Reassessing the Role of *In Vivo* Fish Testing in Chemical Risk Assessment

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PROJECT BACKGROUND & HYPOTHESIS

Regulatory approval and marketing authorisation of chemicals requires the submission of an Environmental Risk Assessment (ERA). This typically includes aquatic ecotoxicity testing of algae, crustaceans and fish to determine the lowest NOECs (No-Observed Effect Concentrations).

High resource demands, limited societal acceptance, and the phase-out of animal testing have driven interest in the 3Rs principles (Reduce, Replace, Refine) and increased focus on new approach methodologies (NAMs).

Computational prediction of species sensitivity is an emerging priority, supporting the replacement of fish with equally or more sensitive non-protected species (e.g., algae or crustaceans) while maintaining environmental protection.

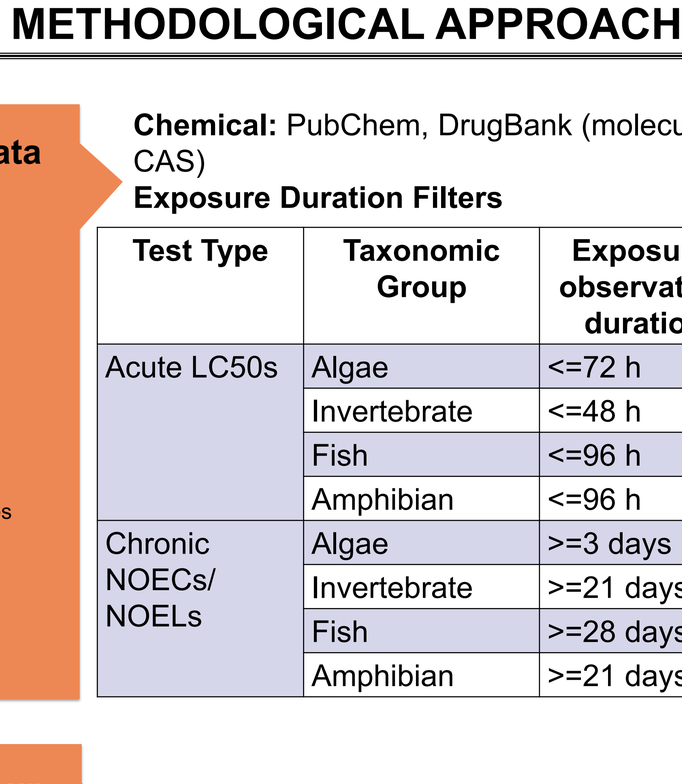

Figure 1: *Environmental risk assessment of chemicals identifies the most sensitive group among algae, Daphnia sp., and fish to determine an environmentally safe concentration. Fish testing may not be required when algae or Daphnia are the most sensitive species.*

METHODOLOGICAL APPROACH

Ecotoxicity Data

ECOTOX
Aquatic species (GNverifier, Taxoniq)

Chronic NOECs, Acute LC50s (Lethal Concentration 50)

Lab-based in vivo studies

Gunnarsson et al.
Chronic NOECs

Chemical: PubChem, DrugBank (molecular weight and CAS)

Exposure Duration Filters

Test Type	Taxonomic Group	Exposure/ observation duration	OECD
Acute LC50s	Algae	<=72 h	Test No. 201
	Invertebrate	<=48 h	Test No. 202
	Fish	<=96 h	Test No. 203
	Amphibian	<=96 h	Test No. 231
Chronic NOECs/ NOELs	Algae	>=3 days	Test No. 201
	Invertebrate	>=21 days	Test No. 211
	Fish	>=28 days	Test No. 210
	Amphibian	>=21 days	Test No. 231

Taxonomic Group Comparison

Grouping by CAS, taxonomic group and endpoint: Median concentration (mg/L)

Ratios across group combinations calculated

Factor of 100 to determine relative fish sensitivity

Taxonomic Group Combinations	Ratio calculation (E ∈ {LC50, NOEC})
Algae vs fish	$\frac{median\ E_{Algae}}{median\ E_{Fish}}$
Invertebrates vs fish	$\frac{median\ E_{Invertebrate}}{median\ E_{Fish}}$
Algae/invertebrates vs fish	$\frac{min(median\ E_{Algae}, median\ E_{Invertebrate})}{median\ E_{Fish}}$
Algae/invertebrates vs Vertebrates	$\frac{min(median\ E_{Algae}, median\ E_{Invertebrate})}{min(median\ E_{Fish}, median\ E_{Amphibian})}$

Similar ratios were calculated for Amphibians as well.
Ratio < 0.1: **Low** fish/amphibian/vertebrate sensitivity
Ratio > 10 : **High** fish/amphibian/vertebrate sensitivity

RESULTS

Vertebrates were found to be tenfold more sensitive than algae/invertebrates for only **9.5%** and **15%** chemicals, respectively, when comparing LC50s and NOECs.

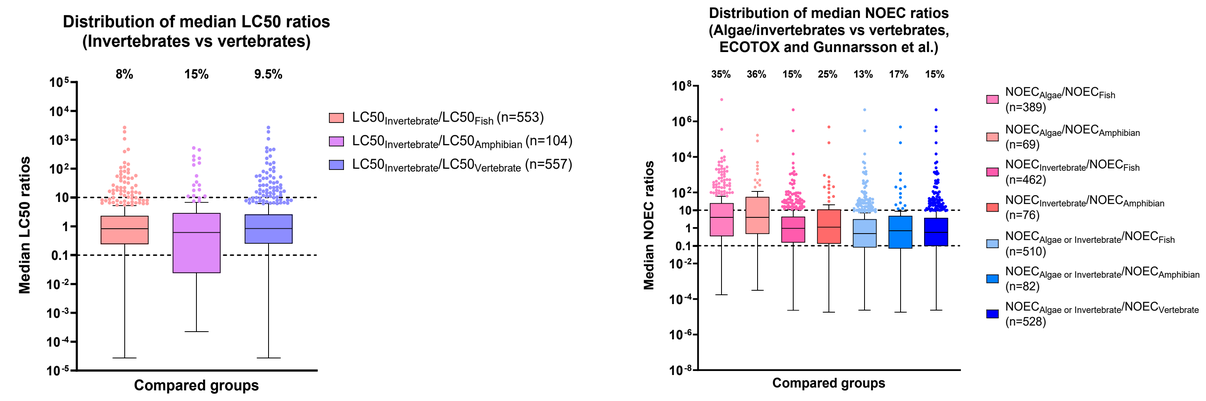

Figure 2: *Distribution of median LC50/NOEC ratios for chemicals tested in algae/invertebrates and vertebrates (fish/amphibians) across different group comparisons.*

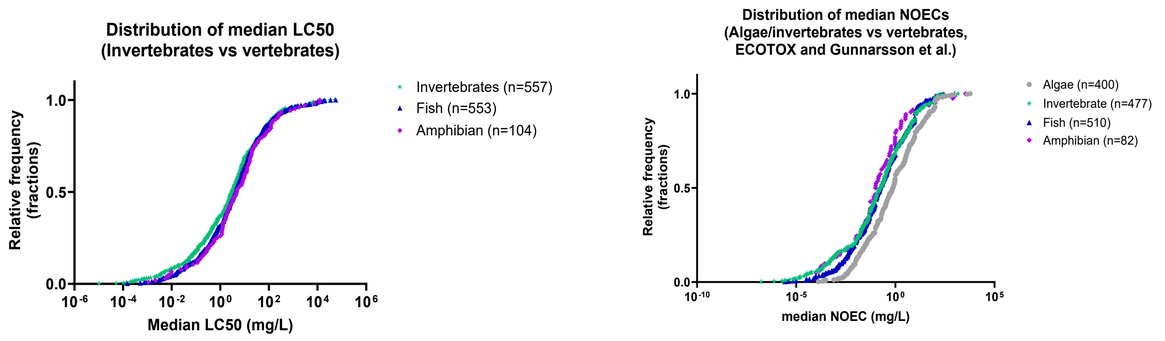

Figure 3: *Cumulative frequency distribution of median LC50/NOEC values for chemicals across different aquatic groups.*

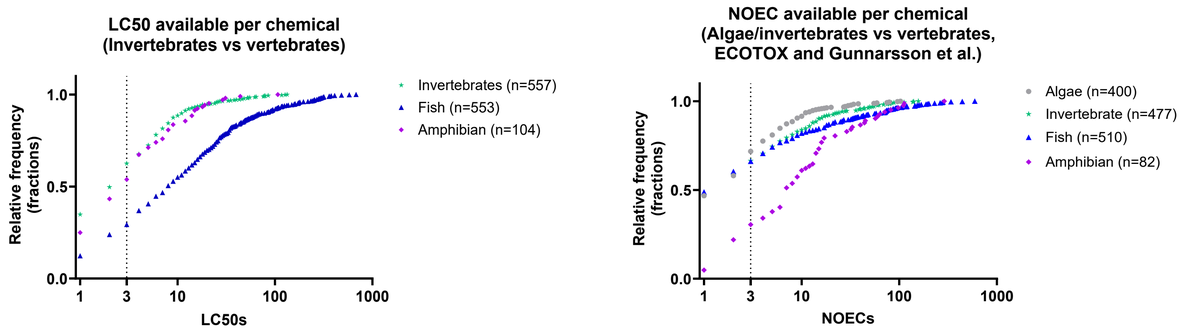

Figure 4: *Cumulative frequency distribution of available LC50/NOEC data points per chemical for different aquatic groups.*

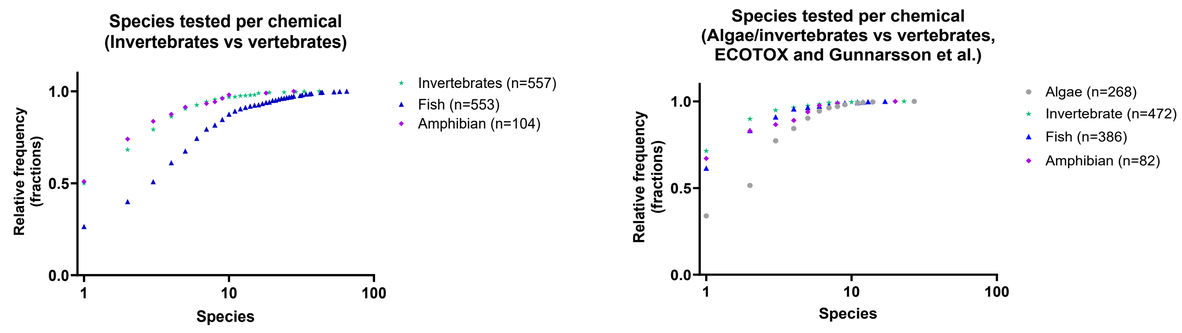

Figure 5: *Cumulative frequency distribution of species tested per chemical across aquatic taxonomic groups.*

AMIRA webapp (**A**nimal-free **M**echanistic **I**nference for species-specific chemical **R**isk **A**ssessment) was created to visualise the results.

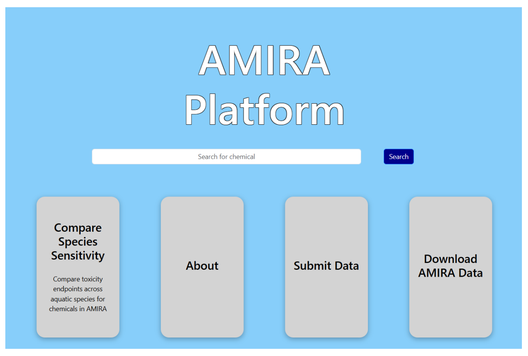

Figure 6: *AMIRA app allows visualisation of ecotoxicity data from ECOTOX and Gunnarsson et al. and helps identify chemicals for which fish might be less/more toxic than other groups.*

TAKE-HOME MESSAGES

For more than **80%** chemicals, data from lower taxonomic groups might be sufficient to determine safe concentrations without the need for vertebrate data.
Understanding the chemical structures and mechanisms of action underlying species-specific sensitivity would enable predictive modelling to classify when fish will be highly sensitive to a new chemical.