MEASUREMENT OF HUMAN LEVELS OF DECHLORANE 602 BY CZC-NCI-HRTOFMS

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Introduction

Dechlorane (Dec) 602 is part of the norbornene derivatives flame retardants family that has quite recently been reported in the environment[1]. Because very little is known about their potential bioaccumulation in human, we developed a GC-MS method for the measurement of 602, 603, 604 and Chlordene Plus (CP) in human serum. The particularity of the 600 family molecules is to exhibit a bicycle [2,2,1]-heptene halogenated structure. During mass spectrometric analysis, they can undergo retro Diels-Alder reactions and typically form hexachlorocyclopentadiene (HCCPD) fragments (m/z = 272). The quantification of those analytes is most of the time carried out on the most abundant ions of this fragment cluster (e.g m/z 271.8102/273.8072 for Dec 602)[2]. In the present report, we further investigated the use of a cryogenic zone compression negative chemical ionization high resolution time-of-flight mass spectrometry (CZC-NCI-HRTOFMS) method previously developed[3] to provide sensitivity and specificity for the target dechlorane compounds, presently focusing on Dec 602. One of the feature of this approach is that the molecular ion cluster is heavily present and the HCCPD cluster that is used for quantification in EI is barely present in NCI, although its dechlorinated product (pentachlorocyclopentadiene – PCCPD – C_5Cl_5) is present in high abundance and therefore both clusters can be used for selective quantification[3]. In addition of using CZC to enhance the instrumental detection limits (iDLs), two different GC phases were combined to reduce the risk of chromatographic co-elutions usually present in the complex blood matrix. Furthermore, the HRTOFMS instrument was equipped with a specific low thermal emission filament, coated with Yttria, that allowed to reproducibly perform in NCI mode at ion source temperatures as low as 140°C. The use of such reduced temperature was ideal to minimize dissociative electron capture (DEC) and enhance resonance electron capture (REC) to favor high MS signal for the parent ion cluster. This is important for isotope dilution (ID) (once ^{13}C labels will be available) accurate quantification, but also for enhanced specificity in the identification of the target molecule, and possibly untargeted emerging analogs. The use of full-scan HRTOFMS additionally allows performing elemental composition calculations.

Materials and Methods

Chemicals and samples

All parameters concerning the quality and potential pre-treatment of the entire chemical used for those analyses are the ones used in routine. The QC pool was made of Central European serum samples that were not artificially fortified in any toxicants. The 51 human serum samples reported were collected in France in 2005.

Analytical procedure

All samples were processed in an ISO17025 BELAC accredited laboratory. Sample sizes of 10 g were extracted using solid-phase extraction (SPE) on non-endcapped C18 cartridges (1g/6 mL). The C18 cartridges were eluted with 3 x 5 mL of hexane. The 15 mL of hexane were then loaded on a multi-layer column made of 1g sodium sulfate / 1g

activated silica / 2g of 22% sulphuric acid silicagel. Further elution with 15 mL of hexane was performed. The evaporation was carried out in 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. Measurements were carried out on a JEOL AccuTOF GC system (JEOL Ltd., Tokyo, Japan). The GC oven (Agilent 6890) was equipped with a ZX1 - LN_2 Cooled Loop Modulation GC x GC System (Zoex Corp., Houston, TX, USA). The ^{1}D and ^{2}D GC columns were respectively a Rtx-PCB (15 m x 0.25 mm ID x 0.25 µm df) and a Rxi-17 (2 m x 0.18 mm ID x 0.18 µm df) (Restek Corp., Bellefonte, PA, USA). The P_M was 4 s with 400 ms of hot pulse duration. The temperature program was 140°C for 2 min, 30°C/min to 250°C then 10°C/min to 300°C hold for 10 min. 1 µL of the final extract in nonane (10 µL) was injected into a split/splitless injector held at 280°C in splitless mode. Helium was used at 1.0 mL/min. The major MS parameters were an ion source temperature of 140°C, an ionisation voltage of 200 V, methane at 1 mL/min as reagent gas, an acquisition range from 30.00 to 700.00 m/z, a recording interval of 0.04 s (25 Hz), an accumulation time of 0.037 s, a data sampling interval of 0.5 ns, and a detector voltage of 2300 V. The mass accuracy of the instrument was ensured by frequent single point calibration checks.

Results and Discussion

Analysis

Isotopic dilution quantification was performed with ^{13}C PCB 209 as internal standard calibration (no ^{13}C-labelled Dec 602 yet available). The calibration curve (range 0.05-10 pg/µL) was achieved with triplicate injections for each level point. Stability over concentration range and time was tested by injecting triplicates of low and high level of Dec 602 standard (0.2 and 5.0 pg/µL) at different times and between unknown sample injections. Mean reported values (n=27) were 0.23 ± 0.03 pg/µL (CV = 13.7%, relative error = 15%) and 5.0 ± 0.6 pg/µL (CV = 12.9%, relative error = -3%), for 0.2 and 5.0 pg/µL calibration standards, respectively. The Youden plot reported in Figure 1 shows the distribution of those calibration points (each point has the 5.0 pg/µL and 0.2 pg/µL coordinates on the y- and x-axis, respectively) in terms of standard deviation units (z-scores). Although limited in time, this indicates a possible deviation of the response over time towards low z-score values. This is an indication on the life time of the calibration curve.

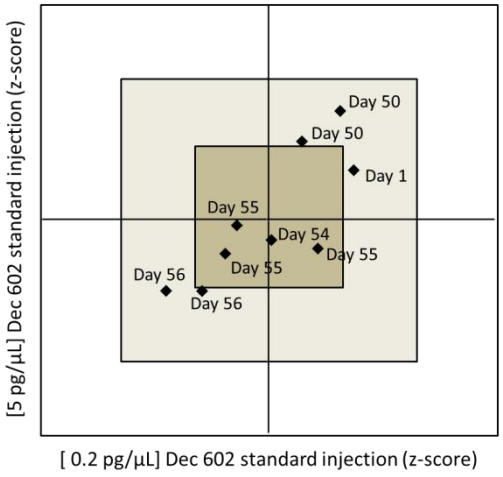

Figure 1: Youden plot for two 602 calibration points (0.2 pg/µL and 5.0 pg/µL) over time. Each marker represents the mean of triplicate injections and their z-score values on both x-axis (0.2 pg/µL) and y-axis (5.0 pg/µL). Centred squares define the limits of 1, 2 and 3 standard deviation units (z-scores).

Dec 602 area calculations for quantification were based on the sum of both the molecular ion and the PCCPD fragment ion to minimize possible variations related to the fact that the internal standard used for quantification doesn't undergo retro Diels-Alder reaction. Better response factors were obtained that way (0.41 for the sum of the molecular ion and PCCPD ion versus 0.11 for the molecular ion area only). Better reproducibility was also observed when those two ions were used (14% CV for the sum of ions versus 21% CV for molecular ion only). Dec 602 recovery rates were calculated using ^{13}C PCB 80. They averaged at 44.3 ± 10.3 % for QC samples.

Both a procedural blank and a serum QC sample were included for each series of 10 unknown samples. No Dec 602 was detected in any of the procedural blanks. QCs were obtained from a non-fortified Central European human serum pool. Figure 2 represents a QC chart for the 6 QC samples that were analyzed during the time of unknown sample analyses. Each QC was included within 2 σ of the total average of Dec 602 QC levels (0.73±0.14 ng/g lipid, CV = 18.7%, n=6). Isotopic ratios were controlled for the molecular ion cluster, the PCCPD fragment cluster, and the internal standard molecular ion cluster. Calculated ratios were within 6% of the theoretical isotopic ratios.

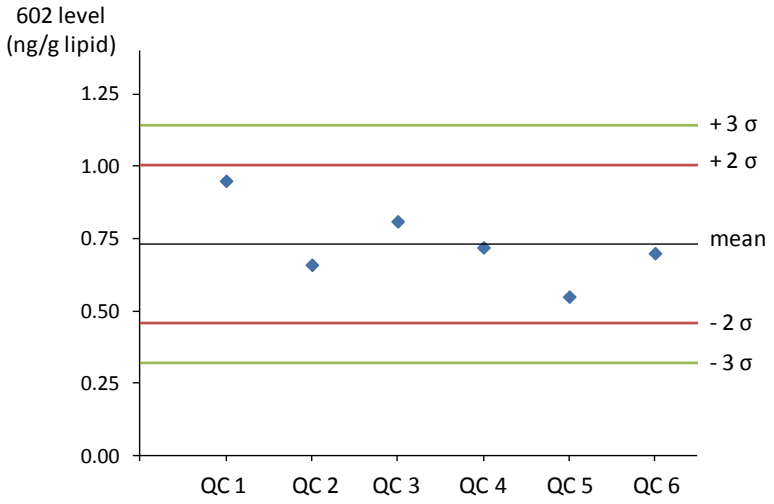

Figure 2: Moving control chart of QC analysis. QC samples were obtained from a pool of Central European human serum and analyzed for each series of 10 samples. The mean QC level was continuously recalculated. The mean Dec 602 level of the unfortified human serum pool was 0.73±0.14 ng/g lipid.

Levels in human

Dec 602 levels were measured in 51 human serum samples collected in France in 2005. The concentration range was 0.21-5.60 ng/g lipid. The mean value was 0.93 ± 0.90 ng/g lipid (no outliers removed). The median value was 0.71 ng/g lipid. Figure 3 illustrates the distribution and frequency of the reported levels.

Independently of the reported concentration, good mass accuracy was observed for all measurements performed on real serum samples. Mass differences were always below 5 mmu (e.g. 3.1 mmu for the highest concentration and 2.8 for the lowest concentration, in real samples, based on cluster ion M of 607.6524 amu). Interestingly, no Dec 602 was recorded in any of the blanks we performed. Other analytes such as Dec 603 and CP, known to be by-products found in Aldrin, Dieldrin, or Chlordane commercial mixtures, were also identified in some samples. Further investigation and quantification of those analytes are under progress.

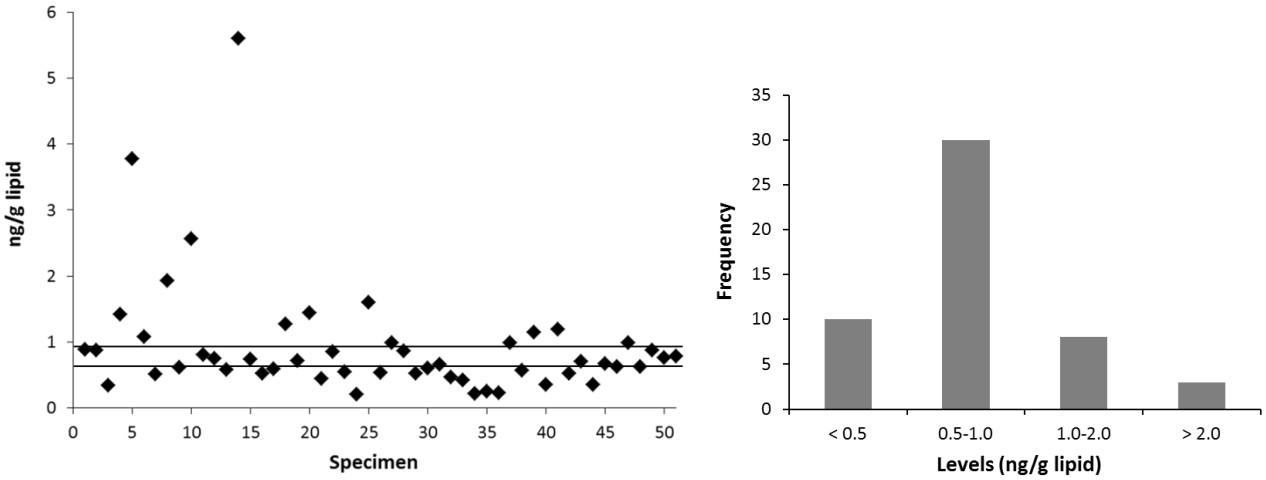

Figure 3: Levels of Dec 602 in human serum samples collected in the general population of France in 2005.

Acknowledgements

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References

1. E Sverko et al., Environ. Sci. Technol. (2010) 44, 574.
2. L Shen et al., Environ. Sci. Technol. (2011) 45, 693.
3. C Brasseur et al., Organohal. Comp. (2011) 73, 1359.