Chemical synthesis, characterisation, analytical method development and control to promote exposure assessments and toxicological testing - Highlights from COMPARE

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Introduction

The issue of endocrine disruptor effects in wildlife and humans grow increasingly important during the 1990s' [1,2]. As part of the focus on endocrine disruptors new contaminants and their metabolites were put forward for studies with endpoints related to hormone disruption. One such large group of chemicals and/or metabolites of neutral semi-persistent or persistent compounds was the substituted phenols, particularly the halogenated phenolic compounds (HPCs) [3-7]. Polychlorobiphenylols (OH-PCBs) were reported to be strongly retained in human blood plasma in 1995 [3] and this article was the first study to point out the general retention of several OH-PCBs in the plasma. The metabolic formation of OH-PCBs was well known [8] and the specific blood retention had been reported for at least one PCB congener, 3,3',4,4'-tetrachlorobiphenyl (CB-77) in some previous studies [9,10]. The identification of OH-PCBs being retained in blood and their specific binding to transthyretin (TTR) has formed much of the basis for two EU R&D programs, first RENCO and now COMPARE.

The present report is aimed to highlight some of the results obtained within the COMPARE program mainly dealing with the chemical synthesis, characterisation and analytical aspects of HPCs.

Result review and discussion

Improved methods for synthesis of OH-PCB congeners: While there is a straight pathway for synthesis of a large variety of 4-OH-PCB congeners from polychloroanisols and chlorinated anilines via the Cadogan diaryl coupling reaction and demethylation [11] it is more difficult to synthesise 3-OH-PCB congeners. Recent efforts to synthesise such compounds have been successfully completed as shown in Figure 1. By applying the methods for synthesis it is today possible to prepare analytical standards and OH-PCBs for toxicological testing. The five most abundant OH-PCB congeners in human blood are thus available. Synthesis of radiolabelled OH-PCBs is more difficult depending on limitations of appropriate ^{14}C-labeled starting materials. Radiolabeled 4-OH-CB107 was synthesised from 3,4-dichloro-iodo[U-^{14}C]benzene and 4-iodo-2,3,6-trichloroanisol in a Ullman diaryl coupling reaction [12]. The PCB methyl ethers are demethylated by borontribromide in dichloromethane.

Lipophilicity (log K_{ow}) and acidity (pK_a) of OH-PCB congeners: Polychlorobiphenylols are slightly acidic chemicals for which the pK_a has a dramatic and natural effect on solubility and partition coefficients. The octanol/water partitioning has been modelled while a UV spectroscopic method was applied for pK_a determination after it was modified for measurements in a water/methanol mixture [13]. The pK_a determined by this method is checked by pK_a determination of pentachlorophenol (PCP) and 2,4,6-tribromophenol..

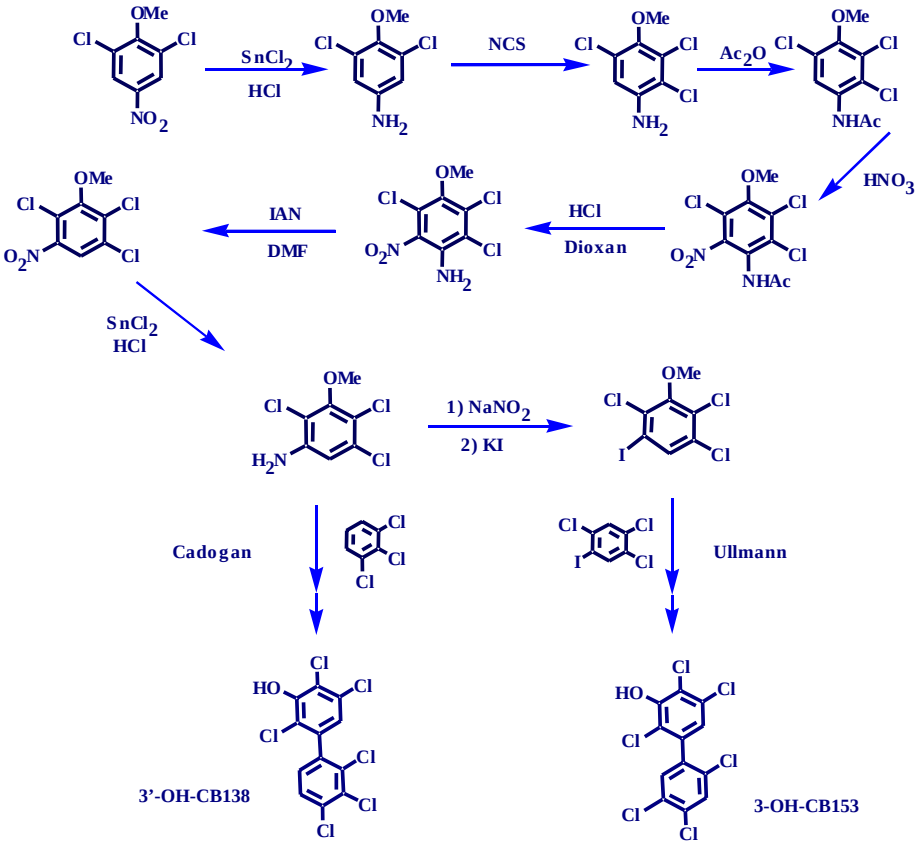

Figure 1. Pathway for synthesis of two *meta*-hydroxy-PCB congeners of environmental relevance; 3'-OH-CB138 and 3-OH-CB153.

The data obtained shows that the OH-PCBs, in general, have pK_a constants between 5 and 7 and log K_{ow} values in the range of 6-7. The data available today on OH-PCBs show constant values of 5-7 (present study, [14,15], modelled pK_a values) with e.g. an experimentally measured pK_a of 4-OH-CB187 of 5.1. The high log K_{ow} values show that the protonated OH-PCB is not dramatically influenced by the hydroxy group that may then lead to bioaccumulation. The pK_a constants shows

however that the OH-PCBs are present in the blood in their ionized form to a major extent; the blood pH being 7.4% and pK_a of most OH-PCBs lower than that indicate 50% or more of the OH-PCB should be ionized in this medium. According to previous studies it seems that the protonated hydroxyl group of e.g. a OH-PCB and pentabromophenol is binding to TTR [16,17]. Interestingly the relative concentration of 4-OH-CB187 is much lower than –O-CB187 concentration but still enough to be extracted form the blood in this way. Alternatively the phenolate ion is protonated o site in the protein.

In vivo half-lives of two OH-PCBs: Ever since the discovery of OH-PCBs being retained in animals it has been a question about their half-lives in vivo. Since OH-PCBs in blood are continuously formed through metabolism of persistent PCB congeners, such as CB-105, CB-118, CB-138, CB153, CB-183 and CB-187, it is difficult to tell the turn-over of these metabolites in vivo. In a recent study [18], two OH-PCBs were given i.v. to rats (1µmol/kg b.w.) and their rates of elimination were determined by chemical analysis. The half-life of 4-OH-CB107 (five chlorine atoms in the compound) and 4-OH-CB187 (seven chlorine atoms in the compound) in rats were 4 and 15 days, respectively. The pharmacokinetics of the two compounds are shown in Figure 2.

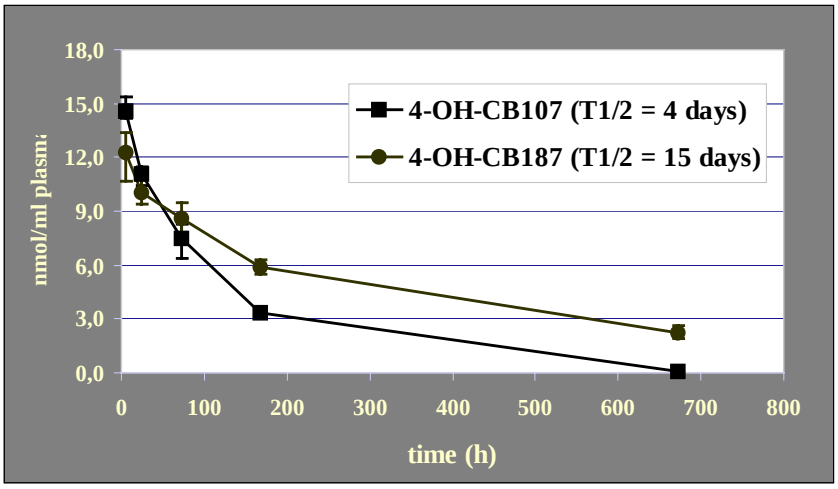

Figure 2. Mean plasma concentrations (nmol/ml plasma) over time for the two OH-PCBs: 4-OH-CB107 and 4-OH-CB187. The two OH-PCBs were administered intravenously.

Method development and control for analysis of HPCs and neutral brominated flame retardants in human serum: The originally developed method for PHC analysis, as described by Hovander and coworkers[19] has been applied for analyses of OH-PCBs by two different laboratories working within COMPARE. To establish the reproducibility of the method and the variation between laboratories was an inter-laboratory calibration performed. Nine samples were prepared and handed out for analysis at the three laboratories. The result was satisfactory with a coefficient of variation between 2-10%.

The same HPC clean up method was also used for controlling any potential differences in the analytical results by using plasma or serum, fresh or frozen, for the analyses. In this study samples were analysed at time 0 and after 2 and 20 months of storage of the plasma and serum samples. The study is described in further detail elsewhere [20]. The study was not optimal but it is still possible to conclude that serum is to prefer for analysis of PHCs. This result was obtained without having complicating clotting of plasma proteins in the plasma being analysed, a complicating factor in other previous studies. It was concluded that plasma had a lower recovery than serum.

Another methodological effort within COMPARE was to further develop analysis of neutral environmental contaminants in human blood. Hence PBDEs and HBCDD were analytes of particular interest. Since HBCDD is labile to base such clean up steps must be avoided. The method for analysis is described in more detail by Weiss et al (Dioxin 2004)[21] but is based on liquid-liquid extraction, conc. sulfuric acid treatments for lipid removal and chromatographic separations for further clean up. All quantifications were performed by GC/MS (ECNI) of the bromide ions m/z: 79 and 81. The recovery of low and high concentration samples for PBDEs were in the range of 80-90%, independent of the sample. Similarly the recovery of HBCDD was 70%. The coefficient of variation was 3-10%. The limit of detection was the highest for HBCDD (4 pg) with the PBDEs in the range of 1-2 pg [21]. The method is valuable for HBCDD quantification leaving out the information on which HBCDD isomer is dominating. This is still to be established but can of course be done by applying another MS technique.

Hexabromocyclododecane (HBCDD) and polybrominated diphenyl ethers (PBDEs) in Dutch maternal and fetal blood: Serum samples were obtained from the Dutch-Groningen-PCB-Infant-Cohort, and contained 8 samples from mothers at the 20th week of pregnancy, 70 samples from mothers at the 35th week of pregnancy and 12 cord blood samples. The blood samples were analysed as described above and by Weiss et al at this symposium [21]. The maternal blood serum was dominated by BDE-153 (mean concentration: 7.0 pmol/g l.w. / 4.5 ng/g l.w.), with BDE-47 at a similar mean molar concentration (6.5 pmol/g l.w. / 3.2 ng/g l.w.). HBCDD and BDE-99 were present at a similar concentration (1.7 pmol/g l.w.). HBCDD was quantified in the majority of all samples, present in the range from not detected (<0.14-7.0 ng/g l.w.) showing also this cycloaliphatic flame retardant to be a pollutant accumulated in humans. Further attention is required for HBCDD contamination in humans. The median PBDE congener and HBCDD concentrations in the fetal blood (cord blood) serum were somewhat different from the concentrations in the maternal blood serum. Hence, BDE-47 was the most abundant (7.4 pmol/g l.w.) compared to half this concentration for HBCDD and BDE-153, with 3.7 and 2.7pmol/g l.w., respectively. While the BDE-47 concentrations were rather similar in the maternal and cord serum, BDE-153 was about half as high in the cord as in the maternal serum, while it was the reverse for HBCDD. More data are needed to confirm these observations. The lower level of BDE-153 compared to BDE-47 in the cord blood have been reported previously [22,23] and may indicate a transplacental barrier for higher brominated diphenyl ethers.

Conclusions

The present program has given valuable contributions to the knowledge of:
Synthesis of polychlorobiphenylols (OH-PCBs).
The acidity (pK_a) and lipophilicity (log K_{ow}) of polychlorobiphenylols (OH-PCBs).
The plasma half lives of polychlorobiphenylols (OH-PCBs).
Serum being preferable over plasma when analysed for organohalogen pollutants and PHCs.
HBCDD is a human blood contaminant being efficiently transferred to the foetus.
HBCDD and PBDE congeners are present in similar concentrations in Dutch women today.

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