Do *Daphnia magna* and *Ceriodaphnia dubia* acute and chronic tests show equitoxic results?

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Philosophy of standard test organisms

- Environmental Risk Assessment relies on standard toxicity tests using standard model organisms
- Each model organism represents an important environmental niche

These species are meant to represent the 8.7+ million species in the world

Development of standard toxicity assays

Qualities of a standard toxicity assay:

- Easy to conduct
- Endpoint(s) related to survivorship, development, reproduction
- Acute tests completed within a business week
- Require minimal amount of test material
- Intra/Inter-laboratory repeatability, reproducible

How did we really get here?

- Guidelines formalized the current testing practices of the time
- Generally speaking, no assay validation was completed. No round robin exercises.
- Assay noise, repeatability, domain of applicability; not well understood.

OECD test guidelines as “gold standards”

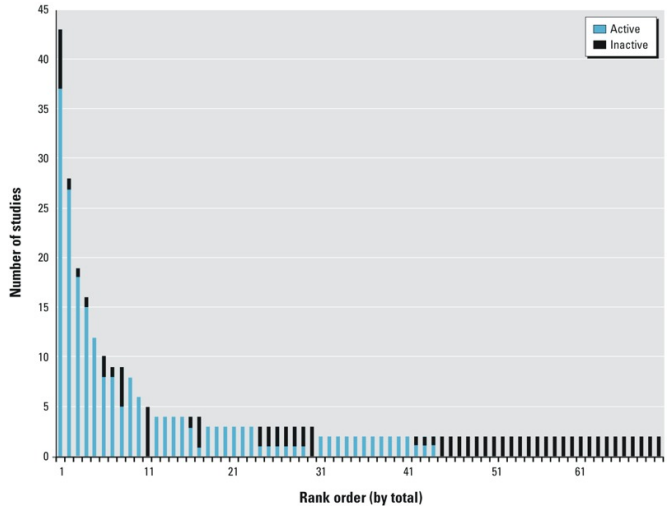
Some assays allow for a diversity of test species, others are more specific. Why?

OECD 203 Fish Acute Toxicity Test
Zebrafish (*D. rerio*)
Fathead minnow (*P. promelas*)
Common carp (*C. caprio*)
Medaka (*O. latipes*)
Guppy (*P. reticulata*)
Bluegill (*L. macrochirus*)
Rainbow trout (*O. mykiss*)

OECD 202 Daphnia Acute Immobl. Test
Daphnia magna Straus
Other “suitable” Daphnia species (e.g. *Daphnia pulex*)

Recent interest in critically evaluating current “gold standard” assays

- Rat uterotrophic assay, LLNA assay, Draize tests
- Similar efforts should be undertaken for ecotox assays



Kleinstreuer et al. 2016. EHP

Daphnid toxicity tests in REACH

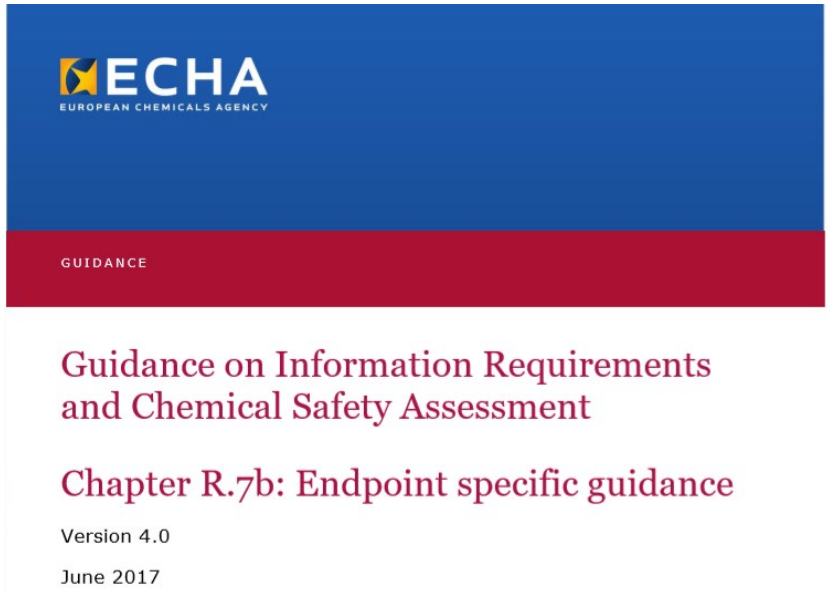
Acute invertebrate (OECD 202, preferred)

Daphnia magna Straus or "other suitable Daphnia species (e.g., *Daphnia pulex*)"

Chronic invertebrate (OECD 211, preferred)

Daphnia magna Straus. "Other Daphnids may be used provided they meet validity criteria"

C. dubia referenced under experimental volume considerations



"In addition to *Daphnia magna*, *Daphnia pulex*, *Ceriodaphnia affinis* and *C. dubia* are commonly tested species. Overall, there is **no significant difference** in sensitivity of *D. magna* and *D. pulex*. Good correlation has been reported between acute toxicities of all three species (ECETOC 2003). **All these can be considered as equally accepted preferred species**."

In practice, *C. dubia* data has **not** been fully accepted by ECHA to fulfil REACH requirements.

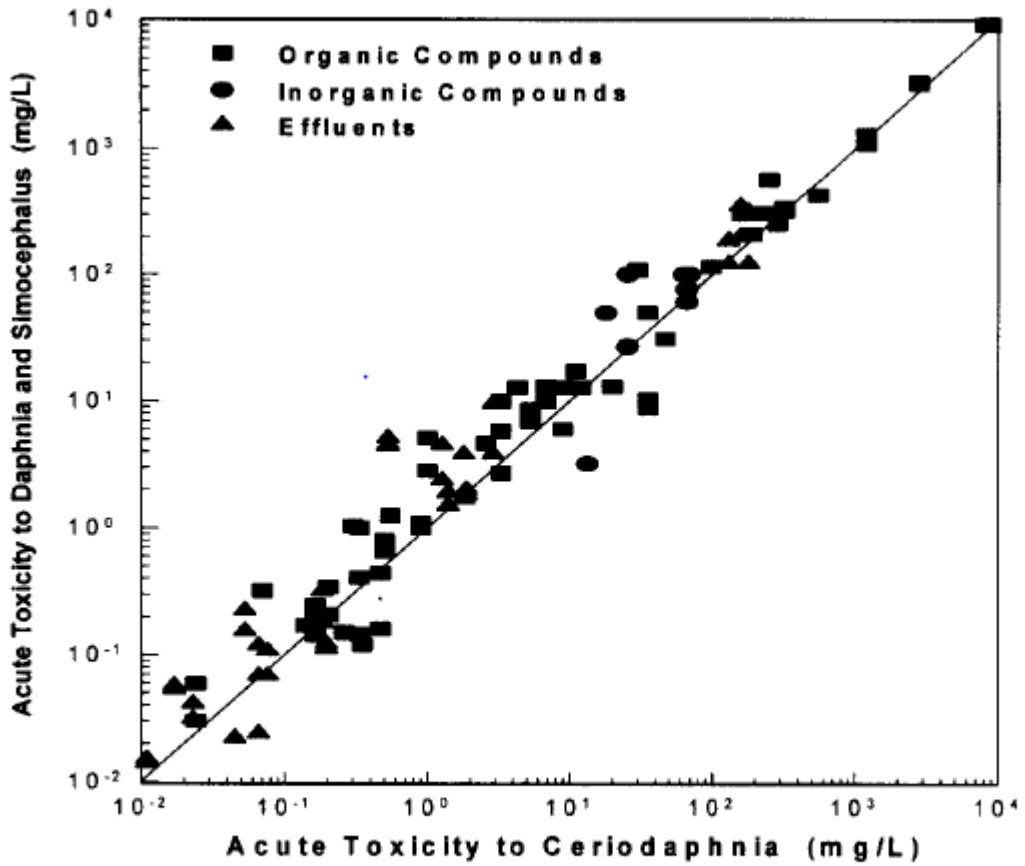
Daphnia magna vs. *Ceriodaphnia dubia*

	Daphnia magna	*Ceriodaphnia dubia*
Habitat	Lakes, ponds, streams	Ponds, slow water, littoral zones
Adult size	5-6 mm (*D.pulex* 3.5mm)	~0.9mm
Time to maturity	6-10d	~3d
Generation time	~4-6d	2d
Clutch size	6-10 neonates	6-10 neonates
Acute duration	48h	48h
Chronic duration	21d	5-8d
Preferred pH	6.5-8.5	6.5-8.5
Temperature	20 ± 2°C	25 ± 1°C
Food	Algae	Algae, YCT

- Both species have broad geographic distribution
- Family: Daphniidae, different Genus and Species
- Both routinely used in Whole Effluent Toxicity (WET) testing, and chemical toxicity testing
  - Ceriodaphnids frequently used USA, Canada, Australia and New Zealand

Image: cfb.unh.edu

Previous work demonstrated 1:1 relationship between *Cerios* and *Daphnids*



Versteeg et al. 1997. Chemosphere

Figure 1 Plot of the acute LC_{50} values of *Daphnia magna*, *Daphnia pulex*, and *Simocephalus vetulus* versus the LC_{50} values of *Ceriodaphnia* species. Greater and less than values have been dropped, 1:1 line shown.

Data-driven approach: Explore species sensitivity relationships

Compare the relationship between *D. magna* and *D. pulex*; *D. magna* and *C. dubia*

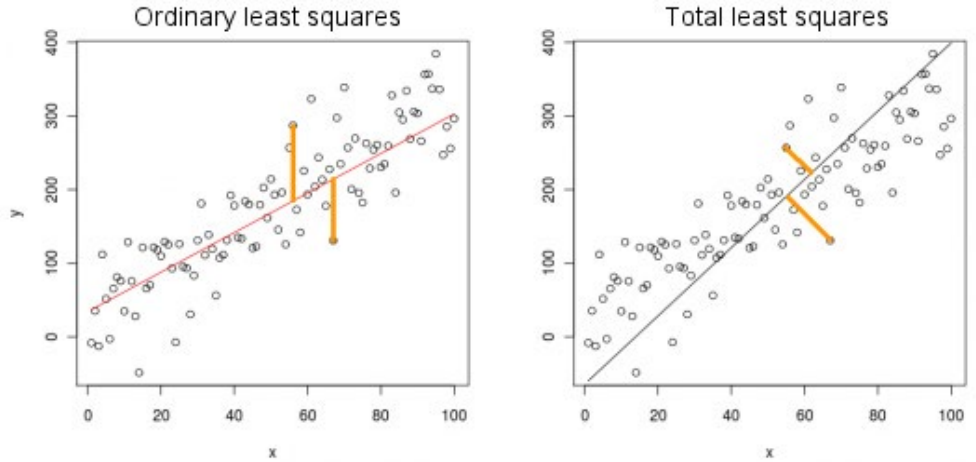
Data collection:

- EnviroTox database
  - ECHA, USEPA ECOTOX, Peer-reviewed literature, ECETOC OASIS, AiiDA, METI, FET, USGS Columbia, ECOSAR training set, EPA Pesticide data, OECD QSAR Toolbox, others..
- P&G internal files

Subset to chemicals that have tested in both species

Geometric means summarize results for chemicals tested more than once

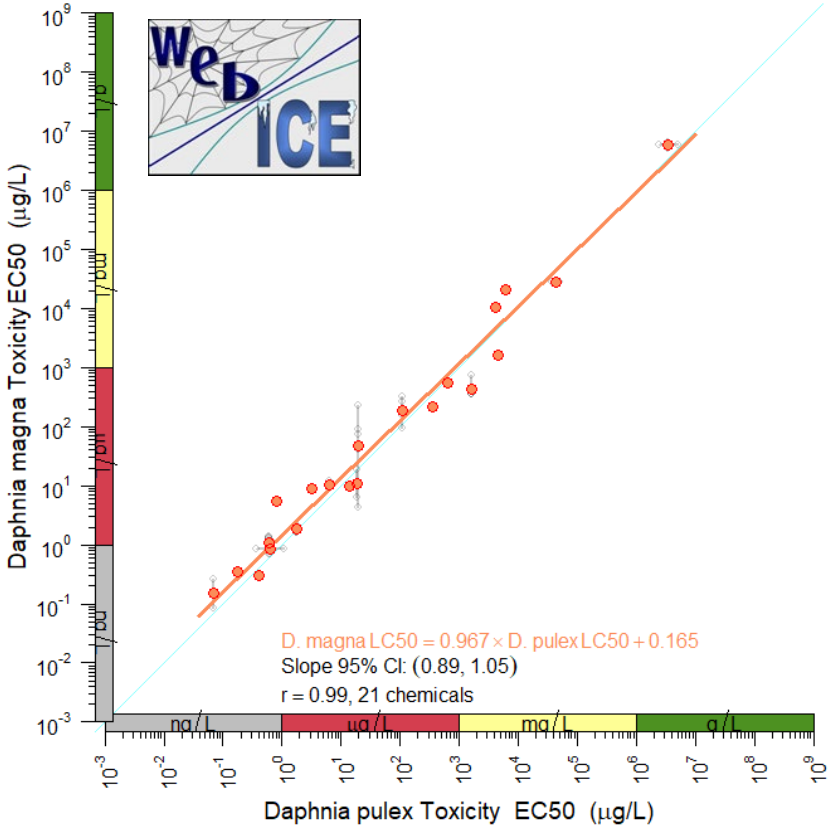
Orthogonal regression

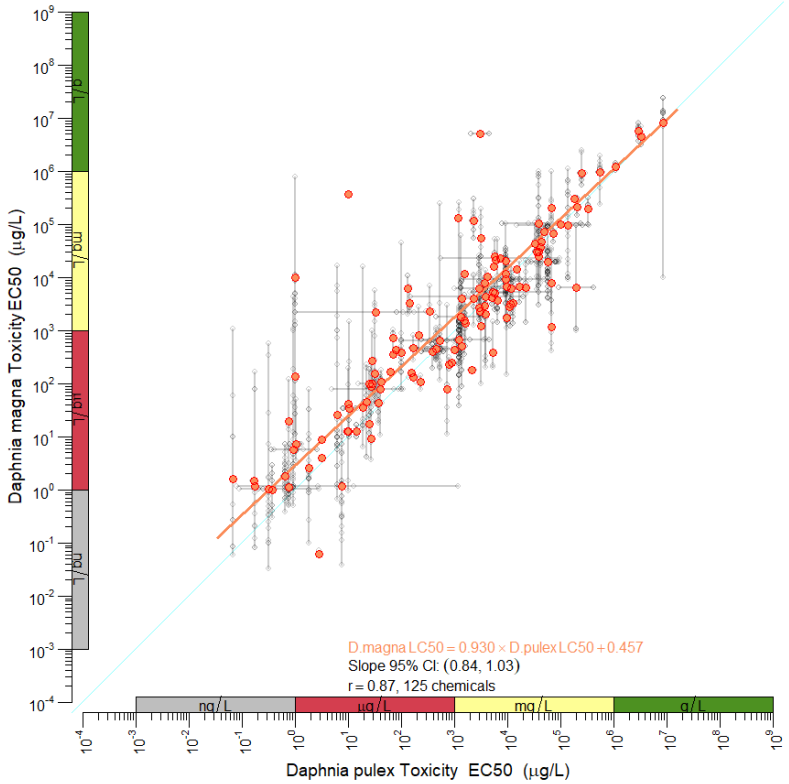


http://www.cerebralmastication.com/2010/09/principal-component-analysis-pca-vs-ordinary-least-squares-ols-a-visual-explination/

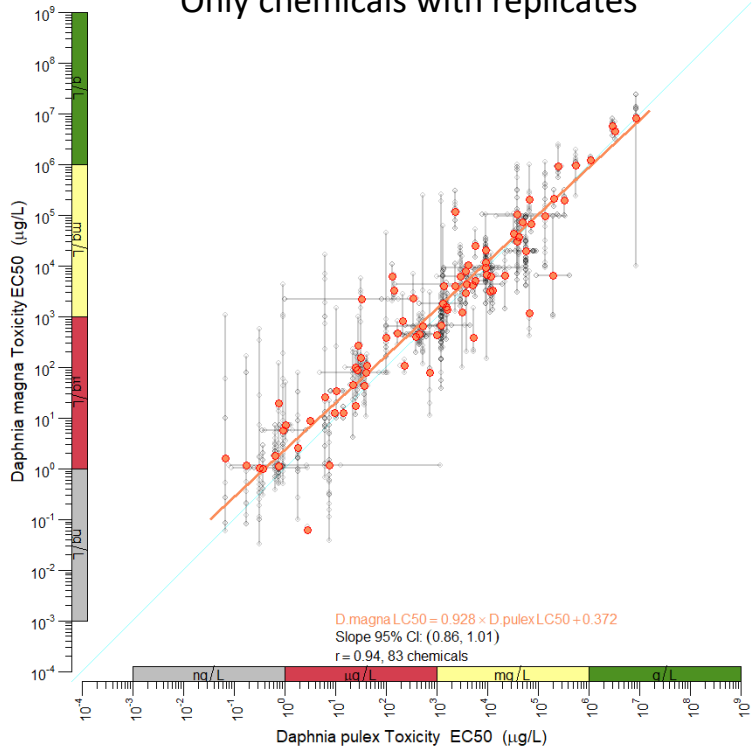
OECD 202 species: *D. magna*, *D. pulex*

This regression captures the level of species-to-species noise that is accepted by the test guideline.



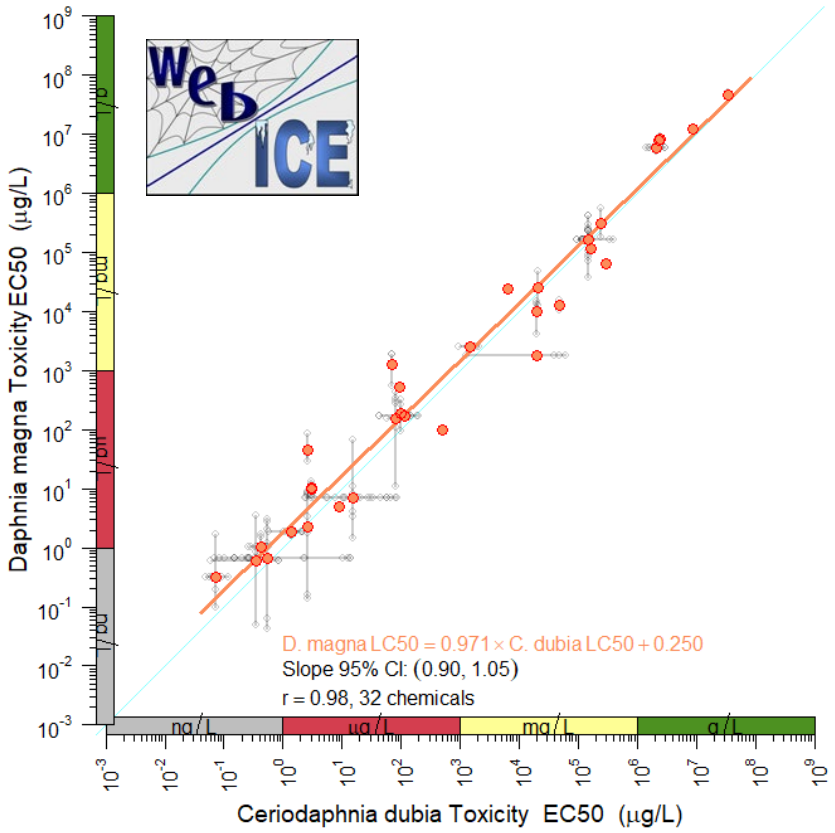


Only chemicals with replicates

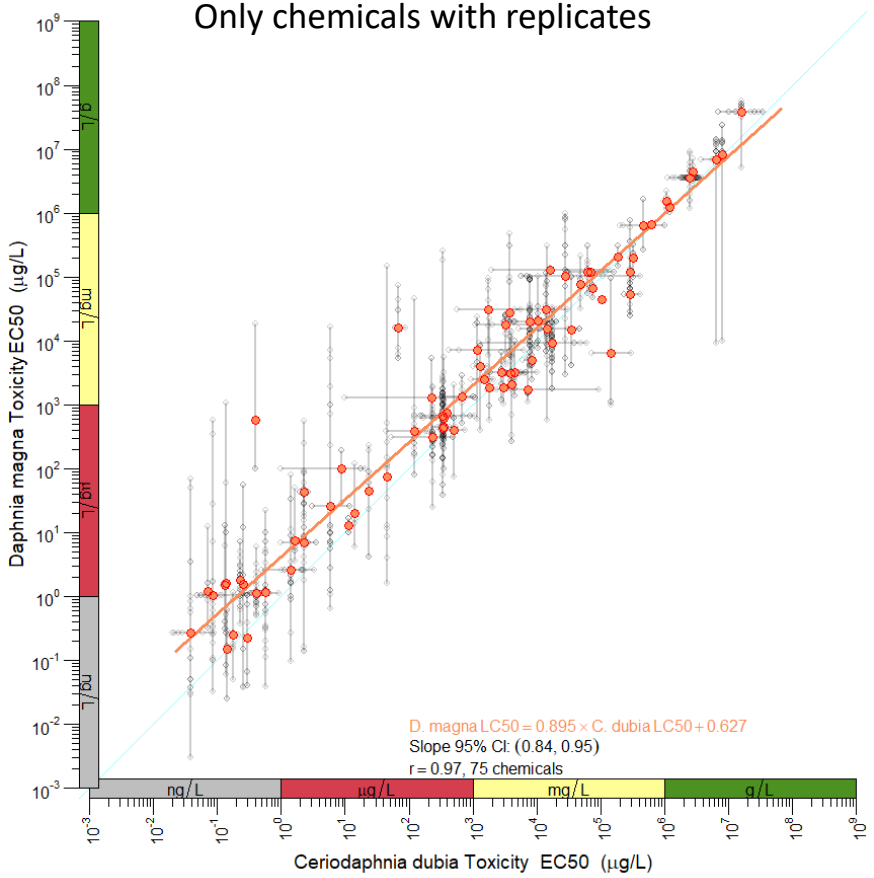


Species comparison	Data source	Chemicals	Entries	Slope (95% CI)	Intercept
D. magna, D. pulex	WebICE	21	78	0.97 (0.89, 1.05)	0.165

D. magna, C. dubia

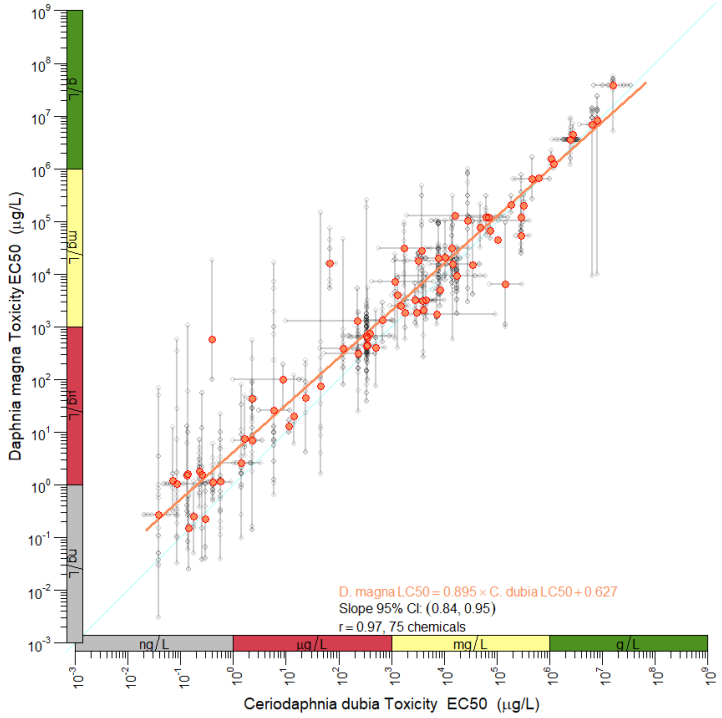


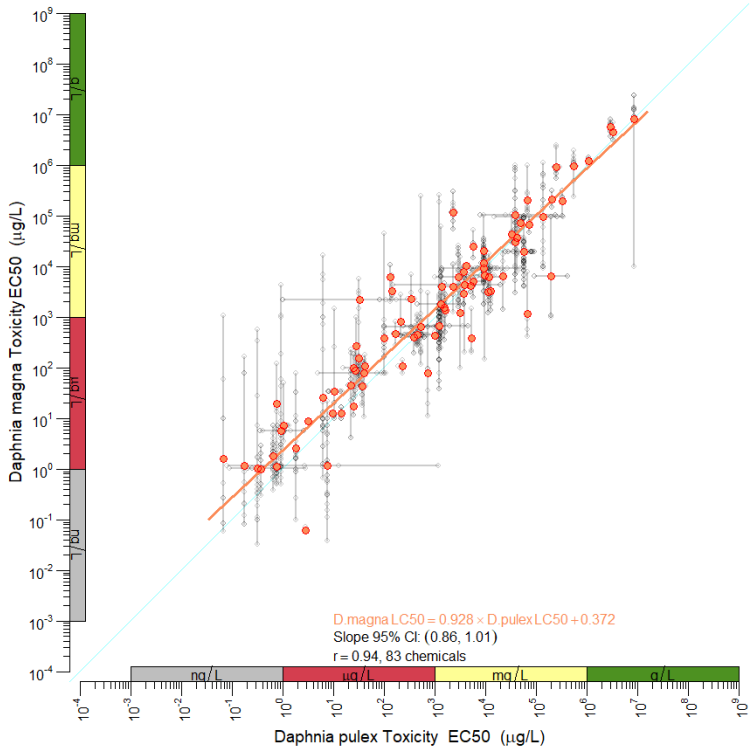
Only chemicals with replicates



Species comparison	Data source	Chemicals	Entries	Slope (95% CI)	Intercept
D. magna, C. dubia	WebICE	32	258	0.97 (0.90, 1.10)	0.250

D. magna, D. pulex, and *C. dubia* are acutely equisensitive

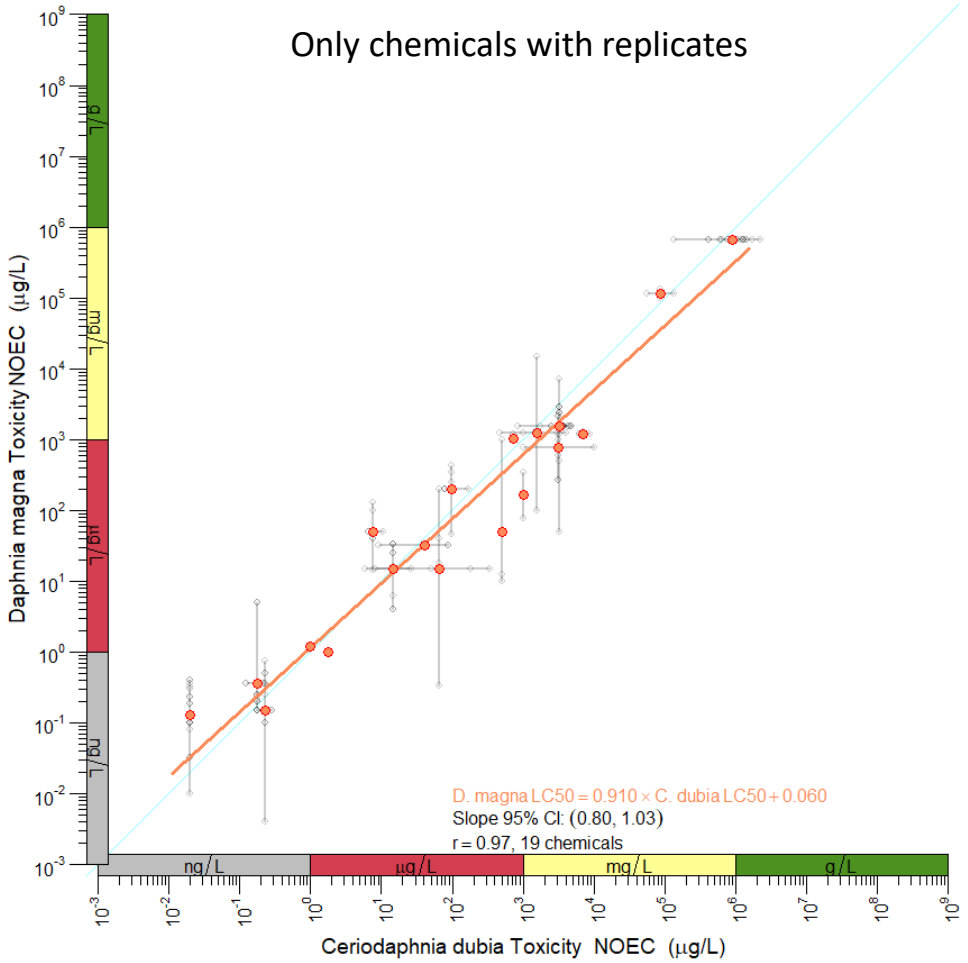




Stats are being finalized.

Will update with Interclass correlation (ICC) values, variance description, etc

Chronic toxicity: *D. magna* vs *C. dubia*



Data source	Chemicals	Entries	Slope (95% CI)	Intercept
Current study	51	307	0.86 (0.71, 1.03)	0.329
Current study, reps	19	184	0.91 (0.80, 1.03)	0.060

Key differences that may impact chronic toxicity results between the two species

- -Duration (3 brood test; DM: 21 days, CD: 5-8 days)
- -Concerns with equilibrium
- -Differences in organism size

Advantages of CD tests:

- -Faster
- -Requires less chemical

Case for developing OECD *C. dubia* guideline

- *Ceriodaphnia dubia* is equisensitive to *Daphnia magna,* acutely and chronically.
- Organisms fulfill similar ecological niche. Both have global geographic distribution.
- *C. dubia* is routinely used in toxicity tests, globally. Testing guidelines already exist.

Consider developing an OECD guideline for *C. dubia* acute toxicity tests, or amending OECD 202 to include *C. dubia*

Broader thoughts on species sensitivity

Remember the protection goals: populations, ecological processes

- Underlying assumption is that if you protect the most sensitive species, the processes at higher levels of biological complexity will also be protected (e.g., communities, nutrient cycling, etc).
- May be better modeled by mesocosms or species sensitivity distributions (SSDs)

Are the current model organisms sufficient to evaluate environmental impacts?

- If no: is the new test species novel taxonomically, physiologically, ecologically?

How well do we understand the variability of non-standard organisms?

- Is the variability due to biological or methodology?

482) Wednesday 10:40AM Room 713A.
The roles for and constraints derived from method standardization in international chemical environmental risk assessment. Scott Belanger.