Chemosphere 139 (2015) 525–533

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Ultra-trace measurement of Dechloranes to investigate food as a route of human exposure

Benjamin L'Homme [a,1], Chiara Calaprice [a,b,c,1], Cosima Damiana Calvano [c], Carlo Zambonin [c,d], Riccardo Leardi [e], Jean-François Focant [a,*]

[a] CART, Organic and Biological Analytical Chemistry Group, Chemistry Department, University of Liège, Allée de la Chimie 3, B6c Sart-Tilman, B-4000 Liège, Belgium
[b] Dipartimento di Ingegneria Civile, Ambientale, del Territorio, Edile e di Chimica, Politecnico di Bari, Via Orabona, 4, 70125 Bari, Italy
[c] Dipartimento di Chimica, Università degli Studi di Bari "Aldo Moro", via Orabona 4, 70125 Bari, Italy
[d] Centro Interdipartimentale di Ricerca S.M.A.R.T., Università degli Studi di Bari "Aldo Moro", c/o Dipartimento di Chimica, via Orabona 4, 70125 Bari, Italy
[e] Department of Pharmacy, Unit of Pharmaceutical and Food Chemistry and Technology, Via Brigata Salerno (ponte), University of Genoa, I-16147 Genoa, Italy

HIGHLIGHTS

- This is the first report on levels of Dechloranes in European Food and feed.
- A specific GC-QQQMS/MS method was developed.
- Most selected Dechloranes were detected in European food and feed.
- Dechloranes are at the pg/g fat levels in food.
- A Dechlorane dietary intake was estimated for the Belgian population.

ARTICLE INFO

Article history:
Received 11 May 2015
Received in revised form 9 July 2015
Accepted 16 July 2015
Available online 24 August 2015

Keywords:
Dechloranes
Ultra-trace measurement
Experimental design
Food analysis
Human intake
Triple quadrupole tandem mass spectrometry

ABSTRACT

Dechloranes, including Dechlorane Plus (syn- and anti-isomers), Dechlorane 602, Dechlorane 603, Dechlorane 604, Chlordene Plus, and Mirex are used as flame-retardants and were recently found in human serum of the European population. In order to investigate if food consumption would possibly be a significant route of exposure, we developed a method for the measurement of Dechloranes in food and feed. We showed that it was possible to extend the scope of the regular polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), dioxin like (DL-), and non-dioxin like (NDL-) regulated PCBs clean-up and fractionation procedure to Dechloranes and that no compound degradation occurred during the strong acidic treatments used for lipid digestion. Dechloranes were measured by gas chromatography coupled to triple quadrupole mass spectrometry (GC-QQQMS/MS). We optimized injection parameters by face centered experimental design (FCD). The electron ionization fragmentation was investigated to set appropriate multiple reaction monitoring (MRM) transitions. Instrumental and method limits of quantitation (iLOQs and mLOQs) were determined following EU guidelines for dioxin analyses in food. A total of 88 samples were analyzed to assess the prevalence of this route of exposure to humans. Average levels of the sum of Dechloranes ranged from 10 to 31 pg/g fat, with the exception of fish, feed additives, and corn that were reported in pg/g wet weight at average levels of 9, 12, and 2 pg/g ww. Based on Belgian food habits, a dietary intake was estimated to be 136 pg/day. The relatively low reported levels indicate that other routes of human exposure should be considered.

1. Introduction

Dechloranes are emerging persistent organic pollutants (POPs) issued from a family containing structural analogue compounds, namely Mirex ($C_{10}Cl_{12}$), 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, $C_{15}H_6Cl_{12}$). They are norbornene derivatives that exhibit flame-retardant and pesticide properties and are used as replacement of regulated compounds such as Mirex (also called Dechlorane) (INCHEM, 1984) and decabromodiphenyl ether (deca-BDE, BDE-209) (Pakalin et al., 2007). DP and other Dechloranes have

* Corresponding author.
E-mail address: JF.Focant@ulg.ac.be (J.-F. Focant).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.chemosphere.2015.07.043
0045-6535/

been extensively used as additive in various synthetic products such as nylon or plastic like polypropylene (OxyChem, 2007) and have recently been reported at low levels in dust collected from various environments (Cao et al., 2014; Dodson et al., 2012). In addition, some break down and dechlorinated products of DP (Muñoz-Arnanz et al., 2012), of Dec 602 (Shen et al., 2012), and mixed halogenated analogues of Dec 604 (Jobst et al., 2013) have also been found in the environment. Besides environmental issues, humans are exposed to these chemicals and, so far, barely any data about toxicity, metabolization, and prevalence in human are available. Nevertheless, very recent human biomonitoring studies have reported levels at the ng/g lipid in human serum from Norway (Cequier et al., 2013) and France (Brasseur et al., 2014), as well as in breast milk from Canada (Zhou et al., 2014).

For the present study, we investigated food consumption as a possible route of human exposure to Dechloranes in Belgium. Such an exercise has only been carried out recently in Korea (Kim et al., 2014), and in Japan (Kakimoto et al., 2014). Both studies were using gas chromatography (GC) coupled to high-resolution mass spectrometry (HRMS) sector instruments for measurements at the pg/g level. As far as we know, no data are currently available regarding Dechlorane exposure from food consumption in Europe, where no Dechlorane production sources have been identified so far, despite the fact that ng/g lipid levels have been reported in humans (Brasseur et al., 2014; Cequier et al., 2013). 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: Mirex, Dec 602, Dec 603, CP, DP syn, and DP anti isomers. We based our analytical approach on one of our recent report on the full validation of a GC isotope dilution (ID) triple quadrupole tandem in space mass spectrometry (QQQMS/MS) method for the measurement of dioxins in food and feed at the low pg level under the European Legislation (L'Homme et al., 2015). To optimize the setup of the Dechlorane dedicated ultra-trace measurement method, we investigated large volume injection (LVI) by using a programmed temperature vaporizing (PTV) injector operated in solvent vent mode. Full factorial design (FFD) and face-centered design (FCD) were used to select optimum inlet parameters. Most efforts were focused on the three most relevant factors such as vent flow, vent pressure, and vent temperature but also on other minor injection parameter, such as purge flow, in order to maximize method sensitivity. On the MS side, specific multiple reaction monitoring (MRM) transitions were selected from typical Dechlorane fragmentation patterns as they can fragment following a retro Diels–Alder reaction of the norbornene moiety of the molecule (Brasseur et al., 2012; Shen et al., 2012). In this paper, we report on the development of the analytical method and its use for the measurement of Dechloranes in selected food and feed samples. It was applied to 88 samples to produce a first estimate of Dechlorane dietary intake for the Belgian population.

2. Experimental

2.1. 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). Solvent batches were tested for contamination before use. 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 Airliquide (Paris, France). Technical N27 grade liquid CO_2 was used for PTV cooling (Airliquide, Paris, France). Sodium sulfate and diatomaceous earth were purchased from VWR International (Radnor, PA, USA). 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). The quantitation of DP isomers was performed using $^{13}C_{10}$-labeled DP syn internal standard, whereas mirex, Dec 602, 603, 604 and CP were quantitated against $^{13}C_{10}$-labeled Dec 602 internal standard. The EC-1414 solution of $^{13}C_{12}$-labeled PCB-80, from CIL, was used as recovery standard. This standard was used to assess the efficiency and the loss of compounds during the sample preparation (internal standard vs recovery standard). The quantitation was however not affected by any loss of compounds since all analytes were quantitated by isotopic dilution (ID) (analyte vs internal standard) during which process analytes and internal standards were lost in the same proportions. Calibration curves were prepared from intermediate stock solutions at 1 ng/μL and consisted in 5–7 calibration points (Supplementary Information, Table S1).

2.2. Samples

A total of 88 samples were collected for analyses. Sample matrices consisted in milk, chicken, pork, eggs, pure animal fat, vegetable oil, salmon, feed additives, and corn. A group of 77 samples of different food and feed matrices were randomly selected from samples entering our ISO17025 accredited routine dioxin laboratory under the EU monitoring program. The remaining 11 samples (5 salmons and 6 chickens) were collected from regular shops and supermarkets in the area of Liege, Belgium, in January 2015, to complete the missing matrices. Samples were frozen and stored until use. During the study, 16 procedural blank samples were also analyzed.

2.3. Sample preparation: extraction and clean up

All samples were prepared in a similar way than for polychlorodibenzo-*p*-dioxin (PCDD), polychloro-dibenzofuran (PCDF), and polychlorobiphenyl (PCB) analysis in an ISO 17025 environment. Details on the method are available in previous reports (Focant et al., 2006, 2001). Briefly, for all the matrices fat extraction was required because of the lipophilicity of Dechloranes and it was performed using accelerated solvent extraction (ASE™ 350, Dionex, Thermo Fisher Scientific). Labeled internal standard spike was carried out before fat extraction for matrices such as salmon and feed, and directly on the extracted fat for the other matrices. After this step, samples underwent a manual acidic silica column pre-clean up and then the automated PowerPrep™ system (FMS Inc, Waltham, USA) was used for deep clean-up and compounds fractionation (Focant et al., 2004). Samples were loaded on a multi-layer acid/basic/neutral (ABN) silica column for lipid breakdown, then passed through a partly deactivated basic alumina column for interference removal and compound fractionation, and finally ended up in a carbon-based column for separation of non-planar from planar species. For PCDD/F and PCB classical clean-up, two fractions were collected and sent to instrumental analysis. Dechloranes were collected with non-planar species (mono-ortho (MO-)PCBs and indicator (I-)PCBs) by forward elution with a mixture of hexane/dichloromethane 50:50. This fraction was evaporated in a dedicated tube using a sensor-equipped TurboVap II Workstation