
Request for Identification of Emerging Perfluorinated Substances in Water and Blood Samples
from Residents of an Industrial Site

Contextualization

Per- and poly-fluorinated compounds (PFAS) are substances containing at least one fully fluorinated carbon (either CF₃- or -CF₂-) and usually a functional (terminal) group that differs between PFAS groups and subgroups.

Since the 1950s, PFAS have been widely used in industrial and commercial applications, due to their various interesting properties. Indeed, they are thermally and chemically stable due to the presence of multiple strong C-F bonds. In addition, the highly fluorinated portion of PFASs is oleophobic (i.e., it repels oil) and hydrophobic because the fluorine atoms are weakly polarizable. Nevertheless, PFASs also contain a hydrophilic portion, which is the terminal polar functional group. This combination of hydrophobicity and hydrophilicity makes them attractive as surfactants and processing additives. PFASs are also widely used as water and oil repellents for textiles, food packaging, cosmetics and release coatings, for example. Another major application of PFASs is in the field of firefighting with their use in water-based foams.

Because PFASs are widely used and stable, they are found and found in all types of environmental matrices. The main sources of contamination are industrial sites and waste disposal leachates, as well as fire training areas, where AFFFs (Aqueous Film-Forming Foams) containing PFAS are used in large quantities.

Wastewater treatment plants are also a source of PFAS release into drinking water, which is one of the primary routes of exposure.

The presence of these compounds in the environment, water and food is of concern because toxicological studies have shown that PFASs may be linked to several health problems such as thyroid disorders, cancers, increased cholesterol levels and immunodeficiency. However, only a small proportion of these compounds are regulated. For example, perfluorooctane sulfonic acid (PFOS), its salts, and related substances were the first to be listed in Annex B of the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2009, after studies showed their presence in wildlife and the blood of the human population. In 2019, perfluorooctanoic acid (PFOA) joined PFOS in the POPs annex. Perfluorohexanoic acid and C₉-C₁₄ perfluoroalkylcarboxylic acids are currently considered for inclusion in the Stockholm Convention and will be subject to restrictions under REACH (the European Regulation on Registration, Evaluation, Authorization and Restriction of Chemicals) starting in February 2023.

In response to growing concerns about PFOS and PFOA, manufacturers voluntarily discontinued their production, as well as that of their related substances, in the 2000s. As a result, several alternatives were used, such as

short-chain PFAS analogues. Because their fluorinated portion is shorter, these analogs tend to be less bioaccumulative, but they perform less well than their long-chain counterparts. The inclusion of ether linkages in the long fluorinated chain is another solution found by industry. The C-O bond may lead to some brittleness and possible degradation to short-chain PFASs, but this is still unclear. Finally, the formation of a fluorinated carbon ring chain, the replacement of some fluorine atoms by hydrogen or halogen atoms and the modification of the production technique are also part of the many replacement processes.

Currently, just over 4,700 PFAS compounds have been identified, but most studies have focused on only a small portion of these compounds. Therefore, the health effects of many of these compounds are still unknown. Large-scale biomonitoring is needed to verify the impact of these new substances in the environment and on exposed populations.

Request from RTBF for the program Investigation

RTBF's Investigation program asked the Analytical and Technological Research Center (CART) of the University of Liège, the National Reference Laboratory for the analysis of persistent halogenated organic pollutants in foodstuffs, to search for emerging perfluorinated compounds in a water sample and in blood samples taken from people living in the vicinity of Solvay's industrial site (fluoropolymer production site) in Spinetta Marengo (Italy).

An extensive literature review was conducted to better understand the demand and objectives of this analytical challenge. Based on the available scientific literature and reports (EFSA and USEPA), we identified a major scientific contribution recently published in the journal *Science* in 2020 (G.W. Washington et al., *Science* 368, 1103- 1107) by a team of scientists from the US Environmental Protection Agency (USEPA). The results of this research identified a family of emerging PFAS by confirming the presence of these compounds in soil samples in southern New Jersey (USA). These samples were collected in an area where two PFOA Stewardship Program signatories are located: Solvay, in West Deptford Township, and Chemours (formerly DuPont), in Pennsville Township. The authors of the publication identified the presence in the soils of a family of chloroperfluoropolyether carboxylate (Cl-PFPECA) congeners which is described as Solvay's product registered under CAS No. 329238-24-6.

A search of the ECHA (European Chemicals Agency, <https://echa.europa.eu/fr/substance-information/-/substanceinfo/100.207.408>) database shows a family of substances registered under the name "1-Propene, 1,1,2,3,3,3-hexafluoro-, telomer with chlorotrifluoroethene, oxidized, reduced, hydrolyzed" (IUPAC name) and known under the trade name of Solvay ADV 7800 and ADV 7850 products. The research also led to a request for an opinion from Solvay in 2010 to the European Food Safety Authority (EFSA) regarding the safety assessment of the substance perfluoroacetic acid, α -substituted by the copolymer of perfluoro-1,2-propylene glycol and perfluoro-1,1-ethylene glycol, terminated by chlorohexafluoropropoxy groups (same CAS number 329238-24-6 listed in ECHA), corresponding to Cl-PFPECA. This substance is a mixture of telomers intended to be used up to 0.5% w/w as an emulsifier and dispersing agent for the processing of fluoropolymers.

The findings of the paper in *Science* led to the elucidation and highly probable identification of the following structure of Cl-PFPECA (Figure 1, in one of these forms) in New Jersey soil samples. The values of e and p (indices of the number of

repetition of the monomer unit) correspond to perfluoro-1,1-ethylene glycol copolymer and perfluoro-1,2-propylene glycol, respectively, and can vary from 0 to 4 ($e=0, 1, 2, 3, 4$ and $p=0, 1, 2, 3, 4$)

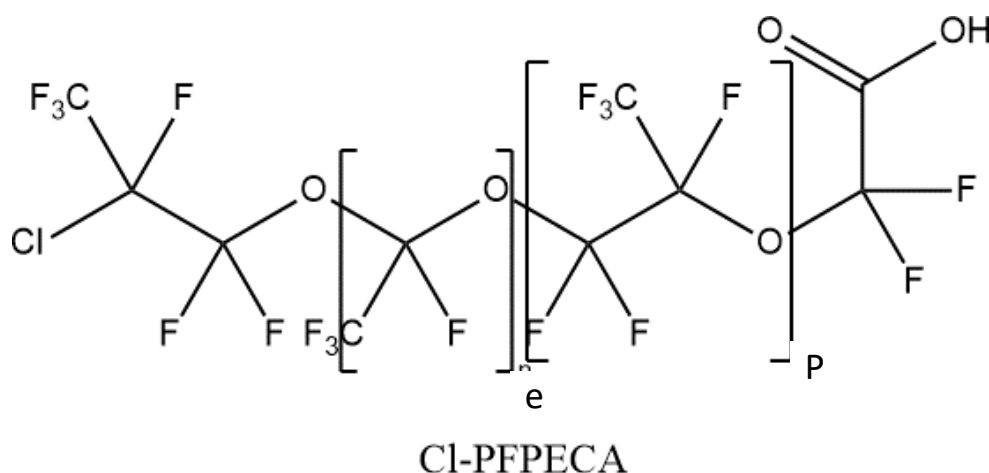


Figure 1: General structure of the oligomeric series of perfluoroether carboxylic acids Cl-PFPECA, representation of the congener ($e, p = 1, 1$)

The findings of the *Science* paper demonstrate the presence in New Jersey soils of 10 of these congeners but also the presence of 5 congeners of Cl-PFPECA in a water sample from the Bormida di Spigno river, downstream of Solvay Specialty Polymers, Italy (Spinetta Marengo, Alessandria, Italy). Subsequently, two new scientific publications in the journal *Environment Science and Technology* (ES&T) by the same authors confirmed the presence of different forms of Cl-PFPECA in soils and in various water samples in New Jersey. Detailed technical information on the analytical methods used in these publications is available online in the supplementary information (SI).

Our laboratory was also asked to confirm or deny the presence of another substance, namely acetic acid, 2,2-difluoro-2-[[2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl]oxy], CAS number (1190931-41-9) and known by the trade name cC6O4. The structure of this molecule is shown in Figure 2.

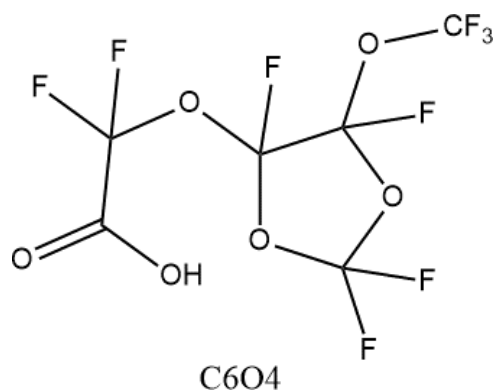


Figure 2: structure of cC6O4

Analytical method used

The CART of the University of Liege has an expertise of more than 20 years in the field of persistent organic pollutants. It is equipped with instruments in mass spectrometry allowing to bring the elements of answer to the raised questions. It is also important to specify at this stage that within the framework of this analytical work, we did not have the reference standards of the molecules (just like in the publications in Science and ES&T). Indeed, both C6O4 and ADV are not commercially available. CART has set up an analytical method based on the scientific publications mentioned above, while adapting the methods to its available instruments. We used mass spectrometry measurement instruments to achieve the highest specificity in order to increase confidence in the identification process. Water and blood samples were extracted by weak anion exchange solid phase extraction (SPE) (Oasis, Wax) according to standard PFAS analysis protocols. Compounds of interest were recovered in 2% methanol/NH₄OH, filtered, and resumed in a 95/5 (v/v) H₂O/CH₃OH mixture.

Non-targeted analysis was performed by ultra-high performance liquid chromatography (UPLC, Acquity I-Class, Waters) using a C18 analytical column (BEH C18, 2.1 x 150 mm, Waters) and coupled to an Orbitrap ultra-high resolution mass spectrometer (Q-Exactive, Thermo Scientific) equipped with an electrospray source operating in negative mode. Chromatography parameters, source settings, acquisition modes in scan mode and MS/MS mode are available on request.

The elution order and chromatographic retention times of the different forms of ADV are identical in water and blood samples. Their constant relative positions with respect to PFOA in the chromatographic elution profiles (see Figures 3 and 4 for C6O4 and ADV in the water sample) allow to confirm the good reproducibility of the elution process in liquid chromatography and to use the relative retention times as a first criterion of identification.

Analysis of the untargeted high-resolution mass spectrometry data from the raw files was processed using XCalibur (Thermo Scientific) and Skyline (MacCoss Lab, Washington) software. Careful analysis of the mass spectra using the exact masses of the majority ions produced in source (M-CF₂COOH) of the different forms of C1- PFPECA (mass accuracy less than 2 ppm in all cases), the fragmentation pathways in MS/MS, and the mass accuracy on the two majority fragments in MS/MS (less than 2 ppm) allowed for greater confidence in the identification process of cC6O4 and the different forms of ADV. Finally, the ADV congeners present in

the analyzed samples fragmented to form a common ion ($\text{ClCF}_3\text{CF}_2\text{O}^-$) of m/z 200.9535 (accuracy below the 2ppm criterion) and verification of the natural isotopic abundance of chlorine via the ratio of $M/M+2$ ions for each of the ADV congeners recovered. All these identification parameters that we have just cited in the context of our analytical development are in line with the above-mentioned scientific literature (*Science* and *ES&T*), articles published in scientific journals with a very high impact factor and peer review process.

The results in the water sampled at the level of the wastewater discharge of the Solvay Spinetta site:

The probable presence of cC_6O_4 in the analyzed wastewater is shown in Figure 3 below. The cC_6O_4 peak appears in the middle of the chromatogram. The identification criteria mentioned above are met.

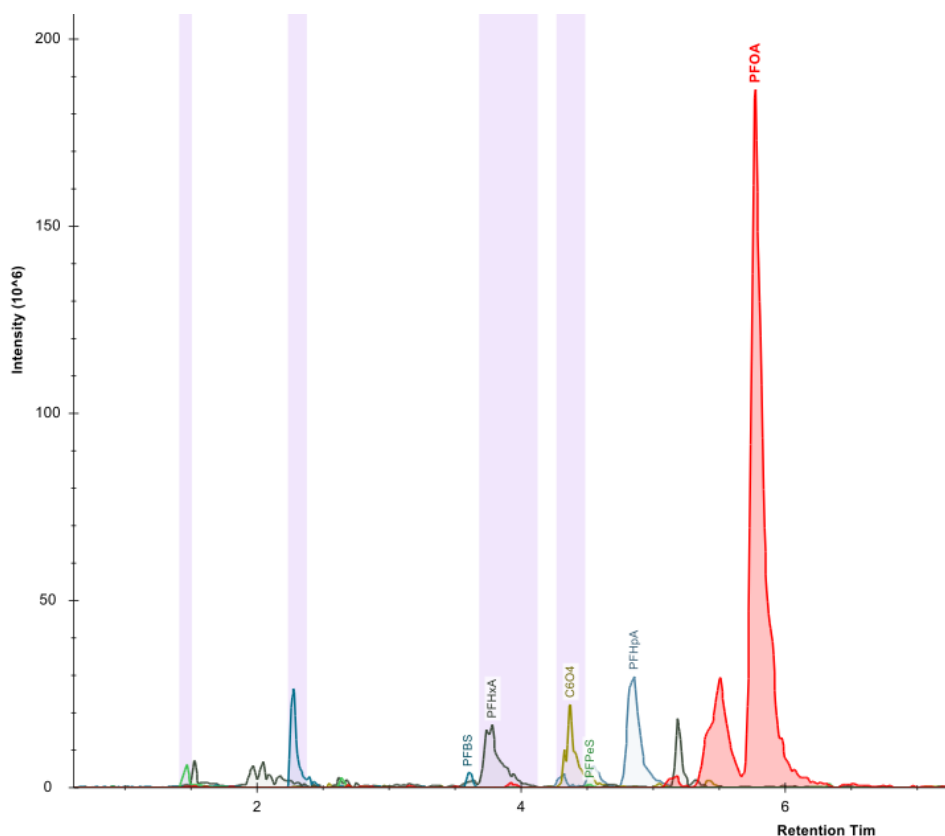


Figure 3: chromatogram of the discharge water at the Solvay Spinetta site, showing the probable presence of cC_6O_4 and other historical PFAS such as PFOA.

Five major oligomeric forms of Cl-PFPECA (ADV) were identified on the basis of the above criteria in the Solvay discharge water. These are the congeners ($e, p = 1.0$; $e, p = 0.1$; $e, p = 2.0$; $e, p = 1.1$; $e, p = 0.2$).

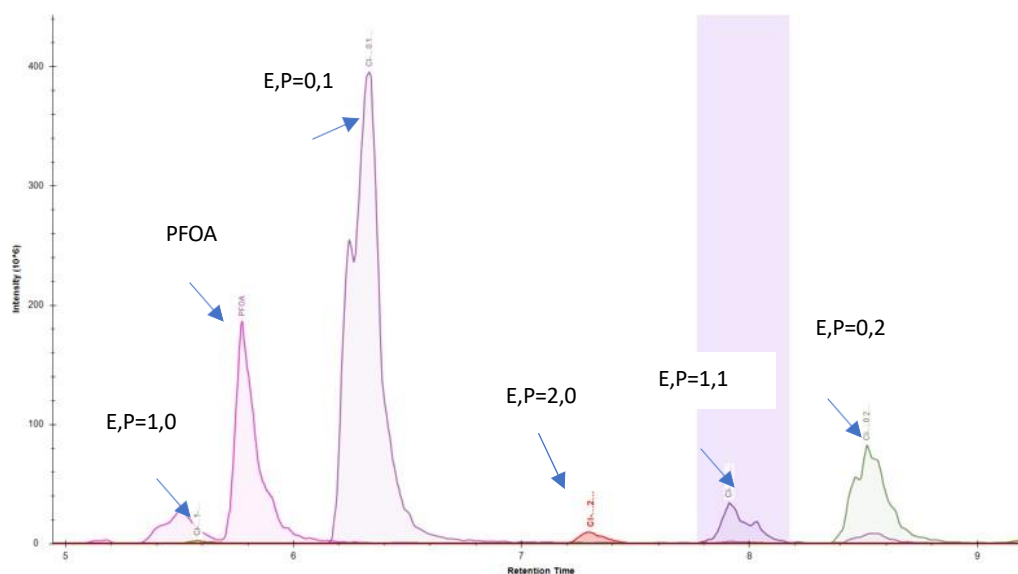


Figure 4: chromatogram of the discharge water at the Solvay Spinetta site, showing the probable presence of five forms of Cl-PFPECA and PFOA.

The paper in *Science* mentions the analysis of a water sample from Spinetta, which is likely to be taken in 2019 or 2020 in view of the scientific publication date. We went to look for the chromatogram in the Supplementary Materials (SI) of the paper by G.W. Washington et al, *Science*, 368, 1103-1107, Figure S3 (MS^e mass chromatogram for Bormida River, Italy sample) whose link is given here because we cannot publish a figure coming from a scientific journal without asking for permission.

(<http://science.sciencemag.org/content/suppl/2020/06/03/368.6495.1103.DC1>).

We observe the perfect correspondence with our analysis performed more than two years later. Not only do we find exactly the same congeners (i.e. e, p = 1.0; e, p = 0.1; e, p = 2.0; e, p = 1.1; e, p = 0.2), an identical chromatographic elution order but also quite similar relative intensities (peak heights) for the different congeners.

The results of blood samples from residents of the Spinetta area of the Solvay site:

Regarding the analysis of the 30 blood samples of the Spinetta residents, our analytical method did not detect the presence of cC604 in the blood samples, above our detection threshold. The scientific literature agrees with this finding, highlighting the less persistent and bioaccumulative nature of this emerging PFSA. A recent publication by Italian colleagues in the journal *Analytical and Bioanalytical Chemistry* (G. Frigerio et al., ABC, 2022, 414:1-20) also shows that their validated analytical method identified the presence of cC604 in a single sample from a sample of 38 blood samples.

To our knowledge, there is no published data in the scientific literature on the presence of Cl-PFPECA congeners in human blood. These results presented publicly in this report are therefore a world first.

Based on all the identification criteria of our analytical method, a rigorous and systematic analysis of our data allowed us to conclude to a strong suspicion of the presence of 5 major oligomeric forms of Cl-PFPECA in the blood samples

of the Spinetta residents. The 5 congeners likely present **are the same as those likely found in the water sample**, namely (e, p =1.0; e, p =0.1; e, p =2.0; e, p = 1.1; e, p =0.2). As in the water sample, the congener (e, p =0.1) is predominantly present **in all blood samples**, and accounts for between 70 - 99% relative abundance, followed in much smaller proportions by (e, p =0.2) and then the other 3 congeners, as clearly shown in Figure 6. According to the Solvay nomenclature, the congener (e, p =0.1) is named N2 and would represent almost 50% by weight of the technical mixture of ADV produced at the Spinetta site, followed by the congener (e, p =0.2) which is named N3 and would represent more than 20% of the ADV. Our results are therefore consistent with these advanced figures. The high proportion of (e, p =0.1) in the blood of the Spinetta residents could be explained by a degradation of the congener (e, p =0.2) into (e, p =0.1), but at the moment no scientific study allows to confirm this advanced hypothesis

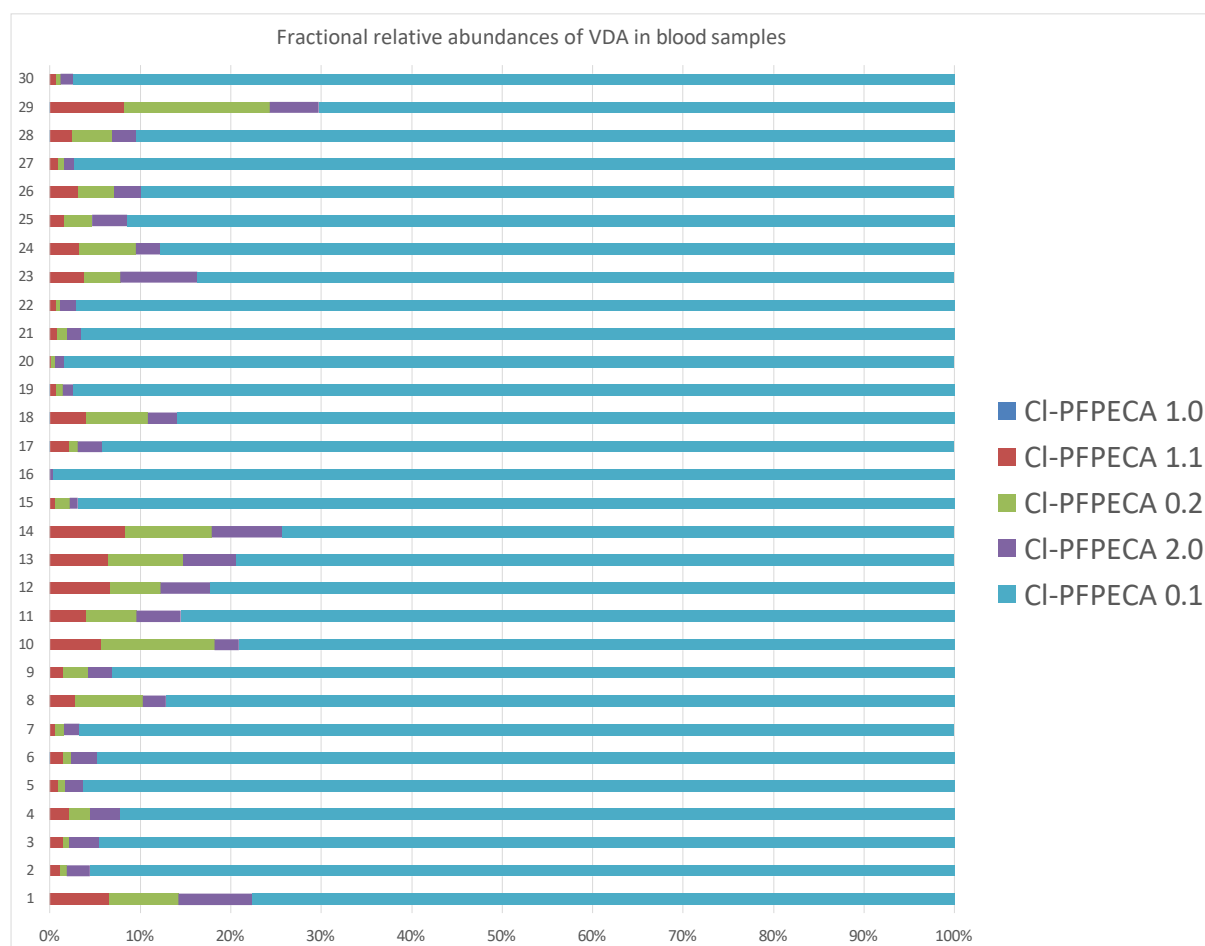


Figure 6: Percentage of relative abundances of different congeners likely present in the blood of Spinetta residents.

Comparison of the relative abundances of the 5 majority congeners of Cl-PFPECA (ADV) in the blood of Spinetta residents

Figures 7 to 11 show the signal intensities measured by high-resolution UPLC-MS for the fragment ion M-CF₂COOH. It can be seen that **differences of several orders of magnitude** in signal intensity are observable on the different graphs. These signals are comparable between them for the same congener of Cl-PFPECA. The tendency is the same for each congener of ADV, namely that the most intense signals correspond to blood sample numbers of former workers of the Solvay factory.

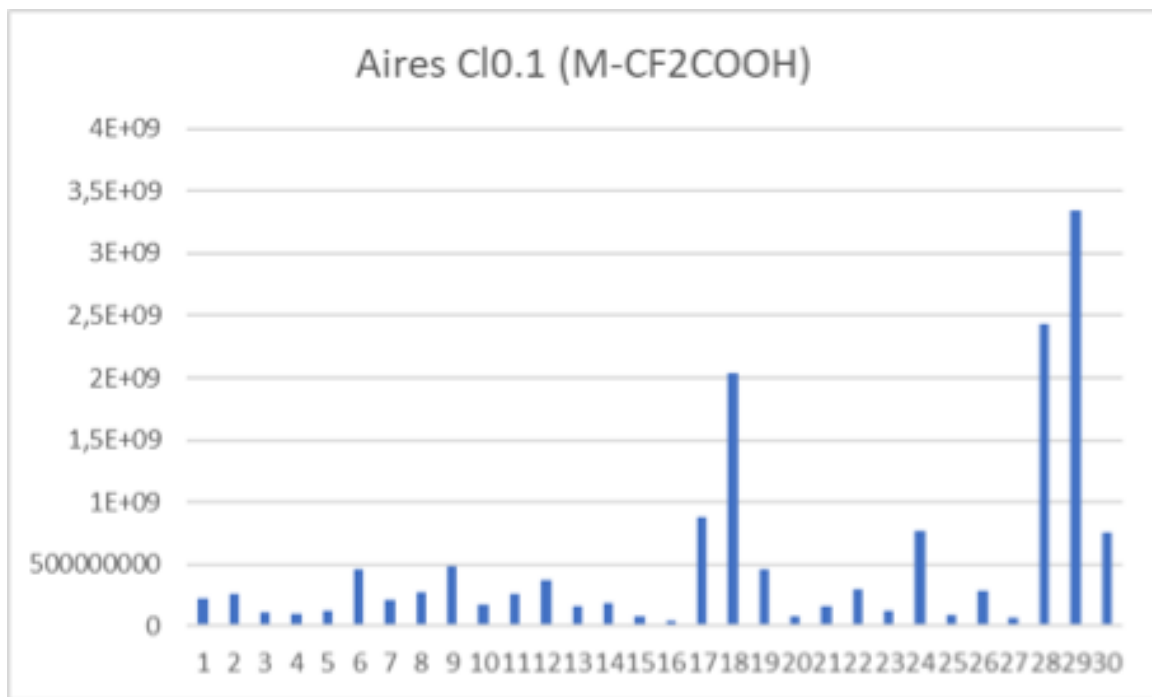


Figure 7: Signal intensities of (e, p =0.1) for the 30 blood samples (1 to 30).

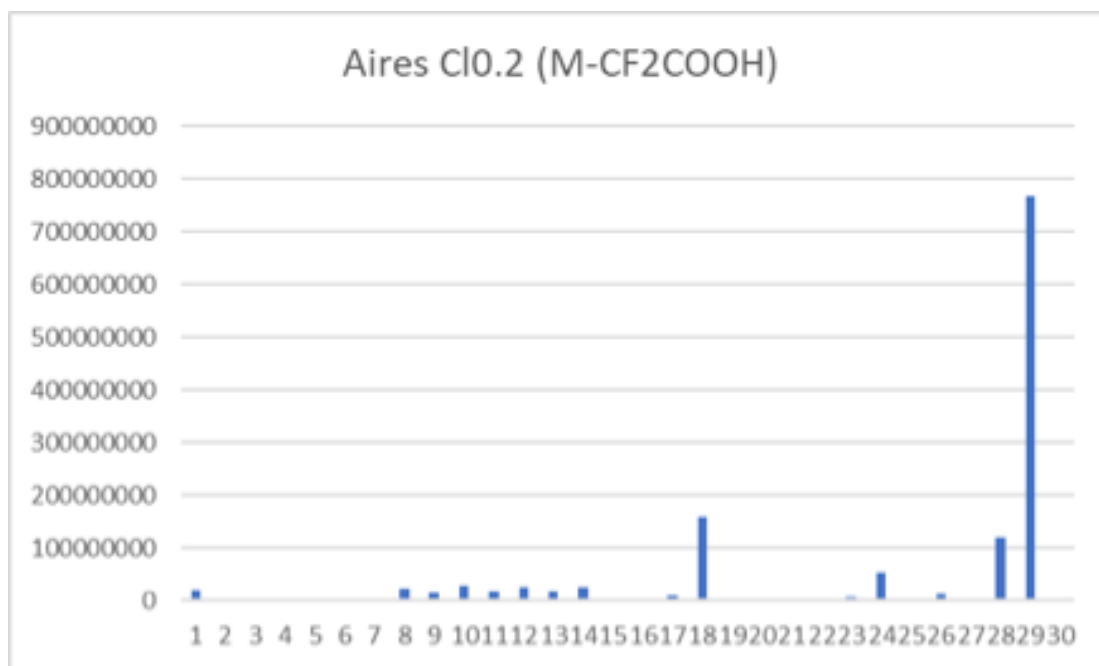


Figure 8: Signal intensities of (e, p =0.2) for the 30 blood samples (1 to 30).

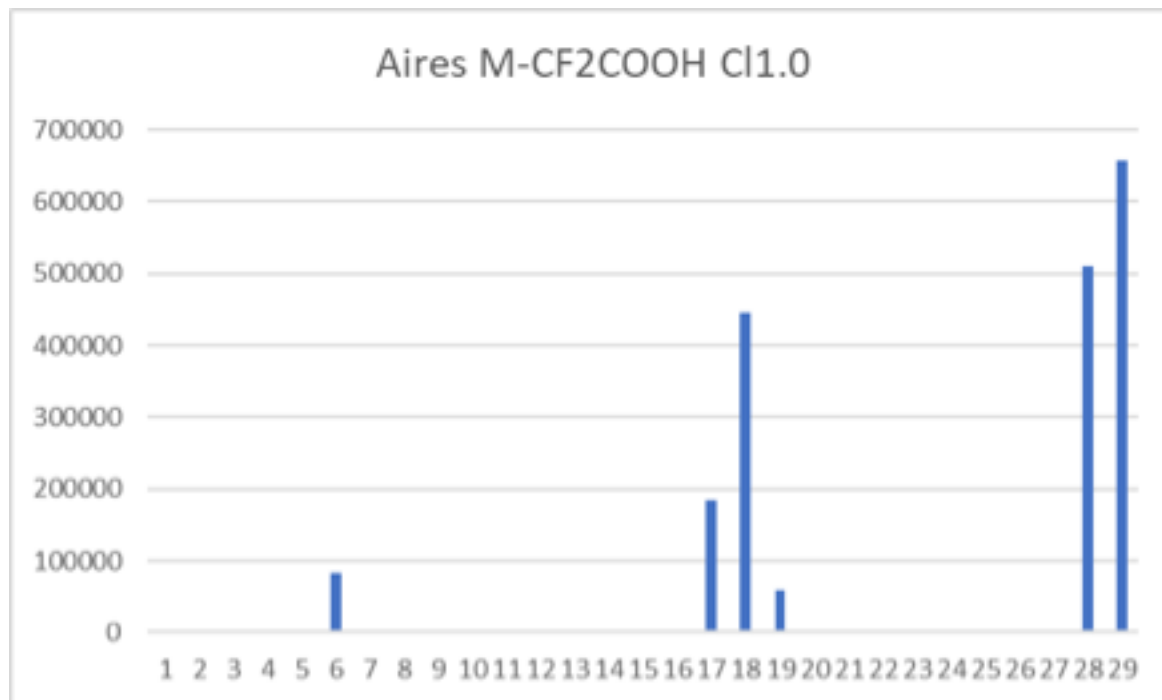


Figure 9: Signal intensities of (e, p =1.0) for the 30 blood samples (1 to 30).

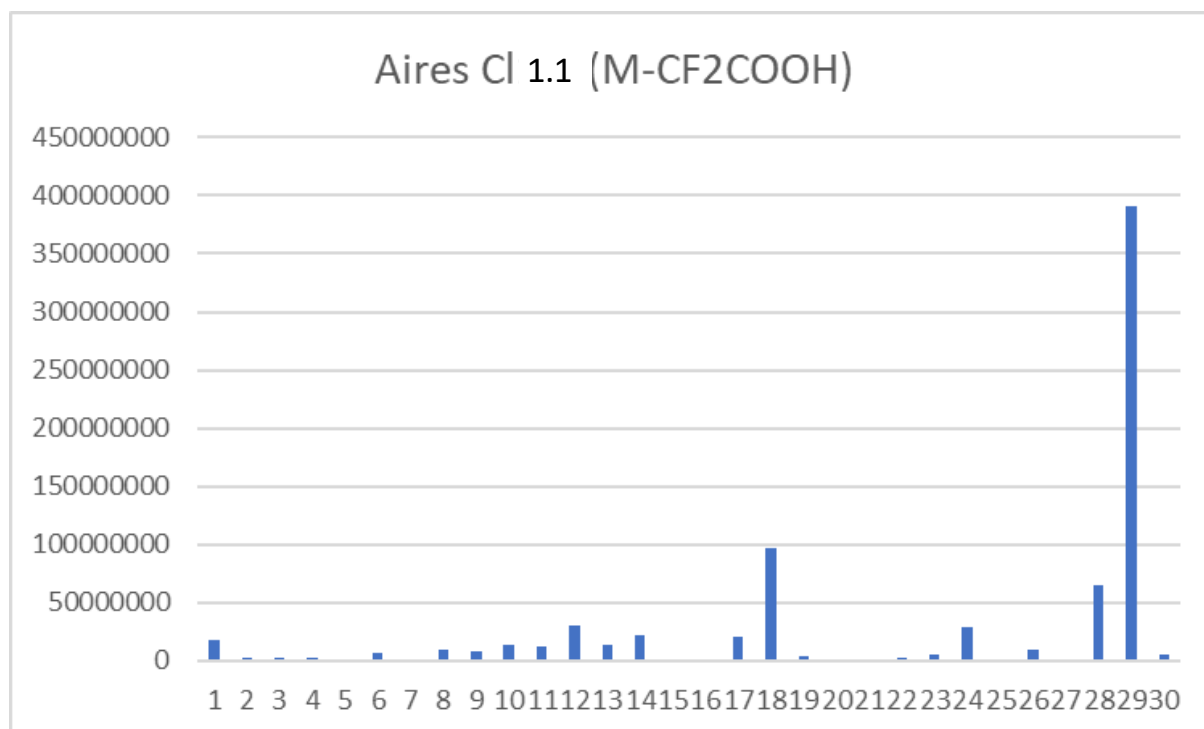


Figure 10: Signal intensities of (e, p =1.1) for the 30 blood samples (1 to 30)

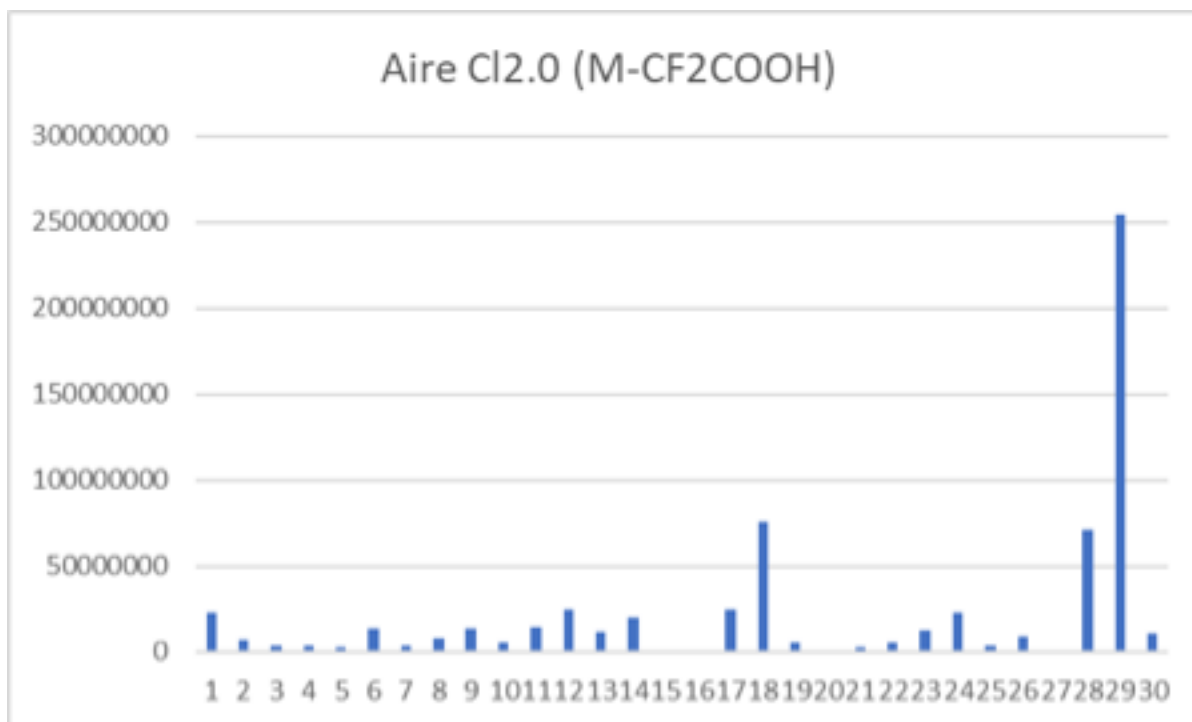


Figure 11: Signal intensities of (e, p = 2.0) for the 30 blood samples (1 to 30).

Conclusions:

The objective of this study was to take up an incredible analytical challenge which consisted in setting up, in record time, a non-targeted analysis method of emerging PFAS by high resolution mass spectrometry. Thanks to a hard and coordinated team work, my laboratory was able to bring in all independence objective and concrete answers to the request formulated by the journalists of the RTBF Investigation program. The expertise of the ULiège CART in the field of POPs analysis allowed, through a set of identification criteria and analytical verifications, to obtain a bundle of concordant and coherent evidence in accordance with the current state of the art of the scientific literature, This has led to a strong suspicion of the presence of cC604 and 5 major oligomers of Cl-PFPECA (ADV) in a sample of water discharged from the Solvay Spinetta Marengo plant, and of the presence of the same 5 oligomers of Cl- PFPECA (ADV), but in different proportions and quantities, in blood samples taken from 30 Spinetta residents. It is important to specify that in no case we can guarantee at 100% that these emerging perfluorinated compounds are indeed present in the samples carried out by the RTBF since we do not have the analytical standards of these investigated molecules.

Liege on September 5, 2022

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