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How well can we predict combined effects?

Rolf Altenburger
21.01.2022

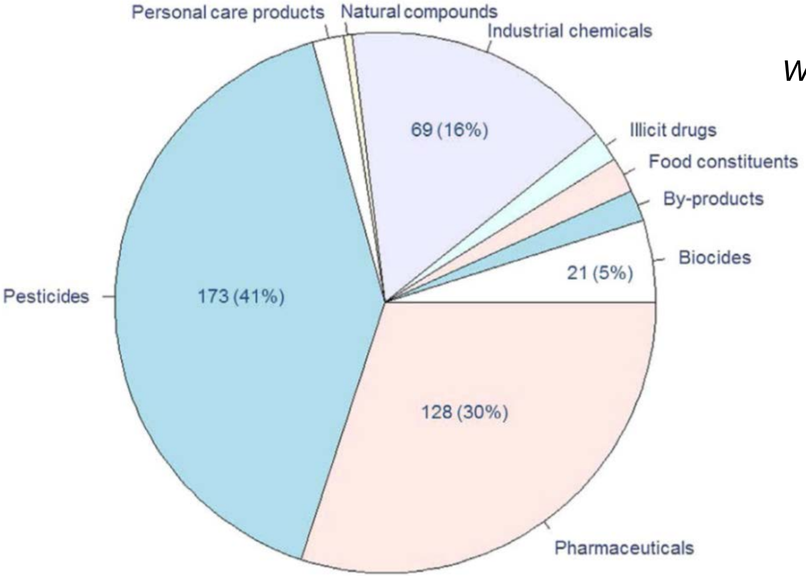
SWEDISH SOCIETY
OF TOXICOLOGY
SINCE 1969

Outline

- The exposome challenge
- Targeted effect-based mixture detection
- Component-based combined effect prediction
- Mixture exposure and multiple responses

Chemicals in the environment → Exposome challenge

e.g. >400 contaminants
in European freshwaters

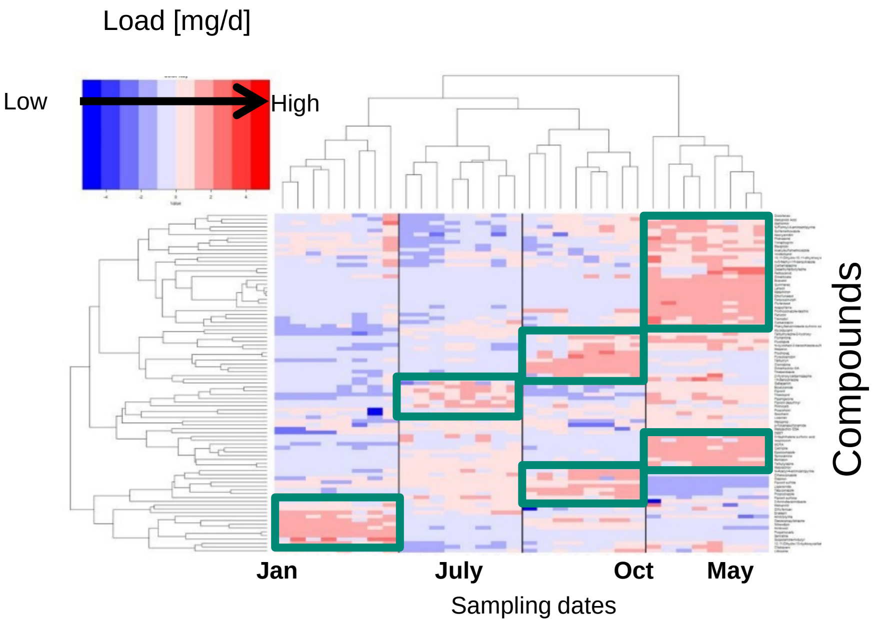


Wibke Busch

Busch, W., et al. (2016): Micropollutants in European rivers: A mode of action survey to support the development of effect-based tools for water monitoring. Environ. Toxicol. Chem. 35 (8), 1887 - 1899

Chemicals in the environment → Exposome challenge

Nontarget analytics in WWTP effluent

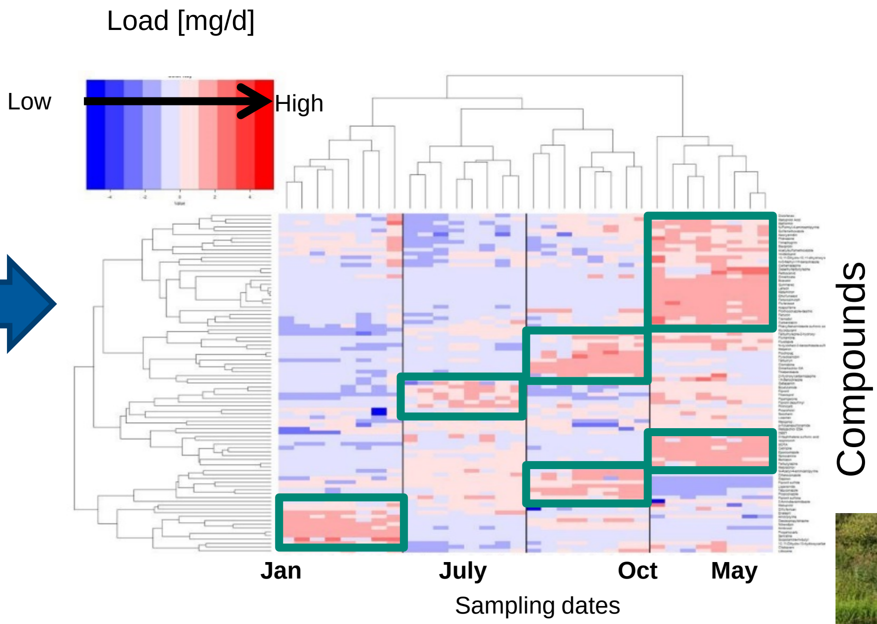


Liza Beckers

*Beckers et al., 2018, **W.**, (2018): Characterization and risk assessment of weather dynamics in organic pollutants mixtures form discharge from a separate sewer system. Water Res. **135** , 122 - 133*

Chemicals in the environment → Exposome challenge

Nontarget analytics in WWTP effluent



Exposure of the population

Exposure of aquatic organisms

Exposome challenge in humans

Chemical universe

Source universe

CAS universe
163M
(CAS RN)

Anthro-
pogenic

Industrial
products
30 k (KEMI)

Non-
anthro-
pogenic

Multiplied by (bio)-transformation and degradation

Exposome

Exposome universe

Proxies:

Blood exposome
50k (Barupal)

Urine exposome
1.4k (HMDB)

Dermal exposome

...

Human biomonitoring

NHANES
319 (1999-2017 campaigns)

GERES

HMB4EU
19 (priority substances and groups)

Sebastian Huhn

Huhn, S., et al (2021): Unravelling the chemical exposome in cohort studies: routes explored and steps to become comprehensive. Environ. Sci. Eur. 33 , art. 17

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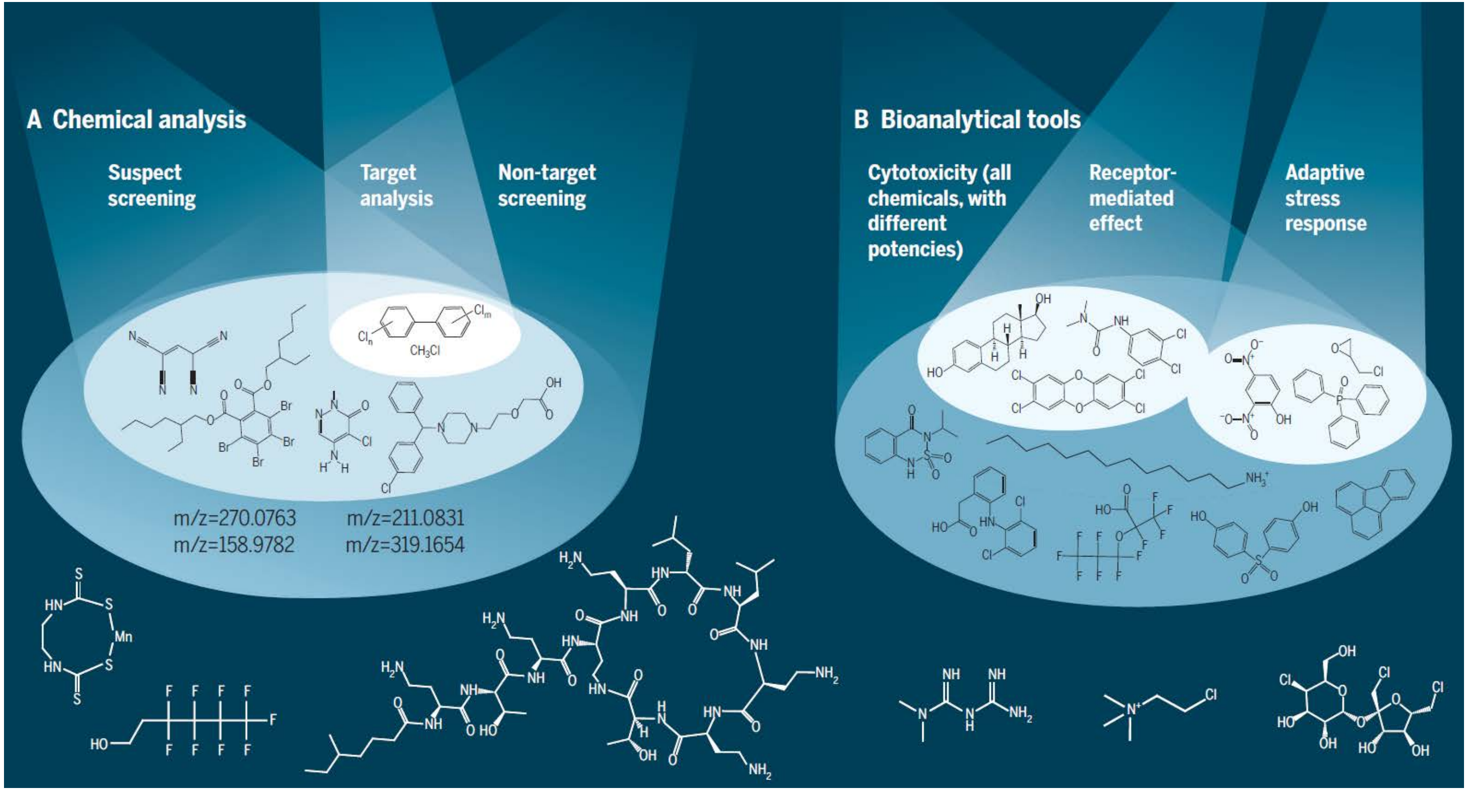
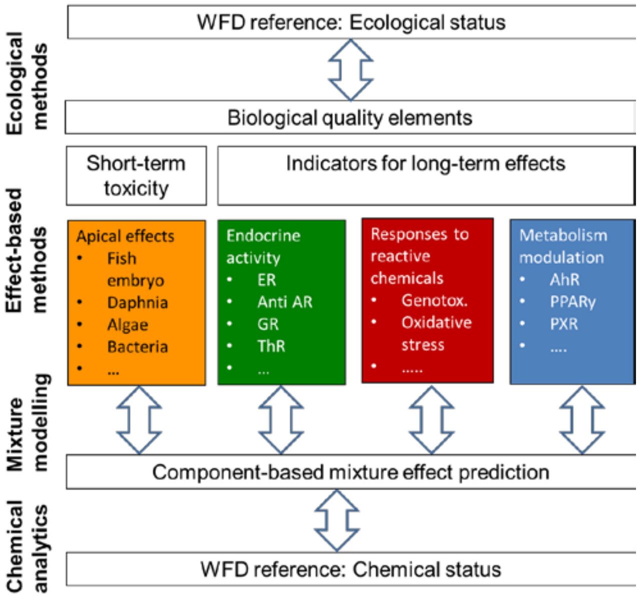
Effect-based mixture detection → bioanalytics



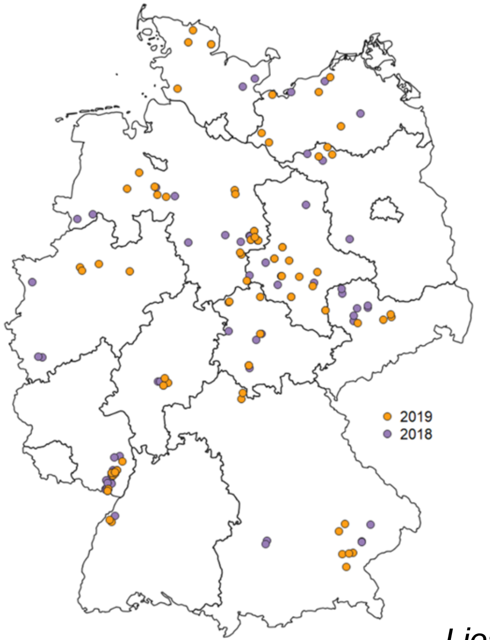
Beate Escher

Escher, Stapelton, Schymanski 2020. Tracking complex mixtures of chemicals in our changing environment. Science 367: 388-393

Effect-based mixture detection → bioanalytics



Altenburger et al. (2019): Future water quality monitoring: improving the balance between exposure and toxicity assessments of real-world pollutant mixtures. Environ. Sci. Eur. 31 , art. 12



Matthias Liess

Liess, et al. (2021) Pesticides are the dominant stressor for vulnerable insects in lowland streams. Water Res 201:117262

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Predicting combined effects → the concepts

LOEWE Additivity - Dose Addition – Concentration Addition

$$1 = \frac{c_1}{EC_{x,1}} + \frac{c_2}{EC_{x,2}}$$

$$ECx_{mix} = \left(\sum_{i=1}^{n} \frac{p_i}{F_i^{-1}(x_i)} \right)^{-1}$$

BLISS Independence - Independent Action - Response Addition

$$E_{(c1,2)} = E_{(c1)} + E_{(c2)} - E_{(c1)} \bullet E_{(c2)}$$

$$X = 1 - \prod_{i=1}^{n} \left(1 - F_i\left(p_i \bullet (ECx_{mix})\right)\right)$$

Journal of the Royal Statistical Society

SERIES B (METHODOLOGICAL)

Vol. XIV, No. 2, 1952

QUANTAL RESPONSES TO MIXTURES OF POISONS

By R. L. PLACKETT

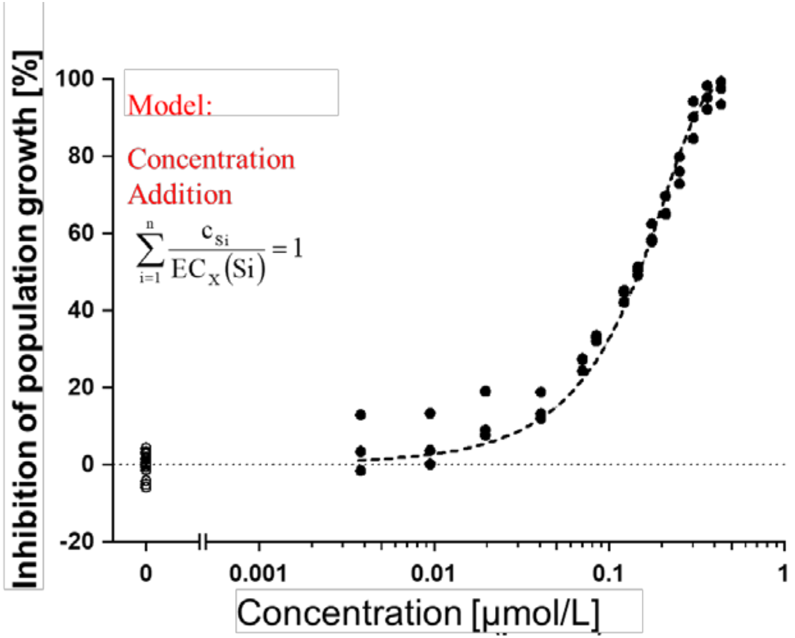
Department of Applied Mathematics, University of Liverpool

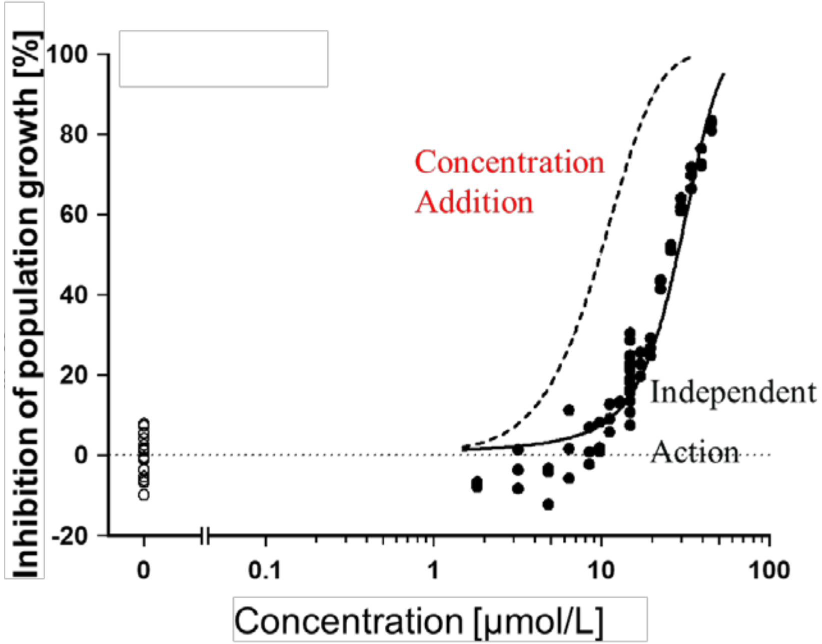
and P. S. HEWLETT

Pest Infestation Laboratory, Slough

		Mode-of-action	
		Similar	*Dissimilar*
Effect	*Non-interactive*	Simple similar	Simple independent
	Interactive	Complex similar	Dependent

Predictability of combined effects → ecotoxicology





Faust et al. 2000
J Environ Qual 29:1063

Predictability of combined effects → rat toxicology

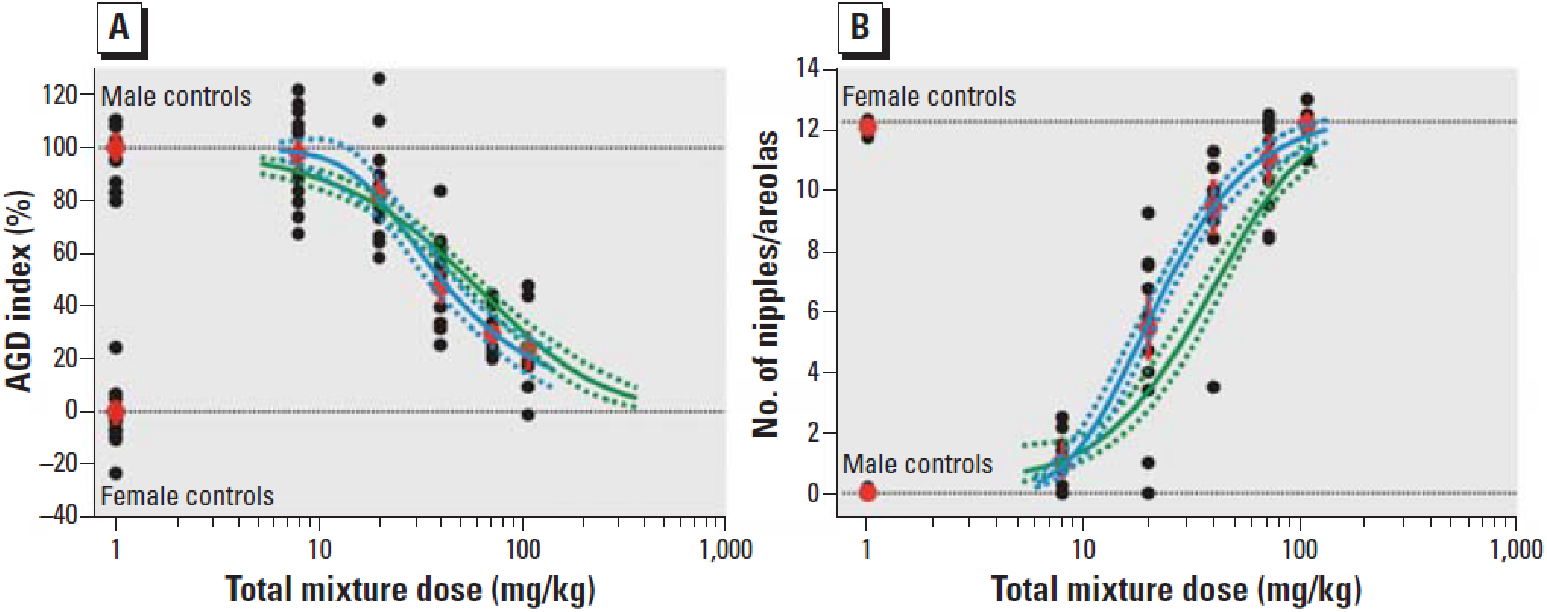


Figure 1. Effects of mixed exposure to vinclozolin, flutamide, and procymidone on AGD (*A*) and NR (*B*).

Combined Exposure to Anti-Androgens Exacerbates Disruption of Sexual Differentiation in the Rat

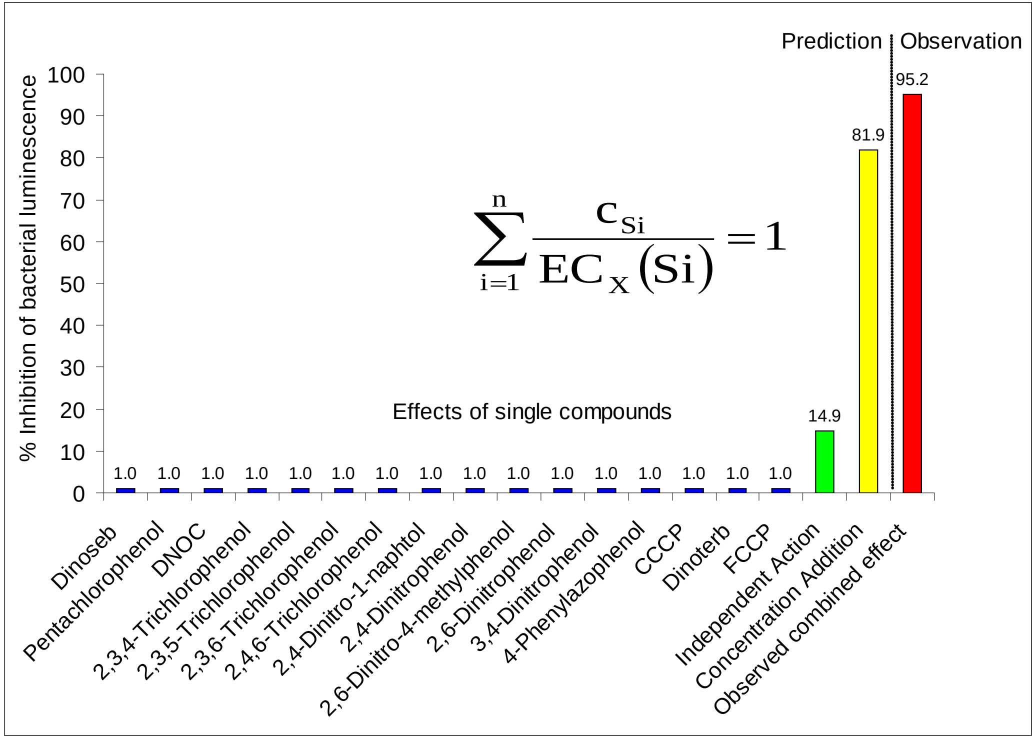
Ulla Hass,[1] Martin Scholze,[2] Sofie Christiansen,[1] Majken Dalgaard,[1] Anne Marie Vinggaard,[1] Marta Axelstad,[1] Stine Broeng Metzdorff,[1] and Andreas Kortenkamp[2]

[1]Danish Institute for Food and Veterinary Research, Department of Toxicology and Risk Assessment, Søborg, Denmark; [2]The School of Pharmacy, University of London, London, United Kingdom

Environ Health Perspec 115 Suppl 1, 122-128 (2007)

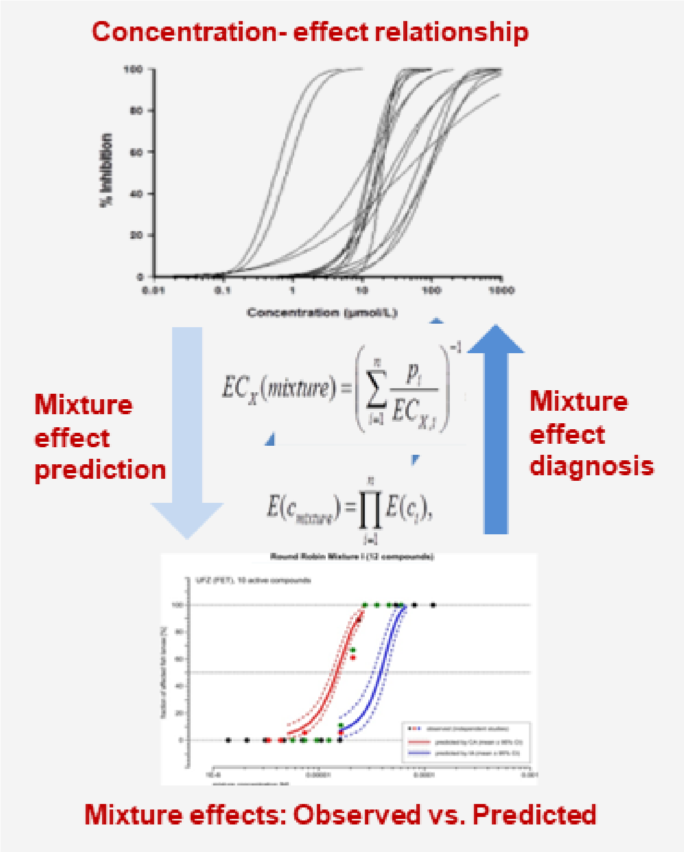
Predicting combined effects at low EC

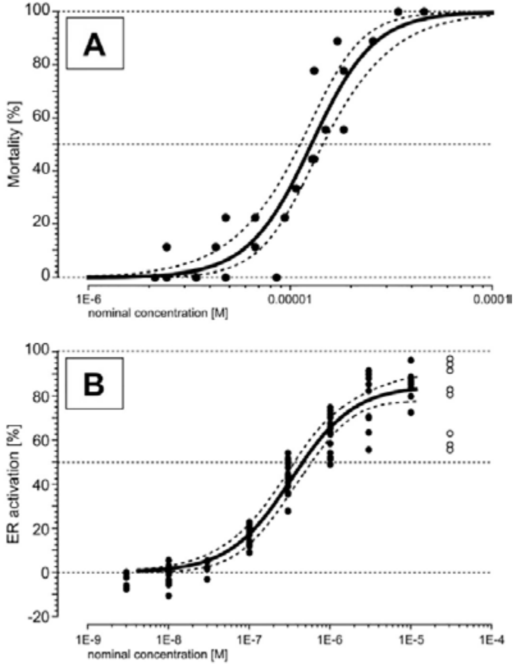
‚Something from nothing‘



Altenburger & Greco,
Integr Environ Assess Manag.
5:62, 2009

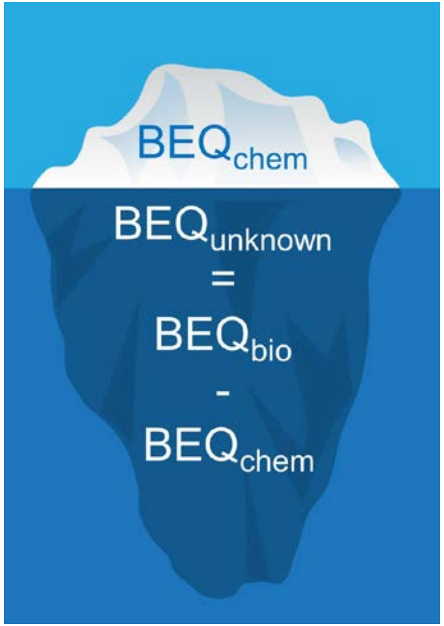
Predictability depends on good quality input data



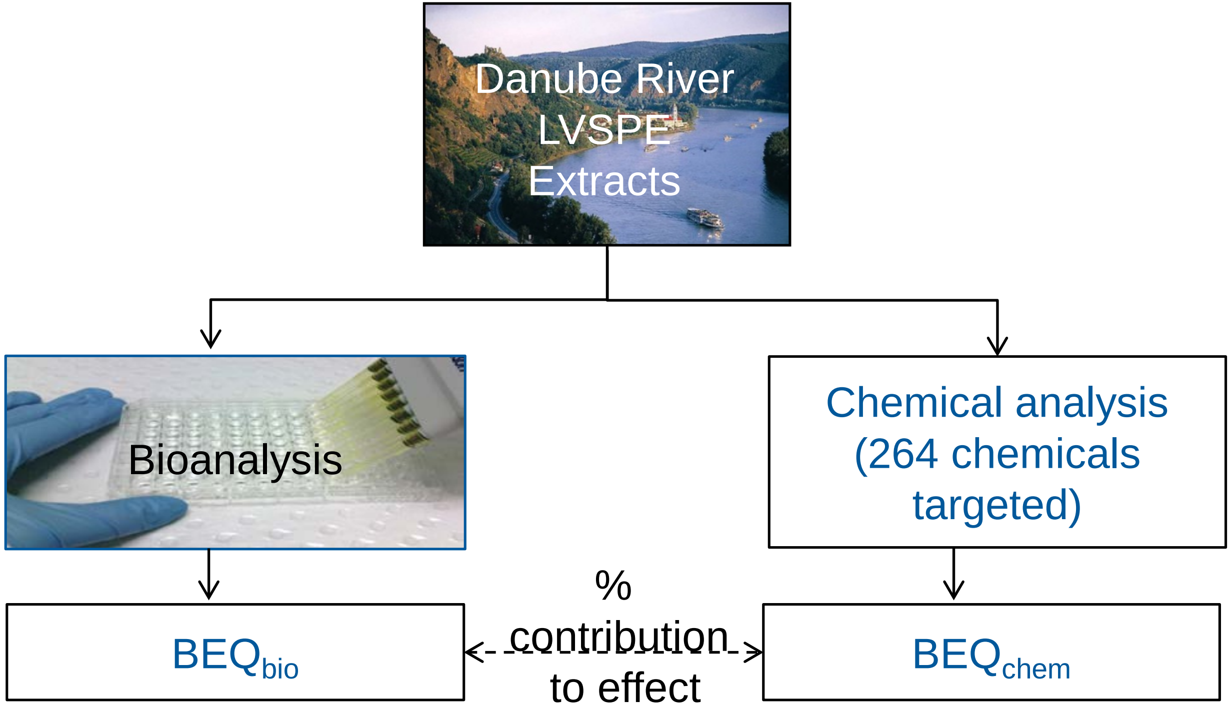


Altenburger et al. (2018): Mixture effects in samples of multiple contaminants – An inter-laboratory study with manifold bioassays. Environ. Int. 114 , 95 - 106

Combined effects – Linking chemical & bio-analytics



„Iceberg“ modelling



Neale, et al. (2015) ES&T 49: 14614-14624

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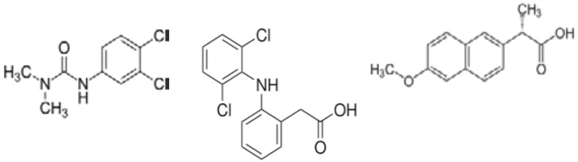
→ towards comprehensive combined effect predictions

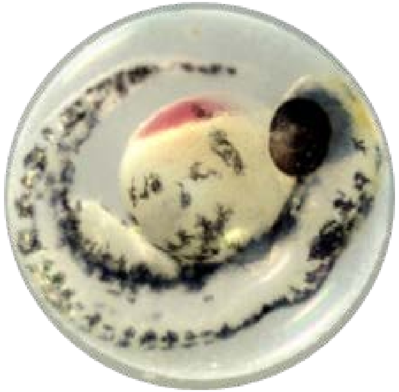
Zebrafish eggs exposed to

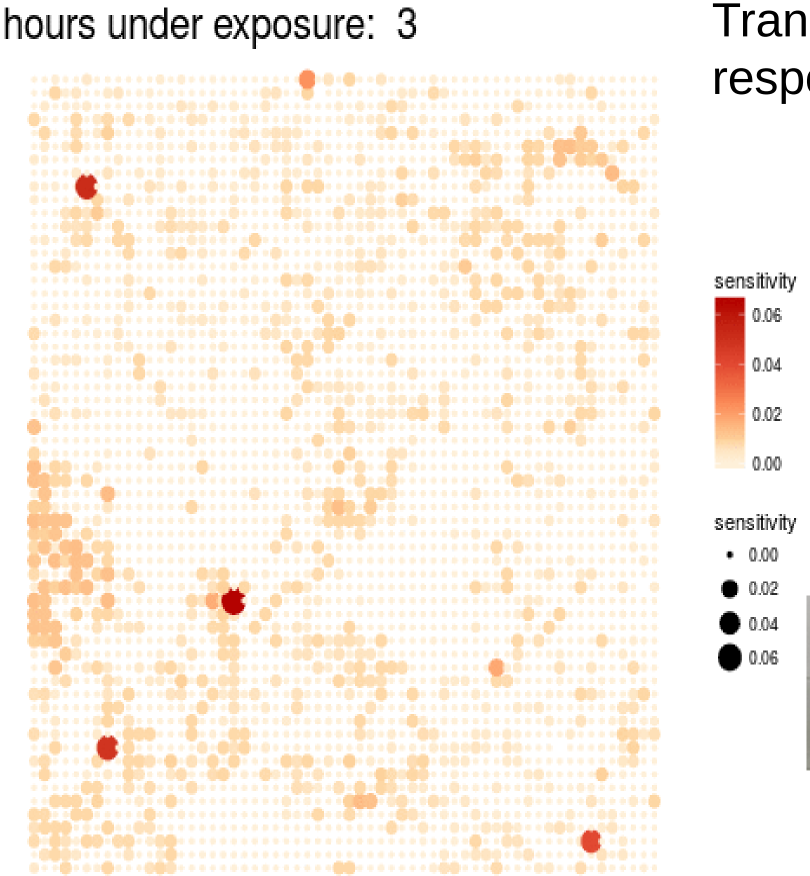


Diuron

Diclofenac

Naproxen

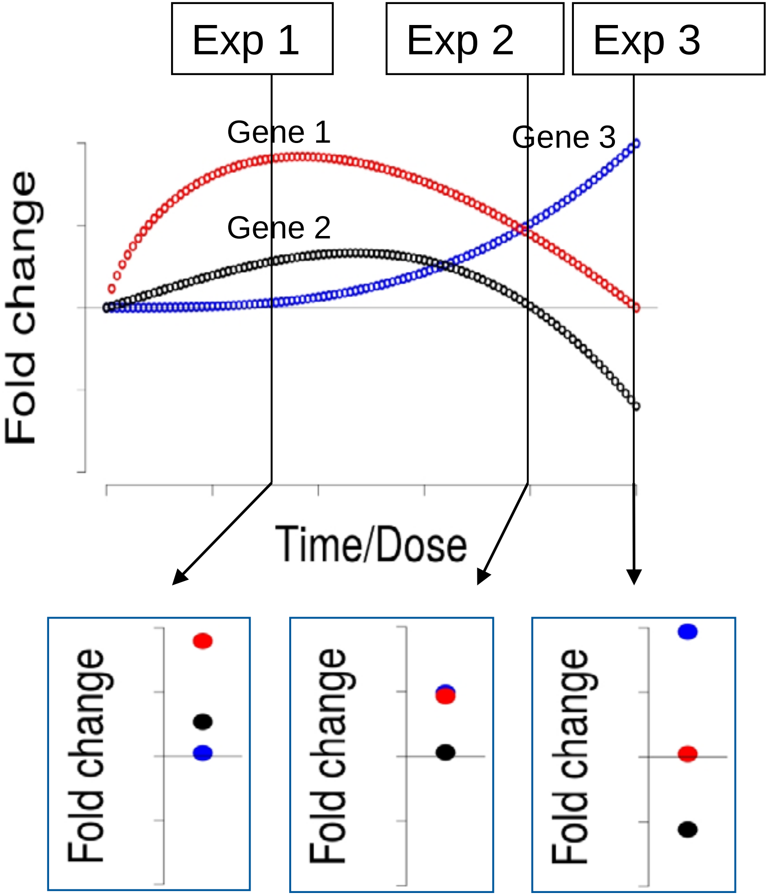




Transcriptome response

Andreas Schüttler

→ towards comprehensive combined effect predictions



→ dense sampling design

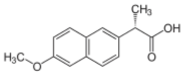
Dose / Time / age ZFE (h of exposure)	0 *Control*	LC25 /x^4	LC25 /x^3	LC25 /x^2	LC25 /x	LC25
24 hpf (0) – Exposure Start						
27 hpf (3)	X X	X	X	X	X	X X
30 hpf (6)	X X	X	X	X	X	X X
36 hpf (12)	X X	X	X	X	X	X X
48 hpf (24)	X X	X	X	X	X	X X
72 hpf (48)	X X	X X	X X	X X	X X	X X
96 hpf (72)	X X	X X	X X	X X	X X	X X

Schüttler et al. 2017,
Toxicological Sciences 157(2): 291–304

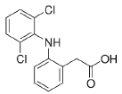
Questions and Approach

- How to see the complete picture?
- How to compare toxicogenomic effects of different substances?
- How to describe those effects mathematically to enable mixture calculations?

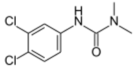
Experiment



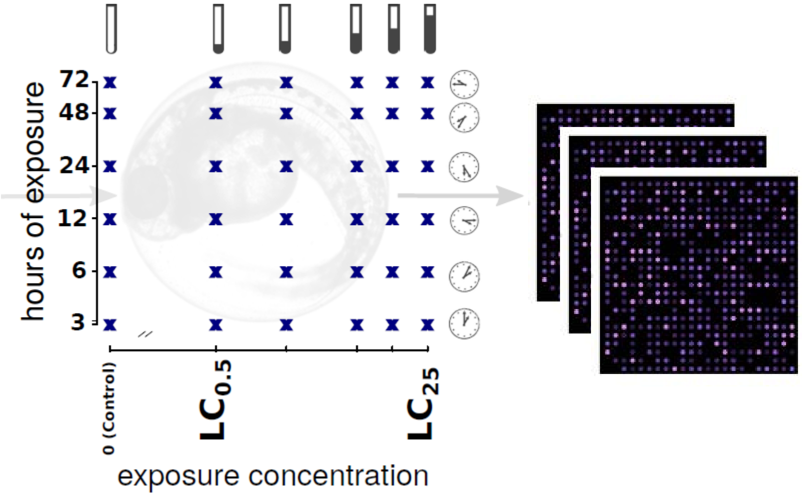
Naproxen



Diclofenac



Diuron



Data analysis

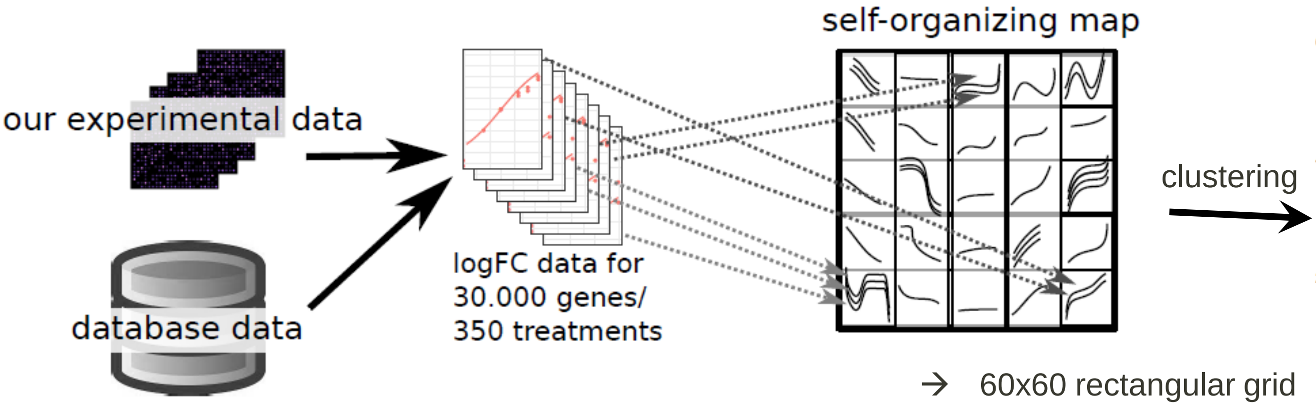
→ ***integration*** **of previous data**

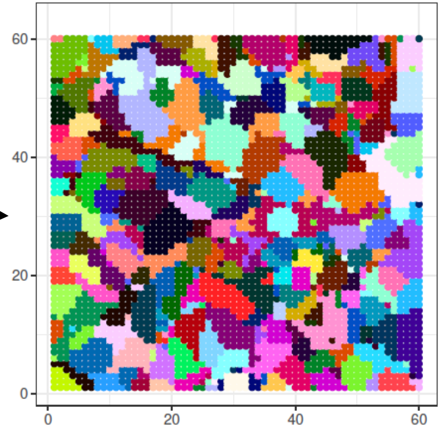
→ ***aggregation*** **of fingerprints**

→ ***modeling*** **of responses**

Schüttler et al., Map and model—moving from observation to prediction in toxicogenomics. GigaScience 8 (6), giz057, 2019

Integration and aggregation of data

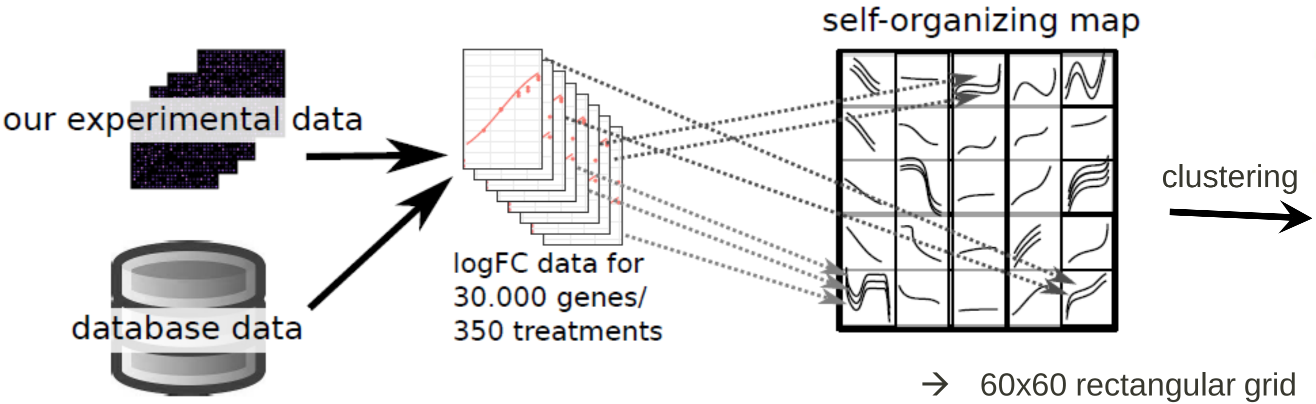




→ 60x60 rectangular grid
→ 3600 nodes
→ 118 clusters

Andreas Schüttler et al.,
GigaScience, 2019

Integration and aggregation of data



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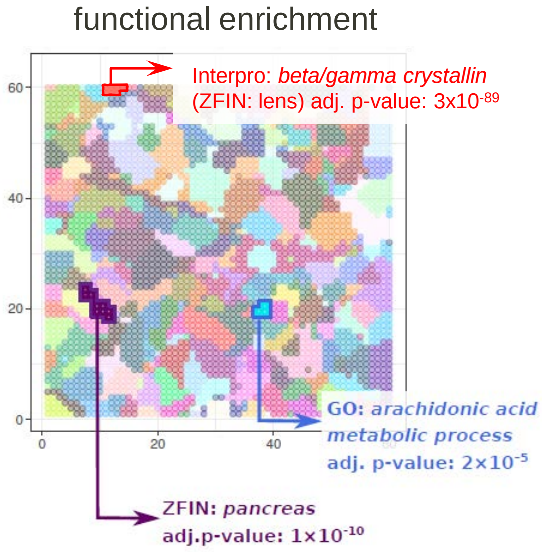
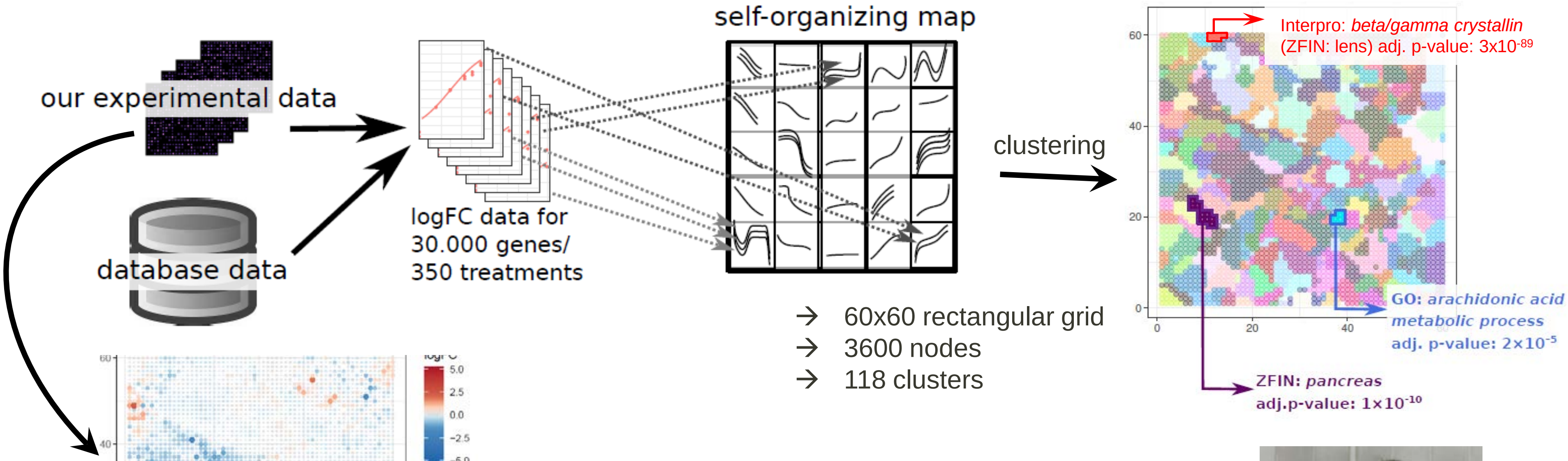


Andreas Schüttler et al., *GigaScience*, 2019

Integration and aggregation of data



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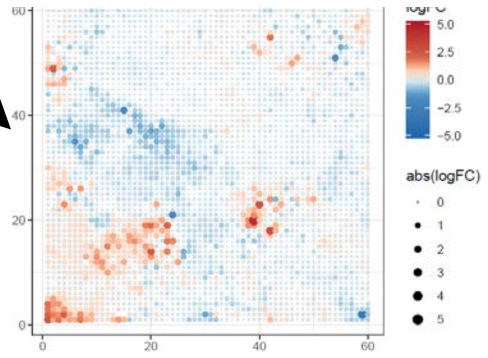
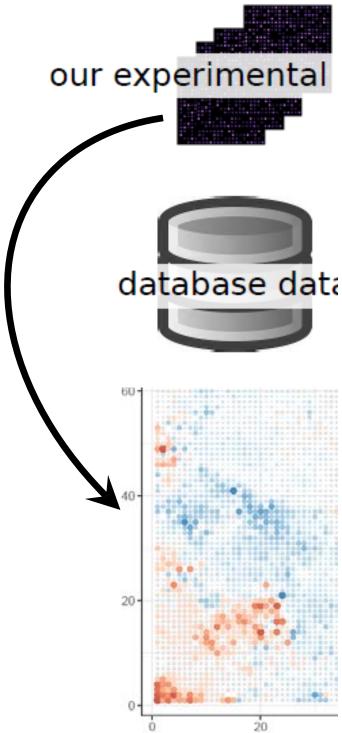


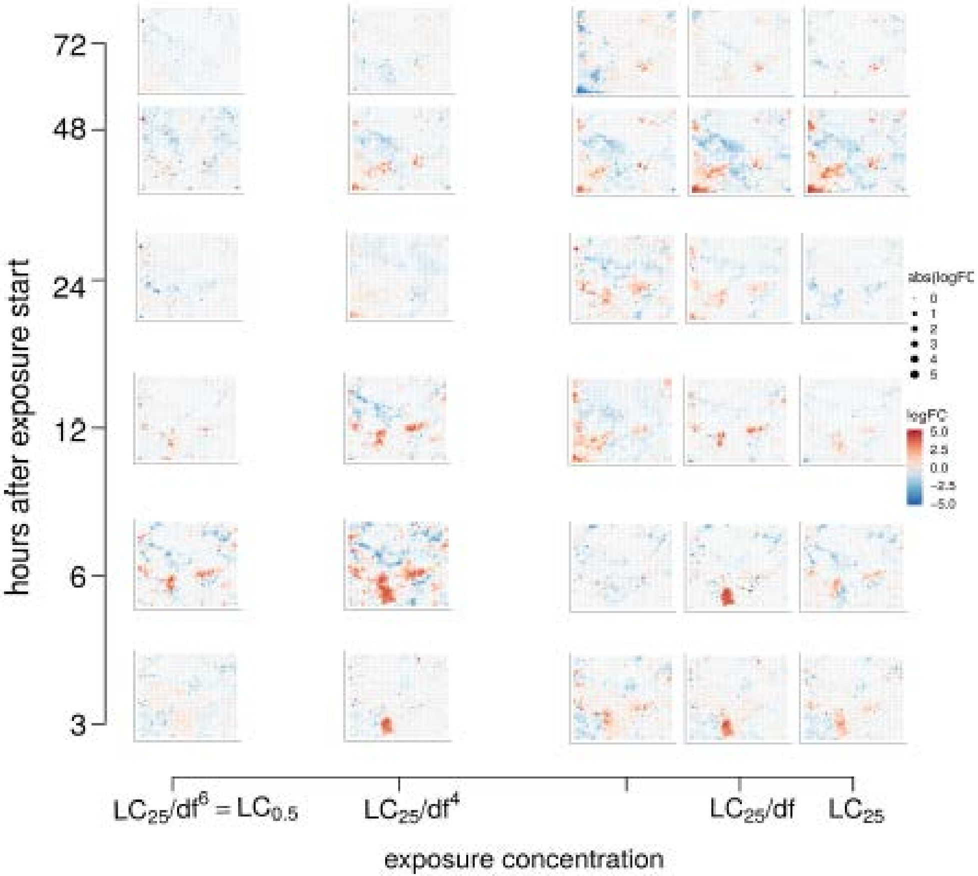
Diclofenac, 48 hours exposure, 7.4 µM

Andreas Schüttler et al., *GigaScience,* 2019

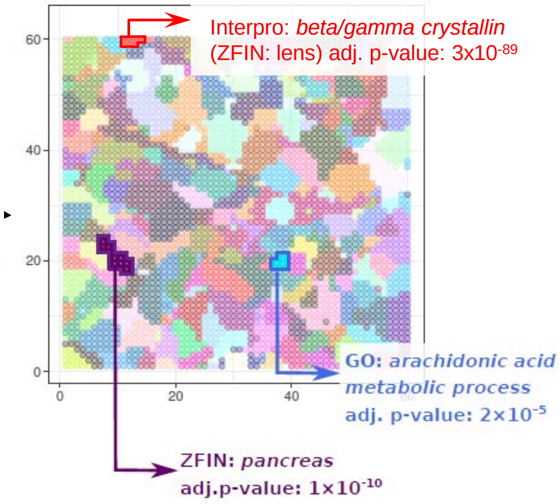
Integration and aggregation of data



Diclofenac, 48 hours

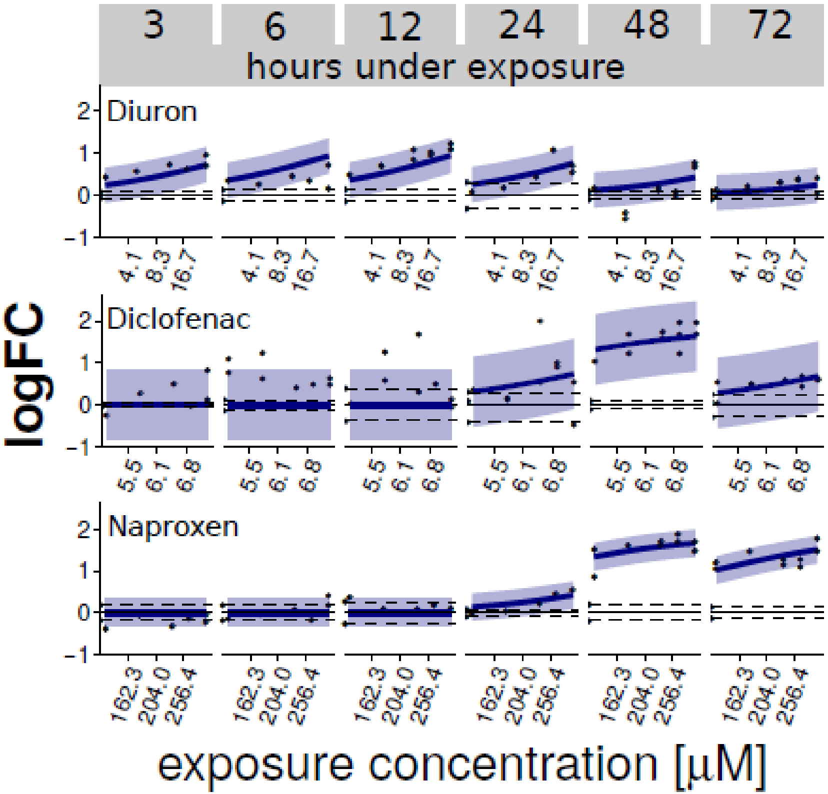


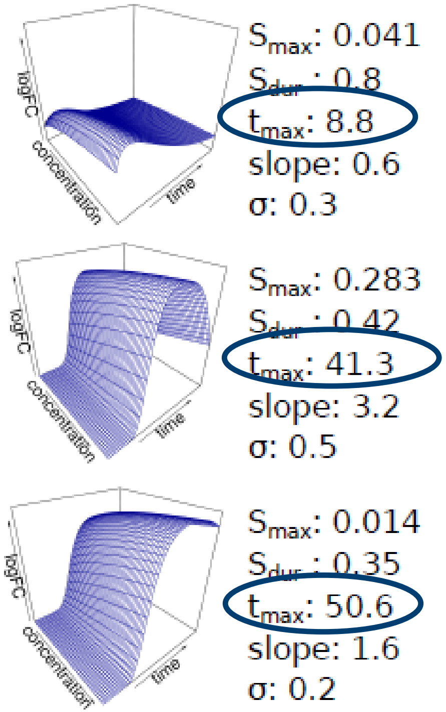
functional enrichment

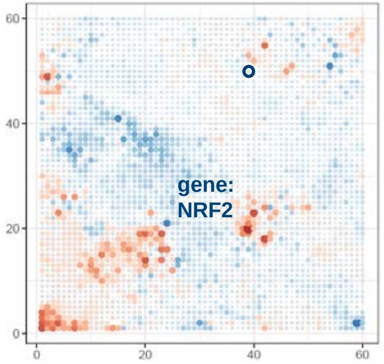


Andreas Schüttler et al., *GigaScience,* 2019

Regression modelling







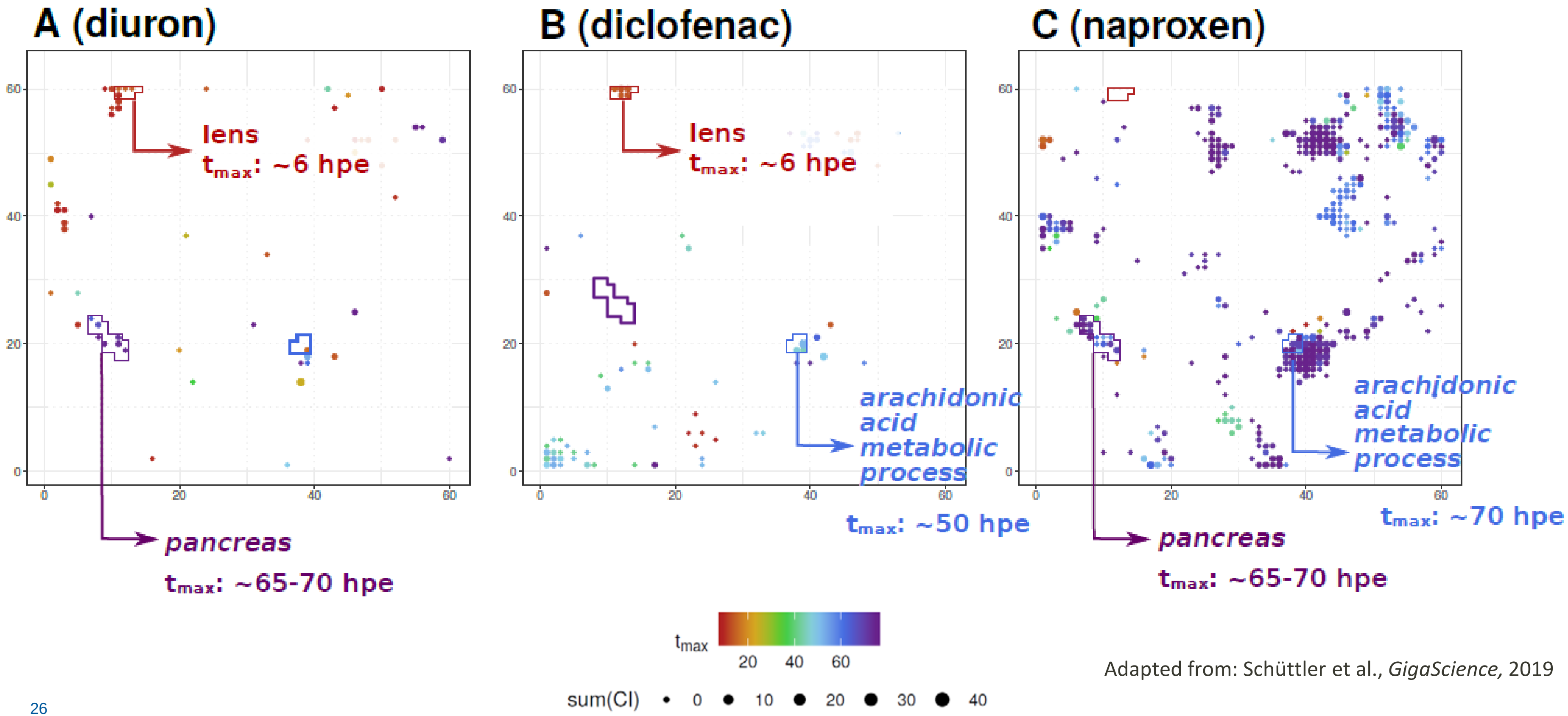
$$logFC(c) = \frac{logFC_{max}}{1 + e^{-slope*(\log(c)-\log(X_{50}))}}$$

$$sensitivity(t) = \frac{1}{X_{50}(t)} = S_{max} * e^{-0.5*\left(\frac{\log(t)-\log(t_{max})}{S_{dur}}\right)^2}$$

Mapping model parameters

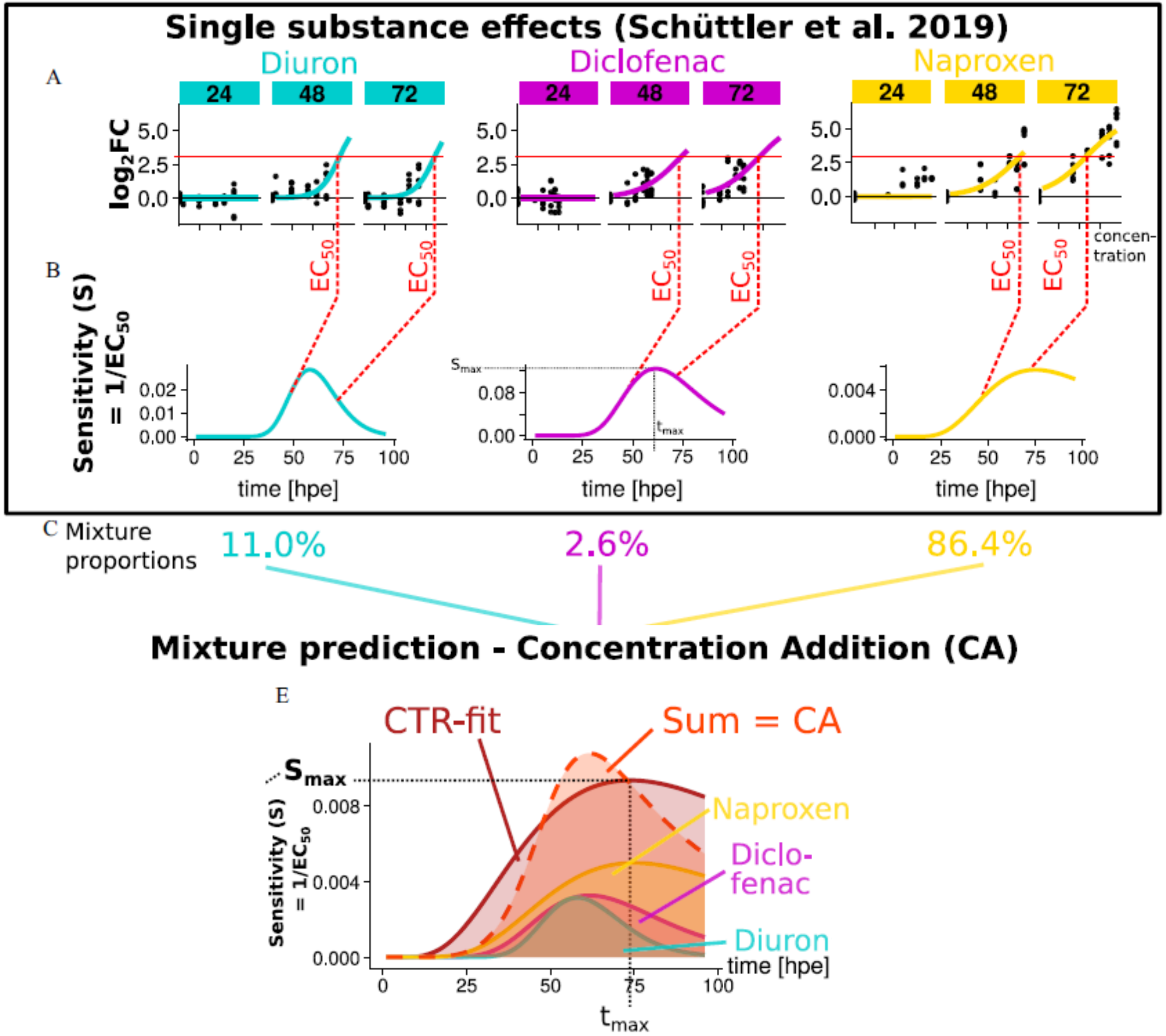
Herbicide (PSII Inhibitor)

Pharmaceuticals (Cox-inhibitors)



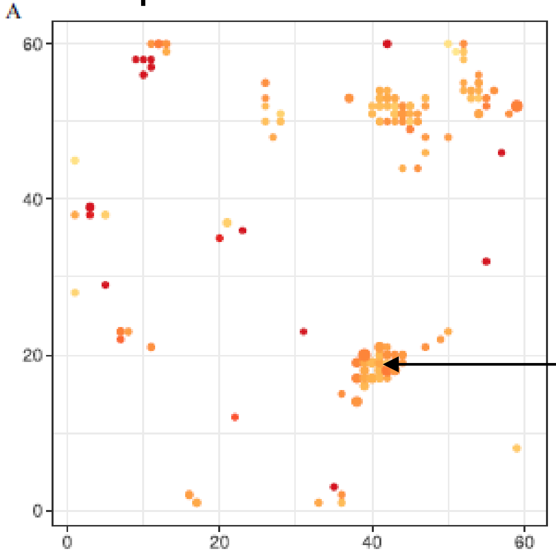
Adapted from: Schüttler et al., *GigaScience,* 2019

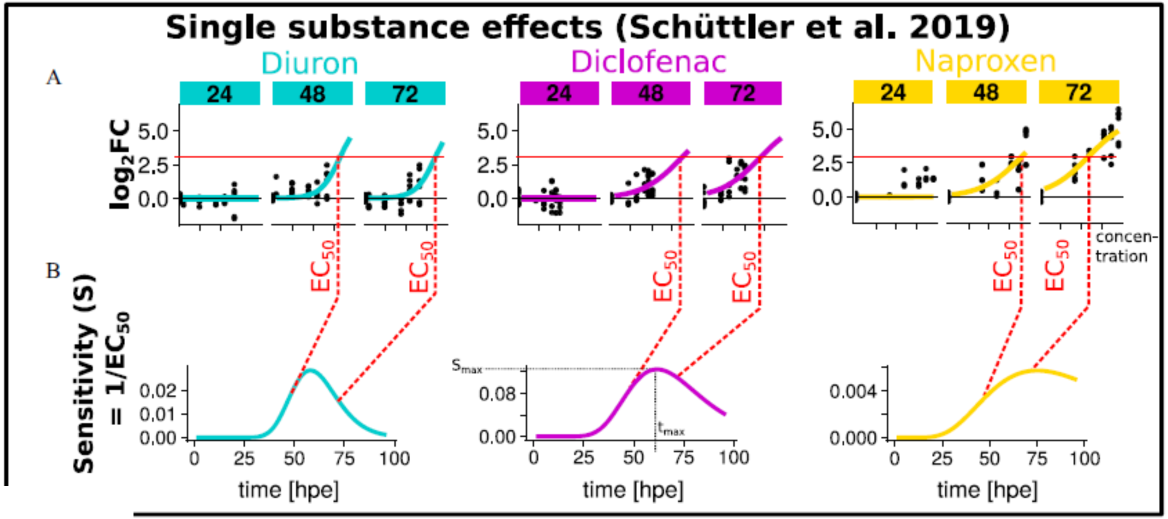
Mixture effect prediction

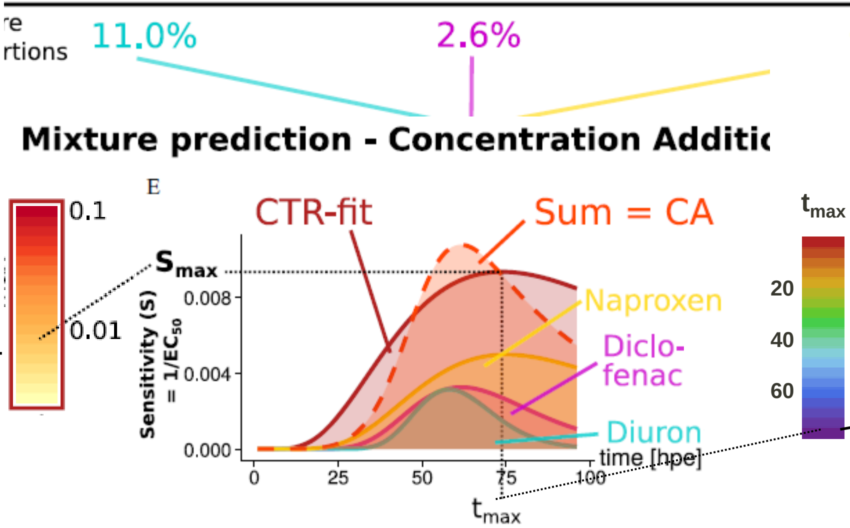


Mixture effect prediction

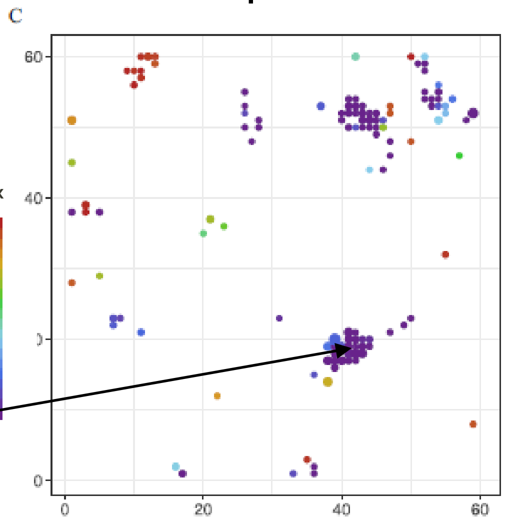
S_{max} map – prediction



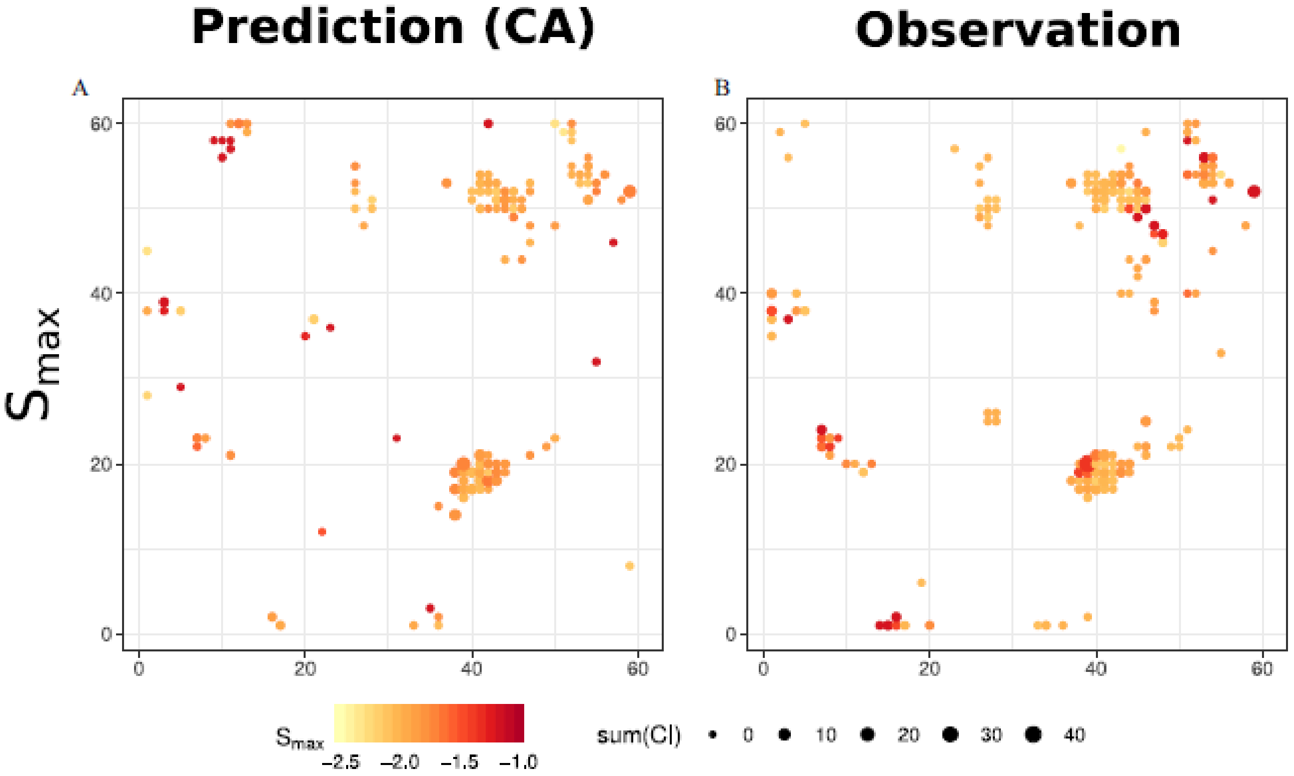




t_{max} map – prediction

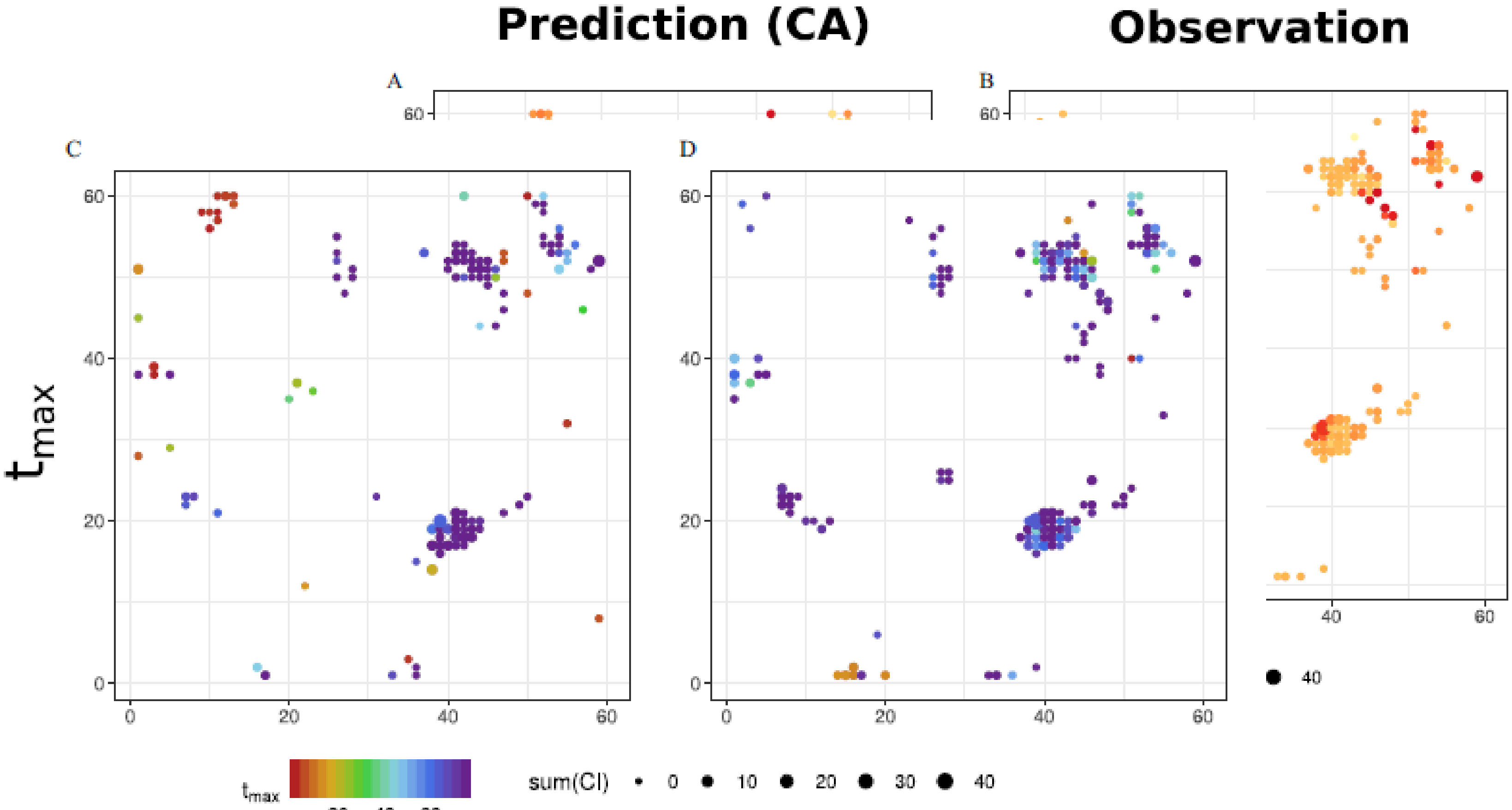


Prediction versus Observation



Schüttler et al., Transcriptome-wide prediction and measurement of combined effects induced by chemical mixture exposure in zebrafish embryos. Environ. Health Perspect. 2021, 129 (4), art. 047006

Prediction versus Observation



Summary (for combined effects in a multiple response system)

- Recovery of single substance effects? **YES**
- Occurrence of combined effects on the transcriptome? **YES**

  → Combined effects observed also for anticipated dissimilar acting compounds
- Can we predict the effects of a mixture on the transcriptome? **YES**

  → CA outperforms other mixture concepts (Schüttler et al. 2021, EHP)
- More-than-additive combined effects occurred rarely,
  - for the pairs of similar and dissimilar acting compounds;
  - for induced and repressed genes and nodes; but
  - can be identified by comparison of observation with CA prediction.

Conclusions and Outlook

- Exposome studies will specify multiple exposure of organisms;
- Analytical characterisation of contamination and mixture exposure can be complemented by bioanalytical approaches;
- Concepts for component-based calculation of combined effects show reasonable predictability accross bioassays;
- Including process knowledge allows access to more complex response patterns.

Drakvik et al. (2020): Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment. Environ. Int. 134 , art. 105267

Thank you for your attention

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https://www.ufz.de