated diphenyl ethers have been reported to form hydroxylated metabolites, primarily in the *ortho*-position but no MAP metabolites have been reported so far [11].

From a toxicological point of view, formation of water soluble metabolites that are excreted is advantageous but occationally biotransformation generates metabolites that are more toxic than the parent compounds[12]. One of the steps that may include increased toxicity is the first oxidative step, generating reactive intermediates such as arene oxides. These metabolites may react with endogenous compounds forming covalent bonds to e.g. DNA and may therefore be genotoxic[13]. More persistent metabolites such as hydroxylated PCB metabolites have been reported to bind to proteins and some fo these may thereby be retained in blood [14-16]. Methyl sulphonyl metabolites of PCB congeners may also bind to proteins and be selectively retained, e.g. in the lungs[17,18]. To assess the risk of exposure to xenobiotics it thus of importance to study the metabolism of persistent organic pollutants and to determine their metabolism and excretion rate, to determine potential selective retention and the formation of reactive intermediates that may bind irreversibly to biomacromolecules. This study reports on metabolism studies of polychlorinated naphthalenes (PCN) by the rat and 2,2',4,4'-tetrabromodiphenyl ether (TBDE) in rat and mouse.

Material & Methods.
^{14}C-Labelled polychlorinated naphthalene (PCN) and 2,2',4,4'-tetrabromodiphenyl ether (TBDE) were synthesized as previously described[19,20].

Generally, adult male rats (Sprague Dawley) and/or mice (C57 Bl) were dosed orally with the radiolabelled compound (ca 30 µmol/kg, 1 Ci/mol, dissolved in corn oil). The animals were kept in metabolism cages and urine and faeces collected daily until day 5 when the animals were sacrificed. Excreta and tissues were monitored for radioactivity content and, based on the results, tissues were selected for chemical analysis.

Extraction of tissues was performed as described by Bergman *et al*[21], using acetone and hexane, followed by hexane and methyl *tert*-butyl ether. The tissue residues from PCN-dosed rats were reextracted with chloroform/methanol in a Soxhlet apparatus[4]. After lipid weight determination of the extracts, the lipids were removed by gel permeation chromatography[22].

Freeze dried and homogenized faecal samples were extracted in a Soxhlet apparatus with chloroform and methanol[4]. Co-extracted water soluble metabolites were partitioned into an aqueous phase (0.1M H_3PO_4, 0.9% NaCl) The co-extracted lipids were removed by GPC, similarly as for the tissue samples. For the remaining analysis, the faecal samples were treated similarly as the corresponding tissues. For the PCN-samples, the GPC-MF was fractionated on a silica gel column into 3 fraction of varying polarity by using solvent of increasing polarity (1: hexane; 2: hexane:dichloromethane (1:1); 3: dichloromethane and finally 4: chloroform:methanol:ammonium (85:10:5).

Analysis was performed on GC/MS (Finnigan ITS40), using electron ionization and GC with an electron capture detector. For both instruments DB5 columns (30m x 0.25 mm i.d., 0.25µm film thickness; J&W) were used and the temperature program essentially the same, starting at 80°C (2 min) and increased with 10°C/min upto 300°C (for CB-101 and PCN - 10 min, for BDE-47 - 25 min).

Results.
Absorption and excretion.
The PCN-exposed rats excreted 99% of the dose during five days, mainly via faeces (94%) and which leaves only approximately 1% in the body of the rats. Approximately 13% of the dose can be calculated to correspond to non-absorbed material, by assuming that the parent compound excreted in faeces the first day is the non-absorbed parent material. For the TBDE-exposed rat, 5% of the does was not absorbed and only 13.6% of the dose excreted in faeces (non-absorbed material included) and 0.2% in urine. For the TBDE-exposed mouse, 32.5% of the dose was excreted in urine and 20.2% in faeces. Included in the latter are 7% of the dose corresponding to non-absorbed material (Figure 1). Thus, the absorption of both these compounds is efficient in those species but the rate of excretion varies considerably.

There were also qualitative differences in the excreted material that varied with compound and species. Figure 1 shows the relative amounts of parent compound and different types of metabolites, as determined by the radioactivity content in the clean-up fractions. Of the faecally excreted radio-labelled compounds from PCN-exposed rats, 22% is not extractable and thus regarded to covalently bound to endogenous molecules, 38% is covalently bound to lipids and 5% water soluble compounds. The remaining radiolabelled compounds (30% of excreted material) mainly corresponds to PCN congeners according to GC/MS, and from these almost half corresponded to more or less polar

Figure 1. Distribution of dose in body and excreta

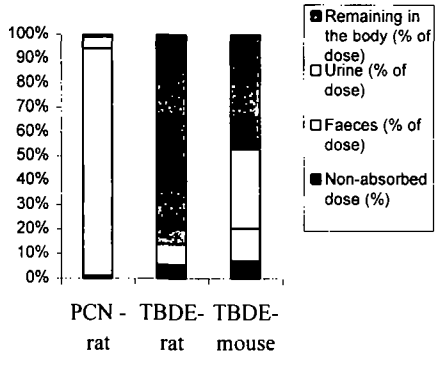
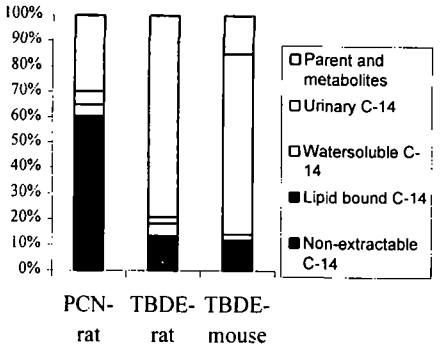


Figure 2. Relative amounts of excreted metabolites



metabolites. For the TBDE-exposed rat on the other hand, 79% of the excreted material was the parent TBDE (including non-absorbed material) and only 21% of the excreted material corresponded to different types of metabolites (covalently bound to macromolecules or lipid, water soluble metabolites, cf Figure 2). From the TBDE-treated mouse, 85% of the excreted material corresponded to various metabolites and only 15% to TBDE.

GC/MS analysis of the GPC-MF from the PCN-dosed rat faeces indicated the presence of several metabolites, hydroxylated tri- to pentaCN congeners, methylthio-tetra- to hexaCNs, methyl sulphoxide tri- and -tetraCNs and a dihydroxy-tetraCN. None of the metabolites could be structurally identified due to the lack of reference compounds. GC/MS analyses of the faecal GPC-MF s from the BDE-treated mice and rats showed that almost the entire material corresponded to parent TBDE. Small amounts of hydroxylated metabolites were indicated from both animals.

Tissues

The 1% of the dose that remained in the PCN-treated rats was fairly equally distributed in the tissue extracts (10-15 nmol/g l.w.) Except in the liver where the concentration was 28 nmol/g tissue. The highest concentration was thus in the liver and the GC/MS analysis showed that this material corresponded mainly to one hexaCN peak in the chromatogram. This peaks most probably correspond to two CNs - 1,2,3,5,6,7- and 1,2,3,4,6,7-hexaCN - as reported by Asplund et al[23]. Trace amounts of a methylthio-tetraCN and a methyl sulphoxide-pentaCN were indicated by GC/MS. More interesting, and quantitatively most important, is the presence of covalently bound metabolites in the liver. As much as 75% of the total ^{14}C-content was firmly bound to the tissue residue and another 10% covalently bound to lipids. In the lung 65% and 59% of the ^{14}C in the kidney were covalently bound to macromolecules and in both tissues, approximately 10% of the radiolabelled was bound to lipids. In the adipose tissue no non-extractable metabolites were present and only traces of lipid bound metabolites were observed.

In the TBDE-treated rats, 86% of the dose remained and the highest concentration was found in adipose tissues - approximately 700 nmol/g both on lipid and fresh weight. That was 3 times higher than in the liver extract (200 nmol/g l.w.) and approximately 5 times higher than in kidney and lung (134 and 128 nmol/g l.w., respectively). In none of the tissues was the covalently bound material more than 5% (liver) of the total ^{14}C, and only traces of lipid bound metabolites were determined. According

to GC/MS, only parent TBDE was present.

In the TBDE-treated mice, the concentration in liver and adipose tissue were approximately similar (122 and 130 nmol/g l.w., respectively). No metabolites were observed in adipose tissue but in the liver, 12% were bound to macromolecules and 16% to lipids. In addition, low concentrations of 3 different hydroxylated metabolites were observed. In the lung and kidney. The ^{14}C concentrations in the extracts were similar in the lung and kidney (63 and 68 nmol/g l.w., respectively) but in the lung, 28% of the total ^{14}C was covalently bound whereas only 4% in the kidney.

Discussion.
PCN is rapidly metabolized and excreted by the rat and only 1% of the dose is retained in the body. However, the retained material is mainly irreversibly bound to macromolecules, including DNA and may therefore potentially be toxic[19]. Another implication of toxicity by PCN is the selective retention of two hexaCN s in the liver. This has previously also been shown by Asplund et al and the induction potency of these hexaCNs is lower than that of TCDD[24].

TBDE is quite different from PCN in the rat, it is only slowly metabolized and excreted. Eightysix % of the dose remained in the rat tissues 5 days after exposure. The retained compound is largely stored as such in the adipose tissue. The excretion of TBDE by the mouse is more rapid than the rat with 53% of the dose excreted. Interestingly, the major excretory pathway is via urine. The water soluble metabolite(s) in the urine has not yet been identified. The high concentration of irreversibly bound metabolites in the mouse lung is notable.

Thus these two representatives of persistent organic pollutants from the environment behave very differently in biotransformation. The results show clearly that it is of importance to include metabolism studies for risk assessment and that the results from such a study may be an indication for what toxicological studies should be performed.

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