Untargeted metabolomics approach to characterize environmental exposure of pregnant women to pesticides by UHPLC-HRMS

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CONTEXT & AIM OF THE WORK

Pesticides are largely used to control pests & diseases in crops but current studies provide only a partial knowledge on the human exposure. Biological measurement is a mean of assessing environmental exposures by an integrated approach accounting for multiple sources/exposure routes. 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, pesticides are generally detected as metabolites whose chemical structures may be unknown. This project aims to develop a new strategy for the characterization of complex pesticide exposures of pregnant women.

MATERIAL & METHOD

➲ **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. These women are living in different areas with more or less agricultural activities (fig.1).

➲ Direct analysis by **UHPLC-HRMS** (C18 stationary phase, Electrospray ionization , LTQ-Orbitrap HRMS or MSn), data processing with the MetWorks software (Thermo Scientific), exposomic method previously described (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 MSn experiments and comparison with metabolites generated *in vivo*.

Figure 1: strategy for the geographical identification of exposure groups based on the PELAGIE cohort and agricultural data

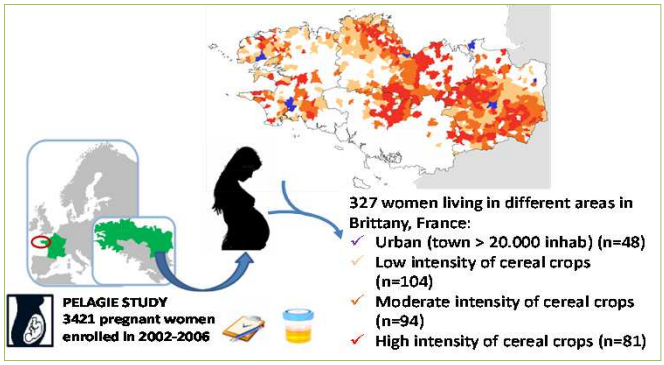

Table 1: list of pesticides for which HRMS urinary signals were studied :

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

Identified Metabolites	Detection frequency (%)
Azoxystrobin_sulf	98,2
Azoxystrobin_gluc metabo 2	79,8
Azoxystrobin_gluc metabo1	68,8
Fenpropimorph_gluc 10	100
Fenpropimorph_OH_sulf 7	97,2
Fenpropimorph_OH 3	93,9
Fenpropimorph_OH 5	91,7
Fenpropimorph_OH 4	89,3
Fenpropimorph_gluc 2	88,7
Fenpropimorph_sulf 8	87,5
Fenpropimorph_OH 6	83,5
Fenpropimorph 1	81,2
Fenpropimorph 9	78,6
Fenpropimorph_gluc 12	70,9
Fenpropimorph_sulf 11	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	99,1
Quizalofop-p-ethyl_gluc 1	94,8
Quizalofop-p-ethyl_sulf 1	64,2
Quizalofop-p-ethyl_sulf 2	60,7
Chlorpropham_OH gluc	2,44
Chlorpropham_OH sulf	1,83

FIRST RESULTS

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 masses, > 70 were detected and **28 pesticide metabolites were identified**. Identifications were achieved by comparison of MSn results with standard compounds when available or with metabolites generated *in vivo* by animal experimentations. Identified metabolites correspond to **7 pesticides used in agricultural lands in 2004**: **3 fungicides: azoxystrobin, fenpropimorph, procymidone; 3 herbicides: quizalofop-p-ethyl, chlorpropham and phenmedipham; 1 insecticide: carbofuran.** 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. Next steps consists in statistical analyses to study association with exposure groups and targeted quantification of metabolites by UHPLC-MS-MS.

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References: Chevrier et al. 2011. Environ Health Perspect 119(7):1034; Jamin et al. 2014. Anal & Bioanal Chem 406: 1149

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