Revolutionizing Chemical Testing

Majority of Substances May **Not** Require Assessing Effects in Fish

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Challenges of traditional environmental safety testing of chemicals

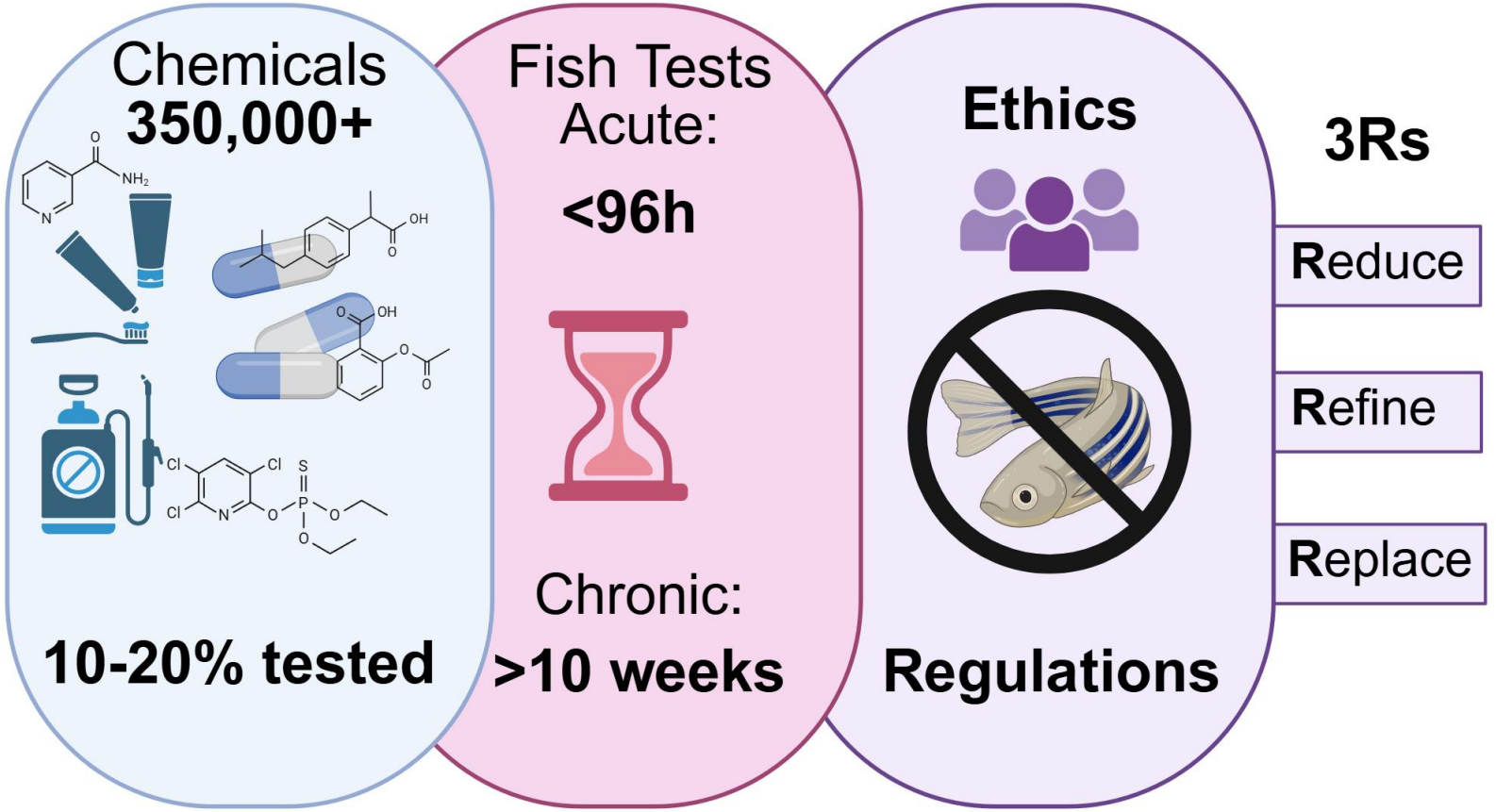
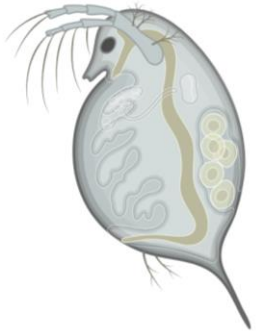


Image made in Biorender

Traditional Environmental Risk Assessment

Algae



Daphnia sp.

Zebrafish

Minimum No Observed Effect Concentration (NOEC)

Most sensitive group?

+AF

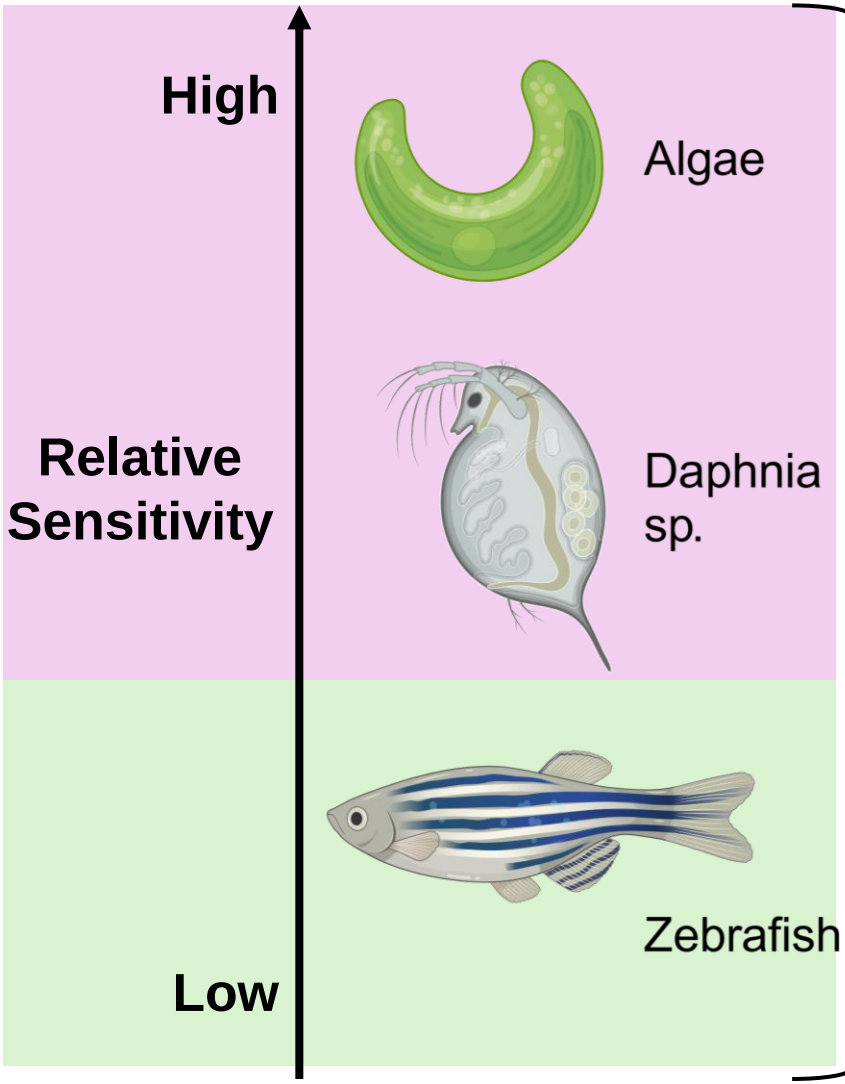
Predicted No Effect Concentration (PNEC)

Safe environmental concentration

AF-Assessment factors, data availability dependent

Images from Biorender

Environmental Risk Assessment



Low NOEC

High NOEC

Minimum No Observed Effect Concentration (NOEC)

Most sensitive group?

Invertebrates or Algae

+AF

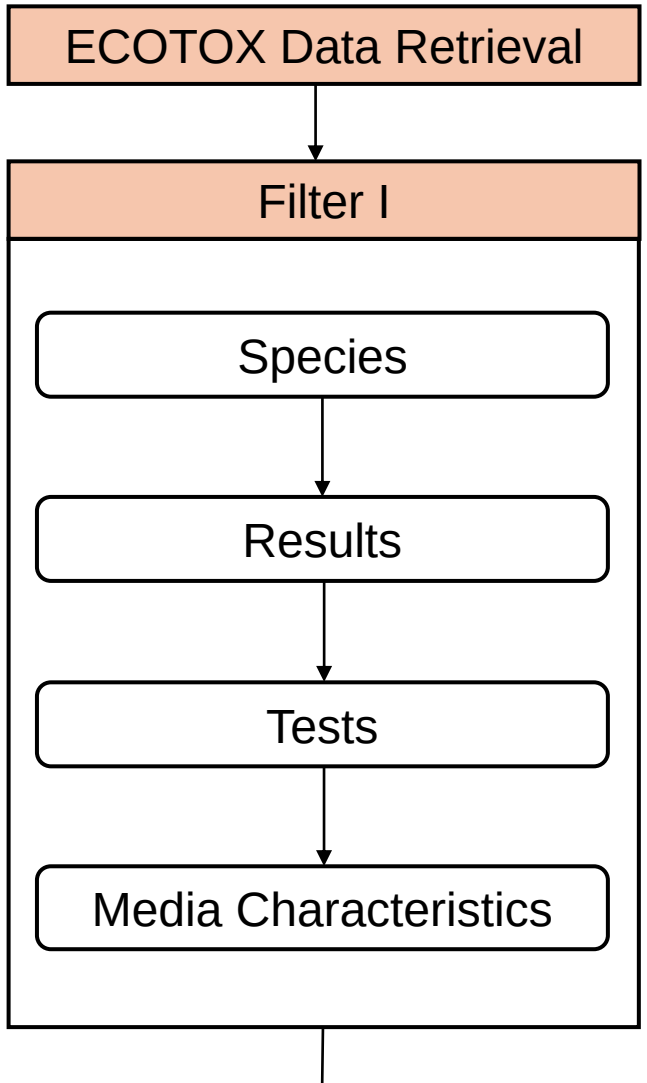
Predicted No Effect Concentration (PNEC)

Safe environmental concentration

!We can also use other non-in vivo based information to support safety assessments!

Images from Biorender

Analysis Pipeline



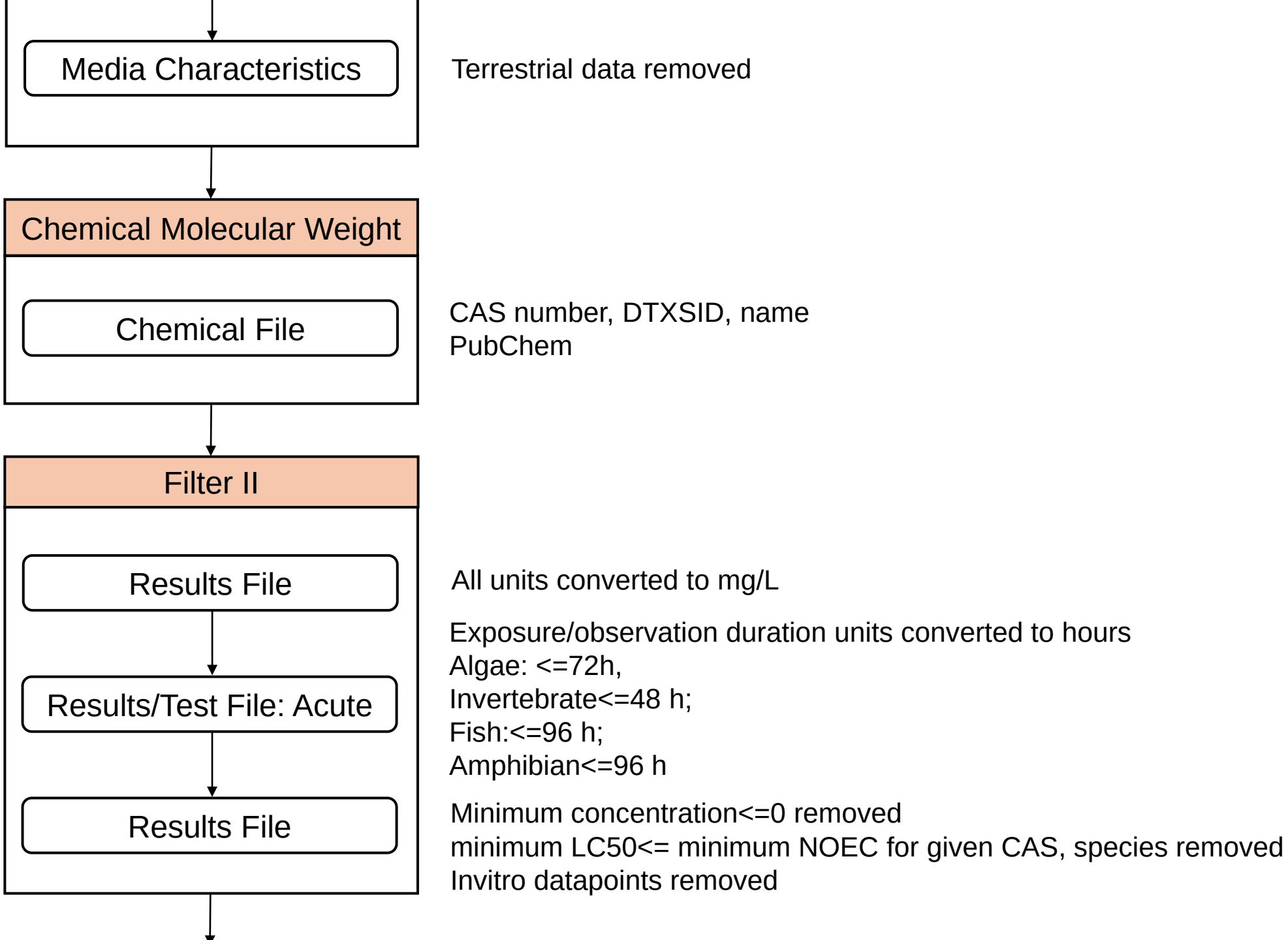
Downloaded:19/03/24, Last Updated:14/03/24

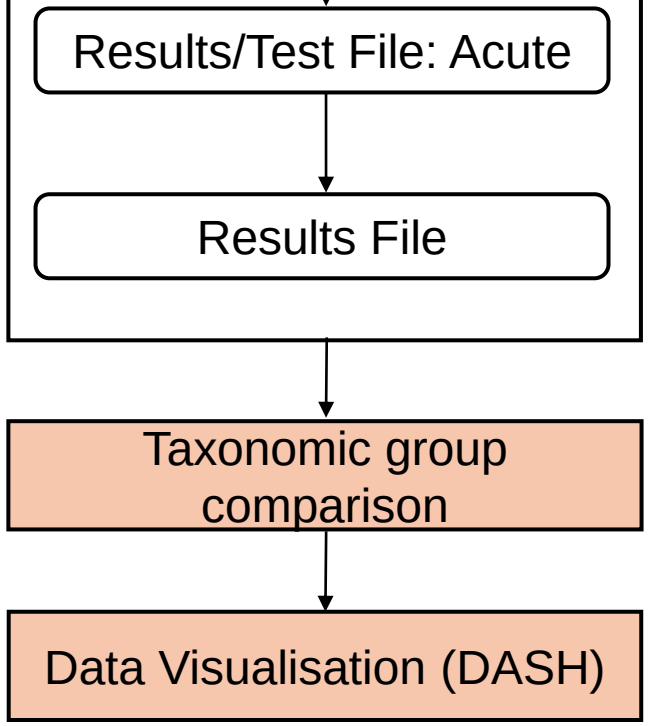
Aquatic species
Species scientific names corrected

Type: active ingredient(A), formulation(F), total(T)
Endpoint: LC50, NOEC, NOEL

Habitat : water, non-soil
Sub habitat: estuarine, riverine, lacustrine, palustrine
Location: Lab

Terrestrial data removed


Media Characteristics
Terrestrial data removed
Chemical Molecular Weight
Chemical File
CAS number, DTXSID, name
PubChem
Filter II
Results File
All units converted to mg/L
Results/Test File: Acute
Exposure/observation duration units converted to hours
Algae: <=72h,
Invertebrate<=48 h;
Fish:<=96 h;
Amphibian<=96 h
Results File
Minimum concentration<=0 removed
minimum LC50<= minimum NOEC for given CAS, species removed
Invitro datapoints removed



Invertebrate<=48 h;
Fish:<=96 h;
Amphibian<=96 h

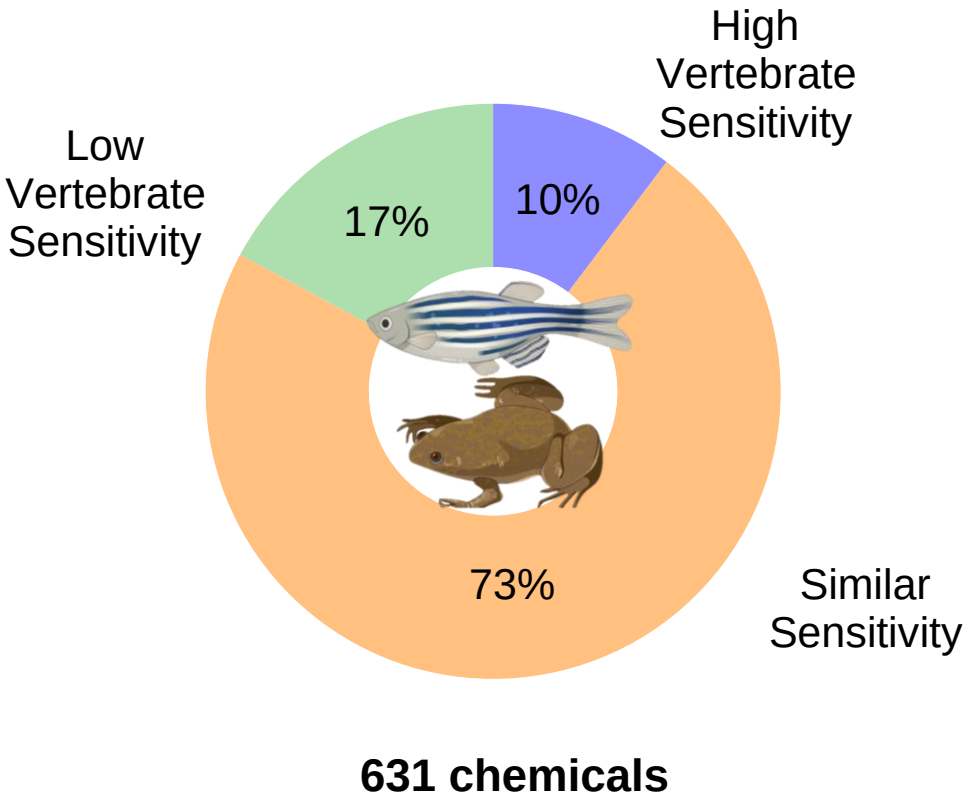
Minimum concentration<=0 removed
minimum LC50<= minimum NOEC for given CAS, species removed
Invitro datapoints removed

LC50, NOEC/NOEL separated
Median concentration (mg/L): Grouping by CAS, taxonomic group

$$\frac{median\ NOEC_{Group1}}{median\ NOEC_{Group2}} = \begin{cases} < 0.1 : Group2\ has\ lower\ sensitivity (compared\ to\ Group1) \\ 0.1 - 10 : Comparable\ Sensitivity \\ > 10 : Group2\ has\ higher\ sensitivity\ (compared\ to\ Group1) \end{cases}$$

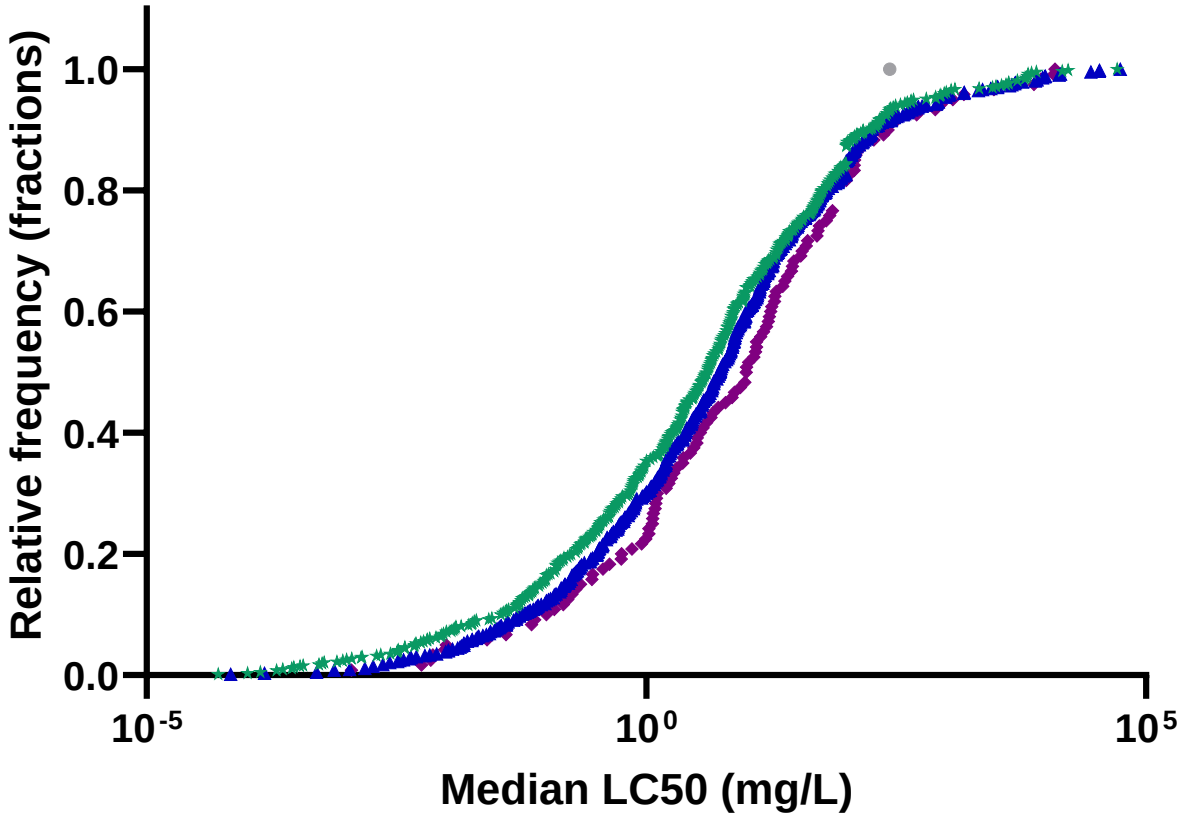
Lethal Concentration 50 (LC50) comparison (lethality)

Vertebrates vs invertebrates/algae (LC50)



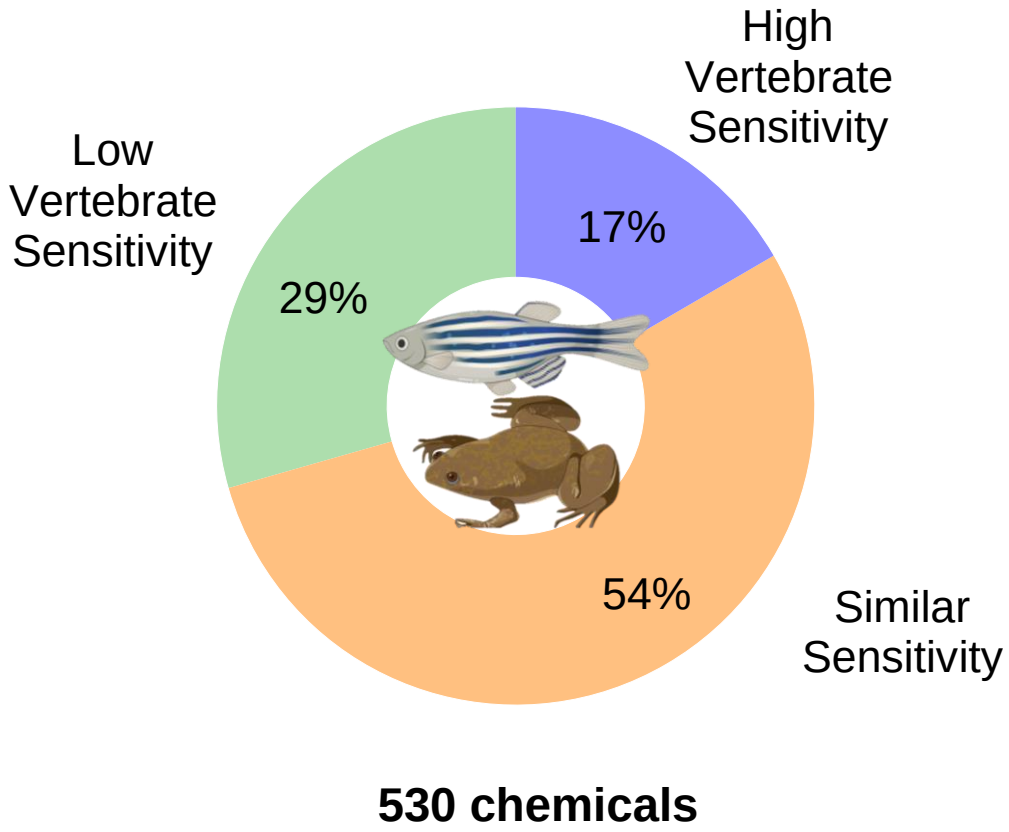
631 chemicals

Median LC50 (mg/L) distribution



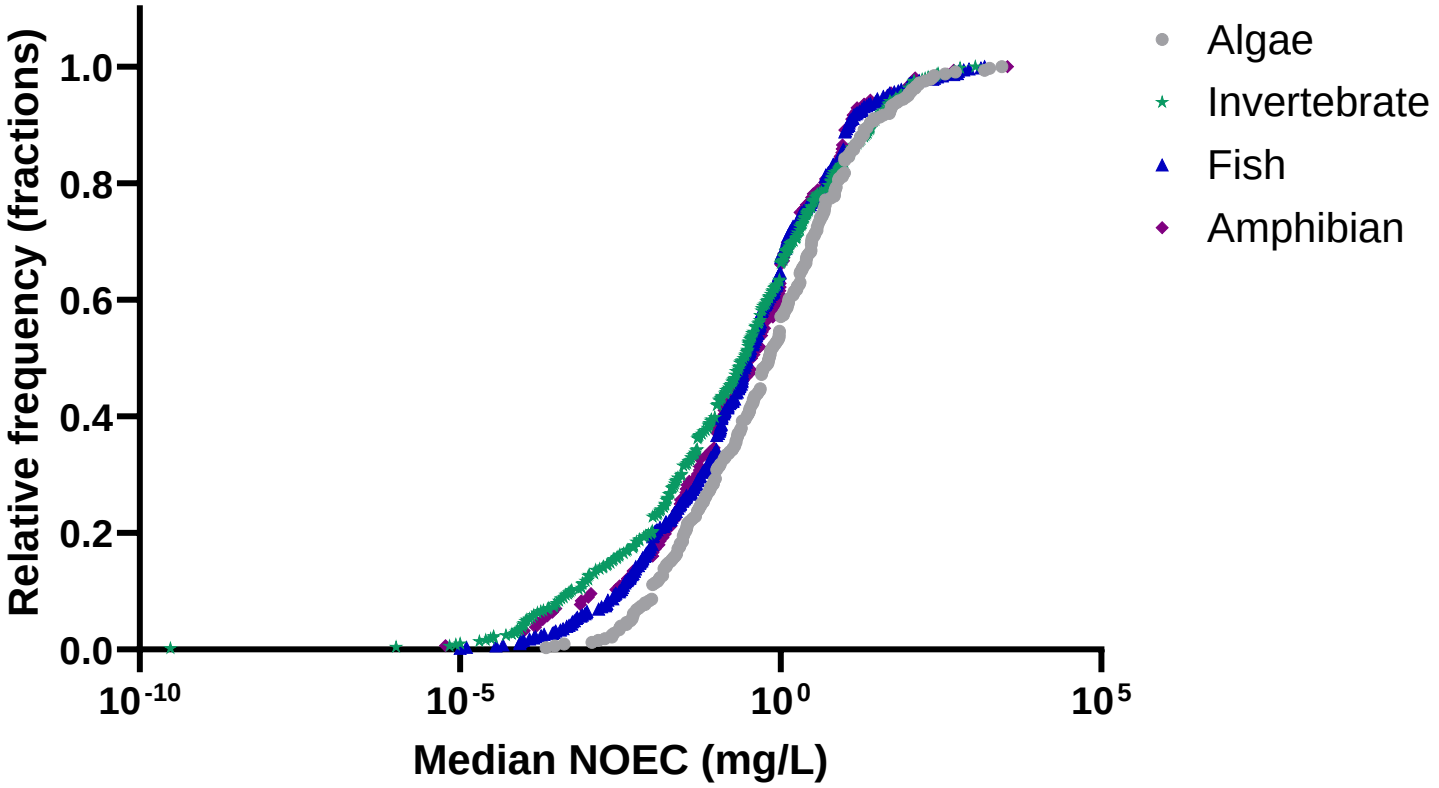
No-observed effect concentration (NOEC) comparison (Sub-lethal endpoints)

Vertebrates vs invertebrates/algae (NOEC)



530 chemicals

Median NOEC (mg/L) distribution



Results: Webapp

Page1 | Page2 |

Compare ECOTOX endpoints across aquatic taxonomic groups

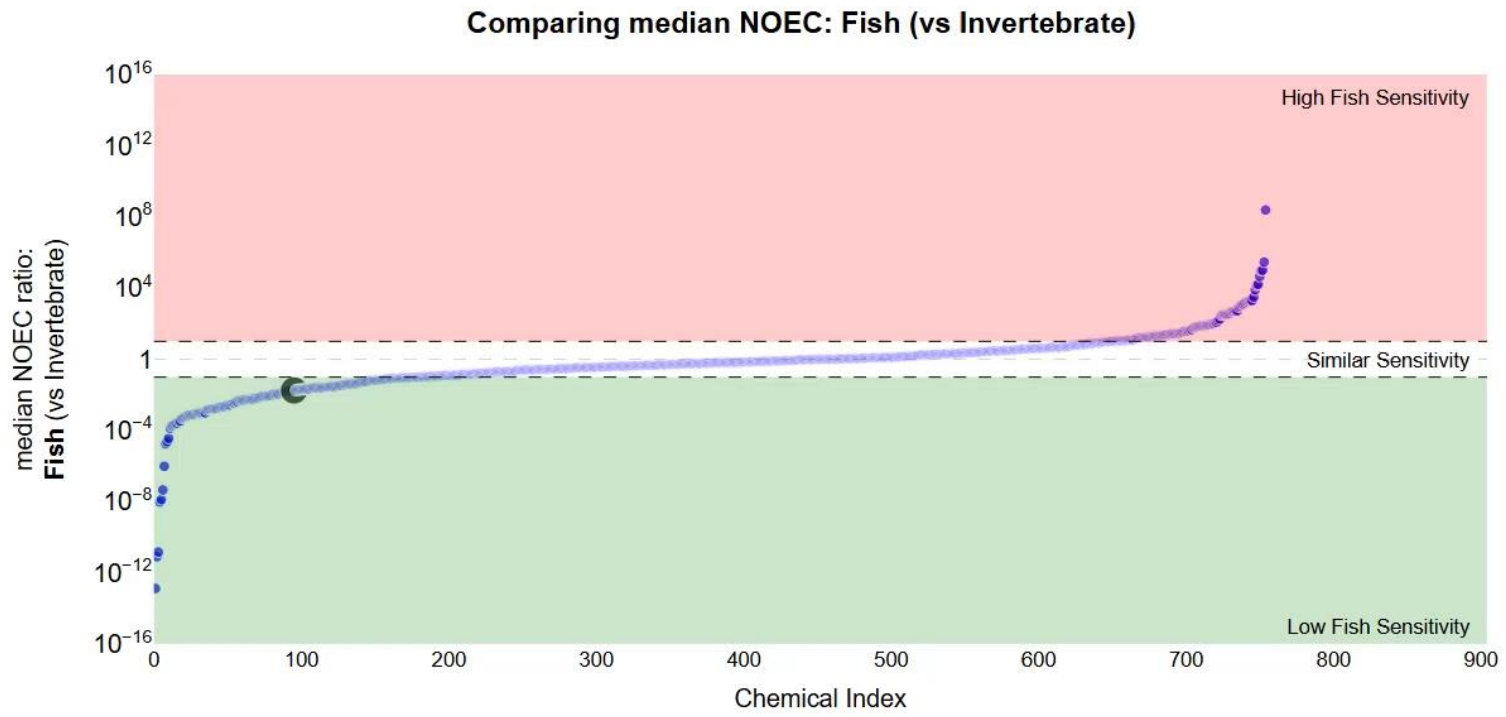
Choose taxonomic group (Numerator)

Invertebrates

/

Choose taxonomic group (Denominator)

Fish



Select ECOTOX Endpoint

- ○ LC50
- ◉ NOEC

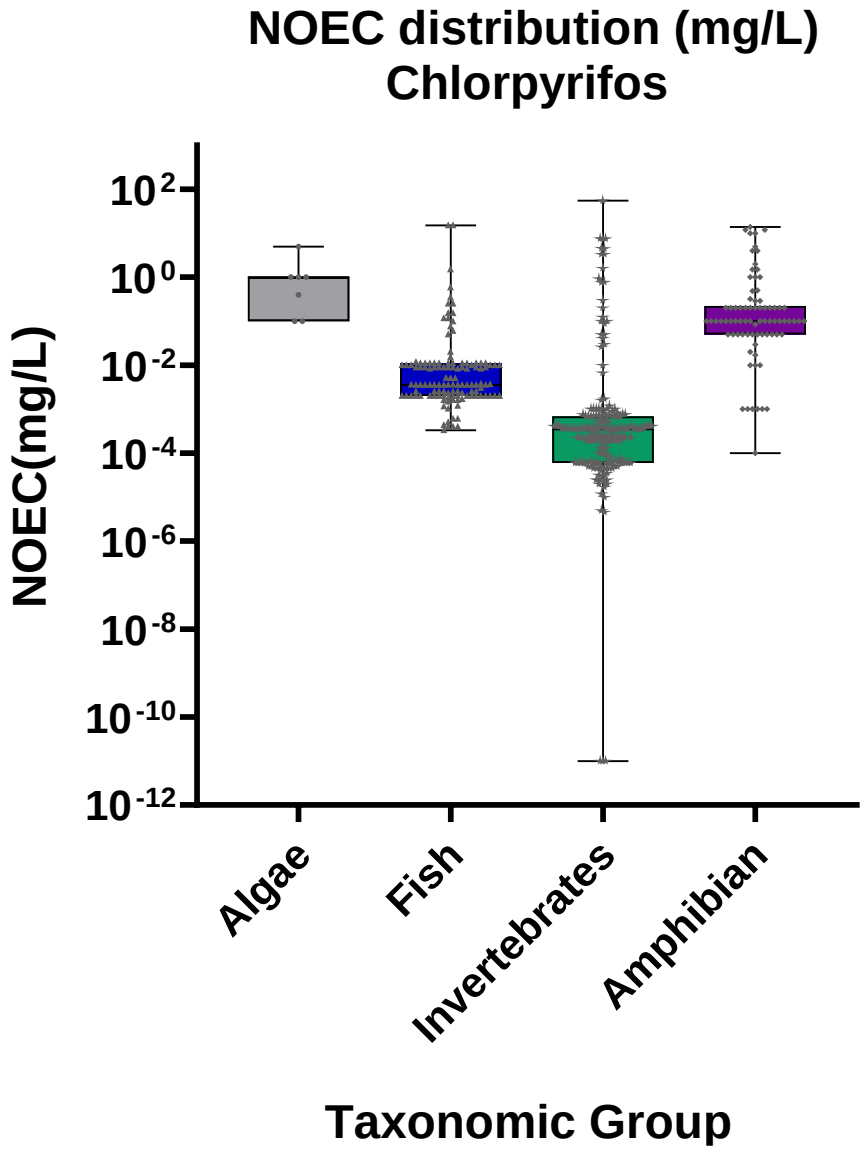
Select measure of central tendency

- ○ mean
- ◉ median
- ○ percentile_95

Filter by use category

All

NOEC distribution : Chlorpyrifos



Median values across groups

Fish: 0.00356

Invertebrate: 0.00035

Algae: 1

Amphibian median: 0.1000

Fish vs Invertebrate: median ratio is 0.098

Fish vs Algae: median ratio is 280

MAC EQS: 0.0001

Conclusion

In more than 80% cases data from lower taxonomic groups might be sufficient to determine safe concentration without the need of vertebrate data. Where fish is the most sensitive, available *in silico* and *in vitro* tools should be able to support such safety assessment.

Only in 10% and 17% chemicals, is Vertebrates median LC50 and NOEC, respectively, ten-fold more sensitive than algae and invertebrates.

Biological pathways conservation / specificity across the groups resulting in the trend observed.

1. T. J. Norberg-King, M. R. Embry, S. E. Belanger, T. Braunbeck, J. D. Butler, P. B. Dorn, B. Farr, P. D. Guiney, S. A. Hughes, M. Jeffries, R. Journel, M. Lèonard, M. McMaster, J. T. Oris, K. Ryder, H. Segner, T. Senac, G. Van Der Kraak, G. Whale, P. Wilson, An International Perspective on the Tools and Concepts for Effluent Toxicity Assessments in the Context of Animal Alternatives: Reduction in Vertebrate Use. *Environ. Toxicol. Chem.* **37**, 2745–2757 (2018)

Conclusion

Mechanistic understanding of the underlying biological pathway of the chemical resulting in toxicity would allow the development of reliable computational predictive models.

Worldwide more than 5 million fish are used for toxicity testing per year[1], potentially without relevant benefits in safety assessments robustness.

We can have relevant, robust and transparent safety assessments, without vertebrate in vivo data, using in silico and in vitro tools in an integrated way, anchored on best available scientific knowledge.

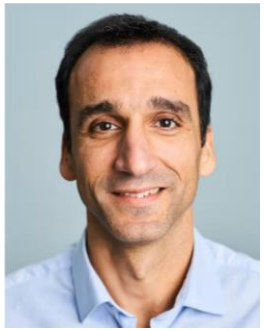
1. T. J. Norberg-King, M. R. Embry, S. E. Belanger, T. Braunbeck, J. D. Butler, P. B. Dorn, B. Farr, P. D. Guiney, S. A. Hughes, M. Jeffries, R. Journel, M. Lèonard, M. McMaster, J. T. Oris, K. Ryder, H. Segner, T. Senac, G. Van Der Kraak, G. Whale, P. Wilson, An International Perspective on the Tools and Concepts for Effluent Toxicity Assessments in the Context of Animal Alternatives: Reduction in Vertebrate Use. *Environ. Toxicol. Chem.* **37**, 2745–2757 (2018)

Acknowledgements

Supervisory Team

Prof Christer Hogstrand

Dr Luigi Margiotta-Casaluci



Dr Bruno Campos

Dr Claudia Rivetti

Prof Peter Kille

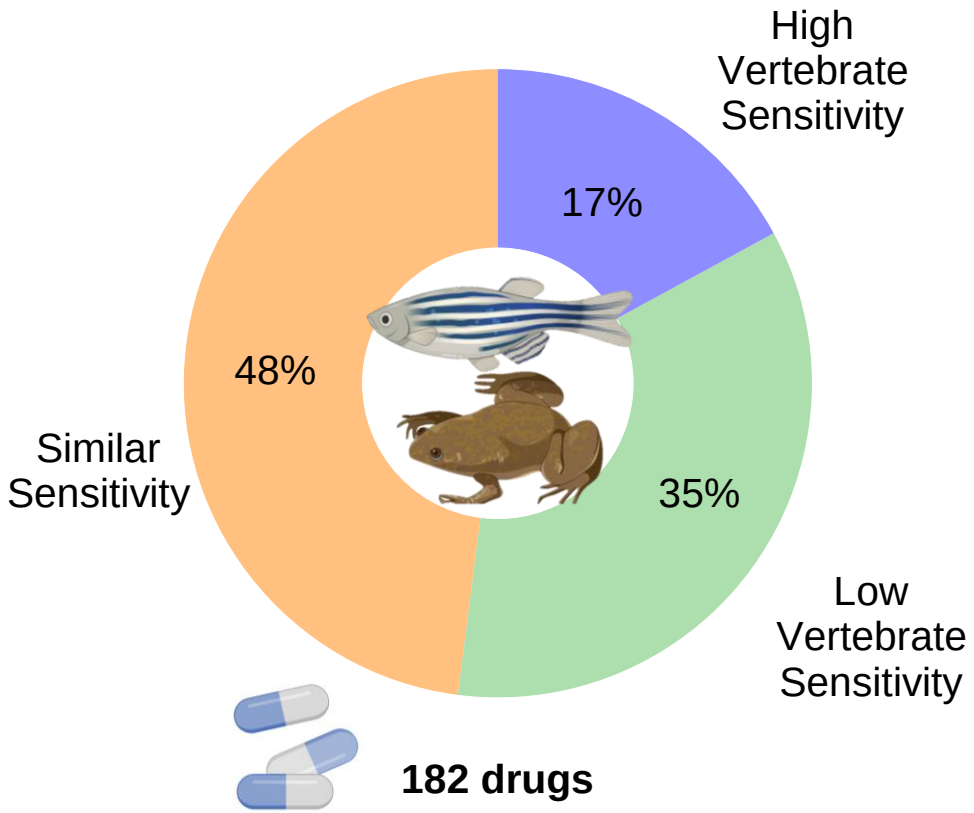
Prof Charles Tyler

Office and Lab mates

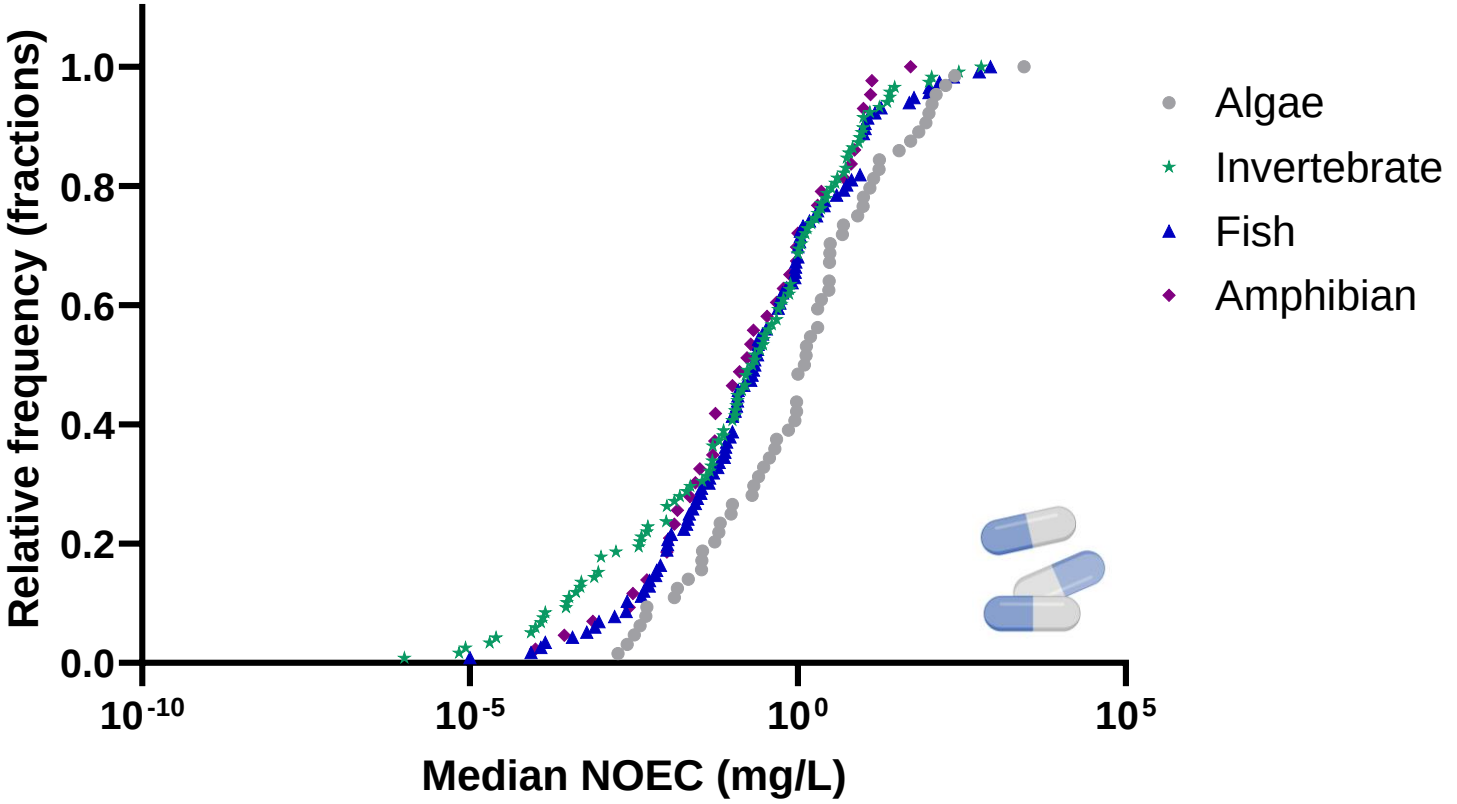
NOEC comparisons

Drugs

Vertebrates vs invertebrates/algae (NOEC)



Median NOEC (mg/L) distribution

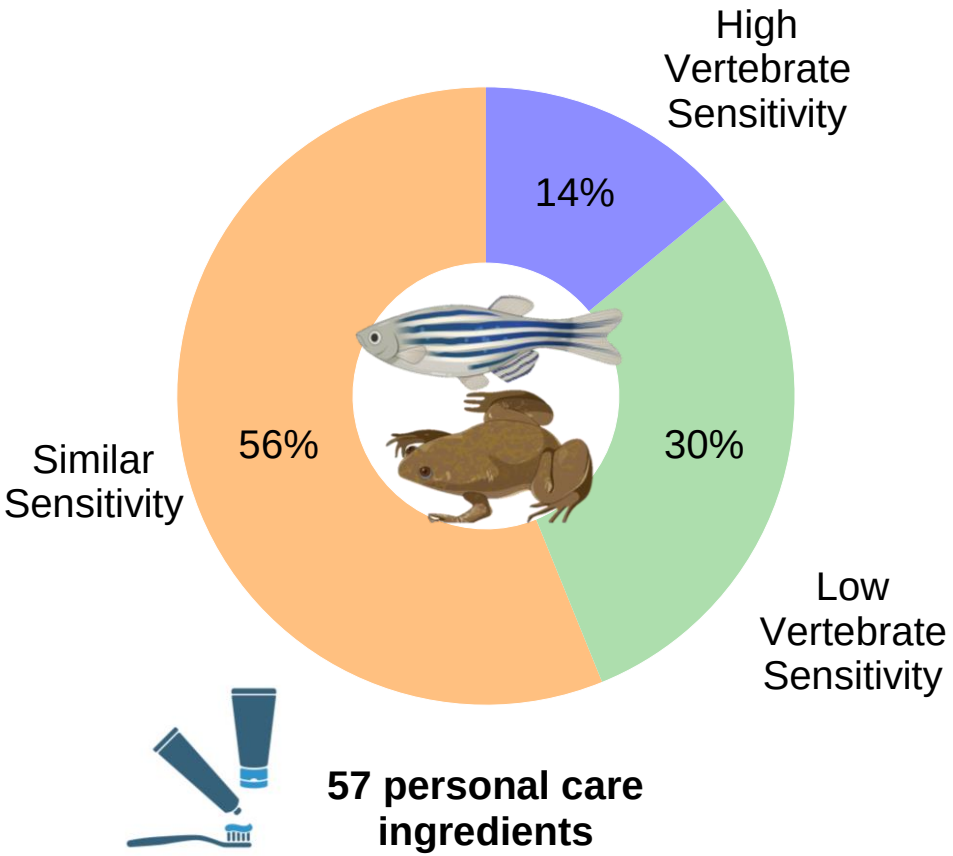


Images from Biorender

NOEC comparisons

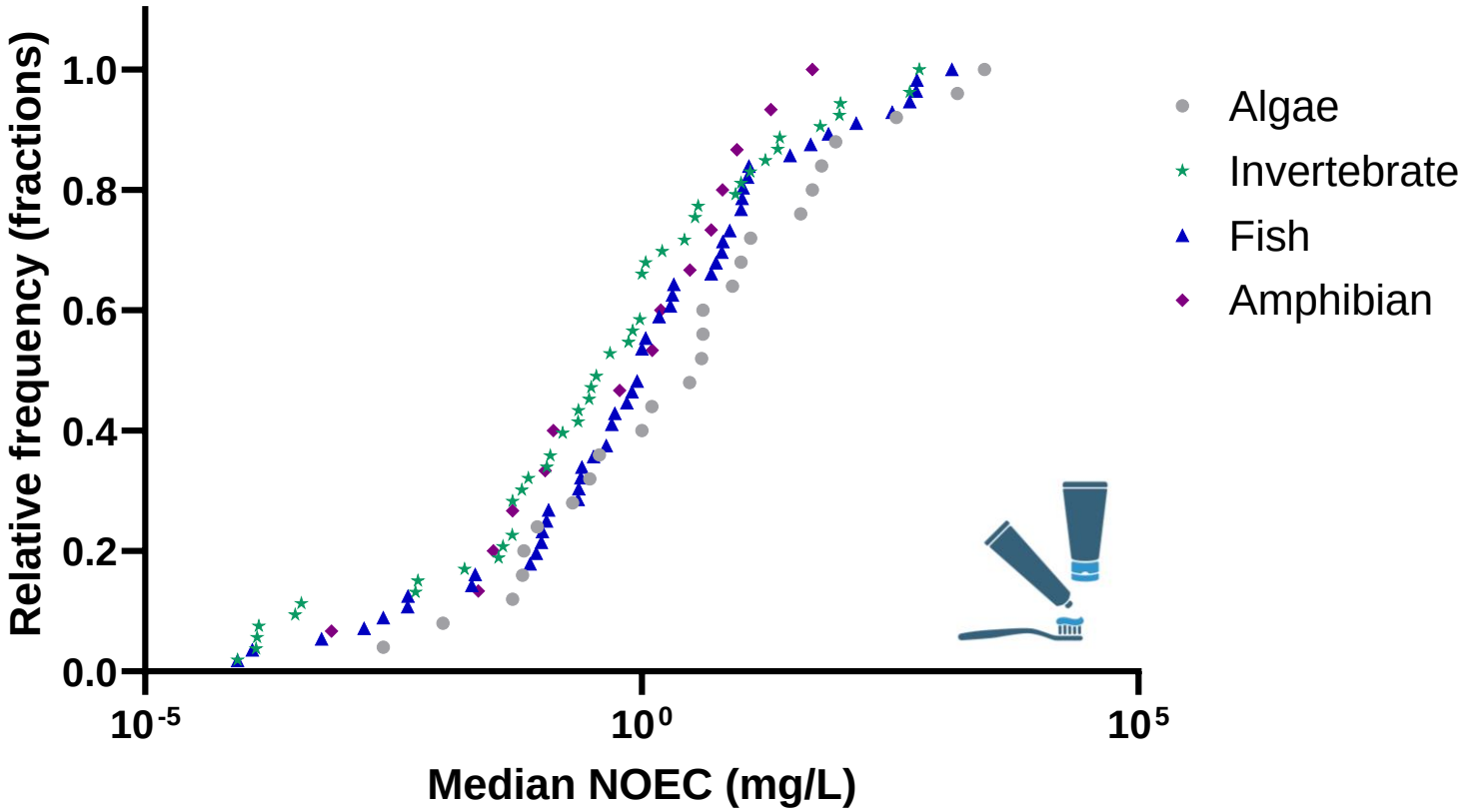
Personal care

Vertebrates vs invertebrates/algae (NOEC)



57 personal care ingredients

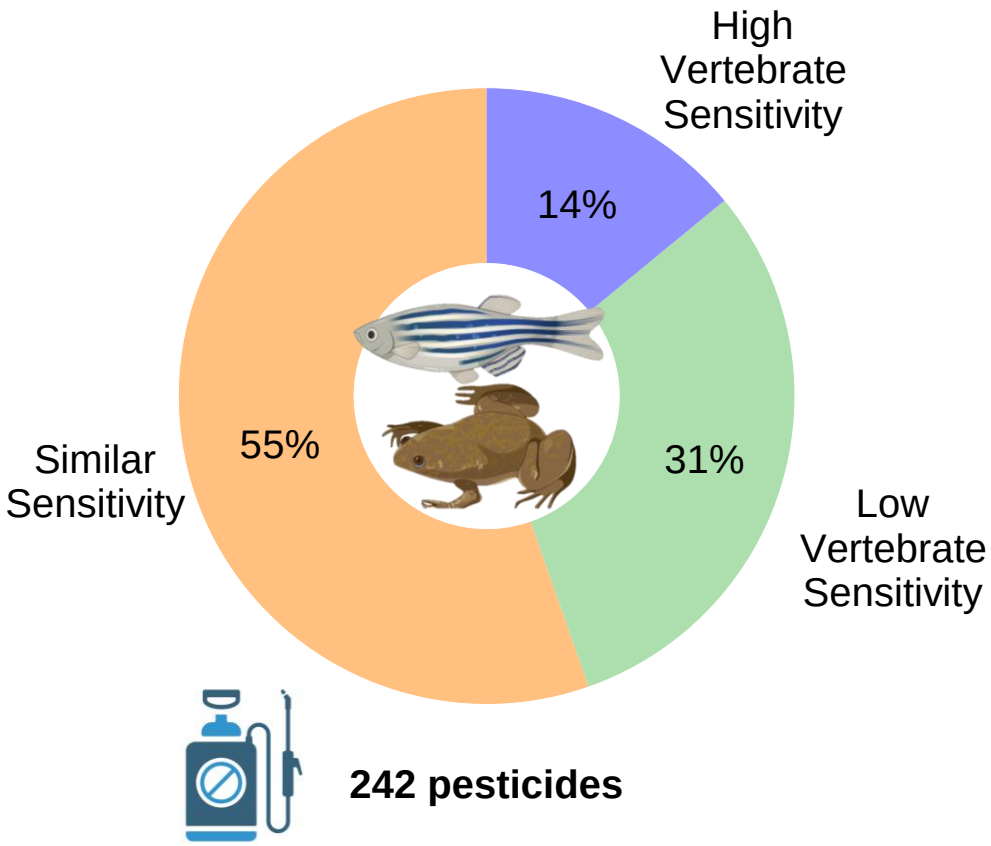
Median NOEC (mg/L) distribution



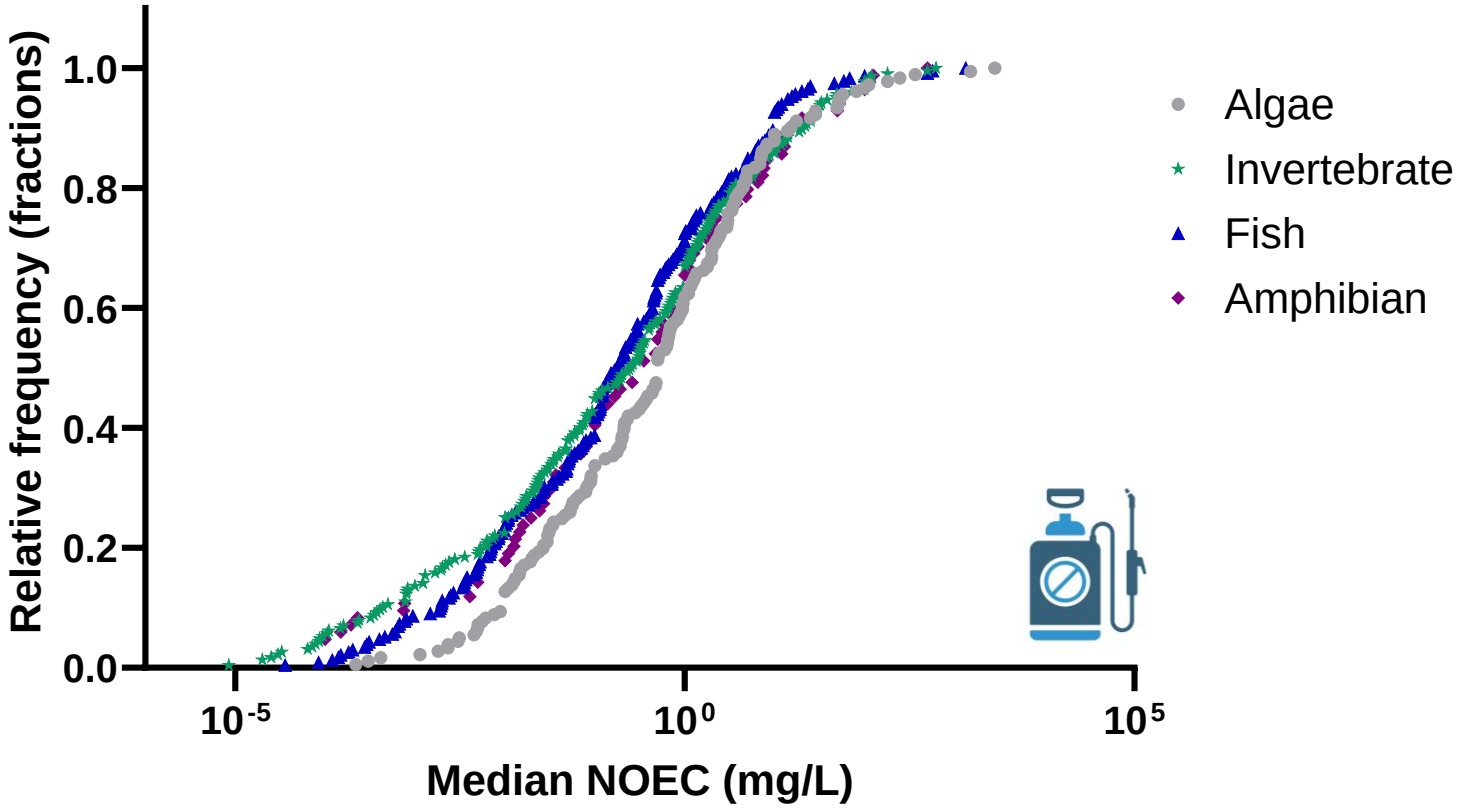
Images from Biorender

NOEC comparisons
Pesticides

Vertebrates vs invertebrates/algae (NOEC)



Median NOEC (mg/L) distribution



Images from Biorender