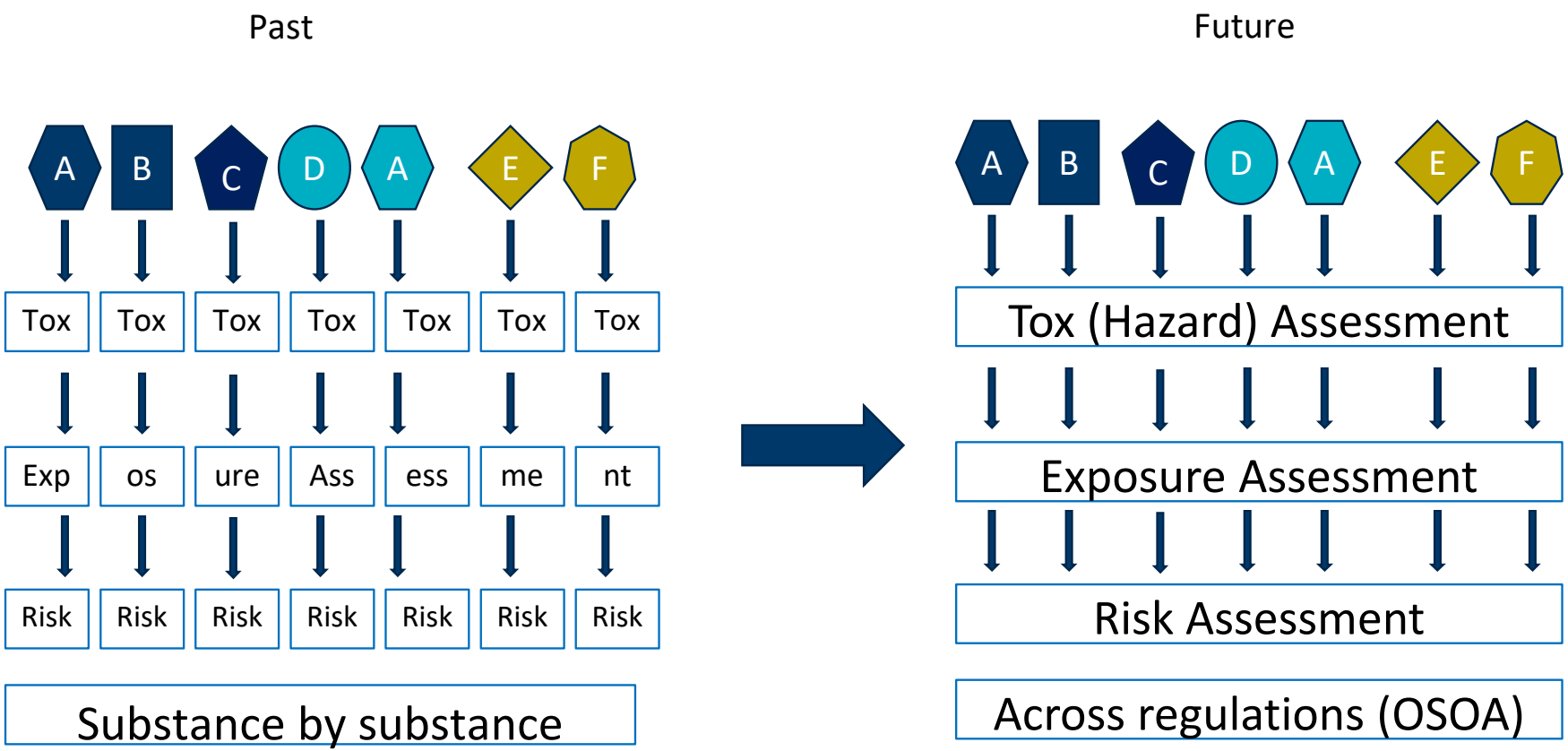
German Federal Institute for Risk Assessment

Mixture risk assessment, CAGs, CKGs…

GPTS, 17.03.2026, Düsseldorf

Dr. Philip Marx-Stölting
Testing and Assessment Strategies
Pesticides Safety

Risk Assessment of Mixtures



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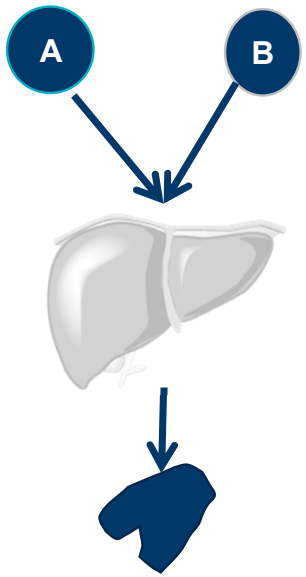
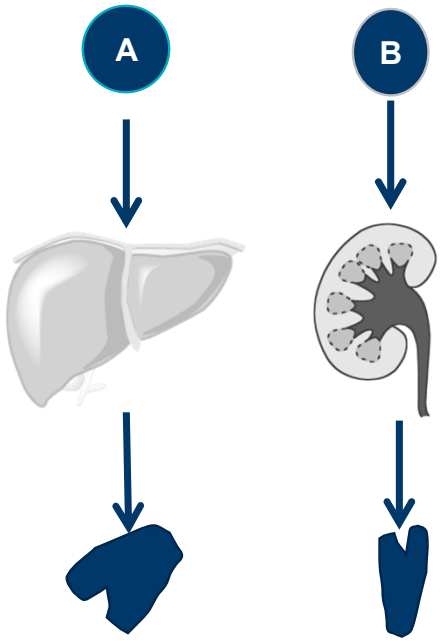
Similar action



$$ECx_{Mix} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i}\right)^{-1}$$

Dose / conc addition

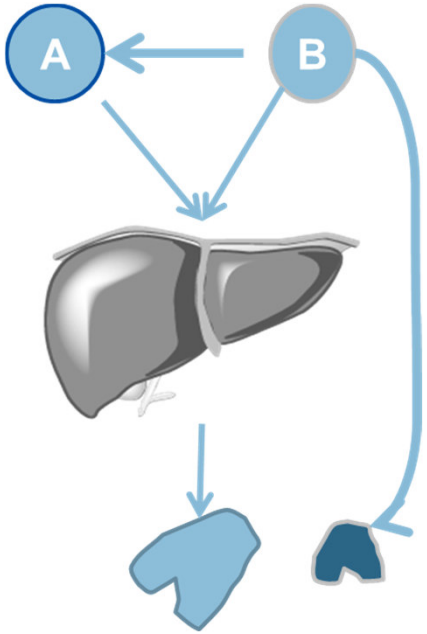
Dissimilar action



$$Edc_{Mix} = 1 - \prod_{i=1}^{n}(1 - Edc_i)$$

Effect addition

Interaction



Antagonism, Synergism

Marx-Stoelting und Rotter 2019, in Marquardt, Schäfer, Barth: Lehrbuch der Toxikologie

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Grouping according to toxicodynamics, target organs (Cumulative Assessment Groups), assumption dose addition

CAG Thyroid

Pesticide A
Pesticide B
Pesticide C
Pesticide H
Pesticide L

CAG Nervous system

Pesticide C
Pesticide D
Pesticide E
Pesticide I

CAG Liver

Pesticide A
Pesticide C
Pesticide F
Pesticide G
Pesticide H
Pesticide J
Pesticide K

CAG Kidney

Pesticide B
Pesticide F
Pesticide H
Pesticide I

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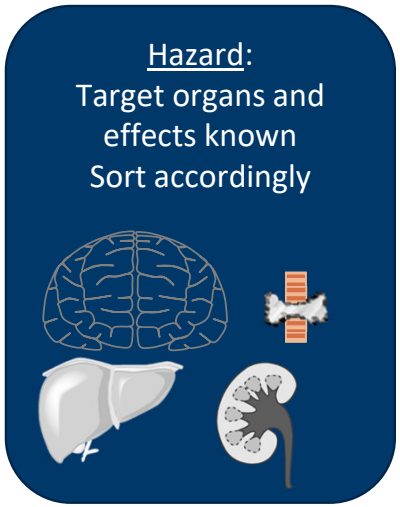
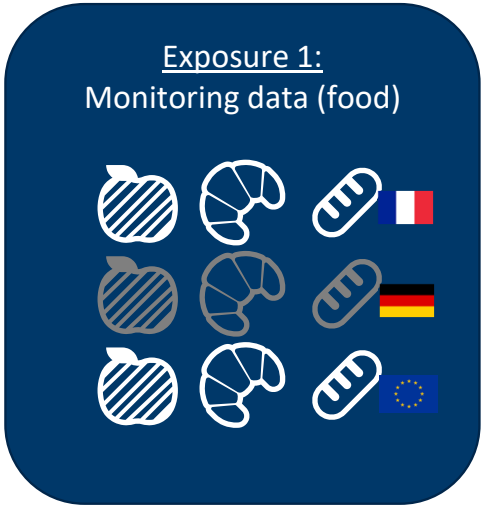
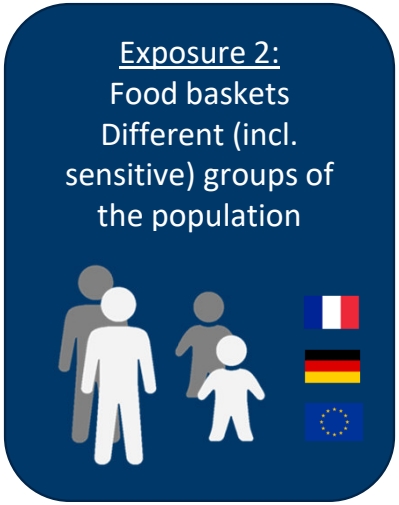
How does mixture risk assessment based on CAG work?

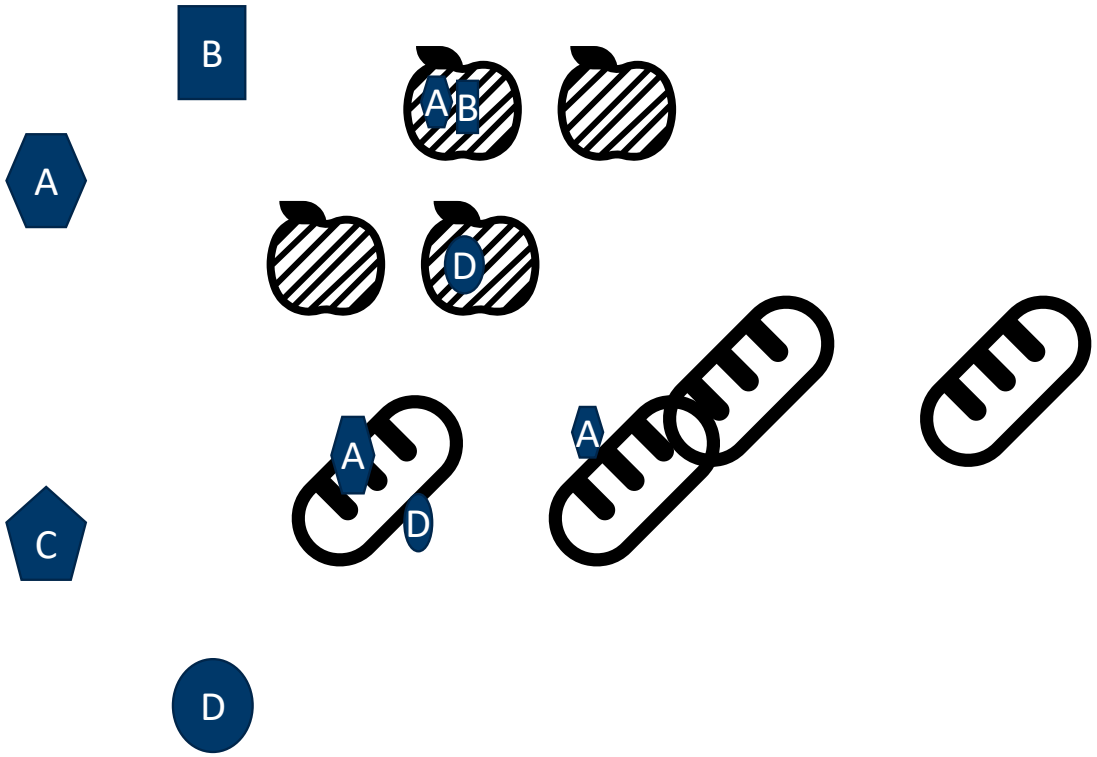


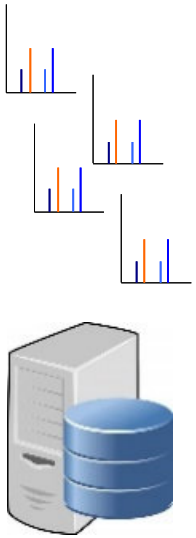




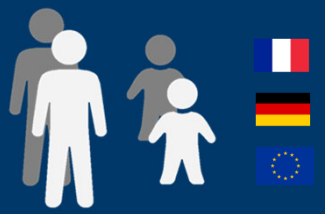
- Group substances according to tox information
- Cummulative Assessment Groups (CAGs)
- Calculate combined exposure based on data
- Succesfully applied for thyroid, nervous system (pesticides)

Exposure to mixtures: a probabilistic approach





Exposure 2:
Food baskets (also for sensitive subpopulations)



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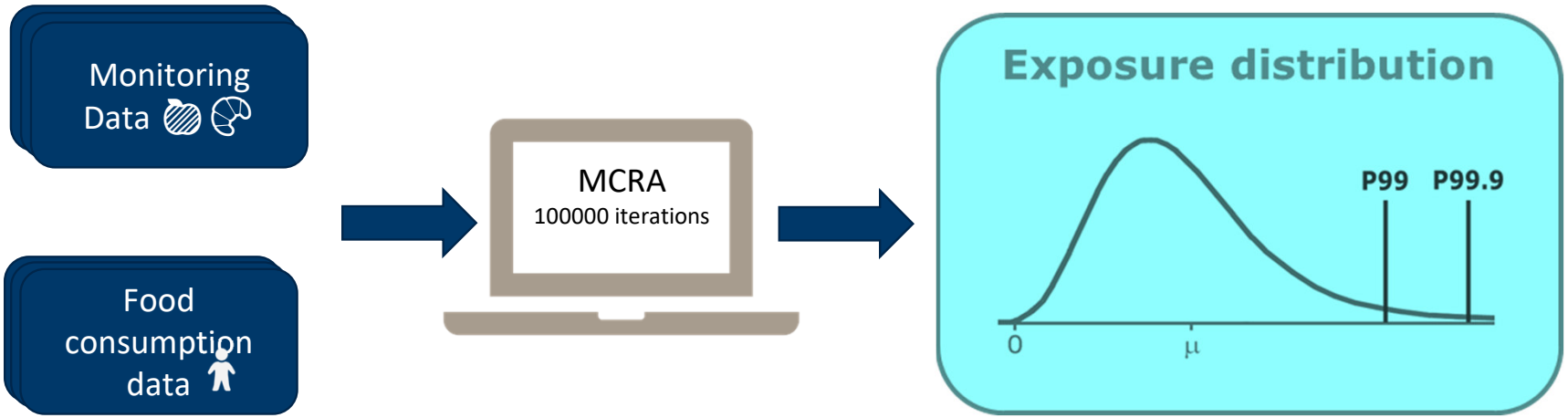
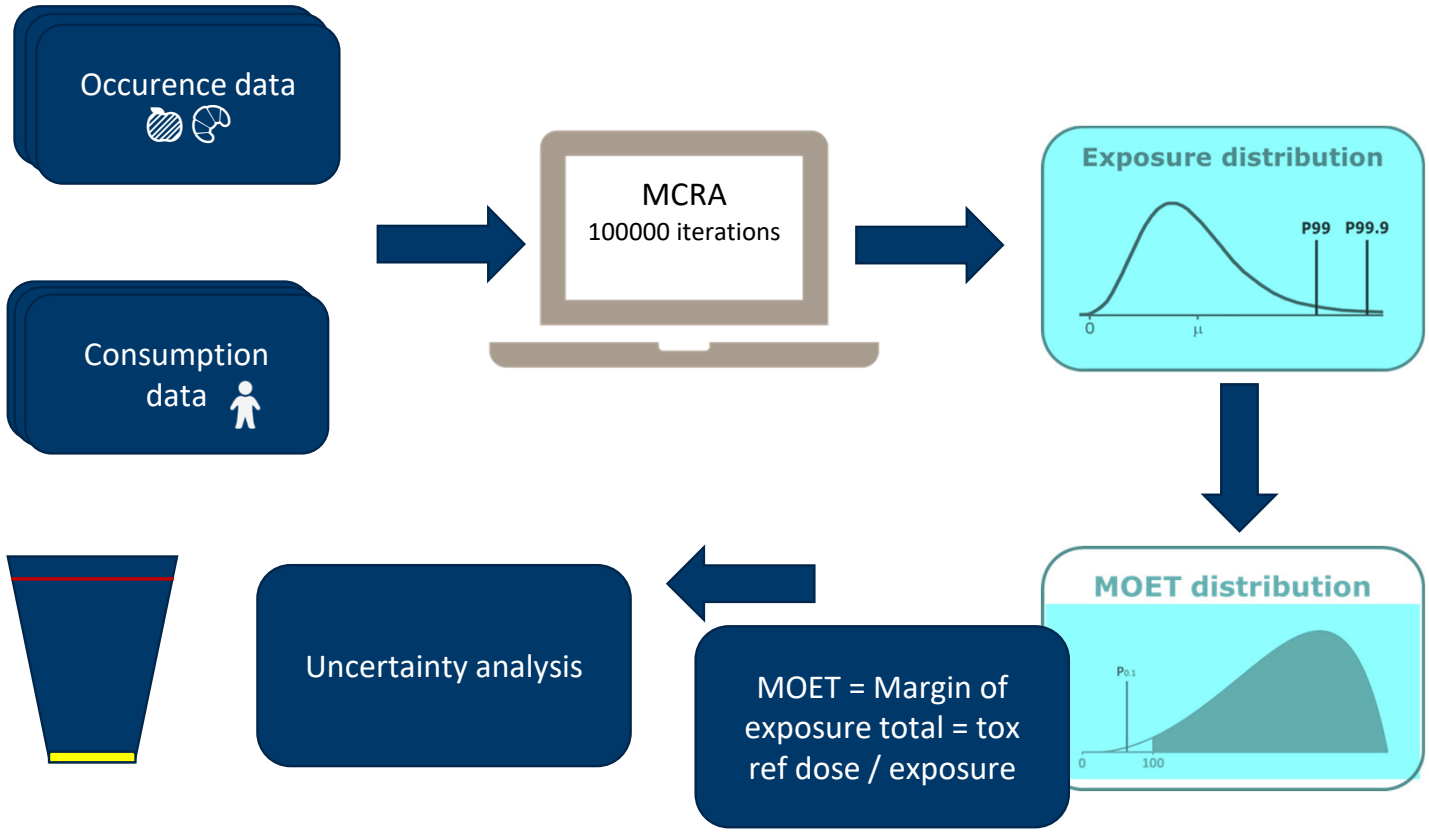
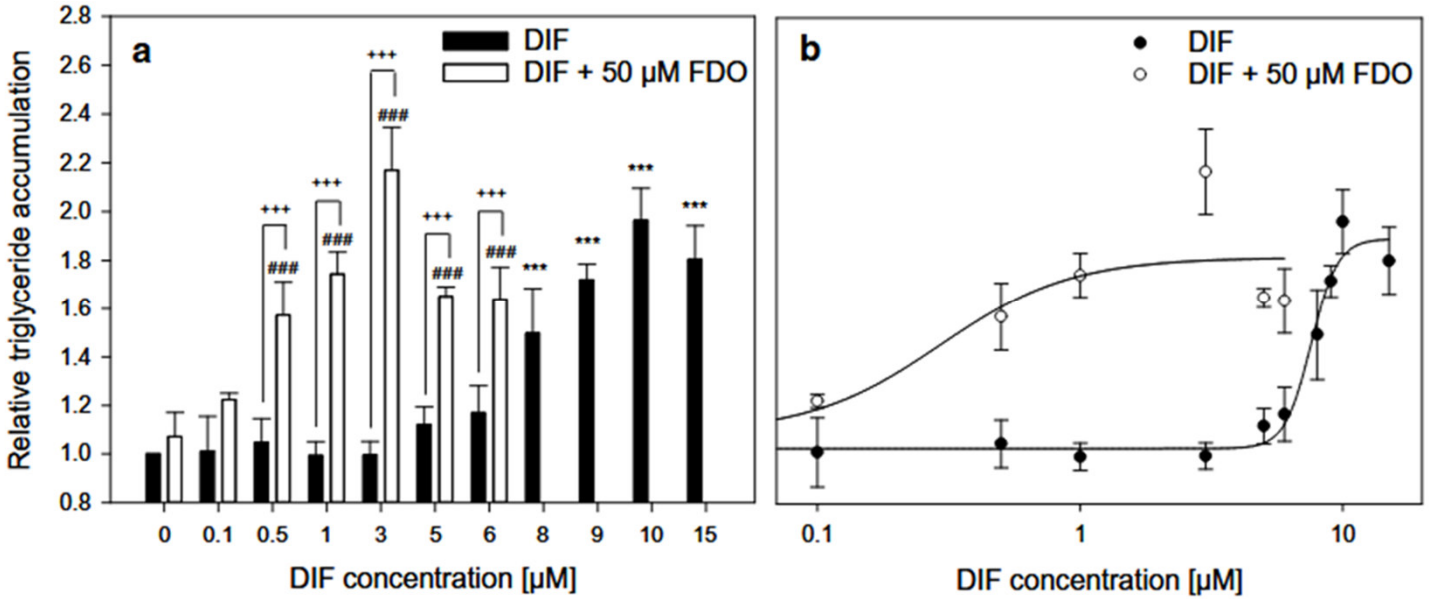
Monte Carlo Risk Assessment (MCRA)





- Cumulative exposure to pesticides with chronic/acute effects on the thyroid/nervous system do not exceed the threshold for regulatory concern (MOET) established by risk managers
- Data-driven mixture risk assessment succesfully applied √

Kinetic interaction



- In interaction generally toxicokinetics plays an important role (Cedergren 2014; Martin et al 2021; Lasch et al 2021, Karaca et al 2021)
- At high dose levels!
- ADME4NGRA (EFSA project, 4 years, 5 Mio, lead ITEM – looking at toxicokinetics)

What about synergism?

Steatotic compounds (triazoles) + non-steatotic compounds (Fludioxonil)

Lasch et al.

More than additive increase in triglyceride accumulation

Primary mechanism:
Inhibition of CYP3A4 by Fludioxonil
Reduced metabolism of Difenoconazole
=Toxicokinetic interaction!!

Archives of Toxicology (2022) 96:1661–1671
https://doi.org/10.1007/s00204-022-03264-8

REGULATORY TOXICOLOGY

An approach for mixture testing and prioritization based on common kinetic groups

Albert Braeuning[1] · Denise Bloch[2] · Mawien Karaca[2] · Carsten Kneuer[2] · Stefanie Rotter[2] · Tewes Tralau[2] · Philip Marx-Stoelting[2]

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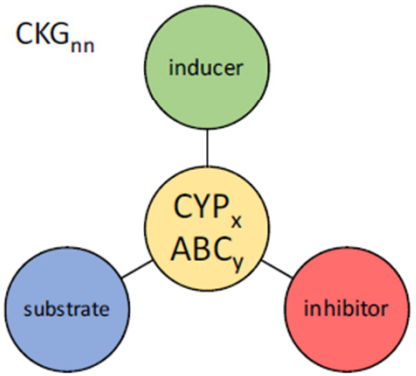
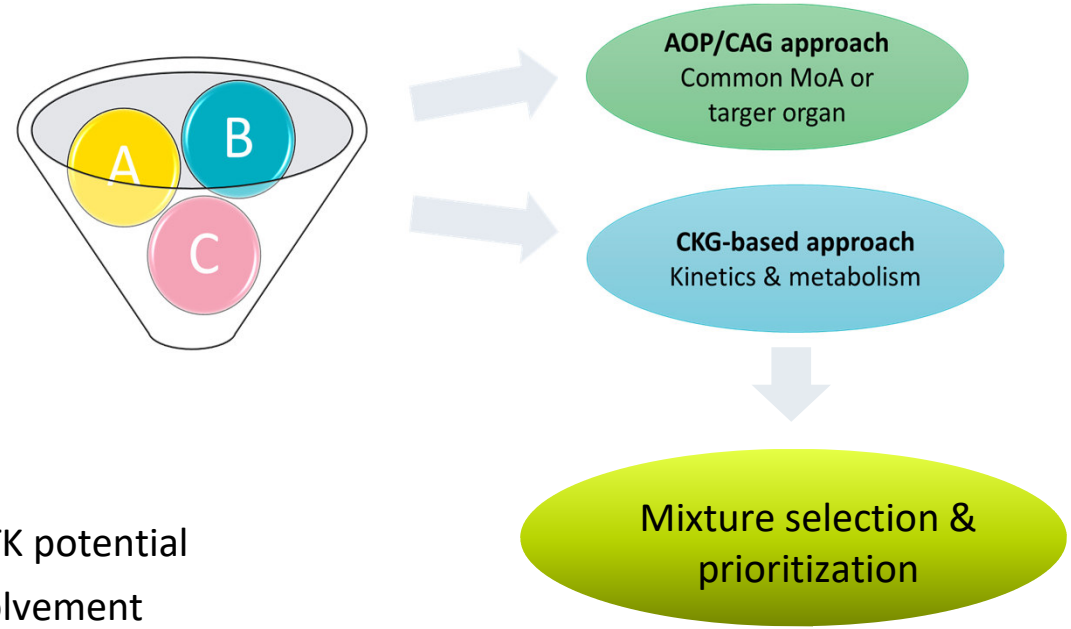


Mixture Assessments

Toxicodynamic approach

- Cumulative Assessment Groups (CAGs)
- Target organs and mode of action (MoA)
- If similar, then dose/concentration addition (DACA) model

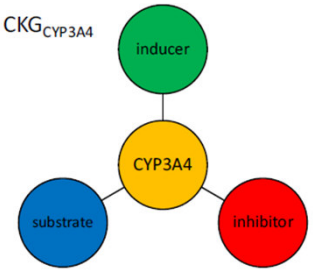


Toxicokinetic information

- Insights into DACA deviations; synergism/antagonism
- Xenobiotic metabolism and transporter interaction
- Prioritize critical mixtures and identify chemicals for TK potential
- Common Kinetic Groups: based on shared ADME involvement

(Figure adapted from Braeuning et al. 2022)

Workflow – Compilation of a first CKG for CYP3A4



PPPs– 400+ substances

- (EC's Pesticide Database)
  - Approved substances (Jan 2024)
  - Microorganisms, mineral oils, etc. excluded
  - ~250 substances

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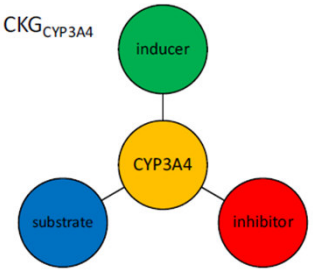

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in silico screening

- Identification of CYP3A4 inhibitors
- SwissADME, ADMET Pred & SuperCYPs Pred
- Tiered approach (based on confidence)
- 6 potential inhibitors + 3 non-inhibitors (proof of principle)

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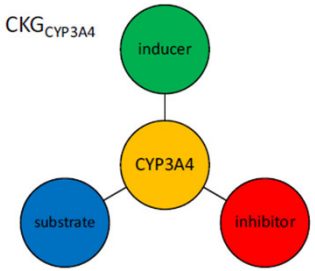

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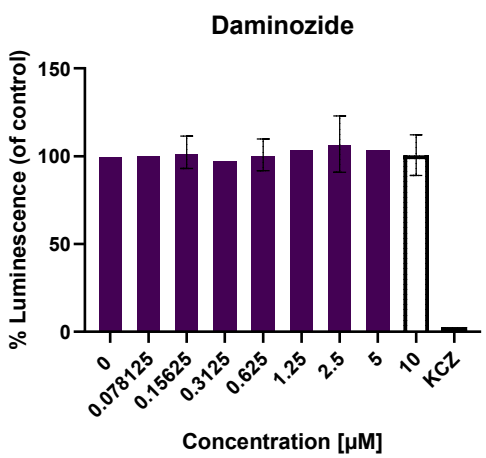
in vitro follow-up

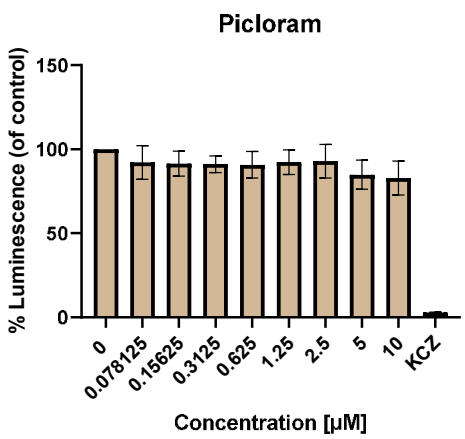
- Promega P450- Glo assay CYP3A4 inhibition
- Luminescence – based
- Binary mixtures

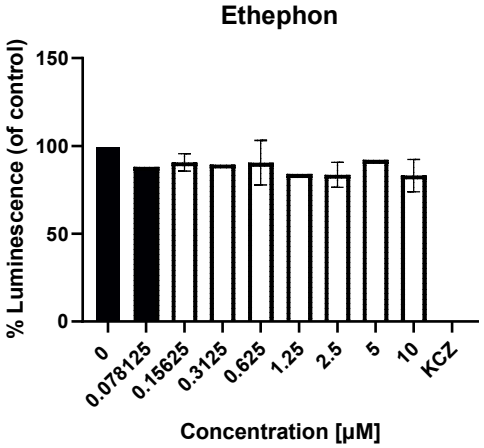
Results Summary – *in silico*

Substance ID	maccs	morgan	ADMET Pred	SwissADME	Literature
Beflubutamid	active(low)	active(low)	Yes (78%)	yes	-
Mandestrobin	active(low)	active(low)	Yes (92%)	yes	-
Mandipropamid	active(low)	active(low)	Yes	yes	-
Tebufenozide	active(high)	active(high)	Yes (55%)	yes	-
Tebuconazole	active(med)	active(high)	Yes (54%)	no	✔
Imazalil	active(high)	active(high)	Yes (73%)	no	✔
Daminozide	inactive(high)	inactive(high)	No (96%)	no	-
Ethephon	inactive(high)	inactive(high)	No (96%)	no	-
Picloram	inactive(high)	inactive(high)	No (96%)	no	-

Results – Non-inhibitors







* <0.05
** < 0.01
*** < 0.001
**** < 0.0001

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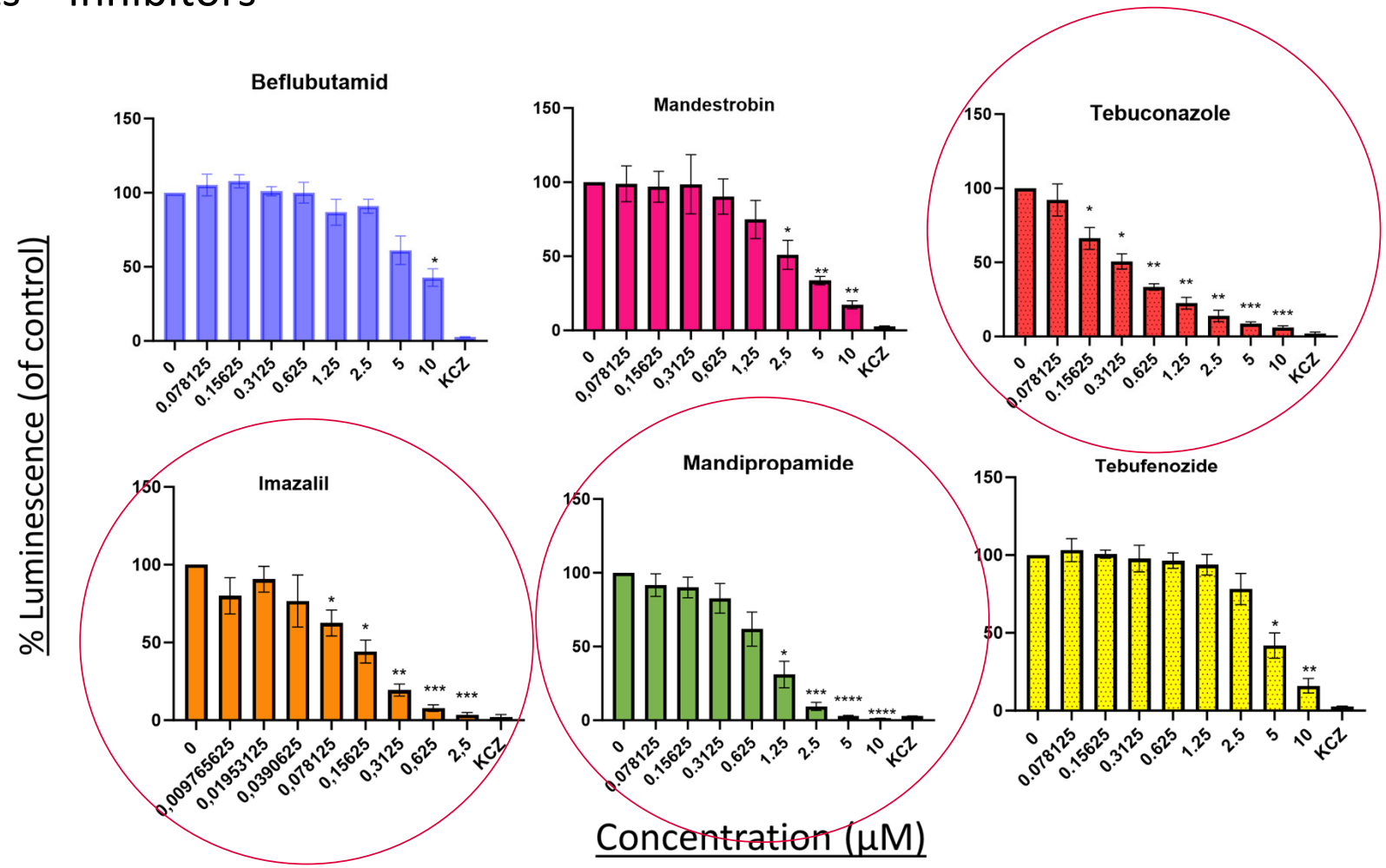
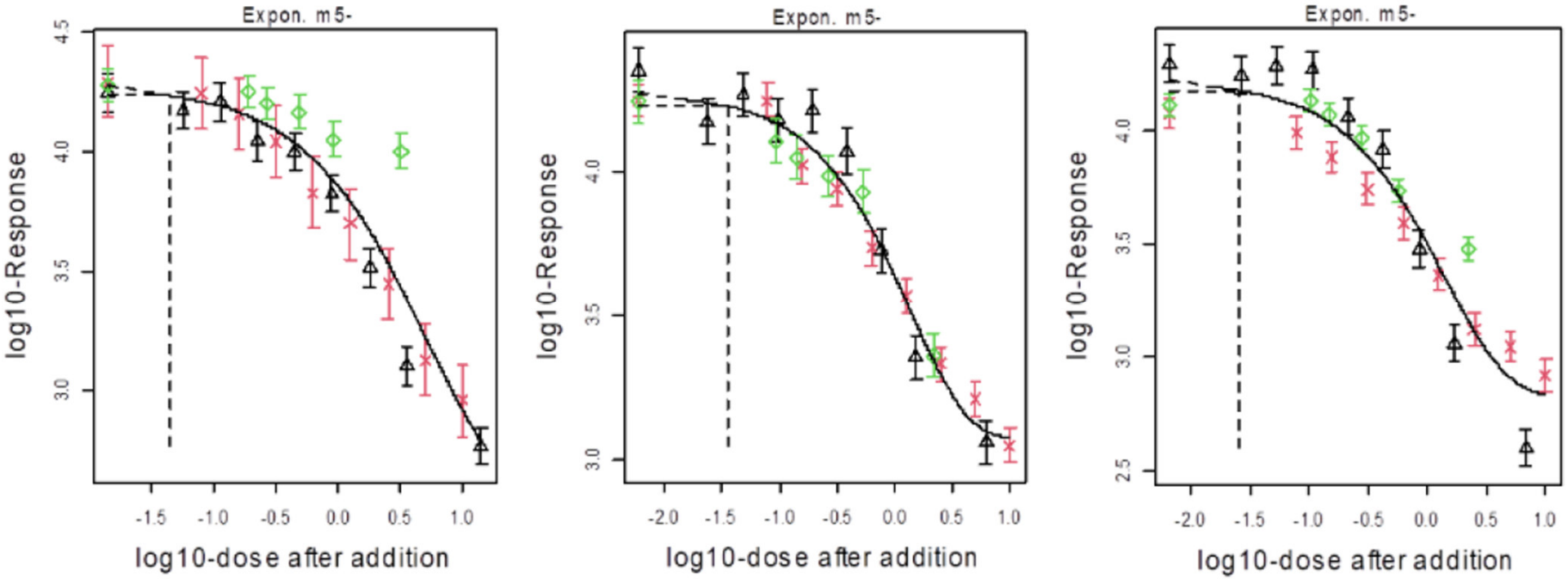
Results – Inhibitors



* <0.05
** < 0.01
*** < 0.001
**** < 0.0001

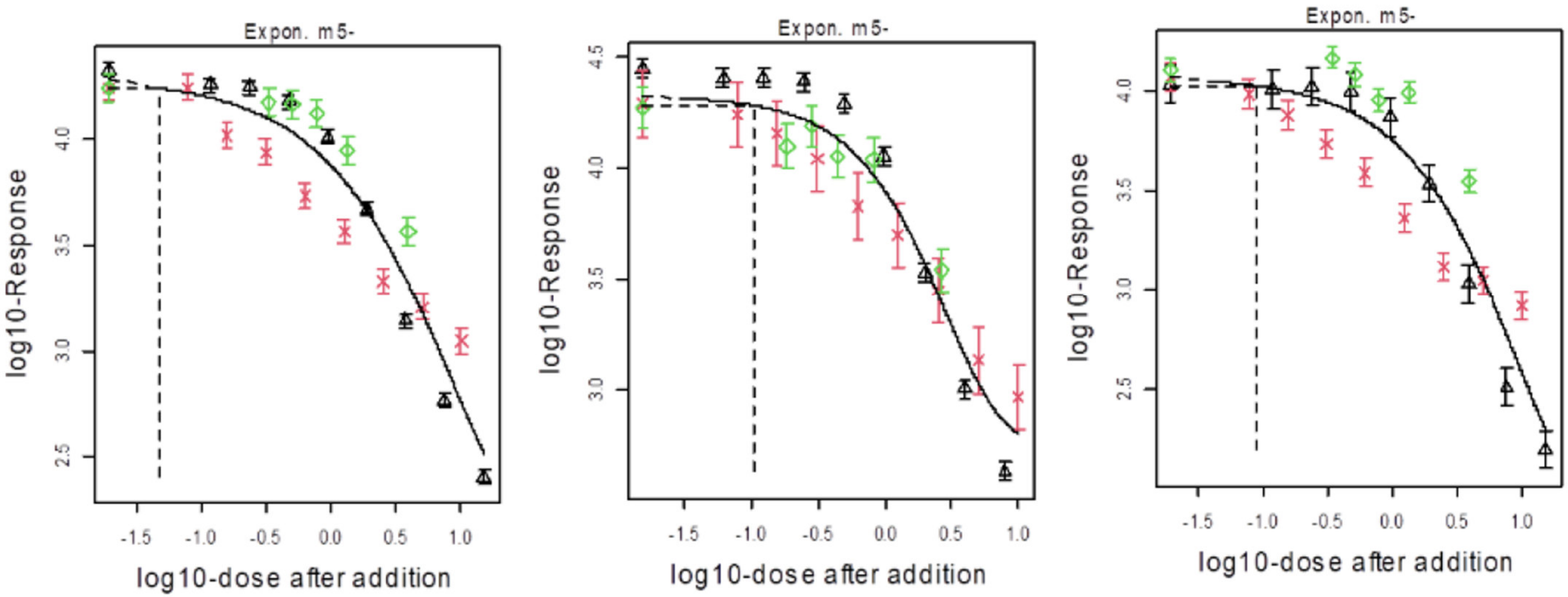
BfR

Mixtures – Tebuconazole + Imazalil



Green – mixtures

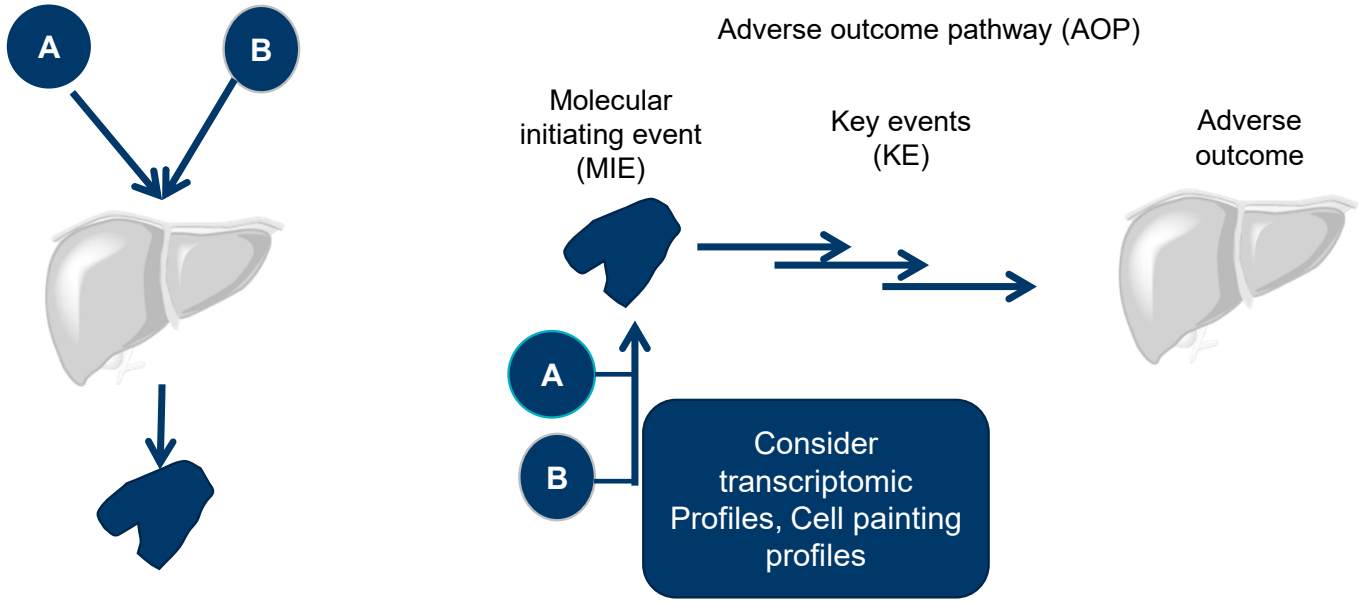
Mixtures – Tebuconazole + Mandipropamide



Green - mixtures

Molecular CAGs

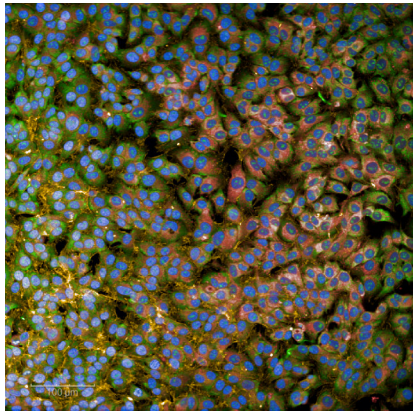
Is there a way to include data poor substances or refine a CAG?



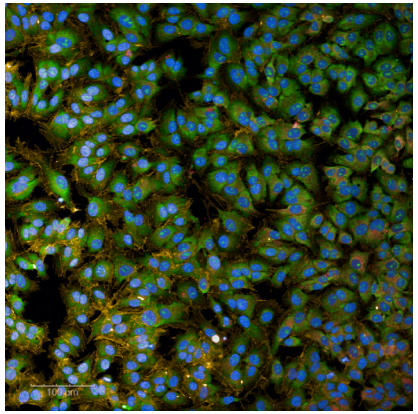
- Group substances according to structure
- Group substances according to cell painting profile
- Shown for 86 pesticiders affecting the liver (and some negative controls)

Examples

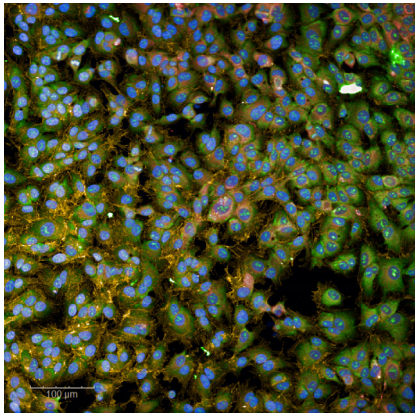
DMSO



Azoxystrobin 40 µM



Glyphosate 0,13 µM



A Conventional Cell Painting (according to Bray *et al.*, 2016)

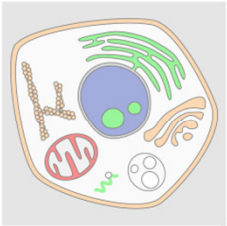
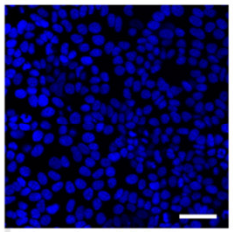


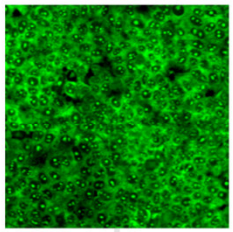
Illustration of stained cellular compartments

DNA



Hoechst 33342

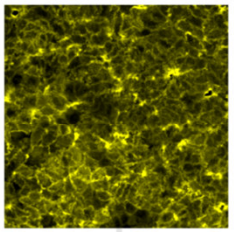
RNA/ER



SYTO™ 14 Green Fluorescent Nucleic Acid Stain

Concanavalin A, Alexa Fluor™ 488

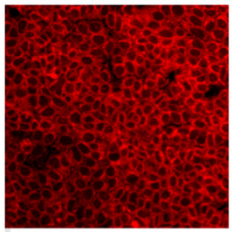
AGP



Phalloidin, Alexa Fluor™ 568

Wheat Germ Agglutinin (WGA), Alexa Fluor™ 555

Mito



MitoTracker™ Deep Red FM

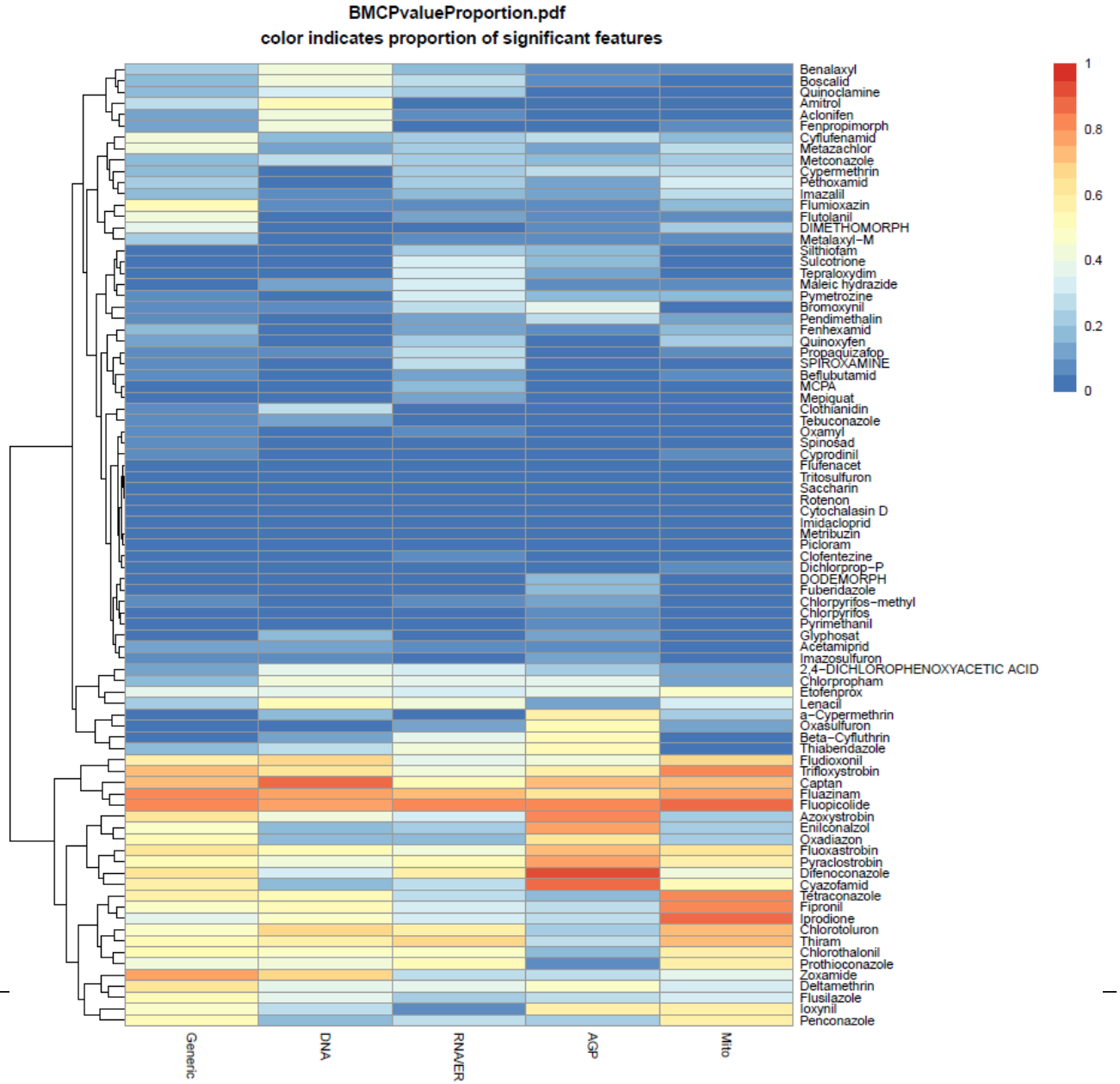
nature communications

Article | Open access | Published: 24 April 2025

Cell Painting PLUS: expanding the multiplexing capacity of Cell Painting-based phenotypic profiling using iterative staining-elution cycles

Elena von Coburg, Marlene Wedler, Jose M. Muino, Christopher Wolff, Nils Körber, Sebastian Dunst & Shu Liu

Nature Communications **16**, Article number: 3857 (2025) | Cite this article


BMCPvalueProportion.pdf
color indicates proportion of significant features
Benalaxyl
Boscalid
Quinoclamine
Amitrol
Aclonifen
Fenpropimorph
Cyflufenamid
Metazachlor
Metconazole
Cypermethrin
Pethoxamid
Imazalil
Flumioxazin
Flutolanil
DIMETHOMORPH
Metalaxyl-M
Silthiofam
Sulcotrione
Tepraloxydim
Maleic hydrazide
Pymetrozine
Bromoxynil
Pendimethalin
Fenhexamid
Quinoxyfen
Propaquizafop
SPIROXAMINE
Beflubutamid
MCPA
Mepiquat
Clothianidin
Tebuconazole
Oxamyl
Spinosad
Cyprodinil
Flufenacet
Tritosulfuron
Saccharin
Rotenon
Cytochalasin D
Imidacloprid
Metribuzin
Picloram
Clofentezine
Dichlorprop-P
DODEMORPH
Fuberidazole
Chlorpyrifos-methyl
Chlorpyrifos
Pyrimethanil
Glyphosat
Acetamiprid
Imazosulfuron
2,4-DICHLOROPHENOXYACETIC ACID
Chlorpropham
Etofenprox
Lenacil
a-Cypermethrin
Oxasulfuron
Beta-Cyfluthrin
Thiabendazole
Fludioxonil
Trifloxystrobin
Captan
Fluazinam
Fluopicolide
Azoxystrobin
Enilconalzol
Oxadiazon
Fluoxastrobin
Pyraclostrobin
Difenoconazole
Cyazofamid
Tetraconazole
Fipronil
Iprodione
Chlorotoluron
Thiram
Chlorothalonil
Prothioconazole
Zoxamide
Deltamethrin
Flusilazole
Ioxynil
Penconazole
Generic
DNA
RNAER
AGP
Mito
1
0.8
0.6
0.4
0.2
0

Summary

Mixture risk assessment requires data-driven approaches

Besides toxicodynamics (as for the CAGs) toxicokinetics has to be considered (e.g. by integrating CKGs)

As mixture toxicity is mechanism driven, molecular approaches and NAM based testing are a key technology

Thank you

Denise Bloch
Asya Kadic
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Regina Puts
Vera Ritz
Team 66 & 6NG2
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Verena Fetz
Shu Liu
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