ULTRA-TRACE MEASUREMENT OF DECHLORANES TO INVESTIGATE FOOD AS A ROUTE OF HUMAN EXPOSURE

Calaprice C[1,2,3], L'Homme B[1], Calvano CD[3], Zambonin C[3], Focant JF[1]*

[1]University of Liège, Chemistry Institute (B6c), Mass Spectrometry Laboratory, Organic & Biological Analytical Chemistry Group, Allée du Six Août, 11, Quartier Agora B-4000 Sart-Tilman, (Liège) – Belgium; [2]Politecnico di Bari, Dipartimento di Ingegneria Civile, Ambientale, del Territorio, Edile e di Chimica, Via Orabona, 4, 70125 Bari – Italy; [3]Università degli studi di Bari, Dipartimento di Chimica, via Orabona 4, 70125 Bari – Italy
*JF.Focant@ulg.ac.be

Introduction

Dechloranes are unregulated emerging persistent organic pollutants (POPs) currently used as replacement of regulated compounds such as Mirex (also called Dechlorane) and decabromodiphenyl ether (deca-BDE, BDE-209). Dechloranes, namely Dechlorane Plus (DP, $C_{18}H_{12}Cl_{12}$, syn and anti-isomers), 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, $C_{15}H_6Cl_{12}$), are issued from a family sharing a norbornene moiety and are produced via Diels-Alder condensation between hexachlorocyclopentadiene and several dienophiles. They exhibit both pesticide and flame-retardant properties and they are currently extensively used as additives in various synthetic products such as nylon or plastic like polypropylene[1], as well as in electronic devices [2]. They have recently been reported at low levels in environmental samples, or in dust collected from various environments [3, 4]. Biota and humans are exposed to these chemicals and in fact very recent human biomonitoring studies have reported levels at the ng/g lipid in breast milk from Canada [5], as well as in human serum from Norway [6] and France [1], even though no production sources have been found in Europe. With the goal of understanding the extent and the origin of human exposure to Dechloranes, in the present study we investigated food consumption as a possible route of exposure for people from Belgium. Because of the emerging character of these analytes, the first part of the study has been dedicated to the development of a specific method for the analysis of 6 Dechloranes (Dec 604 was not detectable at the level of interest). The sample preparation procedure currently applied for dioxin analysis was demonstrated to be suitable for Dechlorane analysis; final extracts were injected and quantitated by means of GC-MS/MS Triple Quad, while usually HRMS instruments have been used for such compounds; the method was validated following the applicable guidelines of the stringent EU Regulation for dioxin analysis (589/2014 and 709/2014), and finally Dechlorane levels have been assessed in 88 selected food and feed samples to produce a first estimate of Dechlorane dietary intake for the Belgian population.

Materials and methods

- *Chemicals and consumables*

Solvents (hexane, toluene, methanol, ethanol and dichloromethane) were Picograde® reagents (LGC Promochem, Wesel, Germany). Nonane puriss analytical-reagent grade standard for GC was purchased from Fluka (Steinheim, Germany). Water was obtained from a Milli-Q Ultrapure water purification system (Millipore, Brussels, Belgium). Sodium sulphate and diatomaceous earth were purchased from VWR International (Radnor, PA, USA). Disposable PTFE columns for the automated clean-up were obtained from Fluid Management Systems (FMS Inc., Waltham, MA, USA). Chromatographic pure grade helium gas, 99.9999% alphagaz 2 was purchased from Air Liquide (Paris, France). Technical N27 grade liquid CO_2 was used for PTV cooling (Air Liquide, Paris, France). Standards of DP syn, DP anti, as well as $^{13}C_{10}$-labeled internal standards DP syn and $^{13}C_{10}$-labeled internal standard Dec 602 were supplied by Cambridge Isotope Laboratories (CIL, Andover, MS, USA). CP standard was bought from Wellington Laboratories (Guelph, ON, Canada). Mirex standard was purchased from Cluzeau Info Labo (France). Dec 602, Dec 603 and Dec 604 standards were purchased from Toronto Research Chemical Inc. (Toronto, ON, Canada). Average response factor calibration curves (5 to 7 calibration points) were prepared from stock standard solutions (100 µg/mL). The quantitation of DP isomers was performed by means of isotope dilution, even though only two labeled standards were commercially available at the time, namely $^{13}C_{10}$-labeled DP syn, used to quantify DP syn and DP anti, and $^{13}C_{10}$-labeled Dec 602, for mirex, Dec 602, 603, 604 and CP quantitation, after assessment on the calibration curve. The EC-1414

solution of $^{13}C_{12}$-labeled PCB-80, from CIL, was used as recovery standard to assess the efficiency or the loss of compounds during the sample preparation (internal standard vs recovery standard). The EDF-4145 solution from CIL was used as the recovery standard for dioxins, furans and coplanar-PCBs.

- *Sample preparation*

A total of 88 samples were collected for analyses, as well as 16 procedural blanks. Sample matrices consisted in milk, chicken, pork, eggs, pure animal fat, vegetable oil, salmon, feed additives, and corn. All samples were prepared in a similar way than for polychlorodibenzo-*p*-dioxin (PCDD), polychlorodibenzofuran (PCDF), and polychlorobiphenyl (PCB) analysis in an ISO 17025 environment. Details on the method are available in previous reports [7, 8]. Briefly, for all the matrices fat extraction was performed using accelerated solvent extraction (ASE™ 350, Dionex, Thermo Fisher Scientific). Labeled internal standards were spiked before (salmon and feed) or after (all the remaining matrices) to allow isotopic dilution quantification. After this step, samples underwent a manual acidic silica column pre-clean up and then the automated PowerPrep™ system (FMS Inc, Waltham, USA) for deep clean-up and compounds fractionation, using the classical ABN silica, alumina and carbon columns. Two fractions are collected for subsequent instrumental detection after evaporation of solvents in a dedicated tube using a sensor-equipped TurboVap II Workstation (Caliper Life Science, Teraflene, Belgium) and after subsequent evaporation in GC vials containing nonane as a keeper (90 and 4 µL respectively for the MO- PCB and for the dioxin fraction) using a RapidVap (Labconco, Kansas City, MO, USA). The related recovery standard solutions, namely $^{13}C_{12}$-labeled PCB-80 for MO-,NDL-PCBs and Dechloranes, and EDF-4145 for dioxins and dioxin like compounds, were added prior instrumental analysis.

- *GC-MS/MS instrumental analysis*

A 7000C gas chromatography triple quadrupole mass spectrometer (GC-QQQMS/MS) from Agilent (Palo Alto, CA, USA) equipped with a 7890B GC oven, a programmable temperature vaporization (PTV) inlet, and a 7693A automated liquid sampler (ALS) was used for instrumental analysis. 5 µL of the final purified extract were injected into the PTV inlet operating in solvent vent mode and cooled by liquid CO_2. More significant parameters were optimized by means of experimental design: inlet initial temperature of 45°C (held for 1.3 min, then ramped up at 720°C/min to 320°C); vent flow was 120 mL/min at vent pressure of 10.5 psi and purge flow at 1200 mL/min after 5 min. All the computations and graphs were performed using multiple linear regression routines written in Matlab (Mathworks Inc., Natrick, USA). The GC column was an Agilent DB-5ms ultra inert (60 m x 0.25 mm x 0.25 µm), the same used in routine for dioxin analysis, for simultaneous detection. The GC oven temperature program started at 140°C for 2.6 min, ramped at 100°C/min to 320°C for a total run time of 25.5 min. The transfer line temperature was held at 320°C. On the MS side, electron ionization (EI) at 70eV energy and temperature of 280°C was used. Quadrupole temperature was set at 150°C and multiple reaction monitoring (MRM) transitions were recorded at "wide" mass resolution (1.2 Da) on the Agilent software. Ultrapure Nitrogen at 1.5 mL/min and Helium at 2.25 mL/min were used respectively as collision gas and quench gas in the collision cell. Dwell times were selected during method optimization to increase the sensitivity as much as possible, providing ten data points per peak as acquisition frequency. Calibration and auto tune were performed in the EI high sensitivity mode. Retention time locking was performed with PCB-105 allowing change and reinstallation of the column while keeping reproducible retention times. Mass Hunter version B.07.00 was used for acquisition and quantitative analyses.

Results and discussion:

- *Suitability of the sample clean-up used for dioxin analysis*

The study aimed at developing a multi-analyte procedure for selected persistent organic pollutant (POP) analysis, based on a preexisting ISO 17025 procedure for polychlorodibenzo-*p*-dioxins (PCDDs), polychlorodibenzofurans (PCDFs), and polychlorobiphenyls (PCBs) analyses in food and feed. We started from the sample preparation steps, since possible Dechloranes degradation could occur after acidic treatment[2]. Our sample clean-up procedure consisted of two steps: a manual high capacity acidic silica column treatment implemented in combination with the automated system involving mixed bed acid/basic silica, alumina and carbon columns. Recovery experiments across each clean-up step were performed to investigate the possible degradation of Dechloranes, that showed strong resistance (all recovery rates close to 100%) throughout the entire process, eluting in the (MO-)PCBs fraction (non planar species). This demonstrated that the classic dioxin clean up and fractionation procedure we reported earlier [7] can be used also for Dechloranes sample preparation.

 Analytical method development and validation

Since no preliminary data on Dechlorane background levels in food and feed matrices were available, we had to work focusing on ultra-trace levels. As the first interface affecting sensitivity, we optimized PTV inlet parameters in order to maximize peak area while taking into account the peak shape, calculating peak symmetry. A value of 1.0 meant the peak was balanced, but in this work we considered as acceptable values in the range 0. 9 and 1.1 because no peak smoothing was applied. We studied 3 effects and their interactions with a Face Centered Design: 1) initial inlet temperature (T), 2) vent flow (VF) and 3) vent pressure (VP), as we worked in solvent vent mode. Significant effects and optimum values to set are summarized in Table 1.

Table 1: Significant effects and optimum PTV inlet parameters.
*VF at 120 mL/min outwards the experimental domain was set after assessing statistically higher peak area by means of a *t*-test.

	Significant effects	Goal	Values to set	Optimum values
Peak area	b_{11} <0	To be maximized	T = 45°C (0)	T = 45°C (0)
	b_2 >0		VF = 100 mL/min (+1)	
	b_3 <0		VP = 1 psi (-1)	
Peak symmetry	b_1 >0	To be held in the range 0.9 – 1.1	T = 60°C (+1)	VF = 120* mL/min (+1.2)
	b_2 >0		VF = 100 mL/min (+1)	
	b_3 >0		VP = 20 psi (+1)	VP = 10.5 psi (0)

The optimum values in Table 1 were the ones leading to highest peak area and very symmetric peaks of the three factors consisted in the compromise between maximum peak area and symmetric peak shape (peak symmetry between 0.9 and 1.1).The best value for vent flow was set at 120 mL/min, outwards the initial experimental domain, as suggested by the response surface (Fig. 1), after establishing statistically higher peak area (*t*-test) increasing the vent flow.

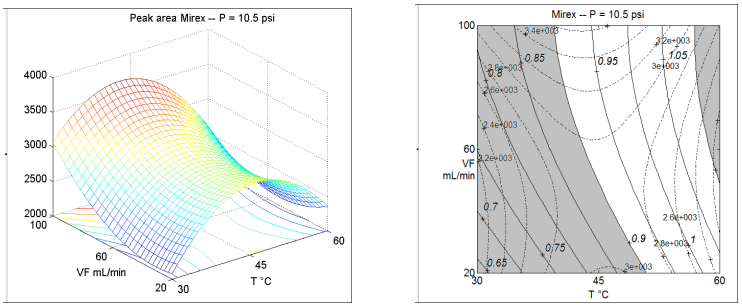

Figure 1: *Left*: peak area response surfaces for Mirex in temperature-vent flow plane at 10.5 psi vent pressure. *Right*: overlapped contour plot of peak area (dashed line) and peak symmetry (continuous line) for Mirex at vent pressure 1 psi and 10 psi. The grey area shows unacceptable values (in italic) for peak symmetry in the experimental domain (outside the 0.9-1.1 range).

The setting of main mass spectrometer parameters were identical than for dioxin analysis as already described in a previous validation study for dioxin analysis[9]. A specific optimization was however necessary and consisted in determining the appropriate MRM transitions. For DP isomers, CP, and Dec 602, the retro Diels-Alder product (the hexachlorocyclopentadiene ion – HCCPD) was the base peak obtained after the electron ionization (EI), so the quantitative MRM transition was 272 > 237 (Cl loss). The same was for Mirex, whereas Dec 603 followed a different fragmentation pattern with the quantitative transition at 263 > 228.
The developed method was validated with the applicable criteria stated in the EU Regulation 589/2014 and 709/2014 for dioxin analysis in food and feed. Method limits of quantitation (mLOQs), different for each sample matrix amount, were assessed either based on procedural blank levels, or, when no signal was recorded from blanks, from the instrumental limits of quantitation (iLOQs) or, when iLOQ was outside the calibration range, from the lowest calibration point, as it was in this case[9]. Dechlorane levels in real samples were calculated according to the reporting method used for dioxin analysis under the EU Legislation, using the approaches of lower-bound (lb, report 0 for the target whenever the level measured in the sample is <mLOQ) and upper-bound (ub, report mLOQ for the target whenever the level measured in the sample is <mLOQ). With the lb approach actual levels are underestimated, with the ub are overestimated.

 Dechlorane levels in food and feed, dietary intake assessment

From what we have seen, at this stage, no class of compound can be highlighted as strongly contributing to human exposure to Dechloranes, but it is interesting to highlight that Mirex is still present in food even if it was banned since the '70s; DP anti is the major contributor for several matrices; Dec 603 is present in almost all the matrices (high levels found in human serum). Figure 2 shows detected levels as the sum of the 6 Dechloranes in 88 samples from different food and feed matrices. The bottom of the box is the lower-bound value, the top is the upper-bound value and real values are within the box. This graph gives the order of magnitude of Dechloranes in

foodstuff, that is few tens of pg/g fat or wet weight. Despite the fact that levels appeared to be lower than those measured in food from Korea[10], they suggest that Dechloranes enter into the food chain and therefore humans are exposed via consumption of food in Europe, where no production plant has been identified.

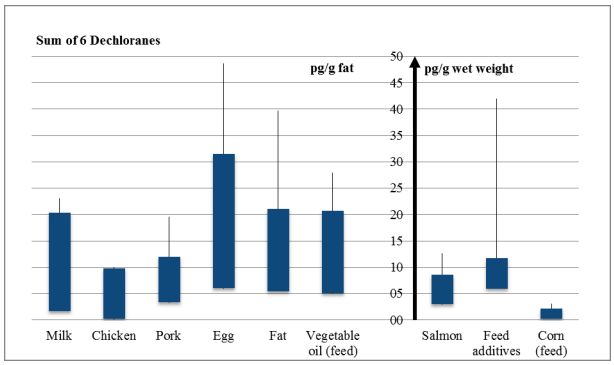

Figure 2: Average levels of the sum of Mirex, Dec 602, Dec 603, CP, DP syn, and DP anti in different food and feed matrices. Boxes represent lower-bound (bottom), and upper-bound (top) results and the error bars are the upper-bound + 2*standard deviation.

Average levels in selected matrices (more commonly consumed goods) were used to estimate an average daily intake of Dechloranes via food for the Belgian population, as an example of European people (Table 2). Average dietary intakes are based on upper-bound levels to produce the worst case scenario.

Table 2: Estimated average dietary intake for the sum of 6 Dechloranes (Mirex, Dec 602, Dec 603, CP, DP syn, DP anti) measured in selected food matrices in Belgium.

	Estimated dietary consumption	Sum 6 Dechloranes	Dechloranes sum intake		
	g/day	g fat/100g	g fat/day	pg/g fat or pg/g ww	pg/day
Salmon	2.7	16.5	0.4	8.6	23.1
Chicken	18.4	9.3	1.7	9.8	16.8
Pork	30.2	9.2	2.8	12.0	33.2
Egg	9.6	11.3	1.1	31.5	34.1
Milk	89.1	1.6	1.4	20.3	29.0
			Estimated Dechloranes dietary intake (pg/day)		**136.2**

Based on levels previously reported in human blood[1], this study is a first point for more extensive and larger studies to estimate the relative contribution of food consumption to global human intake of Dechloranes, that evidently derives from several contributors, as indoor dust can be.

Acknowledgements:
The Research Training Fund for Industry and Agriculture (F.R.I.A) is thanked for providing a Ph.D. bursary to B. L'Homme. C. Calaprice was financially supported partly by Fondo Giovani and from the Inter-polytechnic School (Milan, Turin, Bari). Thanks to Agilent Technologies for instrumental support and to Fluid Management Systems Inc. for providing sample clean-up consumables for this study.

References:

1. Brasseur, C., Pirard, C., Scholl, G., De Pauw, E., Viel, J.F., Shen, L., Reiner, E.J. and Focant, J.F., (2014) *Environment International*, 65, 33-40.
2. Sverko, E., Tomy, G.T., Reiner, E.J., Li, Y.F., McCarry, B.E., Arnot, J.A., Law, R.J. and Hites, R.A., (2011) *Environmental Science and Technology*, 45, 5088-5098.
3. Cao, Z., Xu, F., Covaci, A., Wu, M., Wang, H., Yu, G., Wang, B., Deng, S., Huang, J. and Wang, X., (2014) *Environmental Science and Technology*, 48, 8839-8846.
4. Dodson, R.E., Perovich, L.J., Covaci, A., Van Den Eede, N., Ionas, A.C., Dirtu, A.C., Brody, J.G. and Rudel, R.A., (2012) *Environmental Science and Technology*, 46, 13056-13066.
5. Zhou, S.N., Siddique, S., Lavoie, L., Takser, L., Abdelouahab, N. and Zhu, J., (2014) *Environment International*, 66, 11-17.
6. Cequier, E., Marcé, R.M., Becher, G. and Thomsen, C., (2013) *Journal of Chromatography A*, 1310, 126-132.
7. Focant, J.F., Eppe, G., Pirard, C. and De Pauw, E., (2001) *Journal of Chromatography A*, 925, 207-221.
8. Focant, J.F., Eppe, G., Massart, A.C., Scholl, G., Pirard, C. and De Pauw, E., (2006) *Journal of Chromatography A*, 1130, 97-107.
9. L'Homme, B., Scholl, G., Eppe, G. and Focant, J.F., (2015) *Journal of Chromatography A*, 1376, 149-158.
10. Kim, J., Son, M.H., Kim, J., Suh, J., Kang, Y. and Chang, Y.S., (2014) *Journal of Hazardous Materials*, 275, 19-25.