Protection over Prediction:
Daphnids Safeguard the Use of Alternatives to the Acute Fish Toxicity Test

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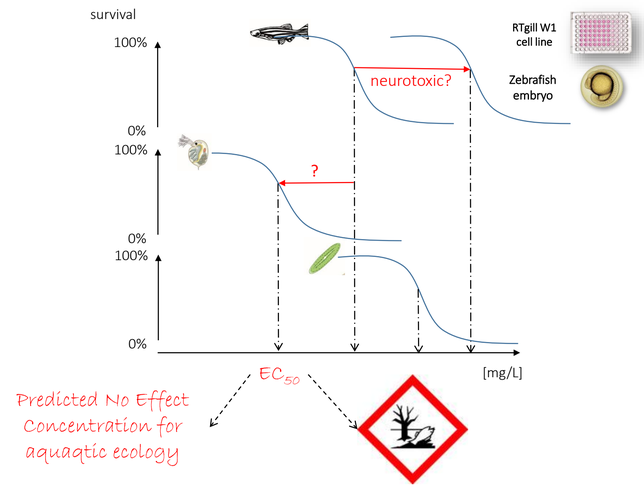
Research Question

~ 45,000 fish / year in Europe for acute toxicity testing

~ 50% of aquatic vertebrates for ecotoxicity testing

→ How to replace acute juvenile fish test with acute Zebrafish-Embryo-Toxicity Test or acute RTgill W1 cell line test, which appear to be less sensitive to neurotoxic chemicals?

→ Do Daphnids safeguard the use of alternative methods for PNEC derivation and GHS classification?



Method

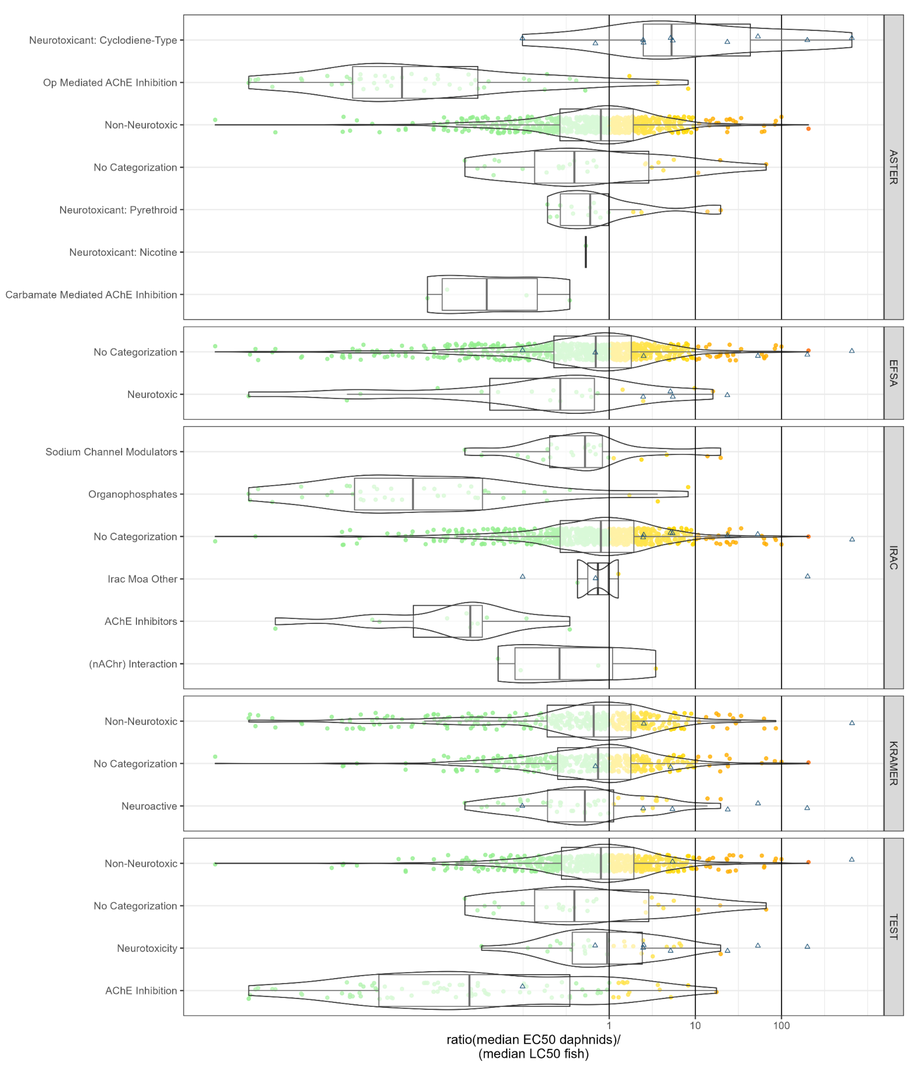
- Employ quality assured **EnviroTox** DB (Connors et al. 2019)
- **Select species & endpoints according to OECD TGs**

trophic level	effect	expo	stat	n
FISH	Mortality, Immobilisation	96h	LC50	11381
FISH	Mortality, Growth, Immobilisation, Intoxication, Loss of equilibrium	96h	EC50	2171
INVERT	Intoxication, Immobilisation	48h	EC50	2885

trophic level	latin name	n	%	trophic level	latin name	n	%
FISH	Oncorhynchus mykiss	4325	31,9	INVERT	Daphnia magna	2367	82
FISH	Lepomis macrochirus	3740	27,6	INVERT	Daphnia pulex	324	11,2
FISH	Pimephales promelas	3344	24,7	INVERT	Ceriodaphnia dubia	123	4,3
FISH	Poecilia reticulata	572	4,2	INVERT	Daphnia carinata	25	0,9
FISH	Oryzias latipes	429	3,2	INVERT	Daphnia sp	13	0,5
FISH	Cyprinus carpio	417	3,1	INVERT	Daphnia laevis	9	0,3
FISH	Cyprinodon variegatus	365	2,7	INVERT	Daphnia longispina	7	0,2
FISH	Danio rerio	306	2,3	INVERT	Daphnia obtusa	6	0,2
FISH	Gasterosteus aculeatus	35	0,3	INVERT	Daphnia spinulata	6	0,2
FISH	prinus carpio ssp. commu	14	0,1	INVERT	Ceriodaphnia lacustris	2	0,1
FISH	Dicentrarchus labrax	1	0	INVERT	Moinodaphnia macleayi	2	0,1
FISH	Pagrus major	4	0	INVERT	Daphnia galeata	1	0

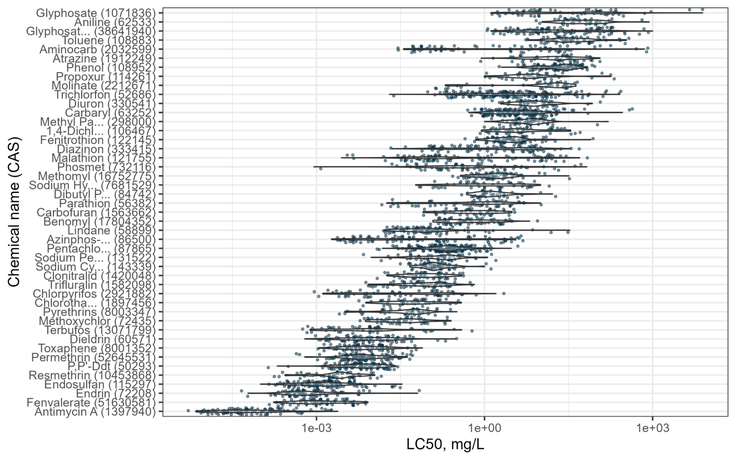
- **Stratify LC50 & EC50 values for neurotoxic MoAs** using different classification schemes, based on QSARs (TEST, ASTER), experimental pesticide MoAs (IRAC database) & expert analysis (EFSA/ Masjosthusmann et al 2021; Kramer et al. 2024)

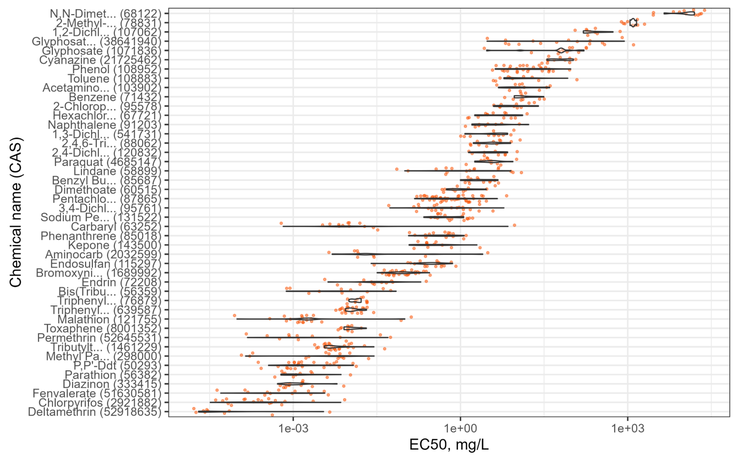
- Chemicals median EC50 daphnids to median LC50 or EC50 fish ratios indicate similar or higher sensitivity of daphnids for all neurotoxic chemicals, except for cylodienes (Δ symbols). This type of chemistry is no longer marketed.



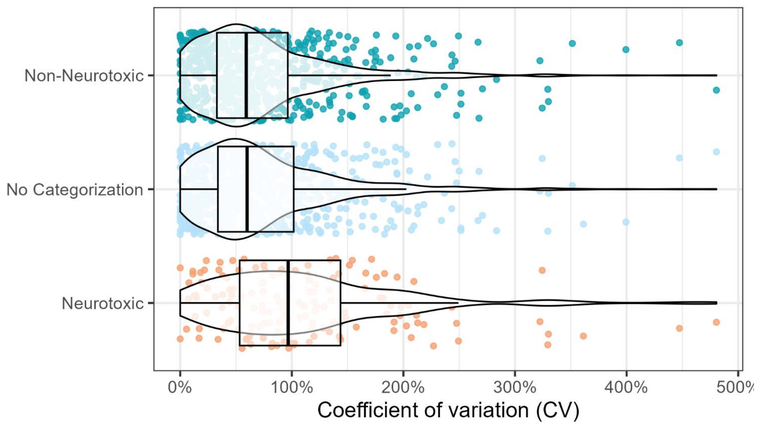
Results

- TG conform LC50 & EC50 values may span several orders of magnitude for fish and daphnids





- On average, chemicals with neurotoxic MoA show more variable LC50 & EC50 values than others
- Use of different fish species can explain the variability in LC50 & EC50 values only for some chemicals



- Chemicals less sensitive in the acute RTgill W1 cell line test compared to the juvenile fish test appear similar or more sensitive in the acute daphnids test, especially if considering the coefficients of variation

chemical name	median EC50 fish	cv fish	n fish	median EC50 daph	cv daph	n daph	median daph/fish EC50 ratio
Allyl alcohol	0,59	57	9	0,25		1	0,42
Permethrin	0,0067	88	109	0,0013	198	42	0,19
Caffeine	151		1	422	88	2	2,79
Lindane	0,086	517	87	1,79	117	40	20,81

- Similar conclusion for chemicals median EC50 ratios compared to all-to-all EC50 ratios
(cyclodiens excluded in this table)

categorization scheme	categories	Median Ratios			All to All Ratios		
		n	% > 10	% > 100	n	% > 10	% > 100
aster	Carbamate mediated AChE inhi	4	0	0	562	0,2	0
aster	Neurotoxicant: Nicotine	1	0	0	2	0	0
aster	Neurotoxicant: Pyrethroid	17	11,8	0	2863	6,8	0,8
aster	Non-neurotoxic	692	3,3	0,1	41498	9,8	1
aster	OP mediated AChE inhibition	49	0	0	9998	0,5	0
busch	Neuroactive	39	0	0	10304	0,4	0
busch	Non-neurotoxic	147	4,1	0	12671	13,9	0,5
efsa	No categorization	29	3,4	0	9077	1	0
efsa	neurotoxic	19	5,3	0	7857	11,1	2
irac	a ch e inhibitors	11	0	0	3143	0,1	0
irac	irac moa other	2	0	0	67	0	0
irac	nAChR interaction	4	0	0	17	0	0
irac	organophosphates	42	0	0	9774	0,5	0
irac	sodium channel modulators	24	8,3	0	5943	3,9	0,4
test	AChE inhibition	87	1,1	0	14854	0,8	0
test	Neurotoxicity	27	3,7	0	3681	6,1	0,5
test	Non-neurotoxic	649	3,5	0,2	36388	10,9	1,2

Conclusions

This research, rooted in decades of efforts to replace the juvenile fish test, shifts the focus from predicting fish toxicity to emphasizing environmental protection. This transition allows completely replacing the juvenile fish test within an integrated testing and assessment approach standardized by the OECD. Furthermore, it paves the way for further eliminating vertebrate animal tests in environmental toxicology.

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