Untargeted high resolution mass spectrometry for characterizing environmental exposure of pregnant women to pesticides

JAMIN E[1,2,3], BONVALLOT N[4,5,*], MARTIN JF[1,2,3], CRAVEDI JP[2,3], MERCIER F[4,5,6], LE BOT B[4,5,6], CHEVRIER C[4,7], DEBRAUWER L[1,2,3]

[1]MetaboHUB-MetaToul-AXIOM platform, Toulouse, France; [2]INRA, UMR1331, Toxalim, Research Centre in Food Toxicology, Toulouse, France ; [3]Toulouse University, INP, France ; [4]INSERM UMR 1085 IRSET, Rennes, France ; [5]EHESP School of Public Health, Rennes – Sorbonne Paris Cité, France ; [6]Environment and health research laboratory (LERES), Rennes, France ; [7]University of Rennes 1, France; * Corresponding email: laurent.debrauwer@toulouse.inra.fr

CONTEXT AND AIM OF THE WORK

Risk assessment related to exposure to chemical cocktails is of growing concern and requires powerful exposure assessment methods. However, human exposure to chemicals (among which pesticides largely used to control pests & diseases) is generally estimated by indirect methods, and human health risks associated with multi-exposure to complex mixtures are currently under-explored. Biological monitoring usually concerns a limited number of compounds monitored in a targeted way, whereas untargeted approaches should provide a wider and more relevant exposure assessment.

Characterization of pesticide exposure still represents a challenge since amounts of biological specimens available are low, and searching for possible compounds has to be as thorough as possible. In addition, from urine samples, contaminants such as pesticides are generally detected as metabolites whose structures may be unknown.

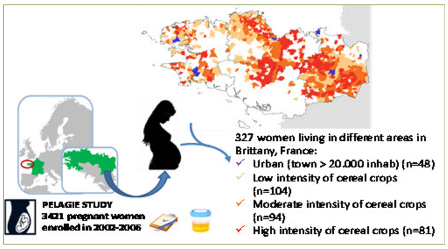
This project aims to develop a new strategy for the characterization of complex pesticide exposures of pregnant women using samples from the French PELAGIE epidemiological cohort. Our workflow integrates (i) untargeted data acquisition using UHPLC-HRMS in both positive and negative ESI modes, (ii) generation of an upgradable list of metabolites to seek from urine samples, (iii) structural identification and / or confirmation of metabolites by comparison of MS/MS spectra with those of standards and metabolites generated by animal experiment, and (iv) statistical treatment of data.

METHODS

➲ **PELAGIE** study: to evaluate the consequences of the exposure to contaminants on pregnancy and child health (Chevrier 2011): 3421 **pregnant women** living in a French area with high agricultural activities (Brittany) → **327 women selected** according to the availability of a **urinary sample** collected at the early pregnancy (2004) and stored in the same conditions. Women living in different areas with more or less agricultural activities (fig.1).

➲ Samples diluted twice in mobile phase A. Analysis by **UHPLC-HRMS** (C18 stationary phase, ESI ionization, LTQ-Orbitrap HRMS or MS^n), data processing with the MetWorks software (Thermo Scientific), previously described method (Jamin 2014). Extraction & integration of HRMS signals of **74 pesticides**/wetting agents (& their known/ theoretical metabolites) identified from agricultural practices and recommendations in 2004 (table 1) → **507 signals monitored**. Confirmation of detected compounds by MS^n experiments and comparison with metabolites generated *in vivo*.

The French PELAGIE study



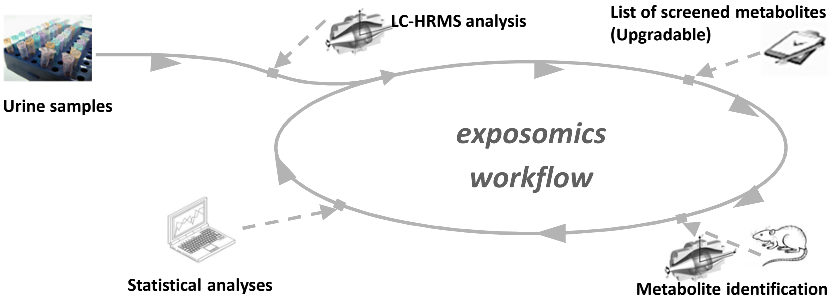
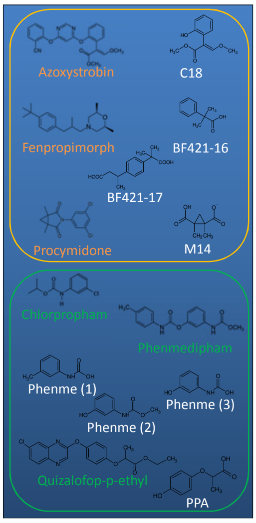


Table 1: list of pesticides for which HRMS urinary signals were studied :

Family/ use	Name
Organophosphorous insecticides	Chlorpyrifos, chlorpyrifos-methyl, dichlorvos, diazinon, malathion, metidathion, omethoate, dimethoate, azinphos-methyl, naled, phosalone, phorate, acephate, fenitrothion, oxydemethon-methyl
Carbamate insecticides or herbicides	Carbendazim, carbofuran, carbosulfan, benfuracarb, pyrimicarb, mancozeb, maneb, chlorpropham
Pyrethroid insecticides	Cyfluthrin, permethrin, cypermethrin, cyhalothrin, deltamethrin, phenothrin, esfenvalerate
Other insecticides	Imidacloprid, teflubenzuron, propargite
Chloroacetamide herbicides	Acetochlor, dimetachlor, metazachlor, propachlor, S-metolachlor
Other herbicides	Quizalofop-p-ethyl, fluazifop-p-butyl, trifluralin, clomazone, glyphosate, linuron, isoproturon, napropamide, phenmedipham, bromoxynil
Triazole fungicides	Epoxiconazole, metconazole, paclobutrazole, tebuconazole
Strobilurine fungicides	Azoxystrobin, krezoxim-methyl
Other fungicides	Fenpropimorph, iprodione, procymidone, pencycuron, thiophanate-methyl, prochloraz, imazalil, captan, thiram, anthraquinone, chlormequat, ethephon, chlorothalonil, cymoxanil
Alkylphenols as wetting agents	Nonylphenol, octylphenol, nonylphenol mono- and di-ethoxylated, octylphenol mono- and di-ethoxylated

RESULTS

Identified Metabolites	Detection frequency (%)
Azoxystrobin C18sulf	98,2
Azoxystrobin C18gluc(2)	79,8
Azoxystrobin C18gluc(1)	68,8
Fenpropimorph BF421.17gluc	100,0
Fenpropimorph BF421.16OHsulf	97,2
Fenpropimorph BF421.16OH(1)	93,9
Fenpropimorph BF421.16OHgluc(1)	91,7
Fenpropimorph BF421.16gluc	88,8
Fenpropimorph BF421.16OH(2)	88,7
Fenpropimorph BF421.16sulf	87,5
Fenpropimorph BF421.16OHgluc(2)	83,5
Fenpropimorph BF421.16	81,2
Fenpropimorph BF421.17	78,6
Fenpropimorph BF421.3gluc	70,9
Fenpropimorph BF421.17sulf	59,9
Phenmedipham_gluc (1)	97,9
Phenmedipham_sulf (1)	94,2
Phenmedipham_gluc (2)	88,4
Phenmedipham_sulf (3)	87,5
Phenmedipham_sulf (2)	82,6
Procymidone M14	99,1
Quizalofop-p-ethyl PPAgluc	94,8
Quizalofop-p-ethyl PPAsulf(1)	64,2
Quizalofop-p-ethyl PPAsulf(2)	60,7
Chlorpropham_OH gluc	2,4
Chlorpropham_OH sulf	1,8



Study population (n=327) is mostly 25-35 years old (~80%) with a high educational level (post-secondary, 60%). Median BMI is 22.5. >70% didn't smoke while 13% stopped during the first trimester. ~15% are living in urban areas and 25% in areas with high intensity of cereal crops.

Among the 507 monitored potential pesticides / metabolites, > 70 were detected and **28 pesticide metabolites were identified**. Identifications were achieved by comparison of MS^n results with standard compounds when available or with metabolites generated *in vivo* by animal experimentation. Identified metabolites corresponded to **6 pesticides used in agricultural lands in 2004**: **3 fungicides: azoxystrobin, fenpropimorph, procymidone; and 3 herbicides: quizalofop-p-ethyl, chlorpropham and phenmedipham.**

Some of these pesticides are characterized according to several metabolites with comparable detection frequencies, reinforcing the confidence in pesticide identification.

The 2 metabolites derived from chlorpropham have a low frequency of detection (~2%) and 2 metabolites of carbofuran were detected below the quantification limits.

This UHPLC-HRMS method allowed to characterize many pesticide metabolites in an untargeted way in urine of pregnant women that are not routinely measured in environmental health studies, while the majority has focused on several well-known pesticides.

These results represent a major step to improve research on mixtures. Statistical analyses are currently in progress to study association with exposure groups and targeted quantification of metabolites by LC-MS/MS.

CONCLUSION

- The untargeted acquisition method developed herein allowed evidencing urinary metabolites resulting from pesticide exposure. These metabolites may constitute new biomarkers of exposure to pesticides.
- Although based on an upgradable list of screened metabolites without need for sample re-analysis, the method is at best semi-quantitative and not fully without a priori yet.
- Some metabolites may be missed due to matrix effects. However, the inclusion of several metabolites for one pesticide in the screened list increases likelihood to evidence exposure to given pesticides.
- Statistical models built from LC-HRMS data are in progress for discriminating women groups according to factors such as women living area (urban/rural), percentage of cereal cultures in living area, etc…

REFERENCES

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