New organohalogens in human plasma - Identification and quantification

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Abstract

Quantitative analysis of a pooled human blood sample showed the presence of more than 100 phenolic organohalogenated substances (OHS), e.g. chlorinated, brominated as well as mixed chloro-brominated phenols, hydroxylated and dihydroxylated PCB metabolites. Among the neutral compounds, six polybrominated diphenyl ethers (PBDEs) were identified.

The identified PBDEs were quantified in 40 human plasma samples and the mean PBDE concentration was 2.1 ±1.4 ng/g lipid weight (l.w.). The total PCB concentration was 660 ±450 ng/g l.w. and OH-PCBs (5 congeners) 200 ±130 ng/g l.w. The OH-PCBs were present in concentrations ranging from 35 to 500 ppb which represented a total OH-PCB to total PCB ratio of 0.03-0.8. The relative peak pattern between the different OH-PCB congeners showed some variation between the 40 samples and were not related to the PCB concentrations.

Introduction

Humans are exposed via food, ambient air etc. (external exposure) to a large number of OHS that are taken up and transported via the blood throughout the body. Environmental contaminants present in blood may thus be a measure of the internal exposure to these substances.

Among the compounds in blood that are of particular interest are those that may contribute to disturbances in the endocrine systems. Compounds giving estrogen-like effects and compounds interfering with thyroxine often have a phenolic structure element, either in the parent compound or to or after metabolism [1-3]. Potential endocrine disturbing substances known to be present in human blood are pentachlorophenol (PCP) and OH-PCBs [4,5]. These compounds compete with thyroxine for the binding site on a transport protein (transthyretin) [3,6]. Some of the hydroxylated PCBs have been reported to also have estrogenic effects [2]. Commonly aromatic compounds are transformed to hydroxylated metabolites to facilitate their excretion but occasionally hydroxylated compounds may bind to e.g. transport proteins in plasma. It is thus of interest to identify any phenolic compounds of anthropogenic origin that may be present in blood. In this presentation qualitative chemical analytical data from a pooled plasma sample is presented. Quantification of some of the identified contaminants was performed individually in 40 human plasma samples.

Experimental methods

Chemicals: As reference compounds, pentachlorophenol (PCP), tri- and tetrachlorophenols, 2,4-dibromophenol, 2,4,5-tribromophenol, 2,4,6-tribromophenol, pentabromophenol, hexachlorobenzene and 3,3',5,5'-tetrabromobisphenol A were used. The GC-standards of the phenolic compounds were methylated with diazomethane [7] prior to injection, similarly as for the samples were treated. For hydroxy-PCBs, the methyl derivatives used were those reported previously [5,8] and also 4-MeO-2,3,5,2',3',4'-hexaCB (4-MeO-CB162[1]). For quantifications the following MeO-PCB congeners were used; 4-MeO-3,5,2',3',4'-pentaCB (4-MeO-CB107) was used as a standard also for the coeluting 4-MeO-2,3,5,3',4'-pentaCB (4-MeO-CB108), 4-MeO-2,3,5,2',4',5'-hexaCB (4-MeO-146), 3-MeO-2,4,5,2',4',5'-hexaCB (3-MeO-CB153), 4-MeO-2,3,5,6,2',4',5'-heptaCB (4-MeO-CB187), 4-MeO-2,3,5,2',3',4',5'-heptaCB (4'-MeO-CB172).

PCB congeners CB-28, 52, 101, 105, 118, 153, 156, 157, 138 and 180 (for structures please see ref 9) were used for PCB analysis [10]. For analysis of PBDEs, the individual brominated diphenyl ether congeners: BDE-17, 28, 49, 47, 66, 100, 99, 85, 154, 153, 138 - using the same numbering system as for PCB) were used [11-13] in addition to Bromkal 70 [14].

All solvents used were of *p.a.* quality or pesticide grade.

Instruments: GC(ECD) analysis was performed as described elsewhere [5]. GC/MS analyses were performed on a TSQ 700 using both electron ionization (EI) and negative ion chemical ionization (NICI) techniques using conditions described by Bergman and coworkers [8], except that the ion source temperatures were 150°C for EI and 120°C for NICI. Mass spectra were collected by scanning from 33 to 800 amu. The GC temperature was programmed starting at 80°C for 1 min and then increased with 10°C/min up to 320°C that was kept for 10 min.

General outline of analytical procedure: A new method for analysis of neutral and phenolic compounds in plasma has been developed, with high recoveries and reproducibility. The project is a collaborative effort between several projects and the detailed description will be published elsewhere [15]. A brief description of the method is given here; Plasma samples (5 g) were denaturated by HCl and iso-propanol after which OHS were extracted with hexane: methyl *tert*-butyl ether. The extract was separated into phenol and neutral compounds by partitioning between KOH (0.5 M in 50% ethanol) and hexane. A non-destructive lipid removal step, using a High Resolution-Gel Permeation Chromatography (HR-GPC) with hexane : dichloromethane as mobile phase, was applied. Phenolic compounds were derivatized with diazomethane prior to HR-GPC separation.

The qualitative analysis of the pooled plasma sample was performed both by GC/MS using both EI and NICI. Quantifications of PCB and OH-PCB were performed by GC(ECD). Quantification of brominated compounds, was performed by GC/MS (NICI) scanning for the ions 79/81 [16,17].

Samples: For the qualitative analysis, ten samples of blood plasma (300 g each) were kindly donated from a blood donor central in Stockholm (Danderyds hospital). The plasma samples were from men between 30 and 40 years old. Aliqoutes of 40 g were taken out from each sample and pooled. Prior to combining the samples, they were analysed individually by

[1] The abbreviation is used consistently for all MeO-PCBs and OH-PCBs. It is based on the numbering system for PCB congeners [9] and the position for the OH- or MeO-group is added prior to the abbreviated name e.g. 4'-OH-CB108 and 4-OH-CB107

GC(ECD) to ensure that the samples were within "normal" contamination levels. The combined sample (400 g plasma) was analysed by GC/MS.
For quantitative analysis, forty plasma samples (the ten individual samples from above included) from a random population, without any known particular exposure to environmental contaminants.

Results and discussion

Qualitative analysis of phenolic compounds; Analysis of the pooled sample of human plasma showed the presence of a large number of phenolic compounds, both by EI and NICI (chromatogram from GC(ECD) in Figure 1). From the GC/MS (NICI), 125 spectra of different phenol type compounds were recorded after methylation. Approximately 50% of these mass spectra has not yet been interpreted. Among the identified compounds, the halogenated phenols are the most abundant. In addition to PCP, 2,4,5-tri-, 2,3,4,6-tetra- and pentabromophenols were identified. Furthermore, lower chlorinated phenols, another tetrabromophenol and at least 10 mixed bromo-chlorophenols were indicated by their molecular weight and isotope pattern.

We have previously reported the presence of OH-PCBs in human plasma, among which 9 were structurally identified [5]. In this study, one more OH-PCB was identified by comparison to the appropriate standard - 4-OH-2,3,5,3',4',5'-hexaCB (4-OH-CB162). In the sample, a total of 30 hydroxylated PCB metabolites were identified or indicated, including four OH-oktaCBs, one OH-nonaCB. Dihydroxylated PCBs, di(OH)-penta-, -hexa- and heptaCBs, were indicated. These metabolites have not yet been structurally identified due to the lack of standards.

In the GC/MS (NICI) analysis, the presence of TBBP-A was indicated by comparison of retention time and bromine content to the authentic standard compound. However, molecular ion and fragments other than 79/81 were small in the standard and could not be seen at all in the sample. Confirming analysis are in progress. TBBP-A is used for the production of flame resistant polymers and has to our knowledge not previously been indicated in humans [13].

Qualitative analysis of neutral compounds; In the neutral fraction of the pooled plasma sample, in addition to the well-known contaminants, e.g. DDE, PCB and HCB, also several PBDE congeners were observed. The PBDE congeners were identified by comparison to individual standards and to the Bromkal 70 DE mixture that recently has been characterized [14]. The identified PBDE congeners are shown in Figure 2.

Quantitative analysis of phenolic and neutral compounds; The mean PCB concentration in the 40 analysed plasma samples was 660 ± 450 ng/g l.w. (CB-153 = 220 ± 160 ng/g l.w.). The sum of 5 OH-PCBs resulted in a mean concentration of 200 ± 130 ng/g l.w., ranging from 35 to 500 ng/g l.w. The ratio of total OH-PCBs to total PCBs ranged from 0.03 to 0.8. The peak pattern of OH-PCBs varied in the samples. Particularly, the 4-OH-CB108 and 4-OH-CB187 varied in relation to each other resulting in a ratio of 4-OH-108 to 4-OH-187 ranging from 0.5-2.2.

According to retention time in the GC/MS(NICI - 79/81) analysis, TBBP-A was present in all 40 samples. Preliminary data indicates concentrations in the low ppb level based on lipid weight. Quantifications are presently being performed.

PBDEs were present in all the samples analyzed. Six PBDE congeners were quantified in the 40 samples; BDE-28, 47, 66, 100, 99 and 153. BDE-47 and BDE-99 were the most abundant, and constituted approximately 70% of the total PBDE (mean value) in each sample. The mean concentrations of PBDE was 2.1 ±1.4 ng/g l.w. and thus at least two orders of magnitude lower

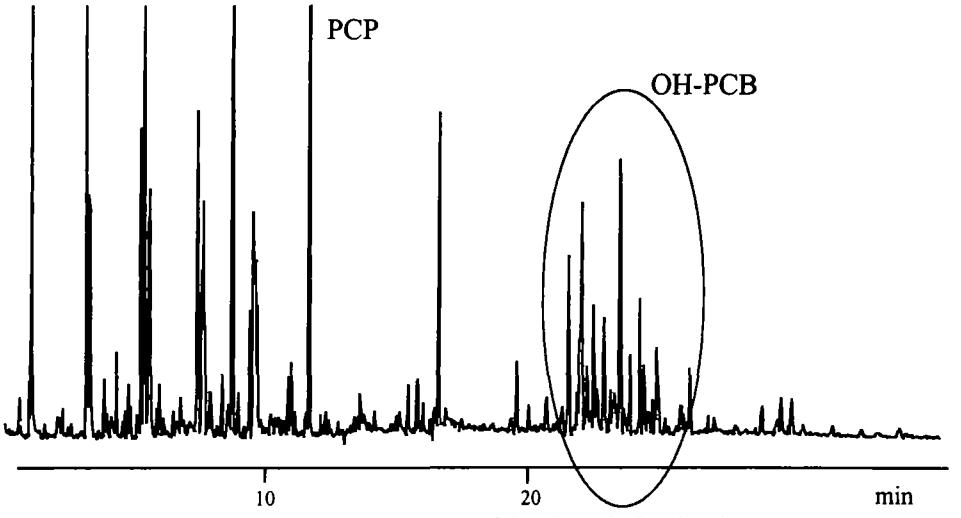

Figure 1. Part of a GC(ECD) chromatogram of the phenolic fraction from a pooled human plasma sample.

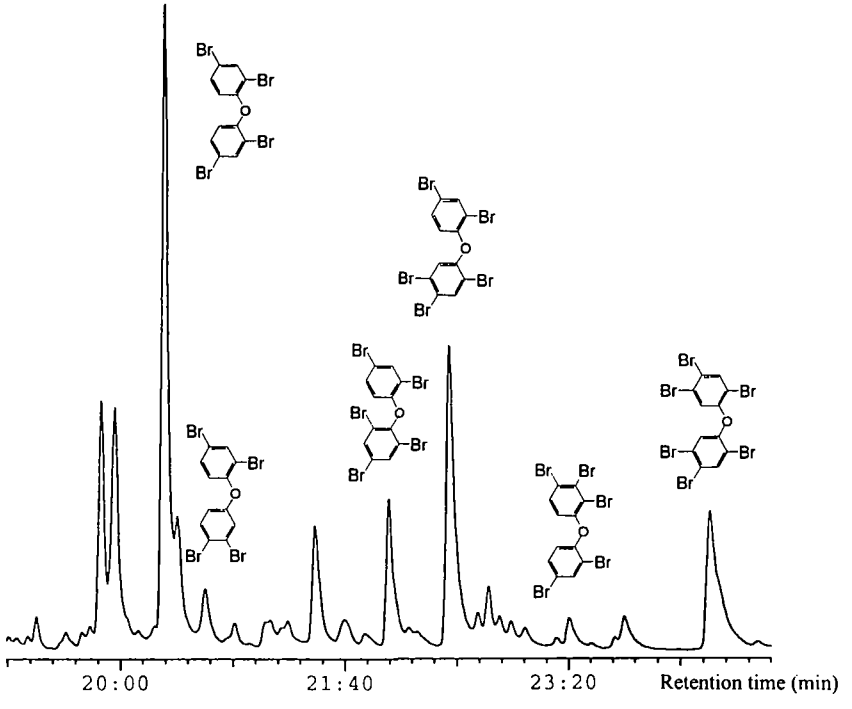

Figure 2. Part of a GC/MS(NICI) chromatogram (m/z=79/81) from the neutral fraction of a human plasma sample.

than PCB.

In the present study we confirm the presence and the levels of OH-PCBs versus PCB in 40 human plasma samples. In general, the same OH-PCB congeners seem to be dominating although there are individual differences in relative peak pattern. Due to lack of standards, all the OH-PCBs indicated by the qualitative analysis could not be structurally identified and consequently not quantified.

Halogenated phenols and benzenes are used industrially and are also formed in incinerators. In addition mixed bromo-chloro phenols are also formed in incinerators but are also registered as flame retardants [19,20]. PCP has previously been reported in human plasma, and also shown to compete for the binding site on a thyroxine transporting protein [4,6].

The presence of PBDE in human blood samples is reported for the first time. The levels are however low, being > 2 orders of magnitude lower than PCB but on the other hand no information on time trends are known in humans and too few toxicological studies have been performed up till now. PBDEs have previously been reported in adipose tissue [21,22] and in human breast milk in Germany [23].

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Literature cited

(1) Korach, K., Sarver, P., Chae, K, McLachlan, J. and McKinney J.D. *Mol. Pharmacol.* **1988**, 33, 120-126.
(2) Kramer, V., Helferich, W, Bergman, Å., Klasson Wehler, E. And Giesy, J. *Toxicol. Appl. Pharmacol.* In press.
(3) Brouwer, A., Morse, D., Lans, M., Schurr, G., Murk, A., Klasson-Wehler, E and Bergman, Å *J. Toxicol. and Industr. Health* **1996**, In press.
(4) Wild, S and Jones, KC, *Chemosphere* **1992**, 24, 847-855
(5) Bergman, Å., Klasson Wehler, E. and Kuroki, H., *Environ. Health Perspec.*, **1994**, 102, 464-469.
(6) Van den Berg, KJ, van Raaij, J, Bragt, P and Notten, W., *Arch toxicol.* **1991**, 65, 15-19.
(7) Fieser, L.F. and Fieser, M. In: *Reagents for Organic Synthesis*, (1967) pp 191-192, vol. 1 New York: John Wiley and Sons.
(8) Bergman, Å., Klasson Wehler, E., Nilsson, A., Kuroki, H. *Chemosphere*, **1995**, 30, 1921-1938.
(9) Ballschmiter, K., Mennel, A. And Buyten, J., *Fresenius J. Anal. Chem.* **1993**, 346,396-402.
(10) Sundström, G. *Acta Chem Scand.* **1973**, 27, 600-604.
(11) Örn, U.; Eriksson, L.; Jakobsson E. and Bergman, Å. *Acta. Chem. Scand.* **1996**, 50, 802.
(12) Hu, J. *Polybrominated diphenyl ethers (PBDE) - synthesis and characterisation.* Licentiate thesis. Dep. of Environmental Chemistry, Stockholm University, Sweden,

1996.
(13) Jakobsson, E.; Hu, J.; Marsh G. and Eriksson L. *Organohalogen Compounds* **1996**, 28, 463. 16th International Meeting, Dioxin '96, Amsterdam, Holland.
(14) Sjödin, A., Bergman, Å, Jakobsson, E., Kierkegaard, A. And Sellström, U., *Organohalogen Compounds* **1997**. 17th International Meeting, Dioxin '97, Indianapolis, USA.
(15) Hovander, L. Athanasiadou, M., Asplund, L. and Klasson Wehler, E. An improved method for analysis of phenolic and neutral organohalogen substances in plasma. Manuscript in preparation.
(16) Buser, H.-R. Anal. Chem. **1986**, 58, 2913-2919.
(17) Jansson, B., Asplund L. and Olsson, M. Chemosphere, **1987**, 16, 2343-2349.
(18) IPCS document, Environment al Health Criteria 172, **1995**, World Health Organization
(19) Tobin, P In: Hexachlorobenzene: Proceedings of an international symposium. (Ed's CR Morris and JRP Cabral), **1986**, pp 3-12. IARC Scientific publications no 77. Oxford university press, UK. ISBN: 92 832 1177 4.
(20) Heeb, NV, Dolezal, IS, Bührer, T, Mattrel, P, Wolfensberger, M. Chemosphere **1995**, 31, 3033-3041.
(21) Krüger, C. Thesis, Westfälischen Wilhelms-University, Münster, Germany, **1988**.
(22) Haglund, P., Zook, D., Buser, H-R and Hu, J. Submitted to Env. Sci. & Technol. **1997**.