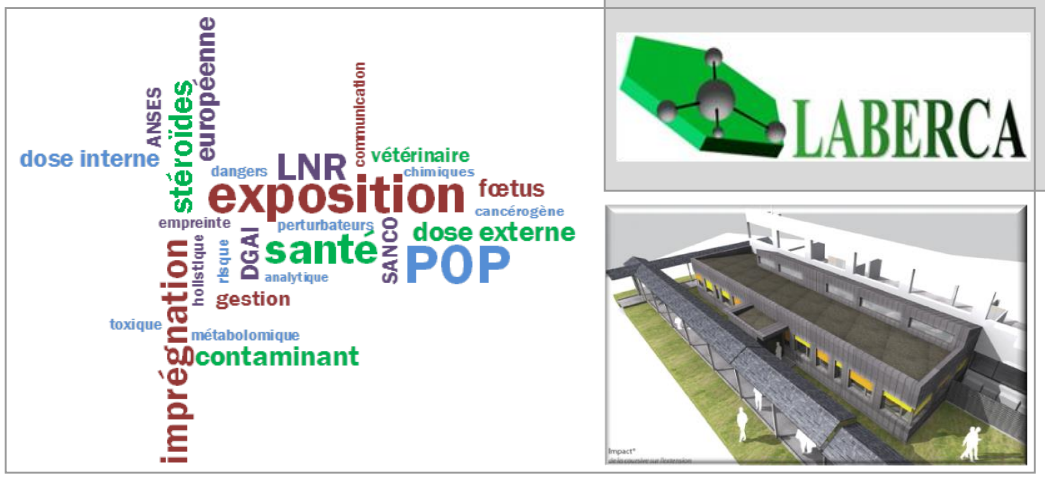
ÉCOLE NATIONALE
VÉTÉRINAIRE, AGROALIMENTAIRE ET DE L'ALIMENTATION

SANTÉ ET ALIMENTATION AU CŒUR DE LA VIE

TUNTWIN Project
9 March 2023, Lyon

L'ANALYSE DANS L'ÉVALUATION DE L'EXPOSITION DE L'HOMME AUX DANGERS CHIMIQUES

ANALYSIS IN THE HUMAN EXPOSURE ASSESSMENT TO CHEMICAL HAZARDS



Emmanuelle Bichon
Ingrid Guiffard, Anaïs Vénisseau,
Frédéric Larvor, Bruno Veyrand,
Gaud Dervilly, Jean-Philippe Antignac,
Philippe Marchand, Bruno Le Bizec

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Introduction

Oniris/LABERCA

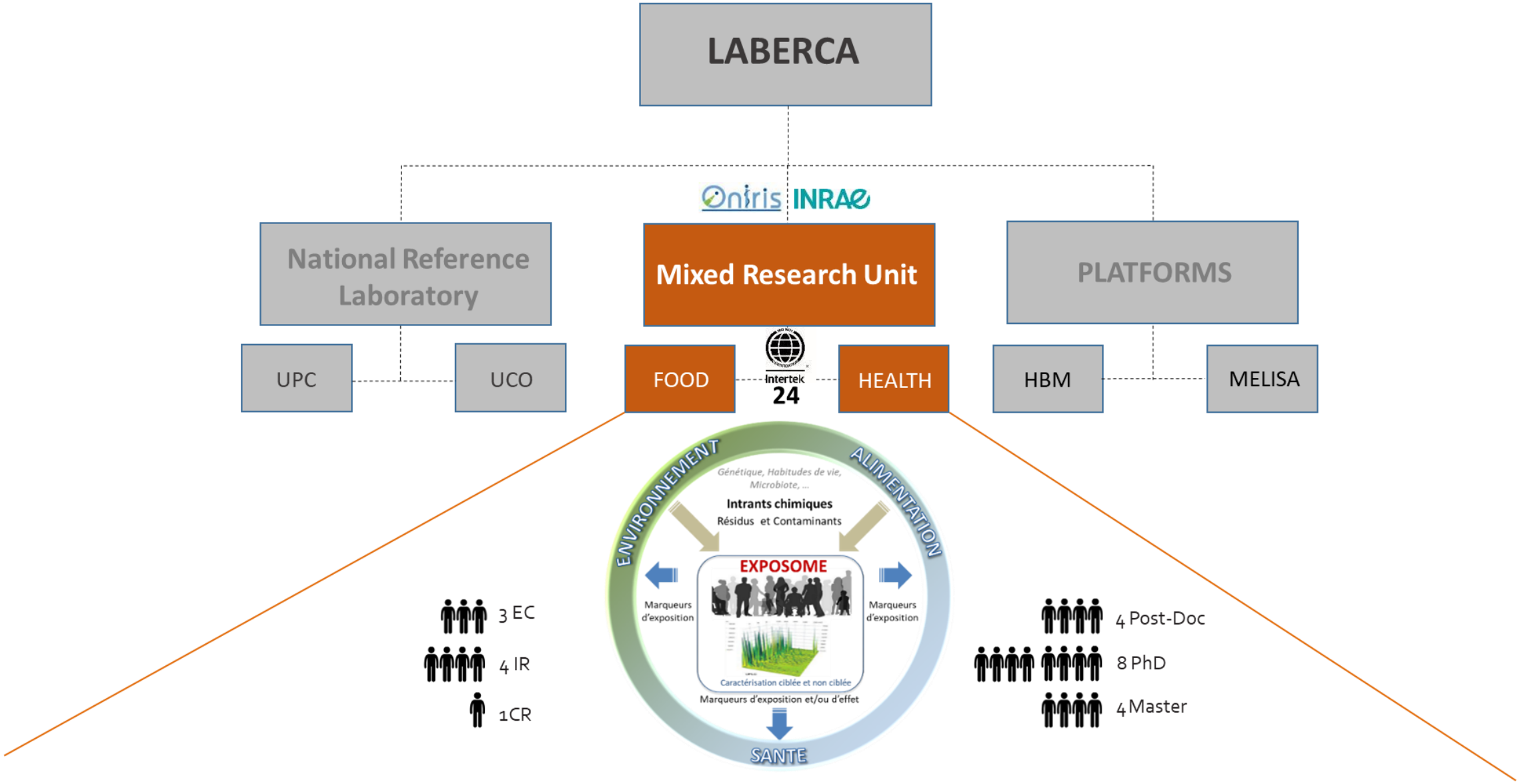
Oniris
École Nationale Vétérinaire, Agroalimentaire et de l'Alimentation
Nantes Atlantique

Vétérinaires

Ingénieurs

LABERCA

Introduction



Global research thematic:
organic chemical residues and contaminants from their environmental sources to their impact on human health **through the food chain**

ENVIRONMENT
Captation of Emerging Risks

- Detect and identify **emerging chemical hazards**
- **Characterise their fate to the human food chain**

FOOD
Chemical Food Safety

- Expand our knowledge of the human **external chemical exposure**
- **Characterise the associated risk**
- Guide and support the **public policy** with **regard to risk assessment and risk management**

TOXICOKINETIC MODELS

- **Model data that are not available or easily accessible (e.g. due to cost, technical, ethical reasons)**
- **Link external and internal exposure**
- **Predict internal dose in given biological compartments**
- **Model lifespan exposure**

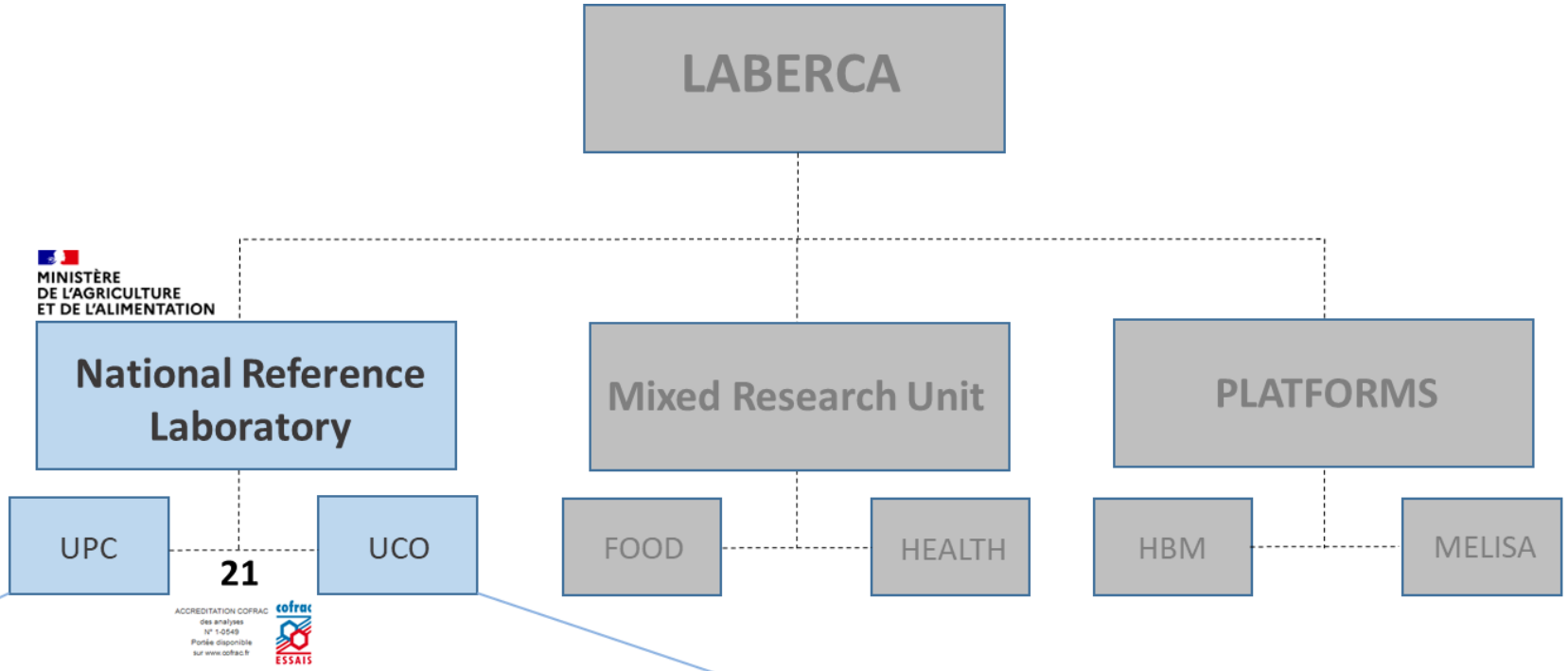
ANIMAL MODEL

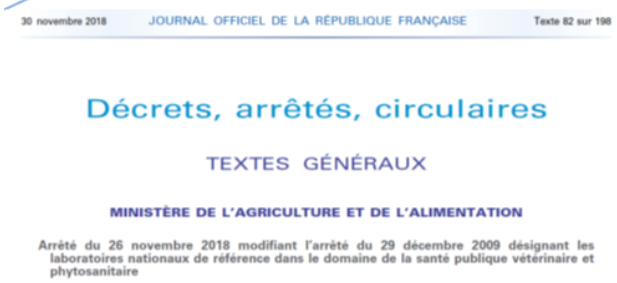
- **Characterize the biological effects induced by chemical exposure**
- **Support human studies through exploratory and/or confirmatory study designs**

HEALTH
Exposure – health relationships

- Expand our knowledge of the human **internal chemical exposure**
- **Link internal exposure to environmental/food determinants**
- **Investigate the associated metabolic profile disruptions (effect markers)**
- **Investigate the link between exposure and health**

Introduction





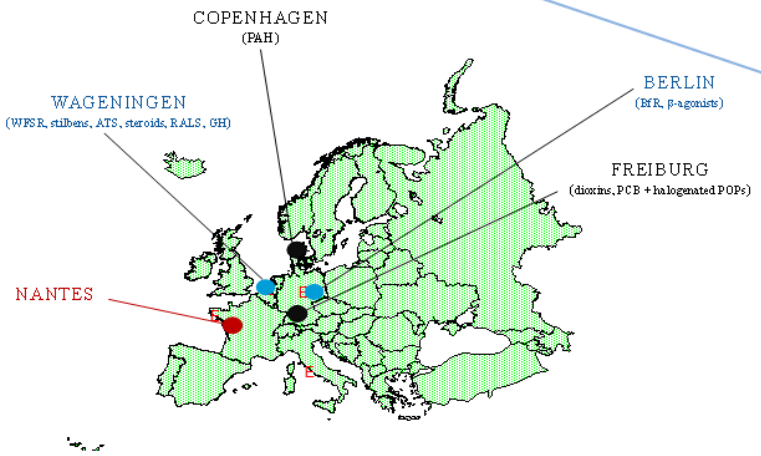
30 novembre 2018 JOURNAL OFFICIEL DE LA RÉPUBLIQUE FRANÇAISE Texte 82 sur 198

Décrets, arrêtés, circulaires

TEXTES GÉNÉRAUX

MINISTÈRE DE L'AGRICULTURE ET DE L'ALIMENTATION

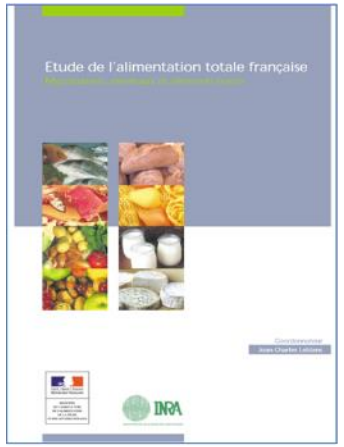
Arrêté du 26 novembre 2018 modifiant l'arrêté du 29 décembre 2009 désignant les laboratoires nationaux de référence dans le domaine de la santé publique vétérinaire et phytosanitaire



Introduction

Characterizing the external (food) exposure
e.g. total diet studies

TDS1
(2000-2004)



TDS2
(2006-2011)

TDSi
(2012-2016)

TDS3
In preparation

Characterizing the internal exposure
e.g. biomonitoring programs

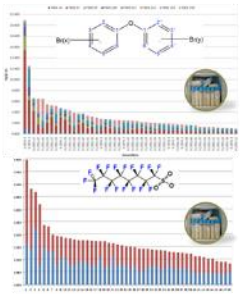
anses
alimentation, environnement, travail

Calipso (2009-11)

ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

Conta-Lait (2017-2019)

Etude pilote cohorte ELFE
(2013)



Antignac et al. Chemosphere 2013;91:802-808.

Santé publique France

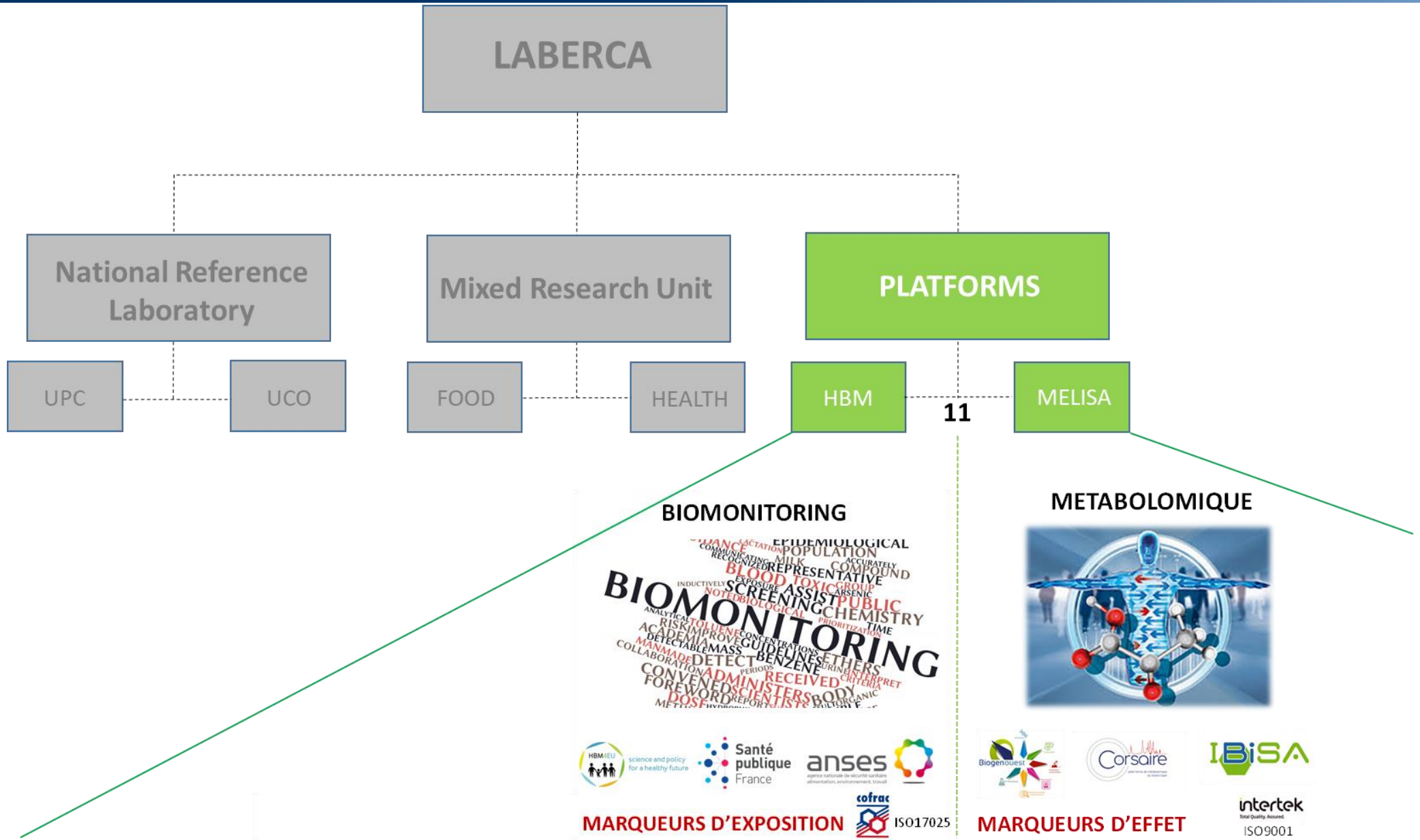
elfe
(2013)

esteban
(2016-18)

FRANCE exposome

EIRENE RI

Introduction



Introduction

LC-QTOF (IMS)

GCxGC-TOF

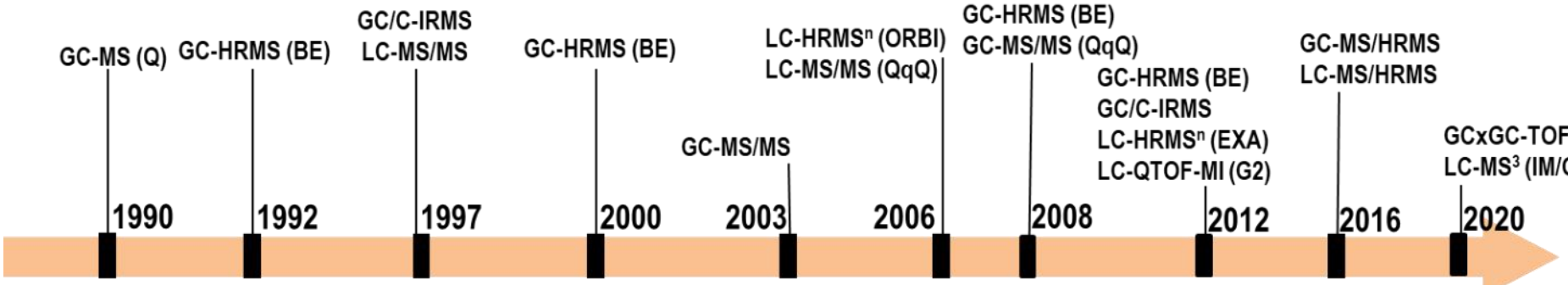
GC- and LC-HRMS^n (x 4, Orbitrap systems)

GC-HRMS (x 3, BE)

GC-C-IRMS (x 2)

GC-MS/MS (x 3, QqQ)

LC-MS/MS (x 3, QqQ)



Context

Communication

Risk assessment

Hazard identification

Hazard characterization

Exposure Assessment

Risk characterization

Risk management

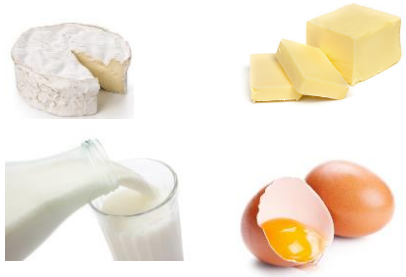
Political options

Preventive measures

Control measures

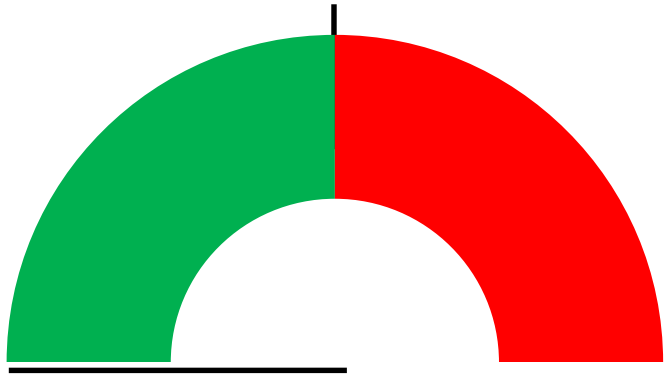
Place of analytical chemsitry

Food



Environment

Acceptable daily Intake



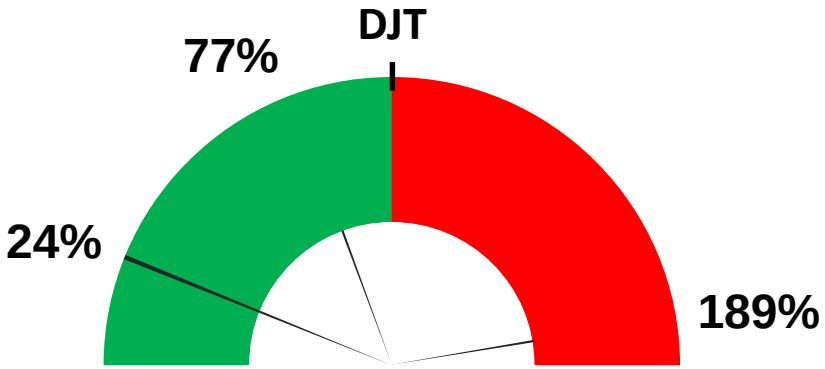
Internal exposure

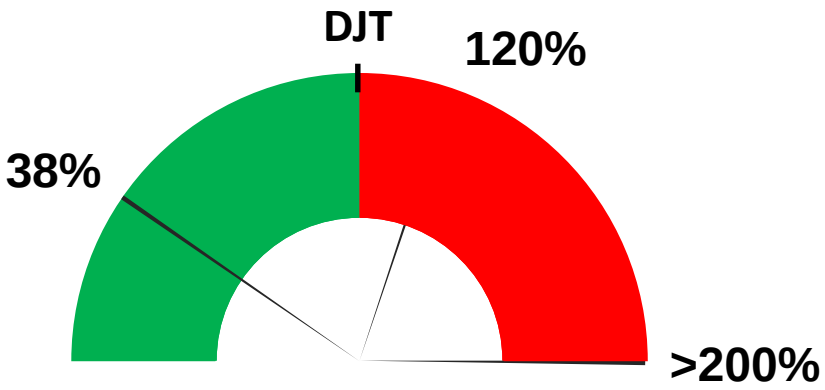
Context

2000

2005

2011





Dioxins and PCB-DL

Etude de l'alimentation totale française 2 (EAT2). Contaminants inorganiques, minéraux, polluants organiques persistants, mycotoxines, phyto-estrogènes. In E. scientifique (Ed.), vol. Tome 1) Anses (2011).

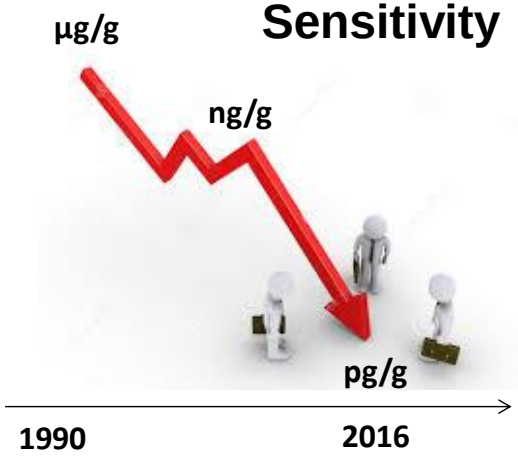
Context

Ideal Analytical method

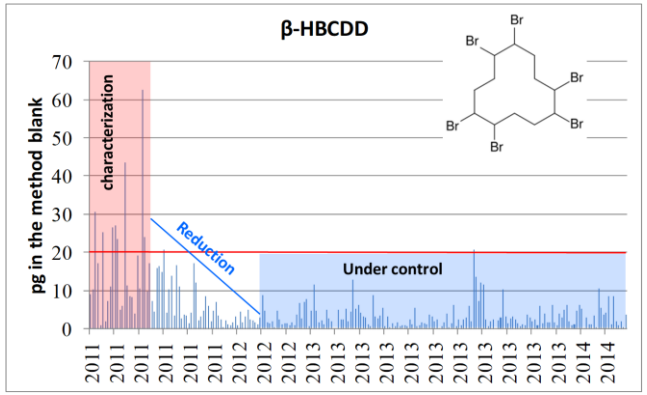
Fast

Robust

Sensitivity



lab contamination



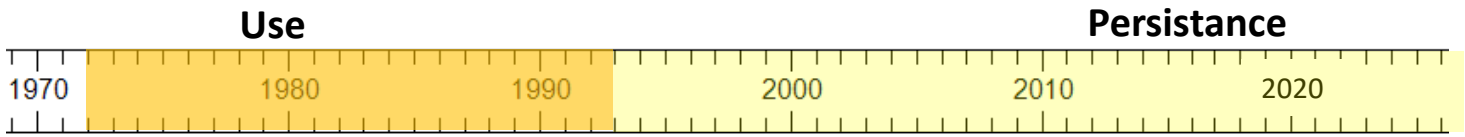
Multiresidues
Emerging and legacy contaminants

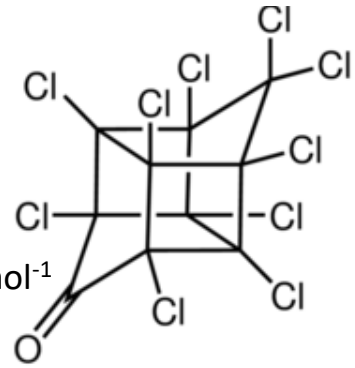
Complex & various Matrices

Data quality

Environmental impact

First illustration : Chlordecone

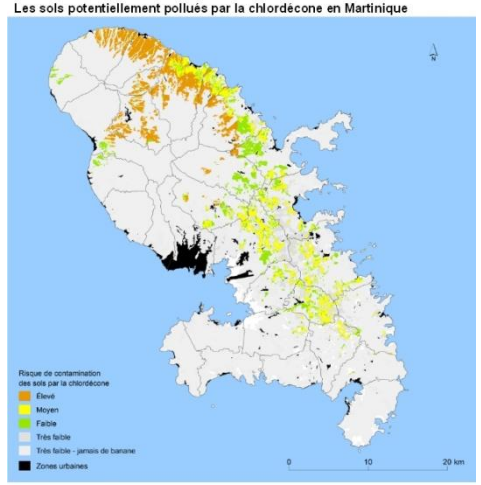




$C_{10}H_{10}O$
MM = 490,6 g.mol^{-1}
Log K_{ow}=4,5

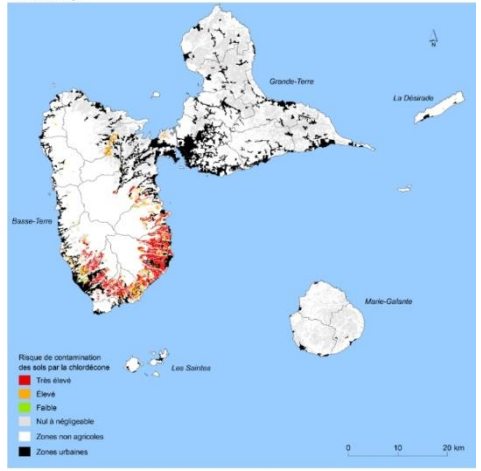
Sensitivity
Difficult compound

Les sols potentiellement pollués par la chlordécone en Martinique



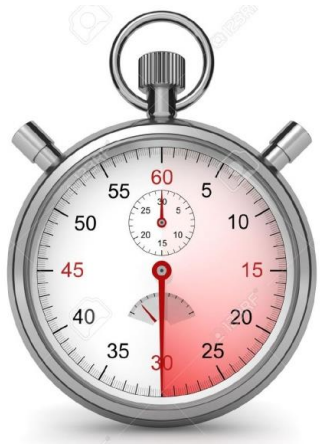
Source : SIG DIREN Martinique – SIG 972 – Chambre d'Agriculture, 2007. Traitements : SOeS, 2013.

Les sols agricoles potentiellement pollués par la chlordécone en Guadeloupe



Source : Source : DAAF Guadeloupe – 2005. Traitements : SOeS, 2013.

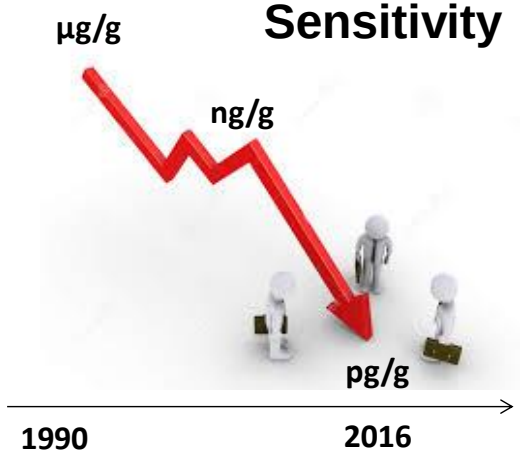
First illustration : Chlordecone



Fast

Robust

Sensitivity



Required LOD min : 60 pg mL^{-1} in blood

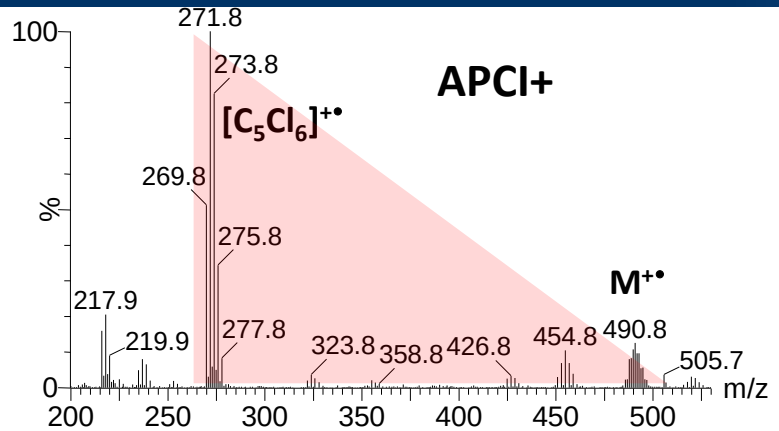
***Emeville, E**. et al. (2013) PLoS One, 8(6), e66460.*

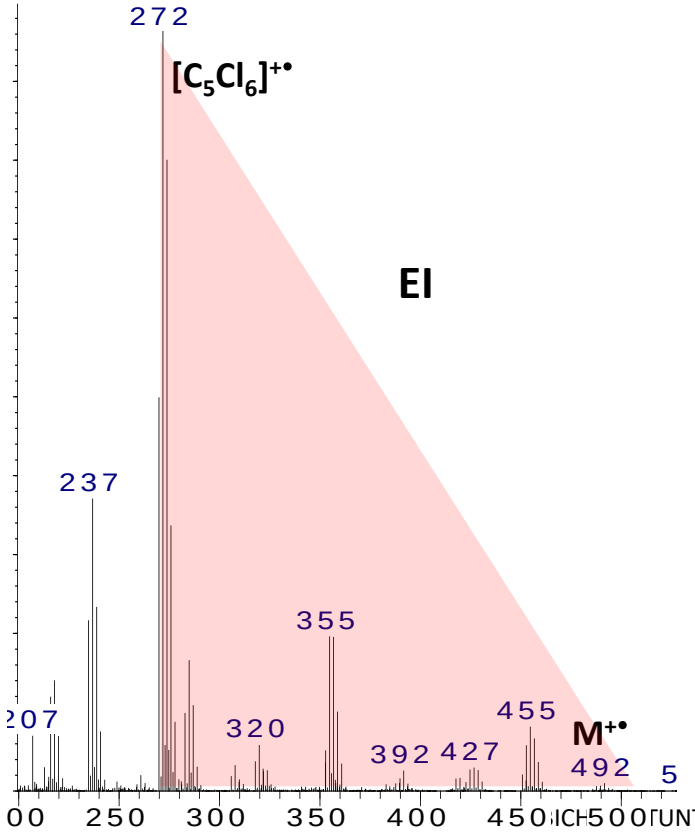
Low levels expected in breast milk

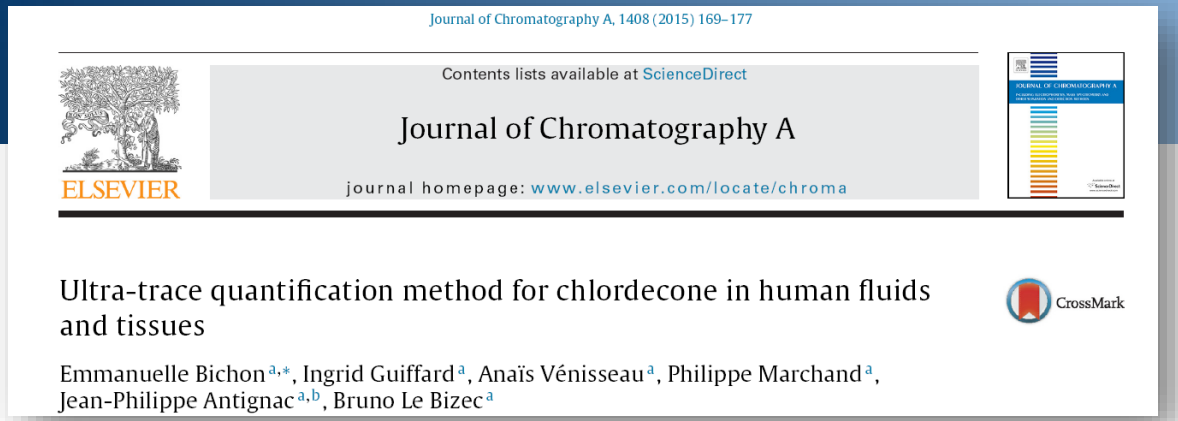
Which Analytical instrument ? chromatography & mass spectrometry

First illustration : Chlordecone

☒ Ionisation assessment






Journal of Chromatography A, 1408 (2015) 169–177

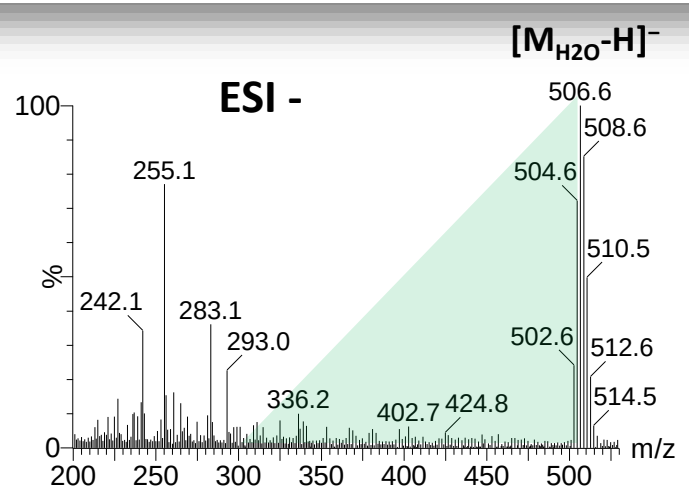
Contents lists available at ScienceDirect

Journal of Chromatography A

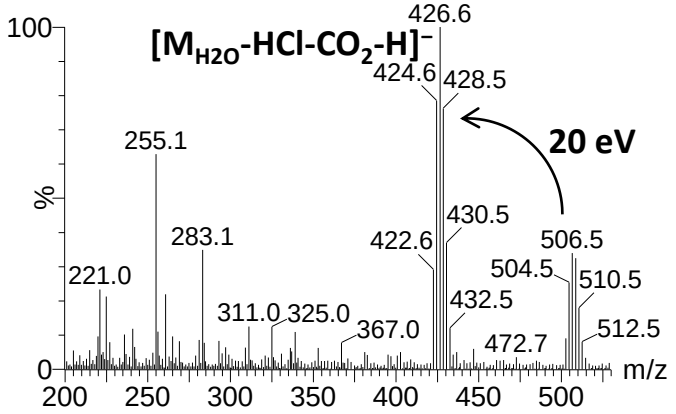
journal homepage: www.elsevier.com/locate/chroma

Ultra-trace quantification method for chlordecone in human fluids and tissues

Emmanuelle Bichon[a,*], Ingrid Guiffard[a], Anaïs Vénisseau[a], Philippe Marchand[a], Jean-Philippe Antignac[a,b], Bruno Le Bizec[a]



MS/MS

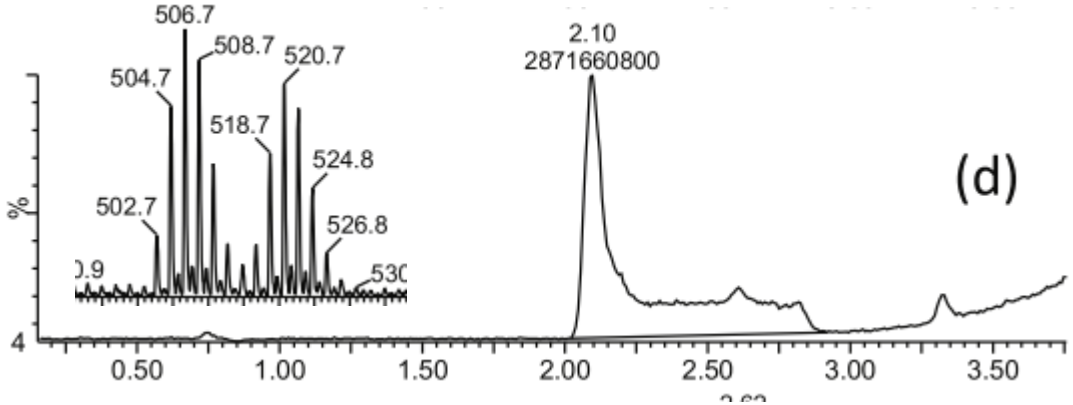


LOQ : 8 pg mL^{-1} in breast milk

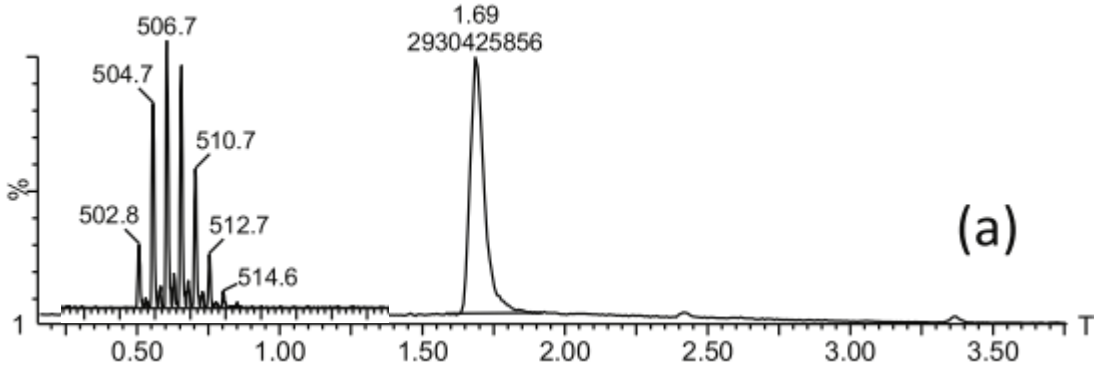
First illustration : Chlordecone

☒ Chromatographic considerations

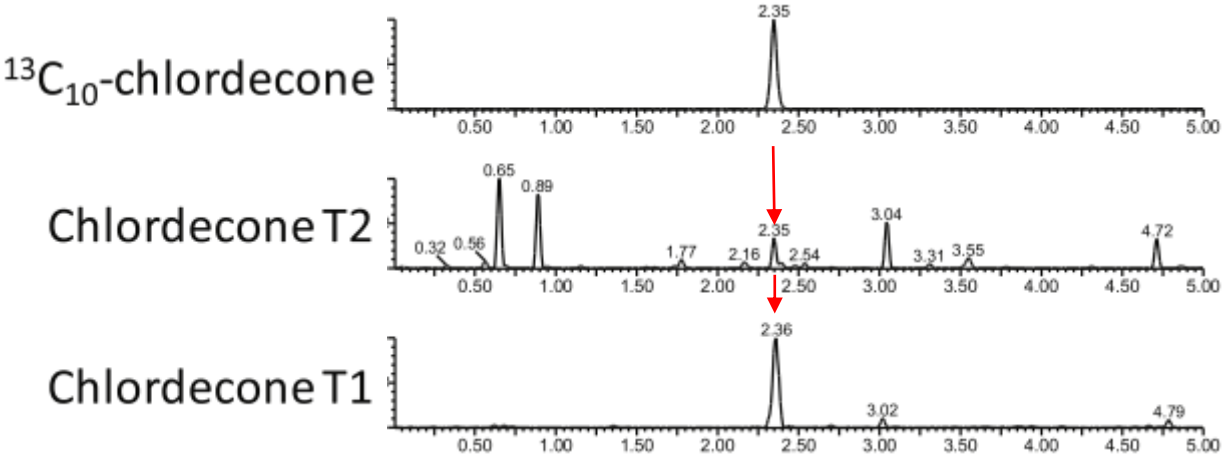
Methanol Based gradient



ACN Based gradient



Spiked Breast Milk at 3,2 pg.mL^{-1}



Thermo Accucore C30 (100 x 2.1 mm, 2.6 µm)

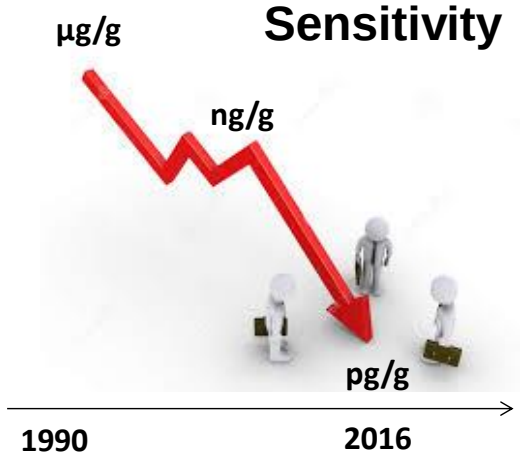
T° = 40 °C, ACN/water gradient, 0,6 mL.min^{-1} flow rate

Second illustration : Brominated Flame Retardant

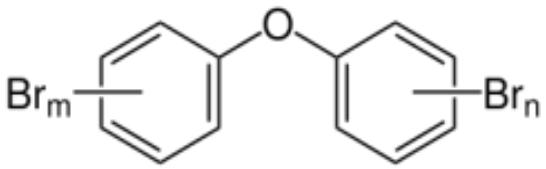
Fast

Robust

Sensitivity



Legacy BFR

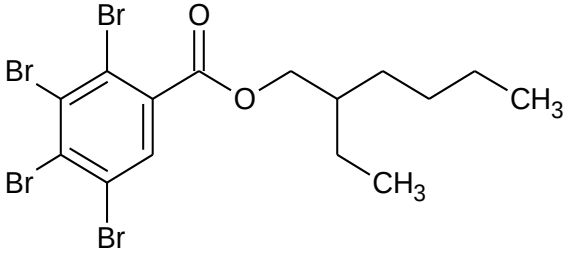


Multiresidues
Emerging and legacy contaminants

Complex & various Matrices

Data quality

Emerging BFR



Second illustration : Brominated Flame Retardant

Mixtures of man-made chemicals that are added to a wide variety of products, including for industrial use, to make them **less flammable**. They are used commonly in **plastics**, **textiles** and **electrical/electronic equipment**.

There are **five main classes of BFRs**, listed here with their common uses:

- Polybrominated diphenyl ethers (**PBDEs**) – plastics, textiles, electronic castings, circuitry.
- Hexabromocyclododecanes (**HBCDDs**) – thermal insulation in the building industry.
- Tetrabromobisphenol A (**TBBPA**) and other phenols – printed circuit boards, thermoplastics (mainly in TVs).
- Polybrominated biphenyls (**PBBs**) – consumer appliances, textiles, plastic foams.
- **Other brominated flame retardants**

[…] In the European Union the use of **certain BFRs is banned or restricted**; however, due to their persistence in the environment there are still concerns about the risks these chemicals pose to public health. BFR-treated products, whether in use or waste, leach BFRs into the environment and contaminate the air, soil and water. These contaminants may then **enter the food chain** where they mainly occur in **food of animal origin**, such as fish, meat, milk and derived products.

https://www.efsa.europa.eu/en/topics/topic/brominated-flame-retardants

Second illustration : Brominated Flame Retardant

GC-EI-HRMS

ELSEVIER

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Journal of Chromatography A, 1100 (2005) 144–152

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

New multiresidue analytical method dedicated to trace level measurement of brominated flame retardants in human biological matrices

Ronan Cariou [a], Jean-Philippe Antignac [a,*], Philippe Marchand [a], Alain Berrebi [b], Daniel Zalko [c], François Andre [a], Bruno Le Bizec [a]

GC-EI-HRMS use in the 2000's

- \+ High chromatographic resolution
- \+ Compounds with mass defect, HRMS is relevant
- \- High m/z DecaBDE (959.17)
- \- Limited Thermostability DecaBDE
- \- Run times until 90 min

A gas chromatography/high-resolution mass spectrometry (GC/HRMS) method for determination of polybrominated diphenyl ethers in fish

M. Alaee [a,*], D.B. Sergeant [b], M.G. Ikonomou [c], J.M. Luross [b]

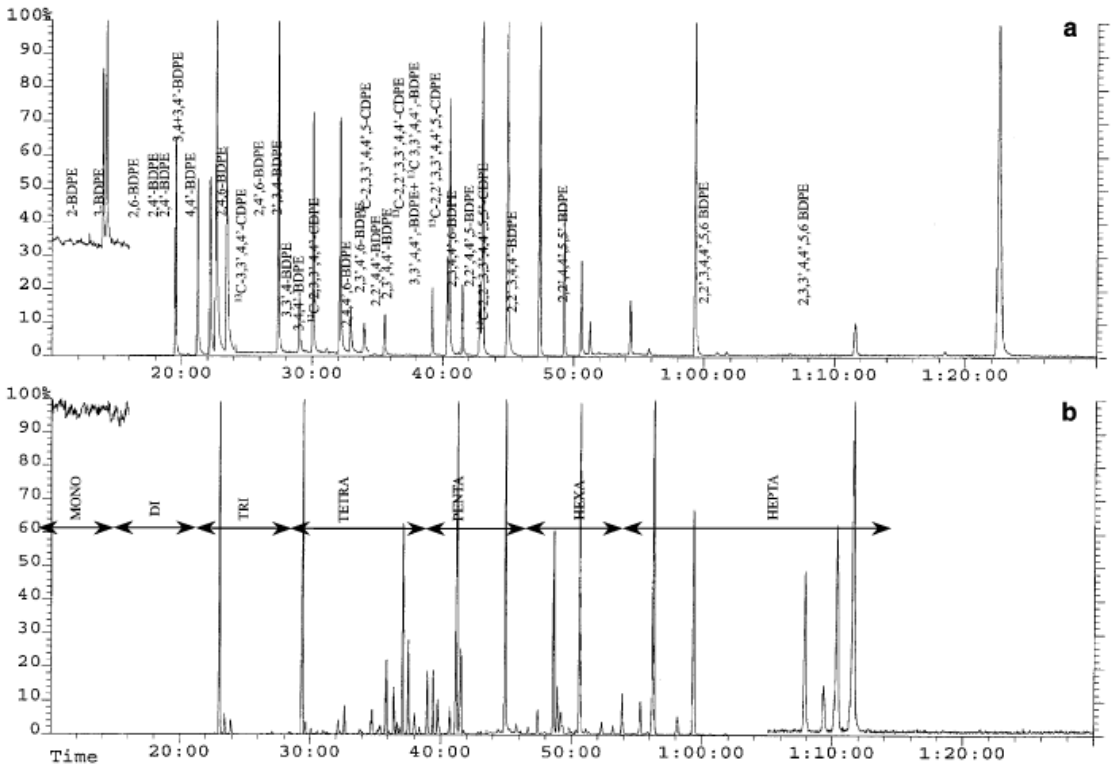
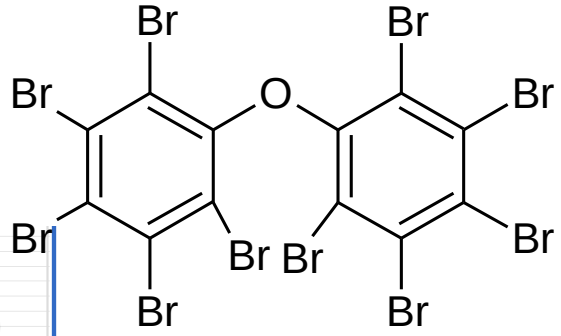
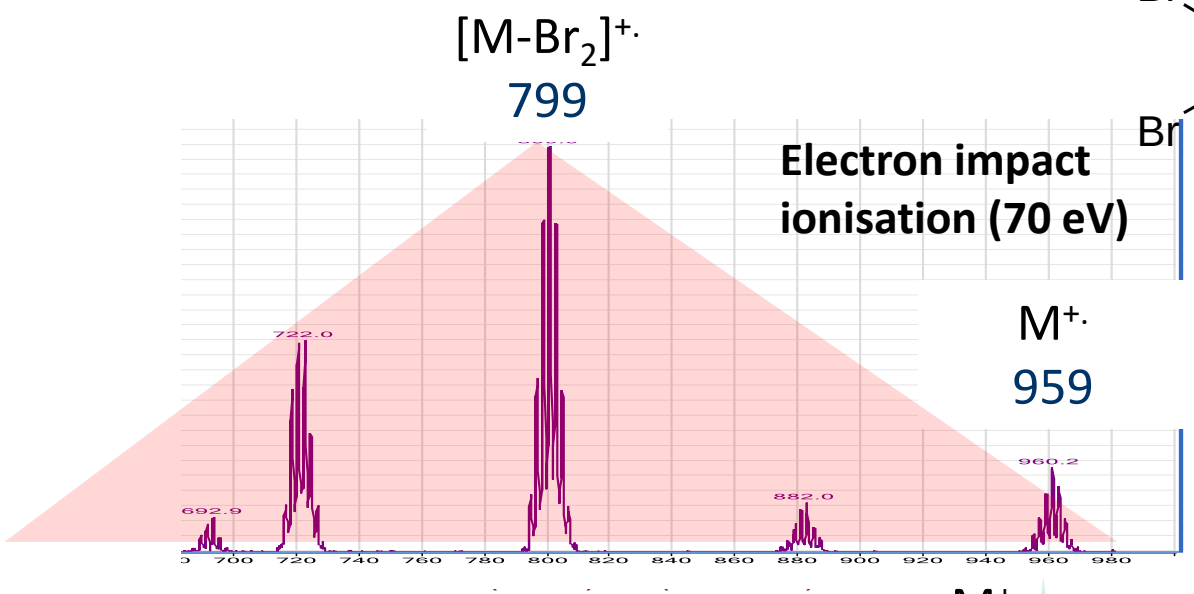


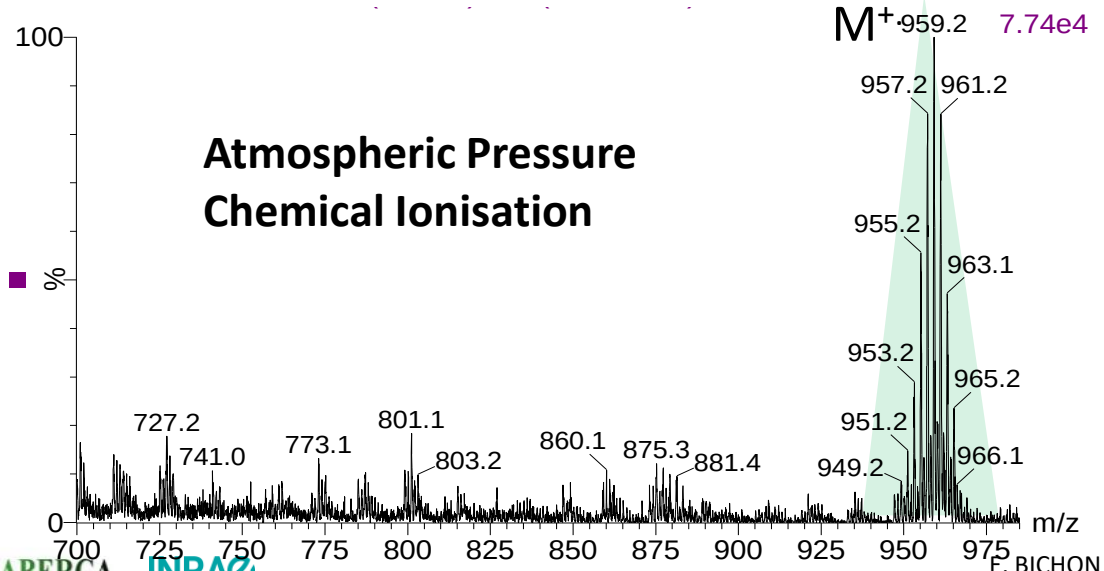
Fig. 1. Total ion current (TIC) of BDPE congeners with ^{13}C CDPEs used as internal standard (a) and in Lake Ontario lake trout (b). In this figure, each function was normalized to 100% to illustrate the presence of various homologue groups and does not show the relative abundance of PBDEs in the lake trout sample.

Second illustration : Brominated Flame Retardant

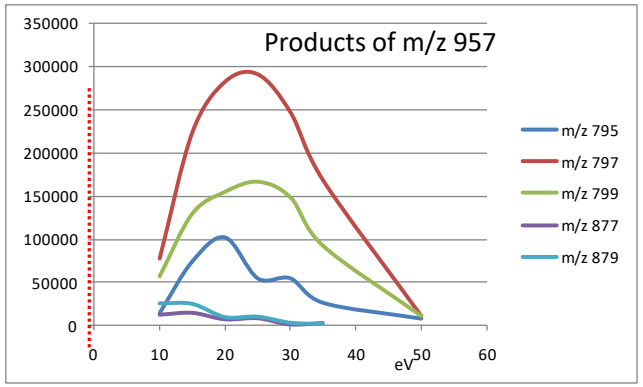
☒ Ionisation assessment

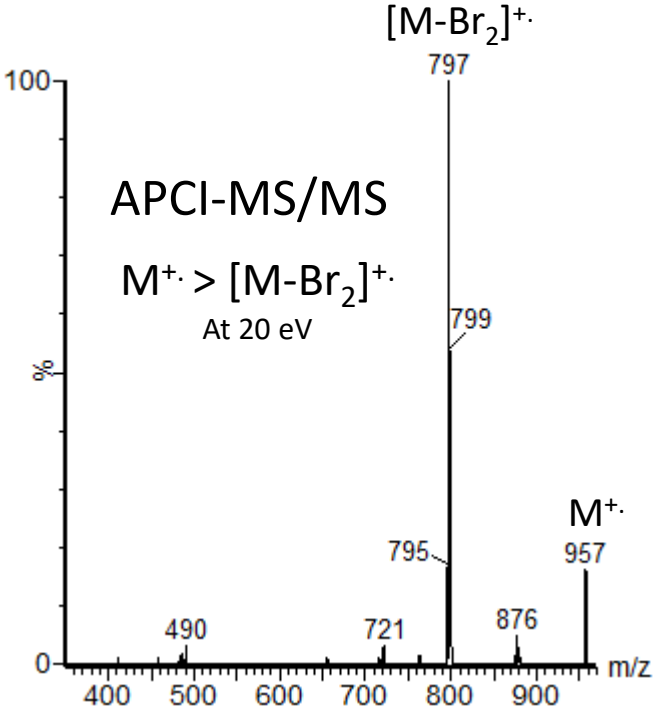






APCI-MS/MS



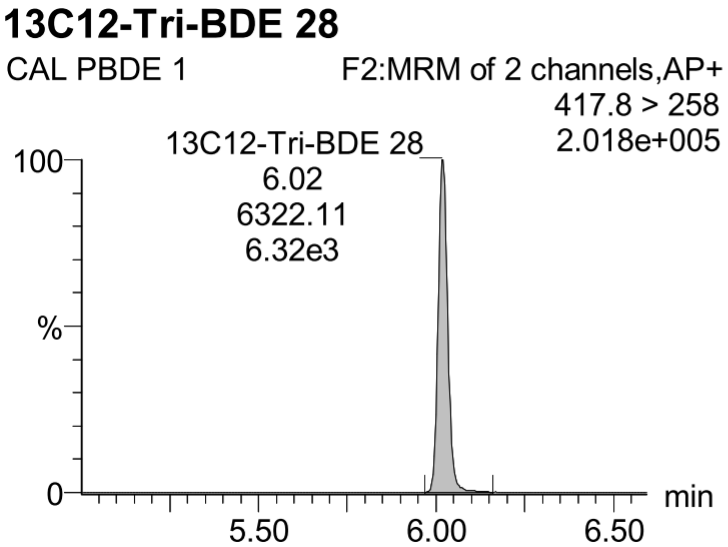


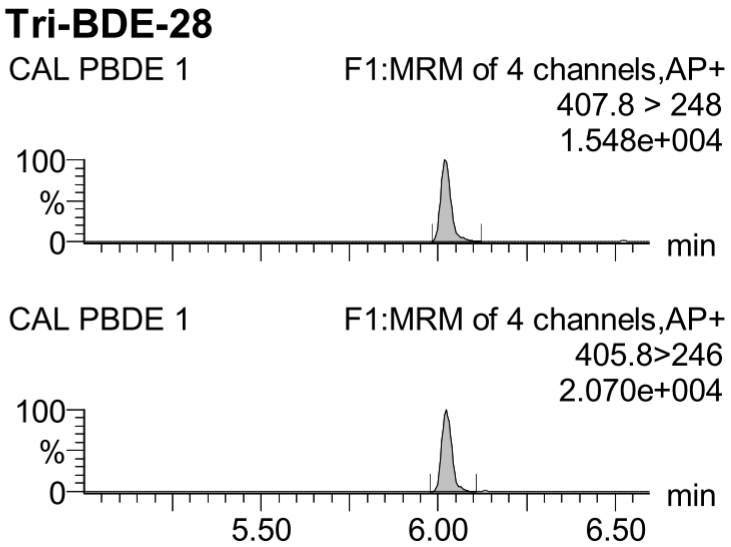
Second illustration : Brominated Flame Retardant

First results in 2013 in APCI

First calibration point

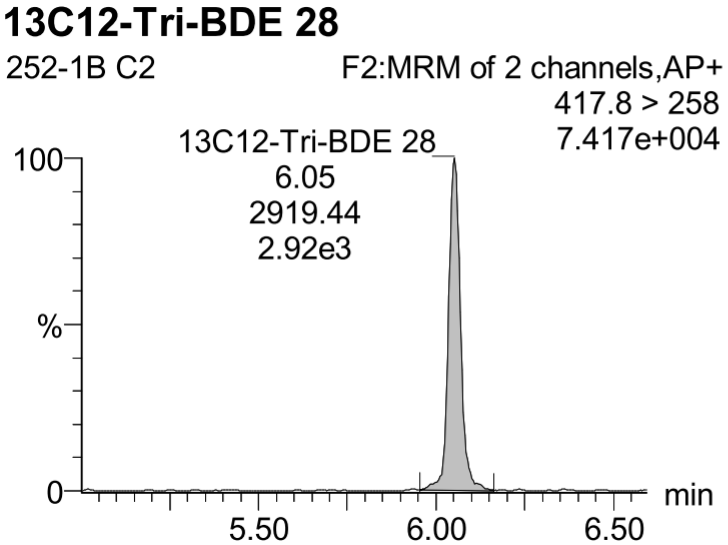
(2 pg injected)

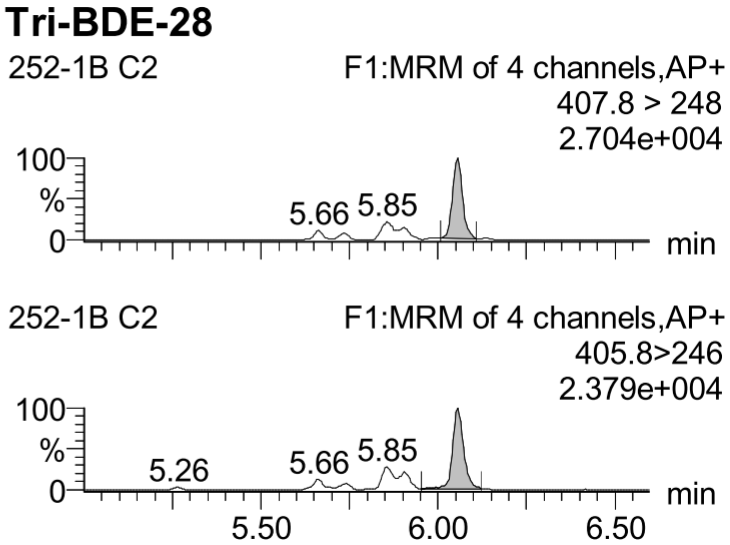




Fish oil

Reference sample

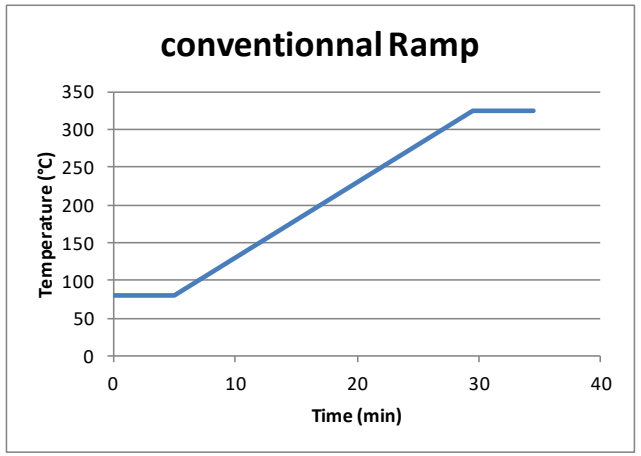


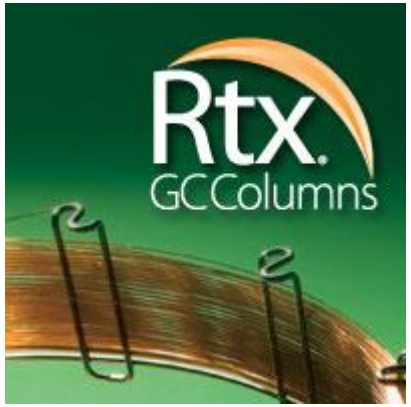


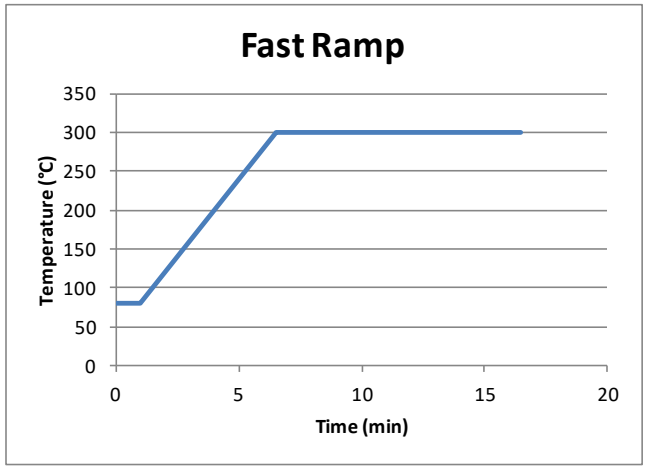
✓ Quantification at ppt level of PBDEs in complex matrices

Second illustration : Brominated Flame Retardant

Through fast GC thanks to APCI







Injector : 250 °C

Column Rtx-1614

15 m x **0.25** mm, 0.1 µm

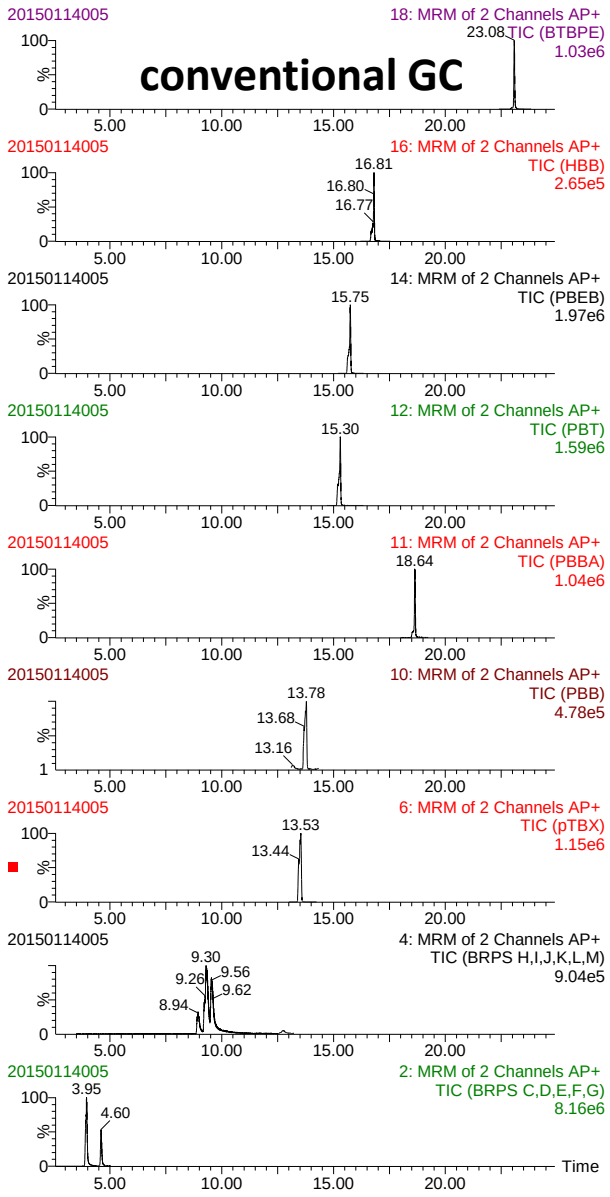
Constant flow rate : 3 mL/min

15 m x **0.32** mm, 0.1 µm

Constant pressure : 30 psi

Second illustration : Brominated Flame Retardant

Through fast GC thanks to APCI



BTBPE

HBBz

PBEB

PBT

PBBAcr

PBBz

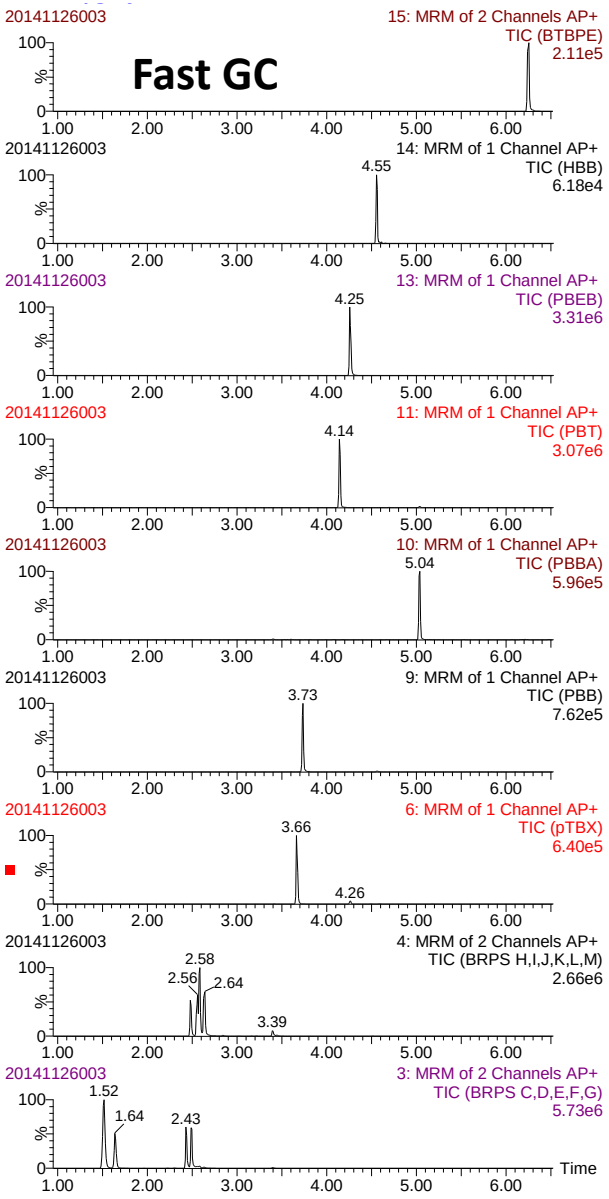
pTBX

triBrPs

diBrPs

Run time/3

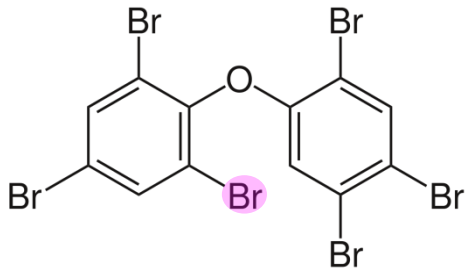
23 min



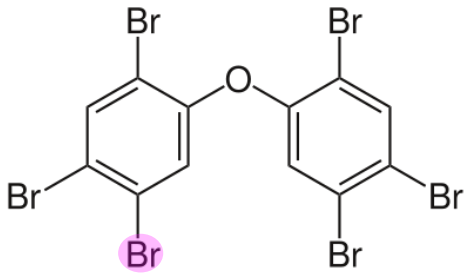
6.3 min

Second illustration : Brominated Flame Retardant

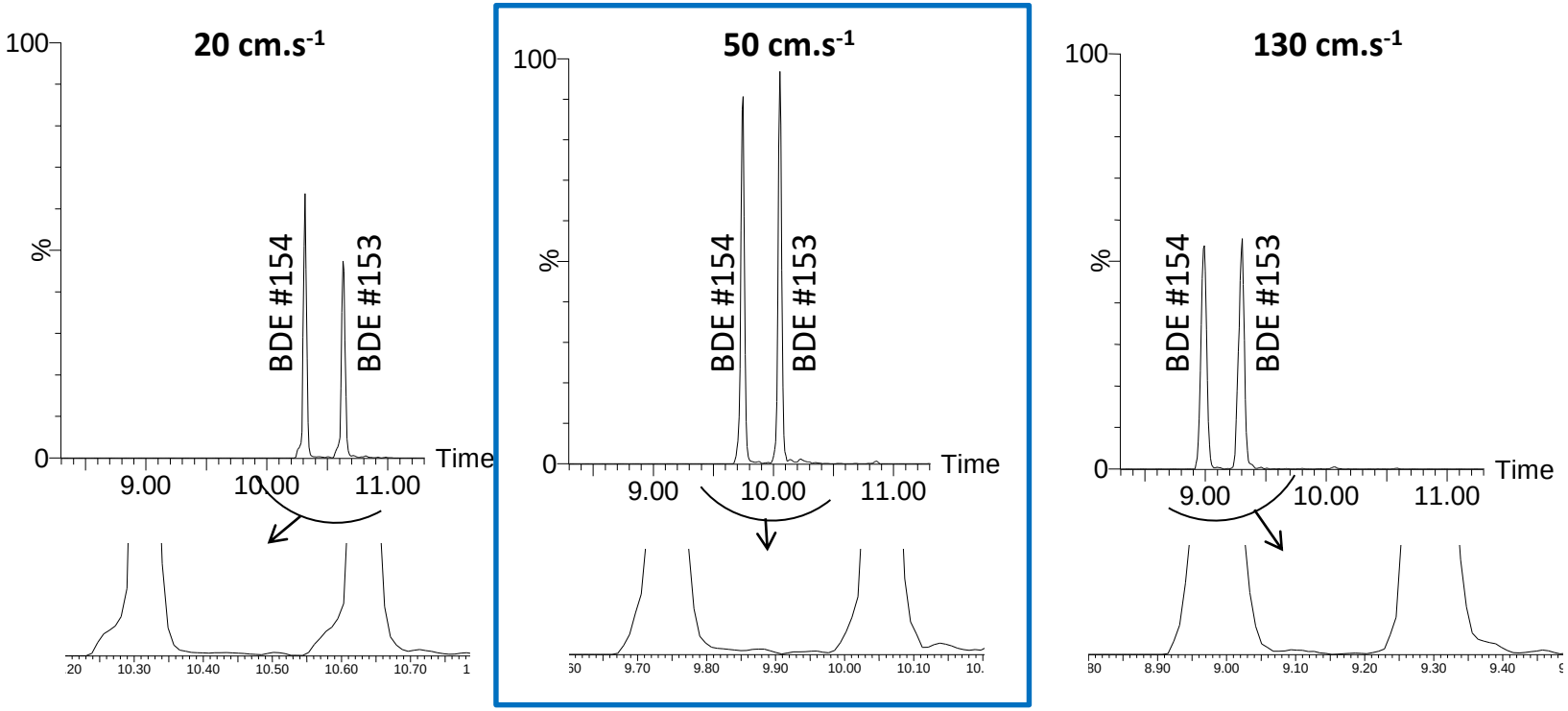
BDE #154



BDE #153



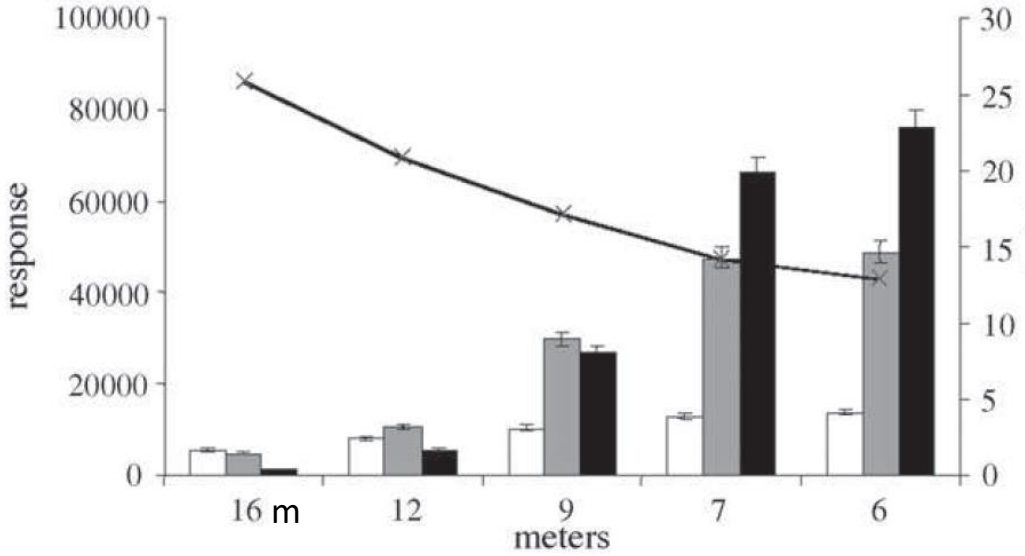
10 pg sur Rtx-1614 15 m x 0.25 mm, 0.1 µm @ 20 °C/min



Second illustration : Brominated Flame Retardant

Rtx-5MS column (0.25 mm i.d.; 0.25 µm f.t.)

A. Binelli et al. / J. Chromatogr. A 1136 (2006) 243–247

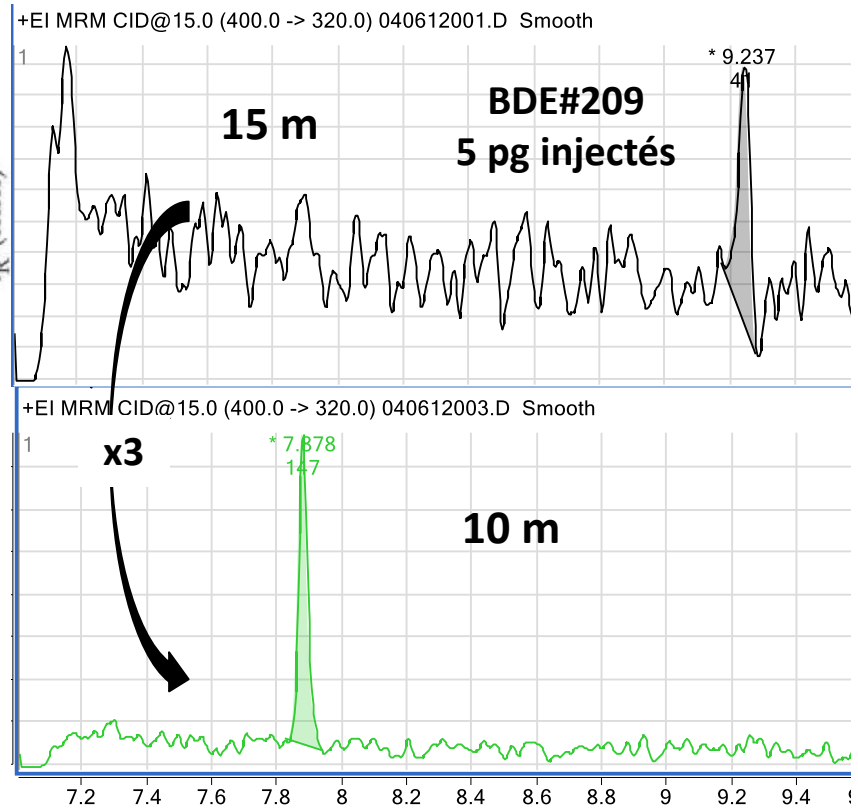


BDE#209

BB#209

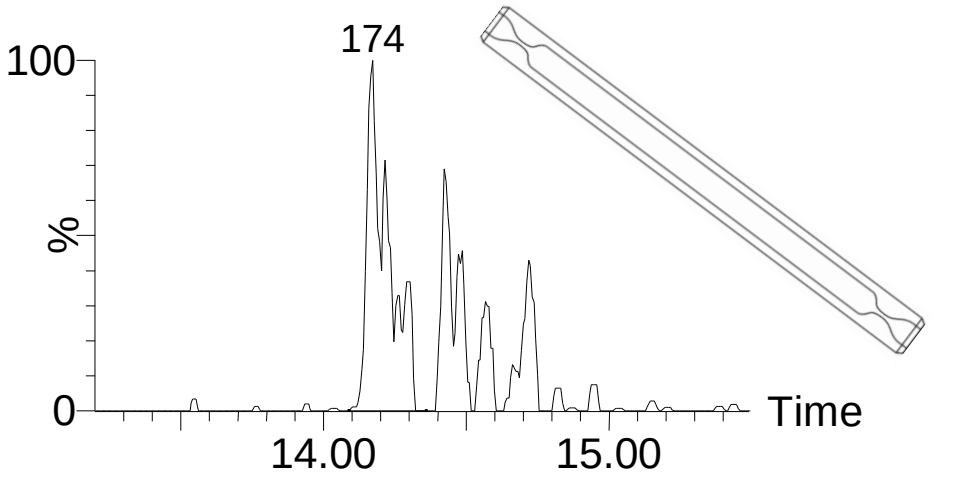
BDE#183

Shorter columns

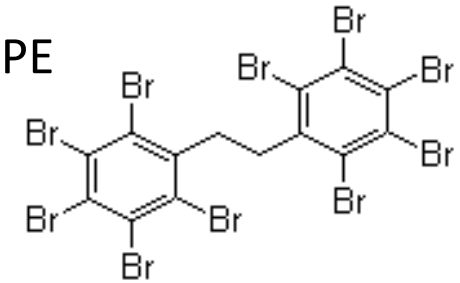


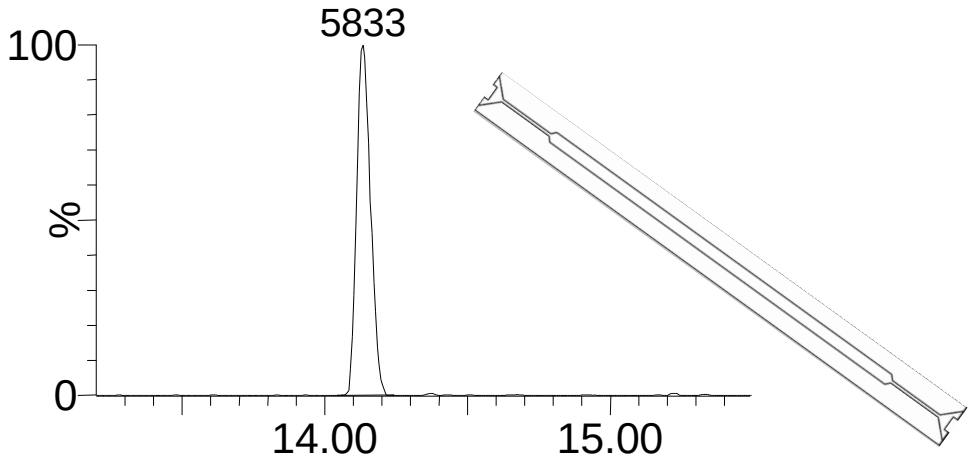
Second illustration : Brominated Flame Retardant

Liner impact



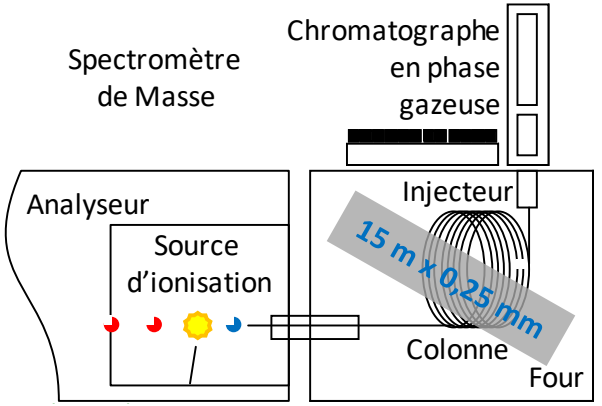
DBDPE

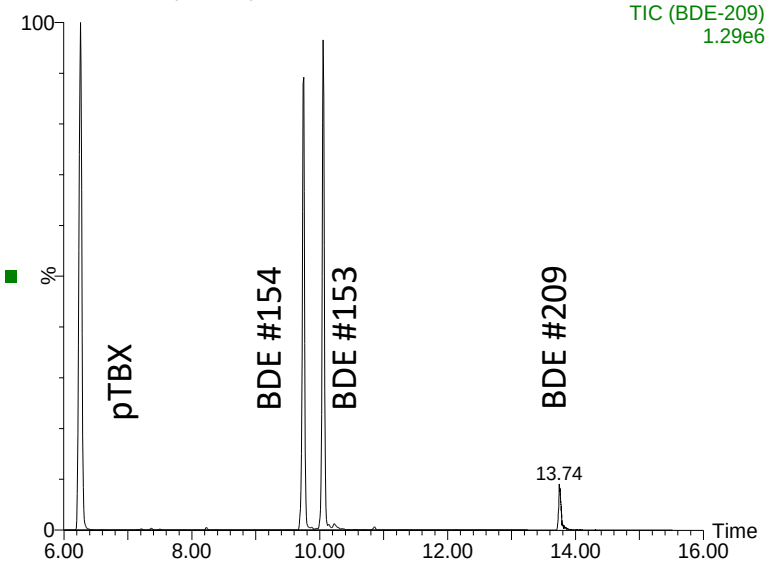




Toluene, 1 µL injected
expansion 114 µL
@ 275 °C/40 psi

APCI
He (20 °C.min^{-1}, 50 cm.s^{-1})

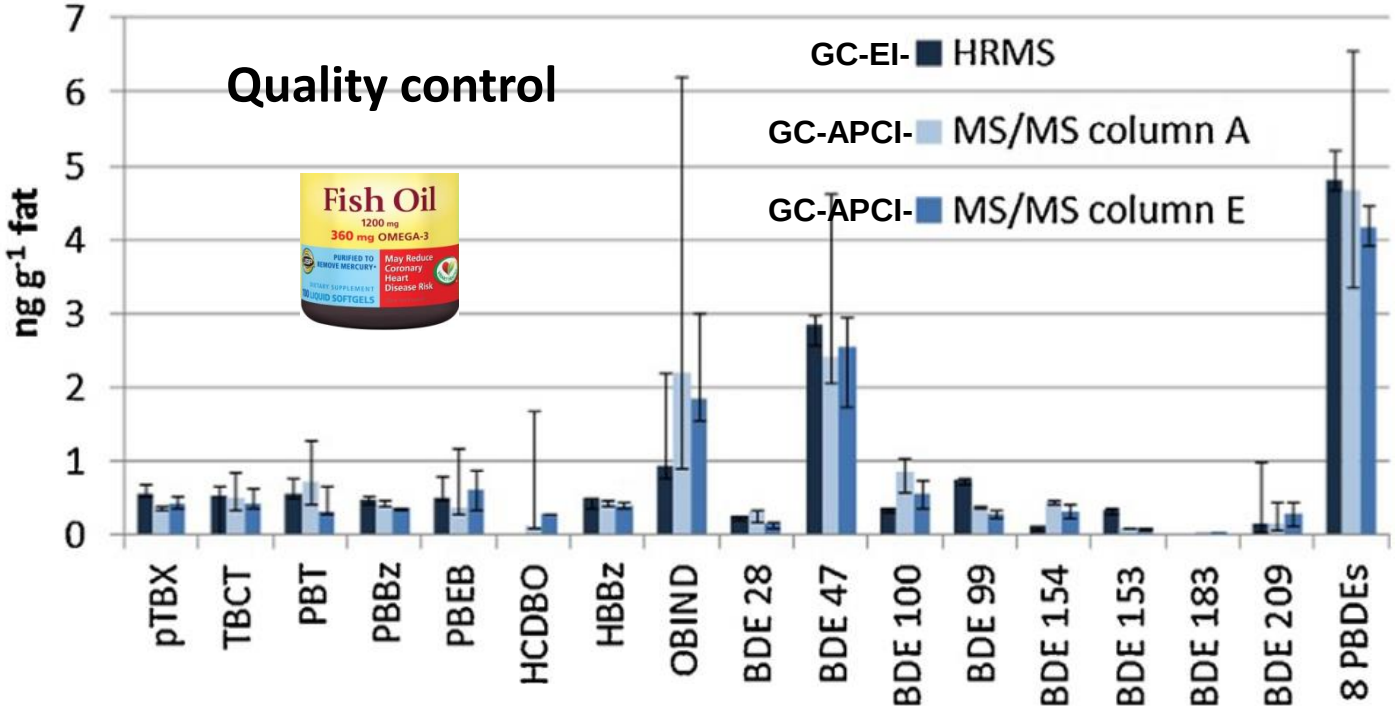




Second illustration : Brominated Flame Retardant

Today

GC-MS/MS vs GC-HRMS

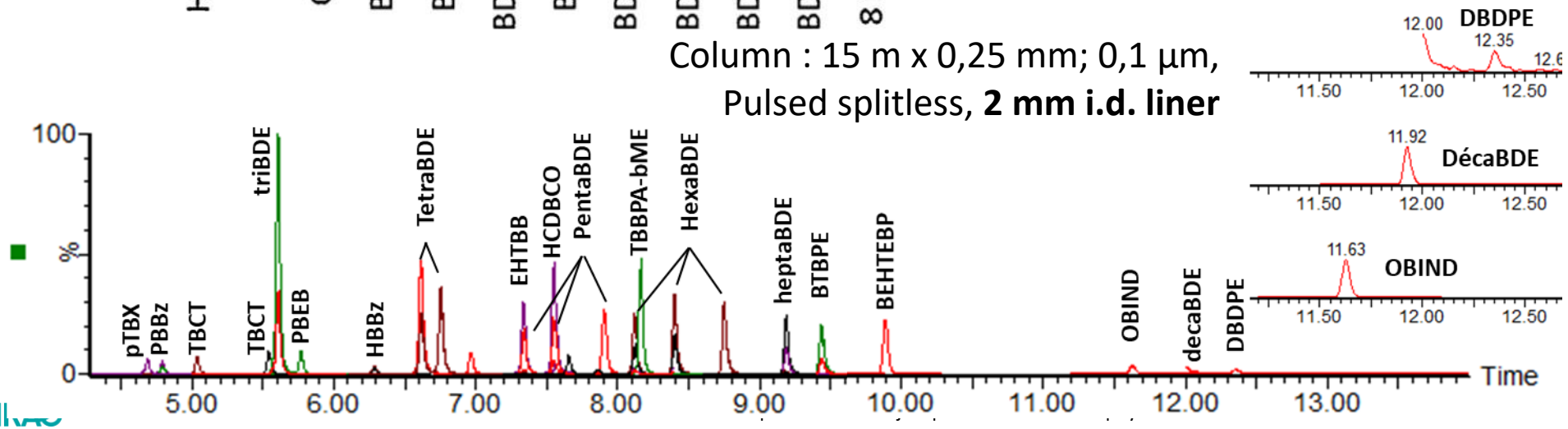


Column A : 15 m x 0,25 mm; 0,1 µm,
Pulsed splitless, 4 mm i.d. liner

Column E : **2,5 m** x 0.1 mm; 0,1 µm,
Pulsed split (1:5), 2 mm i.d. liner

E. Bichon et al. / J. Chromatogr. A 1459 (2016) 120–128

Column : 15 m x 0,25 mm; 0,1 µm,
Pulsed splitless, **2 mm i.d. liner**



Third illustration : Perfluoroalkyl substances (PFAS)

L 316/38 EN Official Journal of the European Union 8.12.2022

COMMISSION REGULATION (EU) 2022/2388

of 7 December 2022

amending Regulation (EC) No 1881/2006 as regards maximum levels of perfluoroalkyl substances in certain foodstuffs

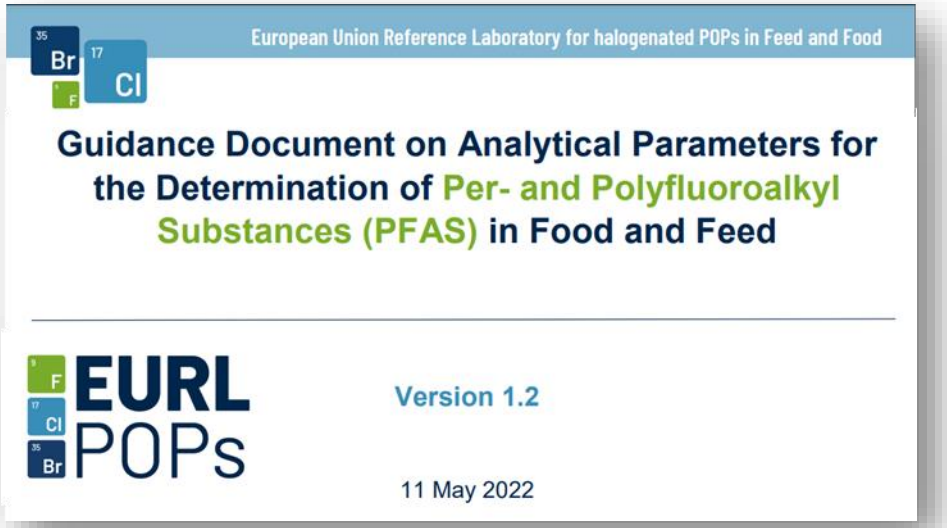
Perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS) [...] are, or were used in numerous commercial and industrial applications. Their **widespread use**, together with **their persistency** in the environment has resulted in a **widespread environmental contamination**. Contamination of food with these substances is mainly the result of bioaccumulation in aquatic and terrestrial food chains and **the diet is the major source of PFASs exposure**. [...]

On 9 July 2020, the European Food Safety Authority […] established a group tolerable weekly intake **(TWI) of 4,4 ng/kg body weight** per week for **the sum of PFOS, PFOA, PFNA and PFHxS**, which is also protective against the other effects of those substances […]

Maximum levels in food for those substances should therefore be set to ensure a high level of human health protection.

Third illustration : Perfluoroalkyl substances (PFAS)

LC-(ESI-)-MS

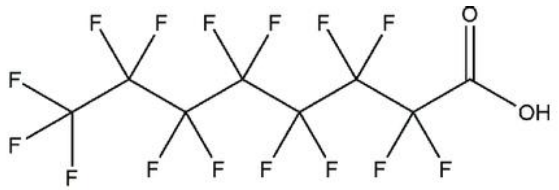

European Union Reference Laboratory for halogenated POPs in Feed and Food

Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed

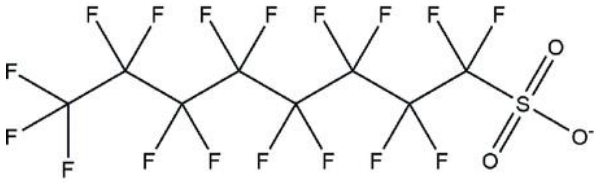
EURL POPs

Version 1.2

11 May 2022



Perfluorooctanoic acid (PFOA)



Perfluorooctane sulfonate (PFOS)

1.10. Instrumentation

(Ultra) High performance liquid chromatography ((U)HPLC) coupled to low resolution or high resolution mass spectrometry (LRMS or HRMS) is recommended for analysis of PFCA and PFSA.

1.10.1. LC-System

The LC system must provide consistent sample injection volumes and be capable of performing binary linear gradients at a constant flow rate. PFAS may build up in PTFE transfer lines when the system is idle for more than one day. To prevent long delays in purging high levels of PFAS from the LC solvent lines, it may be useful to replace PTFE tubing with polyether ether ketone (PEEK) tubing and the PTFE solvent frits with stainless steel frits. In addition a delay column can be installed before the injection valve to reduce the co-elution of PFAS originating from sources prior to the sample loop (e.g. mobile phase, fittings, tubes). Thorough rinse of the LC-needle can reduce the co-elution of PFAS accumulated in sample loop and valves.

1.10.2. Analytical column

The laboratory may select the LC column. Based on previous experience a C18 liquid chromatography column packed with solid phase particles is recommended (see Annex).

1.10.3. Mass spectrometer

The mass spectrometer must be capable of electrospray ionization in the negative ion mode. The system must be capable of producing specific product ions for the method analytes within specified retention time segments.

MANDATORY

Third illustration : Perfluoroalkyl substances (PFAS)

LC-(ESI-)-MS

26.8.2022 EN Official Journal of the European Union L 221/105

RECOMMENDATIONS

COMMISSION RECOMMENDATION (EU) 2022/1431

of 24 August 2022

on the monitoring of perfluoroalkyl substances in food

6. The analyses should be carried out in accordance with Article 34 of Regulation (EU) 2017/625 of the European Parliament and the Council ([6]) using a method of analysis that has been proven to generate reliable results. The limits of quantification of the analytical methods should be below or at:

(a) 0,002 µg/kg for PFOS, 0,001 µg/kg for PFOA, 0,001 µg/kg for PFNA and 0,004 µg/kg for PFHxS in fruits, vegetables, starchy roots and tubers and food for infants and young children;

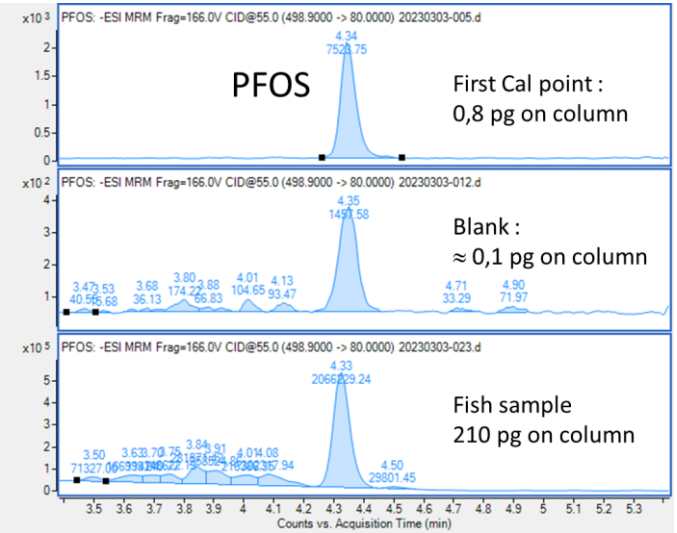
(b) 0,010 µg/kg for PFOS, 0,010 µg/kg for PFOA, 0,020 µg/kg for PFNA and 0,040 µg/kg for PFHxS in milk;

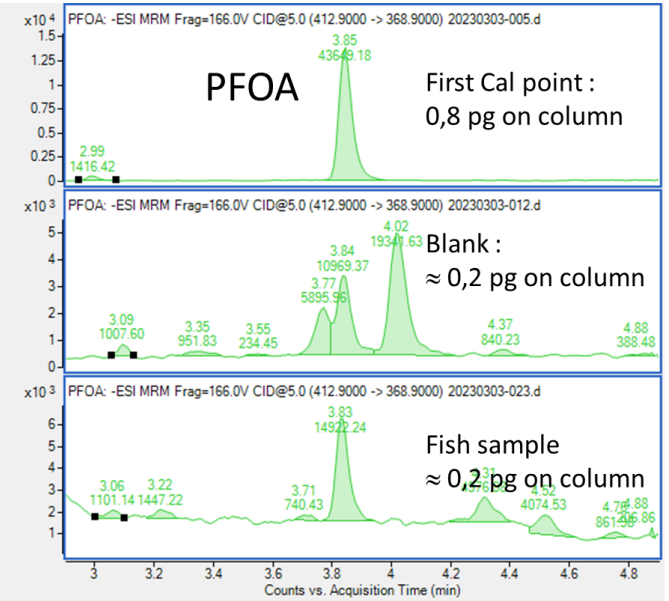
(c) 0,10 µg/kg for PFOS, PFOA, PFNA and PFHxS in fish meat and meat of terrestrial animals;

(d) 0,30 µg/kg for PFOS, PFOA, PFNA and PFHxS in eggs, crustaceans and molluscs;

(e) 0,50 µg/kg for PFOS, PFOA, PFNA and PFHxS in edible offal of terrestrial animals and in fish oil.

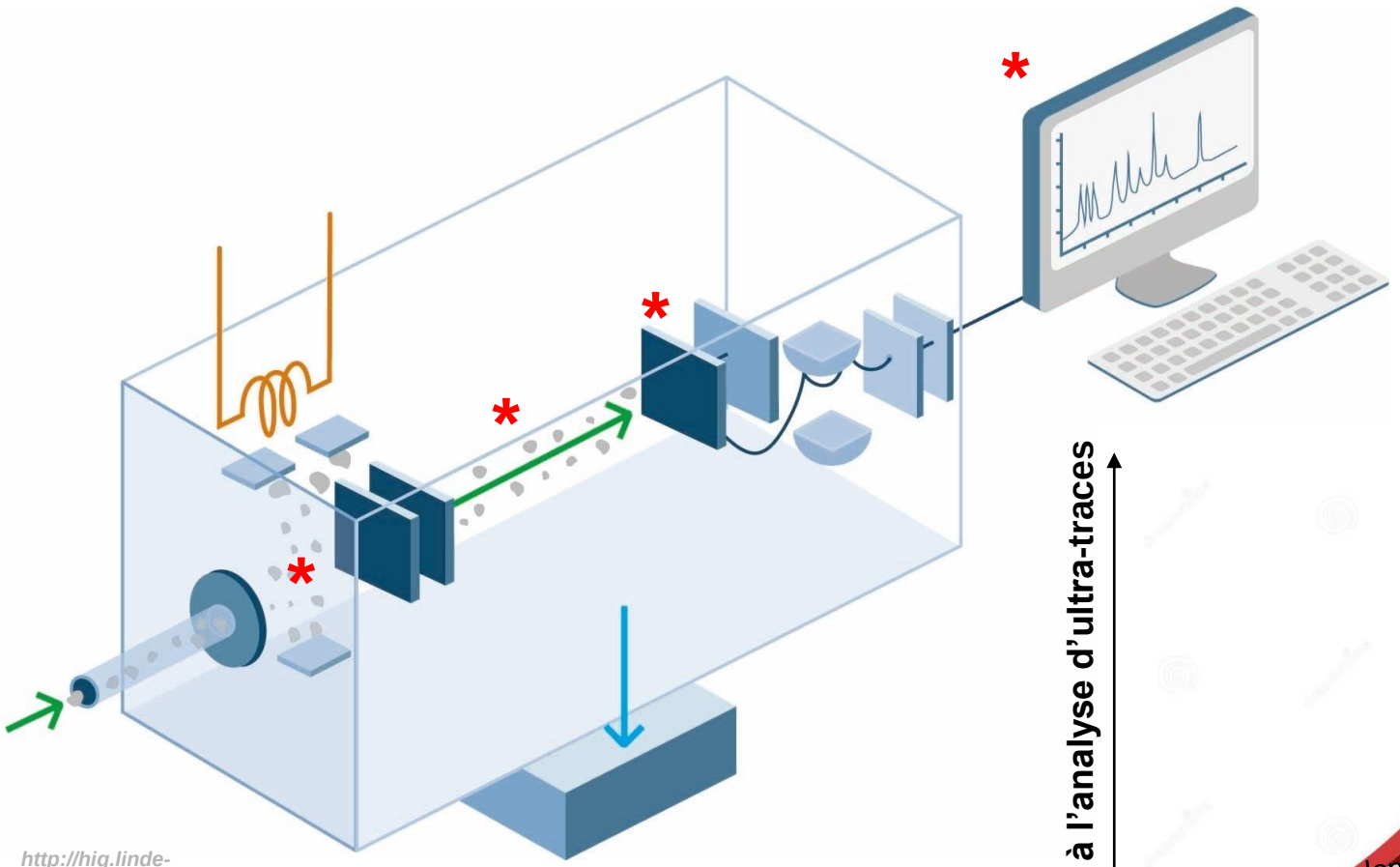
+ **indicative levels** for fruits and vegetables, starchy roots and tubers, wild fungi, milk and baby food, where investigations should be carried out when they are exceeded



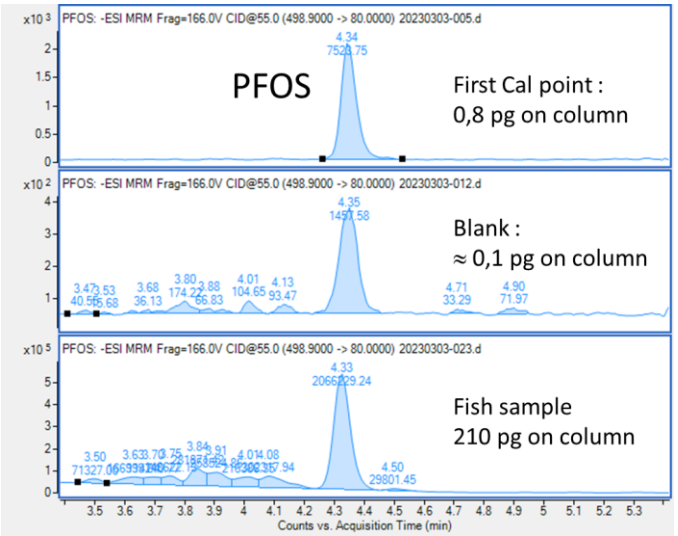


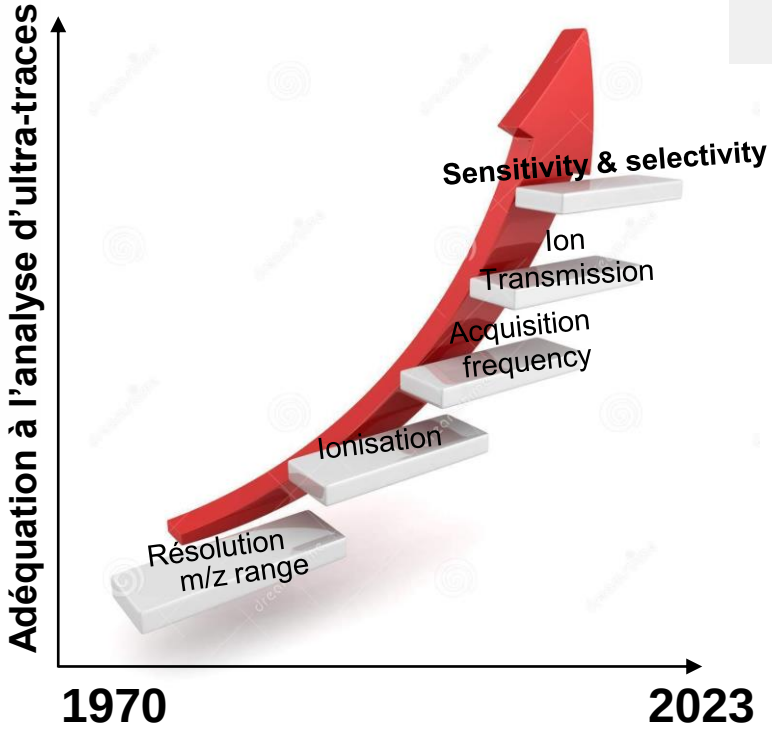
Current Trends in Food Analysis

LOQ & MS sensitivity



http://hiq.linde-gas.com/en/analytical_methods/gas_chromatography/mass_spectrometry.html

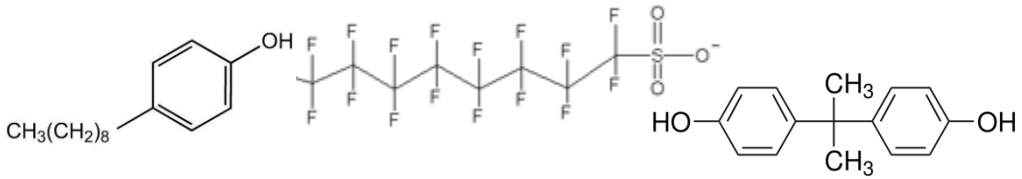


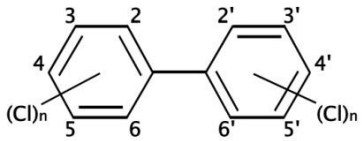


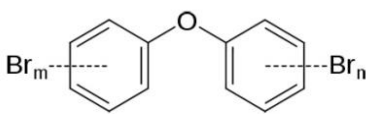
Current Trends in Food Analysis

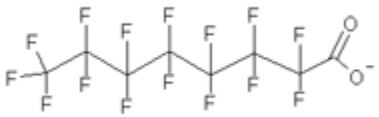
LOQ & background contamination

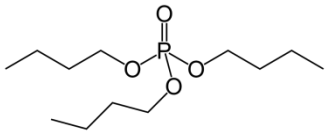
1 compound = several sources
1 source = several compounds

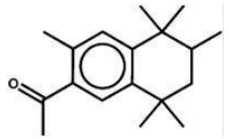






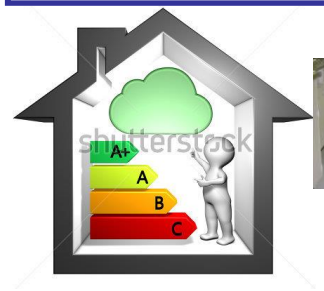






Food Safety | **Biomonitoring**

1998 2000 2005 2010 2015 2020



J. Ferrairio et al. / Chemosphere, Vol. 34, No. 11, pp. 2451-2465, 1997
O. Päpke et al./Talanta 63(2004)1203–1211
R. Loos et al. / Trends in Analytical Chemistry, Vol. 27, No. 1, 2008

N.Salgueiro-Gonzalez et al. / Talanta101(2012) 413–419
H. Gallart-Ayala et al. / Trends in Analytical Chemistry, Vol. 42, 2013
Brandsma et al. / Trends in Analytical Chemistry, Vol. 43 2013

Limit of quantification

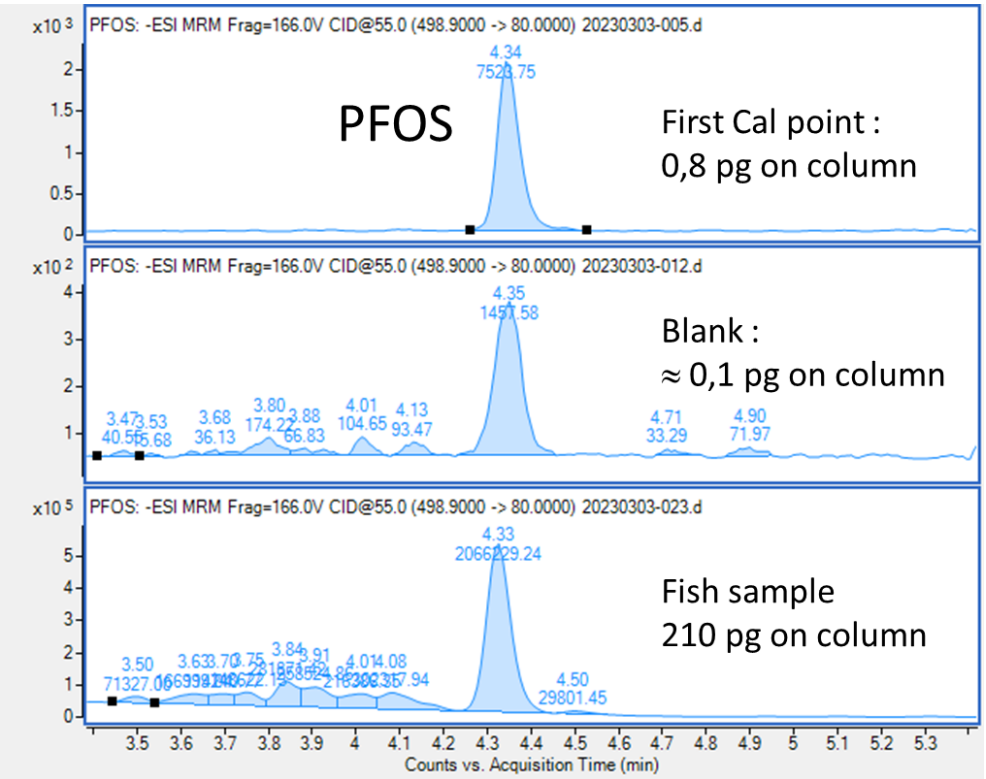
- no longer only associated with the sensitivity of the instrument
- dependent on blank contamination (indoor air + lab material)
- made visible by the increased sensitivity of the systems
- image of the analytical process/environment

In Food Analysis

- Complex matrices
- multiple sample preparation steps
- low expected concentrations (< ng/g)

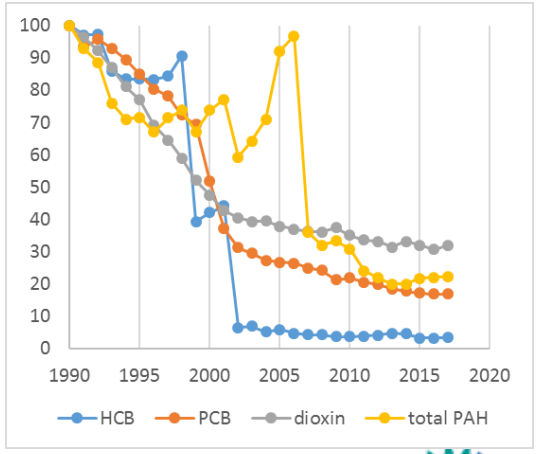
Key to decrease the background contamination

Solvent	Quality improvement	Time consuming/cost
	Provider change	
	Distillation use (additional step)	Easy to carry out
Material (tubes, connections, gloves…)		± Expensive
Material Change		Often efficient
Procedure		
Glass & bench material cleaning		Habits change
Instrumental maintenance		
Indoor environment		
Furniture change		Often expensive
Clean room implementation		But necessary !

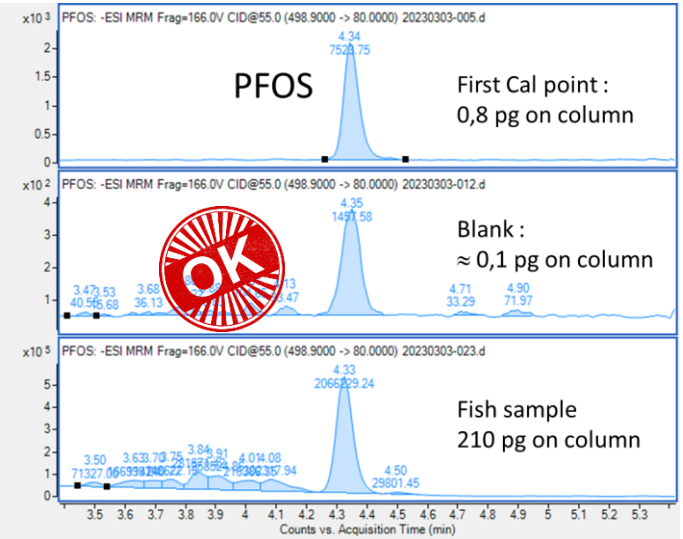


Current Trends in Food Analysis

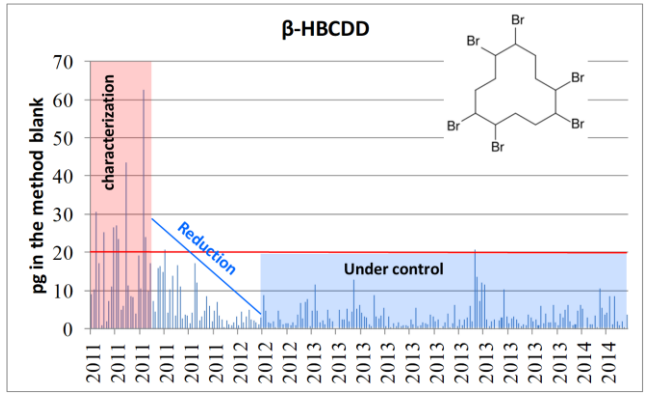
POPs Emission



European Environment Agency



lab contamination must decrease and be under control



Sample size miniaturization

It is also an ethic consideration

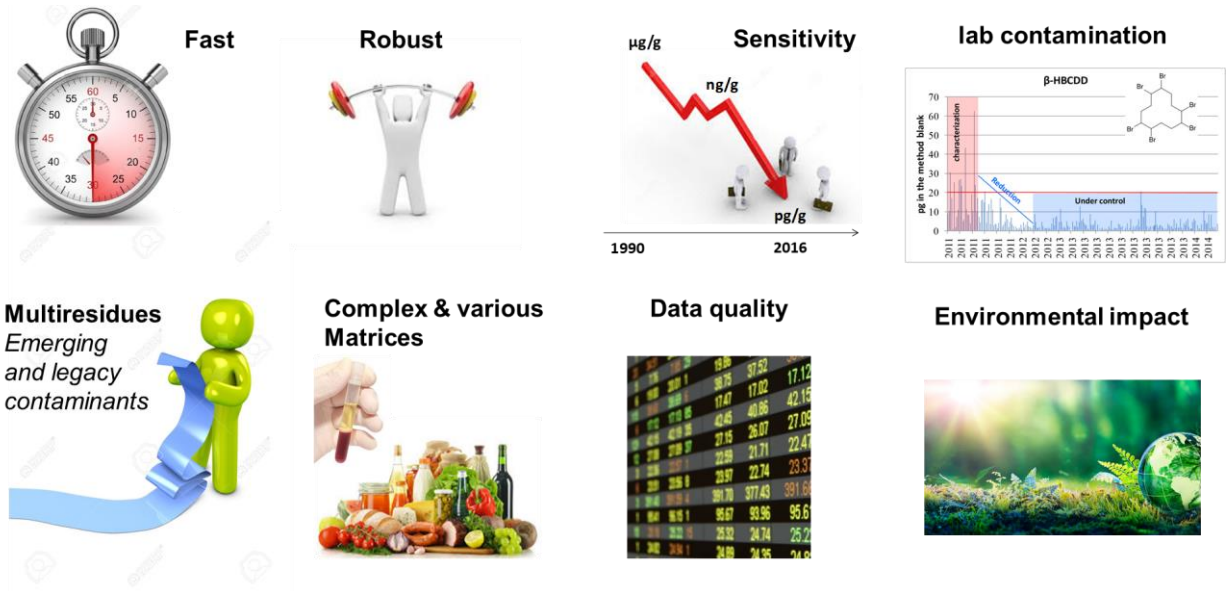
consumables, phases, reagents and solvents miniaturization

To decrease our Environmental Impact

Conclusion and Perspectives

Chromatography coupled to mass spectrometry
is the standard instrument configuration

Lab conception
(automation, clean rooms, …)



An approach to be invented and co-constructed to enable the resilience of analytical laboratories

Always wider multiresidue methods
Following novel and legacy chemical hazard of concern
(i.e. chloroparaffins, chloronaphtalens, additionnal flame retardants, pesticides, …)

Through **suspect and non-target screening** thanks to HRMS

Acknowledgement

And you for your attention !

https://www.laberca.org
https://www.saraf-educ.org