

Toxicity Screening of the ToxCastJ Chemical Library Using a Zebrafish Developmental Assay

Casting About for Correlations and Patterns

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(<http://www.epa.gov/ncct/toxcast/>)



Background on ToxCast_320 Chemicals

There are 309 chemicals: pesticides and pesticide metabolites.

Many of the chemicals have gone through testing in mammals, including guideline tests on development in rat and/or rabbit (ToxRef Database).



Overview on Our Original Goals for Screening for Prioritization

Identify the most toxic compounds in a group of chemicals (send them on for further testing)

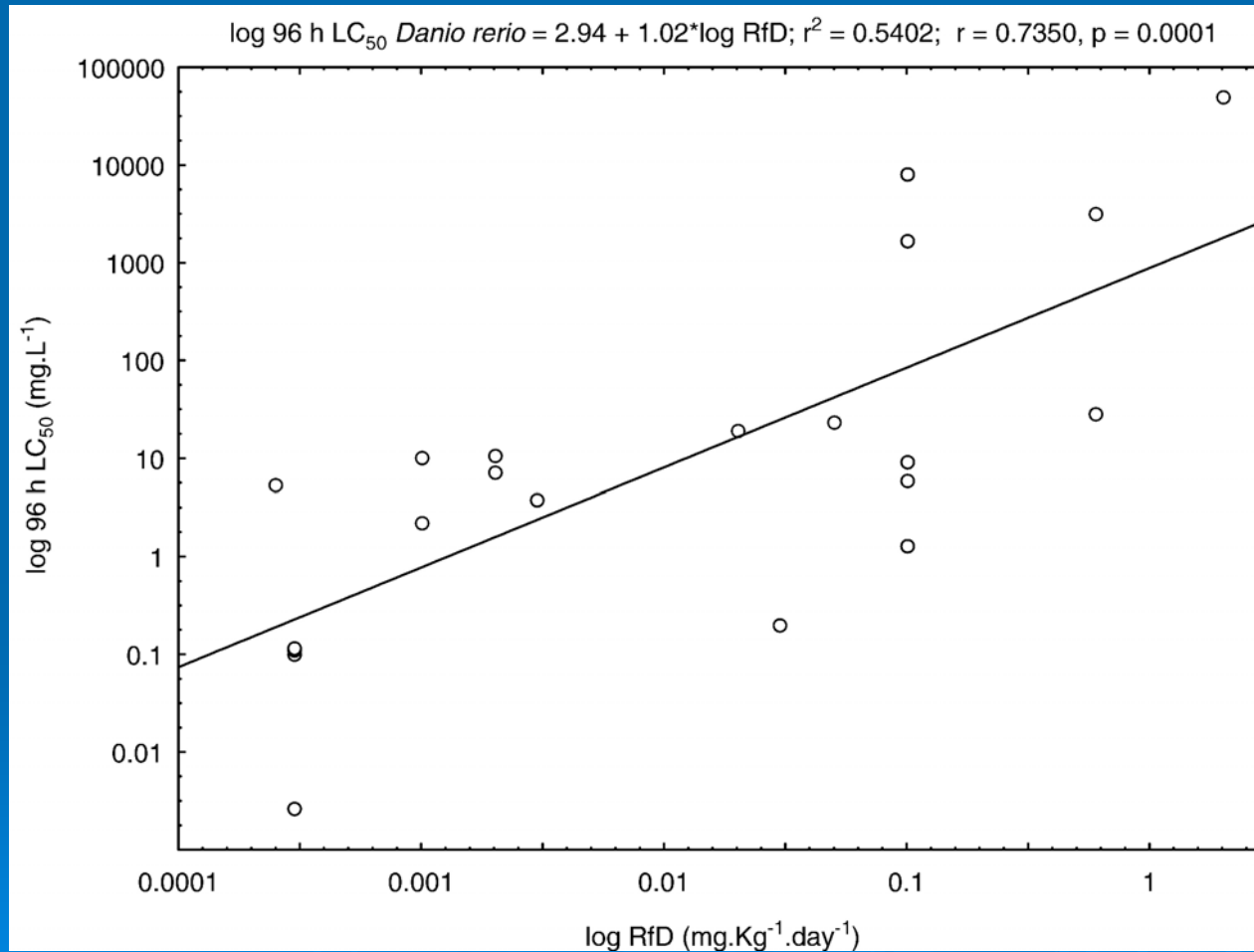
Want to identify the positives.

Not necessarily identify the negatives (willing to accept that there are a lot of reasons for a negative)

There is a difference between drinking a chemical and swimming in a chemical.

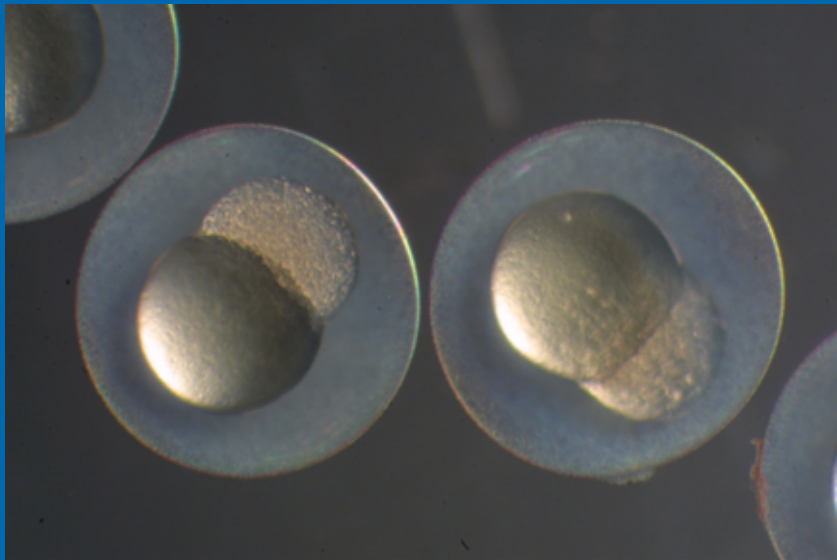


Comparison between 96 hour lethality in zebrafish embryos vs human RfD



From Martins *et al* (2007), *Environ.Internat.* 33:414-425

Zebrafish Development



Egg, About 6 hours after fertilization

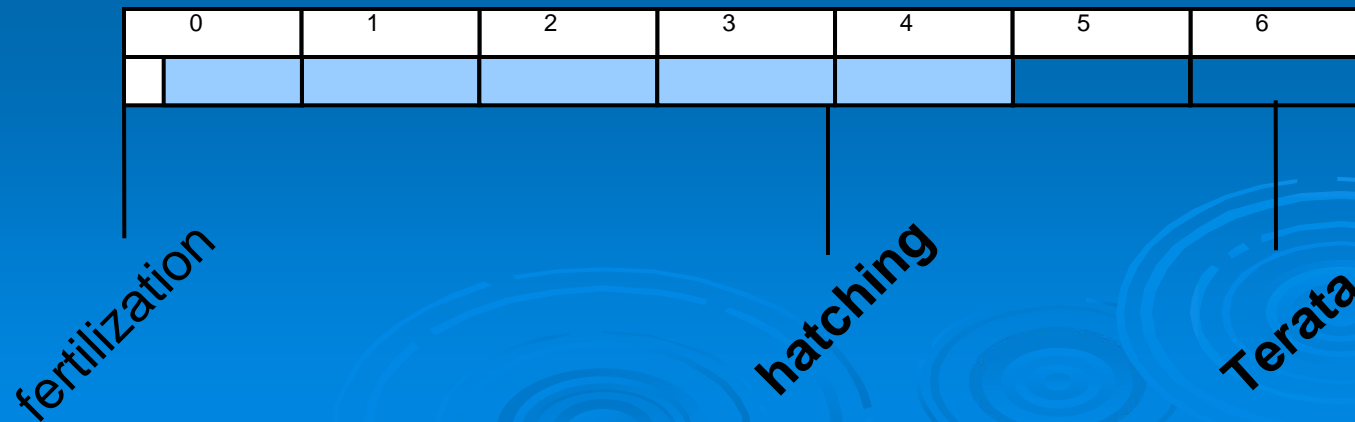


Larva, 6 days post fertilization

Purpose: To assess the toxicity of the ToxCast_320, and then use to the data to see how well it compares to other endpoints

Approach: (n=4) per chemical (each on separate plates and often on separate days).

Only one concentration of each chemical was assessed (80 uM). All chemicals in DMSO (final concentration was 0.4%)





Experimental Questions:

How difficult was the experiment to conduct?

How consistent were the data within plate?

How consistent were the data between plates?

What percentage of hits will we have? (and will it be obvious what a “hit “ is?)

Can we develop a Toxicity Score for each animal?

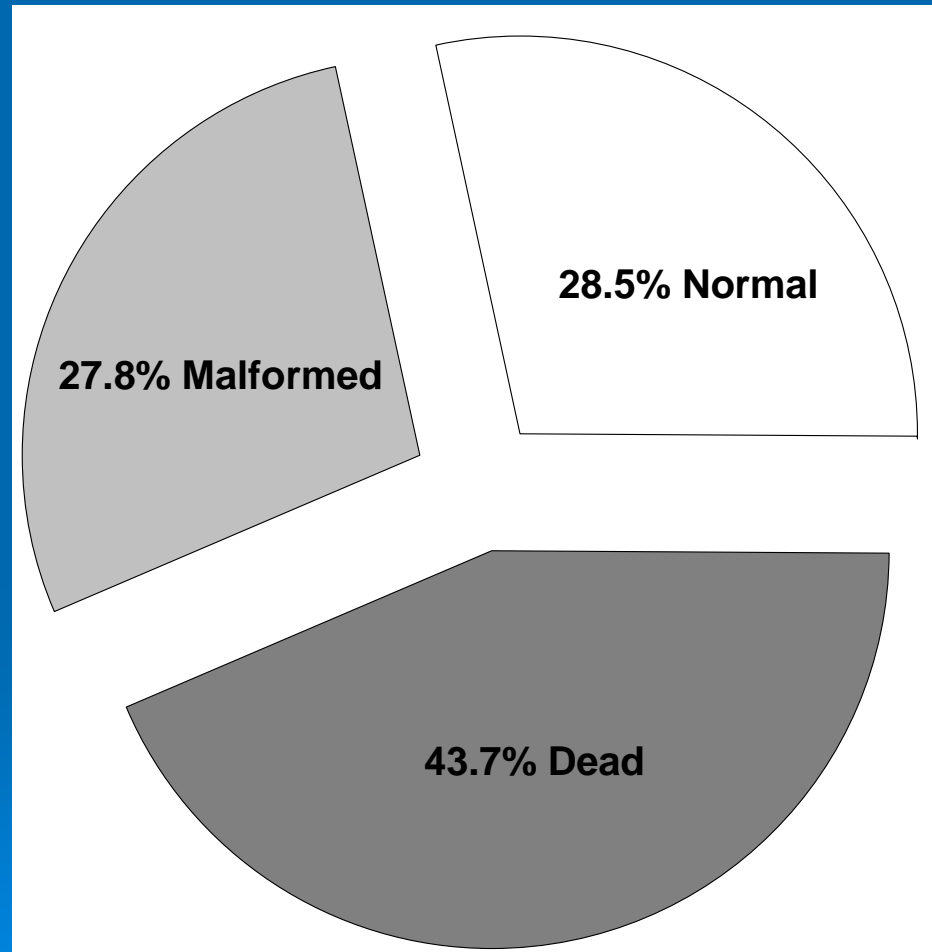
What does a comparison between that toxicity score and the following look like?

physical characteristics of the chemicals

Chemical class or use pattern

ToxRef Database

What percentage of hits will we have? (and will it be obvious what a “hit “ is?)



Can we develop a Toxicity Score for each animal?

- **Toxicity Score:**

- If the animal was alive on Day 6, the Terata Score was used.

If the animal was dead on Day 6 we used to following:

If the animal died on Day 6, the Score was 35

If the animal died on Day 5, the Score was 40

If the animal died on Day 4, the Score was 45

If the animal died on Day 3, the Score was 50

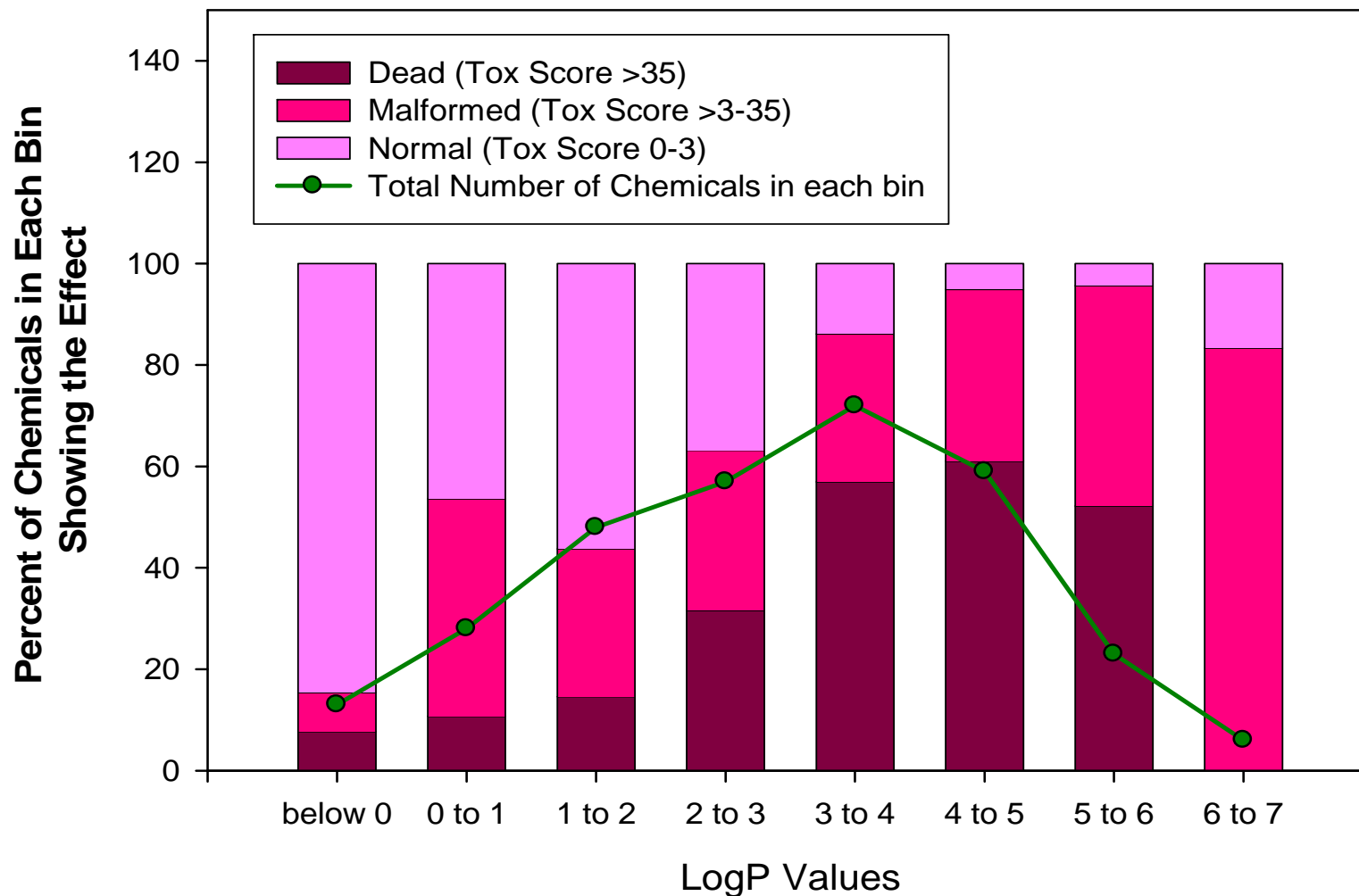
If the animal died on Day 2, the Score was 55

If the animal died on Day 1, the Score was 60

- In other words, it is assumed that the more toxic chemicals will kill the embryo sooner.



Physical Characteristic vs Toxicity



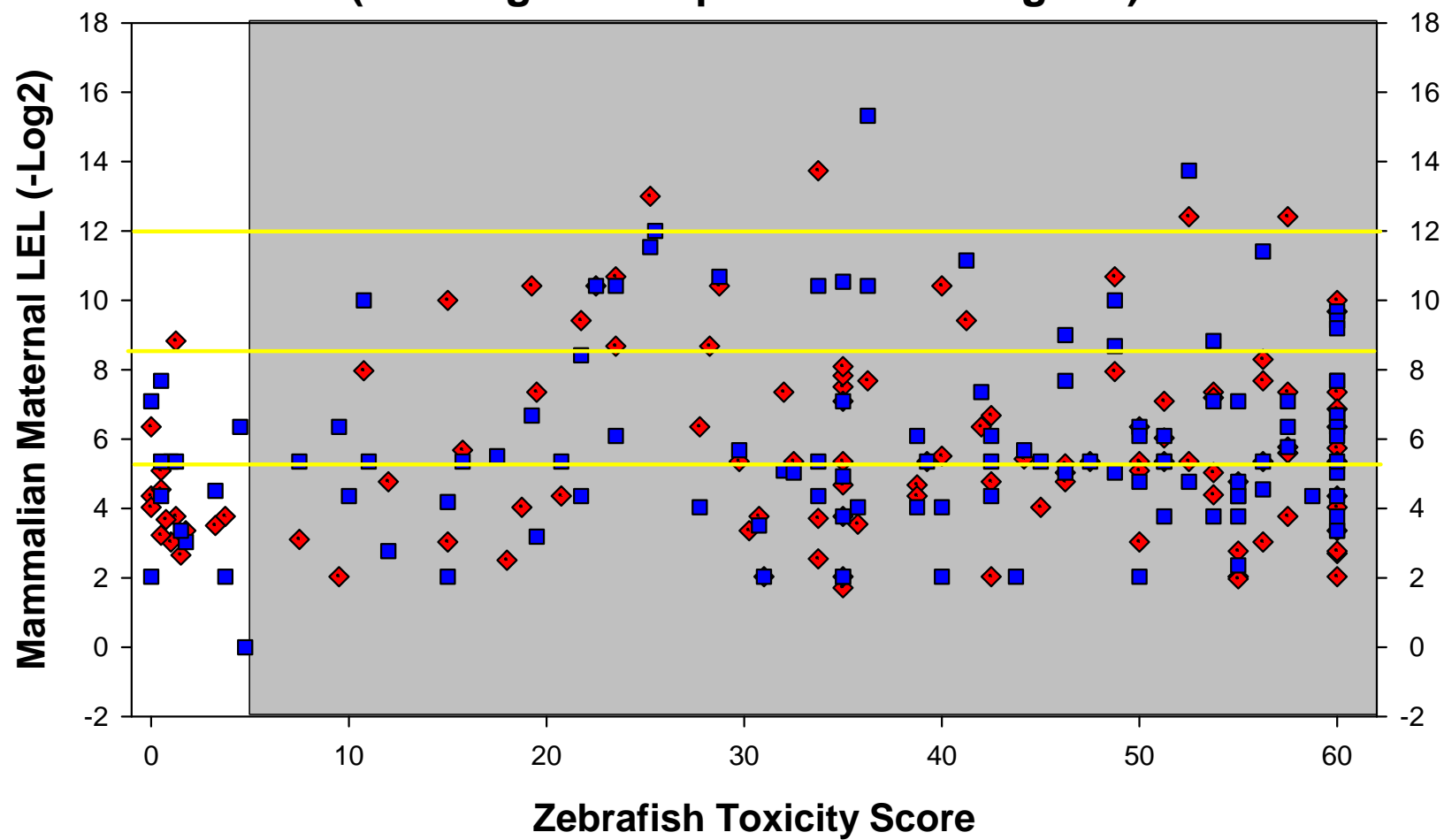


Chemical Class vs. Zebrafish Toxicity

Chemical Class	Toxicity Normal/Total
Pyrethroids	0/11
Mectins	0/7
Conazoles (Triazole & Imidazoles)	0/14
Dithiocarbamates	0/6
Organochlorines	0/3
Aryloxyphenoxypropionic Herbicides	0/7
Organophosphorus Compounds	3/24

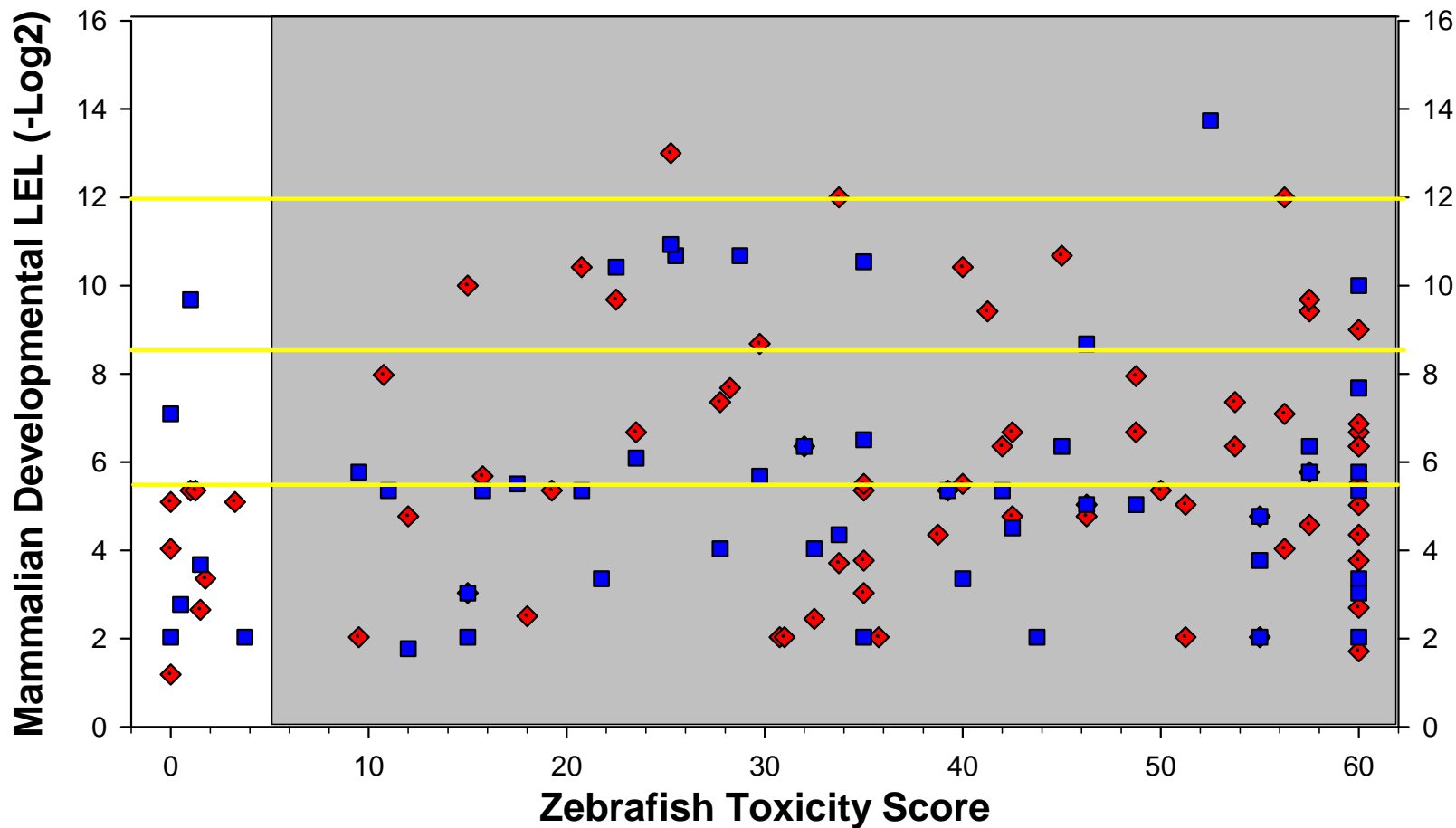
Zebrfish Toxicity Score vs Rat or Rabbit Maternal Toxicity (deleting all compounds with LogP<3)

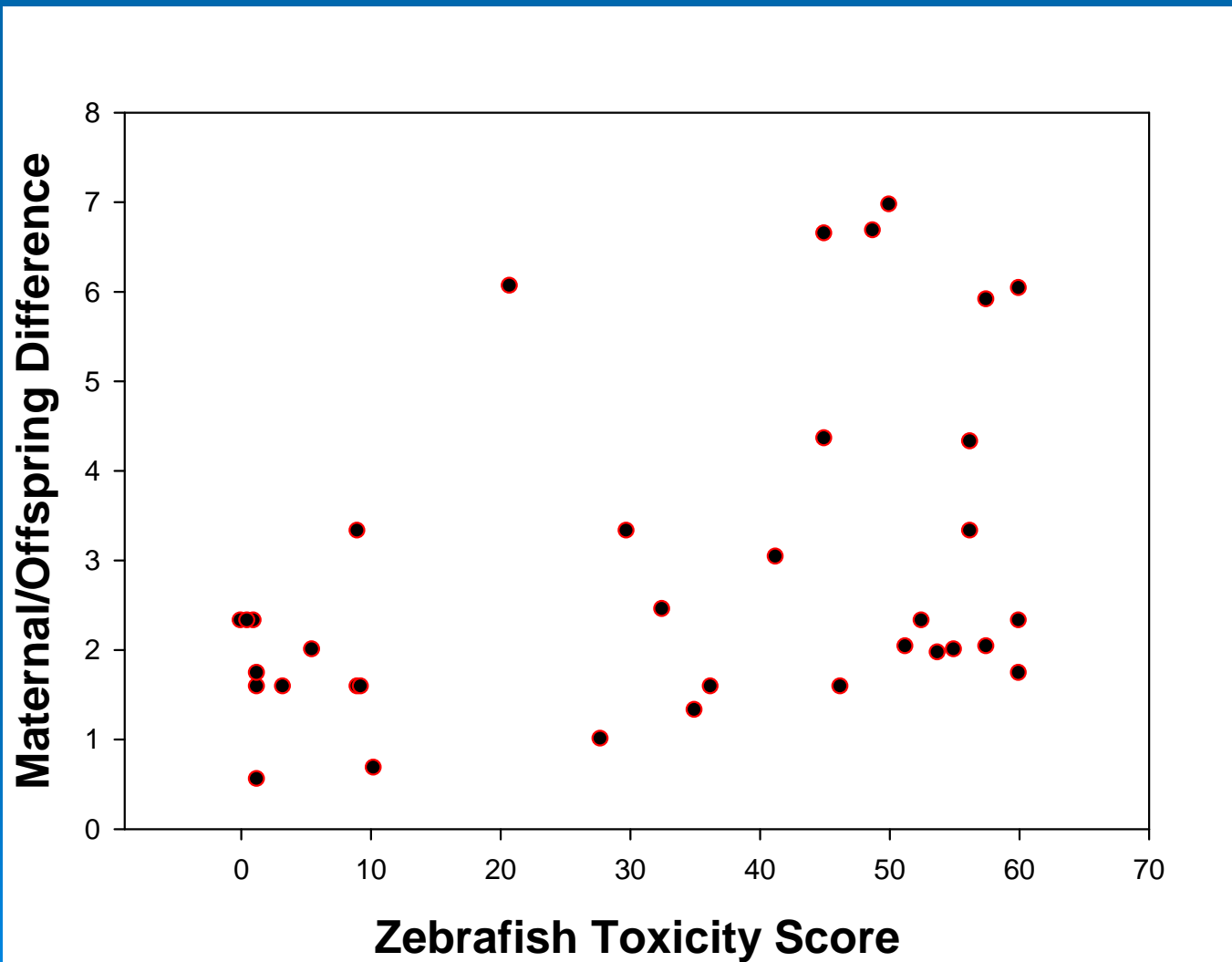
- ◆ Rat Maternal LEL
- Rabbit Maternal LEL



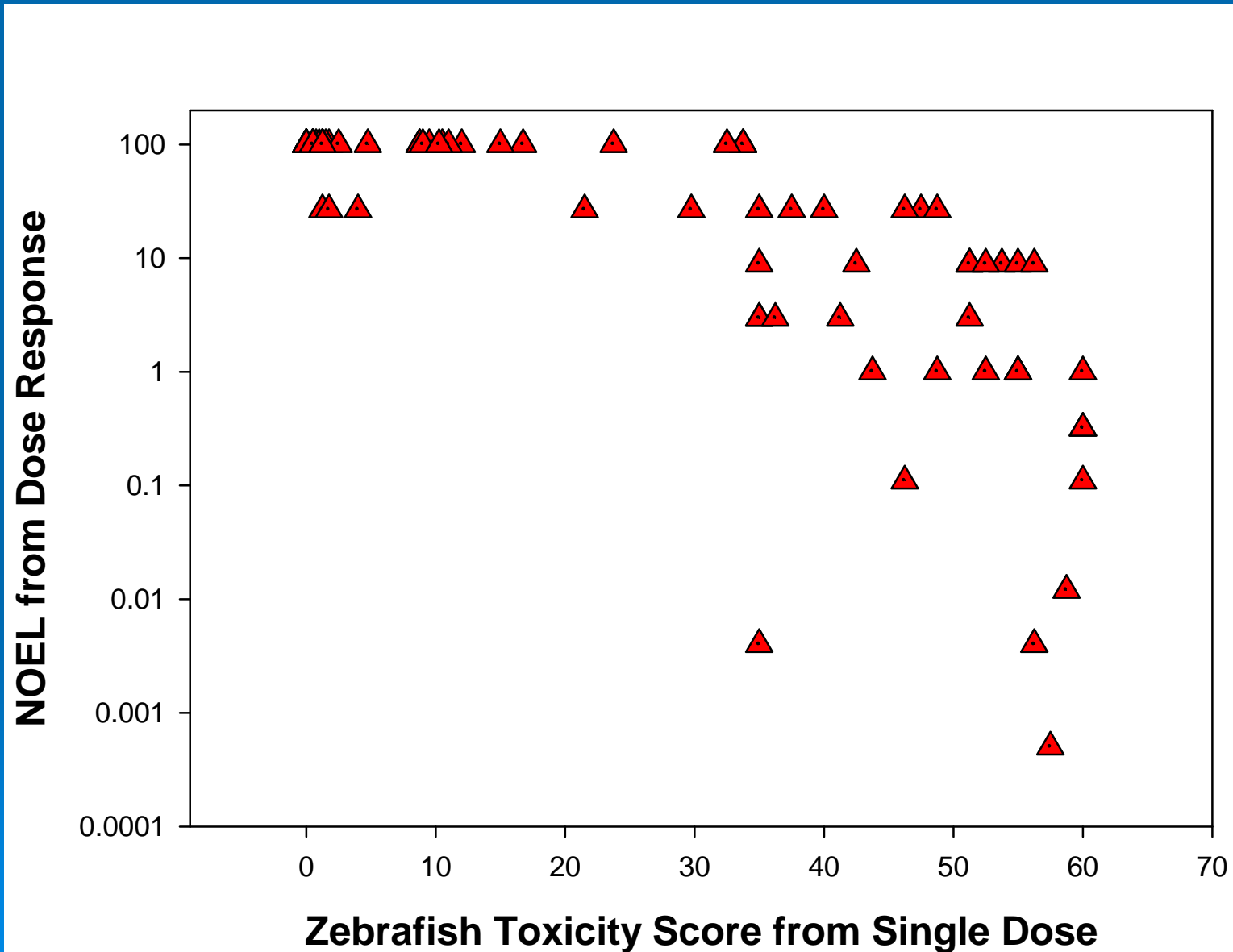
- ◆ Rat Developmental LEL
- Rabbit Developmental LEL

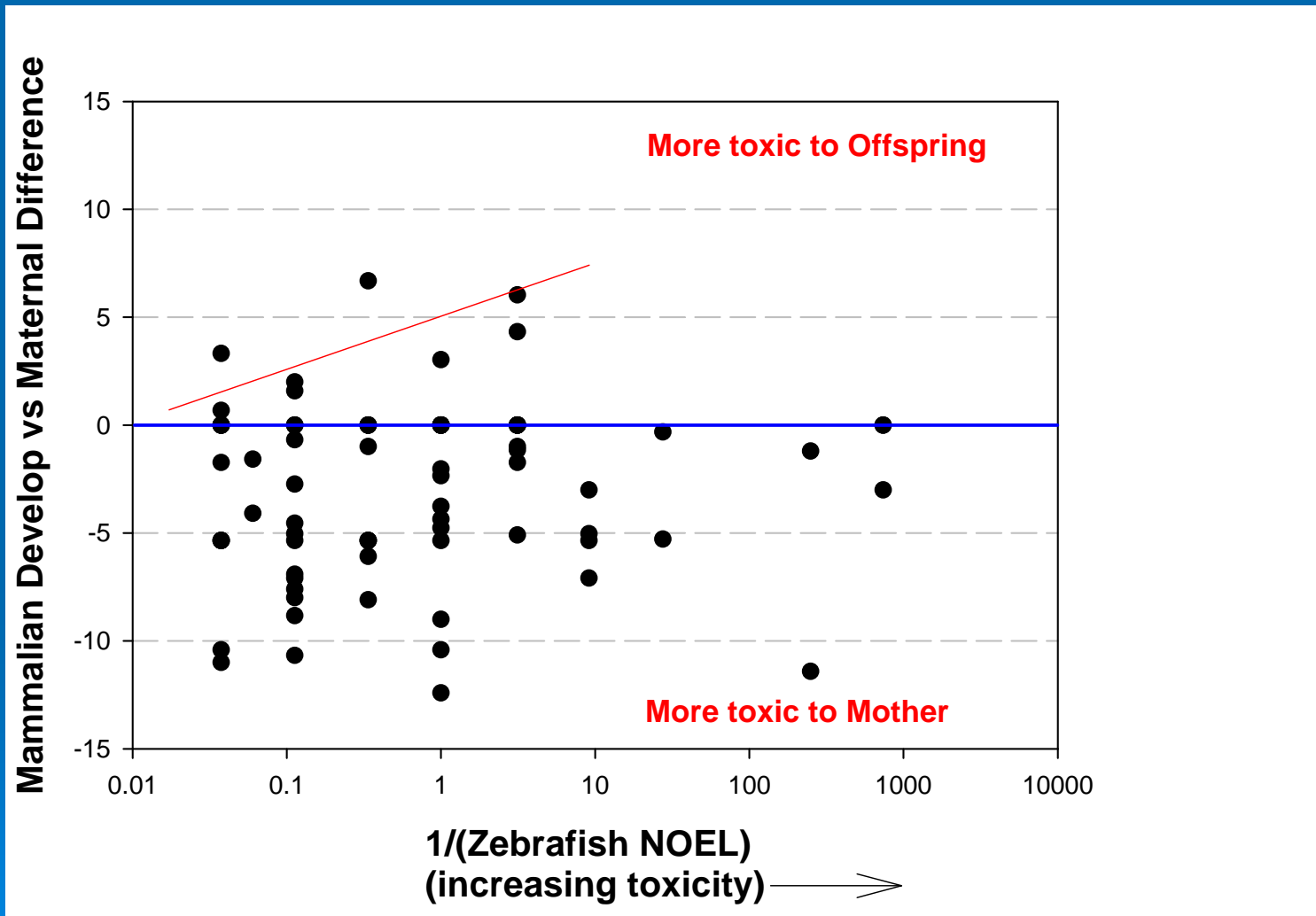
Zebrafish Toxicity Score vs Rat or Rabbit Developmental Toxicity (deleting all compounds with $\text{LogP} < 3$)





Single Dose vs Dose-Response







What Have We Learned so Far?

A group of biologically active compounds gives a high percentage of hits in a zebrafish developmental assay.

Toxicity is related to hydrophobicity of the test compound.

Relationship between maternal potency and zebrafish potency for highly toxic compound

Appears to be a discernable relationship between degree of developmental toxicity and zebrafish toxicity.



Future Directions

Compare zebrafish toxicity with NCCT ToxCast results on mechanisms of action.

Assess pattern of the teratogenic changes

Are there correlations with chemical class?

Are there correlations with mammalian toxicity?