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Zebrafish Biology and Suitability for Developmental Hazard Screening

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Zebrafish, The New Laboratory Rat: Strengths and Weaknesses

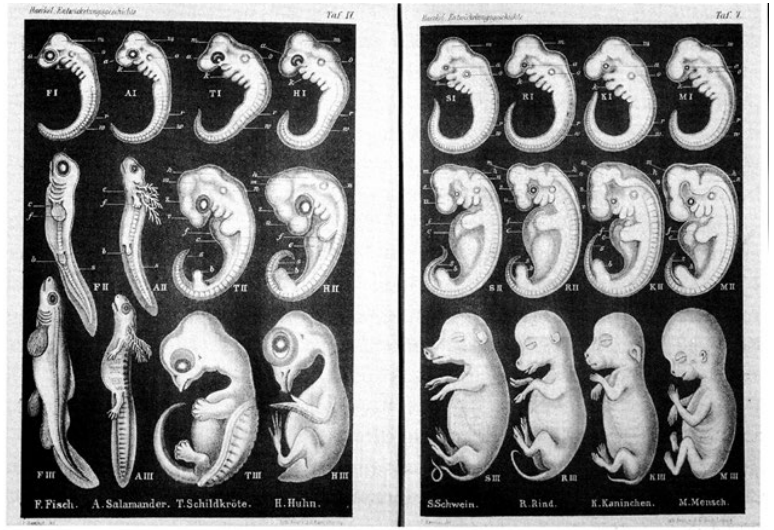
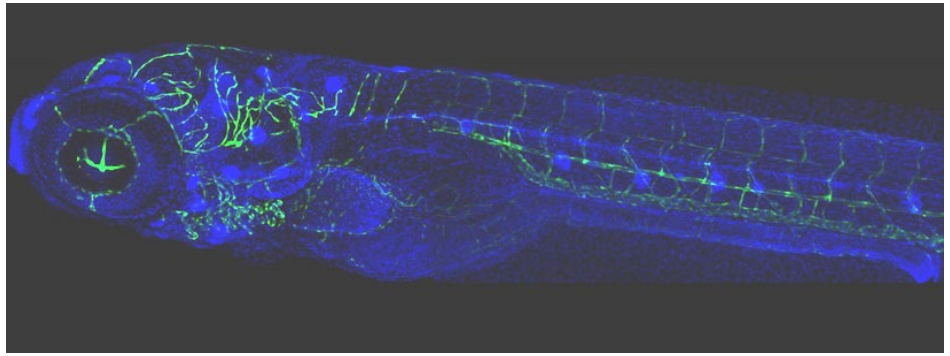


FIG. 41 Comparisons of embryos in three different stages of evolution. Ernst Haeckel, *The Evolution of Man: A Popular Exposition of the Principal Points of Human Ontogeny and Phylogeny* (1883).

- **All vertebrates develop using the same design.**
- **Therefore, we can use "lower" level vertebrates to screen for toxicity in humans.**

- Rapid development (organogenesis is complete within 4 days) (see movie above)
- Transparent embryo
- Developmental pathways are homologous with other vertebrates
- Easy to manipulate genome
- Translational model serving both human- and eco-toxicology
- Apical endpoints, including functional assessments
- Metabolic capability
- Thyroid Axis
- Stress Axis



Courtesy of J. Olin, A. Tennant, and K. Jensen

Concerns:

- difficult to assign mechanism without further tests
- knowing the internal dosage of chemical is not simple

Example of a Zebrafish Developmental Assay

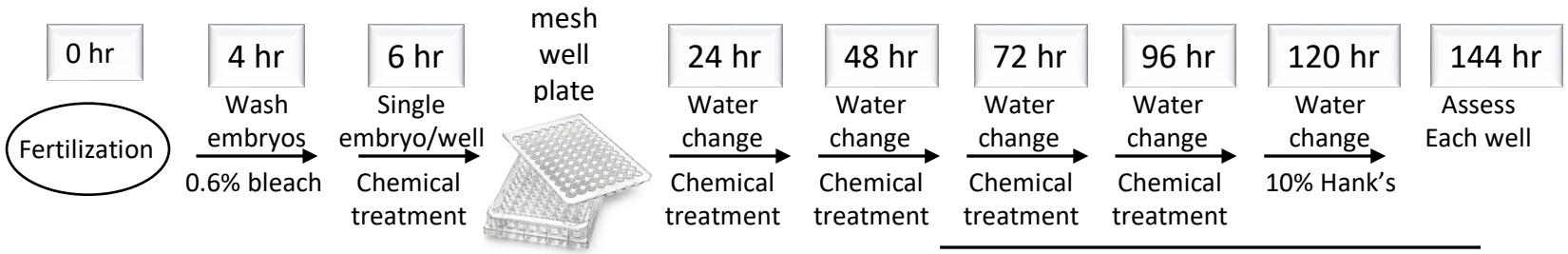
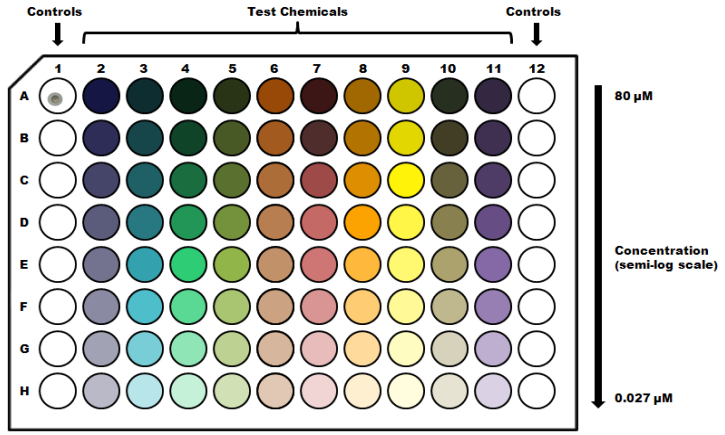
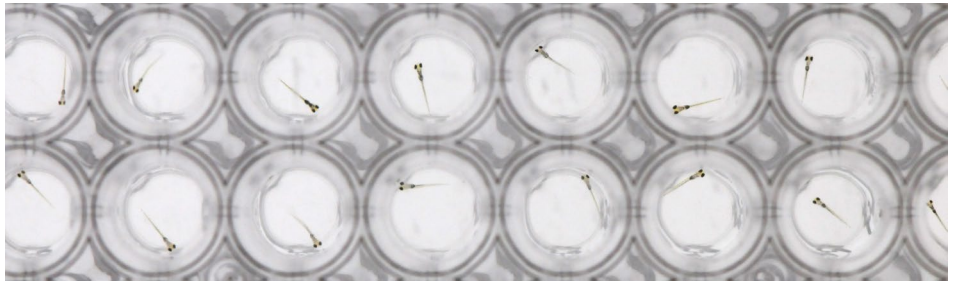


Plate Design

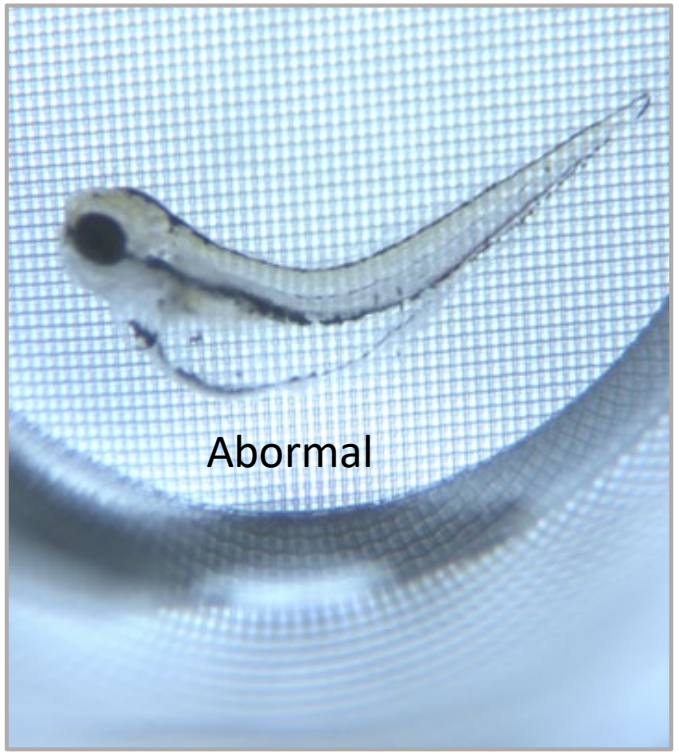


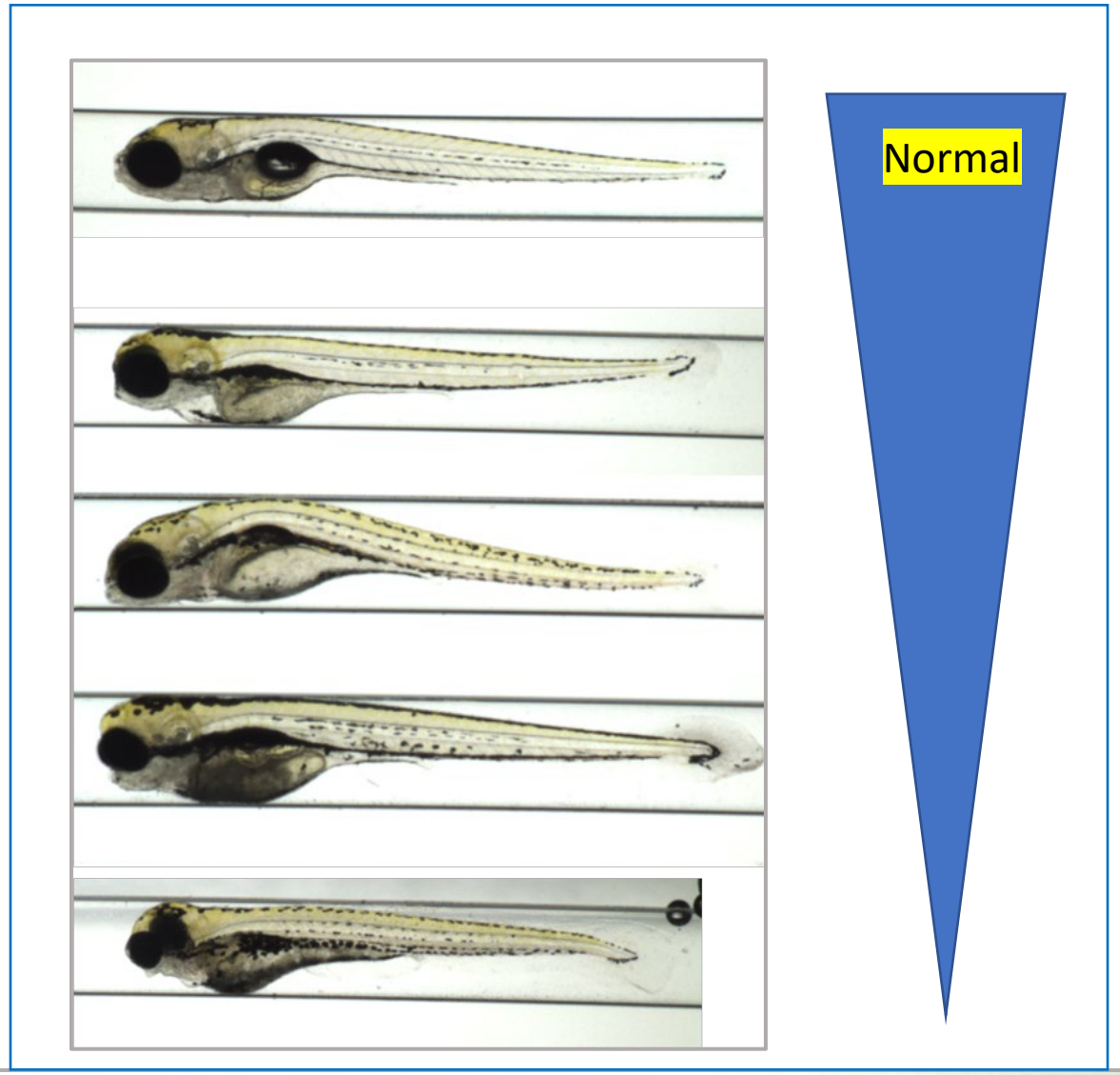
Is the larvae alive?

- No → Record as "Dead"
- Yes → Is the larva hatched?
  - No → Record as "Did not hatch"
  - Yes → *Perform basic malformation* assessment




Normal


Abormal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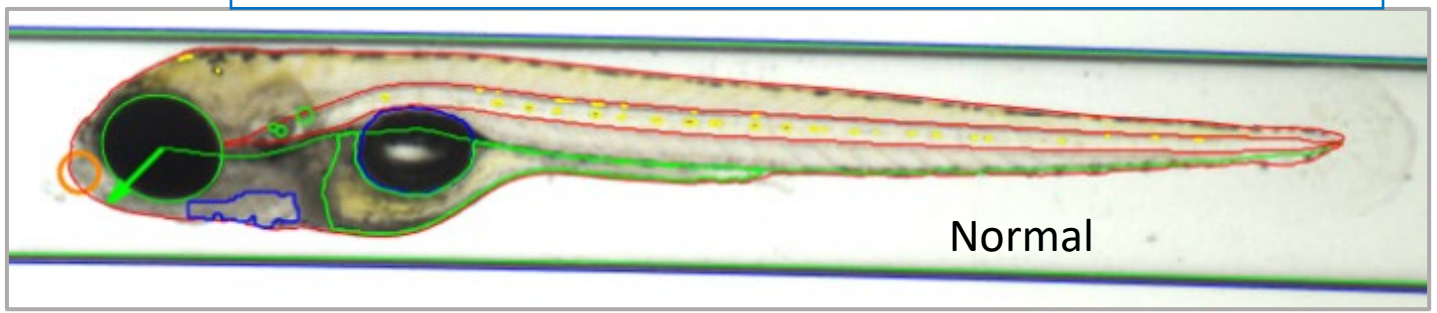
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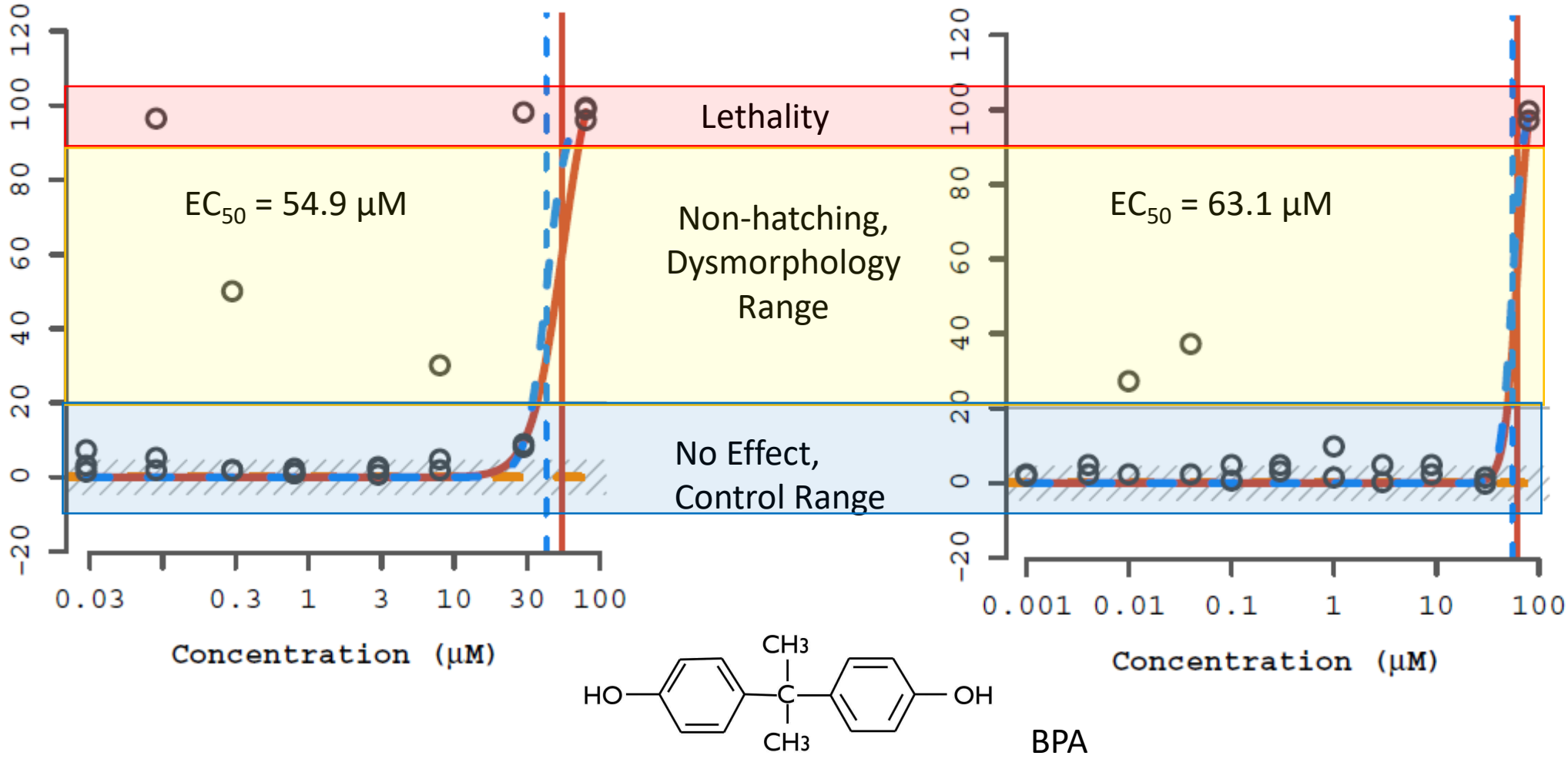
How Good are the Data?

- Consistency Within a Laboratory?
- Consistency over Time?
- Consistency Among Laboratories?

How Consistent are the Data Within a Laboratory?

Same chemical; different sources



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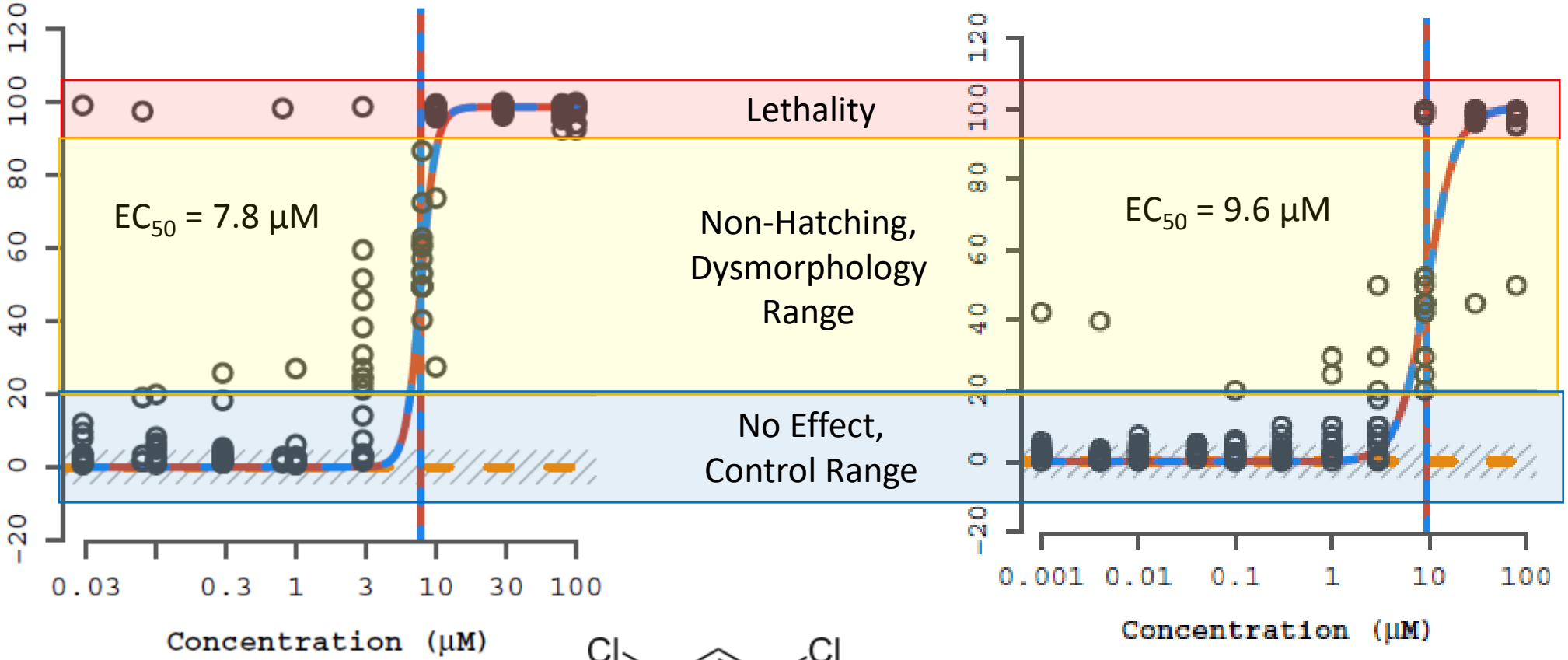
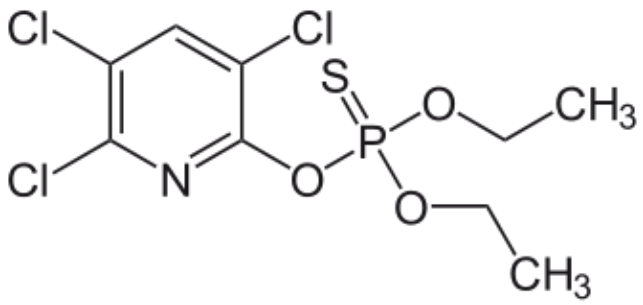




Chlorpyrifos

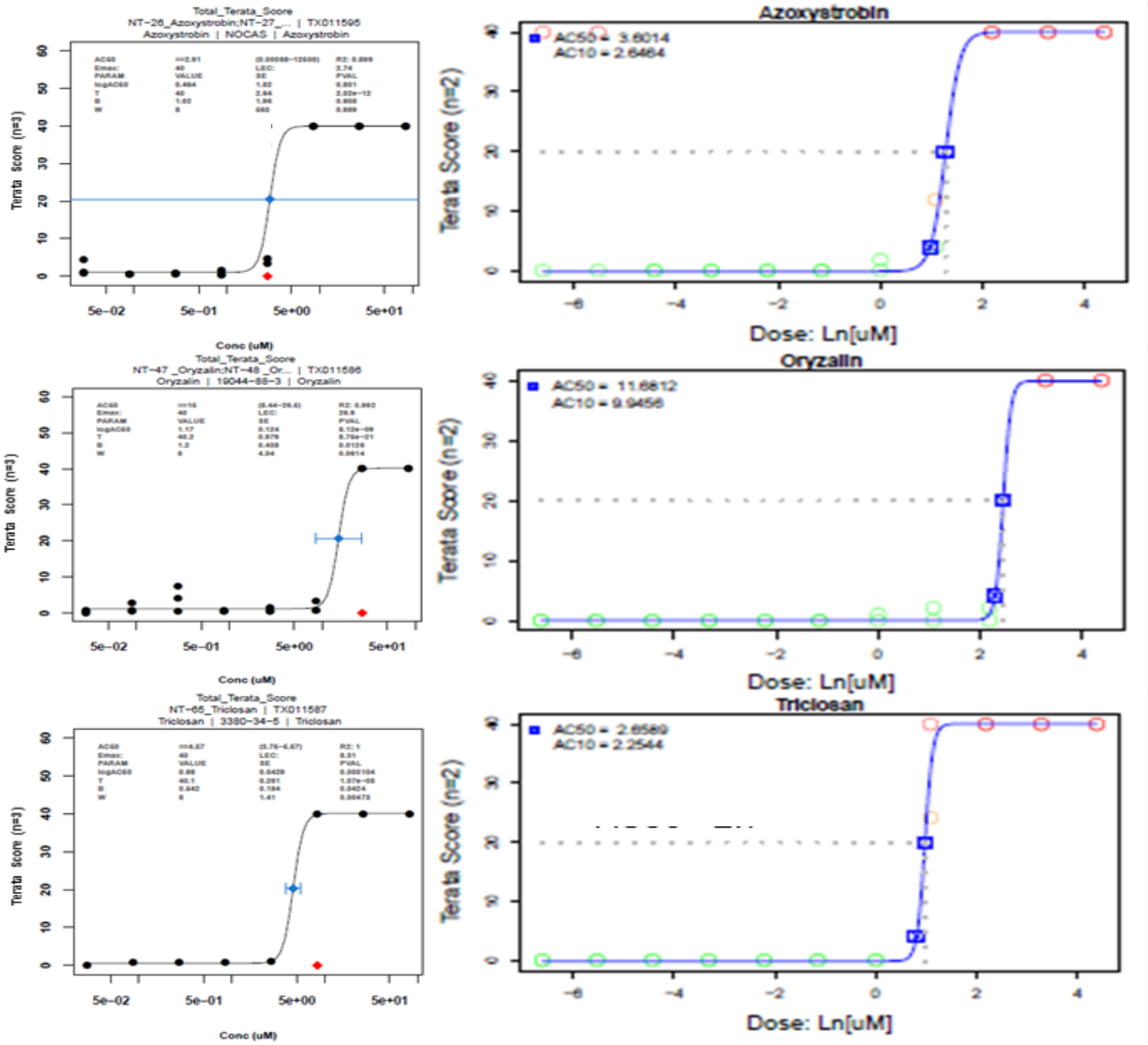
How Consistent are the Data Over Time?

Same Chemical, 3 Years Apart

Azoxystrobin
EC_{50} = 2.9 and 3.6 µM

Oryzalin
EC_{50} = 16.0 and 11.7 µM

Triclosan
EC_{50} = 4.6 and 2.7 µM

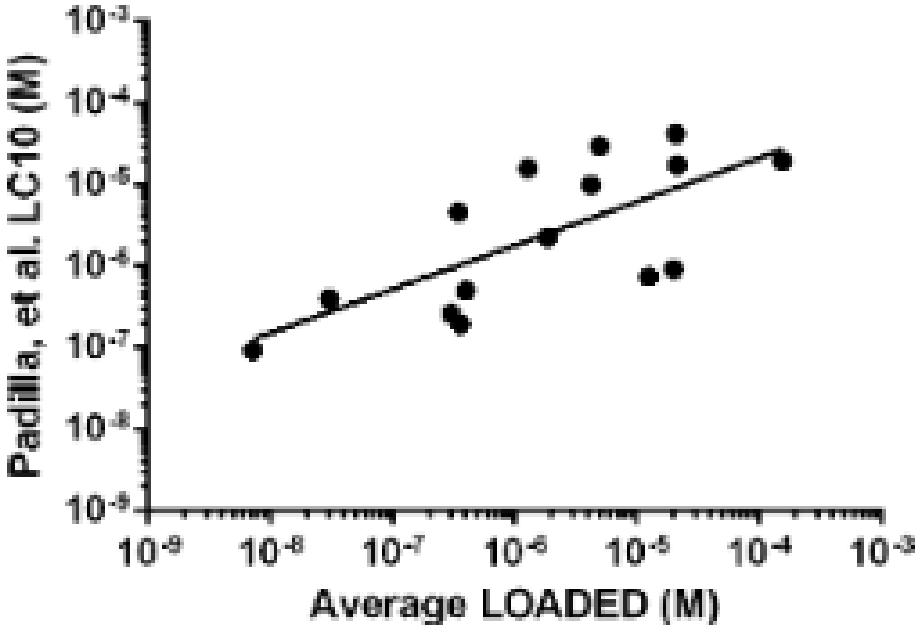


How Consistent are the Data Among Laboratories?

Comparing data from 116 studies to our data

16 chemicals in common:

- Benomyl
- Bisphenol A
- Carbaryl
- Chlorpyrifos
- Chlorpyrifos Oxon
- Cypermethrin
- Dibutyl Phthalate
- Dichlorvos
- Fipronil
- Malathion
- Methoxychlor
- Methyl Isothiocyanate
- Perfluorooctane sulfonate
- Triclosan



N.A. Ducharme *et al*, *Reproductive Toxicology* **41** (2013) 98-108

Concordance Between Organotin Toxicity Ranking for Mammalian and Zebrafish Developmental Toxicity

	Dibutyltin Dichloride	**Dimethyltin Dichloride**	**Monomethyltin Trichloride**	**Monobutyltin Trichloride**
***in vivo*, Mammalian Developmental Toxicity**	Dibutyltin Dichloride>	Dimethyltin Dichloride>	not toxic	no data
Zebrafish Developmental Toxicity	Dibutyltin Dichloride>	Dimethyltin Dichloride>	not toxic	not toxic

From van Woudenberg *et al*, *Reproductive Toxicology*, 2013; **41**:35-44

Concordance Between Mammalian and Zebrafish Developmental Toxicity

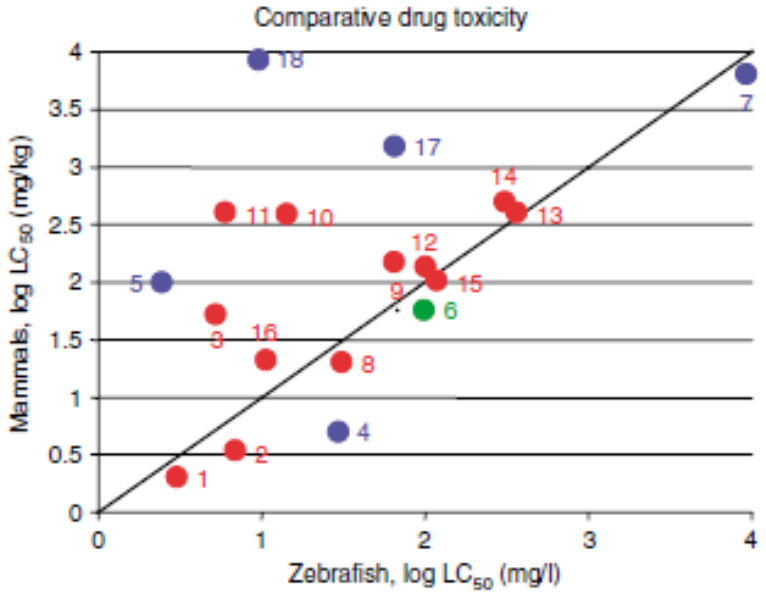


Figure 2 Comparative drug toxicity in zebrafish. Zebrafish embryos were exposed to 18 compounds, including (1) geladanamycin, (2) didemnin B, (3) merbarone, (4) Fujisawa peptide, (5) trithiophene, (6) 4-ipomeanol, (7) ethanol, (8) doxorubicin, (9) cyclosporine A, (10) naproxen, (11) ibuprofen, (12) aspirin, (13) dexamethasone, (14) acetaminophen, (15) caffeine, (16) tacrine, (17) dichloroacetic acid, and (18) polychlorinated biphenyls. Red, blue, or green symbols represent mouse, rat, or rabbit test animals, respectively. The diagonal line represents the perfect regression between the two sets of values. LC_{50} values for mammals were obtained from the NIH TOXNET database, NCI, and others (data courtesy of Patricia McGrath, Phylonix, Boston, MA).

Kari *et al*, *Clinical Pharmacology and Therapeutics*, 2007

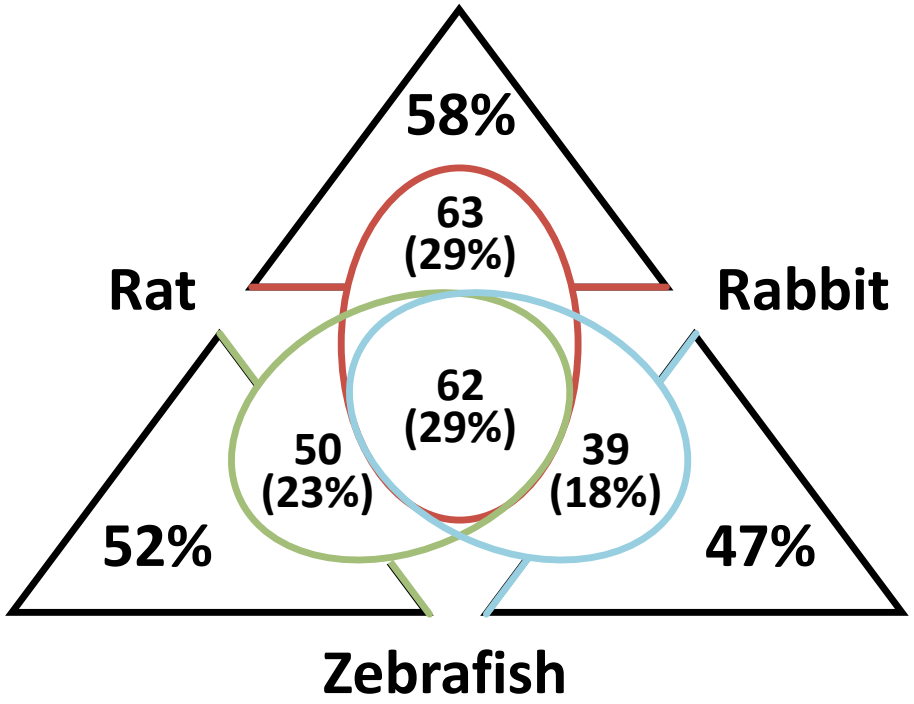
TABLE 1. Mammalian Concordance from Five Zebrafish Embryo Toxicity Studies

	Number of compounds	Mammalian concordance (%)
Brannen et al. (2010)	31	87
Hermsen et al. (2011)	14	64[a]
Hill et al. (2011)	85	89
Selderslaghs et al. (2009)	6	100[a]
Padilla et al. (submitted)	271	55[a]

Four of the most recent zebrafish embryo toxicity studies were summarized in terms of number of compounds in the study with available mammalian data and concordance (both positive and negative concordance).
[a]Indicates values not mentioned in the study, but calculated from the data.

Sipes *et al*, *Birth Defects Research (Part C)*, 2011

Concordance Between Mammalian and Zebrafish Developmental Toxicity



Sipes *et al*, *Birth Defects Research (Part C),* 2011

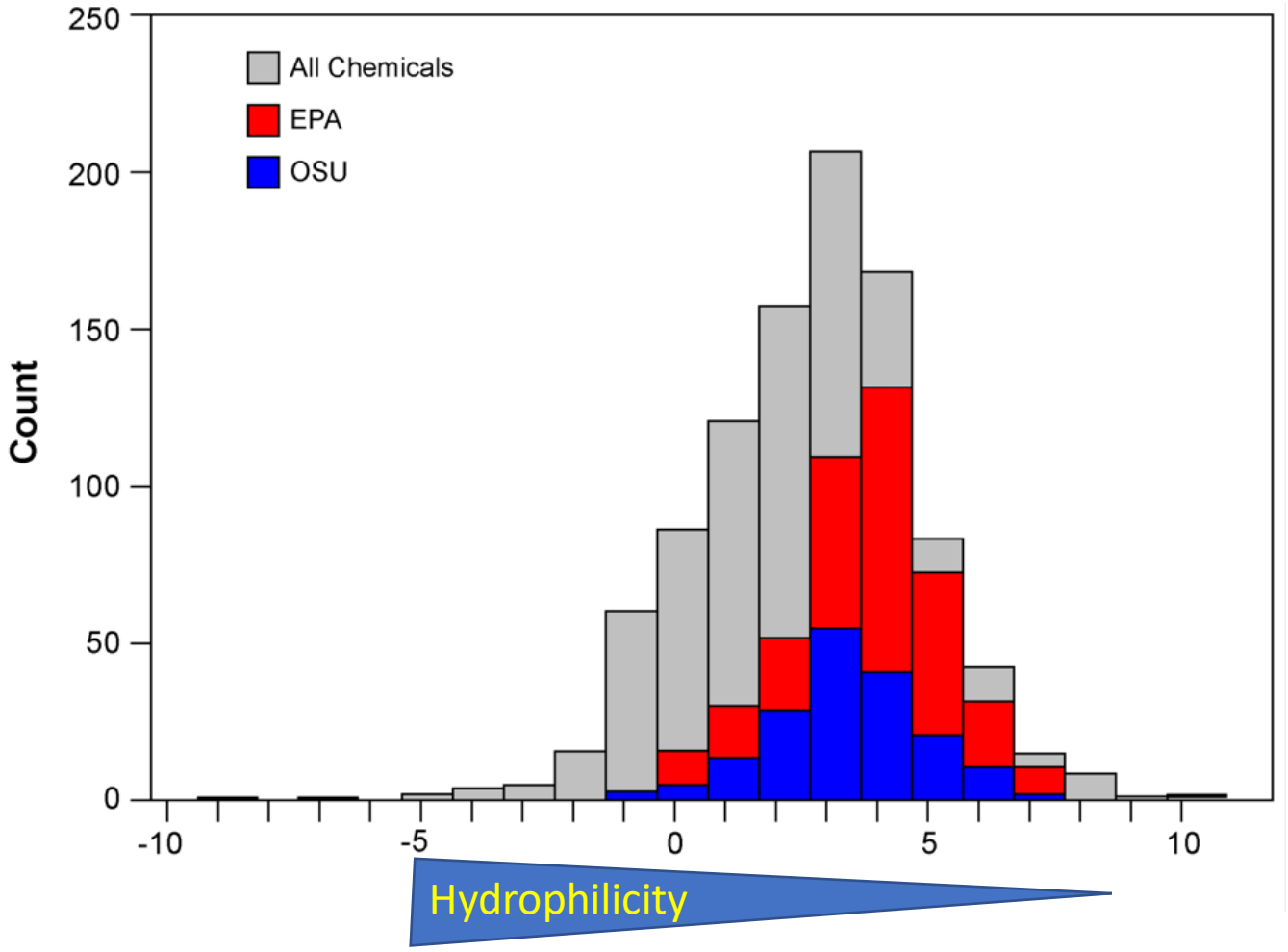
Observed mammalian and zebrafish concordance and endpoints in ToxCast.

A total of 214 chemicals were tested in the rat, rabbit, and zebrafish species in ToxCast. **The number of chemicals that were concordant (positively and negatively) between rat and rabbit and not zebrafish are shown in the red oval, rabbit and zebrafish and not rat are shown in the blue oval, and zebrafish and rat and not rabbit are shown in the green oval.** Data for mammalian *in vivo* endpoints came from prenatal guideline studies listed in ToxRefDB and zebrafish endpoints came from U.S. EPA, Office of Research and Development, National Health and Environmental Effects and Research Laboratory studies.

RISK = HAZARD x EXPOSURE

- Most of what I've talked about so far is HAZARD
- Exposure Considerations
  - Routes for chemical exposure in zebrafish larvae
    - Dermally
    - Partitioning into the yolk and then absorbed
    - After about 3 to 4 days, they can be exposed orally
    - Injection (not practical in a screening context)
    - Not through the gills; gills are not functioning until about 14 days

Physicochemical Characteristics of the Chemical are Related to Exposure



LogP (Octanol/Water Partition Coefficient)

Working Range for Zebrafish Larval Assay appears to be from LogP of -1 to 8.

Other Characteristics that Affect “Dose”

- Presence of Chemical in the Surrounding Solution
- Hepatic Activation
- Hepatic Deactivation
- Age at Time of Exposue
- Duration of Exposure
  - Some chemicals achieve steady state within minutes while others may take days.
- Enzyme Induction
- Even though it is complicated, accurate models are being developed.
  - Siméon *et al*, 2020. PBPK Model for zebrafish embryos/larvae.
  - Klüver *et al*, 2016. General QSAR Model for zebrafish embryos/larvae.

Preliminary Questions Posed by the Committee:

- In setting up an experiment using zebrafish, how many adult fish of each sex would you typically start with as a source of ova and sperm?

- Is potential parental contribution considered in study design or data analysis? For example: Is potential parental contribution considered in test-group assignment, or are all embryos considered equivalent prior to distribution among test groups?

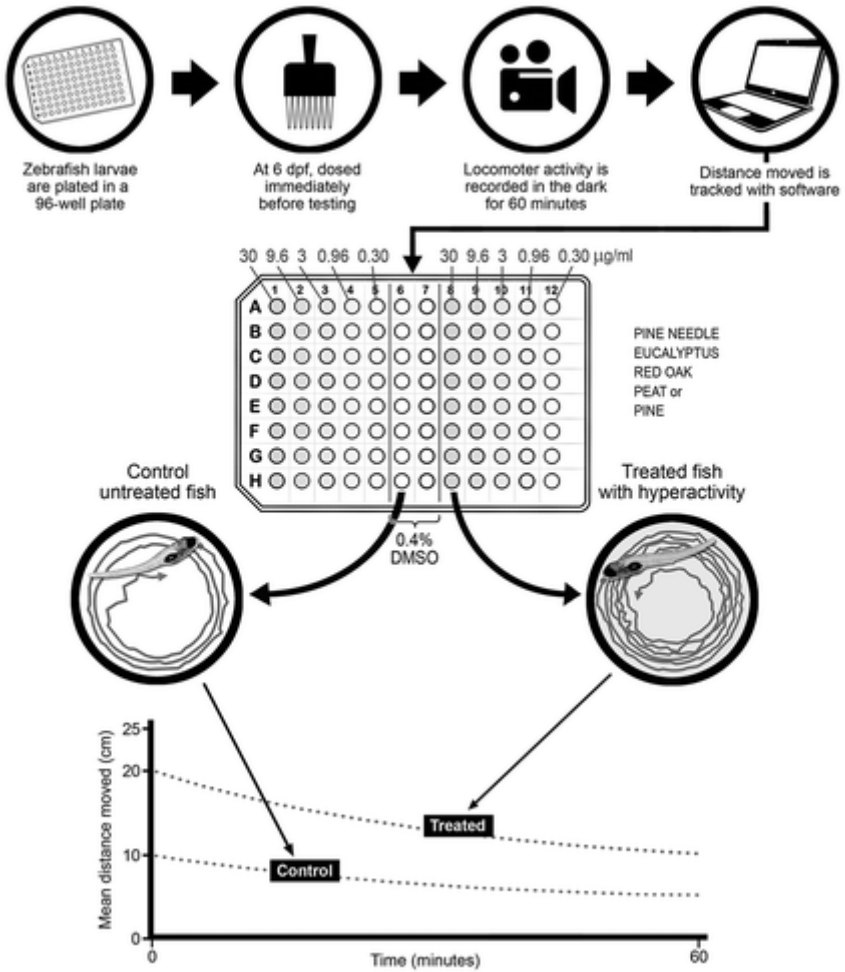
- In analyzing results for offspring, what are statistical considerations, if any, for parental identity (as is done for rodents or lagomorphs)?

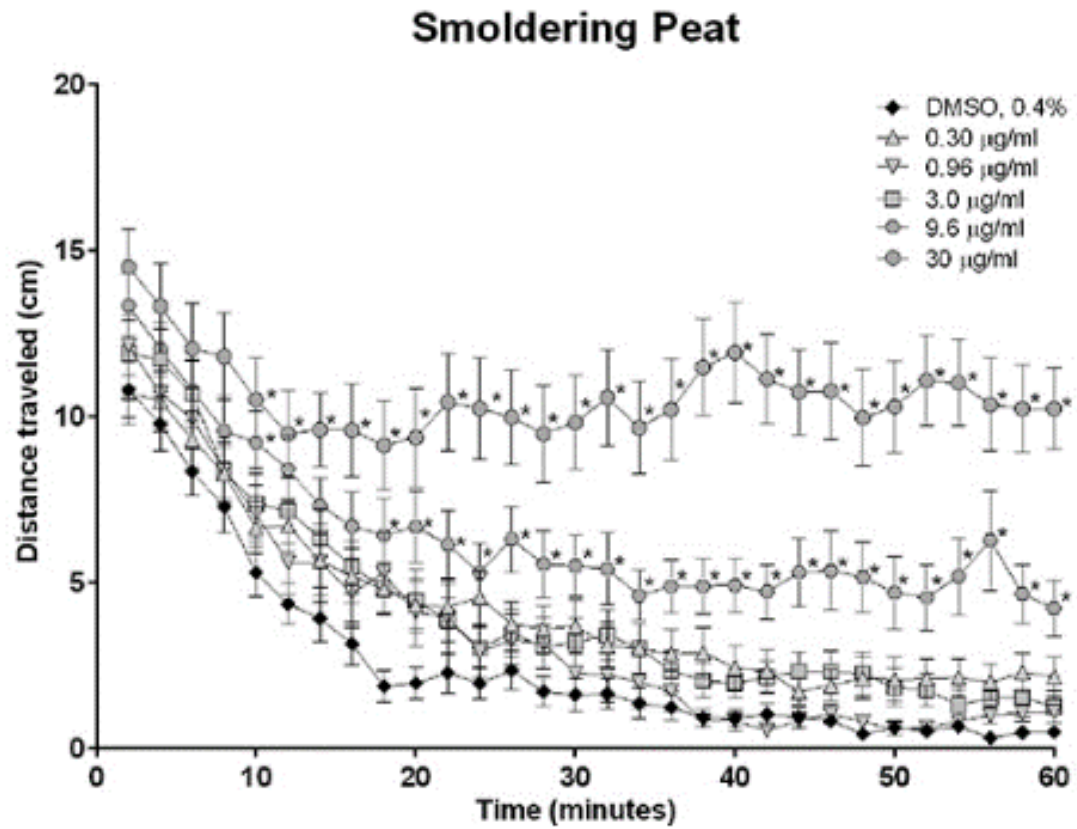
- Studies included in OEHHA's recent hazard identification documents provide examples of similar biological systems or pathways being affected in both zebrafish and mammals by a given chemical, but with different directionality of response or with a different downstream outcome.

- How do we consider the differences as well as similarities between species in evaluations?

Questions?
Comments?

Sequencing of the entire genetic make-up of the zebrafish has revealed that **70 per cent of protein-coding human genes are related to genes found in the zebrafish** and that 84 per cent of genes known to be associated with human disease have a zebrafish counterpart. Howe *et al*, *Nature*, 2013





Martin et al, 2021, J. Tox. Environ. Health A, vol 84.