LEVELS OF DECHLORANES AND PBDEs IN SERUM FROM CENTRAL EUROPEAN POPULATION

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Introduction

Biological monitoring (biomonitoring) can be defined as the assessment of internal dose exposure by measuring a toxicant (or its metabolites or reaction products) in human blood, urine, saliva, adipose, or other tissue. This approach is usually preferred over environmental monitoring for assessing human exposure because the later only provides data on the amount of toxicants that have been released and to which a population might have been exposed at different levels[1]. By-passing the exposure assessment step by directly measuring internal doses via analysis of biological specimens thus reduces the number of uncertainties in assessing health effects of toxicants[2].

Persistent bioaccumulative toxicants are highly lipophilic compounds that have long biological half-lives and are retained in adipose tissues. Equilibration of the lipophilic toxicants takes place between the lipids in adipose tissue and blood[3]. The primary matrix used for biomonitoring of persistent toxicants is blood and, more specifically, serum - typically, 5-75 mL of whole blood are sampled from patients. Even if much less invasive than the classical surgical abdominal fat removals that were performed in the 1980's, the venipunction of several milliliters of whole blood for analytical purpose is still badly perceived by patients. Therefore, either reducing the required volume of serum, or enlarging the list of measured analytes in a defined volume of serum is important.

Dechlorane or Mirex ($C_{10}Cl_{12}$) was extensively used as a pesticide but also as an additive flame retardant in the USA during the 1960s and the 1970s. After its ban, other related compounds such as Dechlorane Plus (DP, $C_{18}H_{12}Cl_{12}$), Dechlorane 602 (Dec 602, $C_{14}H_4Cl_{12}O$), Dechlorane 603 (Dec 603, $C_{17}H_8Cl_{12}$), Dechlorane 604 (Dec 604, $C_{13}H_4Br_4Cl_6$), and Chlordene Plus (CP, $C_{15}H_6Cl_{12}$) became candidates to replace Mirex. All these compounds share a bicyclo [2,2,1] heptene structure, resulting from a Diels-Alder reaction between one or two hexachlorocyclopentadienes (HCCPDs) and various cyclic dienophiles. They present flame retardant properties similar to Mirex[4]. The environmental occurrence of dechlorane-related compounds was first reported in 2006 in North America when DP was detected in air, sediment and fish samples from the Laurentian Great Lakes[5].

Even though the number of studies is still limited to a few, additional data collected in Korea, Brazil, Spain and Germany indicate that DP and related compounds should be considered as possible worldwide contaminants. A collection of the little available data for aquatic and terrestrial biota highlighted the lack of information on the toxicity of DP to aquatic and terrestrial organisms[6]. Two recent review papers described sources, occurrence and behavior of dechloranes in the environment, concluding on the need of more research dedicated to the production of data on exposure and toxicity[7-8]. Additionally to the fact of considering environmental contamination and geographical distribution, a better knowledge of the behavior of DP and related compounds in terms of bioaccumulation and biomagnification is still needed. This is important since structurally similar Mirex was banned and added to the Persistent Organic Pollutant List of the Stockholm convention because of its bioaccumulative potential[9].

Even more importantly, no biological monitoring (biomonitoring) data are available for dechloranes. The assessment of internal dose exposure by mean of measurement of these toxicants in human tissues or fluids is missing so far. Only a couple of reports are available and concern studies conducted in China and Canada. Levels in human serum collected from workers of a Chinese e-waste recycling facility were ranging from 7.8 to 465 ng/g lipid weight (lw)[10]. DP levels in Canadian human milk were also reported to be lower to those of polybrominated diphenyl ethers (PBDEs)[11]. The mean total DP ($\sum$DP) concentration was 0.98 ± 1.34 ng/g lw. None of these studies however reported data for others dechloranes.

The objective of the present study is to determine levels of DP, Dec 602, Dec 603, Dec 604, CP as well as Mirex and PBDEs in human serum samples collected in Central Europe (a region of the world where levels have not

yet been estimated) between 2003 and 2005. A strategy has been developed to separate and measure them in regular volumes of serum. We used GC-HRMS analysis with isotopic dilution. The aim was to provide an overview of the contamination pattern of these compounds in human.

Materials and methods

All samples were processed in an ISO17025 BELAC accredited laboratory for dioxins and PCBs. The procedure for dechlorane and PBDE measurement is not accredited but ISO17025 type criteria were applied. French human serum samples (n=48) were used. All standards were from Cambridge Isotope Laboratories or Wellington Laboratories. Others chemicals and solvents used were of analytical grade. Sample sizes of 10 g were extracted using solid-phase extraction (SPE) on non-endcapped C18 cartridges (1g/6mL). The C18 cartridges were eluted with 3 x 5 mL of hexane. The 15 mL of hexane were then loaded on a multi-layers column made with 1 g of sodium sulfate, 1 g of activated silica and 2 g of 22 % sulphuric acid silicagel. Further elution with 15 mL of hexane was performed. The evaporation was carried out in a PowerVap 6 system (Fluid Management Systems Inc., Waltham, MA, USA) to 500 µL using GC-vial connected evaporation tubes. After gentle room temperature evaporation, the final volume was 10 µL of nonane. Procedural blank samples consisted in 10 mL of Milli-Q water and were included with each series of 8 unknown samples.

Samples were analyzed with a high resolution sector mass spectrometer Thermo MAT95 XL connected by a heated transfer line (275°C) to a CE Trace gas chromatograph (ThermoQuest) equipped with an A2000S autosampler (Thermo). The GC column was a Phenomenex ZB-5 (15m x 0.25mm I.D., 0.25 µm df). Helium was used as the carrier gas at a constant flow rate of 1 mL/min. One or two microliters of the final extract in nonane (10 µL) were injected into a split/splitless injector held at 280°C in splitless mode. For dechlorane measurements, the GC oven temperature was maintained at 140°C for 2 min, ramped at 30°C/min to 280°C then at 5°C/min to 300°C and held for 10 min. For PBDE measurements, the GC oven temperature was maintained at 140°C for 1 min, ramped at 15°C/min to 180°C, then at 10°C/min to 290°C and finally at 80°C/min to 350°C and held for 2 min. The ion source temperature was 250°C and Electron Ionization (EI) was performed with 70 eV. The HRMS instrument was operated in the selected ion monitoring (SIM) mode. Two ions were monitored for both native and labeled isotope ratio check. Calibration stability was ensured by injecting both low and high levels points of the calibration curve every 20 samples. Both instrumental and procedural blanks were monitored.

Isotope ratio between the two monitored ions was checked for variation within 20% of the theoretical value (lower than 15% for PBDEs and between 15 to 30 % for dechloranes). The limit of quantification of the method (mLOQ) was calculated based on a signal-to-noise (S/N) ratio equal to 6. For the compounds detected in procedural blanks, the mean procedural blank value was subtracted from the samples and the limit of quantification was set at 3 times the standard deviation (σ) of the procedural blank. A QC serum sample was included with each series of 8 unknown samples. This QC was constituted with a pool of non-fortified Central European human serum of 1030 adults. QC chart values were normalized as Z-score. Upper and lower control limits (UCL/LCL) corresponded to 3 σ while warning limits were set at 2 σ. For all compounds, each QC was included within 2 σ of the total average. The mean values (ng/g lw) of dechlorane and PBDE levels in the non-fortified pool serum ranged between 0.21 ± 0.03 and 1.65 ± 0.24 ng/g lw, with CVs ranging between 11% and 37%.

Results and Discussion

Levels of DP, Dec 602, Dec 603, Dec 604, CP, and Mirex of general French population are listed in Table 1. Detection frequencies for all investigated dechloranes were high, excepted for Dec 604 that appeared to be below LOQ values for all samples. Dec 603 surpassed all other dechloranes in terms of mean concentration (2.61 ± 2.63 ng/g lw). Mirex, the banned product, was measured in all samples at a mean value of 1.40 ± 0.93 ng/g lw. The mean total DP (∑DP) concentration was 1.40 ± 1.40 ng/g lw, lower than levels reported for Chinese e-waste recycling facility workers, but higher than levels reported for Canadian human milk. As it was the case for the Canadian milk samples, the mean ∑DP level was lower than those of $\sum_5$PBDEs (4.32 ± 2.99 ng/g lw). However, once other dechloranes are included, the mean $\sum_5$dechlorane levels are higher than the mean $\sum_5$PBDE levels (6.24 ± 4.16 ng/g lw versus 4.32 ± 2.99 ng/g lw). Dec 602, Dec 603, and CP therefore appear to be important congeners to measure if one has to estimate a global dechlorane exposure. Focusing on ∑DP only might lead to

an under estimation of the real dechlorane exposure.

Table 1: Mean levels of dechloranes and PBDEs in human serum (ng/g lw) (n=48).

	LOQ	Detection frequency (%)	Mean	Median	Min	Max	SD
Mirex	0.03	100	1.40	1.06	0.14	4.30	0.93
Syn-DP	0.08	75	0.34	0.22	nd	2.30	0.42
Anti-DP	0.16	94	1.20	0.89	nd	5.09	1.12
Total DP	-	-	1.40	1.20	nd	7.04	1.40
Dec 602	0.04	100	0.64	0.44	0.15	4.21	0.63
Dec 603	0.4	90	2.61	2.01	nd	12.10	2.63
CP	0.08	92	0.20	0.16	nd	0.71	0.16
Σ_5 Dechloranes	-	-	6.24	5.21	1.33	20.09	4.16
BDE-47	0.75	100	2.06	1.56	< LOQ	8.72	1.80
BDE-99	0.27	100	0.49	0.27	< LOQ	4.69	0.68
BDE-100	0.15	100	0.34	0.26	< LOQ	1.57	0.26
BDE-153	0.05	100	1.39	1.14	0.46	5.83	0.97
BDE-154	0.02	58	0.05	0.04	nd	0.26	0.06
Σ_5 PBDEs	-	-	4.32	3.46	1.63	15.02	2.99

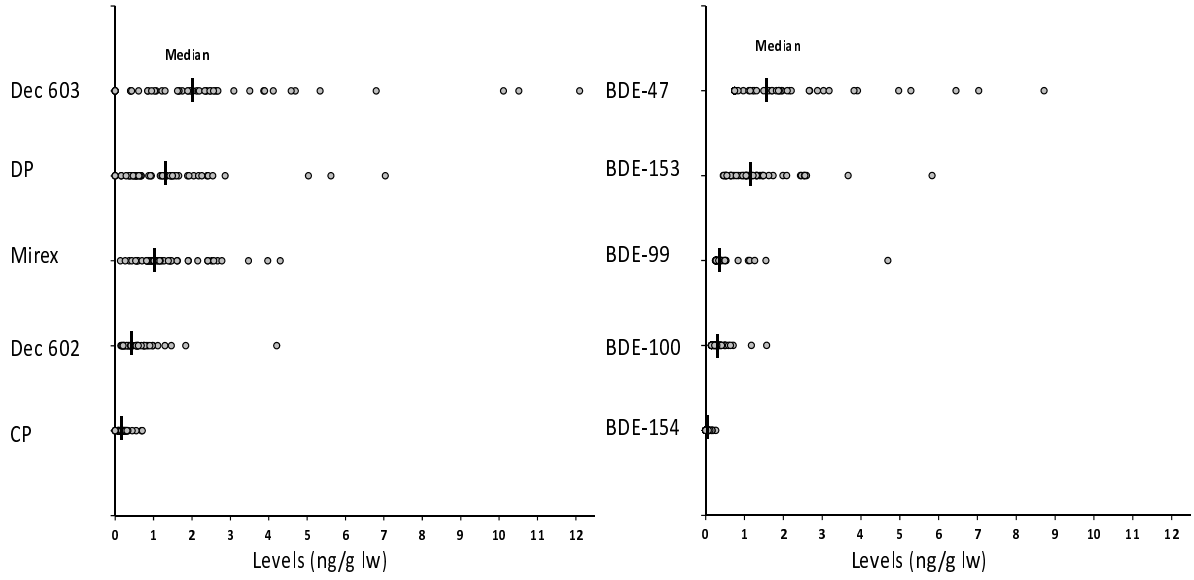

Figure 1. Distribution of analyte levels in human serum samples and estimation of the relative concentration pattern based on median values (n=48).

As expected, for PBDEs, BDE-47 and BDE-153 were the major congeners with mean levels at 2.06 ± 1.80 ng/g lw and 1.39 ± 0.97 ng/g lw, respectively. The mean $\sum_5$PBDE levels (4.32 ± 2.99 ng/g lw) is in the range of typical Central Europe levels.

Figure 1 shows the distribution of the recorded levels for each analytes. Dechlorane distributions are

compared to PBDE distributions. The larger distribution, as well as the higher levels, were recorded for Dec 603 and BDE-47. Levels of both families are similar. Such data indicate that the attention currently given to PBDEs should be extended to dechloranes, at least until more toxicological data are available.

This study is the first report on levels of dechloranes compounds in European human serum. DP as well as others related dechloranes were detected, while no production source has been identified yet in Europe. The hypothesis of long term transport has to be considered. A specific pattern of contamination was found, and Dec 603 was reported with high levels, compared to others biota samples that have been analyzed from Europe. These results indicate that bioaccumulation properties should be further investigated and taken in consideration when considering human exposure to dechloranes.

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