DEVELOPMENT OF ANALYTICAL METHOD FOR THE DETERMINATION OF DECHLORANES AND RELATED COMPOUNDS IN ENVIRONMENTAL AND BIOTA MATRICES

Barón E[a], Corcellas C[a], Eljarrat E[a*], Barceló D[a,b]

[a]Dep. of Environmental Chemistry, IDAEA, CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain; [b]Catalan Institute for Water Research (ICRA), Parc Científic i Tecnològic de la Universitat de Girona, Pic de Peguera 15, 17003 Girona, Spain.

Introduction

Dechloranes 602 (Dec 602; $C_{14}H_4Cl_{12}O$), 603 (Dec 603; $C_{17}H_8Cl_{12}$), 604 (Dec 604; $C_{13}H_4$ Br_4C_{l6}), and Dechlorane Plus (DP or Dec 605; $C_{18}H_{12}Cl_{12}$) are halogenated flame retardants introduced as replacements of Mirex (Dechlorane; $C_{10}Cl_{12}$)[1], which use was banned in 1978[2]. All of these replacements were designed and synthesized to avoid bioavailability of the chlorinated norbornene. However presence of these emerging pollutants has been observed in sediments and biological matrices such as fish and eggs[3] showing their bioaccumulation potential.

Despite the increasing number of recent scientific publications, the knowledge about the environmental occurrence, fate and behavior of dechloranes and related compounds is still limited. The purpose of this study was to develop analytical methodologies able to determine the occurrence of these emerging flame retardants in a wide range of matrices, including environmental samples (sediment, sludge, air), biota samples (fish) and human samples (human breast milk).

Materials and methods

Chemicals

Dec 602 (95%, CAS# 31107-44-5), Dec 603 (98%, CAS# 13560-92-4), and Dec 604 (98%, CAS# 34571-16-9) were purchased from Toronto Research Chemical Inc. (Toronto, ON, Canada). DP (CAS# 13560-89-9), syn-DP and anti-DP standards, were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). Mirex (CAS# 2385-85-5) and DPMA were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA). Hepta-BDE-181 was used as quantitative standard and was purchased from Wellington Laboratories Inc. (Guelph, ON, Canada).

Instrumental analysis

Chromatographic separation was carried out by Gas Cromatography (GC) fitted with a 15 m DB-5-MS capillary column (0.25 mm i.d. x 0.10 µm film thickness), and detection was carried out by Mass Spectrometry (MS) working in Negative Chemical Ionization (NCI). The GC-MS experimental conditions were optimized in order to obtain maximum signal to noise ratio. Different parameters were studied, starting with the selection of the chemical ionization moderating gas (ammonia and methane). Once methane was established as the better reaction gas, other instrumental parameters were optimized such as: source temperature, system pressure, emission energy and electron energy.

Sample preparation

Sample methodologies for Dec 602, Dec 603, Dec 604, CP, Mirex and DP analyses were optimised based on previous methods for PBDE determinations. Prior to extraction, samples were spiked with 50 ng of each compound.

Sediments. To reduce time of analysis, a selective pressurized liquid extraction (SPLE) method was used[4]. SPLE was carried out using a fully automated ASE 200 system (Dionex, Sunnyvale, CA). A 22mL extraction cell was loaded by inserting two cellulose filters into the cell outlet, followed by 6 g of alumina (0.063-0.200 mm, from Merck, Darmstadt, Germany). Spiked sediment samples were ground with alumina and cooper (<63 ím, from

Merck, Darmstadt, Germany) (1:2:2). Mixtures were loaded into the extraction cell on top of alumina. The dead volume was filled with Hydromatrix (Varian Inc., Palo Alto, U.S.A.), and the cell was sealed with the top cell cap. The extraction cell was heated to 100 °C and filled with hexane:CH_2Cl_2 (1:1) mixture until the pressure reached 1500 psi. After an oven heat-up time of 5 min under these conditions, two static extractions of 10 min at constant pressure and temperature were developed. After this static period, fresh solvent was introduced to flush the lines and cell, and the extract was collected in the vial. The flush volume amounted to 80-100% of the extraction cell. The extraction was cycled twice. The volume of the resulting extract was about 35 mL. Extracts were finally concentrated to incipient dryness and redissolved with 50 µL of toluene prior to the analysis by GC-NCI-MS.

Sewage sludge and Air samples. One gram dw of sample was spiked with internal standard (hepta-BDE-181). Spiked samples were kept overnight to equilibrate. Then, samples were extracted by PLE, using the same conditions applied to sediments. After extraction, samples extracts were treated with concentrated sulphuric acid and subsequently purified with five grams of alumina SPE cartridges. Samples were finally concentrated to incipient dryness and re-dissolved 50 µL of toluene prior to the analysis by GC-NCI-MS.

Biota and human samples. One gram dw of sample was spiked with internal standard (hepta-BDE-181). Spiked samples were kept overnight to equilibrate. Then, samples were extracted by PLE, using the same conditions applied to sediments. Resulting extracts were solvent removed for gravimetric lipid determination and subsequently redissolved in hexane. The solution was then treated with concentrated sulphuric acid and then cleaned by alumina SPE cartridges. Samples were finally concentrated to incipient dryness and re-dissolved 50 µL of toluene prior to the analysis by GC-NCI-MS.

Quality Control

Recovery tests were carried out by addition of each analyte to the different matrices. Five replicates were prepared for evaluation of the reproducibility of the methods. Method detection limits (LODs) defined as the minimum amount of analyte which produces a peak with a signal-to-noise ratio equal to 3, as well as method quantification limits (LOQs) defined as the minimum amount of analyte that produces a peak with a signal-to noise ratio equal to 10, were determined for each analyte.

Results and discussion

Instrumental method optimization

Different parameters were studied, starting with the selection of the chemical ionization moderating gas. Two different reagent gases, methane and ammonia, were tested in the NCI system. For all the selected analytes, the sensitivity obtained using methane was much higher than those obtained with ammonia.

Once methane was established as the better reaction gas, other instrumental parameters were optimized such as: source temperature, system pressure and electron energy. The abundances of selected analytes *vs* system pressure is given in Figure 1a. Maximum abundances were obtained at the highest system pressure tested. Higher values were not recommended by the instrument consumer. Regarding the source temperature, 175 °C was the optimal value, with sensitivity decreasing with increase of the temperature. Regarding the electron energy (Figure 1b), the maximum sensitivity was obtained for 100-150 eV, with the minimum values obtained for 50 eV. When the energy was increased from 150 eV to 200 eV, also a decrease in the sensitivity was observed.

Analytical parameters

In order to evaluate the instrumental method developed, different quality parameters such as linearity, intra- and inter-assay variation, and sensitivity were studied. Instrumental LODs and LOQs are presented in Table 1. LODs varied from 24.4 to 454 injected fg for DPMA and Dec 604, respectively. LOQs ranged between 81.2 and 1513 fg.

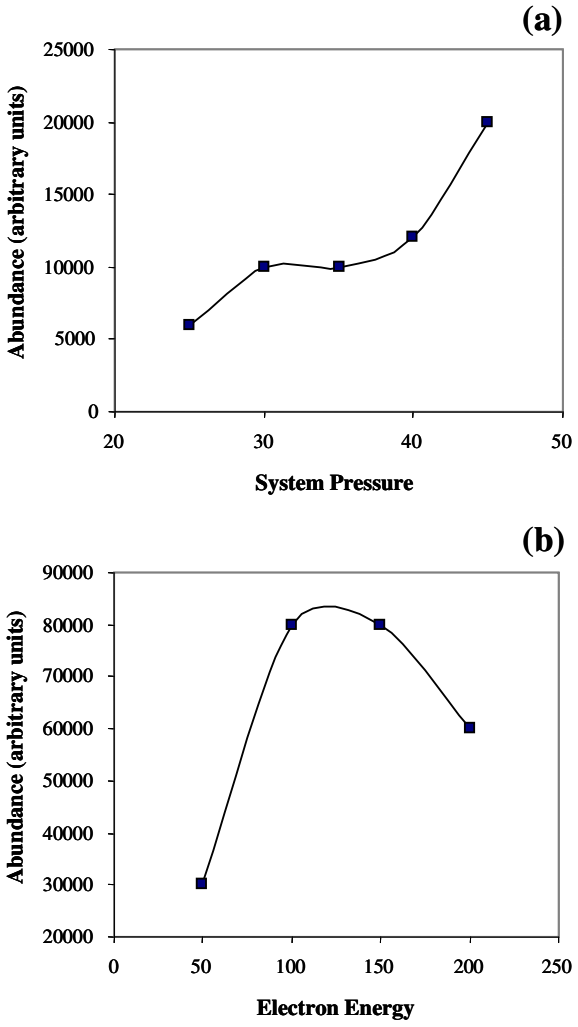

Figure 1. Variation of the abundance of Dechlorane plus vs the system pressure and electron energy, using methane as moderating gas.

Table 1. Instrumental limits of detection and limits of quantification (expressed as injected fg) of the developed method by GC-NCI(methane)-MS

	t_R (min.)	*m/z*	LOD	LOQ
DPMA	13.6	35	24.4	81.2
Mirex	16.6	368	73.1	244
Dec602	18.0	614	408	1361
Dec603	20.9	638	43.7	146
Dec604	21.3	79	454	1513
sDP	22.8	654	27.5	91.6
aDP	23.4	654	32.0	107

On the other hand, method LODs and LOQs obtained for the different matrices analysed are shown in Table 2. As expected due to the matrix characteristics, the best values were obtained for sediment, with LODs ranging between 2.47 to 70.2 pg/g dw. Similar values were calculated for fish samples, with LODs between 11.8 and 108 pg/g dw. Finally, higher values were obtained for sewage sludge samples, with LODs in the range of 29.4-743 pg/g dw.

Table 2. Limits of detection and limits of quantification (expressed as pg/g dw) of analytical methods developed for different matrices.

	Sediment		Sludge		Fish	
	LOD	LOQ	LOD	LOQ	LOD	LOQ
DPMA	45.0	150	743	2475	89.8	299
Dec603	70.2	234	49.0	163	79.0	263
Dec604	46.4	155	277	924	46.6	155
sDP	2.47	8.25	16.8	56.1	108	360
aDP	11.3	37.6	29.4	98.2	11.8	39.4

Acknowledgements
This research project was founded by the Fundación BBVA under the BROMACUA project (Evaluación del impacto ambiental de los retardantes de llama bromados en ecosistemas acuáticos de América Latina), by the Spanish Ministry of Environment and Rural and Marine Affairs through the project IMPAR (Ref. 106/2010) and by the Spanish Ministry of Science and Innovation through the projects CEMAGUA (CGL2007-64551/HID) and SCARCE (Consolider Ingenio 2010 CSD2009-00065).

References
1. WHO, (1984). Mirex, ICPS*, Environmental Health Criteria, 44.*
2. Persistent Bioaccumulative and Toxic (PBTs) Chemical Program*. http://www.epa.gov/pbt/pubs/mirex.htm* (Accessed April 2011).
3. Guerra P, Fernie K, Jiménez B, Pacepavicius G, Shen L, Reiner E, Eljarrat E, Barceló D, Alaee M. (2011); *Environ. Sci. Technol.* (45): 1284 – 1290.
4. De la Cal A, Eljarrat E, Barceló D. (2003); J. of Chromatogr. A (1021): 165-173.