MassTwin Workshop
Mass spectrometry in support of the environment, food, and health interaction and disease
Antwerp, Belgium, 18th-20th April 2018

MASS SPECTROMETRY FOR CHARACTERIZING HUMAN INTERNAL CHEMICAL EXPOSURE: STRENGTHS AND CHALLENGES

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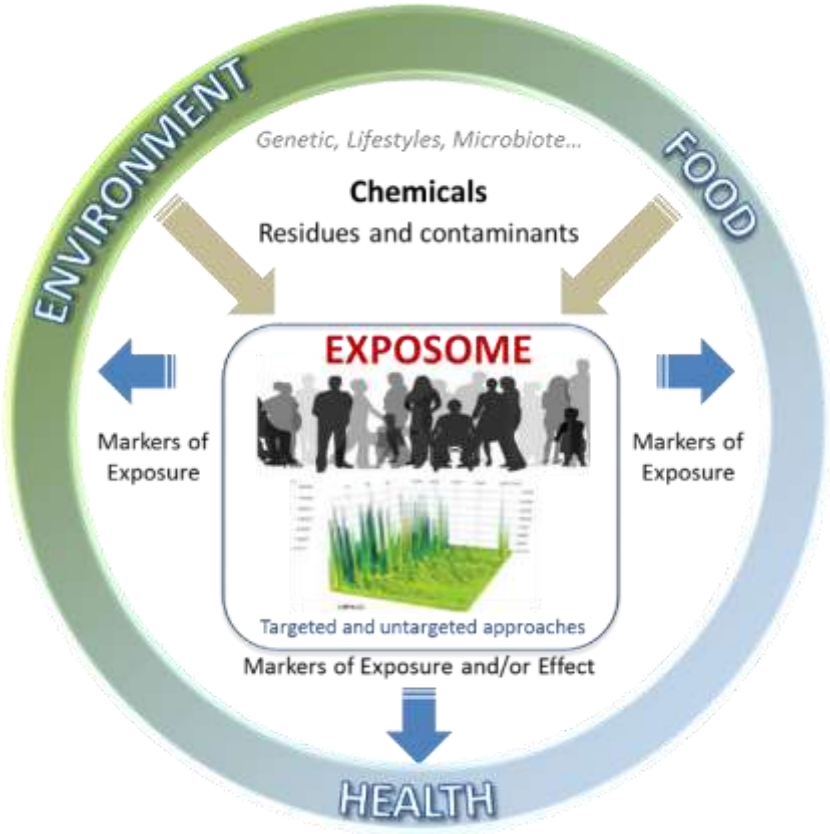


- Public laboratory (50 people)
- Research (UMR 1329 Oniris-INRA)
- Method development & innovation
- Support to public authorities (NRL)
- MS based analytical platform
- Training and pedagogical engineering

SARAF
School for Advanced Residue Analysis in Food

Outline

- Introduction
- How measuring ?
- Where measuring ?
- When measuring ?
- What measuring ?
- Conclusion

Temporal increase of human exposure to environmental chemicals

Coincidence ?
Association ?
Causality ?

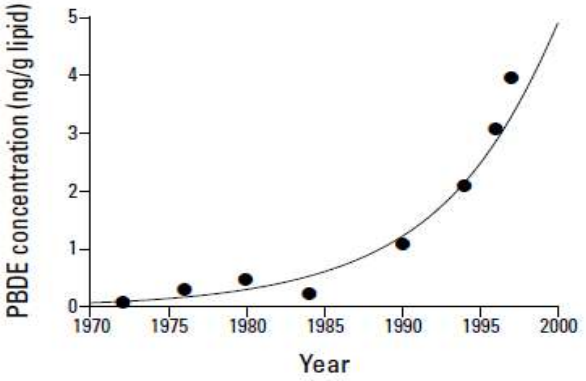
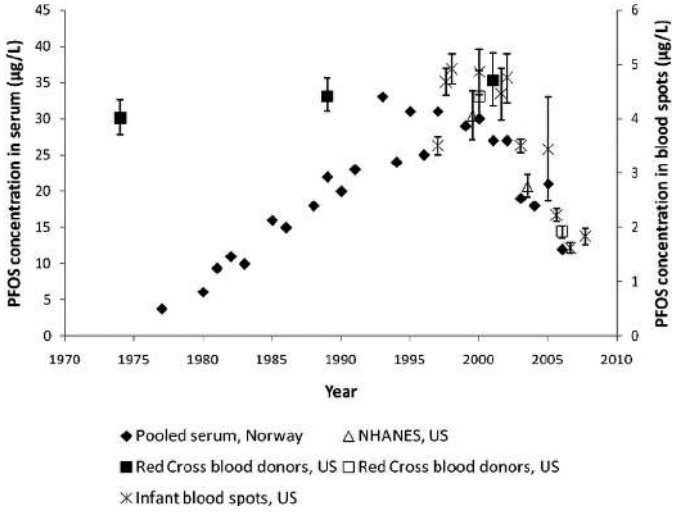


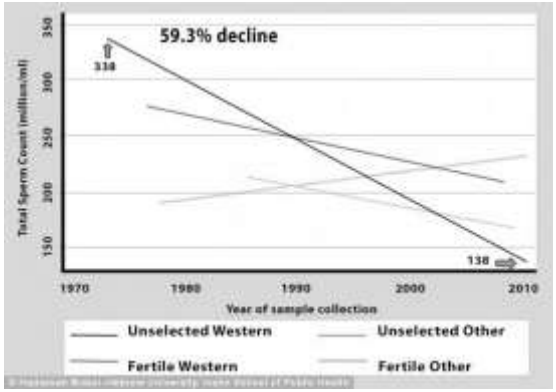
Figure 5. Time trend of the sum concentrations of 8 PBDE congeners in pooled mother's milk samples from Swedish mothers living in the Stockholm region. Data from Norén and Meironyté (*51*).



Temporal increase of some human health outcomes

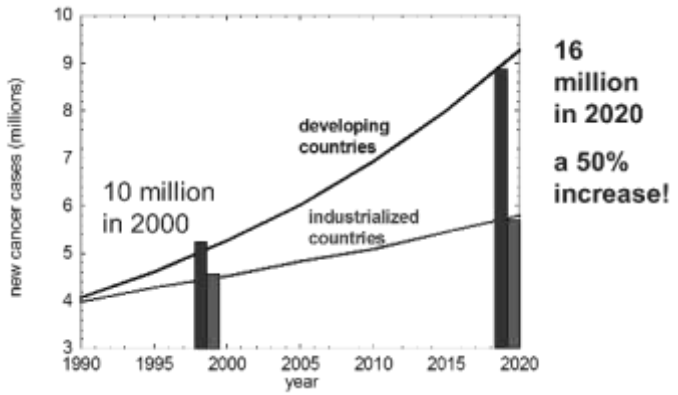
Reproduction & development

(↘ sperm quality & age of puberty)



Cancer

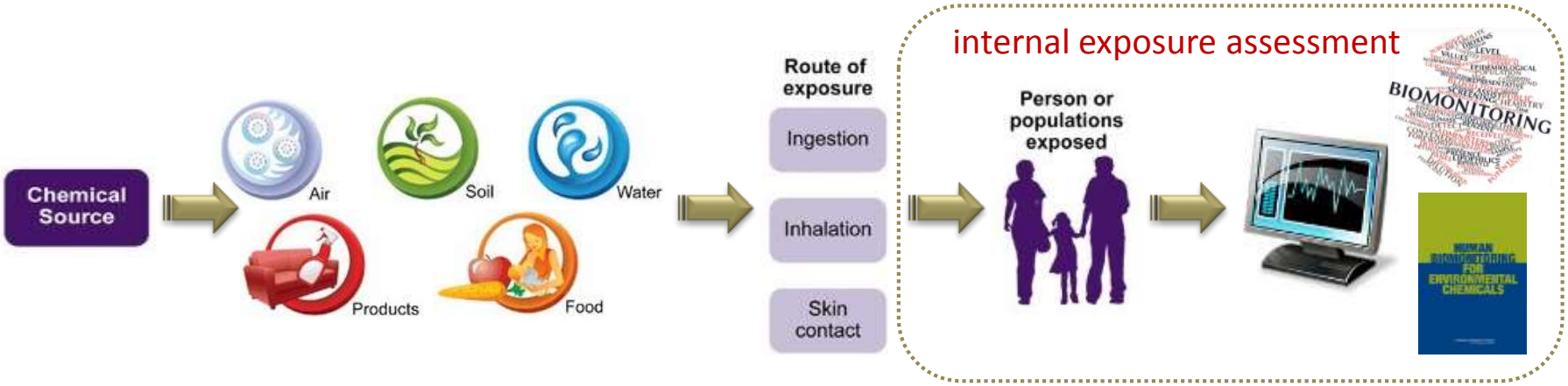
(↗ breast & prostate cancers incidence)



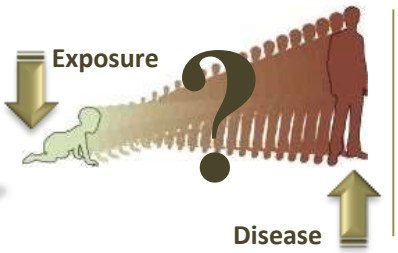
Metabolism & immune system

(obesity, metabolic syndrome, allergy…)

Need for human chemical exposure data



Now… how, where, and when measuring what ?

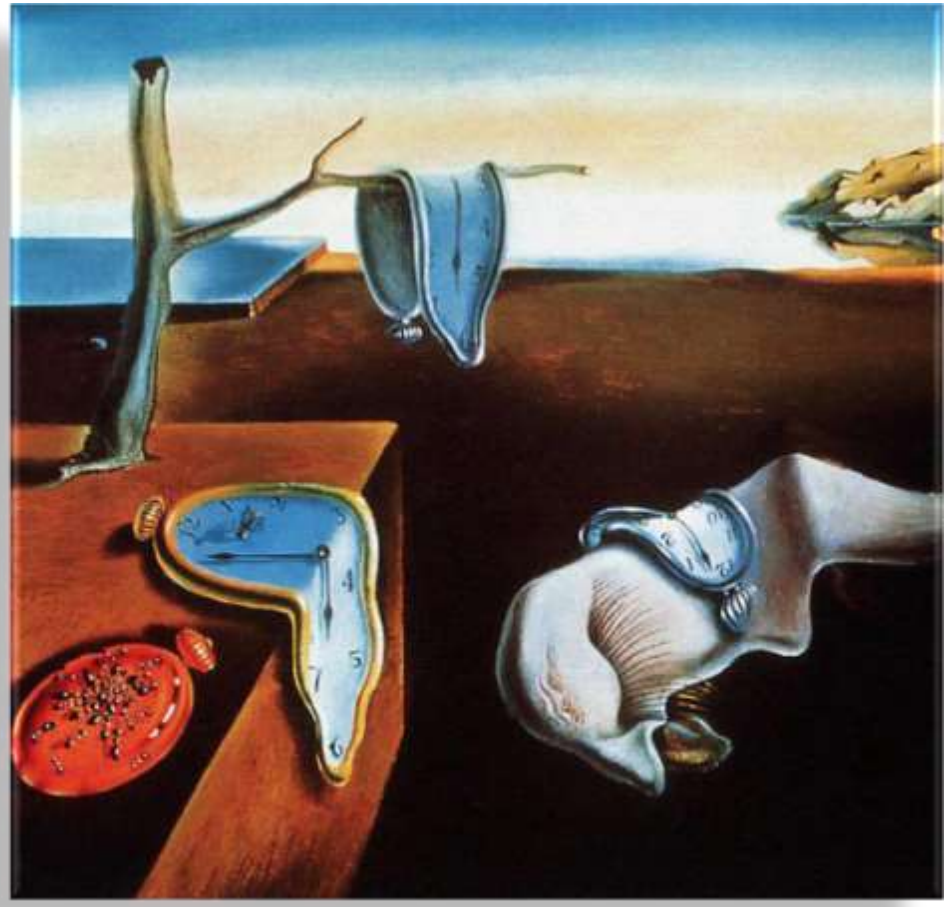


Parent compounds?
Direct metabolites?
Indirect biomarkers?

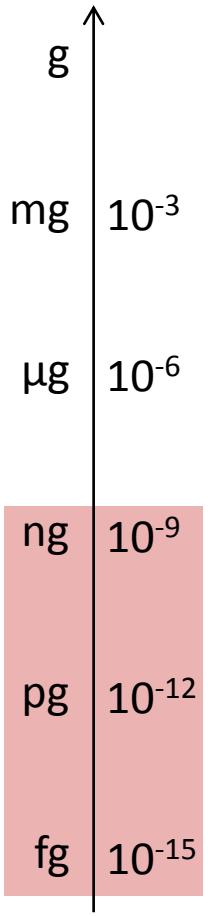
Dioxins Phytosanitary
PCBs PAHs Drugs
BFRs PFAS Bisphenols
Metals Phtalates …

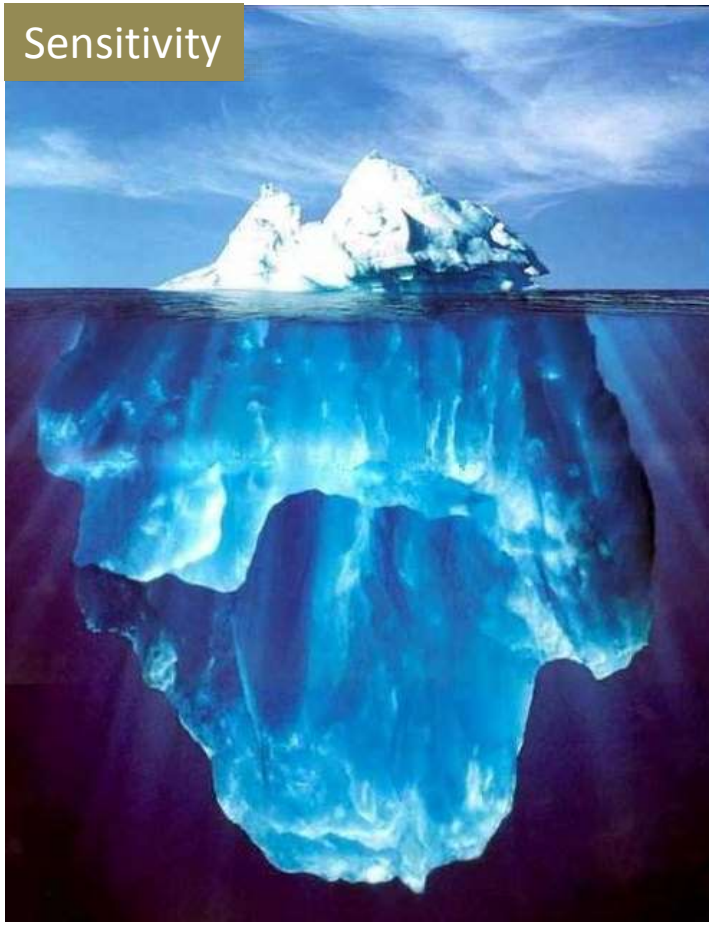
Outline

- Introduction
- How measuring ?
- Where measuring ?
- When measuring ?
- What measuring ?
- Conclusion



- Extremely low concentration levels





- Very complex biological matrices

- Need for unambiguous identification and precise/accurate quantification

UPLC/APGC-MS/MS (x 2)

GC-MS (x 3)

HPLC-UV/DAD (x 2)

LC-HRMS^{n} (x 3)

GC-MS/MS (x 2)

LC-MS/MS (x 2)

UPLC/SFC-IM-HRMS (x 1)

GC-HRMS (x 3)

GC-C-IRMS (x 3)

Dioxins/PCB/PBDE

Serum

Extraction
SPE C18

Purification 1
Florisil column

Purification 2
Carbon/Celite

Purification 2
Carbon/Celite

Purification 2
Liquid/liquid

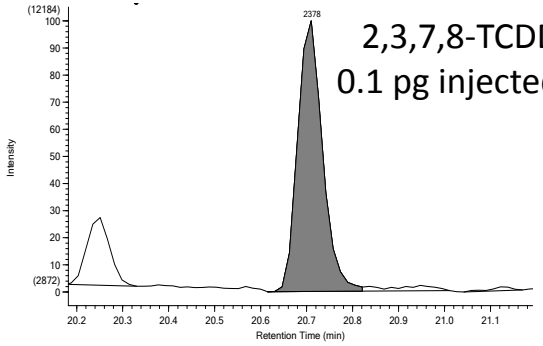
PCB, PBB, PBDE
n=12 n=3 n=8

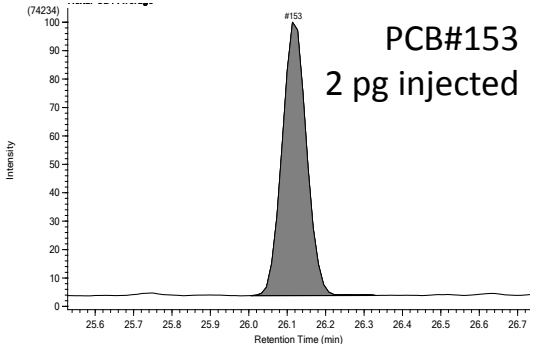
PCDD/F
n=17

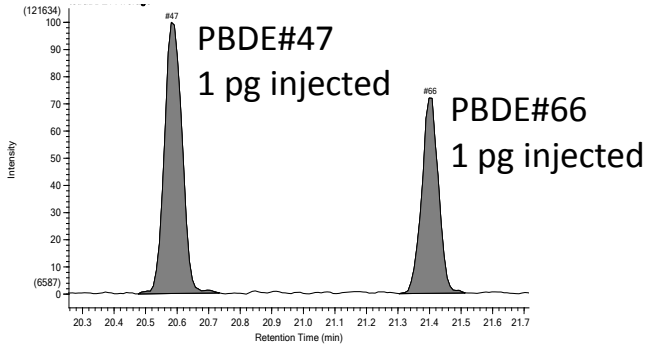
HBCD
n=3

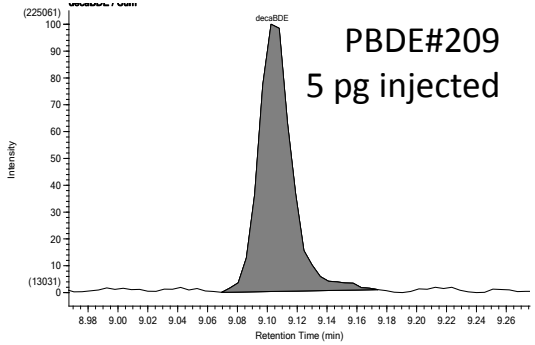
GC-HRMS

LC-MS/MS

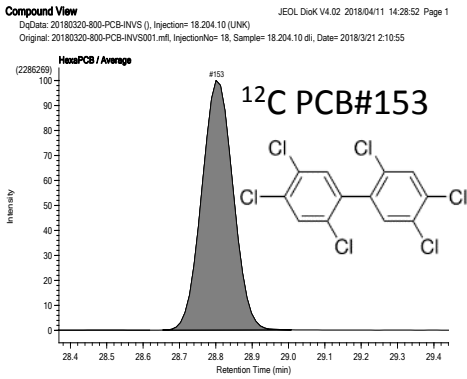




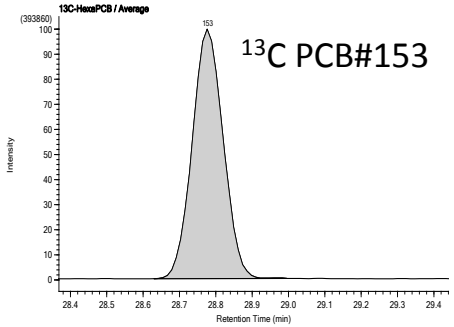




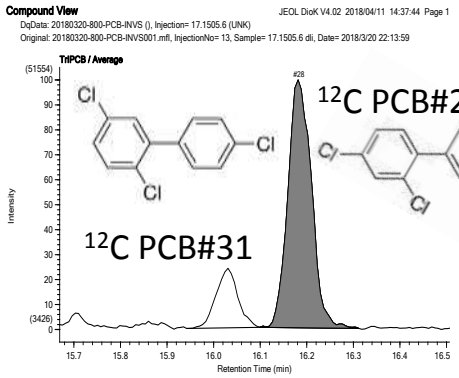
Dioxins/PCB/PBDE



^{12}C PCB#153

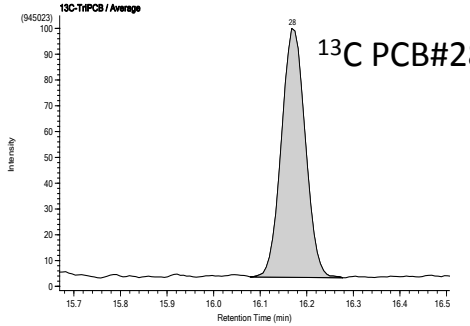


^{13}C PCB#153

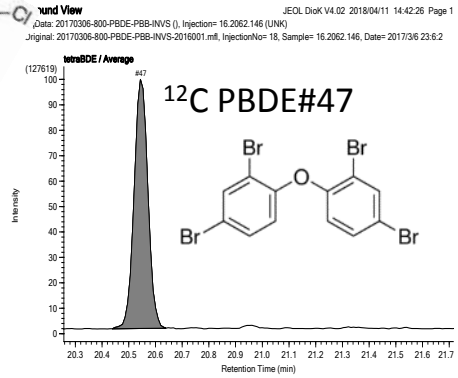


^{12}C PCB#28

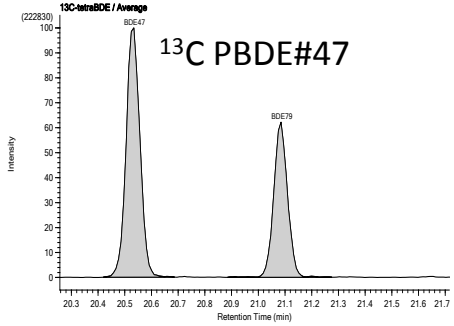
^{12}C PCB#31



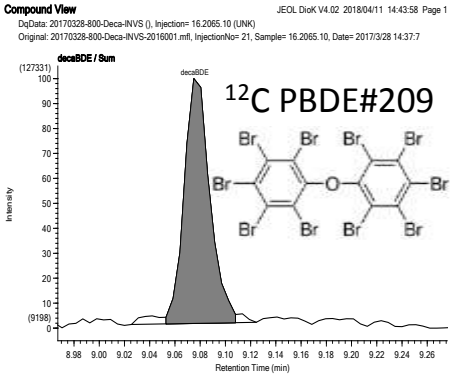
^{13}C PCB#28



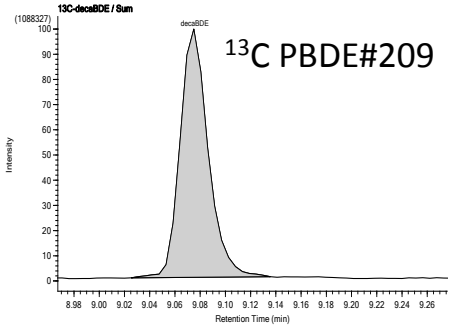
^{12}C PBDE#47



^{13}C PBDE#47



^{12}C PBDE#209



^{13}C PBDE#209

Organochlorine pesticides

Adipose tissue

Extraction
ASE/PLE

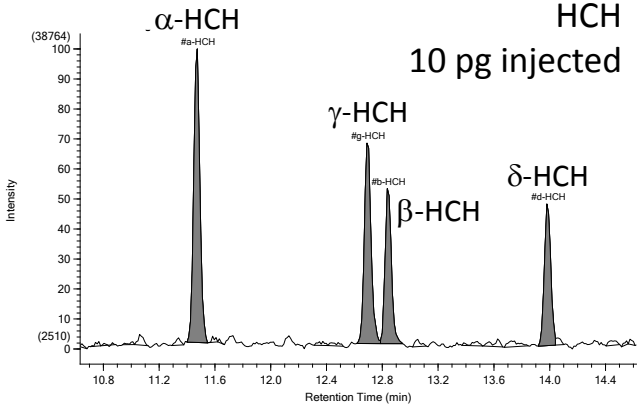
Purification
GPC

Other OCs
n=28

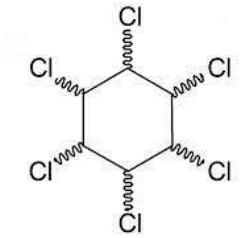
Chlordecone

GC-HRMS

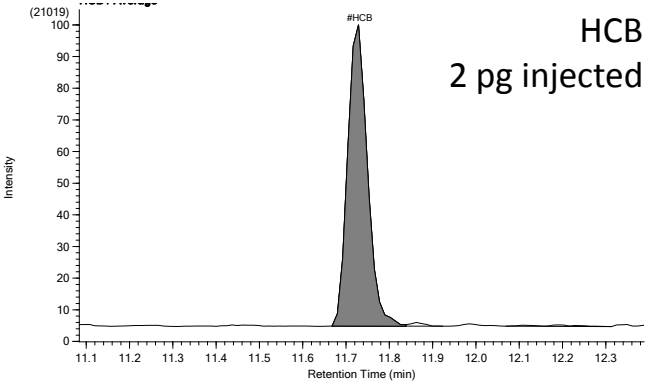
LC-MS/MS



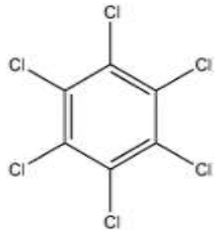
HCH
10 pg injected



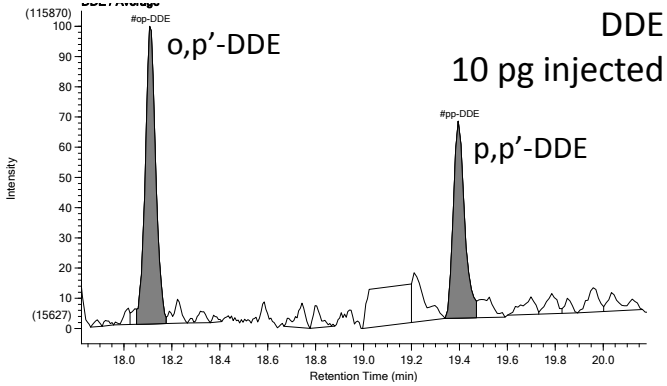
Hexachlorocyclohexane



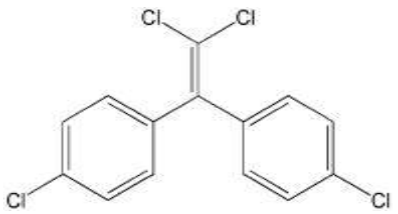
HCB
2 pg injected



Hexachlorobenzene

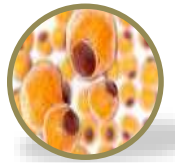


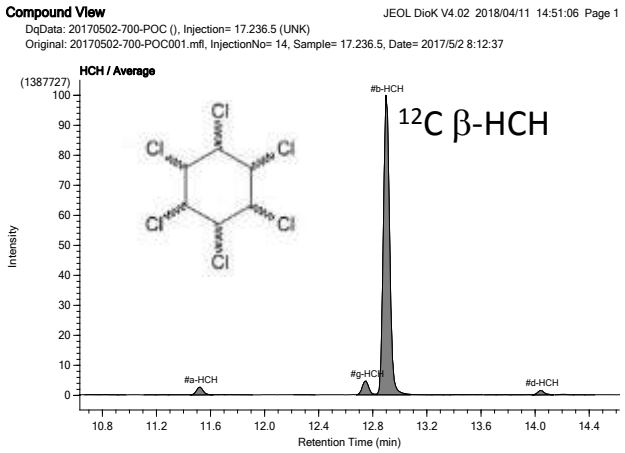
DDE
10 pg injected

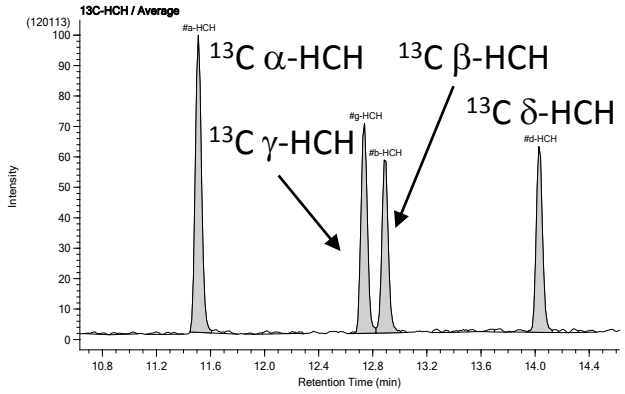


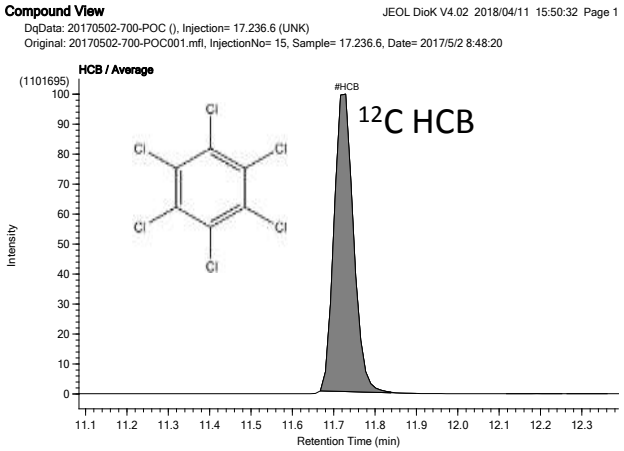
Dichlorodiphenyl-
dichloroethylene

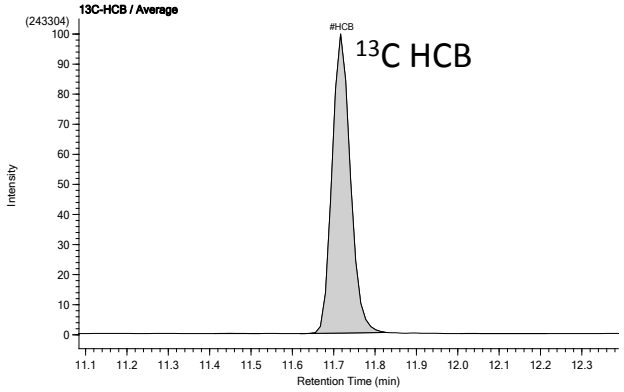
Organochlorine pesticides

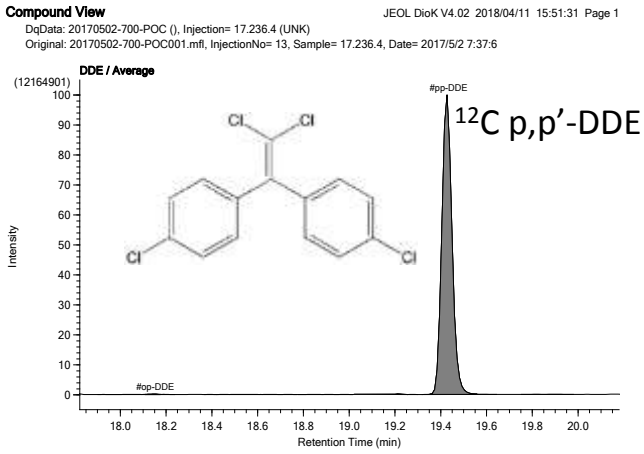


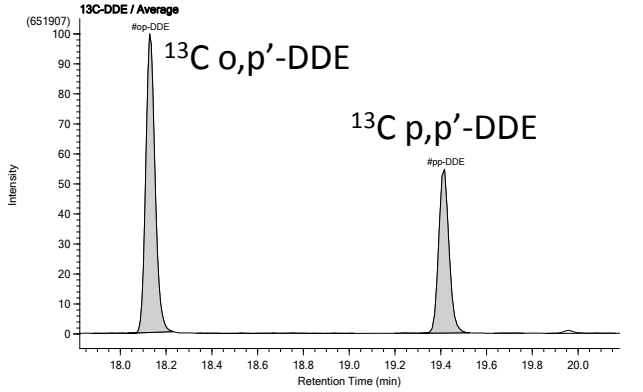






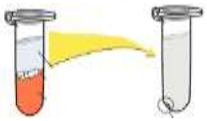






Perfluorinated alkylated substances (PFAS)

Serum



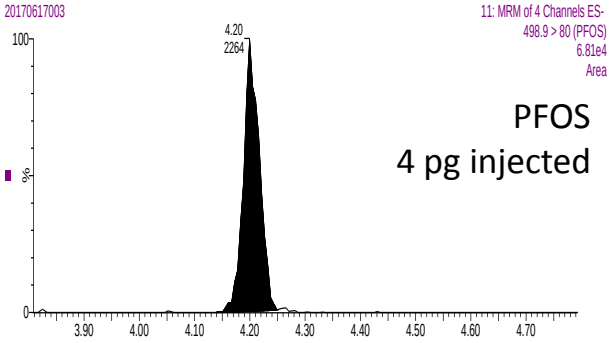
Extraction
Liquid/liquid

Purification 1
SPE Oasis HLB

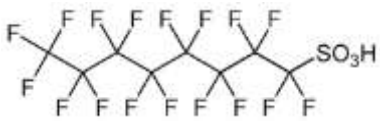
Purification 2
SPE EnviCarb

PFAS
n=16

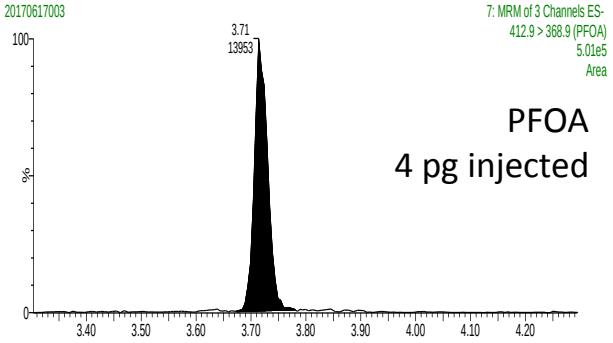
LC-MS/MS



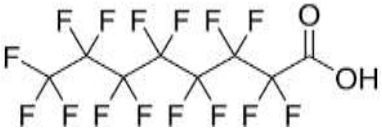
PFOS
4 pg injected



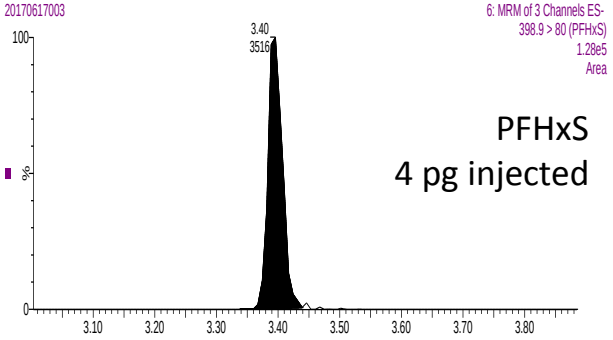
Perfluorooctanesulfonic acid



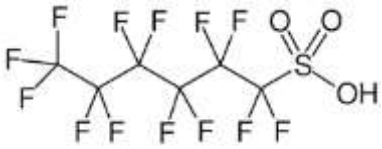
PFOA
4 pg injected



Perfluorooctanoic Acid

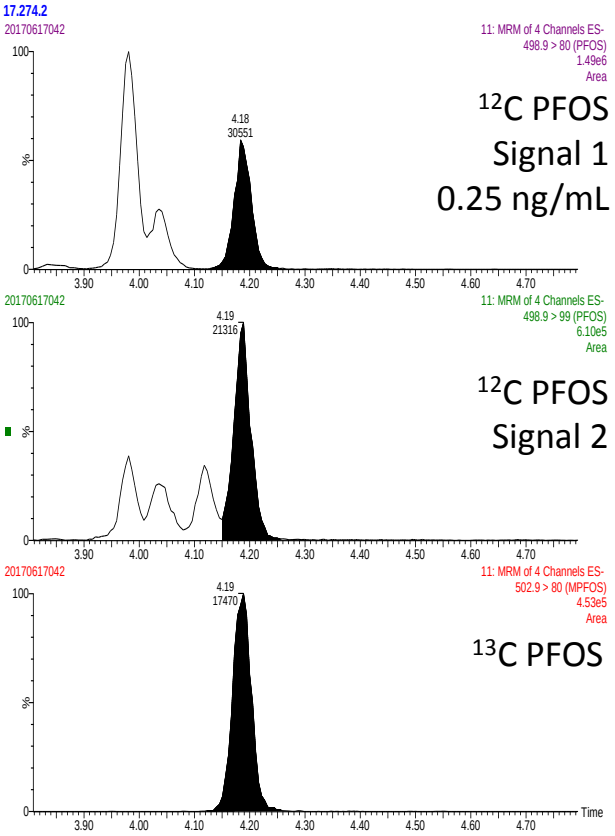


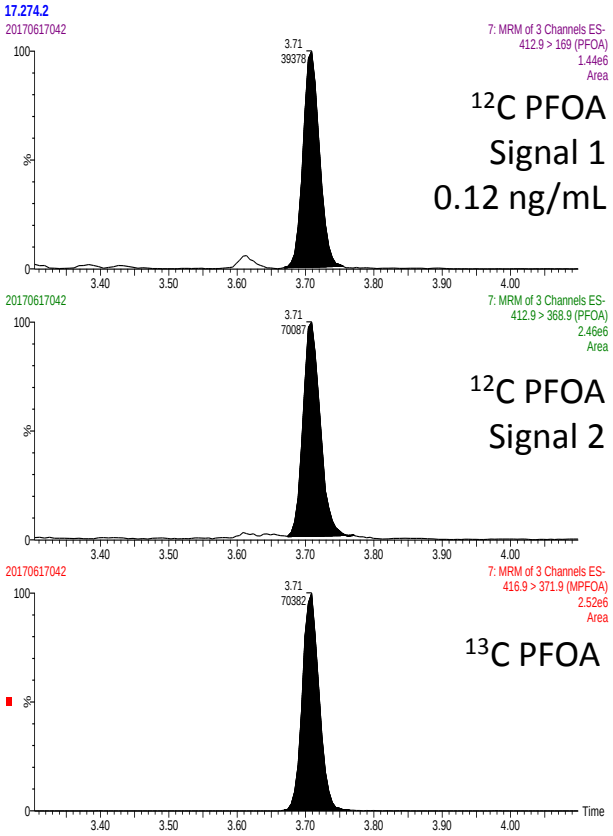
PFHxS
4 pg injected

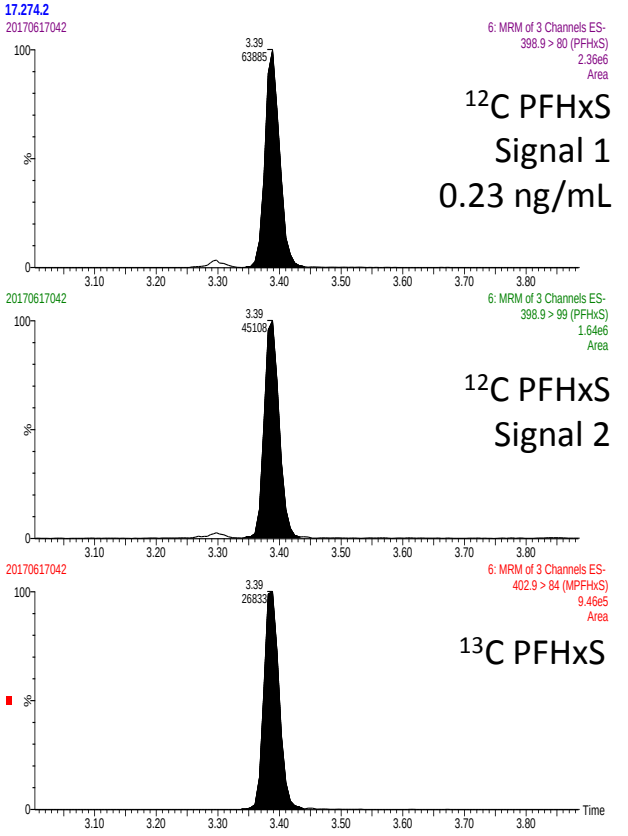


Perfluorohexane
sulfonate

Perfluorinated alkylated substances (PFAS)

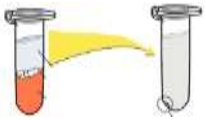






Bisphenols

Urine



Hydrolysis
Enzymatic

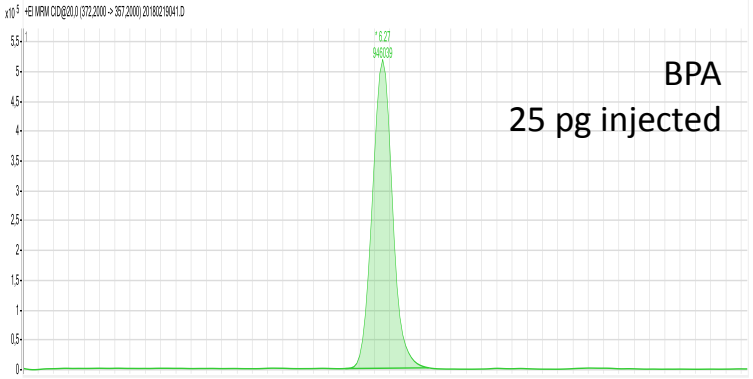
Extraction
SPE HR-X

Purification
MIP

Bisphenols

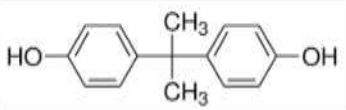
n=18

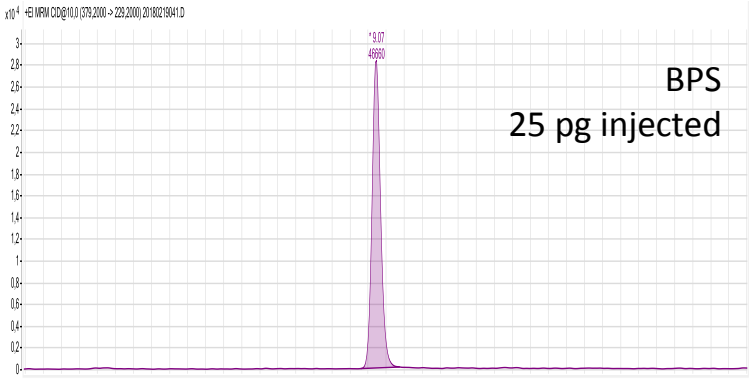
GC-MS/MS



BPA
25 pg injected

Bisphenol A
(BPA)





BPS
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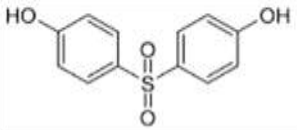
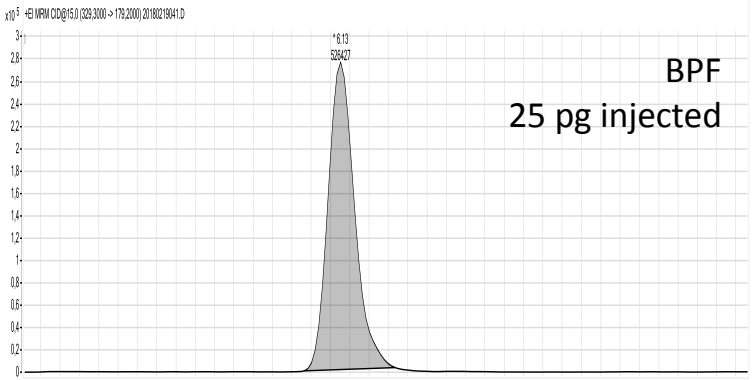
Bisphenol S
(BPS)





BPF
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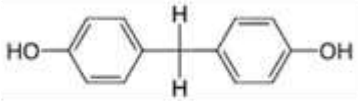
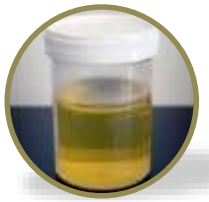
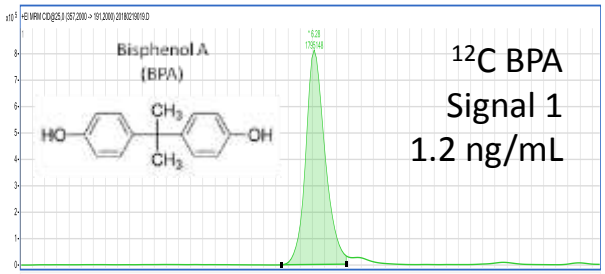
Bisphenol F
(BPF)

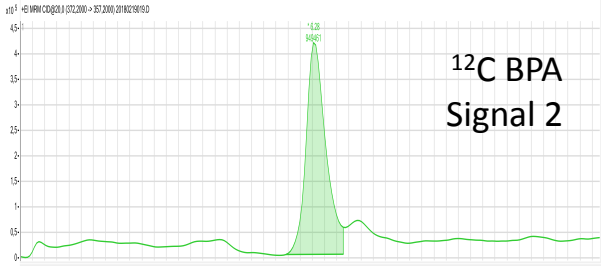


Bisphenols

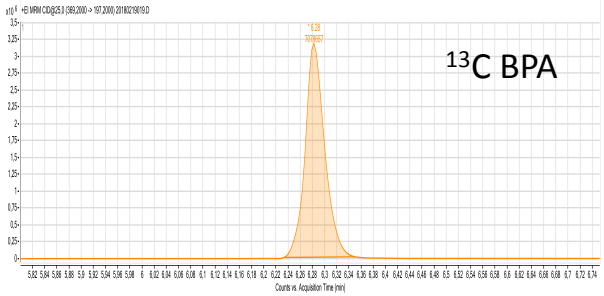




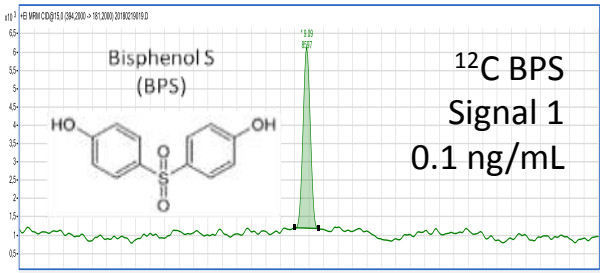
^{12}C BPA
Signal 1
1.2 ng/mL



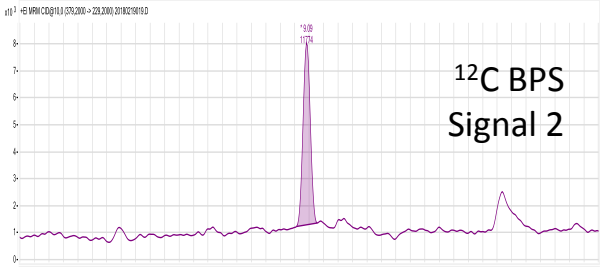
^{12}C BPA
Signal 2



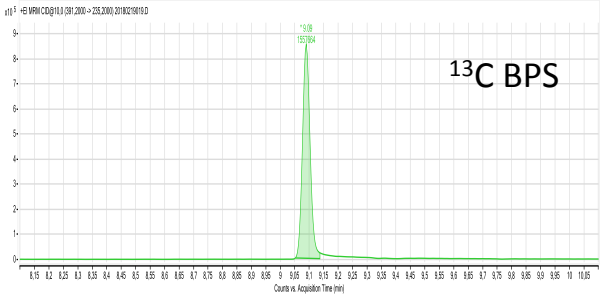
^{13}C BPA



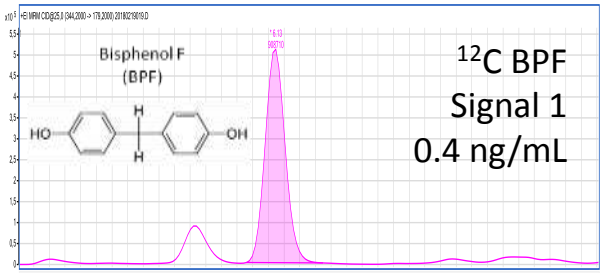
^{12}C BPS
Signal 1
0.1 ng/mL



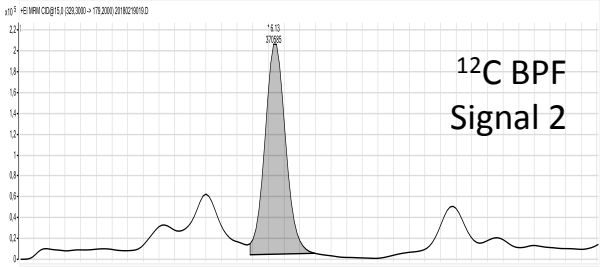
^{12}C BPS
Signal 2



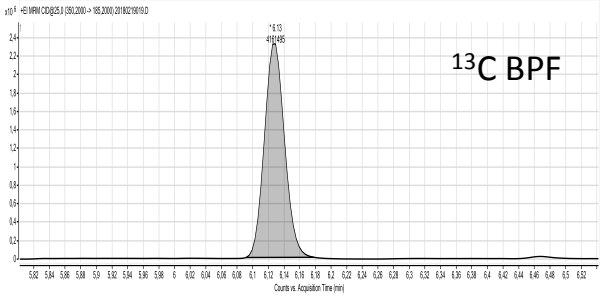
^{13}C BPS



^{12}C BPF
Signal 1
0.4 ng/mL



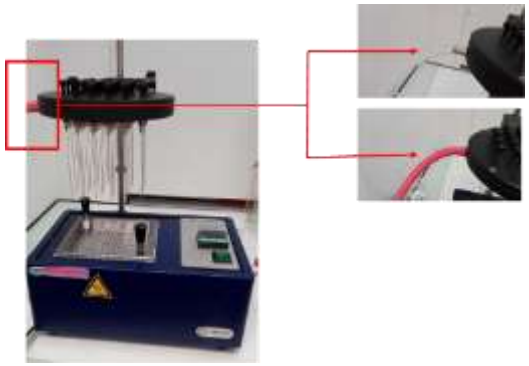
^{12}C BPF
Signal 2

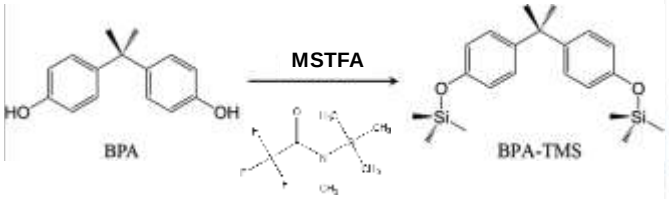


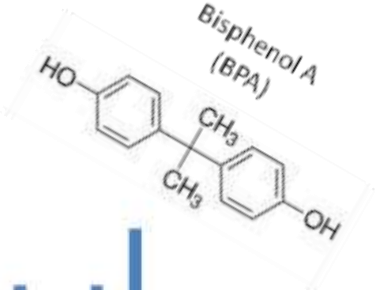
^{13}C BPF

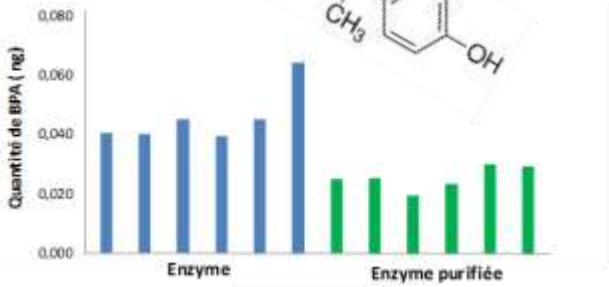
The external contamination for ubiquitary contaminants

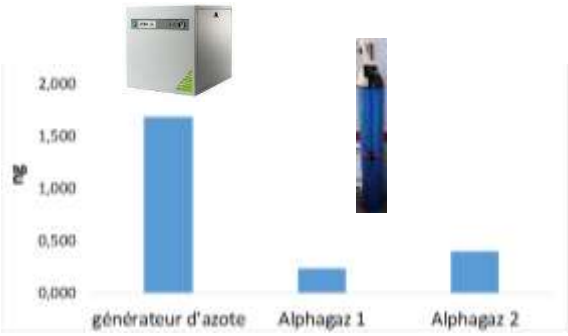
- Enzymatic hydrolysis
- SPE HR-X
- SPE MIP
- N_2 evaporation
- MSTFA derivatization
- GC-MS/MS measurement

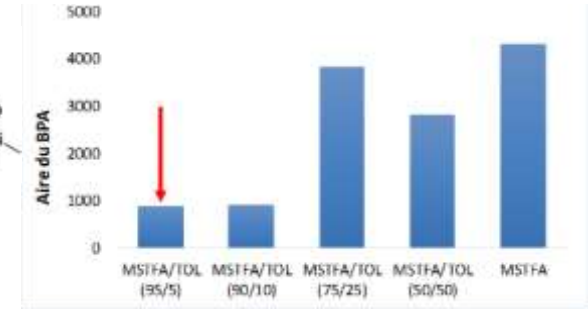












The sensitivity

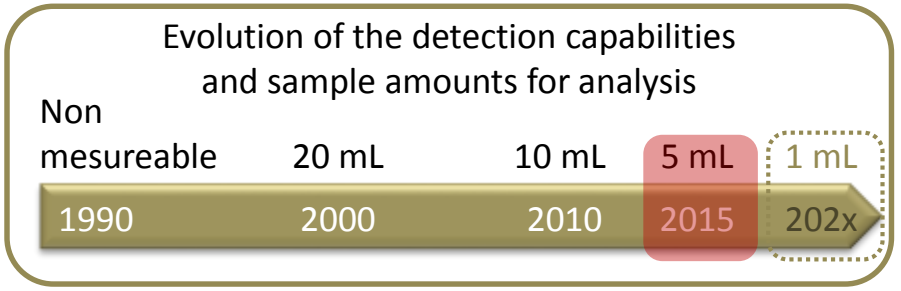
- Very restricted samples amounts typically available for human studies
- Global decrease of environmental exposure for historical substances
- Exposure levels even lower for particular sub-populations (children)
- Low dose and mixture effect studies also push toward more sensitivity
- Low fat content of serum increase again the analytical challenge for POPs

1 g adipose tissue

% fat

200 mL serum

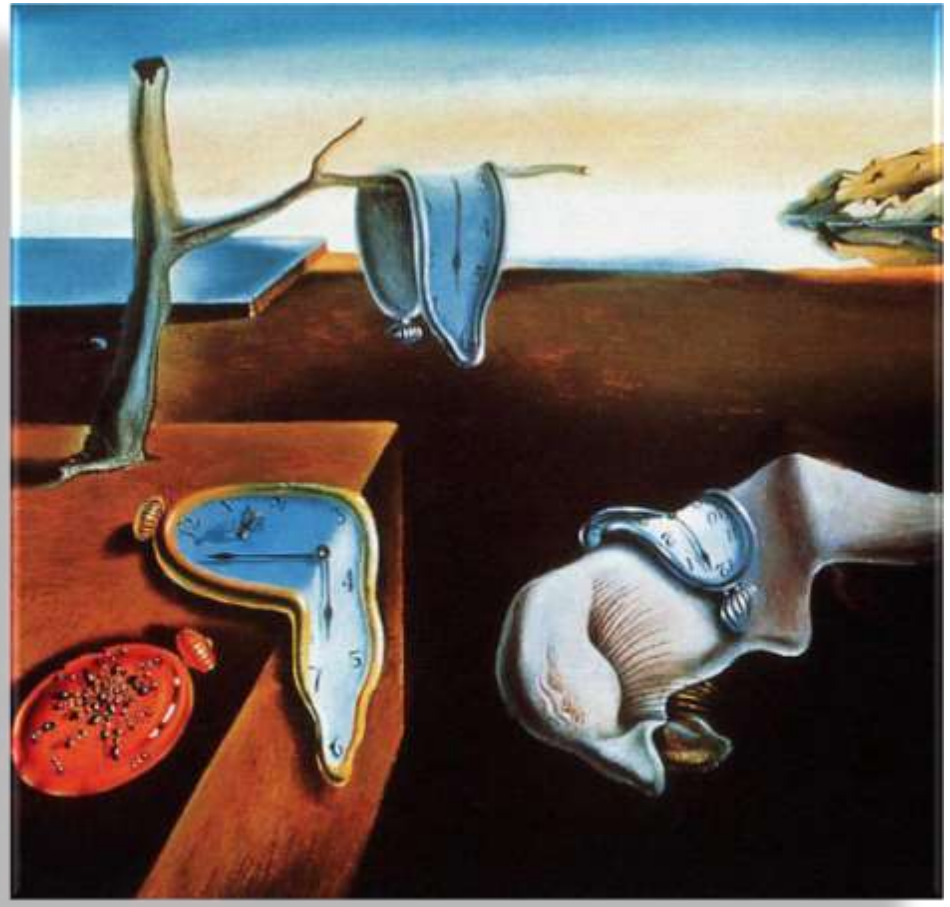
Example of dioxins and PCBs



→ Continuous methodological and technological developments still useful and necessary (High selectivity of sample preparation + high sensitivity of MS measurement).

Outline

- Introduction
- How measuring ?
- Where measuring ?
- When measuring ?
- What measuring ?
- Conclusion



What rationale for considering a target biological matrix in environment-health studies?

- Representativeness / internal exposure level
- Relevance / considered health outcome
- Precise sampling localization and conditions
- Multicompartment investigations = multiple analytical challenges
- Cartography of chemical residues = practical and cost limitations

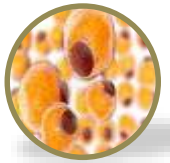
Hair | Adipose tissue | Blood | Milk | Urine | Meconium

Reservoir compartments | Circulating compartments | Excretion compartments

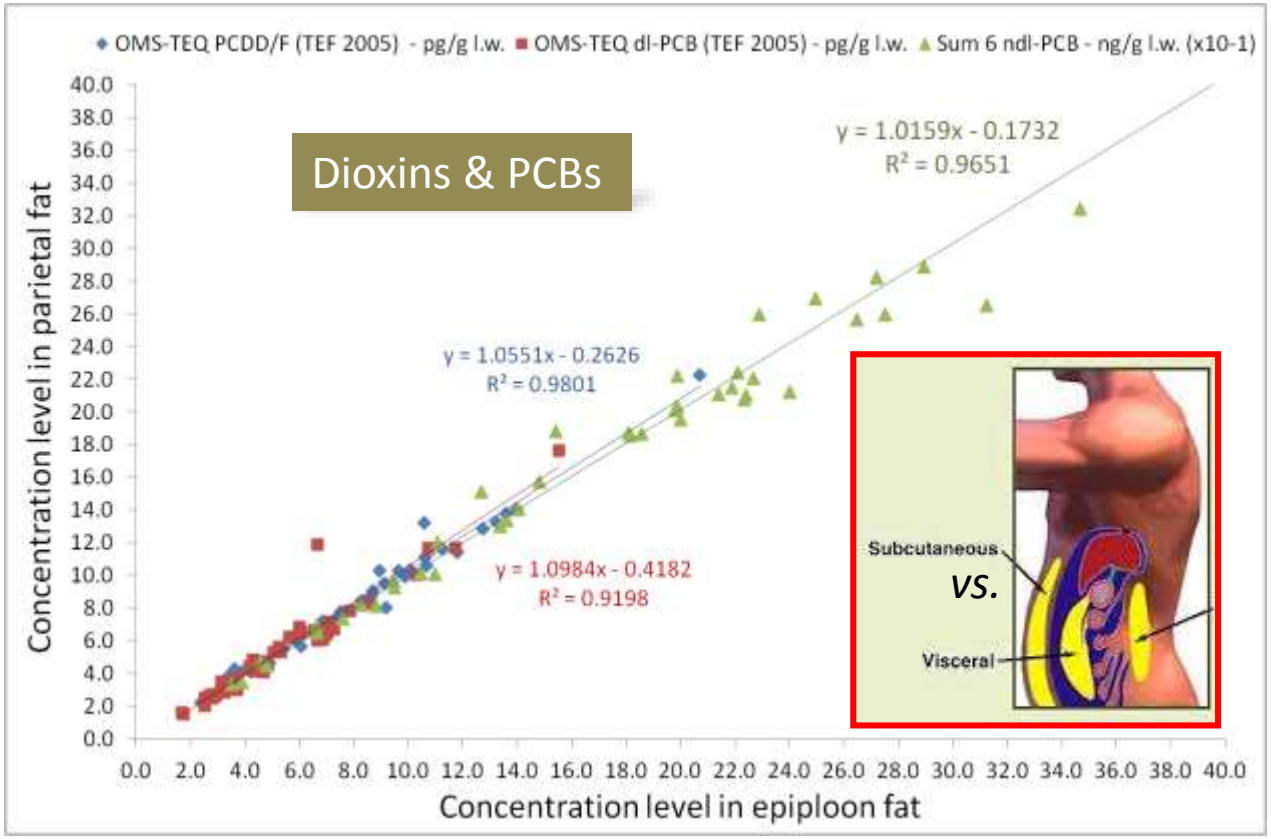
ENDOMET project - "Association between POP exposure and endometriosis"

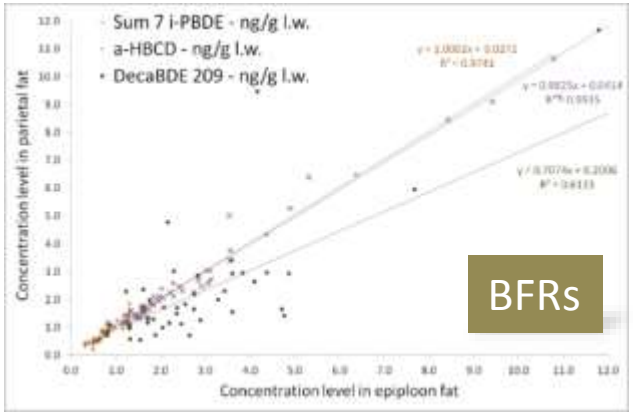
CHU Nantes (PhD thesis Dr. Stéphane Ploteau)

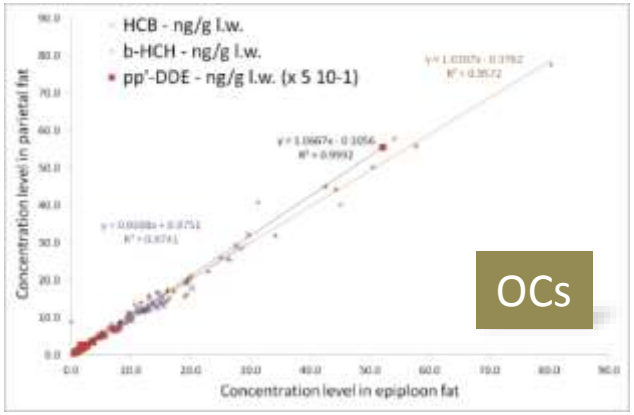
Are different adipose tissue depots equivalent in terms of POPs concentration levels?



- POPs levels in superficial and deep AT appeared equivalent in steady-state individuals
- No necessarily the case for all chemicals and depends on individual's weight stability







Ploteau et al., Environ. Int. 97 (2016) 125-136.

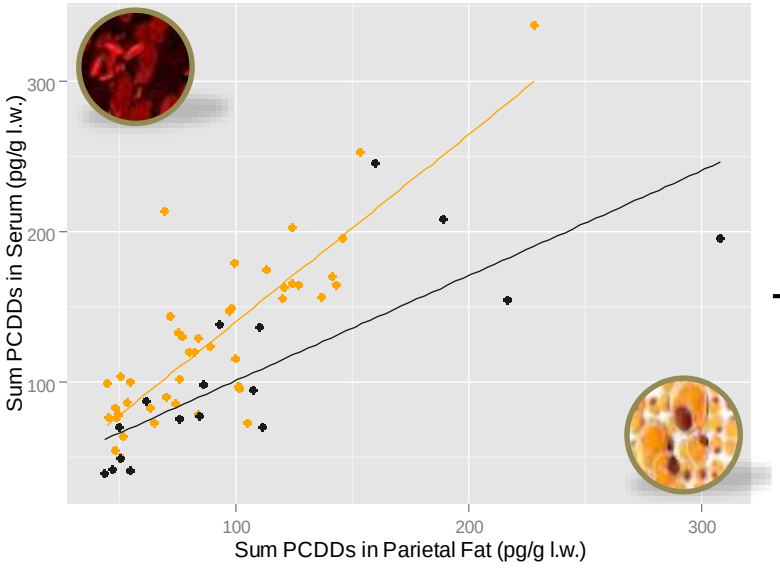
ENDOMET project - "Association between POP exposure and endometriosis"

CHU Nantes (PhD thesis Dr. Stéphane Ploteau)

Are adipose tissue and serum equivalent in terms of POPs information / health outcome?

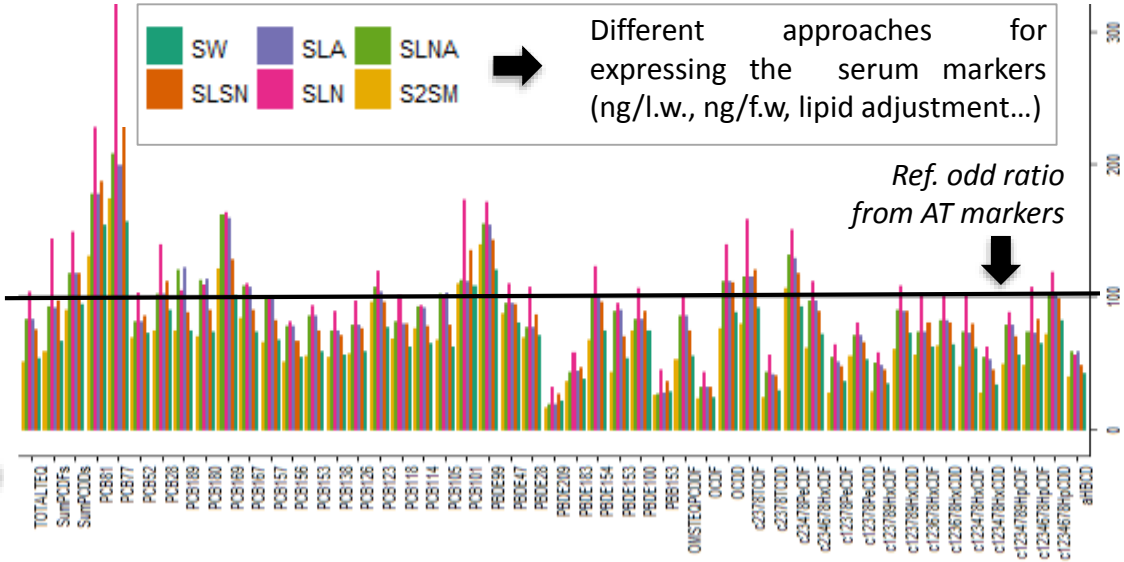
- Good correlation serum/AT usually observed for POPs but not for all chemicals
- Higher variability typically observed in serum (analytical + biological)
- Stored/circulating ratio as new integrative marker
- Still non definitive approach for relying exposure and health outcome

Cases (endometriosis) *vs*. Controls



Ploteau et al., Environ. Int. 108 (2017) 195-203.

Association between exposure and endometriosis (odd ratio)

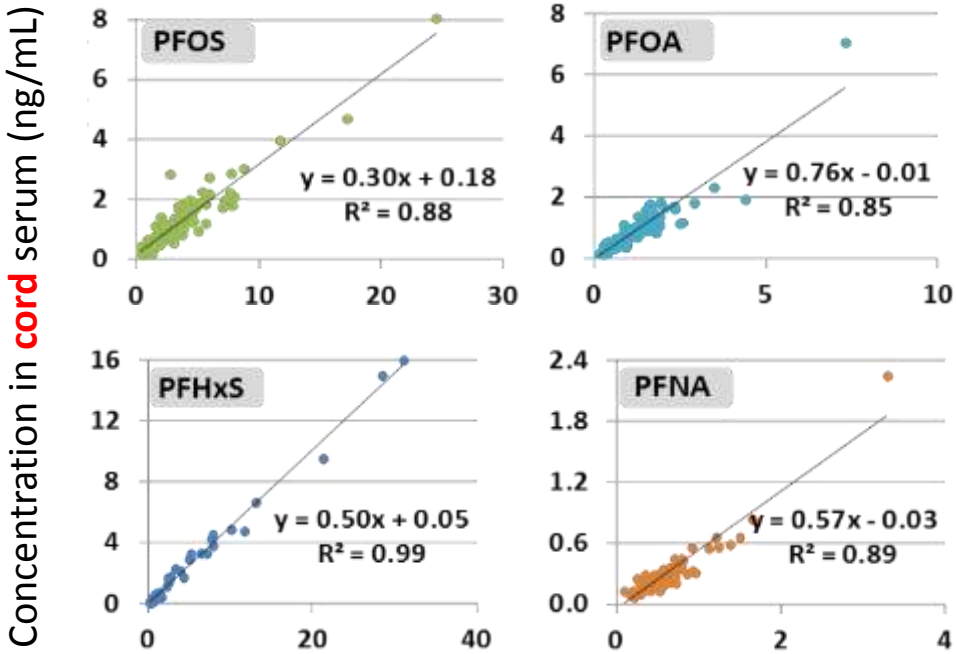


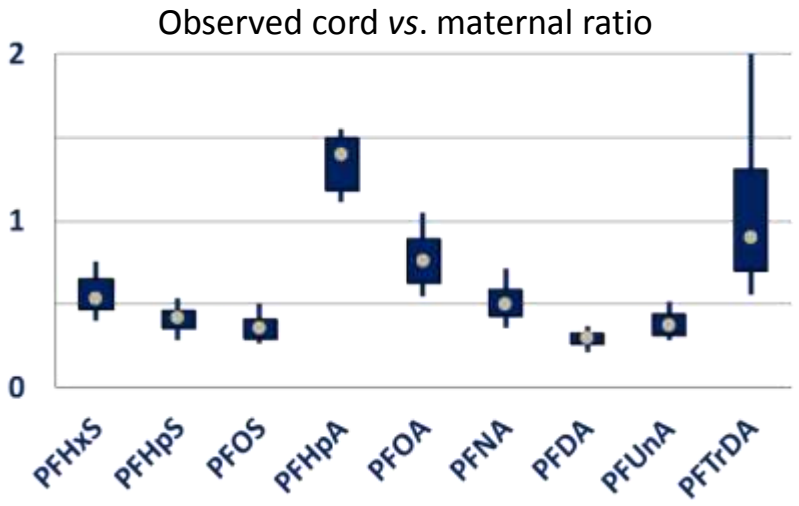
Cano-Sancho et al., Chemosphere 2018 in press.

CONTREPERF project - "Contribution to the human exposure assessment to PFAS" - ANR-10-CESA-008
Collab. CHU Toulouse (Alain Berrebi), INRA UMR 1331 Toxalim (Daniel Zalko)

Are maternal and cord serum correlated in terms of POPs concentration levels?

- Good maternal/cord serum correlation observed for PFAS
- Cord vs. maternal ratio depends on the considered compound





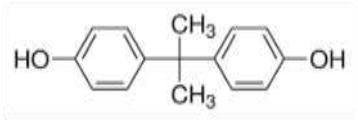
Cariou et al, Environ. Int. 84 (2015) 71–81.

NEWPLAST project - “Contribution to the human exposure assessment to Bisphenols” - ANR-13-CESA-0012-01 - Collab. CHU Toulouse (Alain Berrebi), INRA UMR 1331 Toxalim (Daniel Zalko)

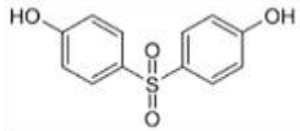
Are maternal and cord serum correlated / non persistent chemicals concentrations?

- Poor maternal/cord serum correlation observed for bisphenols

Bisphenol A (BPA)

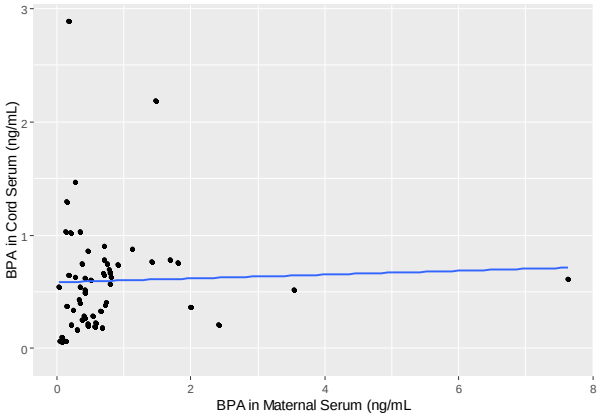


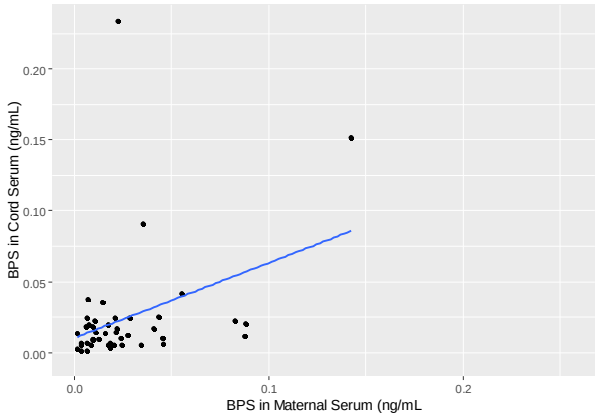
Bisphenol S (BPS)

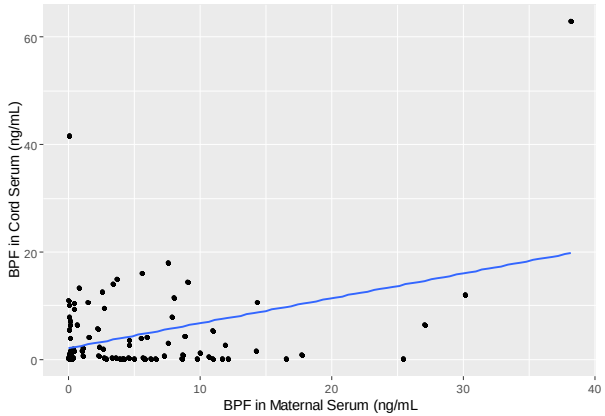


Bisphenol F (BPF)

Concentration in **cord** serum (ng/mL)







Concentration in **maternal** serum (ng/mL)

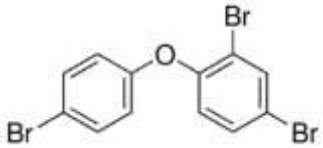
Antignac et al., manuscript in preparation.

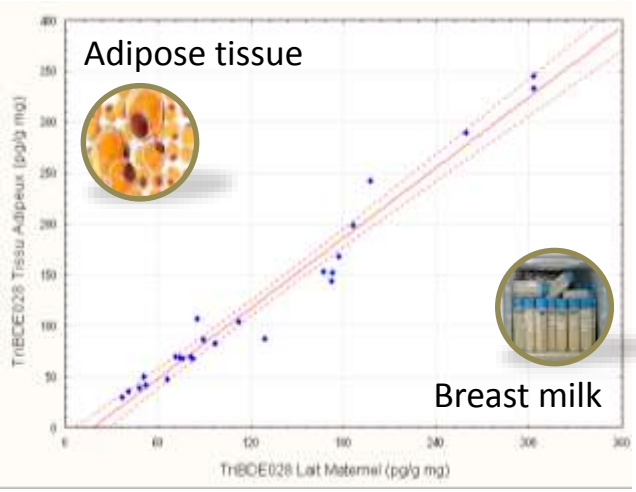
CONTREPERF/NEWPLAST projects - "Contribution to the human exposure assessment to PFAS / Bisphenols." - Collab. CHU Toulouse (Alain Berrebi), INRA UMR 1331 Toxalim (Daniel Zalko)

Does breast milk a good option for assessing maternal and fetal exposure?

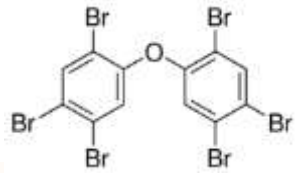
- Global significant correlation between levels in adipose tissue and breast milk for POPs
- Stronger association for lowest *versus* highest brominated PBDE congeners

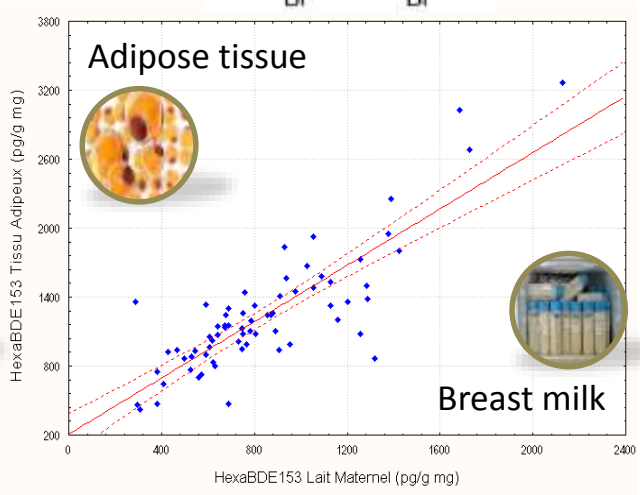
PBDE#28 (tri-BDE)



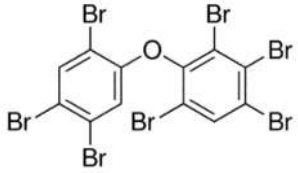


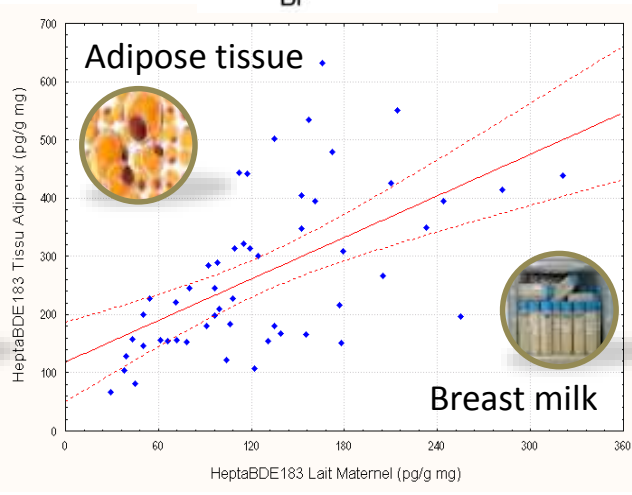
PBDE#153 (Hexa-BDE)





PBDE#183 (Hepta-BDE)



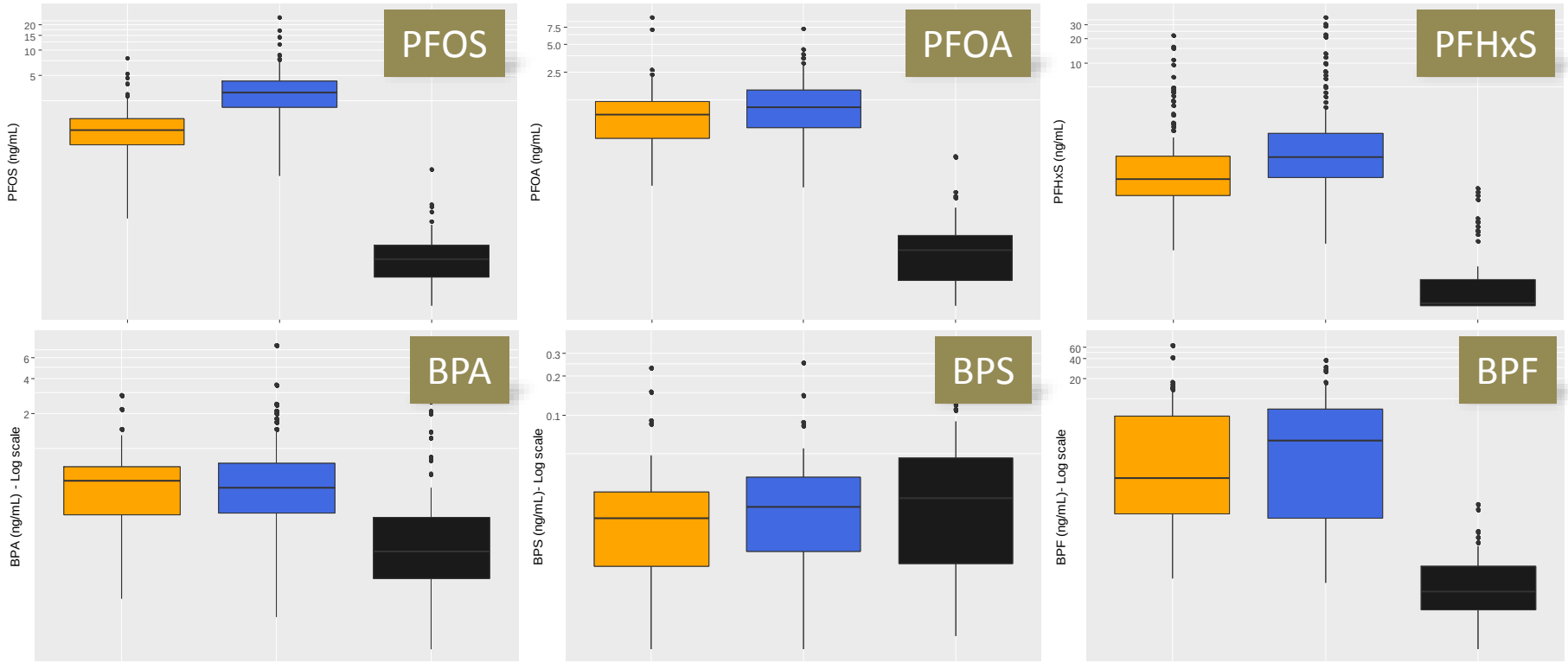


Antignac et al., Environ Pollut. 157 (2009) 164-173.

CONTREPERF/NEWPLAST projects - "Contribution to the human exposure assessment to PFAS / Bisphenols." - Collab. CHU Toulouse (Alain Berrebi), INRA UMR 1331 Toxalim (Daniel Zalko)

Does breast milk a good option for assessing maternal and fetal exposure?

- Milk transfer rate depends on substance chemistry and other factors (BMI, weight gain/loss, diet…)



Outline

- Introduction
- How measuring ?
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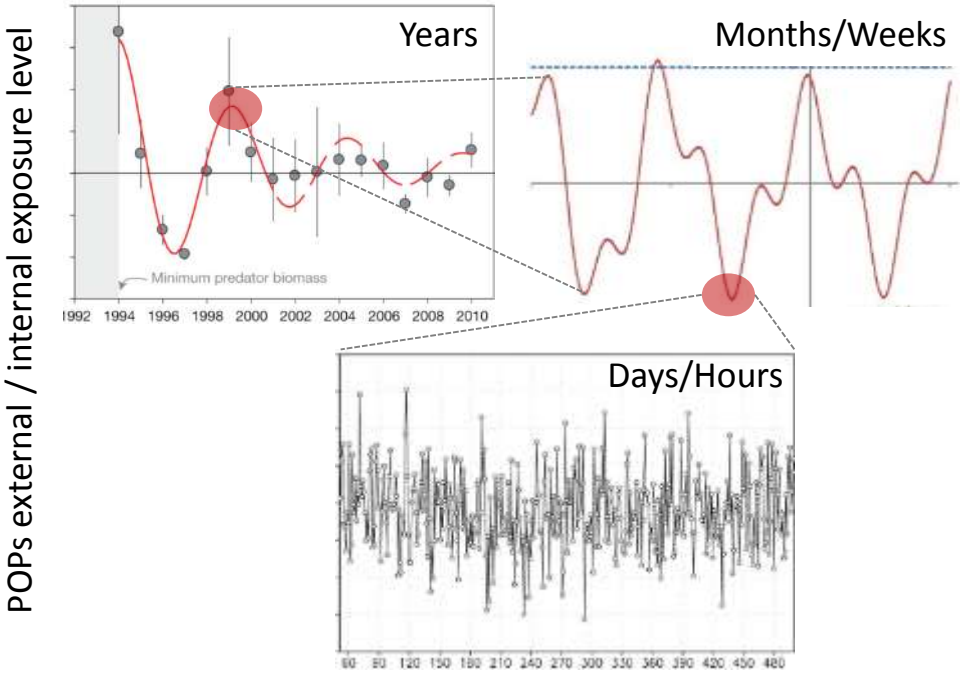
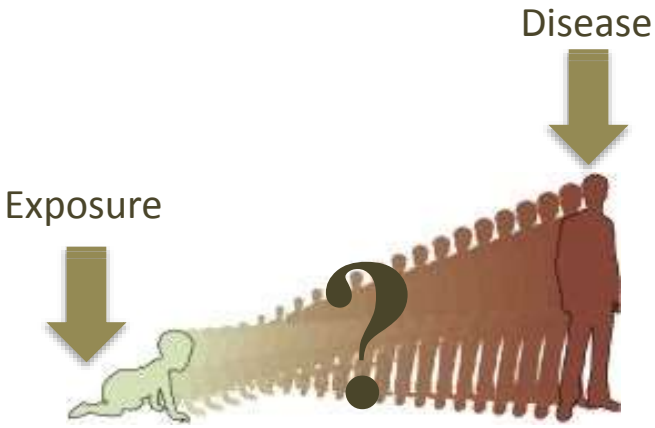
What rationale for getting a fix snapshot from a moving process?

Intra-individual temporal variability in terms of chemical exposure and related internal dose



Long term effects of early exposure "Developmental Origins of Health and Disease (DOHaD)"



Chemicals hypothesized to be a factor of:

- Disease induction/apparition?
- Disease development/progression?
- Disease programming?

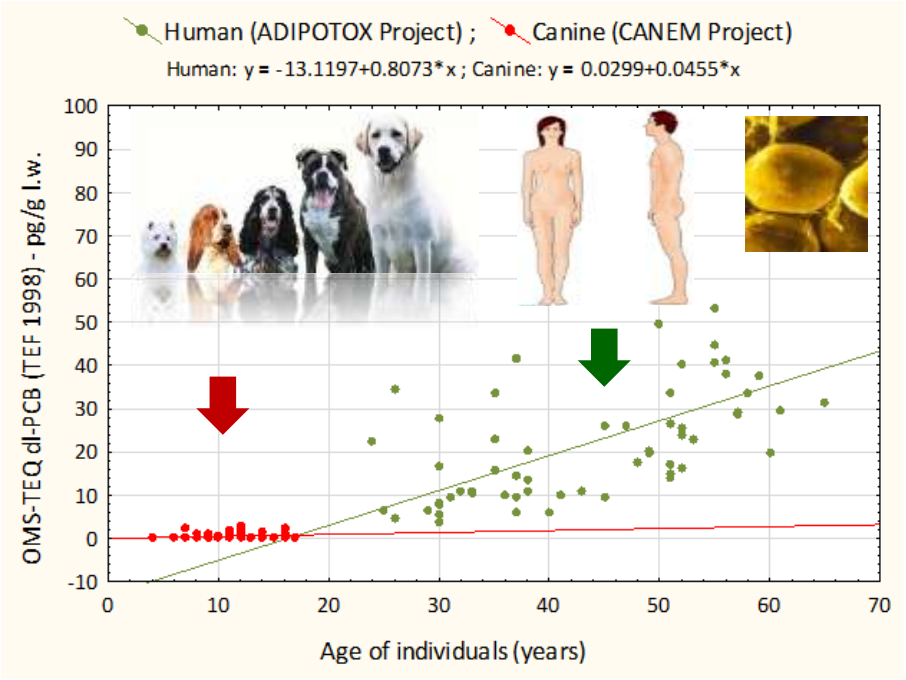
Determining the exposure levels:

- At the time of the disease diagnostic?
- At one earlier (critical) time windows?
- At birth?

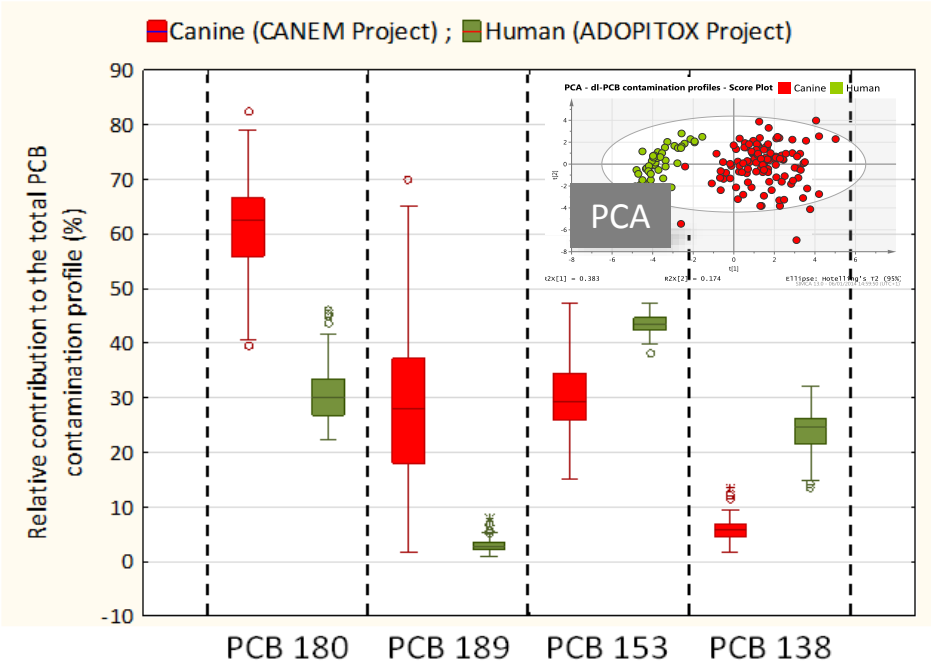
CANEM project "cancer and environment, the dog model"
Coord. : Oniris AMaROC (J. Abadie), F. Morio, F. Sauvaget

Use a full life animal model

Investigating the POPs internal exposure levels and contamination profiles in dogs *versus* human



Concentration levels of dl-PCB (OMS-TEQ) determined in dog *versus* human according to the age of individuals.



Relative contribution of some PCB congeners to the total PCB internal exposure observed in dog versus human individuals.

Sévère et al, manuscript in preparation.

→ Pet dog model not fully satisfying as sentinel of human chemical exposure.

ERSaViEnt project – "Estimation of a full life food exposure to chemicals"
PhD Marion Pruvost-Couvreur, Collab. ANSES (Gilles Rivière)

Model a full life exposure from one or several isolated measurements

Physiologically Based Pharmacokinetic Modeling of Persistent Organic Pollutants for Lifetime Exposure Assessment: A New Tool in Breast Cancer Epidemiologic Studies

Marc-André Verner,[1] Michel Charbonneau,[2] Lizbeth López-Carrillo,[3] and Sami Haddad[1]*

[1]Département des sciences biologiques, Université du Québec à Montréal, Montréal, Québec, Canada; [2]INRS-Institut Armand-Frappier, Université du Québec, Laval, Québec, Canada; [3]Instituto Nacional de Salud Publica, Cuernavaca, Mexico

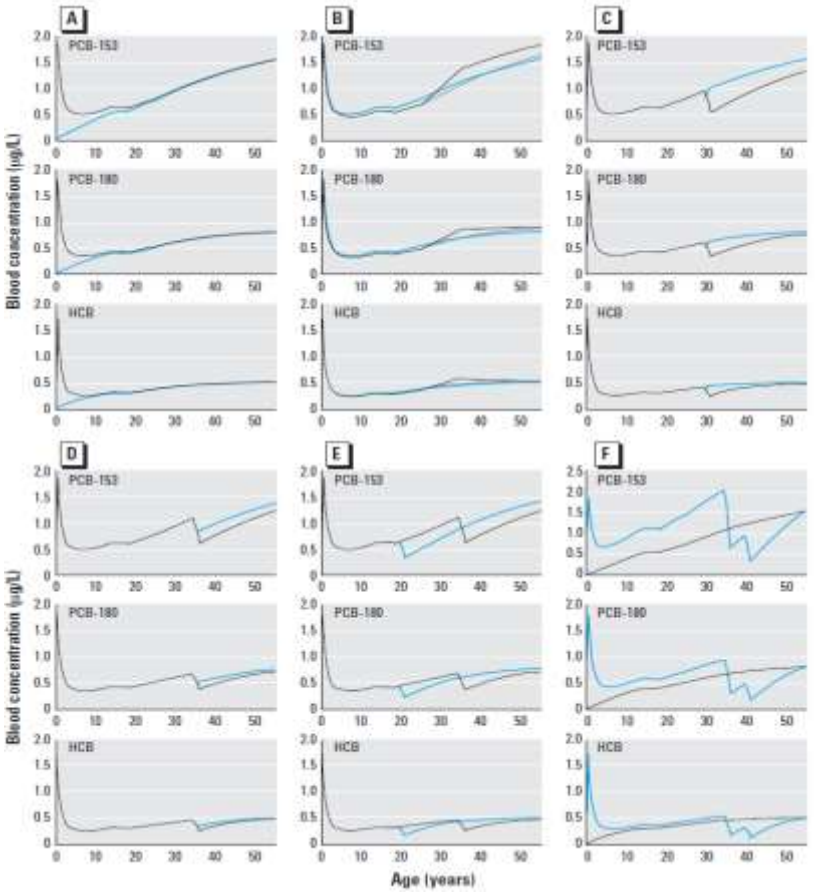


Figure 3. Toxicokinetic profiles for PCB-153, PCB-180, and HCB blood concentration for (*A*) normal body weight history and 10 ng/kg/day exposure to each of these chemicals for a woman who was not breast-fed in childhood (blue line) or breast-fed for 6 months (black line); (*B*) normal weight (gray line), weight loss (black line), or overweight (blue line) profiles and 10 ng/kg/day exposure to each of these chemicals for women who were breast-fed for 6 months in childhood; (*C*) normal body weight history and 10 ng/kg/day exposure to each of these chemicals for a woman who was breast-fed for 6 months in childhood and had a pregnancy at 30 years of age followed by no lactation (blue line) or a 12-month lactation period (black line); (*D*) normal body weight history and 10 ng/kg/day exposure to each of these chemicals for a woman who was breast-fed for 6 months in childhood and had a pregnancy followed by a 12-month lactation period at 20 years of age (blue line) or 35 years of age (black line); (*E*) normal body weight history and 10 ng/kg/day exposure to each of these chemicals for a woman who had a pregnancy at 35 years of age followed by a 6-month lactation period (blue line) or a 12-month lactation period (black line); (*F*) normal body weight history for a woman who was exposed to 10 ng/kg/day of each of the three chemicals and had no pregnancy (black line) or was breast-fed for 6 months in childhood, was exposed to 18.7 ng/kg/day PCB-153, 13.8 ng/kg/day PCB-180, 11.6 ng/kg/day HCB, and who had two pregnancies at 35 and 40 years of age followed by 12-month lactation periods (blue line).

→ Statistical / conceptual developments running but pieces of knowledge still missing.

Outline

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- How measuring ?
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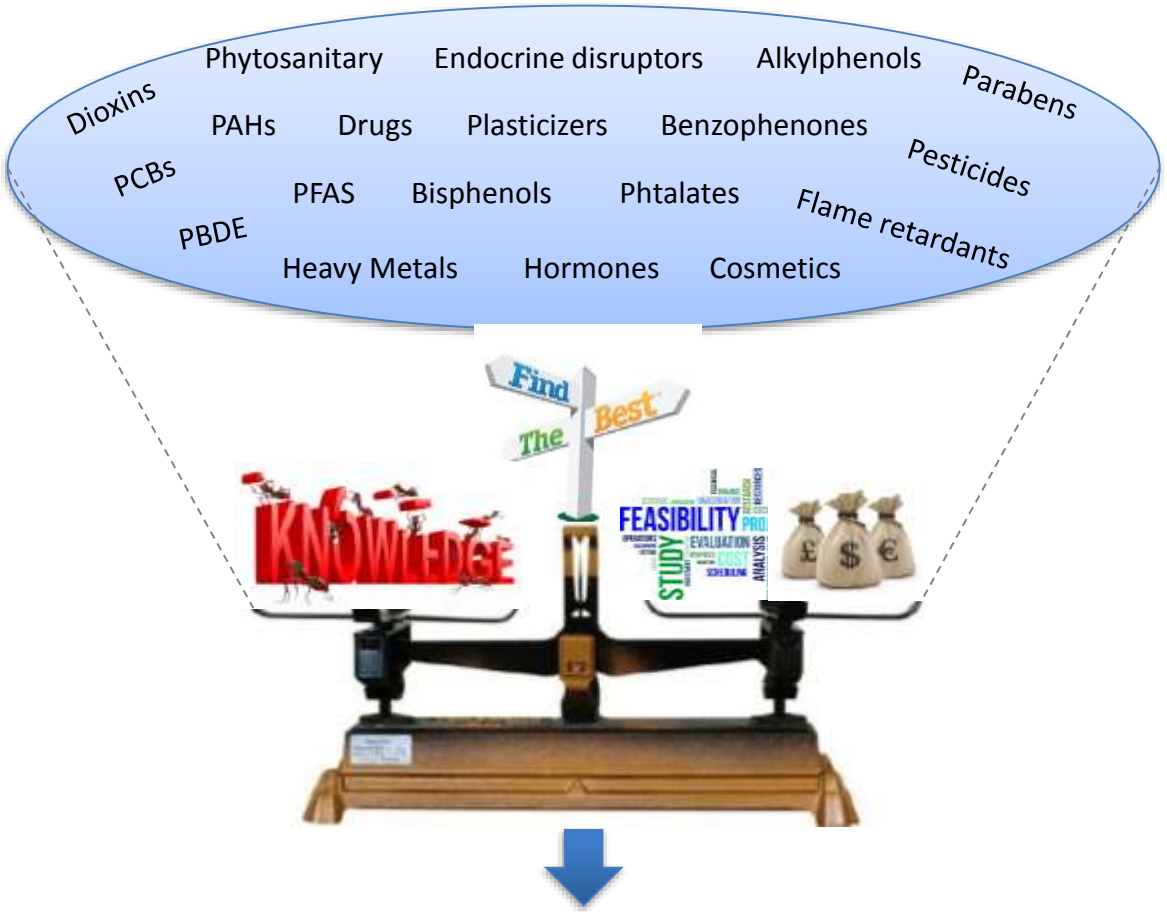
More and more chemicals in our environment to be considered for risk assessment

Scientific vs. practical and economical issues is the main constraining frame of environmental-health studies

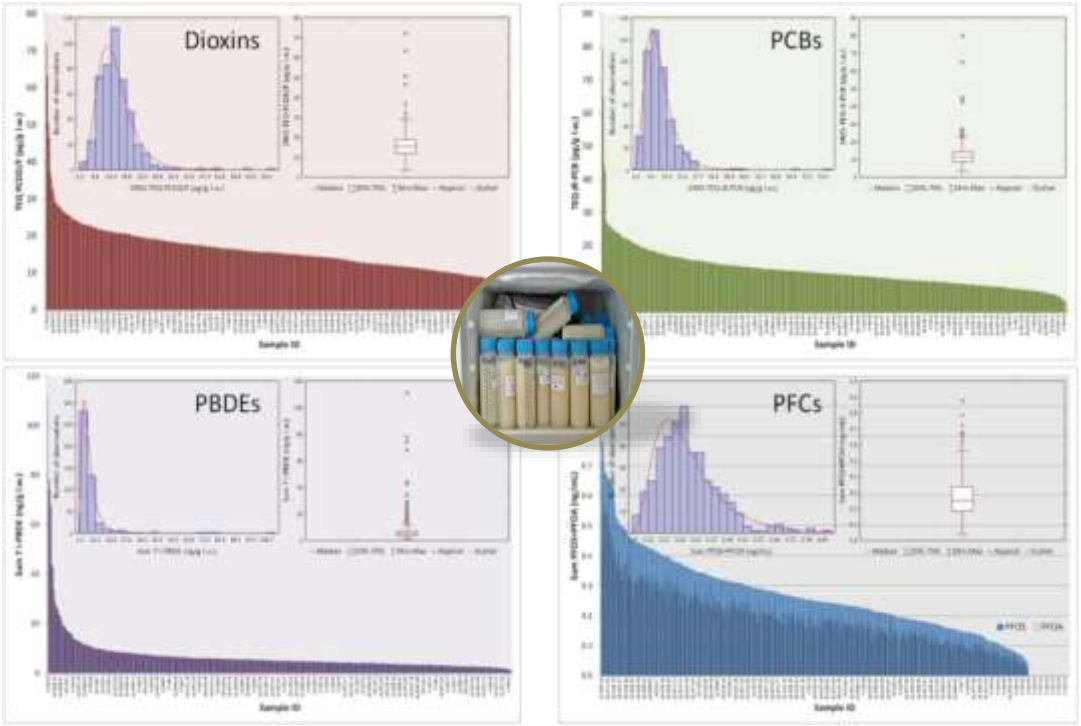
Several options may be envisaged

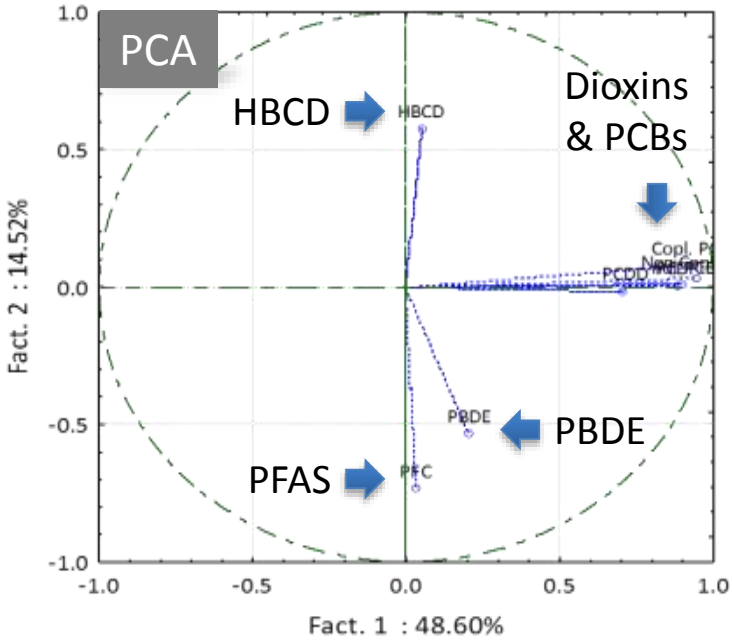


- Limit the number of targeted POPs families?
- Limit the number of targeted congeners among each POPs family?
- Increase the methodological capabilities?
- Develop new approaches based on integrative biomarkers?

DEER project – "Developmental Effects of Environment on Reproduction", Coord. : Univ Turku, J Toppari
Collab. Righospitalet, Copenhagen, DK (Katharina Main, Niels E Skakkebaeck)

Can the exposure levels determined for some POPs classes be predictive of the other classes?





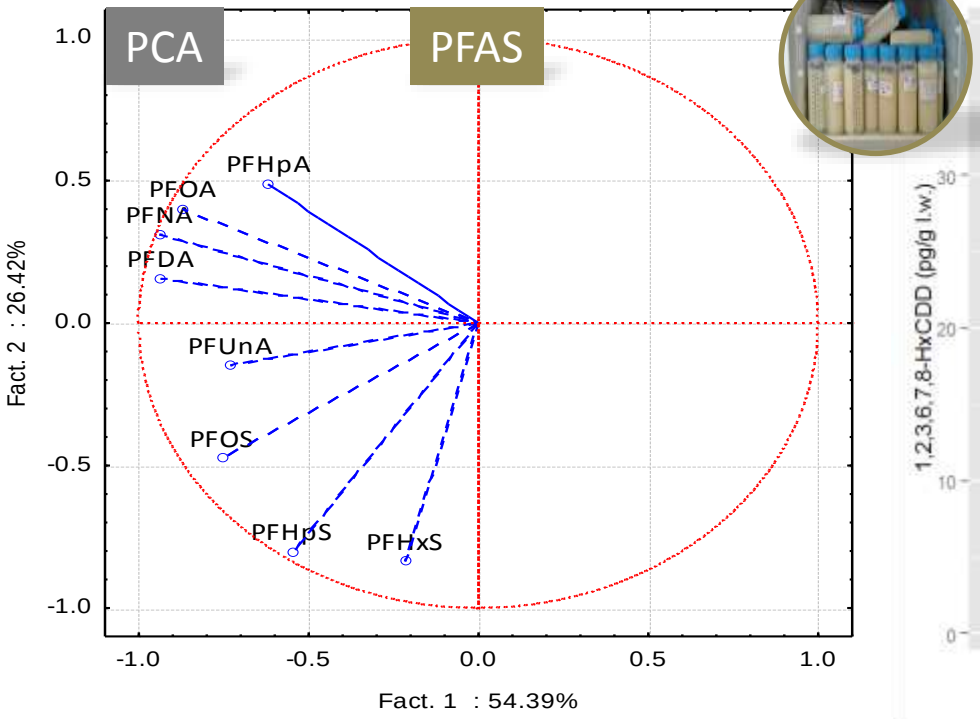
Antignac et al. Organohalogen compounds 2012.
Wohlfahrt Veje et al. EHP 2014;147(4):391-399.
Antignac et al., Environ Pollut. 218 (2016) 728-738.

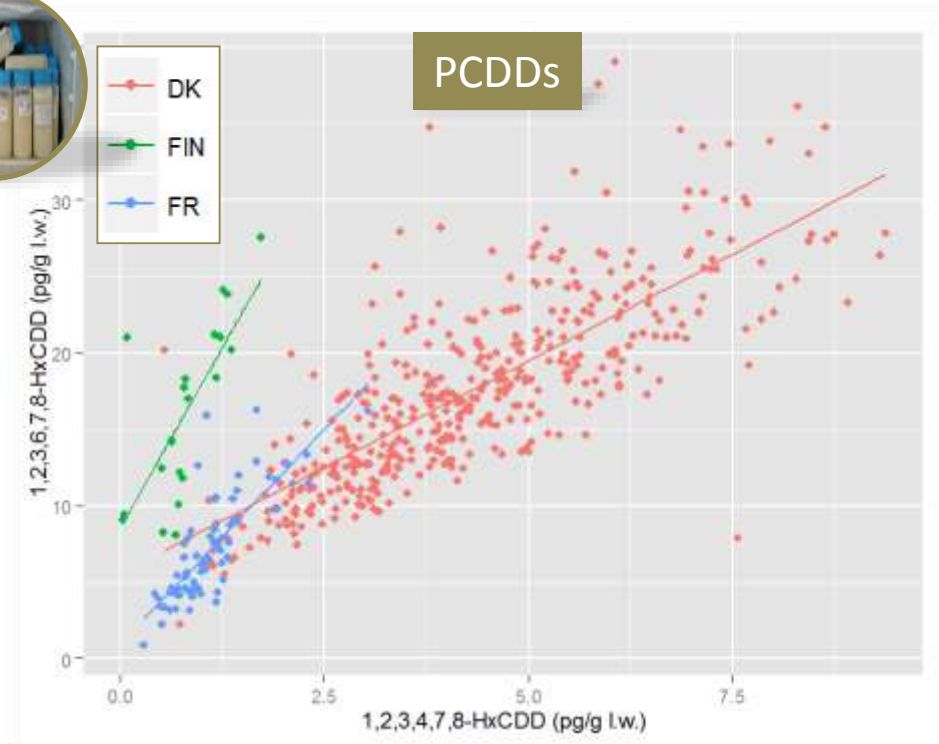
→ Global POPs contamination profiles can not be restricted to a limited number of families.

CONTREPERF project - ANR-10-CESA-008
Collab. A. Berrebi, D. Zalko

DEER project - FP7-ENV-2007-1 (212844)
Collab. K. Main, Niels E Skakkebaeck

Can the exposure levels determined for particular POPs congeners be predictive of the others?





- No correlation relation between ≠ PFAS compounds

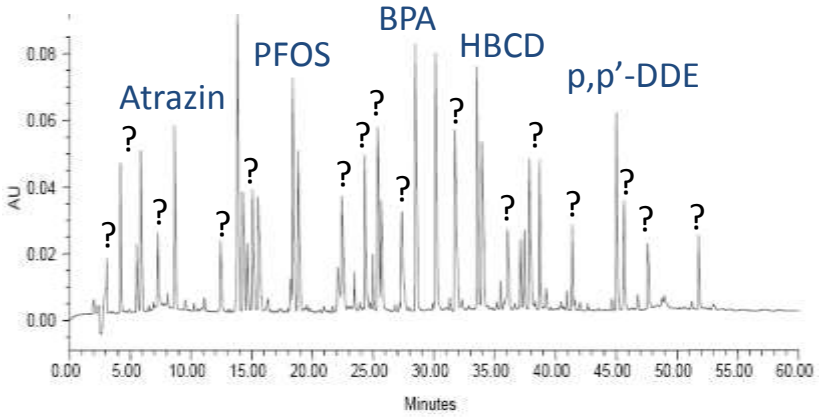
Cariou et al, Environ. Int. 84 (2015) 71–81.

- Country dependent correlation between 2 PCDD congeners

Antignac et al., Environ Pollut. 218 (2016) 728-738.

→ Global POPs contamination profiles can not be restricted to a limited number of congeners.

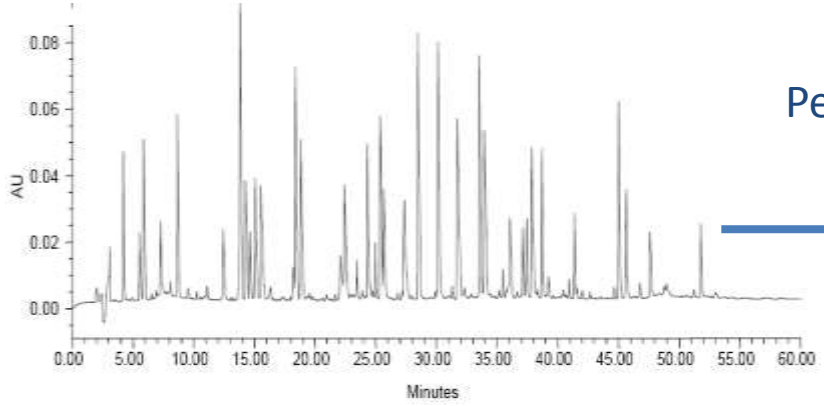
- Trend observed with the relative democratization of HRMS
- Wider coverage but lower quantitative performances
- Still depends on sample preparation
- Need for extended and QA/QC consolidated ref. library



Annotated profile

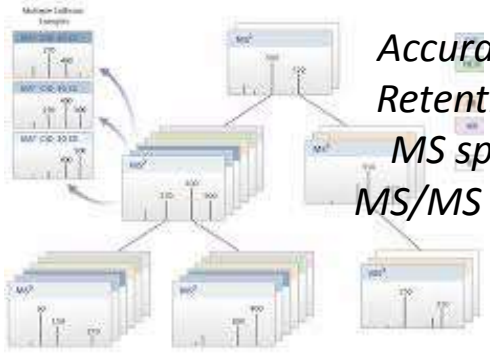
Human samples

High Resolution Mass Spectrometry Profiling
(LC-HRMS, GC-HRMS, GCxGC-HRMS)



Peak annotation

MS Reference Library



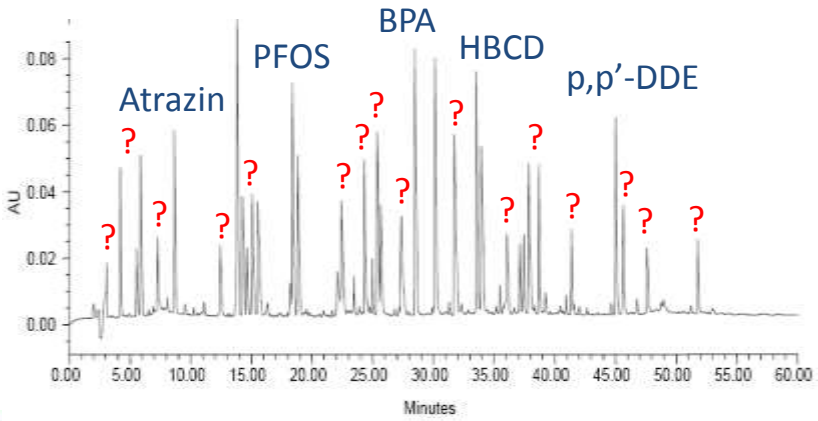
Accurate mass
Retention time
MS spectrum
MS/MS spectrum

- "Fishing" emerging substances & new markers discovery
- Still depends on sample preparation
- Need for advanced expertise for data processing & analysis
- Need for advanced expertise for structural elucidation

Human samples

Raw or annotated HRMS Profile

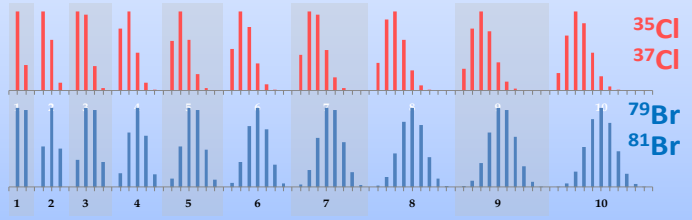
(LC-HRMS, GC-HRMS, GCxGC-HRMS)



Look for unknowns

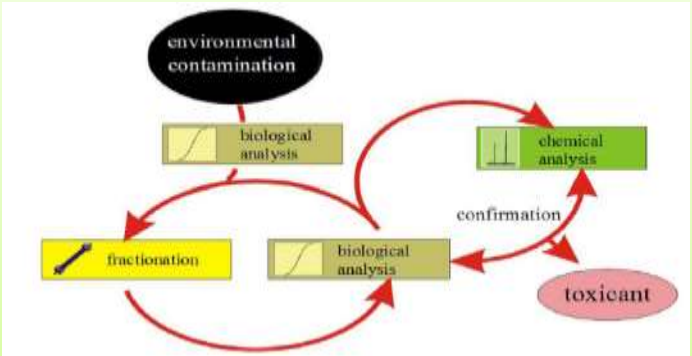
- Chemistry driven approach

(look for particular signatures, e.g. halogens)



- Biology driven approach

(look for biological activity / toxicity, EDA)



- Statistic driven approach

(look for temporal trends, differential patterns)

Samples A (e.g. old, lower exposed) ≠ Samples B (e.g. recent, highly exposed)

Contents lists available at ScienceDirect

Analytica Chimica Acta

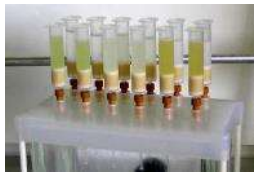
journal homepage: www.elsevier.com/locate/aca

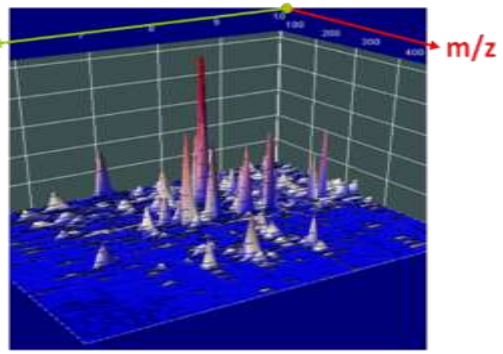
Screening halogenated environmental contaminants in biota based on isotopic pattern and mass defect provided by high resolution mass spectrometry profiling

Ronan Cariou*, Elsa Omer, Alexis Léon, Gaud Dervilly-Pinel, Bruno Le Bizec

LUNAM Université, ONIRIS, Laboratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), Nantes, F-44307, France

Sample prep. and HRMS strategies





analytical chemistry

Subscriber access provided by UNIV OF OREGON

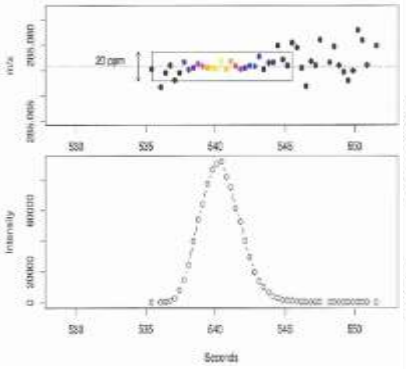
Article

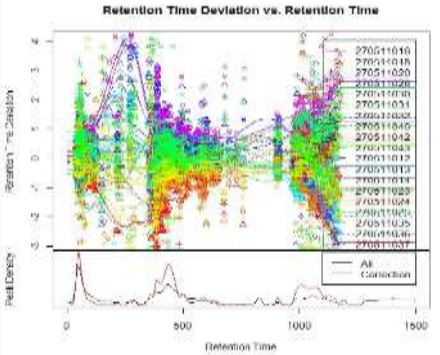
Automated detection of natural halogenated compounds from LC-MS profiles – Application to the isolation of bioactive chlorinated compounds from marine-derived fungi

Catherine Roullier, Yann GUITTON, Marine Valery, Séverine Amand, Soizic Prado, Thibaut Robiou du Pont, Olivier Grovel, and Yves François Pouchus

Anal. Chem., Just Accepted Manuscript • DOI: 10.1021/acs.analchem.6b02128 • Publication Date (Web): 18 Aug 2016

Biocomputering data processing solutions

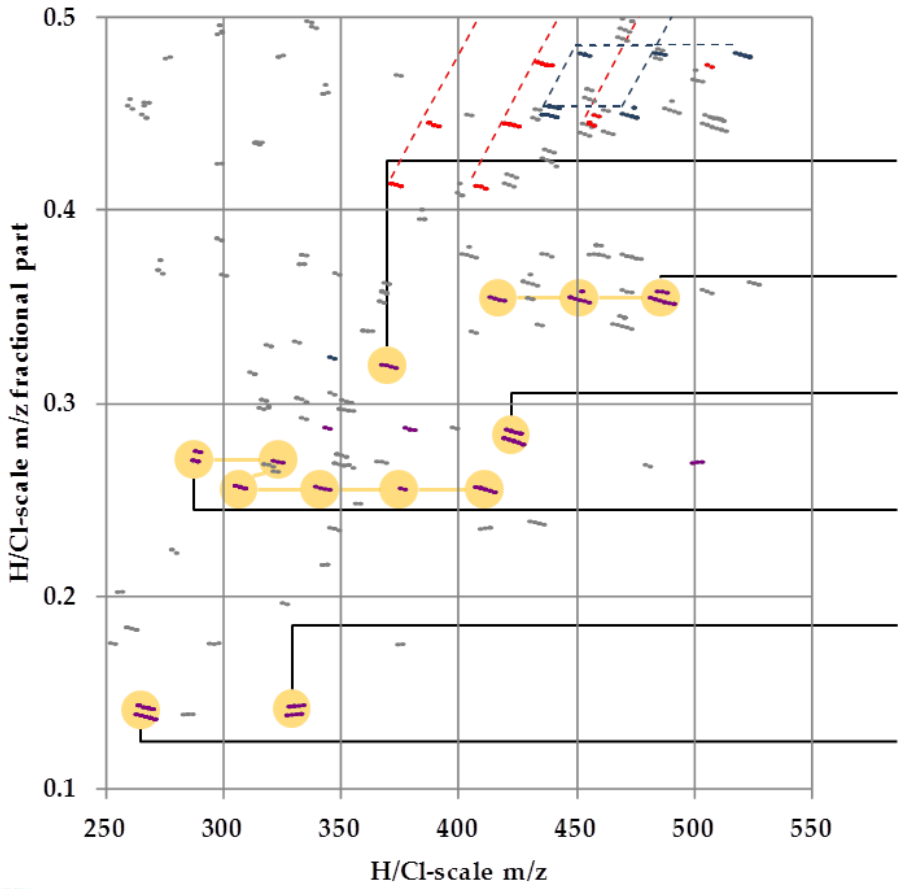




→ Already successful stories in environmental and food matrices.

Output: mass defect based maps of the detected features permitting to point out (and further identify) halogenated substances. Possible application to screen for not yet known novels BFR, pesticides metabolites, Br/Cl phenols, BR+Cl POPs…

« HaloSeeker » user friendly application



Putative formula

$[C_{14}H_9OCl_5]^-$ -8.00 ppm

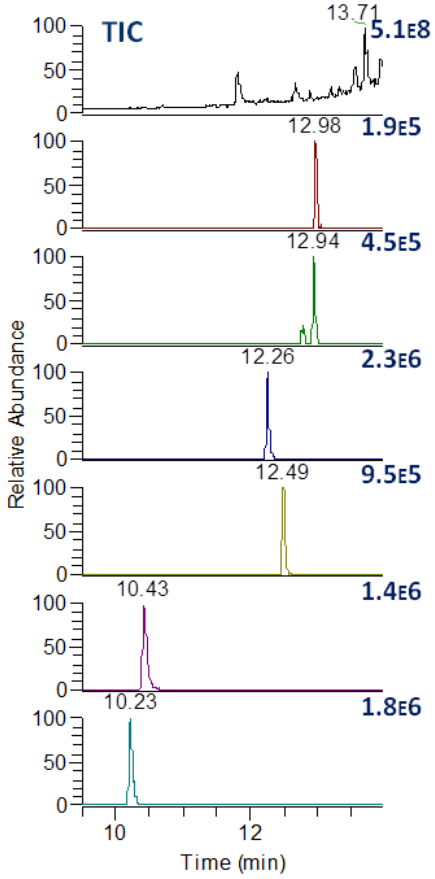
$[C_{12}H_{10}O_3Cl_8]^-$ -6.51 ppm

$[C_9H_6O_4Cl_6S]^-$ -6.61 ppm

$[C_{12}H_7O_2Cl_3]^-$ -7.37 ppm

$[C_6H_3OBr_3]^-$ -7.06 ppm

$[C_6OCl_5]^-$ -7.44 ppm



Putative structure

Dicofol

$C_{12}H_9O_3Cl_8$ Unsat. = 4

Endosulfane Sulfate

diOH-triCB

Br_3-Phenol

Cl_5-Phenol

HBM4EU

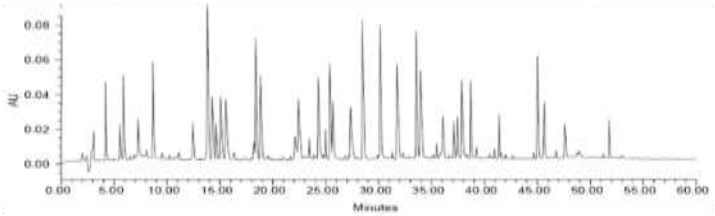
Human Biomonitoring for EU

EJP Cofund H2020-SC1-2016-RTD, 733032, SC1-PM-05-2016

WP16
"Emerging chemicals"

Human samples
(conventional vs. innovative storage, circulating and/or excretion compartments)

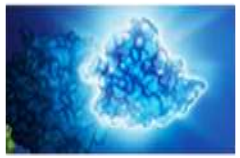
High Resolution Mass Spectrometry Profiling
(LC-HRMS, GC-HRMS, GCxGC-HRMS)

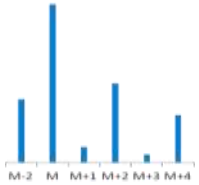


1. Suspect screening
(look for particular target signals)

New human exposure data
Feed associated databases/libraries

2. Untargeted screening
(look for unknown)





Chemistry driven data processing strategies
(e.g. focus on halogenated chemical patterns…)

Structural elucidation work
(signal annotation, multidimensional MS,…)

Fractionation-biological activity testing
(look for endocrine disrupting character, interaction with nuclear receptors e.g. AhR…)

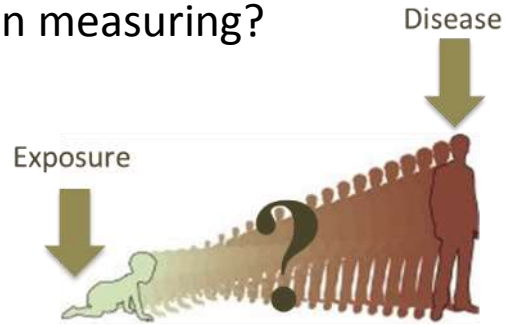
Outline

- Introduction
- How measuring ?
- Where measuring ?
- When measuring ?
- What measuring ?
- Conclusion

How measuring?

- Sample preparation remains a corner stone
- Targeted quantitative methods still needed
- Untargeted profiling methods now the trend

When measuring?

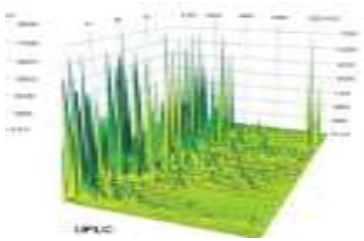


- Need for longitudinal data and PBPK modeling
- Need for better experimental models
- MS profiling in personalized medicine

Where measuring?

- Need for more multi-compartment studies
- Stored vs. circulating ratio as integrative marker
- Non identical strategies for HBM / health studies

What measuring?



- Need for more extended characterizations…
- … Following the exposome and mixture issues
- Need for integrating exposure / effect markers

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www.saraf-educ.org

• Financial supports

SEVENTH FRAMEWORK PROGRAMME
HORIZON 2020
FEDER Union Européenne
ANR AGENCE NATIONALE DE LA RECHERCHE
anses
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MassTwin Workshop
Mass spectrometry in support of the environment, food, and health interaction and disease
Antwerp, Belgium, 18th-20th April 2018

MASS SPECTROMETRY FOR CHARACTERIZING HUMAN INTERNAL CHEMICAL EXPOSURE: STRENGTHS AND CHALLENGES

Jean-Philippe ANTIGNAC

Philippe Marchand, Ronan Cariou, Bruno Veyrand, Anaïs Venisseau, German Cano-Sancho, Stéphane Ploteau, Emmanuelle Bichon, Ingrid Guiffard, Yann Guitton, Fabrice Monteau, Bruno Le Bizec

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