Colloque

Améliorer le diagnostic des polluants organiques environnementaux :

Mise en œuvre d'approches d'analyses non-ciblées

mardi 6 février 2018

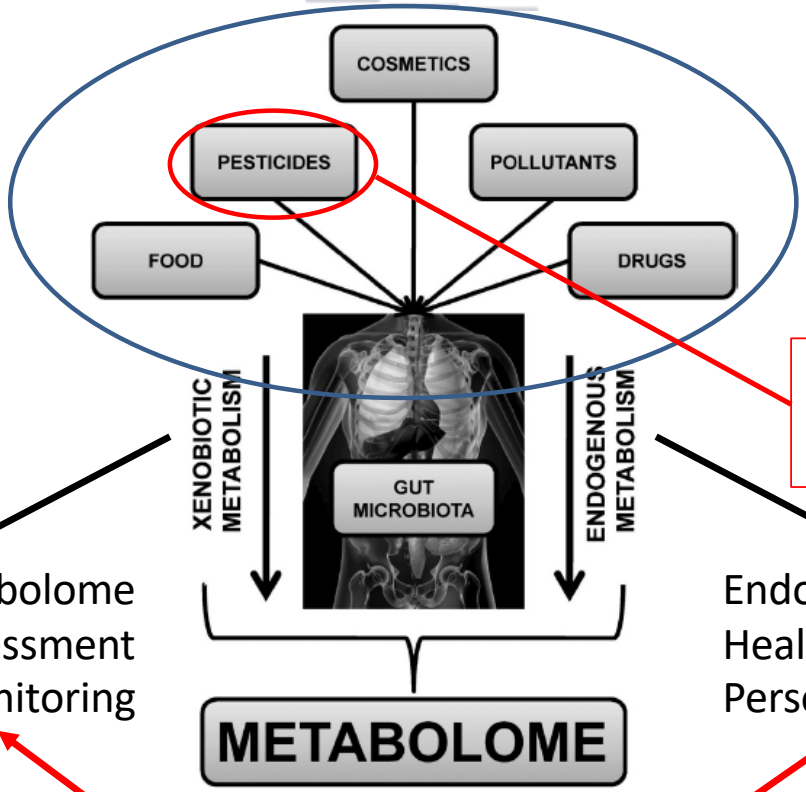
Approches non ciblées en spectrométrie de masse pour la caractérisation de l'exposome chimique : le cas des pesticides

L. Debrauwer

INRA Toxalim UMR1331, MetaboHUB-MetaToul-AXIOM platform
TOULOUSE, France

http://www.metatoul.fr
http://www.metabohub.fr

Exposome, metabolomics and toxicology



Journal of Toxicology and Environmental Health, Part B, 17:21–44, 2014

ISSN: 1093-7404 print / 1521-6950 online
DOI: 10.1080/10937404.2013.860318

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POTENTIAL INPUT FROM METABOLOMICS FOR EXPLORING AND UNDERSTANDING THE LINKS BETWEEN ENVIRONMENT AND HEALTH

Nathalie Bonvallot[1,2,3], Marie Tremblay-Franco[3], Cecile Chevrier[1,4], Cecile Canlet[3], Laurent Debrauwer[3], Jean-Pierre Cravedi[3], Sylvaine Cordier[1,4]

Exposure sources: domestic / occupational / dietary / environmental

Xenometabolome
Exposure assessment
Biomonitoring

Endometabolome
Health impact
Personalized nutrition / medicine

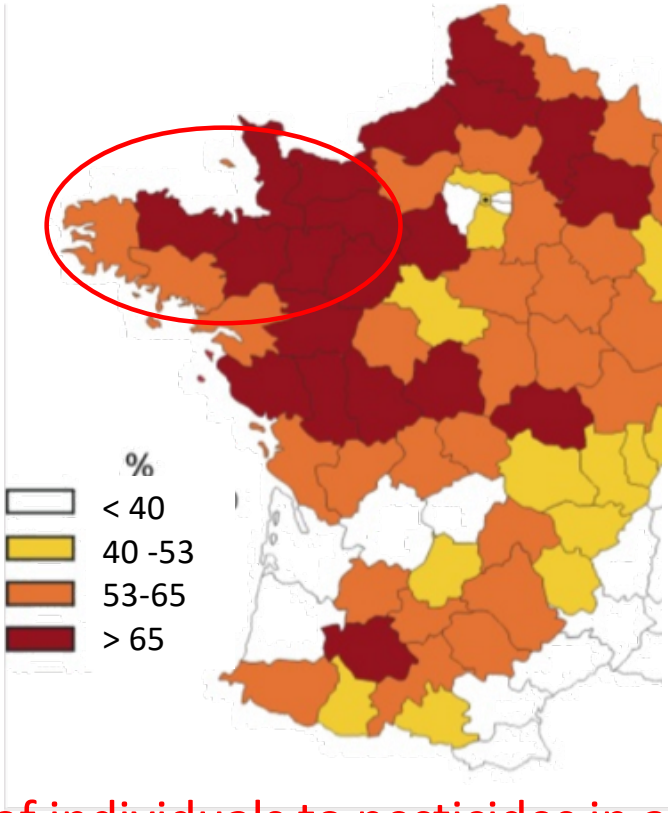
Targeted analysis
Suspects screening
Non-target screening

First case study: the PELAGIE Cohort (*n*=3,421)

Study of consequences of environmental exposure to xenobiotics on children development [1]

Ca. 3500 pregnant women (<19th week of pregnancy)

Urine samples collected 2004



60% surface devoted to agricultural activities (mainly cereal / corn)

1400 tons pesticides / year used in early 2000's

60% of plots receiving at least 4 different treatments

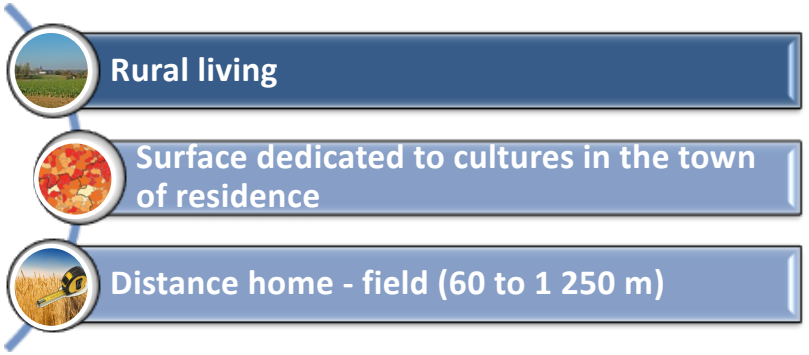
Can we assess the exposure of individuals to pesticides in a non targeted way ?

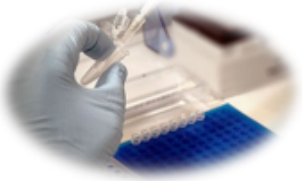
[1] Petit C. *et al*. Am. J. Epidemiol. (2002) 175:1182–1190

Development of a suspect screening approach

Materials & methods

Urinary samples selection:
% of land devoted to cereal cultures in the city of residence
40 samples selected for proof of concept



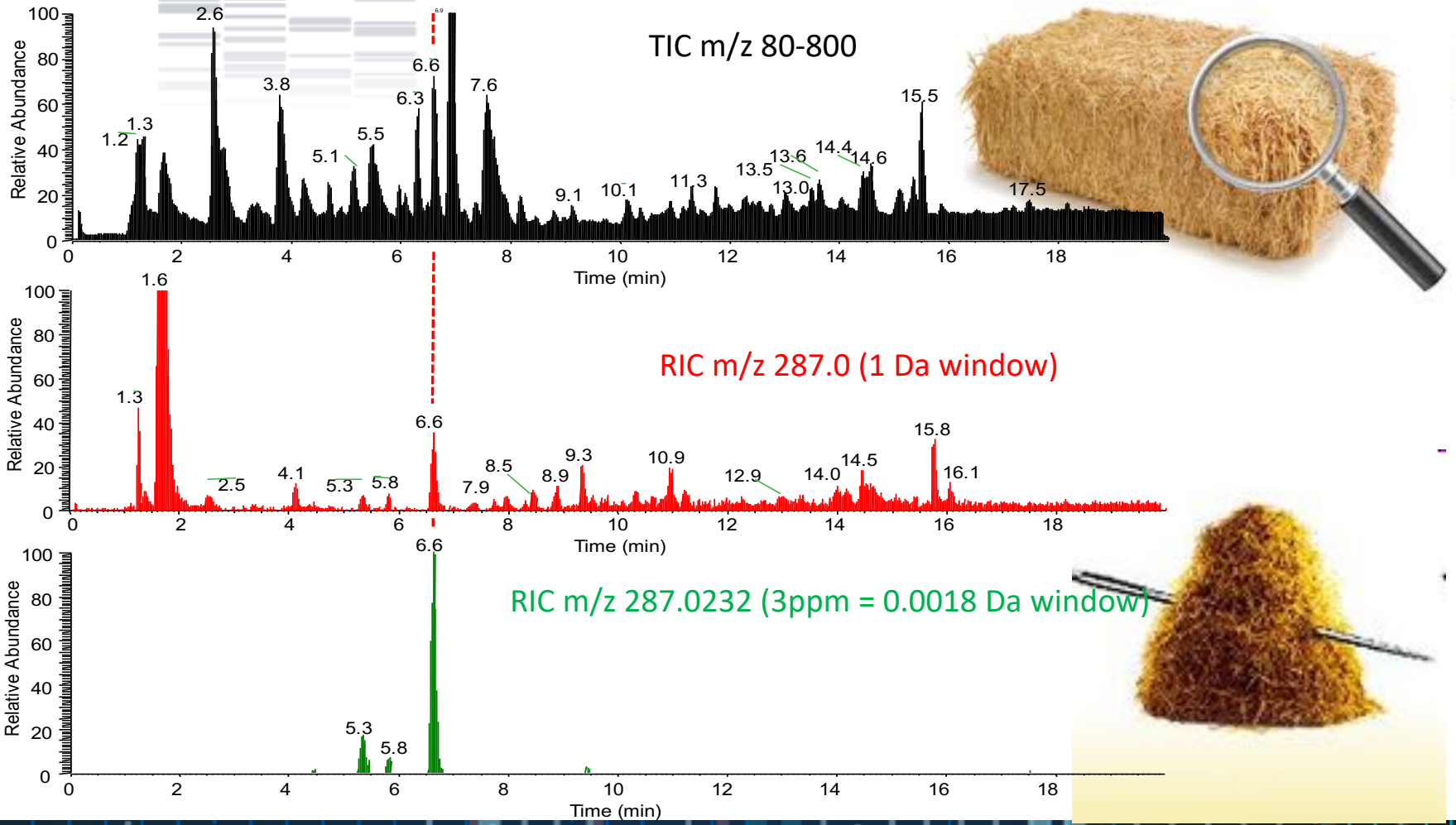


Sample preparation: dilution 2x in mobile phase A

UHPLC: Hypersil Gold C18, gradient elution $CH_3OH/H_2O/CH_3CO_2H$
HRMS: ESI(+) and ESI(-), LTQ-Orbitrap XL, *m/z* 60-800

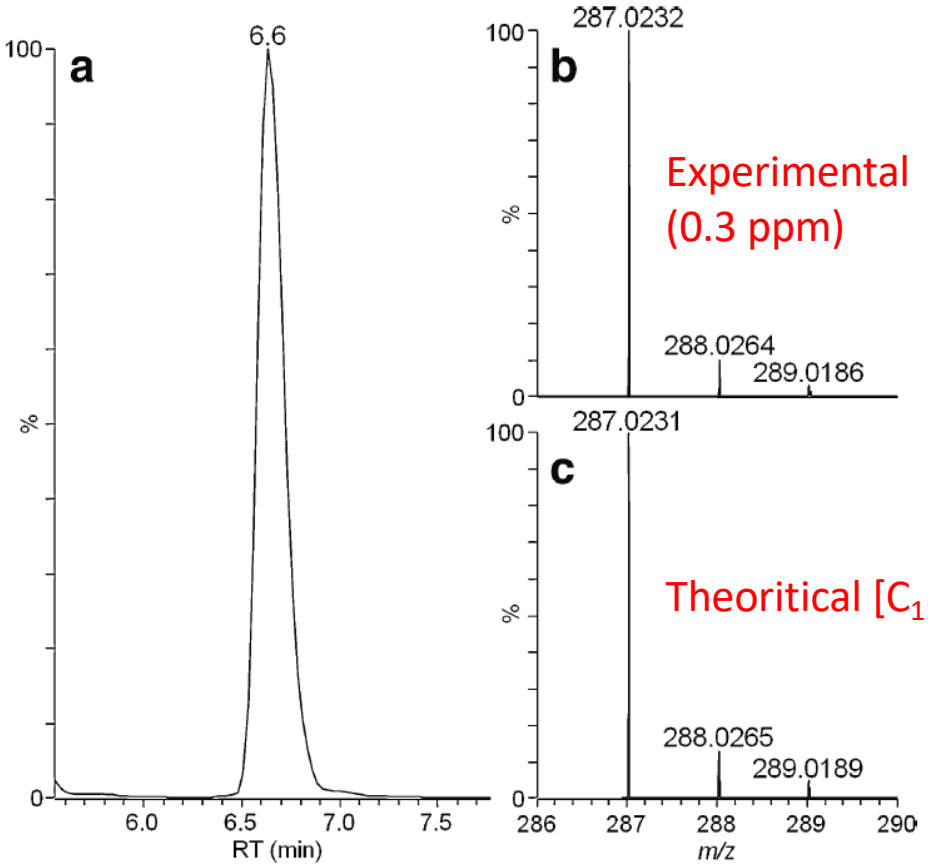
47 Pesticides: culture cartography, agricultural practices/surveys, period, region
459 Metabolites: according to: - litterature
- databases (EFSA)
- putative phase II metabolites

Taking advantage of high resolution MS analysis



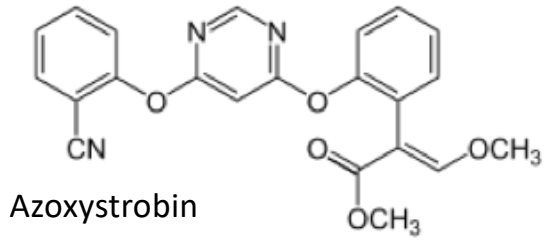
Detection of a suspected metabolite

Methyl-2-(2-hydroxyphenyl)-3-methoxyacrylate sulfate



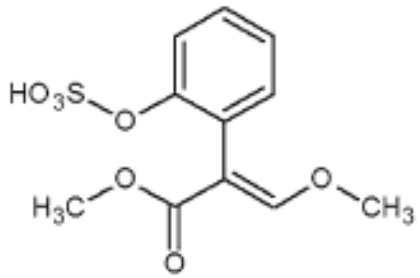
Experimental
(0.3 ppm)

Theoritical $[C_{11}H_{12}O_7S]^-$



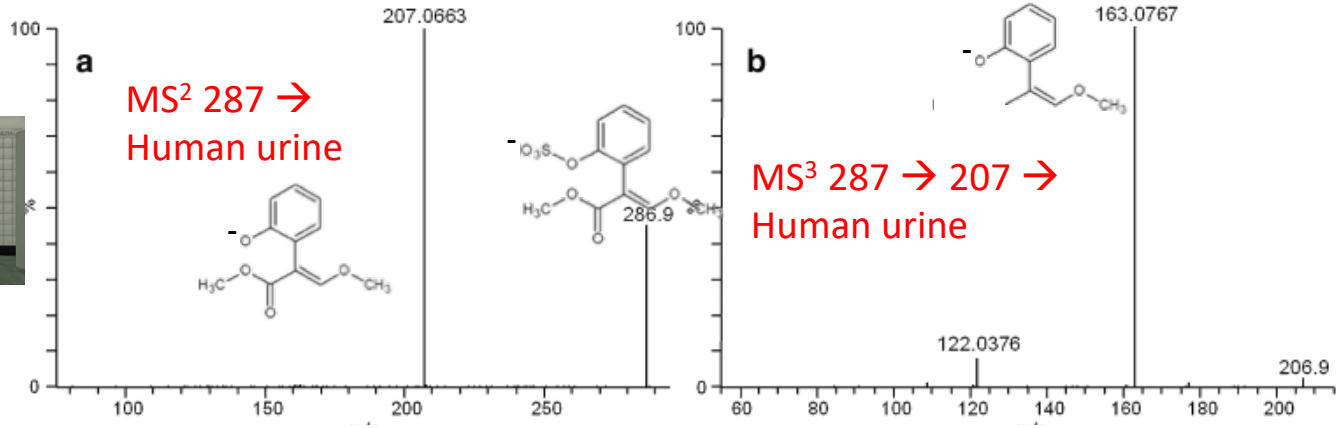
Azoxystrobin

?



Jamin E.L., Debrauwer L. *et al.* Anal. Bioanal. Chem. (2014) 406:1149-1161

Structural confirmation (“standard” compound for comparison)



Jamin E.L., Debrauwer L. *et al.* Anal. Bioanal. Chem. (2014) 406:1149-1161

Identifications from suspect screening

HRMS screening (±5ppm): 33 features in ESI(+)
128 features in ESI(-)

Identification: 1st step by MS^n (level3) [1] -> 24 putatively characterized compounds

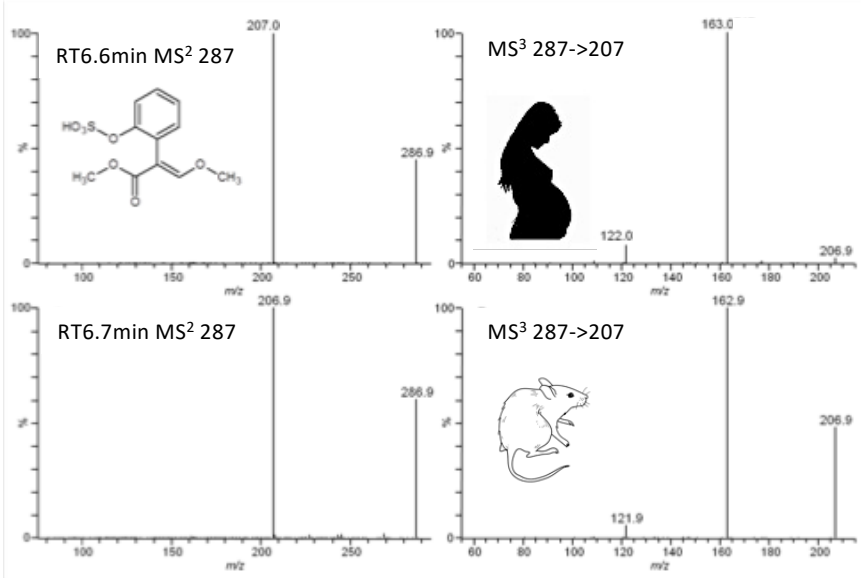
2nd step by comparison with standard (level1)
same RT + same HRMS + same MS/MS

- commercially available compounds

-> 1 validated and 1 invalidated

- not commercial metabolites

-> *in-vivo* biosynthesis of metabolites using a rat exposed to suspected pesticides



23 metabolites identified in ESI(-)

20 level1 & 3 level3

17 metabolites detected in more than 50% of samples

[1] Sumner L.M. *et al.* Metabolomics (2007) 3:211-221

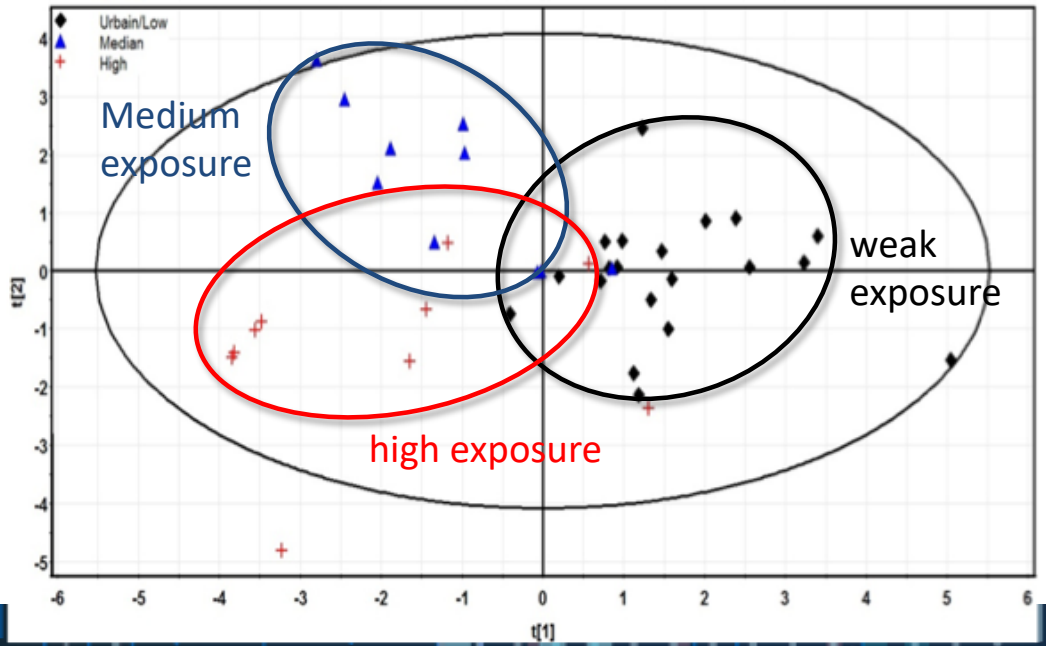
Statistics

Statistics on 23 identified metabolites in ESI(-):

1st step PCA (QC validation)

2nd step PLS-DA (OSC)

3 groups : “weak exposure (urban)” (*n*=20) ; “medium” (*n*=10) ; “high exposure (rural)” (*n*=10)



R^2=51.9%

Q^2=0.359

Validated (permutation test)

PELAGIE: results

VIP>1 ; KW<0.05

High p-value
Weak contribution to groups discrimination
(carbofuran metabolites)

Metabolite	Pesticide	*p*-value	Weak->Medium	Weak->High	Medium->High
methyl-2-(2-hydroxyphenyl)-3-methoxyacrylate sulfate	**Azoxystrobin**	**6.5E-06**	↗	↗	=
2-methyl-2-phenylpropanoic acid	**Fenpropimorph**	**2.2E-05**	↗	↗	=
methyl-2-(2-hydroxyphenyl)-3-methoxyacrylate glucuronide (1)	**Azoxystrobin**	**9.6E-05**	↗	↗	=
methyl-2-(2-hydroxyphenyl)-3-methoxyacrylate glucuronide (2)	**Azoxystrobin**	**6.3E-05**	↗	↗	=
3,3-dimethyl-2,3-dihydro-1-benzofuran-7-ol sulfate	Carbofuran	0.0197	=	↗	=
3,3-dimethyl-2,3-dihydro-1-benzofuran-7-ol glucuronide	Carbofuran	0.0409	↗	=	=
7-hydroxy-2,2-dimethyl-1-benzofuran-3(2H)-one glucuronide	Carbofuran	0.0033	=	↗	↗

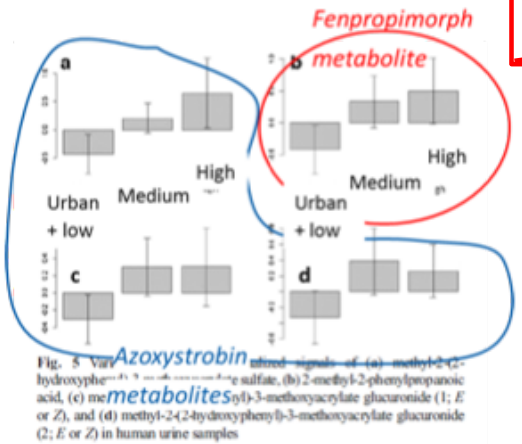
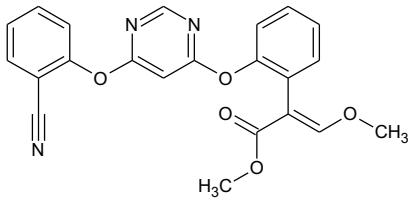
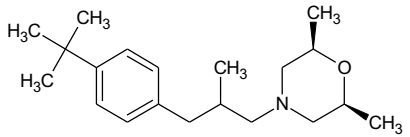


Fig. 5 Variance-normalized signals of (a) methyl-2-(2-hydroxyphe…e sulfate, (b) 2-methyl-2-phenylpropanoic acid, (c) me…yl)-3-methoxyacrylate glucuronide (1; *E* or *Z*), and (d) methyl-2-(2-hydroxyphenyl)-3-methoxyacrylate glucuronide (2; *E* or *Z*) in human urine samples

Azoxystrobin



Fenpropimorph



azoxystrobin: (*strobilurin*) fungicide for **cereals/vegetable crops**

fenpropimorph : (*morpholin*) fungicide for **cereals**

Azoxystrobin and fenpropimorph present as a mixture in some commercial formulations

[1] Jamin E.L. *et al.* Anal. Bioanal. Chem. (2014) 406:1149-1161

Second study case: the BioNutrinet cohort (*n*=28,245)

BioNutrinet:
Contribution of organic food to the Diet [1]
Conducted in France since 2009

Urinary samples: *n*=300
150 organic food *versus* 150 conventional food consumers
(subjects matched according to propensity score)
210 women and 90 men

Signal decrease: cleaning of ESI source
8 batches of ~40 samples + QC

Suspect list: 102 pesticides including molecules allowed in organic cultures + 1146 metabolites (known + putative)

[1] Baudry J. *et al.* Nutriments (2015) 7:8615–8632

Same suspect screening approach

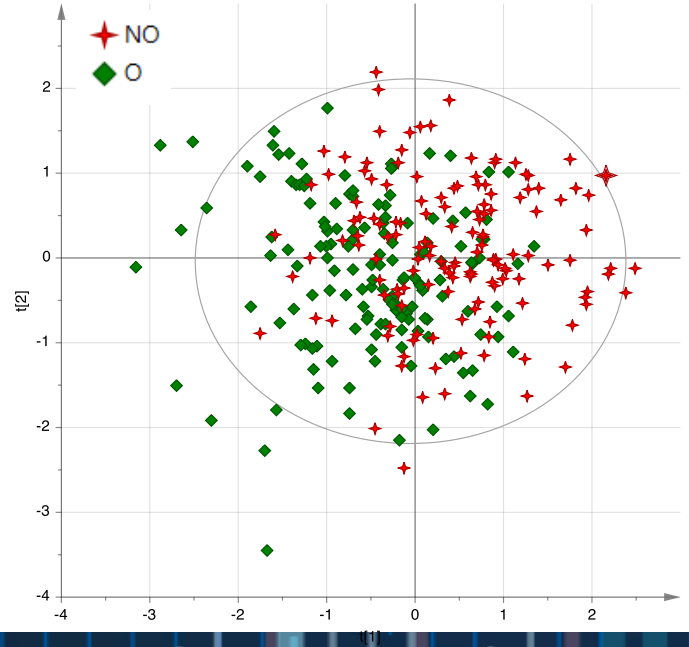
BioNutrinet: suspect screening

Statistics on 68 identified metabolites (level1 & 3):

1st step: batch correction using QC

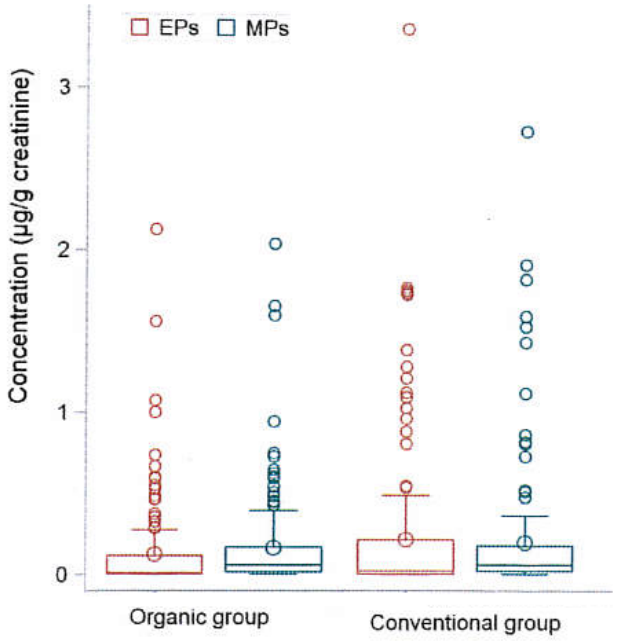
2nd step: PLS-DA (OSC)

2 groups: "organic" (*n*=150) *versus* "conventional" (*n*=150)

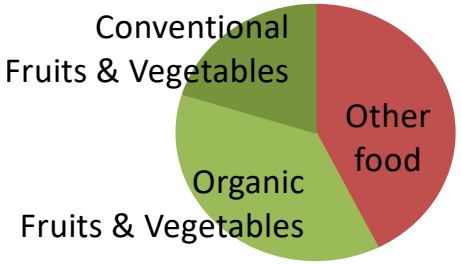


BioNutrinet: targeted analyses

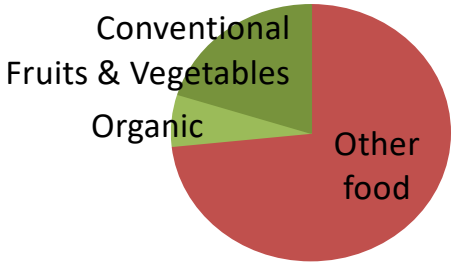
Quantification of metabolites of organophosphorus pesticides [1]



-> significant but weak differences



Organic food consumer



Conventional food consumer

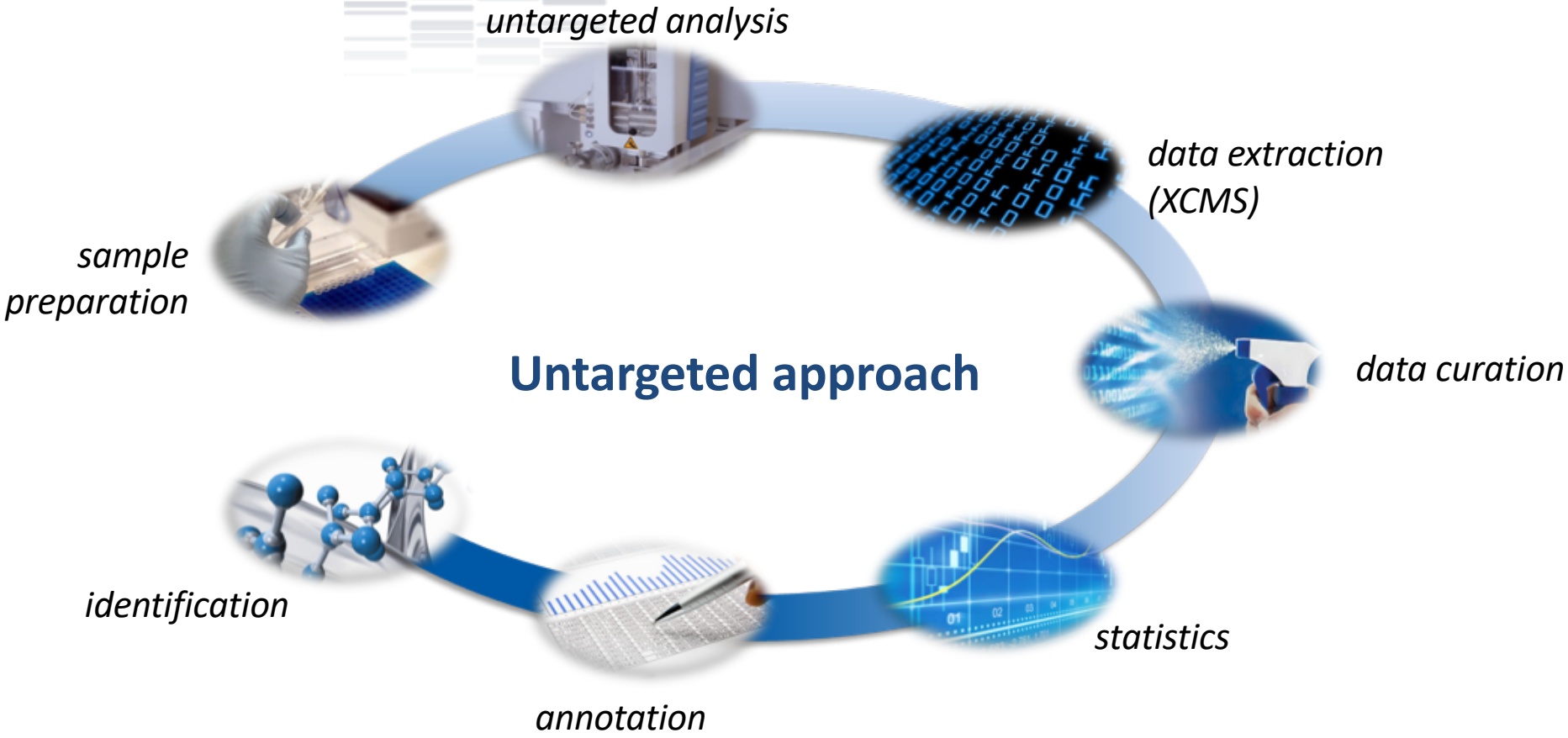
Fruits and vegetables consumption higher in organic food consumers

Similar absolute consumption of conventional fruits and vegetables

-> both groups should be differentiated according to other determinants (diet ?)

[1] J. Baudry *et al.* Environ. Health (submitted)

BioNutrinet: untargeted approach

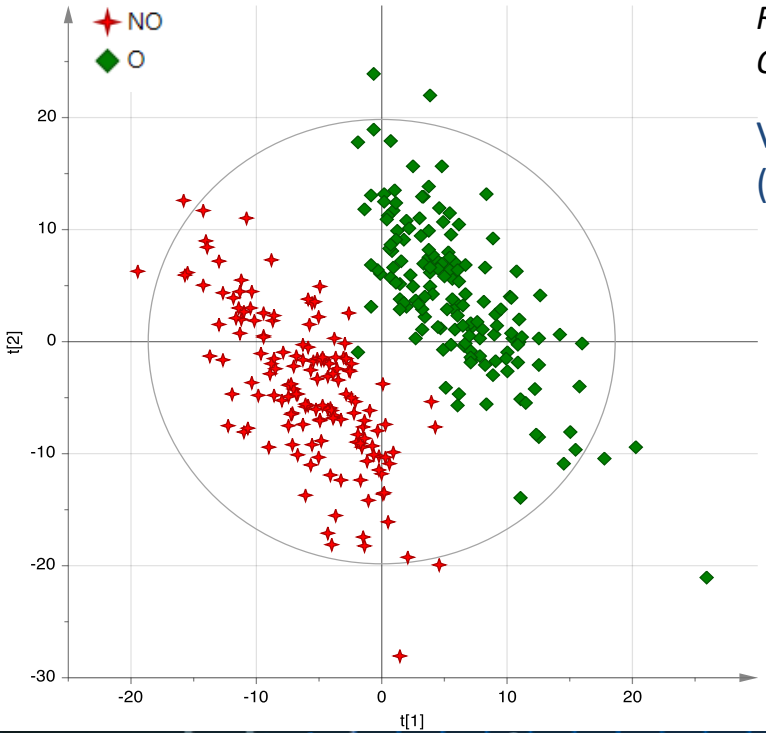


BioNutrinet: results

Statistics on 10420 features in ESI(-) and ESI(+):

1st step: batch correction using QC

2nd step: PLS-DA (OSC)



R^2=94,9%

Q^2=0.492

Validated
(permutation test)

Identification of significant metabolites

under progress

-> some endogenous metabolites
(carnitines, dimethylguanosine, etc.)
→ Suggests different metabolic status

-> metabolites from food
(citrus, cocoa, plant hormones, etc.)
→ Highlights different diet habits

-> only 2 metabolites of pesticides
(azoxystrobin, napropamide)
→ Relevance for biomonitoring ?

Take home messages

- **Suspect screening: setup of a powerful workflow allowing**

the characterization of a particular exposure:

- environmental exposure to pesticides according to the distance to the fields
- dietary exposure to pesticides according to organic / conventional food consumption

the detection of unexpected pesticides (for possible inclusion in biomonitoring lists):

- e.g. azoxystrobin, fenpropimorph, napropamide

the validation of identifications by "*in-vivo* biosynthesis":

- metabolism study of suspected pesticides using *e.g.* rodents

- **Untargeted analysis of the same datasets allowing**

a wider characterization of the exposome including other contaminant classes as well as food metabolites:

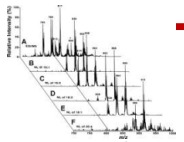
- differentiation of organic food consumers according to the proportion of fruits and vegetables in their diet

the detection of endogenous metabolites (input of complementary NMR analyses):

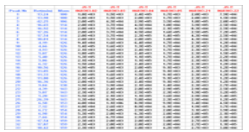
- access to the metabolic status of the studied population groups

Towards an integrated workflow

NMR
600 MHz
Cryoprobe



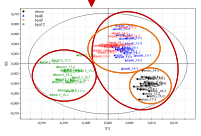
Data treatment



Workflow4metabolomics

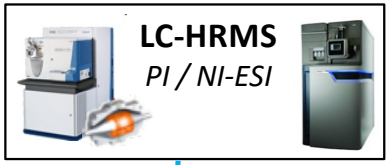
Metabolic fingerprints

Multivariate statistical analyses



Discriminating variables (biomarkers)

LC-HRMS
PI / NI-ESI



Metabolite suspect screening

"exposomics"

Structural identification

List of identified metabolites

Classification of groups according to their metabolic status

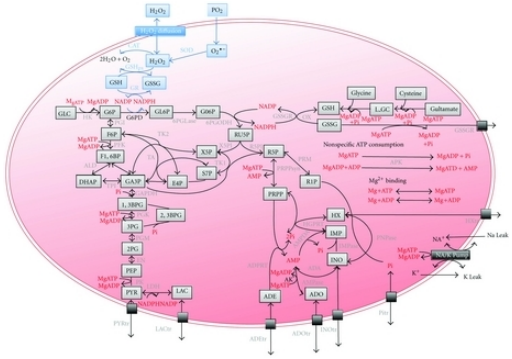
Classification of groups according to their impregnation by contaminants

Conclusion : contribution of (NMR/MS)metabolomics for:

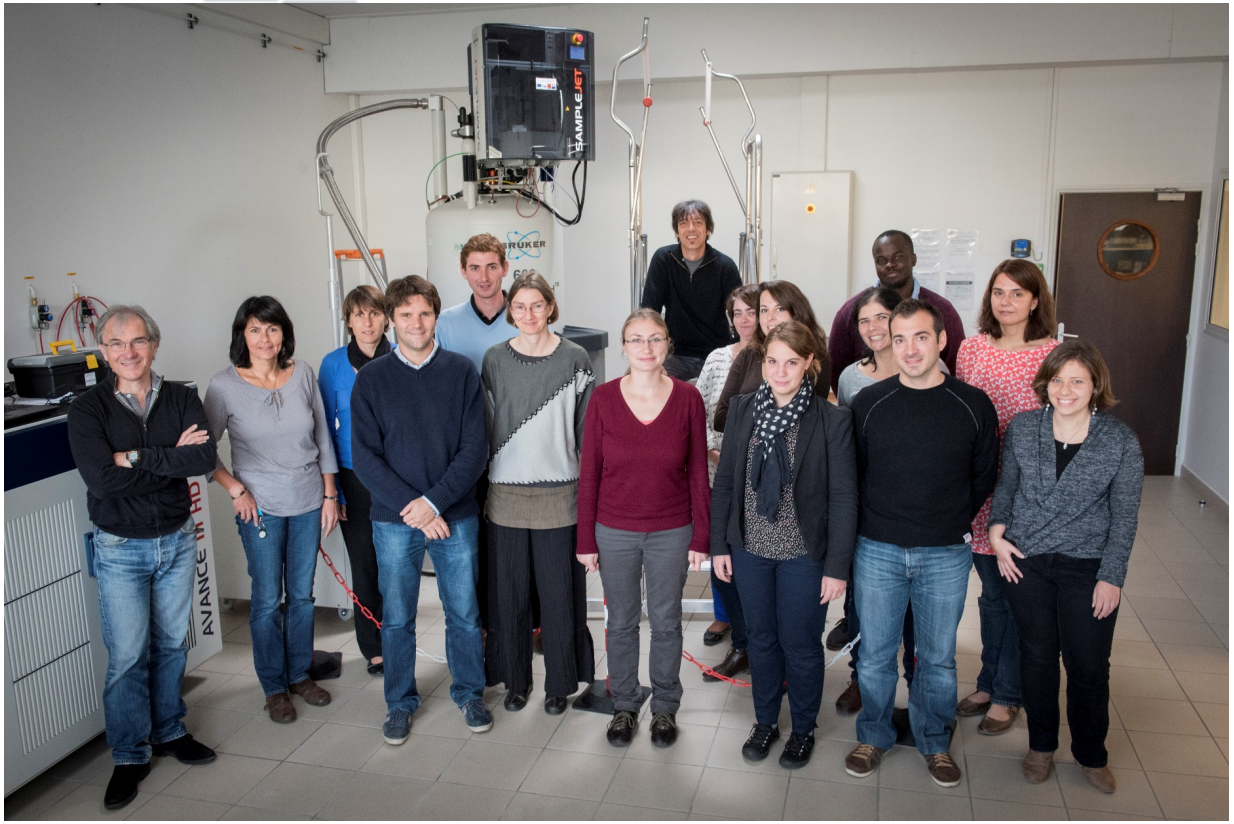
Assessing the exposure to pesticides from biofluids in an untargeted way

Studying the influence of exposure to multiple pesticides on the organism

Deciphering mechanistic pathways which could be involved in the metabolic changes observed



Thank you for your attention



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Thanks to : E. Jamin, A. Delcambre, J.F. Martin, N. Bonvallot, E. Kesse-Guyot, J.P. Cravedi

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