High-**T**hroughput **Ec**otoxicology (HITEC)

October 2025

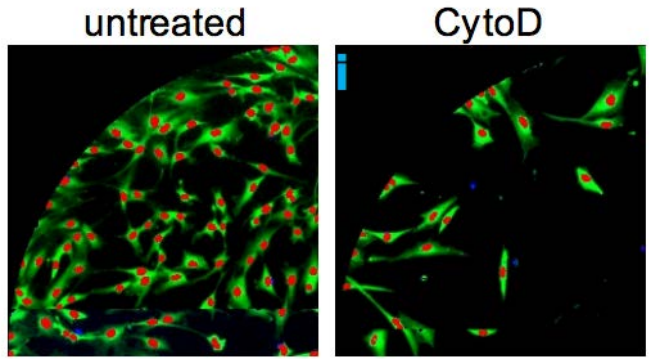
Jo Nyffeler

Gender-neutral

Niffeler

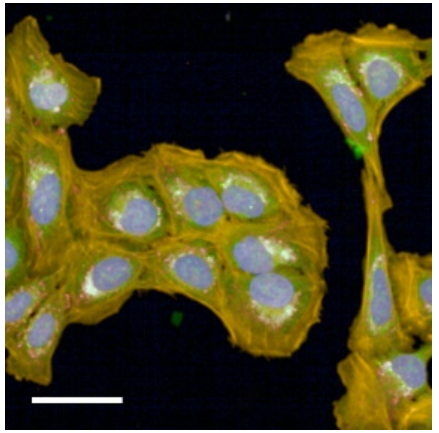
Pronouns:
- they/them
- Jo

Introduction: Dr. Jo Nyffeler



- **PhD at University of Konstanz, Germany (2012-2017)**
  - group of Dr. Marcel Leist
  - development of high-content assays for *in vitro* developmental neu

- **PostDoc at Center for Computational Toxicology & Exposure (CCTE), US EPA (2017-2022)**
  - group of Dr. Joshua Harrill
  - high-throughput imaging-based profiling ('Cell Painting'), computational toxicology



Helmholtz Young Investigator Grant

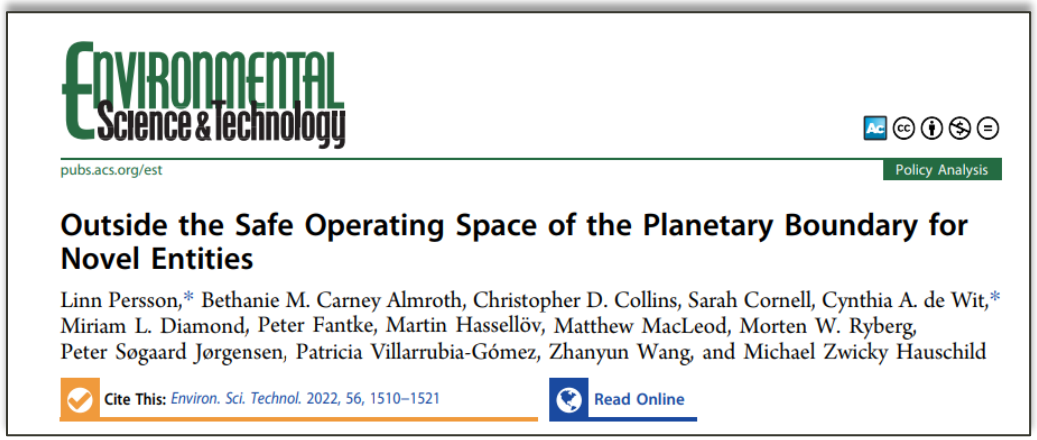
- Goal: establish and lead my own research group
- 1.5 Mio € over 5 years
  - salaries
  - experiments
  - travel, training, …
- Jan 2023 – Dec 2027
  - evaluation for tenure in spring 2026

High-**T**hroughput **Ec**otoxicology

HiTEc

GOETHE UNIVERSITÄT FRANKFURT AM MAIN

Too many chemicals – too little toxicity information



Environmental Science & Technology

pubs.acs.org/est

Policy Analysis

Outside the Safe Operating Space of the Planetary Boundary for Novel Entities

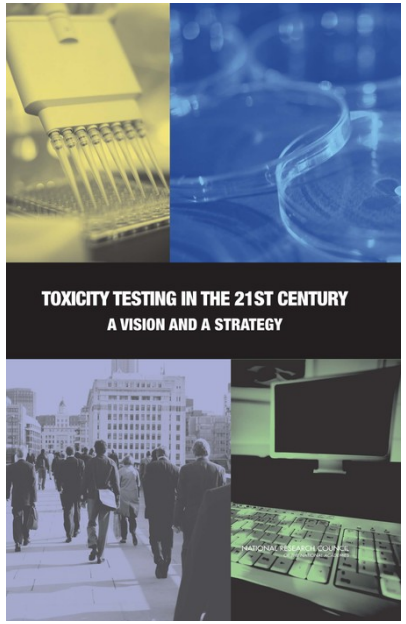
Linn Persson,* Bethanie M. Carney Almroth, Christopher D. Collins, Sarah Cornell, Cynthia A. de Wit,* Miriam L. Diamond, Peter Fantke, Martin Hassellöv, Matthew MacLeod, Morten W. Ryberg, Peter Søgaard Jørgensen, Patricia Villarrubia-Gómez, Zhanyun Wang, and Michael Zwicky Hauschild

Cite This: *Environ. Sci. Technol.* 2022, 56, 1510−1521 Read Online

>300.000 chemicals worldwide

Traditional test methods:

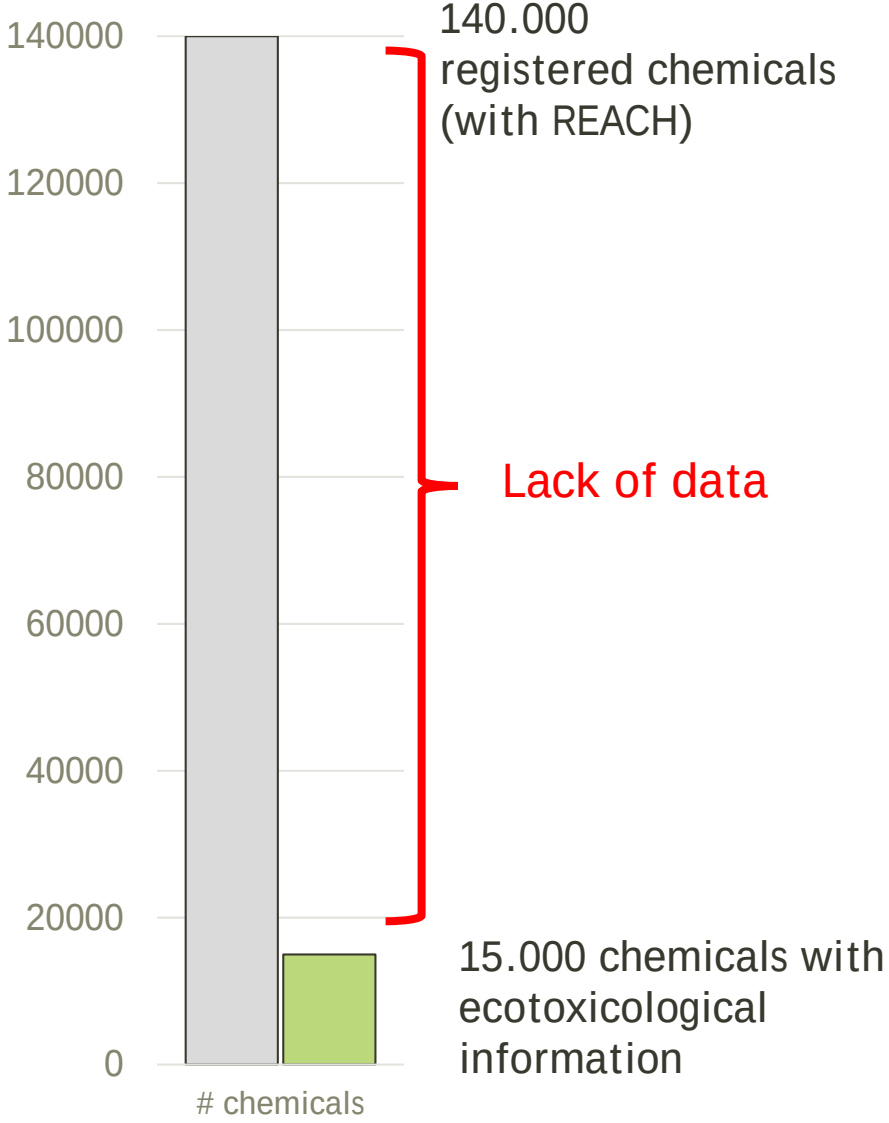
- Rely on animal experiments
- Time-consuming (1 year/chemical)
- Cost-intensive (1 Mio €/endpoint)



National Research Council 2007

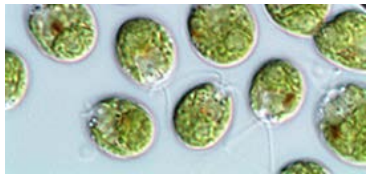
New
Approach
Methods

Challenges in ecotoxicology



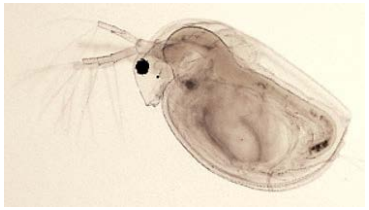
Typical test battery

Algae



www.eawag.ch

Invertebrate



www.hydrotox.de

Fish

www.tnaqua.org

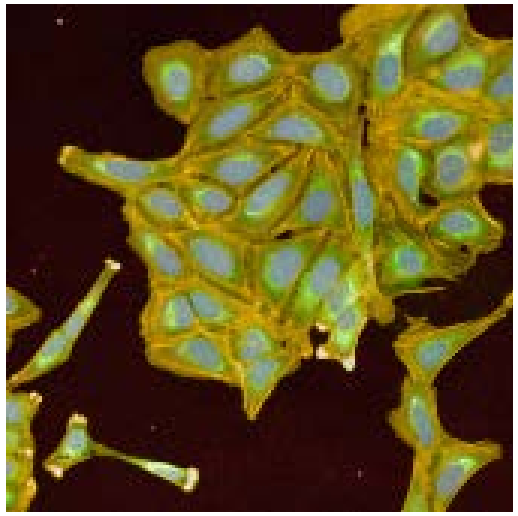
Chemicals with data in ECOTOXdb:

Taxon	Number of chemicals
Fish	6833
Crustaceans	3587
Insects/Spiders	2968
Mammals	2196
Molluscs	1843
Other Invertebrates	1797
Worms	1510
Birds	1490
Amphibians	1168
Reptiles	168

Underrepresented taxa

Aim: Transform ecotoxicological hazard assessment

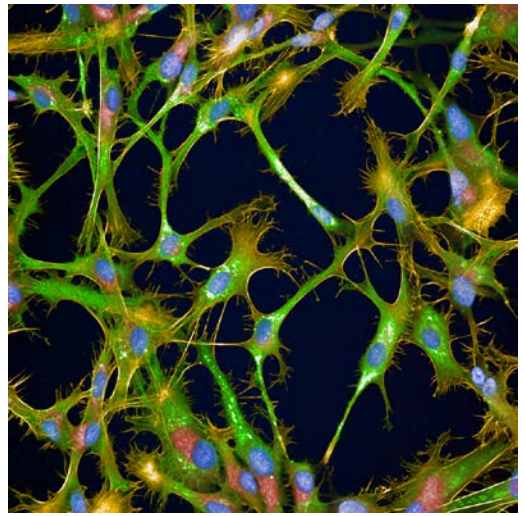
human-centric



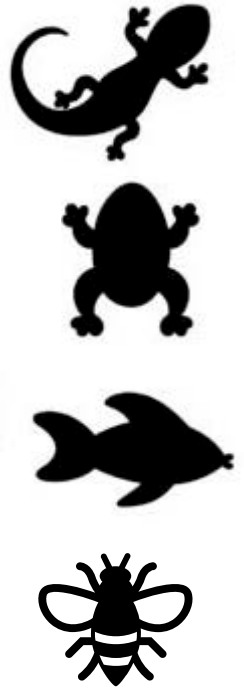
Human osteosarcoma cells
Adapted from Nyffeler *et al.* 2020

- image-based methods
- high-throughput

ecologically relevant species



Rainbow trout gill cells
Adapted from Nyffeler *et al.* 2022

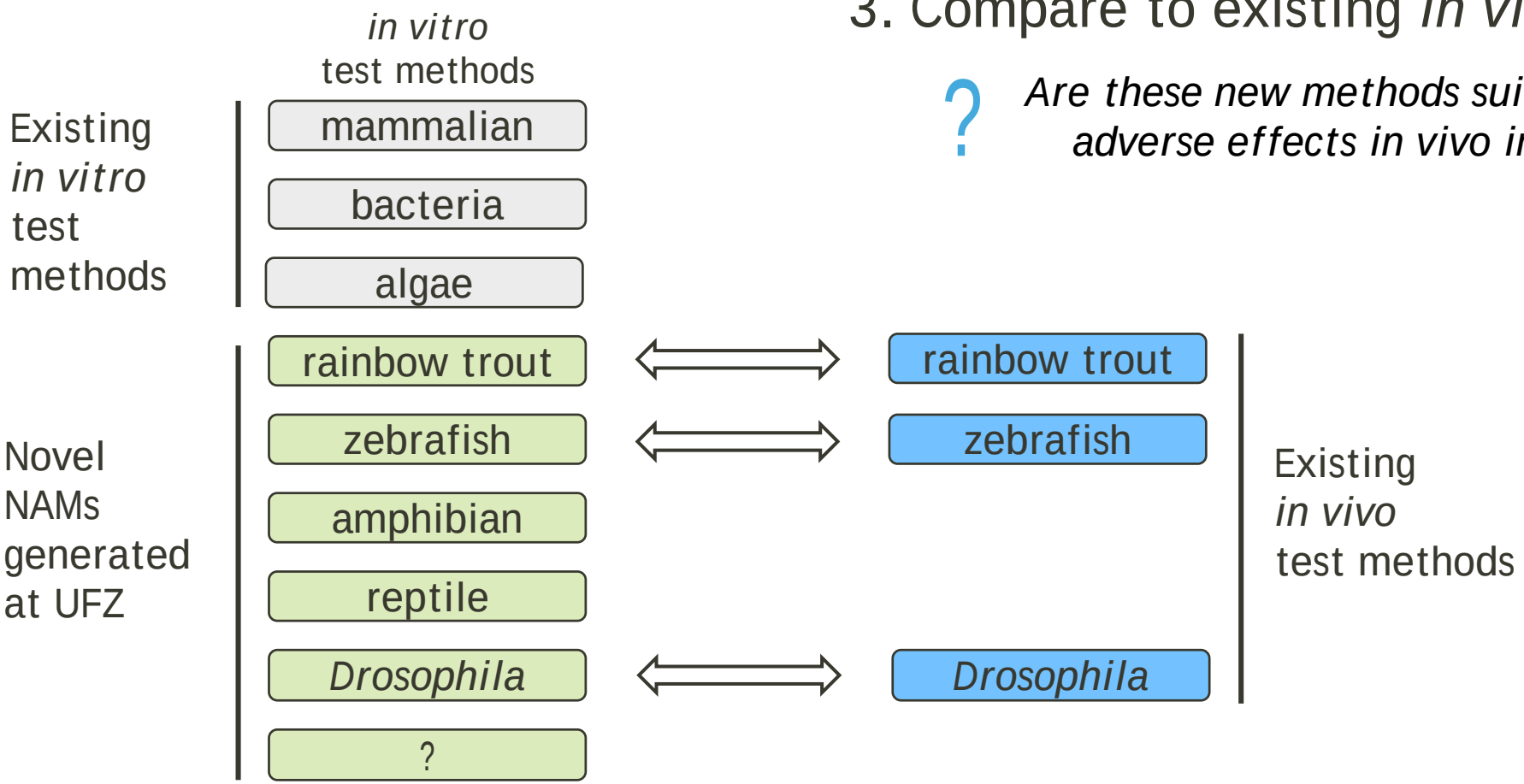


 # tested chemicals ↗
 # represented species ↗

Research Strategy

1. Develop new test methods for six taxa
2. Test ~240 chemicals with each test method
3. Compare to existing *in vivo* data

? *Are these new methods suitable for predicting adverse effects in vivo in various species?* ?



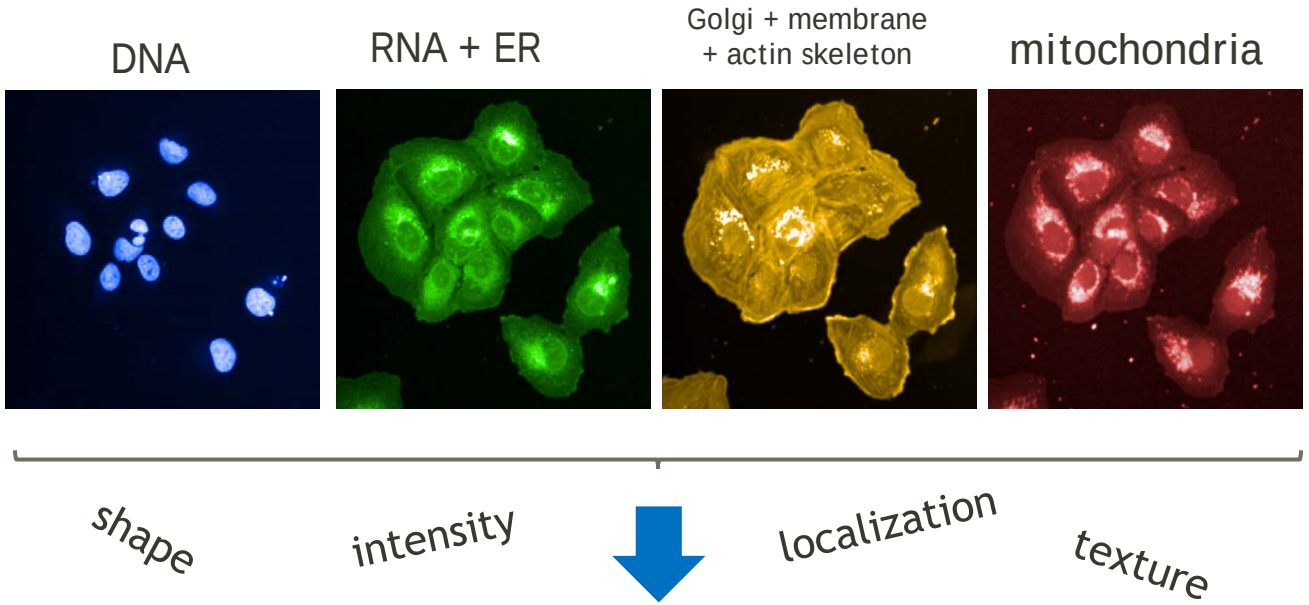
What is Imaging-Based Phenotypic Profiling?

- labeling of various cell organelles with fluorescent probes in *in vitro* cultures
- assessing a large variety of morphological features on individual cells

‘Cell Painting’ assay

Gustafsdottir *et al.* 2013

Bray *et al.* 2016



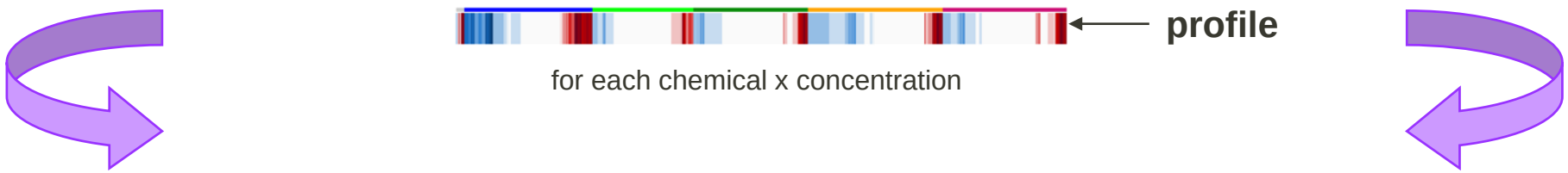
Flourescent labels
DNA: H-33342
RNA: SYTO14
ER: Concanavalin A-488
Actin: Phalloidin-568
Golgi + Membrane: wheat germ agglutinin (WGA) -555
Mitochondria: MitoTracker

Nyffeler *et al.* 2020

Cell Painting = Phenotypic Profiling

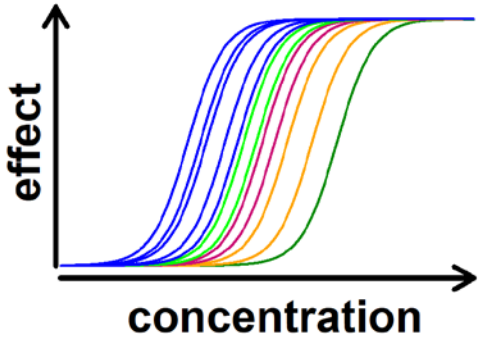
High-Throughput Phenotypic Profiling = HTPP

Two Applications

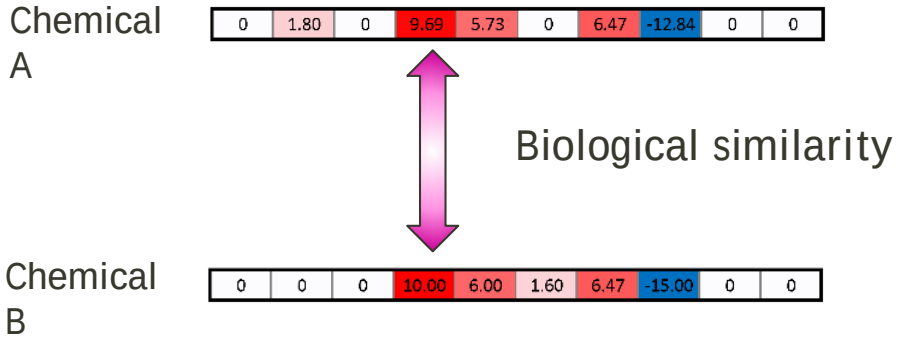


Application 1

concentration-response modelling



Application 2



Potency estimation:
***in vitro* point-of-departure (POD)**

- Nyffeler *et al.* (2020). Toxicol Appl Pharmacol. PMID: 31899216
- Willis *et al.* (2020). SLAS Discov. PMID: 32546035
- Nyffeler *et al.* (2021). SLAS Discov. PMID: 32862757
- Nyffeler *et al.* (2022). Toxicol Appl Pharmacol. PMID: 35483669

Compare profiles with annotated reference chemicals
→ putative mechanisms

- Nyffeler *et al.* (2022). Toxicol Appl Pharmacol. PMID: 35483669
- Nyffeler *et al.* (2023). Toxicol Appl Pharmacol. PMID: 37044265

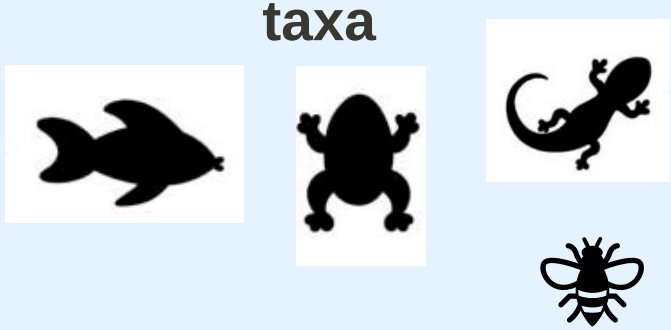
Research Projects

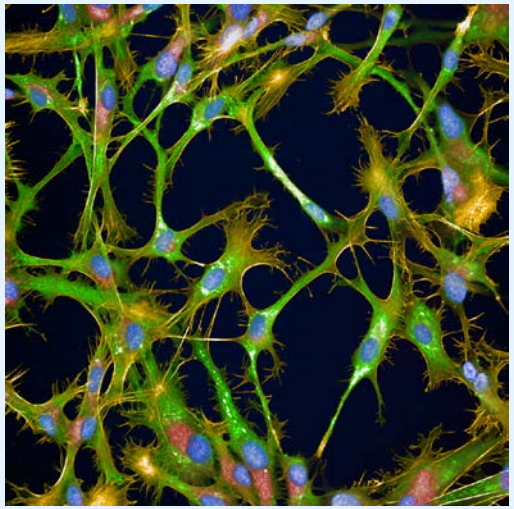
Helmholtz Young Investigator Grant (1.5 Mio €)

Cooperation with Bayer Crop Science
- funding of a PhD student (~ 200.000 €)

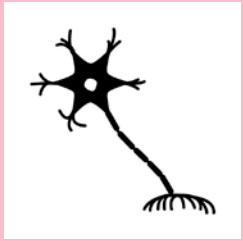
+ large interest from industry

Application of Cell Painting to environmentally relevant taxa





Parkinsonian Neurodegeneration Rapid Assessment using NAMs (PANDORA)



AOP-PD

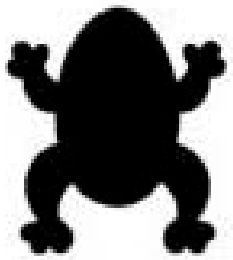
efsa

1.3 Mio €
2024-2027

15.000 €
7 months

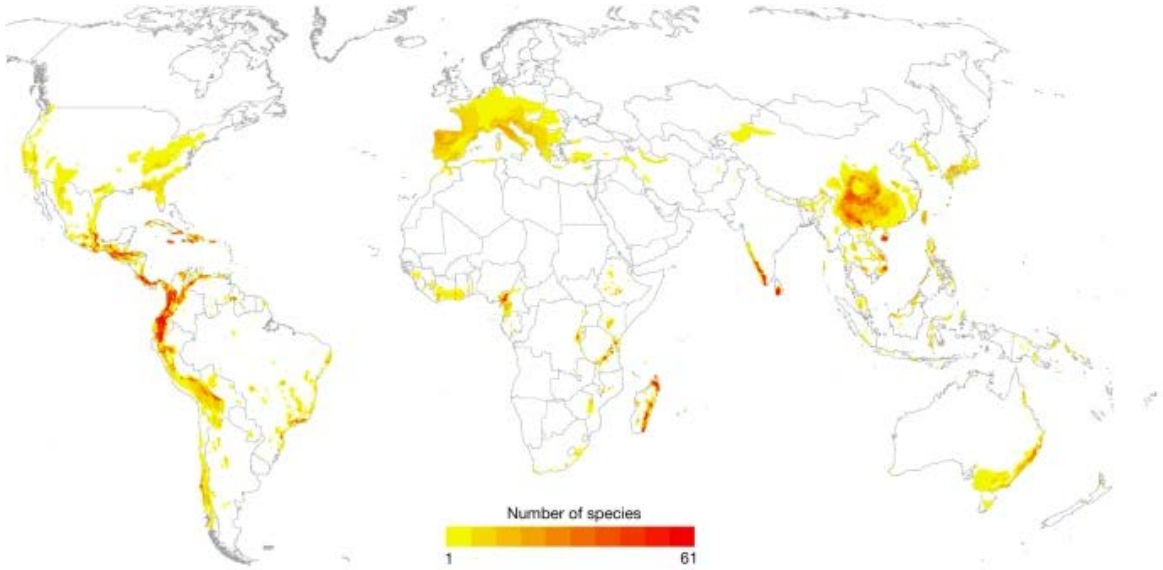
Collaborative Research on Avian NAMs for Ecotoxicology (CRANE)

7 months
~100.000 €



Developing NAMs tailored to amphibians

Luisa Reger, PhD student



Luedtke et al., 2023

Research Gap

- **Regulatory Gap**

  Current pesticide risk assessments may not adequately address unique amphibian biology.

- **Testing & Data Gaps**

  Standard tests focus on aquatic life stages with no established methods for terrestrial stages

  Crucial need for *in vitro* endpoints

- **Proposed Approaches**

  Develop a tiered risk assessment framework

  Consider combining multiple exposure routes (dermal, oral, overspray) for a realistic worst-case scenario

→ **Action is needed!**

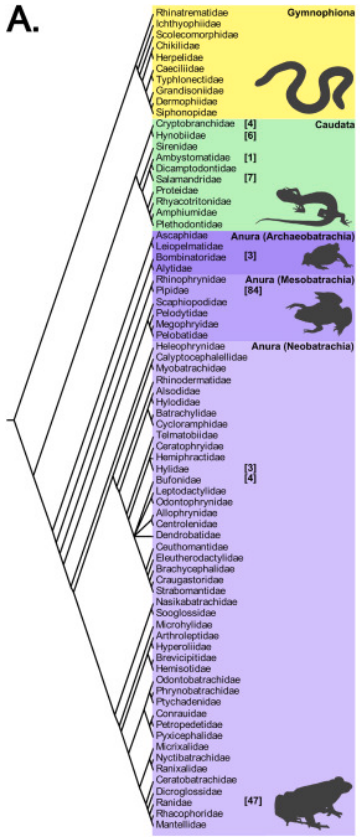

SCIENTIFIC OPINION

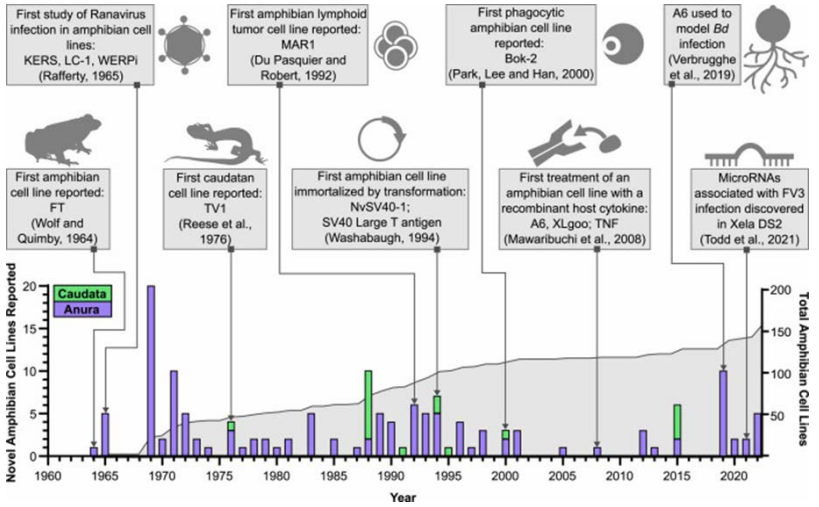
EFSA Journal

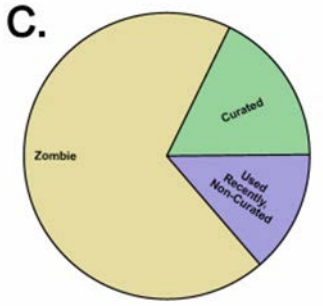
ADOPTED: 22 November 2017

doi: 10.2903/j.efsa.2018.5125

Scientific Opinion on the state of the science on pesticide risk assessment for amphibians and reptiles







Scope of the Project

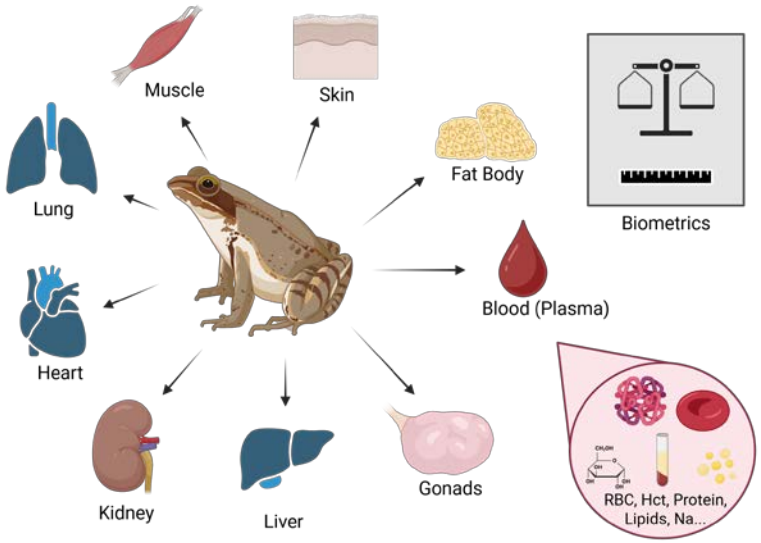
- Establish High Throughput Phenotypic Profiling (“Cell Painting) in amphibian cell line
  → Test ~ 200 chemicals in both cell lines
- Quantitative In Vitro-In Vivo Extrapolation (QIVIVE)

  translates *in vitro* toxicity data to nominal effect concentrations
- Physiologically-Based Toxicokinetic Models (PBTK)

  breaks down absorption, distribution, metabolism,

  and excretion using amphibian-specific physiology

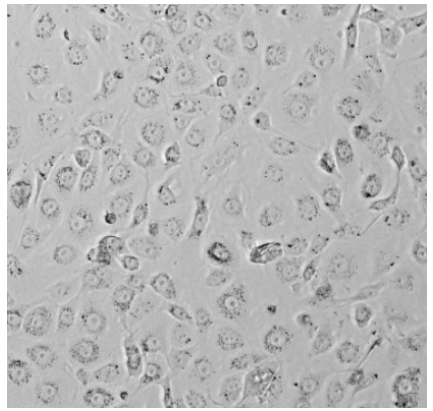
  → Collect physiological parameters for amphibians



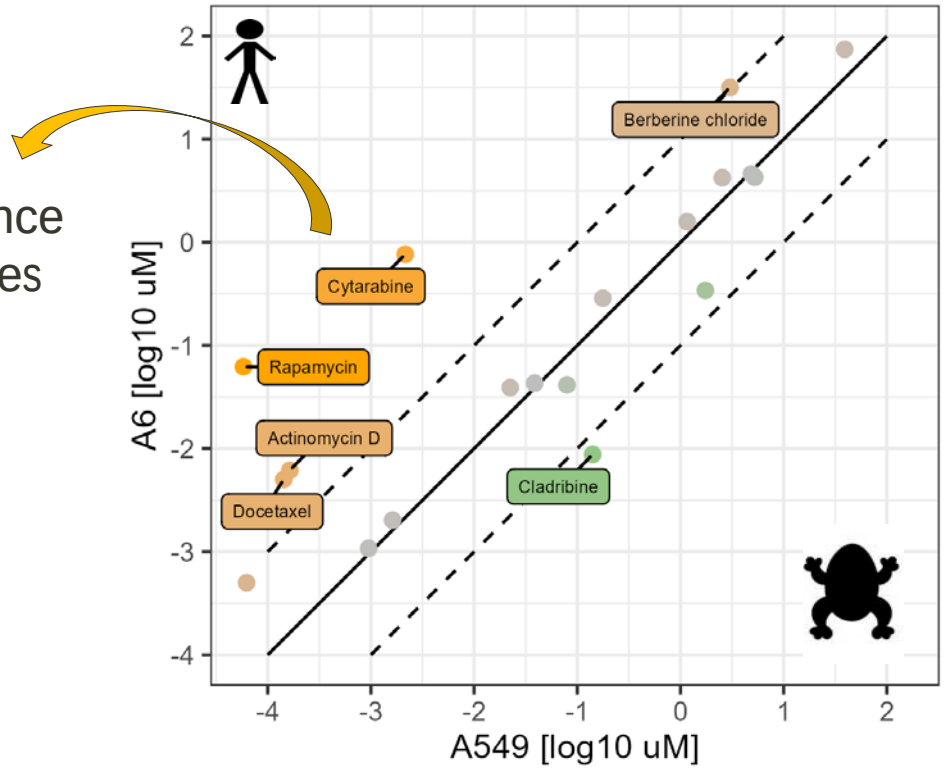
→ Include more ecology relevant species through modelling or through *in vitro* systems

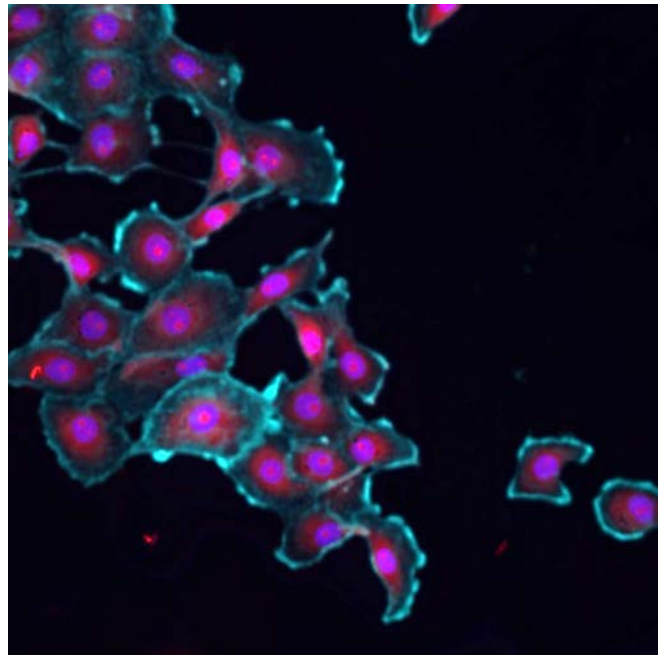
Insight into ongoing work

- Established two amphibian cell lines successfully in our lab
- Performed Cell Painting with set of reference chemicals in one and compared it to human cell line



chemicals with
>10-fold difference
between cell lines





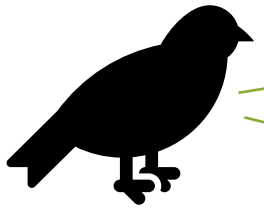
Collaborative Research on Avian NAMs for Ecotoxicology (CRANE)

Nisa Nurdhy, PhD student

Amine Aouini, HiWi

Collaborative Research on Avian NAMs for Ecotoxicology (CRANE)

- Long-term goal: alternative methods to toxicity testing with living birds
- Short-term goals:
  - collect existing *in vivo* data
  - evaluate existing *in silico* and *in vitro* methods



Sensitive indicator and informative species

OECD TG233 acute oral toxicity and TG205 avian dietary toxicity

Low throughput, time consuming, against animal welfare

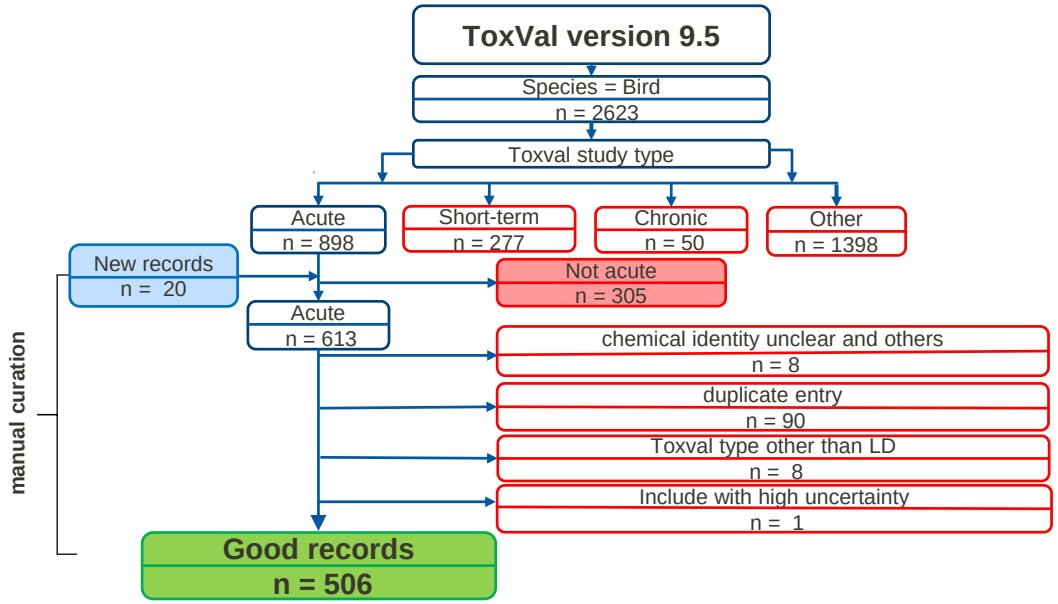
New Approach Methodologies (NAMs) in avian toxicity testing

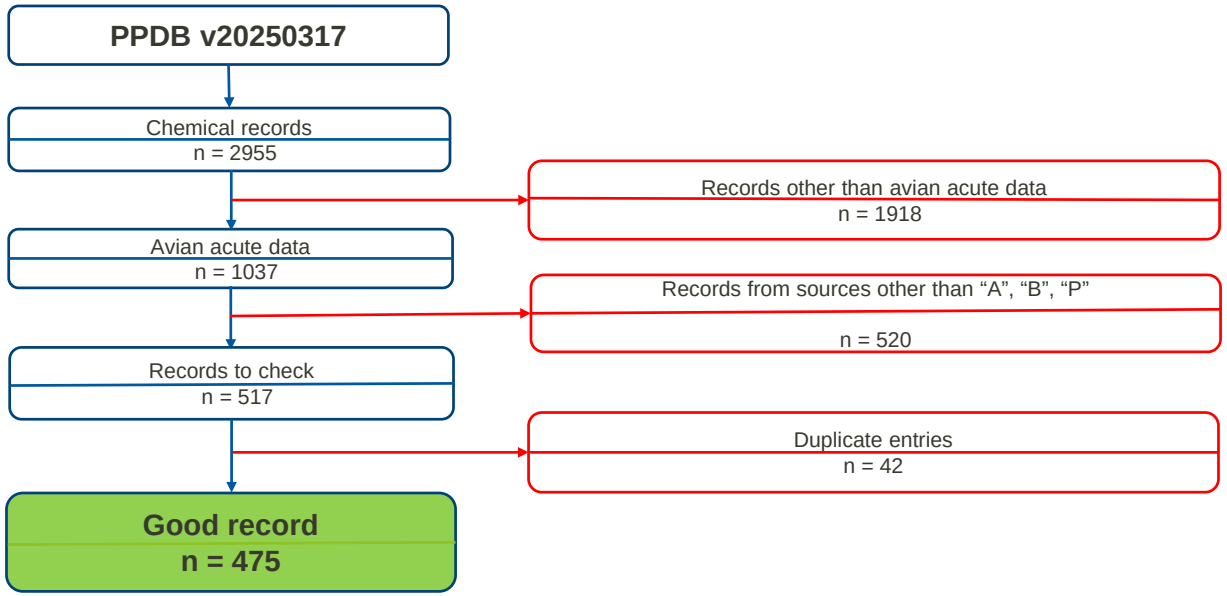
NAMs implementation challenges: *in vivo* data as reference for validation

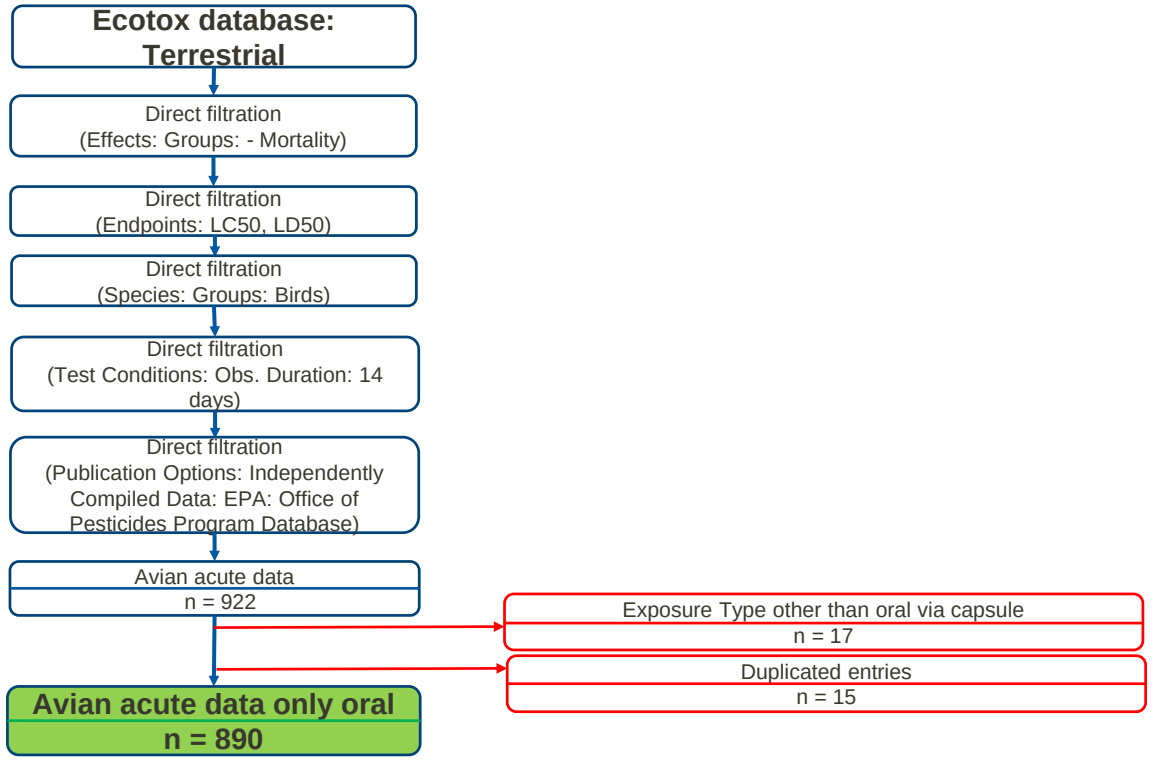
- Scattered *in vivo* data in different databases
- Lack of important details and incorrect entries

Better curation for *in vivo* database is required

Data curation process

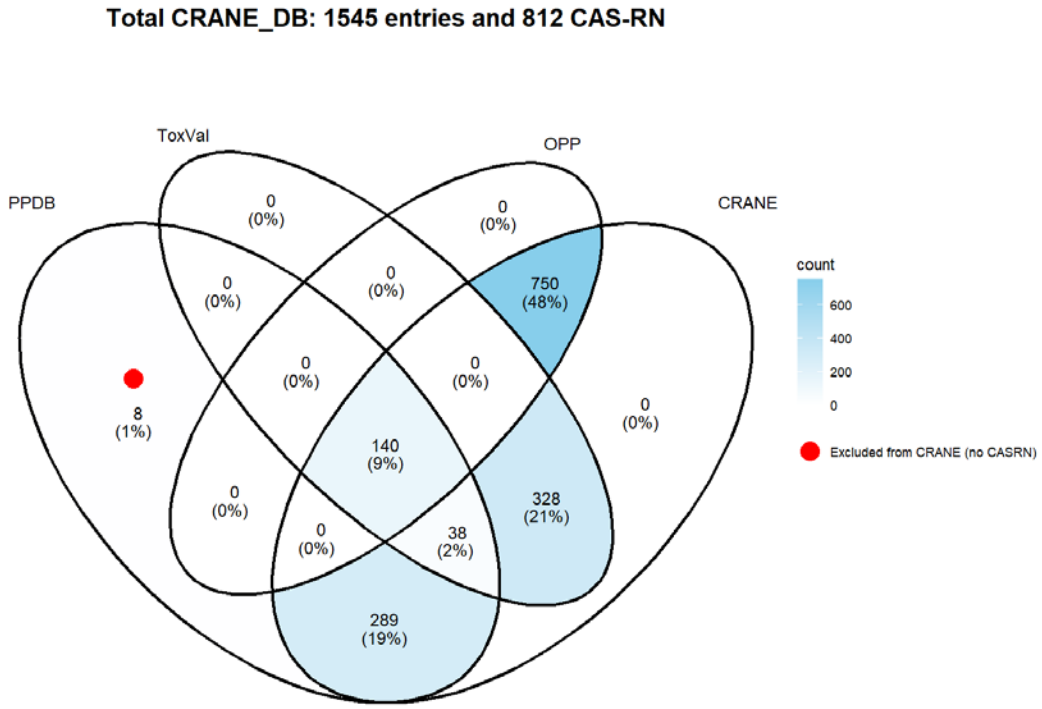


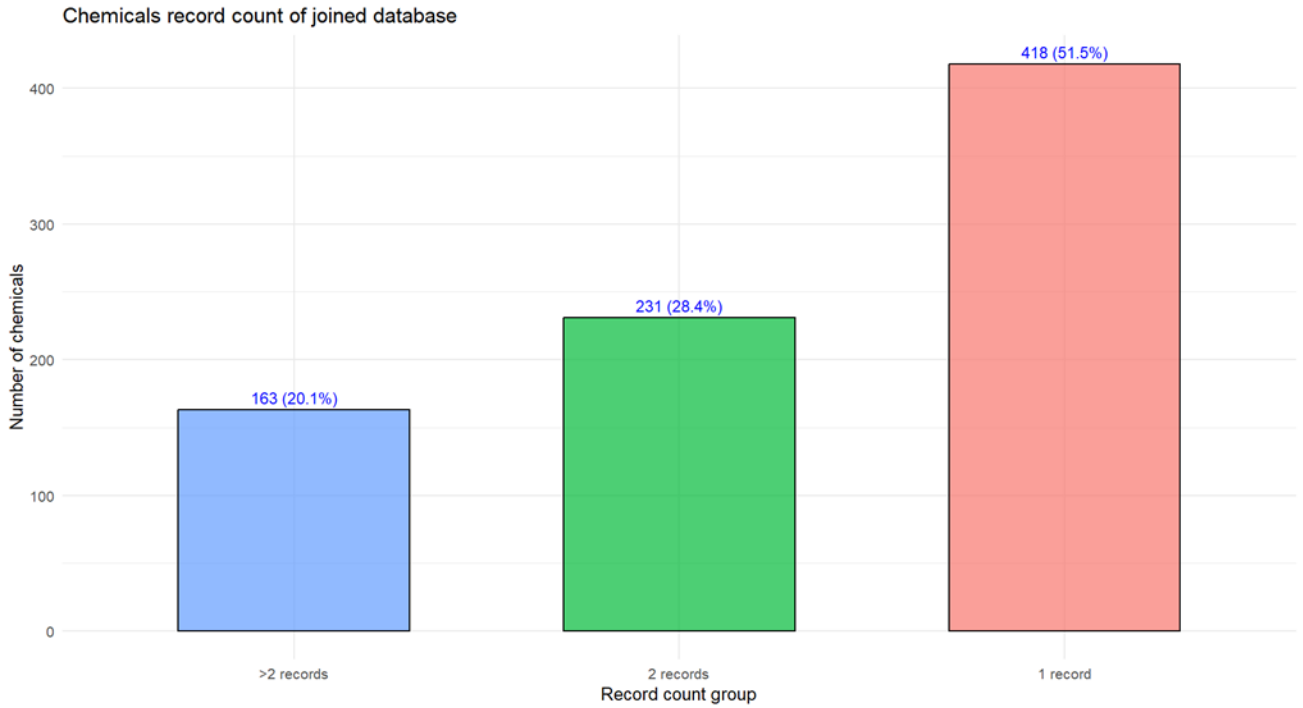




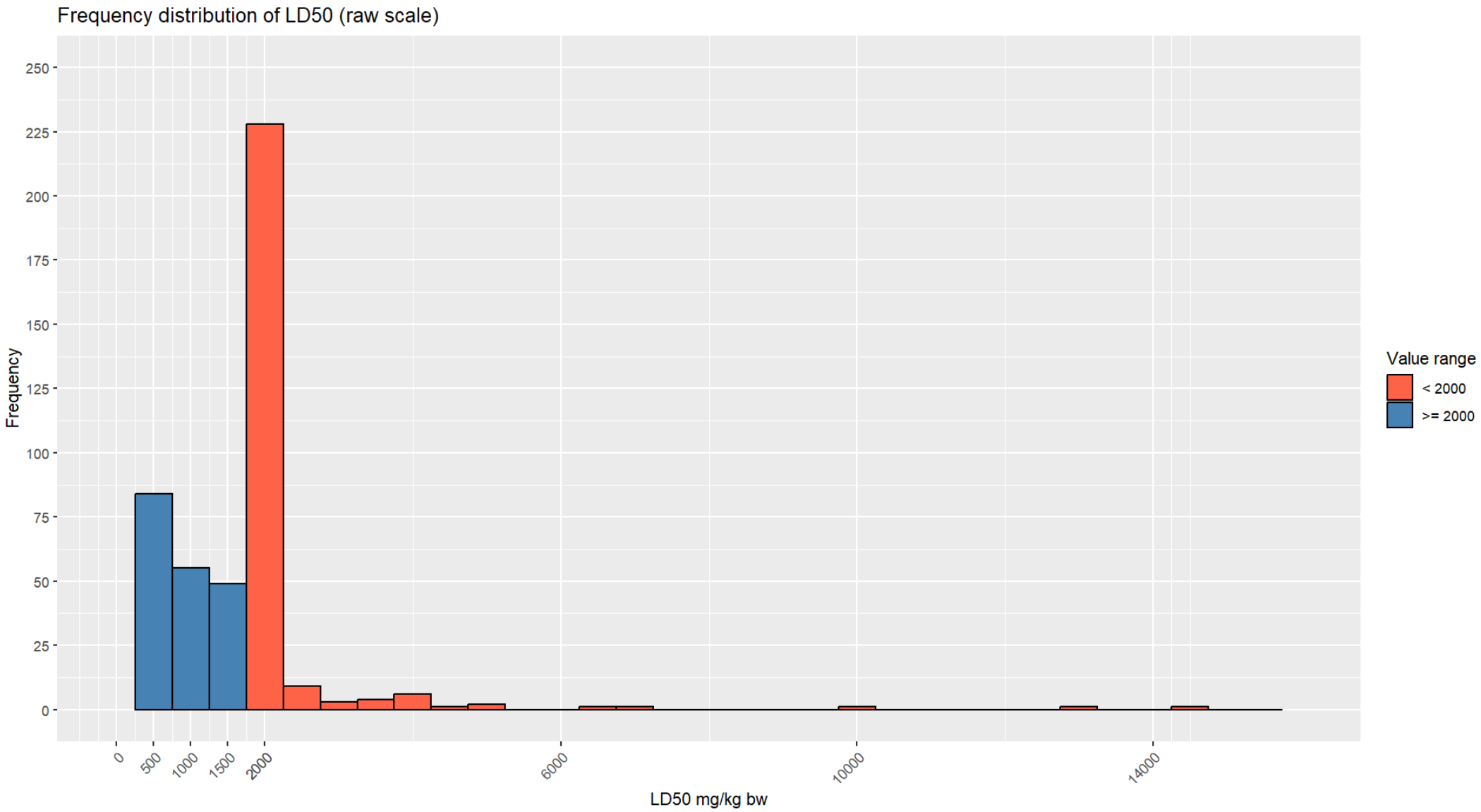
Overall:
1545 entries of 812 unique chemicals

Curated new database : CRANE DB composition

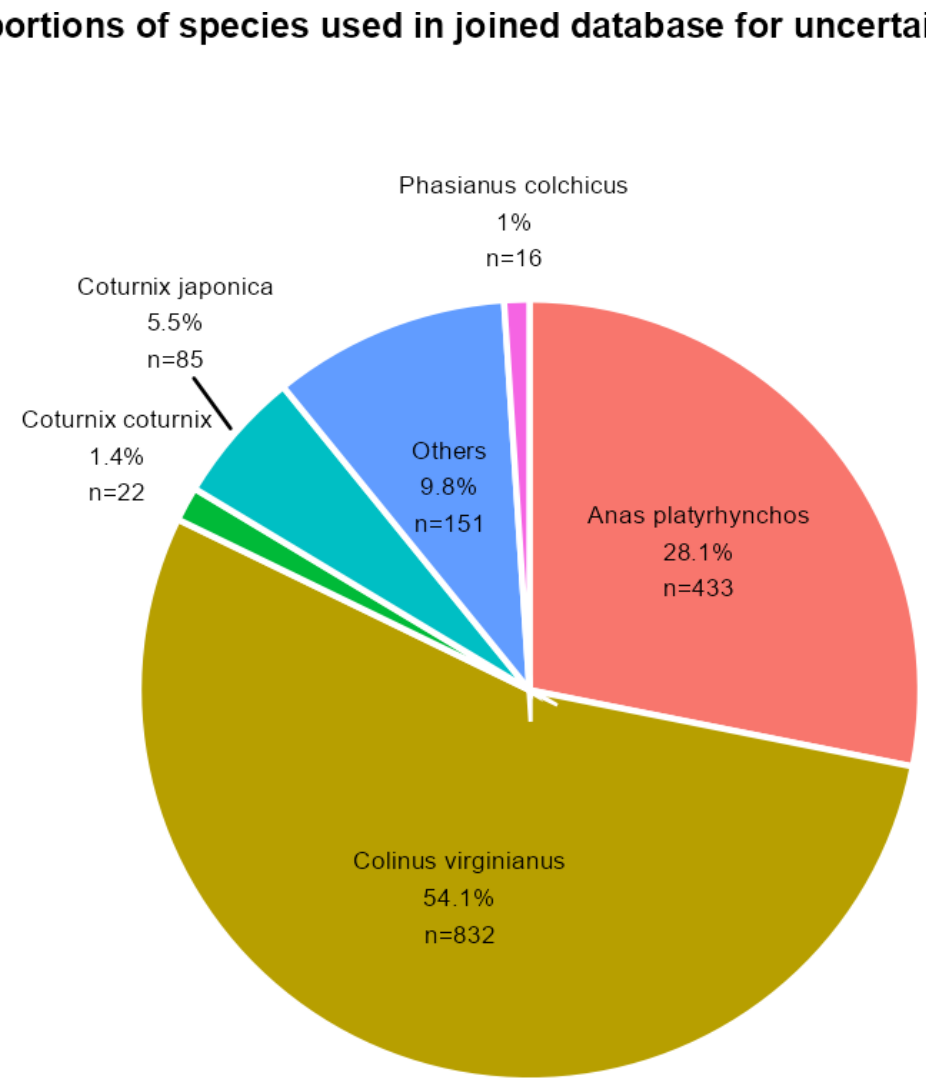




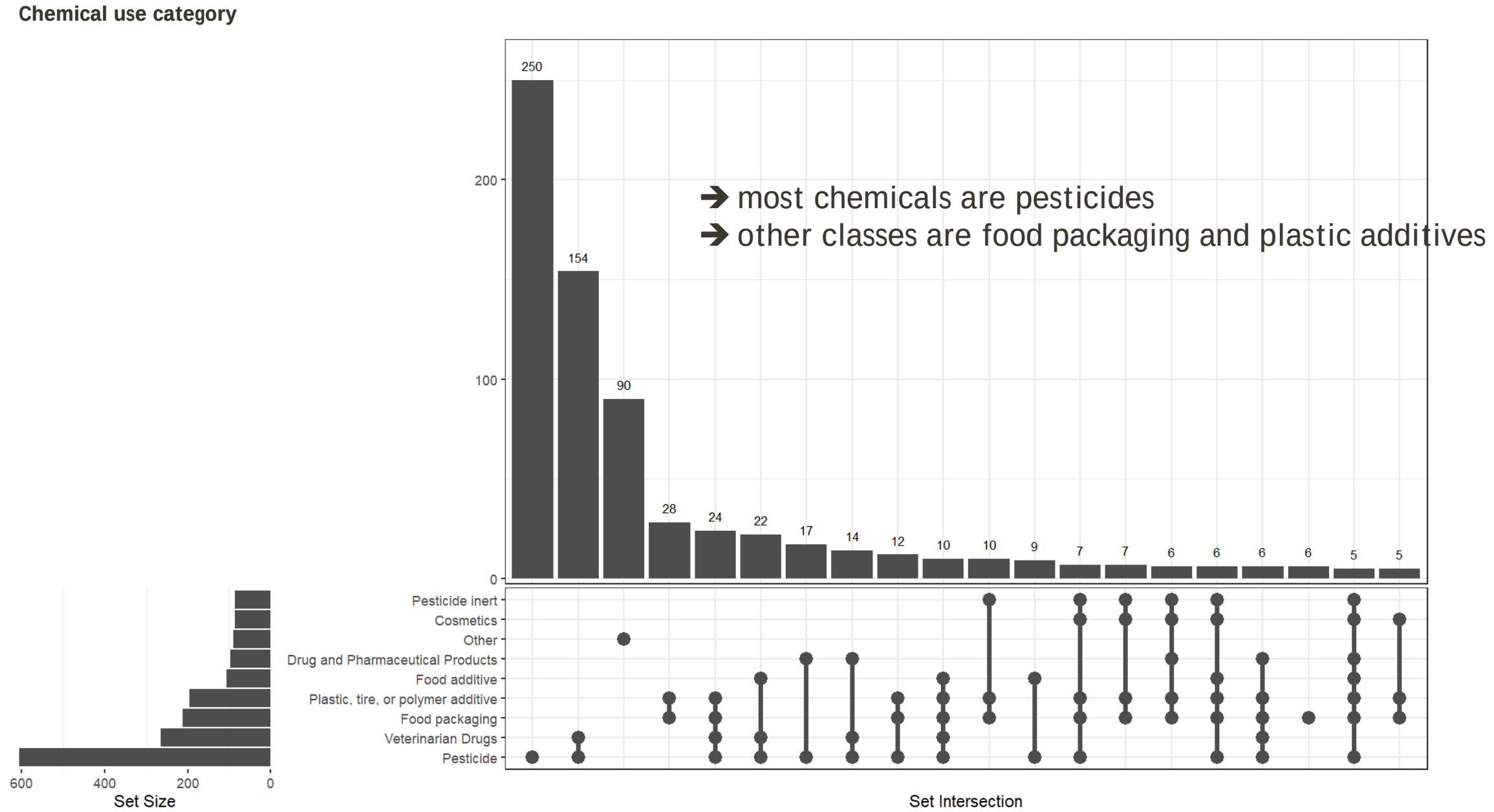
Curated new database : CRANE DB composition



Curated new database : CRANE DB composition



Curated new database : CRANE DB composition



➔ most chemicals are pesticides

➔ other classes are food packaging and plastic additives

Variability Analysis Results

Study	Metric	Published	CRANE Data
Pradeep et al.	R^2 (↑)	0.57	**0.77**
	RMSE (↓)	0.70	**0.42**
Pham et al.	Variance Explained (↑)	68.9%	**90.6%**
	Uncertainty (↓)	126-fold	**17-fold**

➔ **CRANE data shows significantly better results than published studies.**

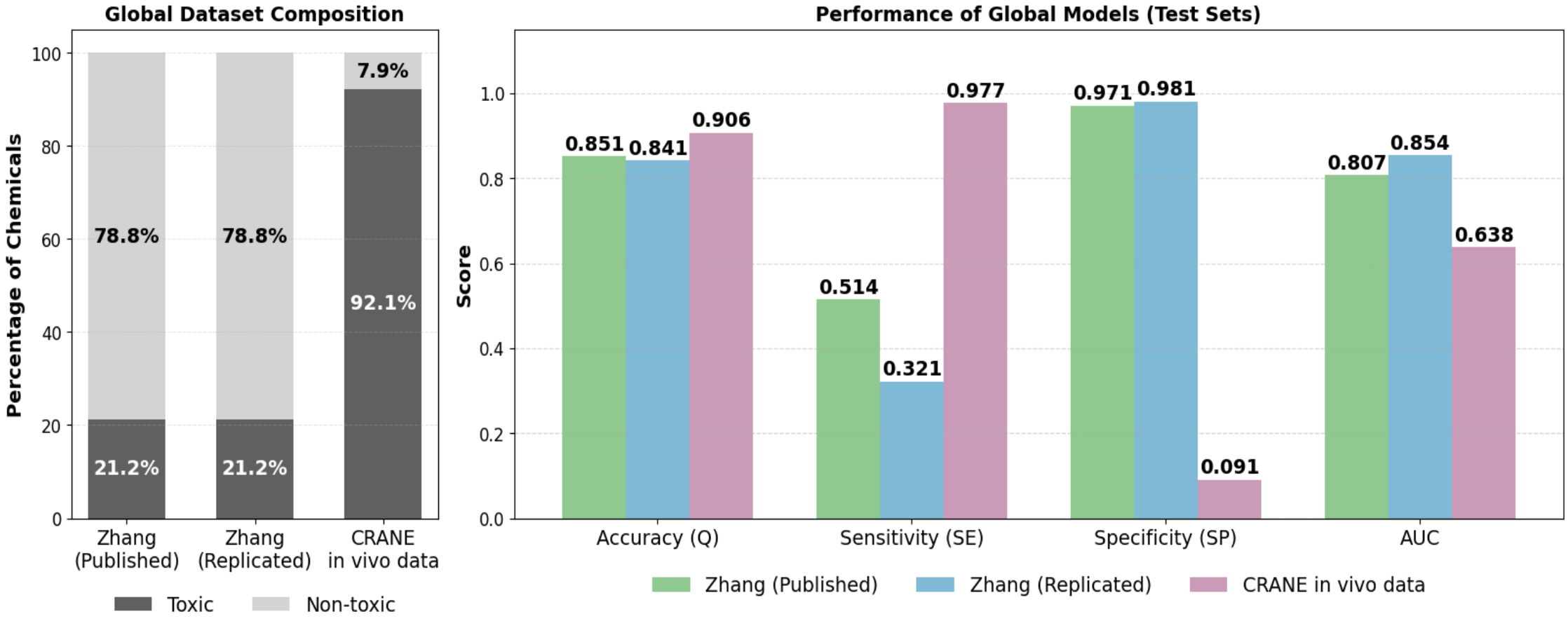
Collaborative Research on Avian NAMs for Ecotoxicology (CRANE)

- Long-term goal: alternative methods to toxicity testing with living birds
- Short-term goals:
  - collect existing *in vivo* data
  - evaluate existing *in silico* and *in vitro* methods

Review of existing QSAR methods

- **Goal:** Evaluate existing methods for avian toxicity prediction to identify best practices for CRANE
- **8 QSAR papers reviewed** (predictive modeling).
- **Key weaknesses identified:** Small datasets, limited species diversity, incomplete method reporting, class imbalance not addressed.

QSAR Model Replication - Zhang et al.



➔ Our dataset has higher proportion of toxic chemicals (92% vs 21%); this improves the model's sensitivity from 51% to 98% at the cost of the loss of specificity

PANDORA

Milad Mohammadi, PostDoc

Amrutha Edassery, post-Master

Parkinson‘s Disease

BfR
German Federal Institute for Risk Assessment

Opinion 063/2023

doi https://doi.org/10.17590/20231204-163651-0

30 November 2023

Plant protection products and Parkinson’s: confirmation of previous findings

The German Federal Institute for Risk Assessment (BfR) reassesses the possibility of occupational diseases in agriculture

Reuters

World Business Markets Sustainability Legal More

Farmworkers, enviros sue EPA over paraquat herbicide authorization

By **Sebastien Malo**

September 24, 2021 9:38 PM GMT+2 · Updated 3 years ago

Aa

Mitochondrial dysfunction

↓

Loss of dopaminergic neurons

↓

Parkinson‘s Disease

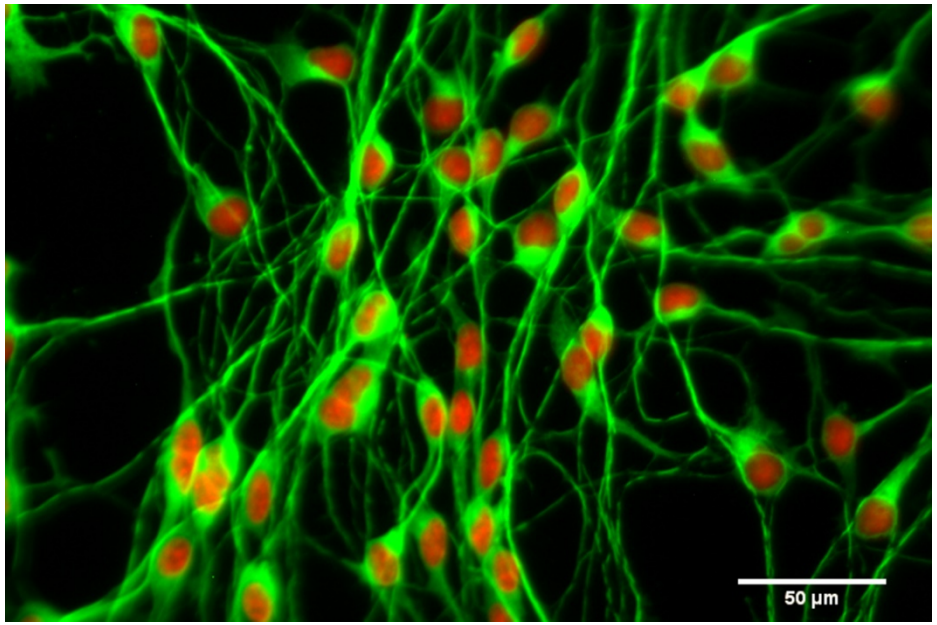
⇨ **Rotenone and paraquat associated with Parkison‘s Disease**

⇨ **Other pesticides are suspected to share this mechanism**

An *in vitro* Battery for Parkinson‘s Disease

European Food Safety Authority

1.3 Mio €
2024-2027
Lead: Jo Nyffeler

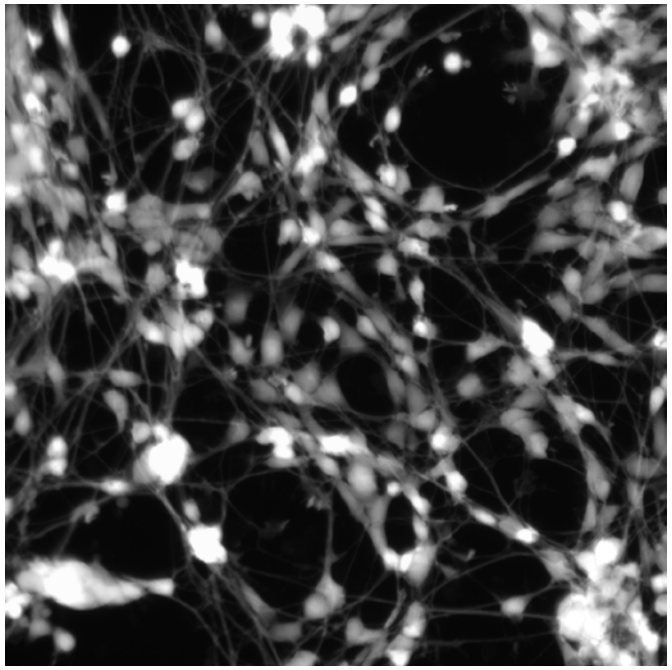


human dopaminergic neurons
Credit: Marcel Leist, University of Konstanz, Germany

- Testing ~ 100 pesticides registered in Europe
- Combination of novel cell-based and zebrafish assays
- Goal: identification of pesticides that induce mitochondrial toxicity and potentially lead to Parkinson‘s Disease

⇨ **Generated data will be used by regulators (i.e., EFSA) for decision making**

Parkinsonian Neurodegeneration Rapid Assessment using NAMs (PANDORA)



human dopaminergic neurons

- LUHMES cells
- set up the assay to test for neurodegeneration upon treatment with test chemicals
- Goal: screen 140 chemicals (at 8 concentrations)
  → > 200 plates
  → ca 5 months

Up and down sides

Positive

- excellent infrastructure
  - new labs
  - Grossgeräte
- support from the department
  - funds for smaller equipment

Negative

- would be beneficial to have more information on instruments in other groups/departments (e.g., CitePro)
- no option for tenure (for anyone else but Jo)

Thank you for your attention!