SPRINT
SUSTAINABLE PLANT PROTECTION TRANSITION

da Silva Fernandes, Sylvina
University of Antwerp

16-weeks of exposure to a real-world, low-dose pesticide mixture in rats

Introduction & reasoning

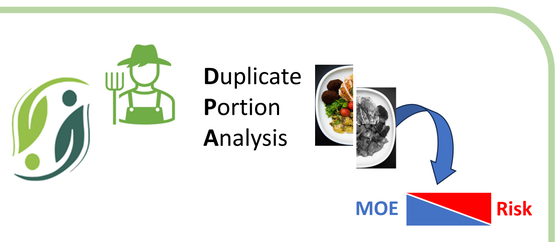
Farmers and their households are continuously exposed to **multiple Plant Protection Products** (PPP's) not only through occupational exposure, but also via the consumption of PPP-containing foods.

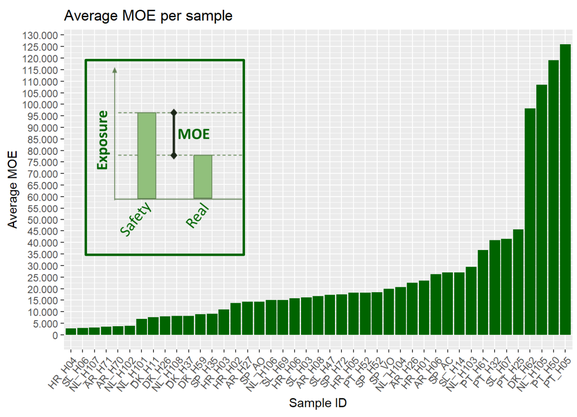
To investigate the potential health effects of such a real-world exposure situation, we interrogated the **Duplicate Portion Analysis** (DPA) data generated within SPRINT (WP2, WP3). This data contains information on what PPP's and at which doses these PPP's were present in the analyzed food.

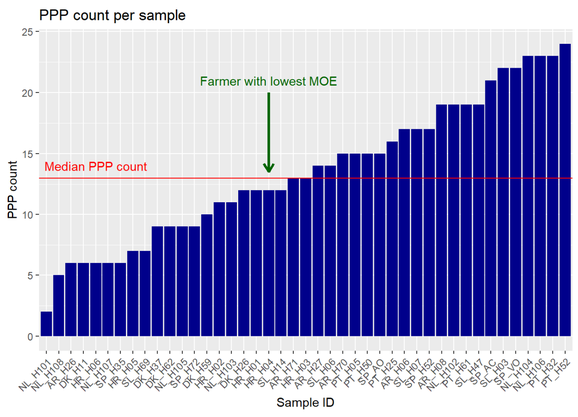
Using this data we identified the **most at risk farming household**, i.e. with the lowest average margin of exposure (MOE). According to the DPA this household was exposed to 12 different PPP's through food intake, which is close to the median of 13 PPP's. While Glyphosate was not detected in the food, we included this as a 13th PPP as it was detected in the faecal samples, suggesting oral intake.

Based on this list of 13 PPP's we set up a study with **three increasing exposure doses** including the real-world exposure (lowest) and two regulatory values (Acceptable Daily Intake (ADI) and ADI divided by 25).

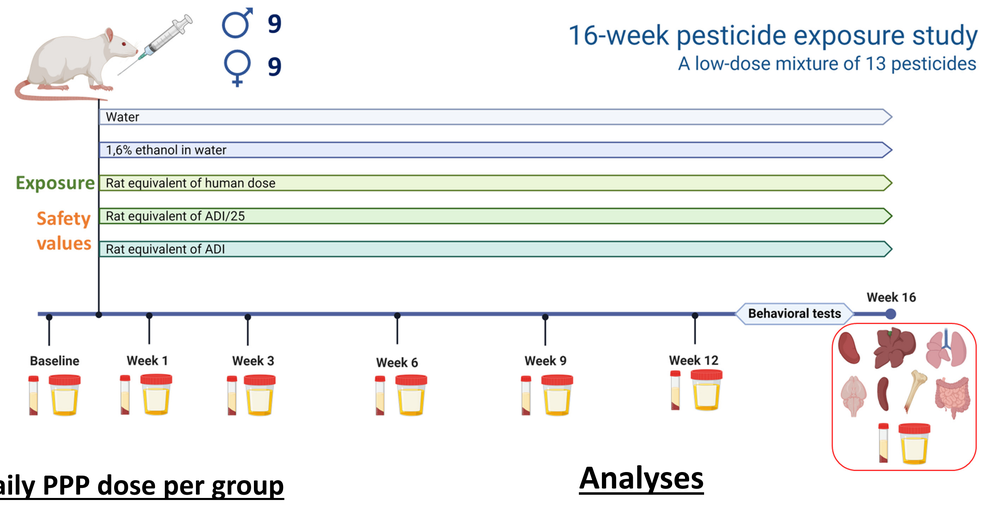
For each exposure group we re-calculated the human exposure values to an **Animal Equivalent Dose** (AED) for rat.







Study set-up & Methods



Daily PPP dose per group

PPP	AED exp	AED ADI/25	AED ADI
Acetamiprid	0,232	5,3	132
Boscalid	0,811	8,5	211
Dimethomorph	0,108	10,6	264
Fluopyram	0,060	2,5	63
Mandipropamid	0,063	31,7	793
Metalaxyl-M	0,050	16,9	423
Metamitron	0,237	6,3	159
Oxadixyl	0,027	2,1	53
Pirimicarb	0,556	7,4	185
Prothioconazole	0,707	2,1	53
Tebuconazole	0,097	6,3	159
Thiamethoxam	0,040	5,5	137
Glyphosate	1,599	105,7	2643

**Values = µg/kg bodyweight*
AED, Animal Equivalent Dose;
ADI, Accepted Daily Intake

Analyses

General clinical data: After 16 weeks of exposure animals were killed followed by blood and organ collection for analysis and evaluation of **general health effects** of PPP exposure.

Pathomics: We applied **Next-Generation Morphometry** on PAS-stained whole-slide images of **kidney** tissue to perform a high-throughput analysis on size and shape features of kidney structures, i.e. glomeruli and tubules.

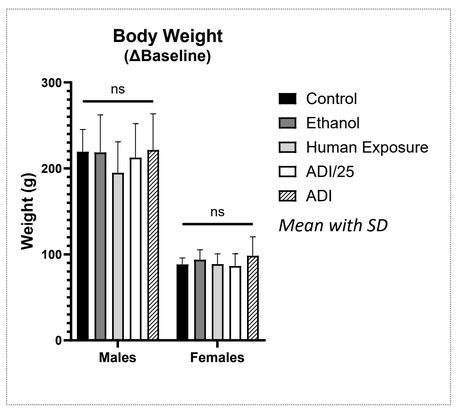
Open Field Test: As part of a battery of behavioural tests performed around week 13, we included the open field test to evaluate the general **explorative behaviour** of the rats upon PPP exposure.

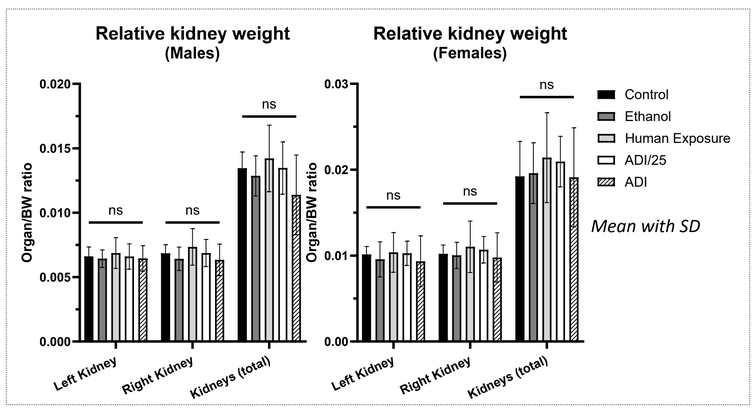
Preliminary Results

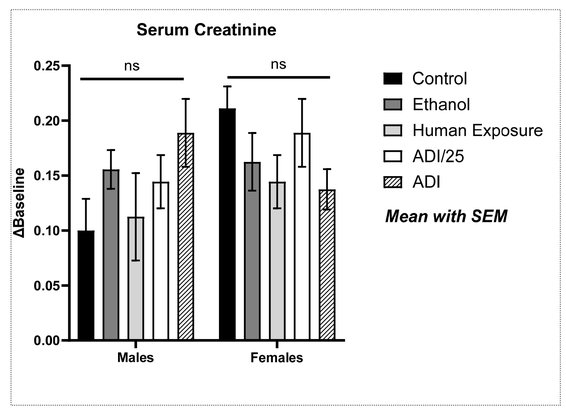
General health parameters

In blood samples we analyzed Na, Cl, K, iCa, HCO_3, Glucose, creatinine, Blood Urea Nitrogen (BUN), liver enzymes (ALP, AST, ALT) and Hemoglobin/Hematocrit. In general there were no major deviations from physiological control values in pesticide-exposed animals.

We highlight the lack of significant difference in body weight increase since the start of the exposure period, relative kidney weight and kidney function (creatinine). The absence of major clinical changes is not unexpected as the doses of exposure were relatively low.

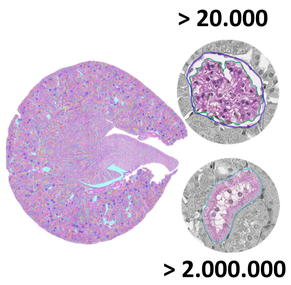


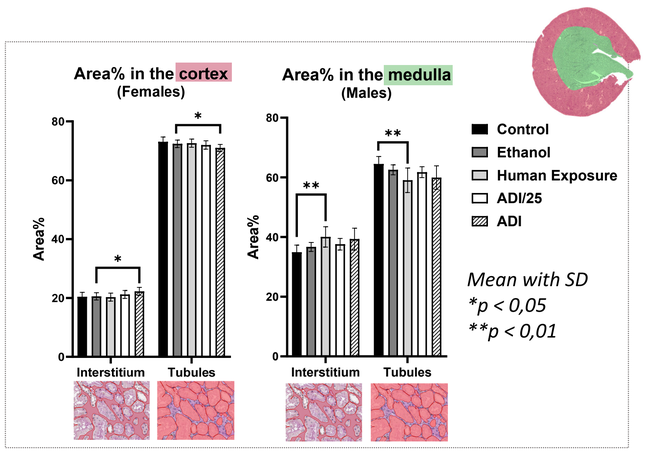


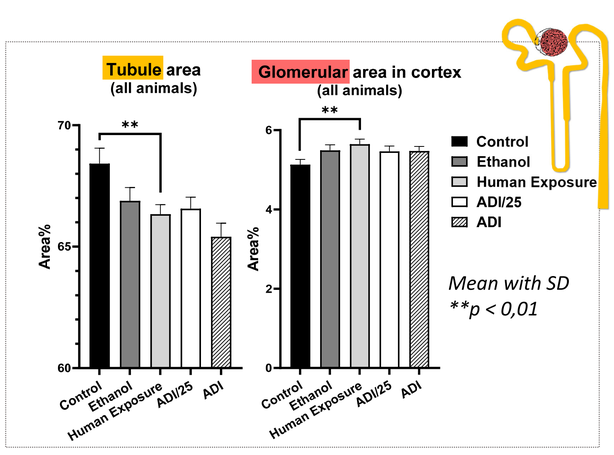


Kidney Next-Generation Morphometry

Parameter	Animals	Region	Comparison	Change
Tubular area	Males	Medulla	Human Exposure vs Control	↓
	Females	Cortex	ADI vs Ethanol	↓
	All	Overall	Human Exposure vs Control	↓
Interstitial area	Males	Medulla	Human Exposure vs Control	↑
	Females	Cortex	ADI vs Ethanol	↑
Glomerular area	All	Cortex	Human Exposure vs Control	↑

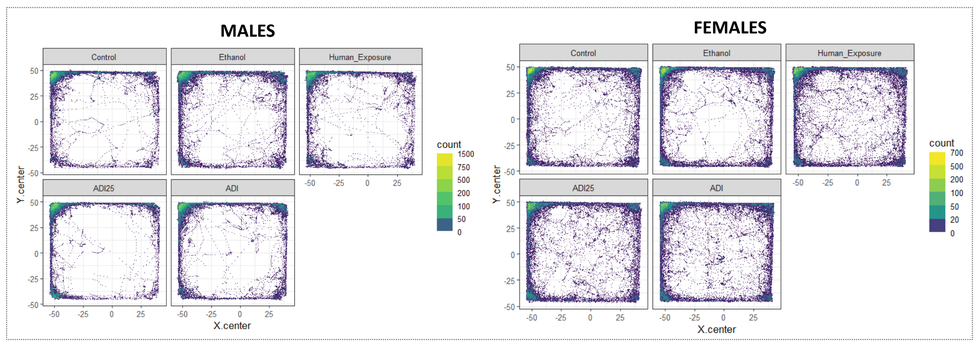


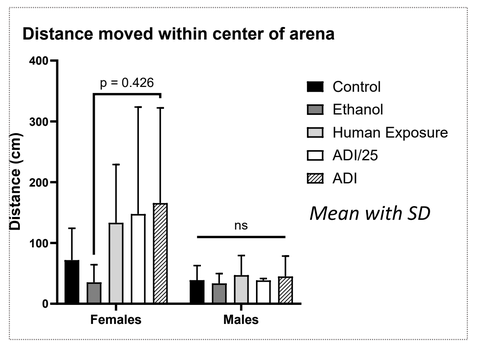




Open Field Test

The **heatmaps** on the right represent the **time** the rats spent in different **locations** of the **arena**. The more bright yellow/green the area, the longer or more often rats were in this region. In general all animals seem to prefer the **home corner** = the corner where animals are introduced to the set-up at the start of the test. Females also tend to travel more within the center of the arena as compared to males ($p < 0.05$, Two-way ANOVA).





Conclusion and Perspectives

Here we present preliminary results on the **potential health effects of a low-dose, real-world mixture of 13 PPP's** following 16 weeks of oral exposure in rats. While no negative effects were noted on a preliminary subset of general health parameters, it is clear that sub-clinical changes have occurred because of PPP exposure.

Next steps include histological analysis of other collected organs (bone, liver, spleen, lung, gut) and data-driven selection and analysis of organ-specific biomarkers in serum, urine and/or plasma to detect more subtle molecular changes. In the future, we aim to provide a full report on the potential health effects of this real-world PPP mixture.

UNIKLINIK RWTH AACHEN Institut für Pathologie
University of Antwerp
SPRINT SUSTAINABLE PLANT PROTECTION TRANSITION
LaBooratory of Nephropathology
University of Antwerp
Cell death signaling lab
Kidney team
fwo Research Foundation Flanders
Opening new horizons