SPRINT Conference

Brussels – 25 June 2025

SPRINT

SUSTAINABLE PLANT PROTECTION TRANSITION

Human Toxicology Studies

Daniele Mandrioli, MD, PhD
Cesare Maltoni Cancer Research Center
Ramazzini Institute, Italy

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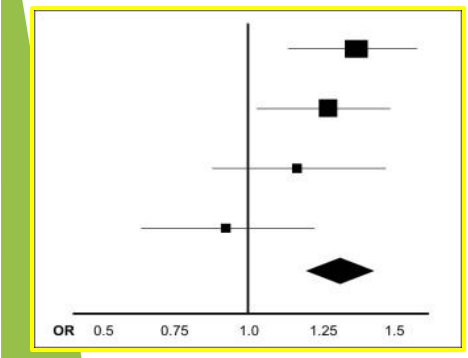
Work Done and Results: Tiered Approach



- Tier 1: Assessment of the available data and in silico modelling

- Tier 2: In vitro testing

- Tier 3 In vivo Texting

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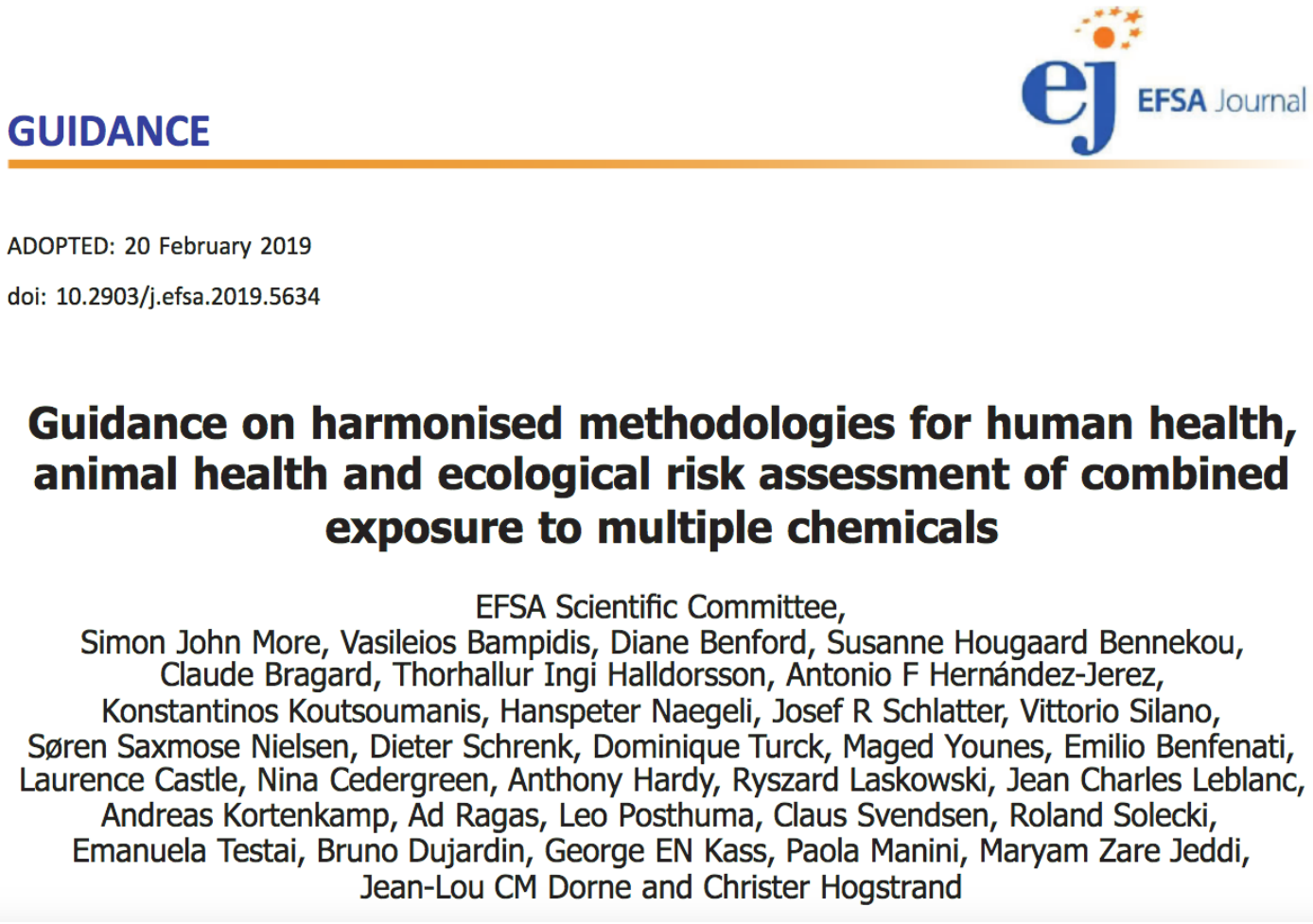
Work Done and Results: Tiered Approach (Component-based)



GUIDANCE

EFSA Journal

ADOPTED: 20 February 2019

doi: 10.2903/j.efsa.2019.5634

Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals

EFSA Scientific Committee,
Simon John More, Vasileios Bampidis, Diane Benford, Susanne Hougaard Bennekou, Claude Bragard, Thorhallur Ingi Halldorsson, Antonio F Hernández-Jerez, Konstantinos Koutsoumanis, Hanspeter Naegeli, Josef R Schlatter, Vittorio Silano, Søren Saxmose Nielsen, Dieter Schrenk, Dominique Turck, Maged Younes, Emilio Benfenati, Laurence Castle, Nina Cedergreen, Anthony Hardy, Ryszard Laskowski, Jean Charles Leblanc, Andreas Kortenkamp, Ad Ragas, Leo Posthuma, Claus Svendsen, Roland Solecki, Emanuela Testai, Bruno Dujardin, George EN Kass, Paola Manini, Maryam Zare Jeddi, Jean-Lou CM Dorne and Christer Hogstrand

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WP 4.3 Toxicology (Human Health)

Tier 1: Assessment of the available data and in silico modelling (RAM, WR, RU):

- Top 20 components of concern. PPPs prioritized using weighted hazard quotient (wHQ), based on occurrence data from SPRINT CSS data and EU Pesticide Database (ADI and AOEL).

Tier 2: in vitro testing (RAM, WR, WU):

- Top 10 components of concern from Tier 1
- EFSA TESTS: cytotoxicity (ISO 10993-5), in vitro mammalian micronuclei test (OECD TG 487).
- NEW SPRINT INDICATORS: organoid models, kidney cell lines.

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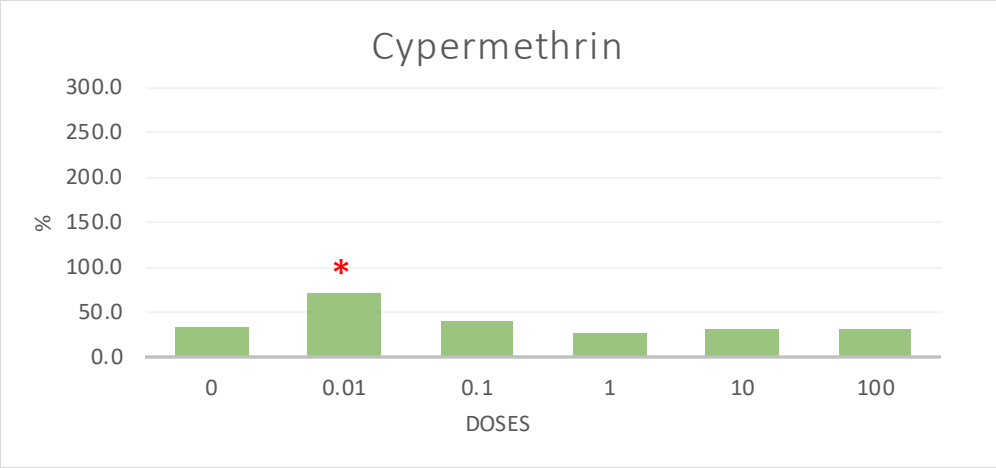
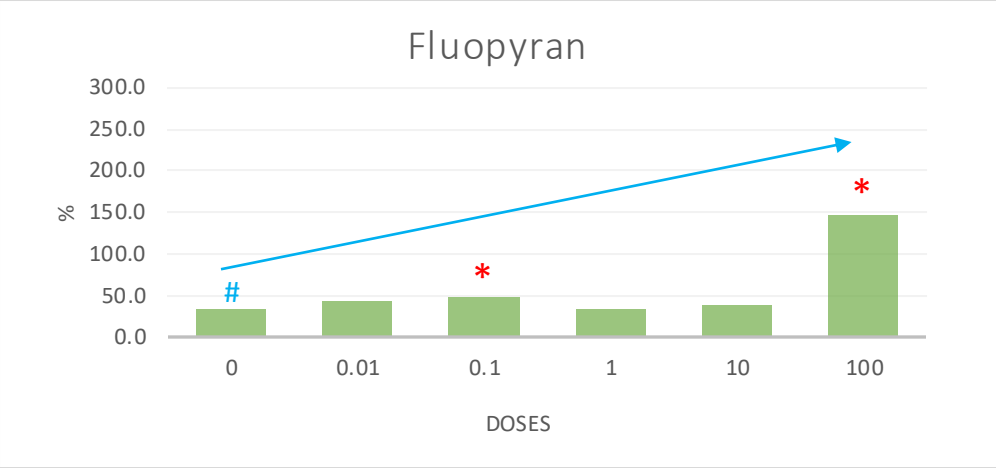
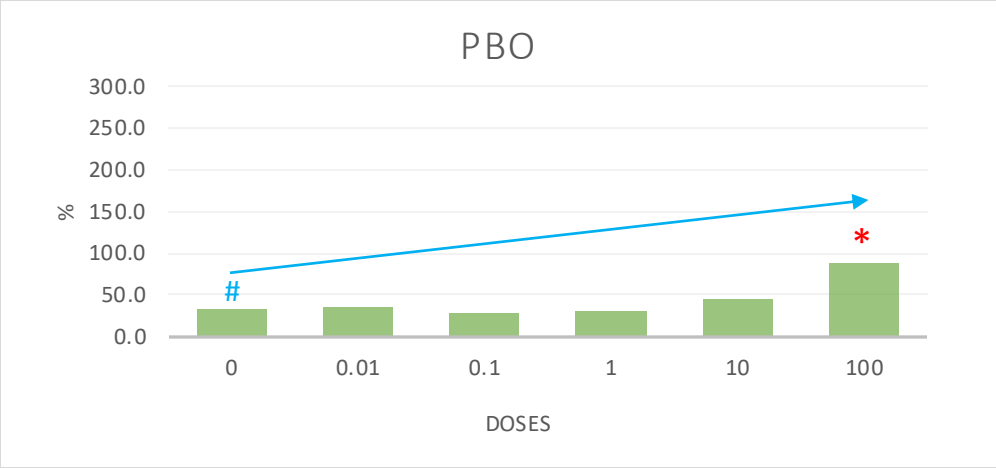
MICRONUCLEI







Substance	Dose	Binucleated cells	Micronuclei	% Cytotoxicity
Ctrl CYTO B	0	1027	34	
Imazalil	0.01	1008	14	38.82
Imazalil	0.1	1024	12	39.34
Imazalil	1	1010	13	38.66
Imazalil	10	1011	12	42.00
Imazalil	100	128	13	82.61

No statistical analysis was performed due to the high citotoxicity and low number of micronuclei observed

Statistical analysis:

one-sided Cochran-Armitage trend test;

* pairwise comparisons of the incidence of micronuclei over binucleated cells (1-sided Fisher's exact test)

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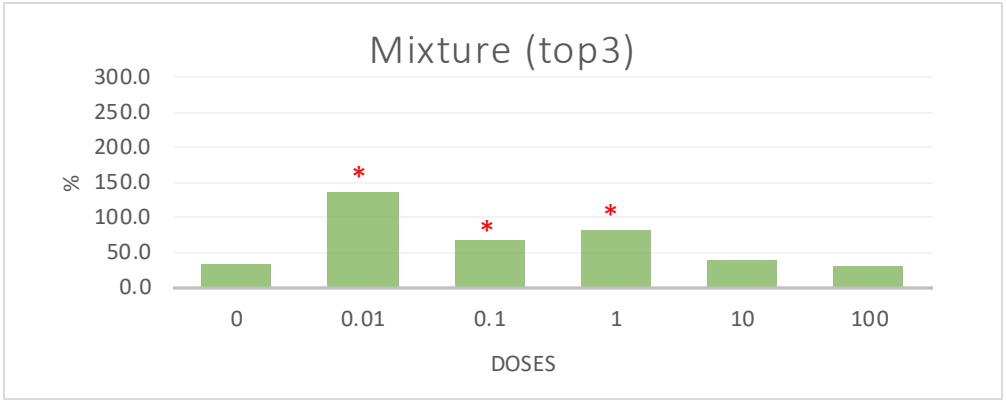

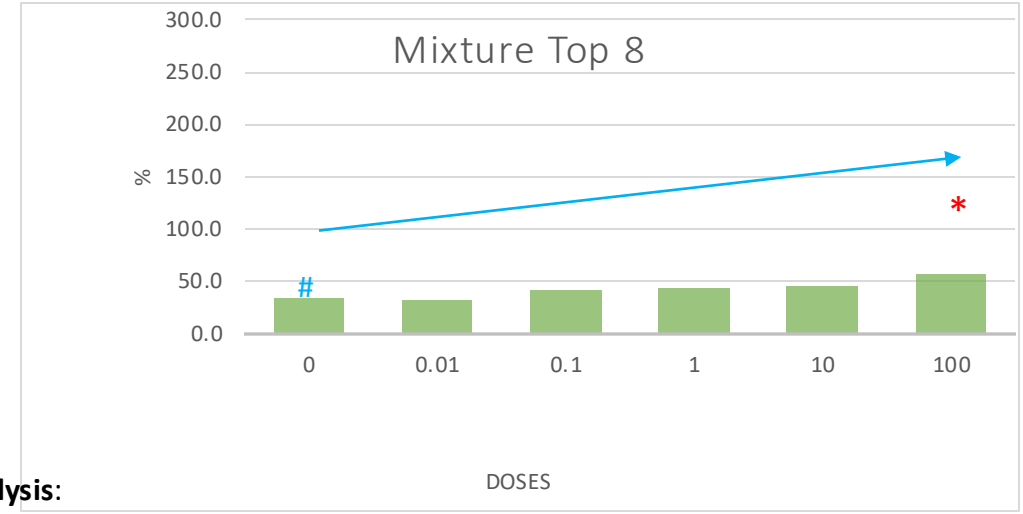

Statistical analysis:

one-sided Cochran-Armitage trend test;

* pairwise comparisons of the incidence of micronuclei over binucleated cells (1-sided Fisher's exact test)

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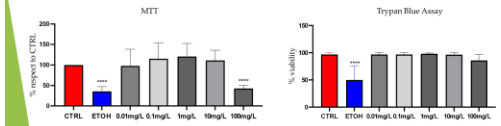
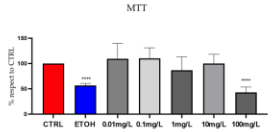
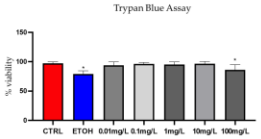
CITOTOXICITY

LAMBA-CYHALOTHRIN

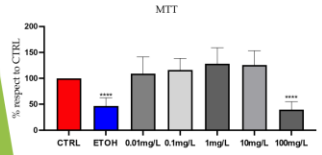


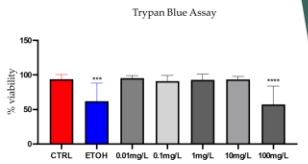
CYPERMETHRIN



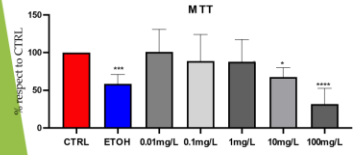


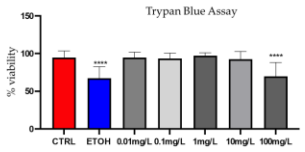
DELTAMETHRIN





TEBUCONAZOLE





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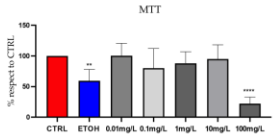
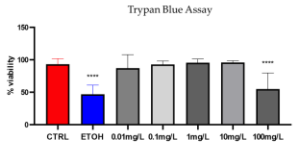
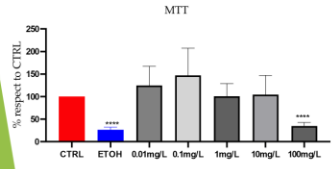
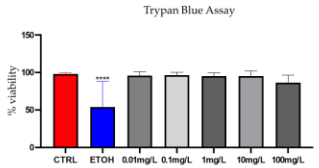
GLYPHOSATE



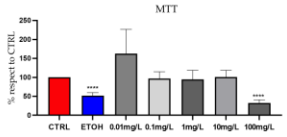


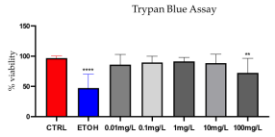
ACETAMIPRID



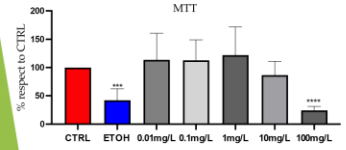


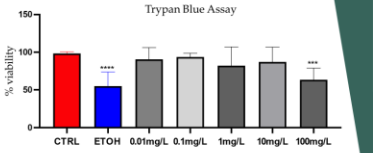
CYPRONIDIL





PYPERONIL BUTOXIDE





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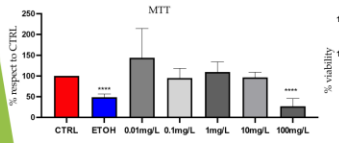
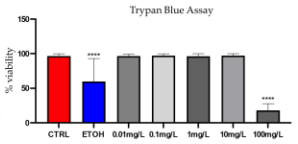
IMAZALIL





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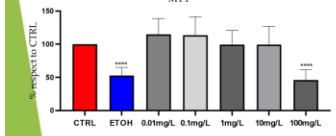
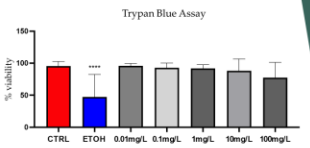
FLUOPYRAN





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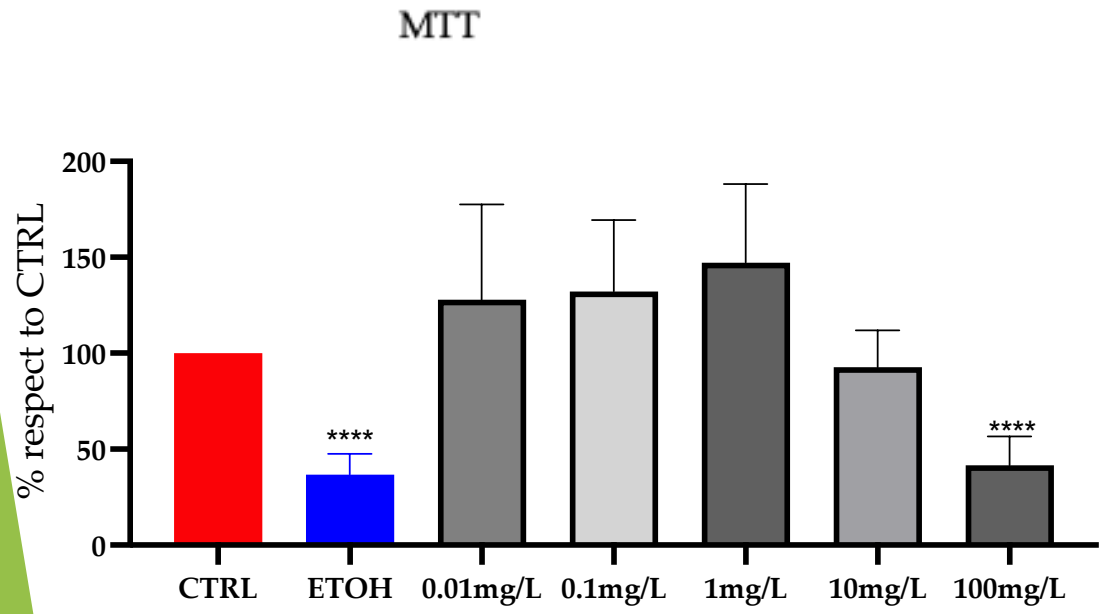
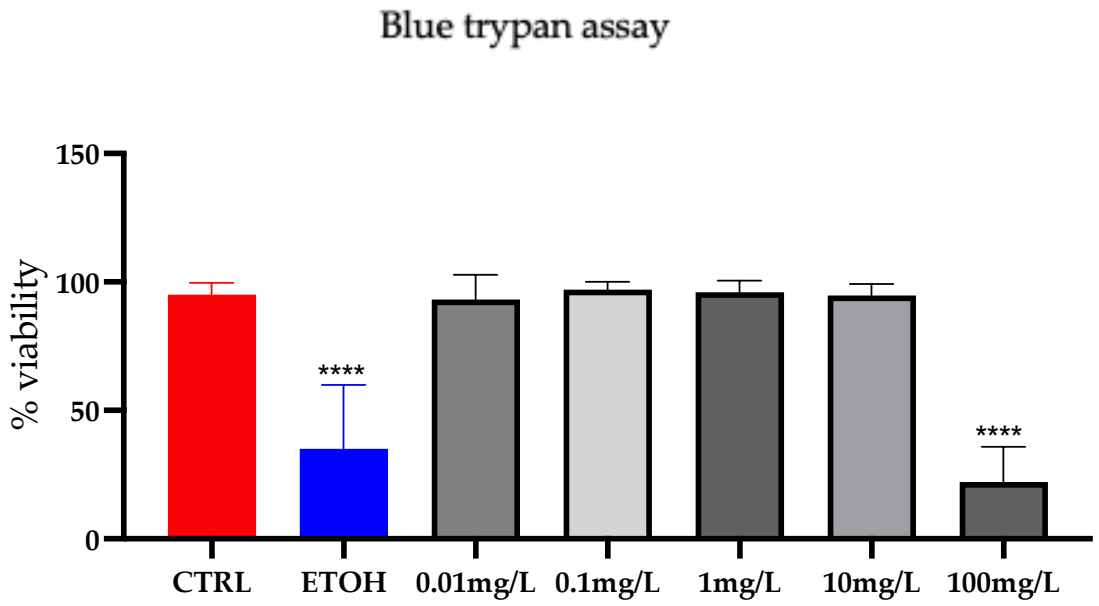
TOP 3 (Acetamiprid, Tebuconazole, Glyphosate)





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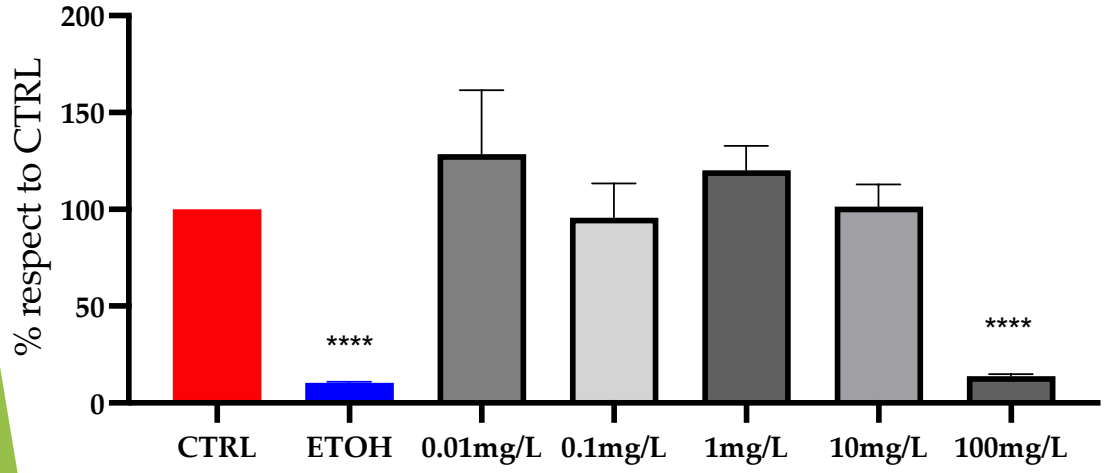
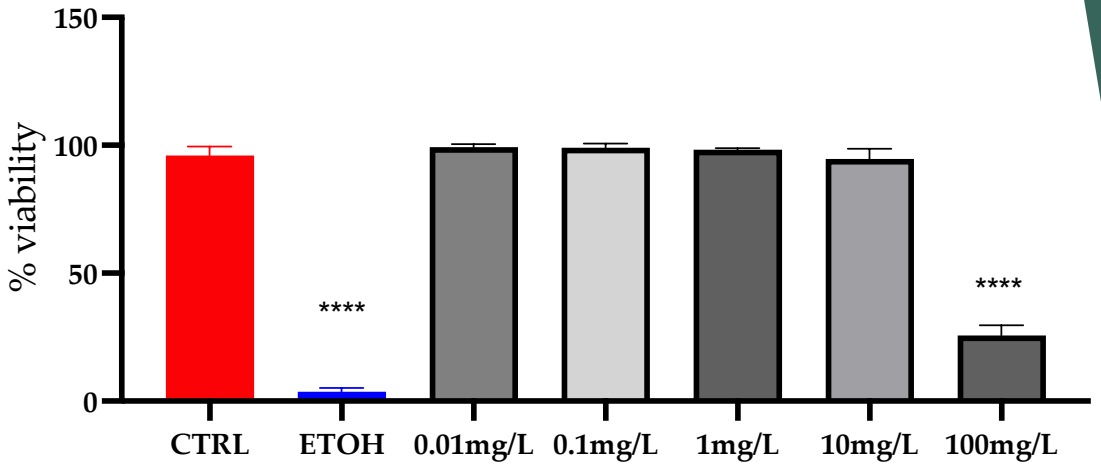
TOP 8

MTT



Blue trypan assay



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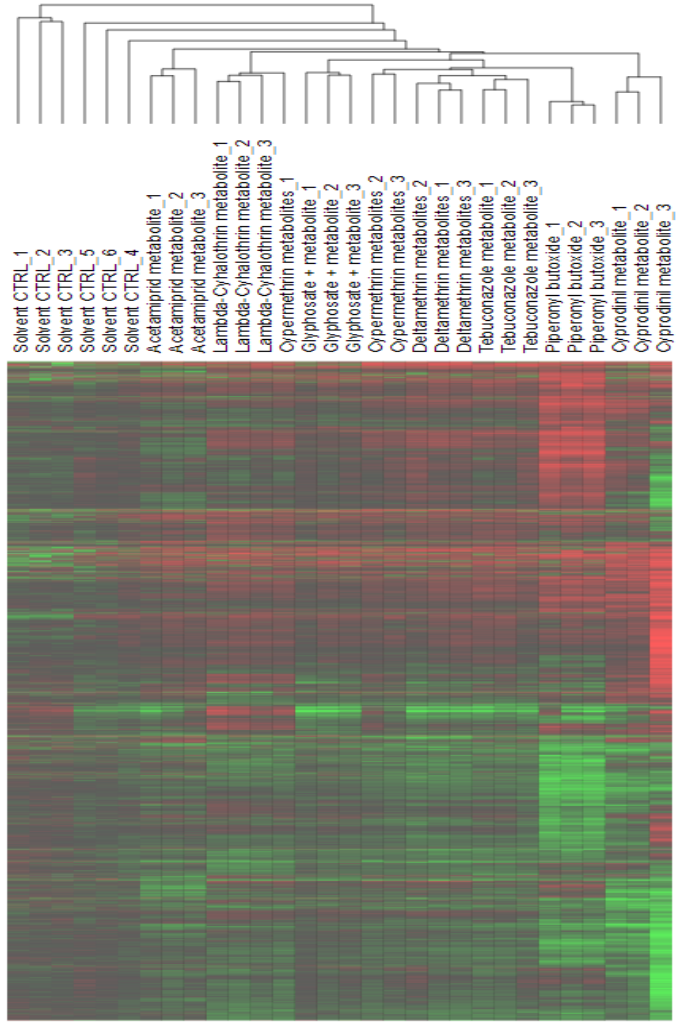
KIDNEY CELL LINES



Log2 ratio vs solvent control

-1 0 1

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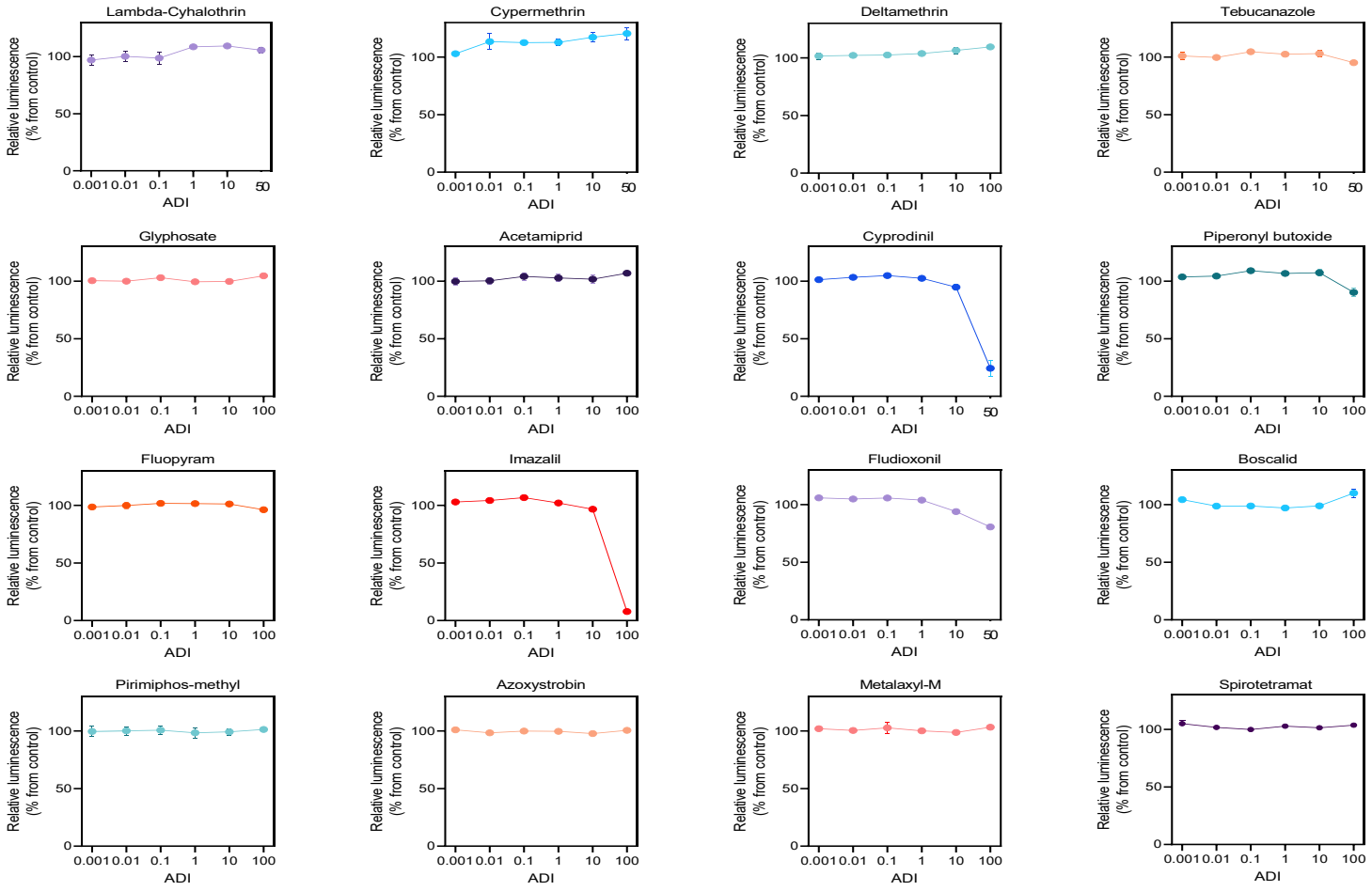
ORGANOIDS



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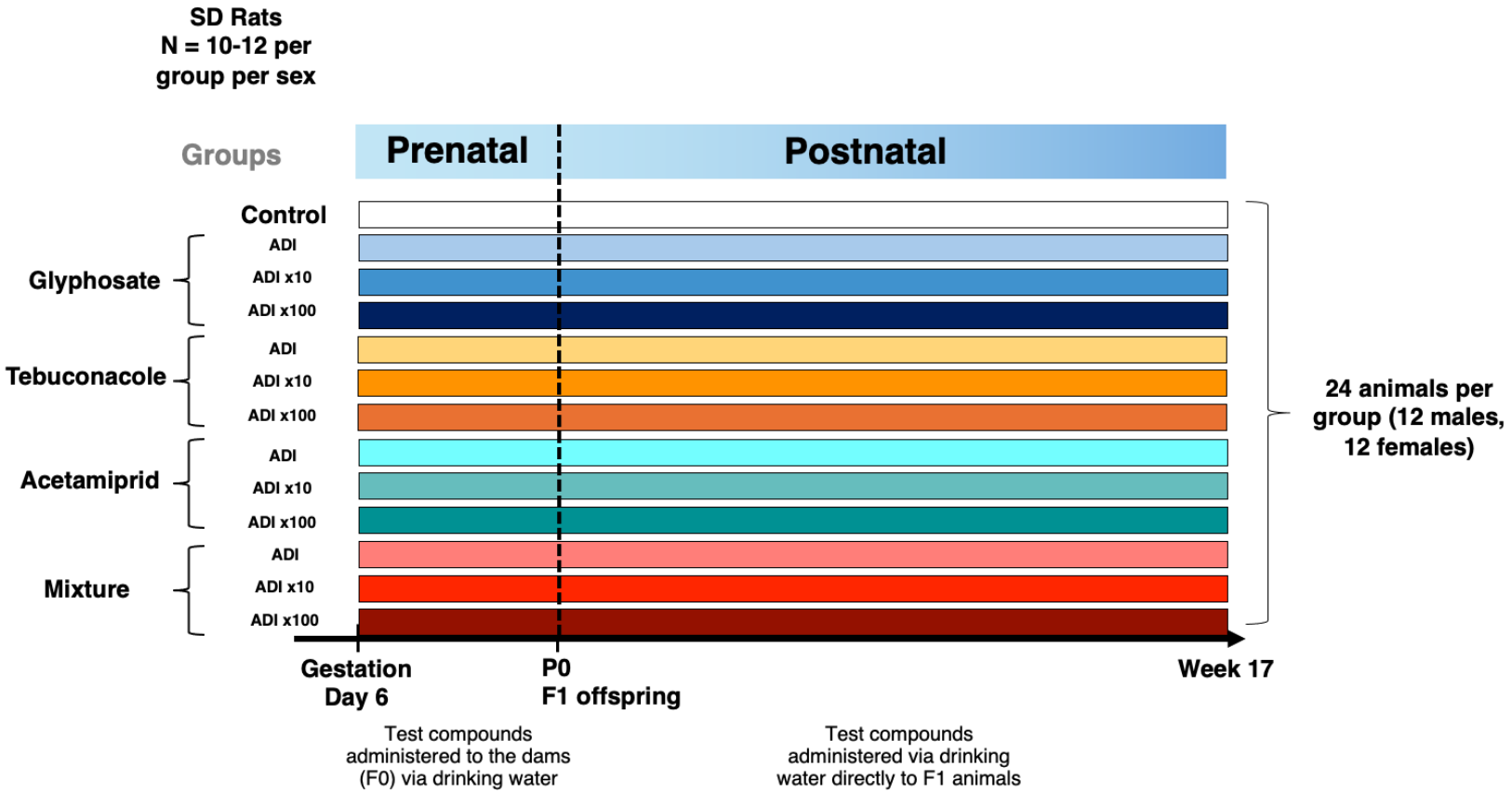
IN VIVO EXPERIMENT (Rats)



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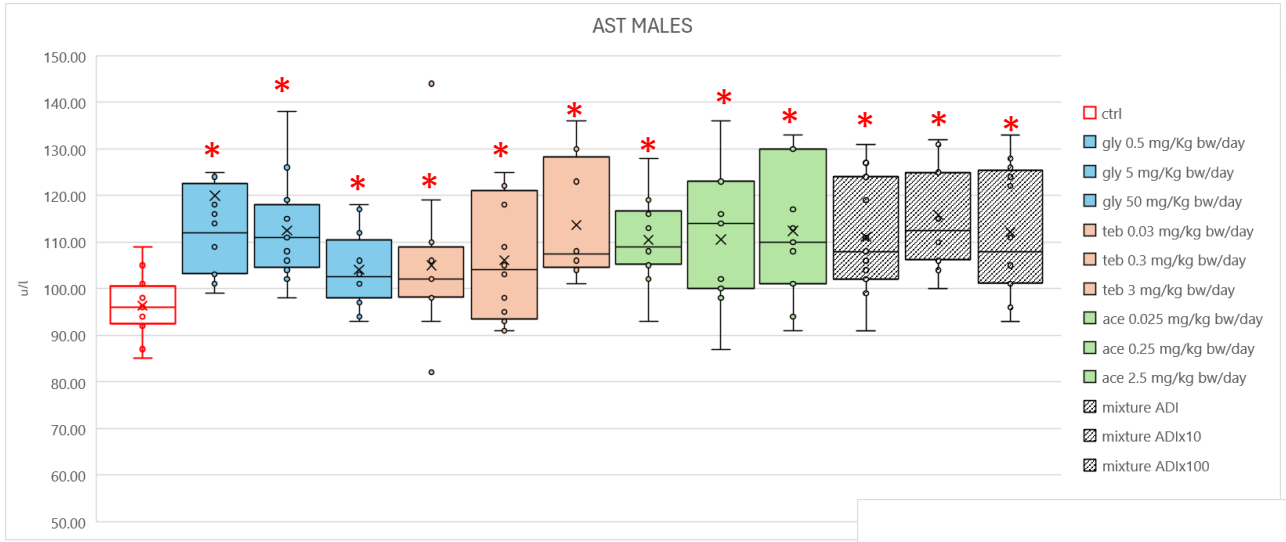
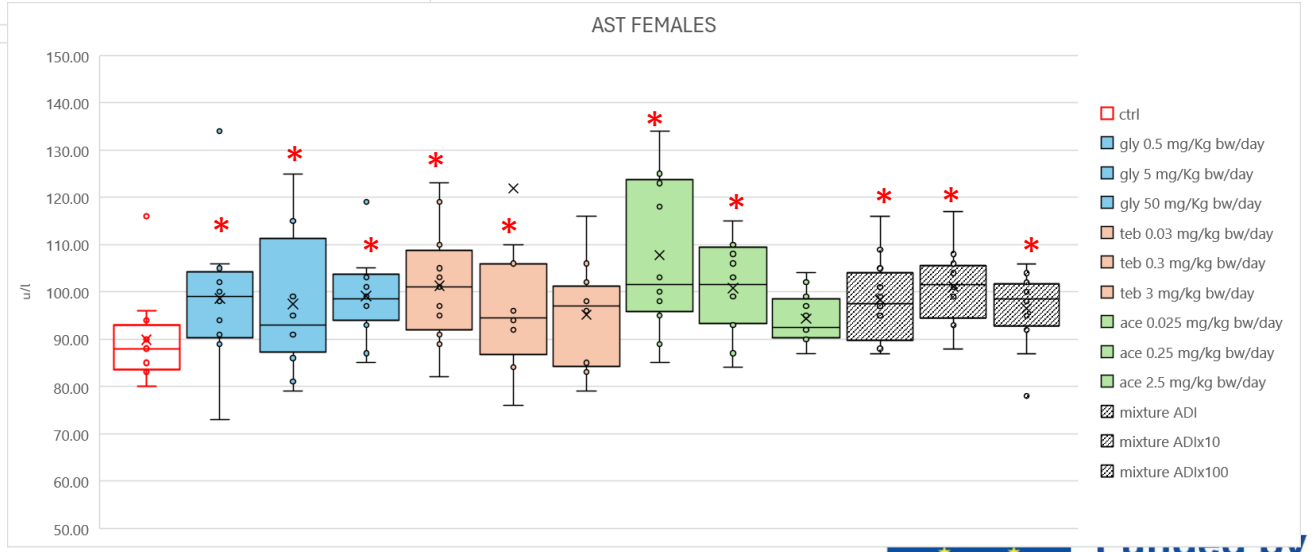
Liver Enzimes



♂

Aspartate aminotransferase (AST):

increase concentration in all the treated groups compared to the mean value in the control group (** statistically significant with Dunn's test)*



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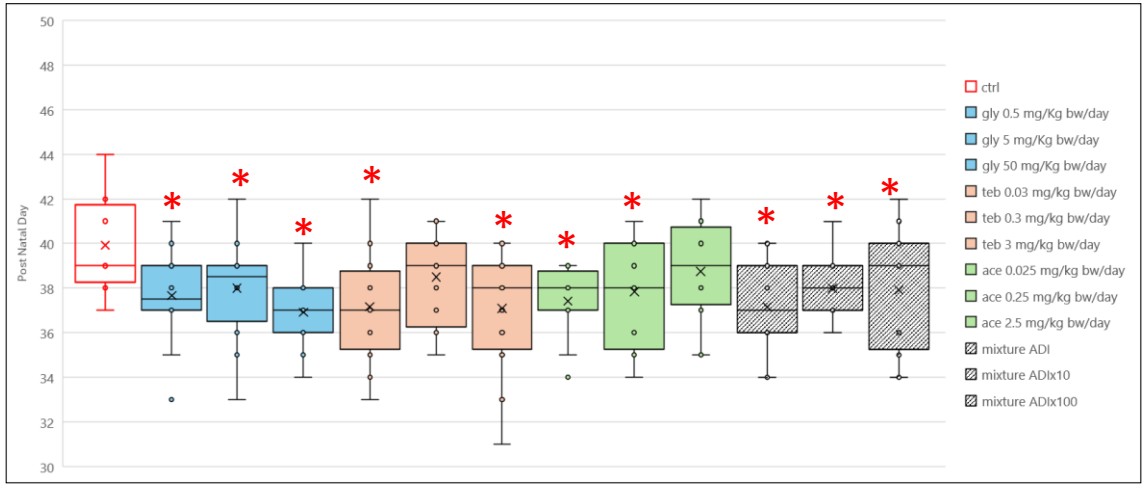
Sexual Maturation Indexes

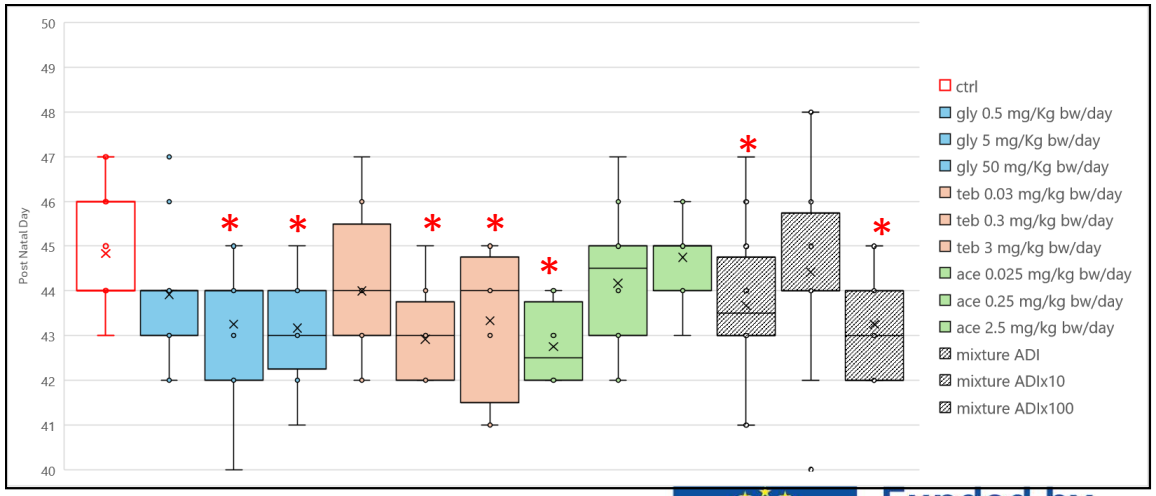
♀

VO: A statistical significance anticipation of day (PND) of occurrence of sexual maturation in females was detected *(* statistically significant with Dunn's test) . No* significant difference between weights at VO was observed.



♂

BPS: A statistical significance anticipation of day of occurrence of sexual maturation in males was detected *(* statistically significant with Dunn's test).* no significant difference between weights at BPS was observed.



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SPRINT findings

- A tiered approach, has been used in the SPRINT project based on the EFSA guidelines for combined exposure to multiple chemicals using a component-based approach (EFSA, 2019).
- In Tier 1, based on the available data from the SPRINT CSS on over 200 PPPs and in silico modelling, the top 20 pesticides of concern were assessed and prioritized for in vitro testing based on occurrence and wHQ.
- In Tier 2, standard in vitro models (EFSA tests) along with new models (New Sprint Indicators) were used to test the Top 10 pesticides and mixtures of concern, showing cytotoxic and genotoxic effects in a linear and additive dose-response (no synergistic effects observed) along with alterations of kidney cells viability and transcriptome.
- In Tier 3, standard in vivo models (EFSA tests) along with new models (New Sprint Indicators) were used to test the Top 3 pesticides of concern and their mixtures, showing sex-related effects on endocrine disruption, liver toxicity, kidney toxicity, microbiome and behavioural alterations, even doses that are currently considered safe in humans or without adverse effects in animals (between ADI and NOAEL).

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SPRINT findings

- The new models used (new SPRINT indicators), both in vitro and in vivo, proved to be sensitive and relevant for different toxicological endpoints of interest. The mechanistic information provided by the New SPRINT Indicators could inform hazard assessment on standard (in vitro kidney toxicity, organoids, in vivo behavioural effects) and new (in vivo microbiome effects) endpoints, but further validation is required for their use for risk assessment and pre-market evaluations.
- The tiered approach was successful and allowed to optimize the resources on the pesticides of greatest concern, since all the pesticides that have been tested in Tier 2 and 3 showed at least one positive signal. Pesticide mixtures proved to be consistently more detrimental, consistenly showing additive (non-synergistic) and linear dose-responses.
- Therefore, our findings in in vivo human toxicology confirm that a component-based approach, accounting also for sex-related effects and early life exposures, is predictive and adequate for hazard and risk assessment of mixtures.

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