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Supplementary file

Environmental exposure to POPs and heavy metals in urban children from Dhaka, Bangladesh

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POP analysis

Chemicals

The organochlorine pesticides and the PCB standards were either synthesized in house or purchased from Larodan Fine Chemicals AB, Malmö, Sweden. PBDE standards were purchased from LGC Standards (Borås, Sweden). Solvents, acids and salts were of highest quality available and the methyl *tert*-buthyl ether was distilled. The silica gel (200-400 mesh, 60 Å, Aldrich, USA) was baked at 300°C over night before use.

Instrumental details

Chlorinated POP analyses were performed on a gas chromatograph (GC) from Varian (CP-450) equipped with a CP-8400 autosampler and an electron capture detector (ECD). A large volume injector was used. An injection volume of 5 µl was used for PCBs and HCHs whereas 1 µl injection volume was used for 4,4'-DDE and 4,4'-DDT analysis. The temperature program for the injector was as follows: 80°C, 0.35 min; 200°C/min to 300°C. A non-polar column (CP-SIL 8CB 25 m × 0.15 mm × 0.12 µm) Varian, Middleburg, the Netherlands, was used. Helium was used as a carrier gas and nitrogen as the make up gas. The column oven temperature was programmed from 80°C, 2.5 min; 20°C/min to 300°C; hold for 5 min.

The PBDEs were analyzed on a SSQ 710 mass spectrometer (ThermoFinnigan) connected to a Varian 3400 GC. A septum equipped temperature programmable injector (SPI) was used together with a DB-5 HT capillary column (15 m × 0.25 mm × 0.1 µm) from J&W Scientific, Folsom, USA. The temperature program for the GC was 85°C, 1 min; 15°/min to 300°C and then 2°C/min to 310°C which was held for 5 min. Electron capture negative ionization (ECNI) with methane (scientific 5.5, AGA Stockholm, Sweden) as a reagent gas and an electron energy of 70 eV was used. Helium was the carrier gas, the ion source temperature was 200°C and the transfer line temperature was set to 290°C. Selected ion monitoring (SIM) mode (isotopes m/z 79 and 81) was used.

Clean-up procedure

Surrogate standards (CB-200, 1 ng and BDE-138, 0.4 ng) were added to the serum samples before extraction. The serum (2.7-4.5 g) were extracted and cleaned-up according to Hovander et al. 2000[1] with some modifications. In brief, hydrochloric acid and 2-propanol were added to the samples before extraction with cyclohexane : methyl *tert*-buthyl ether (MTBE) (1:1). The organic phase was washed with 1% potassium chloride. Neutral and phenolic compounds were separated by partitioning with potassium hydroxide in 50% ethanol. The clean-up step consisted of a sulfuric acid partitioning and a sulfuric acid treated silica gel column. The column (0.1 g silica gel + 1 g sulfuric acid : silica gel column 1:2) was eluted with cyclohexane : DCM, (1 : 1, 10 ml). CB-189 (1 ng) was added to the samples as an injection standard prior the GC/ECD analysis for recovery calculations of the surrogate standard (CB-200). The final volume was set to 0.5 ml for PCB and organochlorine pesticide analysis by GC/ECD. Prior to analysis of the PBDEs by GC/MS, the samples were fractionated on a silica gel column (1 g). The first fraction, containing PCBs and pesticides, was isolated with cyclohexane (6 ml) as the mobile phase. A second fraction, containing PBDE congeners, was isolated with DCM (6 ml) as mobile phase. After evaporation of the solvents and exchange to n-hexane, BDE-139 (0.3 ng) was added as an injection standard. The final volume was corrected to 50 µl prior to PBDE analysis by GC/MS-ECNI.

QA/QC

Solvent blank samples and laboratory reference samples were run with each batch of samples. The mean recovery of the surrogate standards were 83% for CB-200 (relative standard deviation 15%) and 88% for BDE-138 (relative standard deviation 7.5%). All chlorinated compounds were above the limit of quantification (LOQ) in all samples except for β-HCH.

LOQ for β-HCH was estimated to 4 ng/g fat and half the limit of quantification was used for calculations. The limit of quantification for the PBDEs was set to ten times the signal to noise or, if occurring in the blank samples, three times the level in the blank samples. LOQ for the PBDE congeners were as follows; BDE-28 (1.5 pg), BDE-47 (15 pg), BDE-99 (6.5 pg), BDE-100 (3.1 pg), BDE-153 (0.29 pg), BDE-154 (1.2 pg) and BDE-209 (42 pg). LOQ is expressed as total amount in the samples. The background levels of PBDE congeners found in the solvent blank samples were subtracted from the levels measured in the serum samples.

Metal analysis

Instrumental details

Blood selenium (B-Se), blood cadmium (B-Cd), and blood lead (B-Pb) were determined by inductively coupled plasma mass spectrometry (ICP-MS; Thermo X7, Thermo Elemental, Winsford, UK). A sample volume of 500 µL whole blood was diluted ten times with an alkaline solution according to Bárány et al.[2]. Using the dilute solution as a carrier/rinsing fluid, the samples were introduced in a segment-flow mode. The samples were analyzed in peak-jumping mode, 70 sweeps and 1 point per peak, 30 ms dwell time for ^{114}Cd, 20 ms for ^{82}Se, 15 ms for $^{206, 207, 208}$Pb and 10 ms for the internal standards ^{115}In ^{205}Tl and ^{209}Bi. The intensity of the isotopes 206, 207 and 208 were summarized for Pb. Interference corrections were made for ^{114}Cd for the spectral overlap of Sn.

QA/QC

The detection limits, calculated as three times the standard deviation (SD) of the blank were for Se, Cd, and Pb, 1.6, 0.02, and 0.06 µg/L, respectively. All samples analyzed were prepared in duplicate and the method imprecision (calculated as the coefficient of variation for duplicate preparations measurements), were 3.9, 3.6, and 3.0%, for Se, Cd, and Pb, respectively. The analytical accuracy was checked against reference material by use of Seronorm Trace Elements Whole Blood (SERO AS, Billingstad, Norway) and human blood reference samples from Centre de Toxicologie du Quebec, Canada (CTQ). The obtained values for Se, Cd and Pb in the seronorm samples were 123±11 (mean±SD), 6.2±0.01 and 388±1.9 µg/L versus recommended 123±10, 6.0±0.4 and 393±21 µg/L and for Cd and Pb in the Quebec samples 0.76±0.02 and 23.9±0.10 µg/L versus recommended 0.79±0.28 and 22.8±1.1 µg/L.

References

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