SEAZIT Webinar Series:
Using Informatics to Improve Data Analysis of Chemical Screening Assays Conducted in Zebrafish

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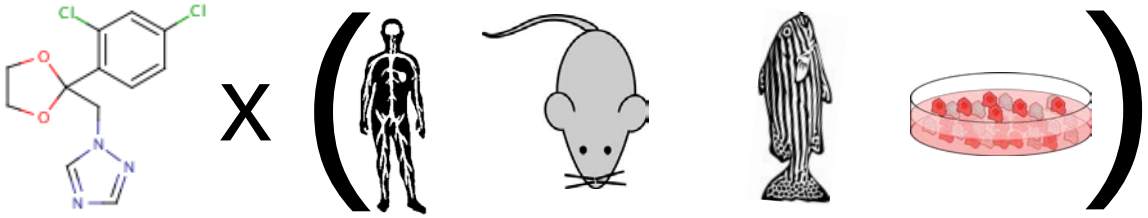
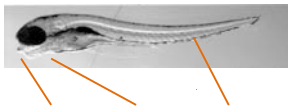
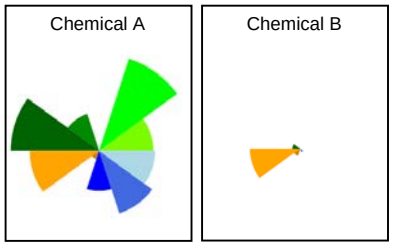


Behavior

Development



Integrated Health Effects



Introduction to Zebrafish Screening: Survey of variability in design, analysis, and applications

Scope of this introductory survey

- Limited to medium or high-throughput screening (HTS) of chemicals
- Covers embryonic developmental period, with a focus on morphological phenotypes (Note that behavior is often measured concurrently)
- Not discussing growing body of literature on "Zebrafish as a model for *<insert disease/phenotype here>*" nor "Zebrafish screening reveals role of *<insert gene name here>*"

Key elements affecting harmonization | Informatics considerations

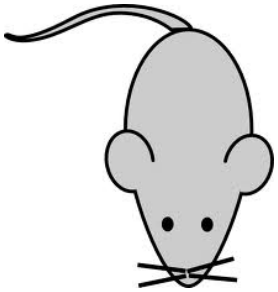
- Environment: Chemical exposure schemes, concentration spacing, and dechorionation
- Phenotype: What is (are) the assay endpoint(s) of interest?
- Resolution: Pooled vs. individual zebrafish wells and time points for evaluation(s)
- Reproducibility: Chemical delivery, automation, throughput, and historical data

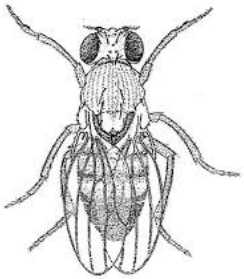
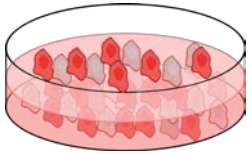
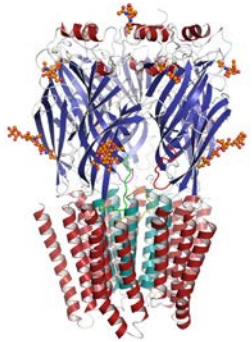
Conclusions and Next Steps

- What might harmonization require, and would it be worth the effort?
- How can informatics help?
- Behavioral analysis (preview)

What options are available to assay the hazard of environmental chemicals?

Humans | Mammals | Vertebrates | Invertebrates | Cells | Biochemicals

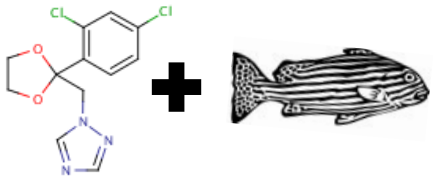




Each offers pros/cons in terms of: throughput, cost, human relevance, specificity (targets), complexity (development, systemic interactions).

Toxicological endpoints such as abnormal behavior or development are difficult to measure using purely *in vitro* systems.

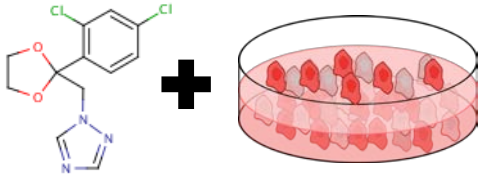
High-throughput studies using embryonic zebrafish complement targeted approaches and provide systematic data that can be used for integrated analysis across *in silico, in vitro,* and multi-scale *in vivo* endpoints.

Zebrafish HTS generates data complementary to *in vitro* systems

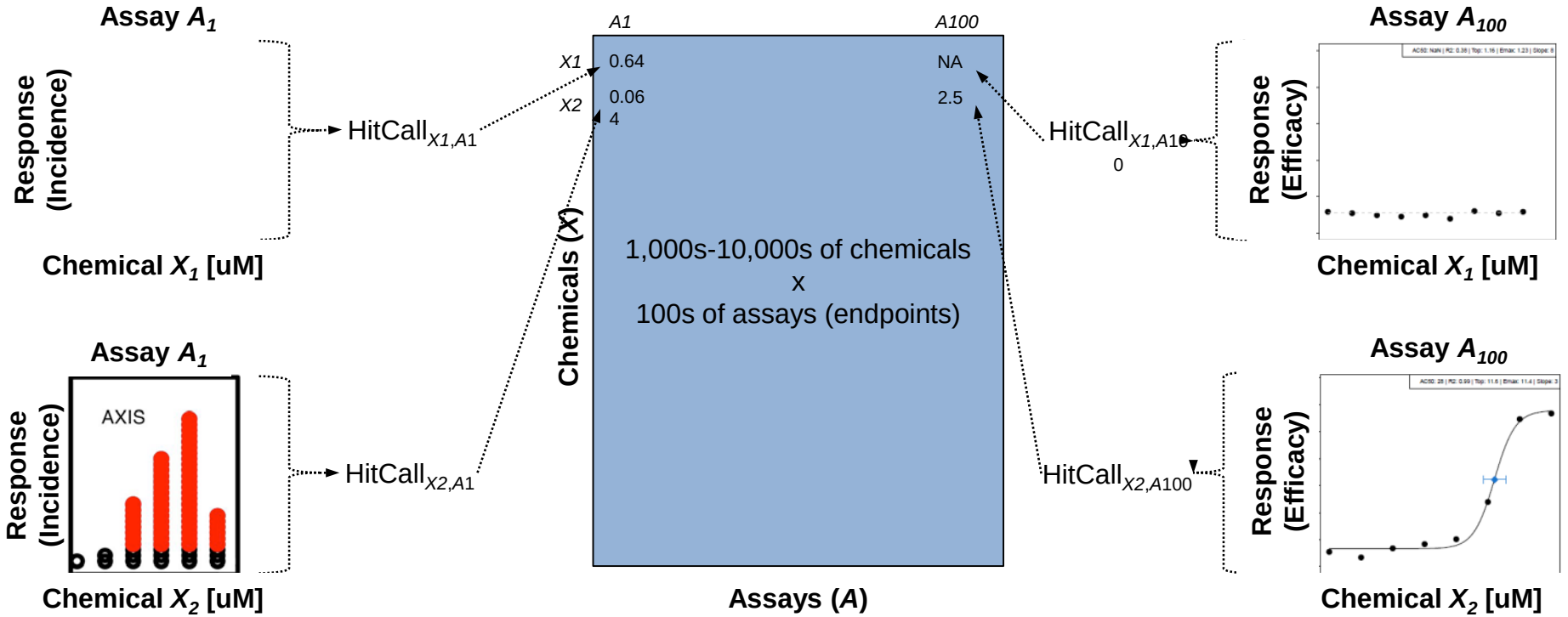


zebrafish assay systems

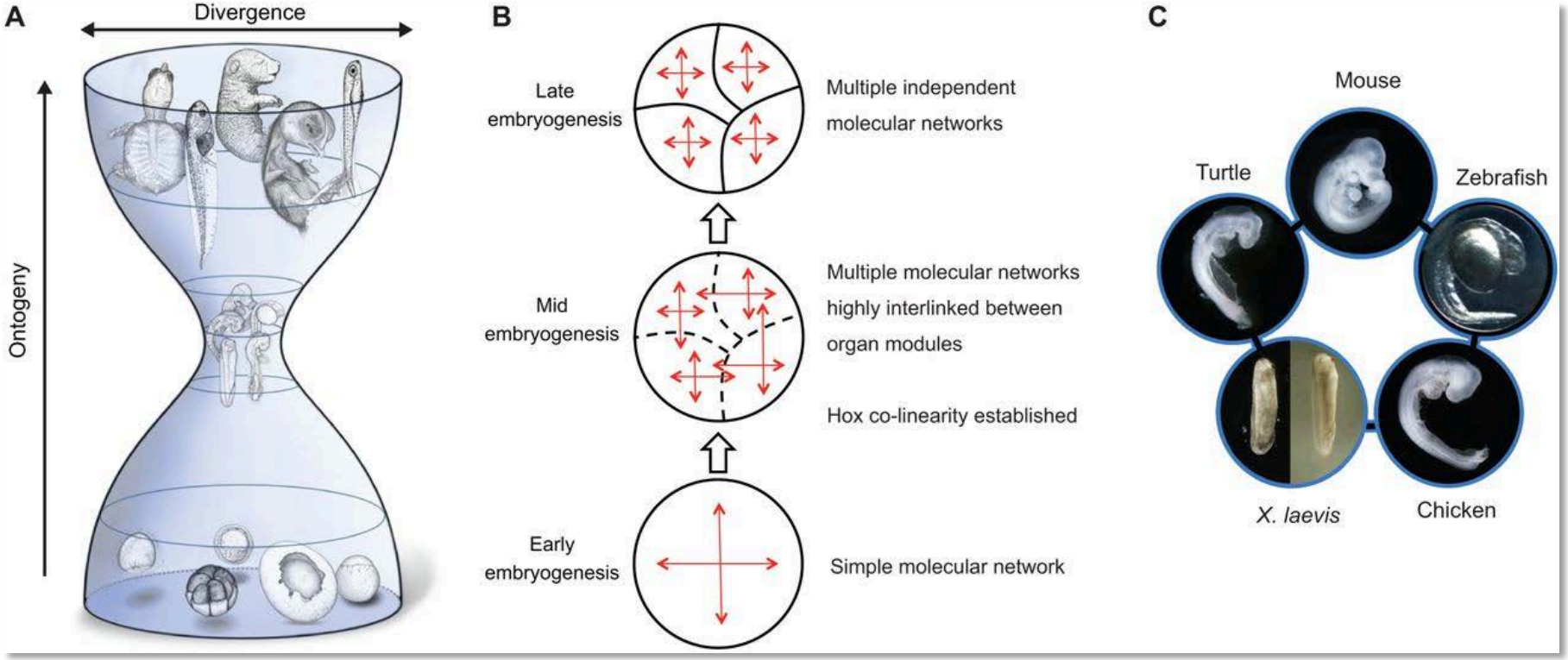
Chemicals (*X*) are tested in concentration-response mode in all assays (*A*) to generate massive Chemical-Assay data.



***In vitro* assay systems**

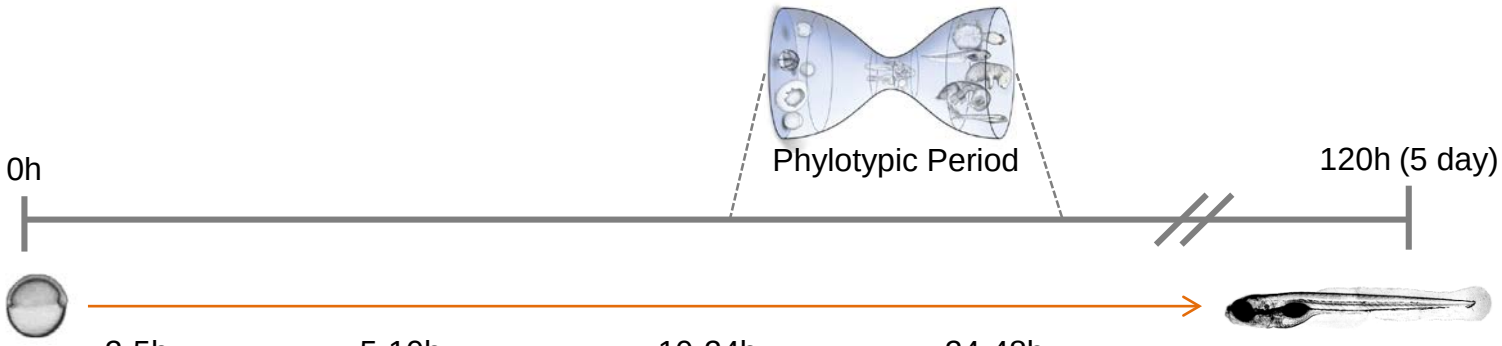


Developmental processes are conserved during the vertebrate “phylotypic” period



[Irie et al. (2011) *Nature Communications*; Irie et al. (2014) *Development*]

Zebrafish HTS experiments cover time periods during which key developmental processes take place



	~2-5h	~5-10h	~10-24h	~24-48h	
Developmental landmarks	• Yolk syncytial layer forms	• Axis and neural plate • Brain and notochord rudiments • Tail bud prominent • Early polster	• First somite • Polster prominent • Optic vesicle • Kupffer's vesicle • Neural keel • Pronephros forms • Otic vesicle • Brain Neuromeres	• Early pigmentation • Heartbeat • Early touch reflex • Retina pigmented • Early motility • Tail pigmentation • Rudiments of pectoral fins	• 3.9mm total body length • 6 teeth
Morphologically Observable Phenotypes	• Yolk Sac	• Body Axis • Notochord & Bent Tail	• Somite • Eye Defect • Otic Vesicle • Mortality	• Pigment • Touch Response • Pectoral Fin • Caudal Fin • Mortality • Circulation • Truncated Body • Brain	• Snout • Jaw • Mortality • Swim Bladder • Pericardial Edema

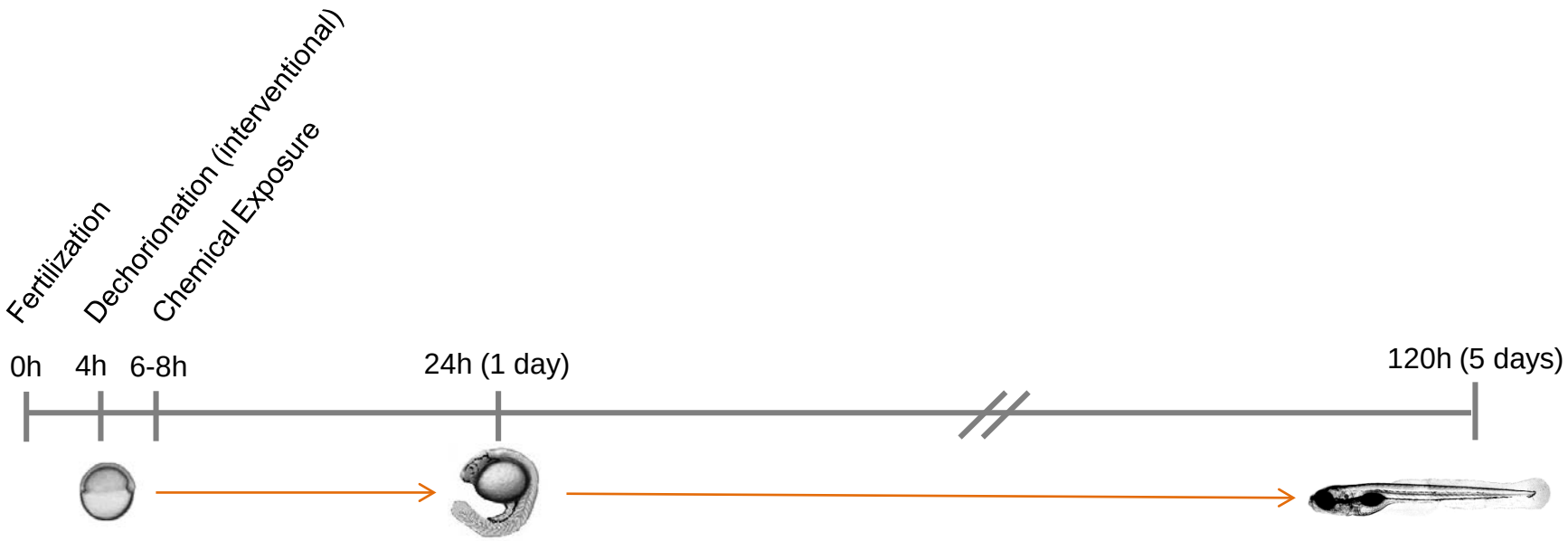
[Zhang et al. (2016) *Toxicology and Applied Pharmacology*]

Key elements affecting harmonization

Environment:

- Chemical exposure schemes: static vs regular renewal
- Concentration spacing: broad (spanning several orders of magnitude) vs narrow
- Dechorionation: early automated vs later natural hatching

Example design: Early dechorionation followed by early (static) chemical exposure

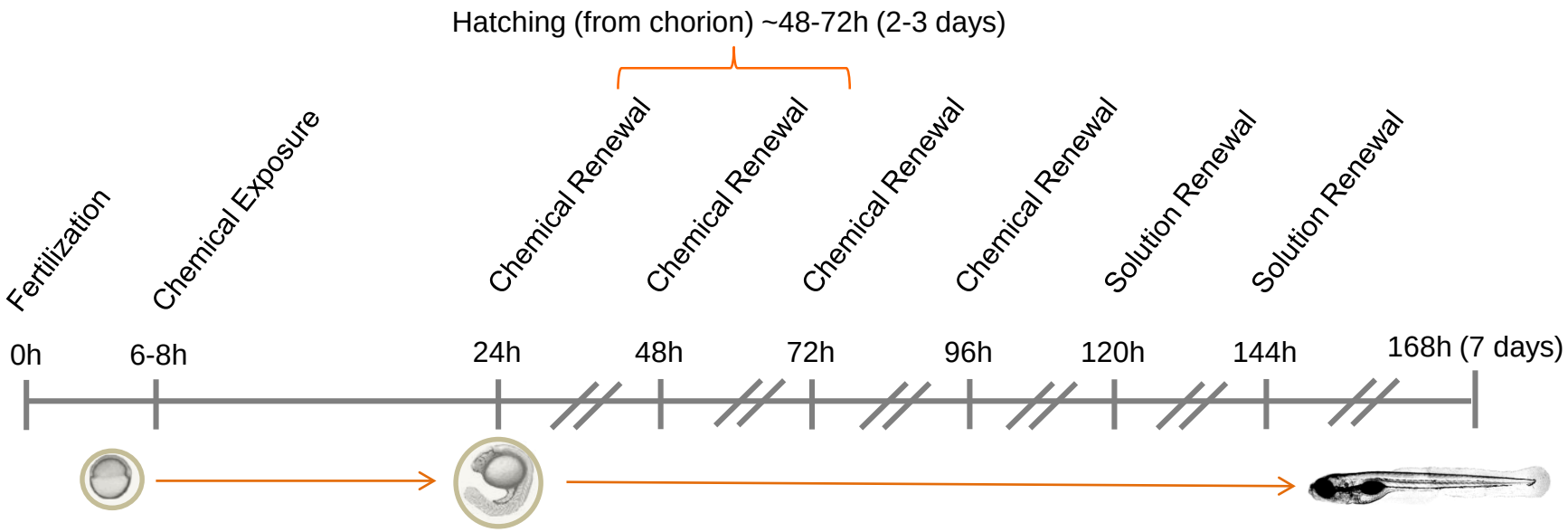


Key elements affecting harmonization

Environment:

- Chemical exposure schemes: static vs regular renewal
- Concentration spacing: broad (spanning several orders of magnitude) vs narrow
- Dechorionation: early automated vs later natural hatching

Example design: Repeated exposure (renewal); Chorion remains until hatching

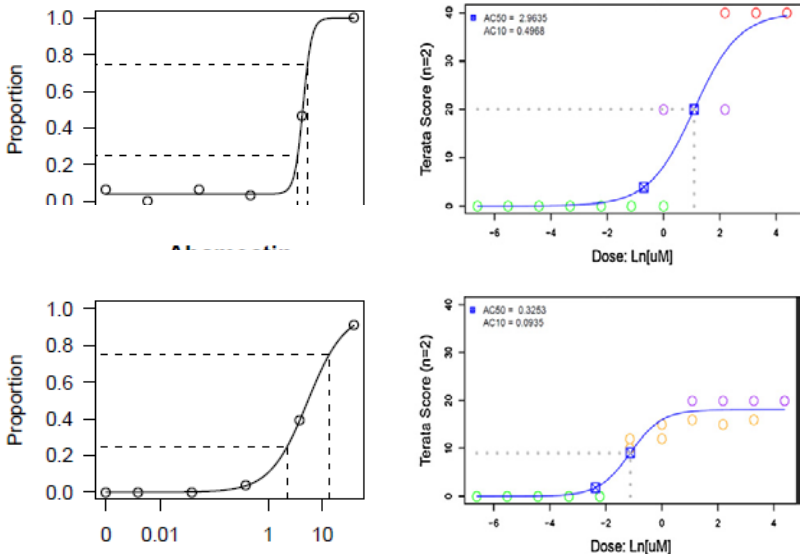


Key elements affecting harmonization | Informatics

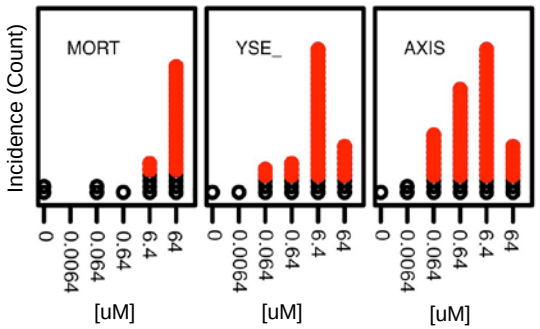
Environment:

Concentration spacing & number of replicates affects analysis methods

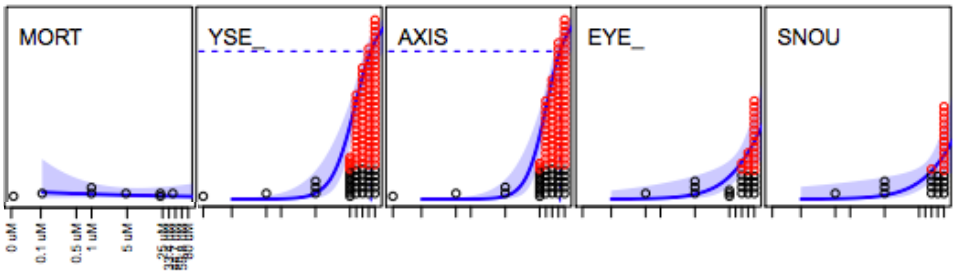
Response range (e.g. proportion affected vs score)



Mortality “censoring”

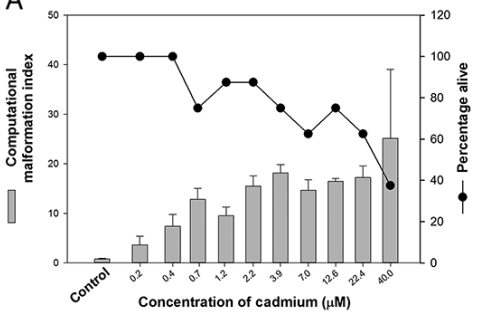


Concentration range/spacing affects fit-based (curve) methods



Non-monotonicitv

A



[Padilla et al. (2011) *Reproductive Toxicology*]
[Truong et al. (2014) *Toxicological Sciences*]
[Reif et al. (2015) *Archives of Toxicology*]
[Deal et al. (2016) *Applied Toxicology*]

Key elements affecting harmonization

Phenotype:

What is (are) the assay endpoint(s) of interest?

Whether captured via automated systems or detailed visual inspection, most endpoints collected cover some combination of the following phenotypes:

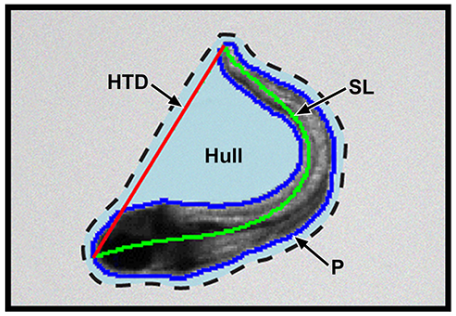
- Size (length, width, or area)
- Axis (curvature of body axis)
- Craniofacial (defects in eye, snout, or jaw)
- Edema (swollen pericardial tissue or yolk sac)
- Trunk (abnormal length)
- Pigment (abnormal coloration)
- Mortality

The unit of analysis can be specific endpoints → <u>What</u> did this chemical affect?

OR

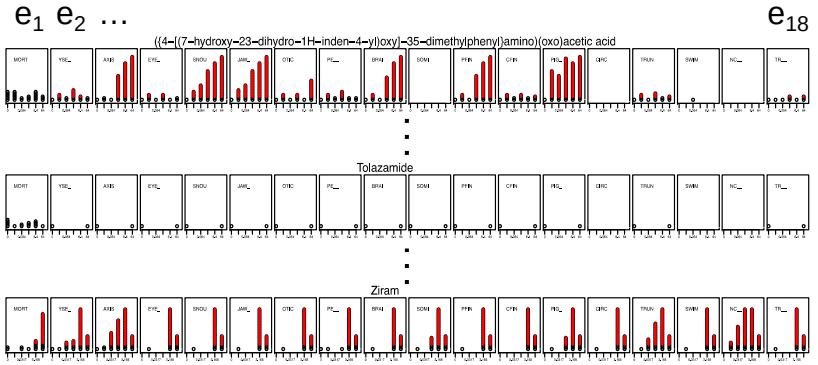
Recombinations of endpoints into summary scores → <u>Did</u> this chemical have an effect?

A



B

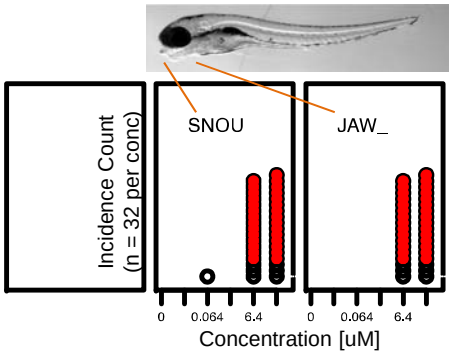
Parameter	Description
Area	Area within the mask drawn around the fish, calculated as pixel count or micrometers
Perimeter–area (P)	A ratio of the outer perimeter of the fish to the area
SL	A line drawn approximately down the middle of the fish from the tip of the larvae's head to the tip of its tail
Width	The maximum distance perpendicular to the Spine Length
Length–width ratio	A ratio of SL to width
HTD	A direct line drawn from the tip of the larvae's head to the tip of the tail
Straightness	A ratio of HTD to SL
Convexity	A ratio of the fish area to the area of the hull



[Deal et al. (2016) *Applied Toxicology;* Truong et al. (2014) *Toxicological Sciences*]

Key elements affecting harmonization | Informatics

Developmental Assessment



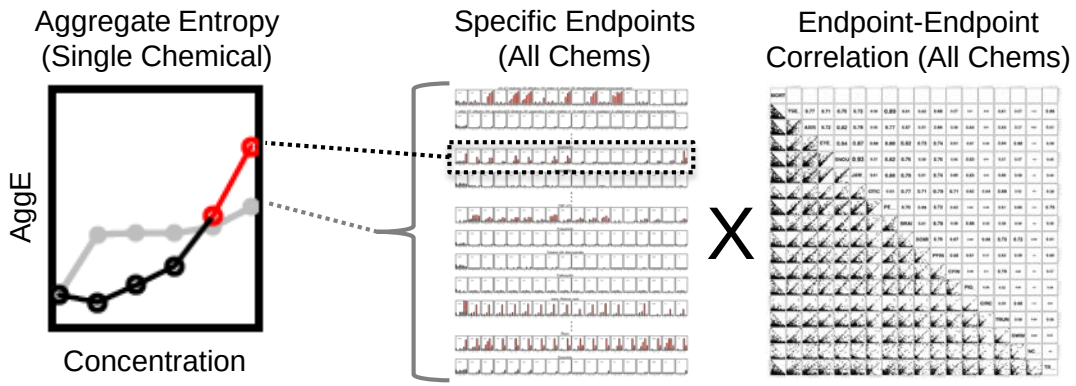
The magnitude of the HTS data can be used to explore relationships amongst endpoints:

- How should these patterns be utilized to summarize effects (specific endpoints versus summary “badness” scores)?
- How can these patterns inform targeted follow-up hypotheses?
- What do these patterns say about vertebrate development?
- Can we use this knowledge to integrate data from multiple labs/sources?

Correlation structure across all morphological data for 1,060 chemicals

[Zhang et al. (2016) *Reproductive Toxicology*]

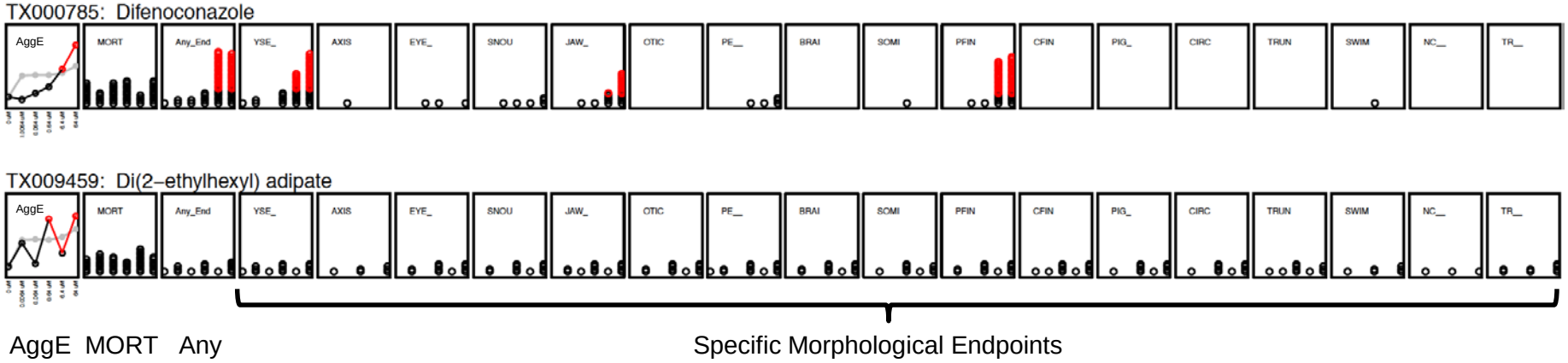
Key elements affecting harmonization | Informatics



AggE provides a metric for assessing "aggregate" activity over (sets of) specific morphological endpoints that leverages all data to account for underlying correlation structure for individual dose-response estimates.

All data (for all chemicals) are used to set the empirical significance **threshold**. Data for an **individual chemical** are compared to this threshold to determine **significant AggE**.

Example response vectors for all endpoints are presented for two chemicals. A summation "Any" has been added to represent a positive response in any specific endpoint. Responses turn red when the stack (incidence count) at a given concentration surpasses the statistical significance threshold. AggE is plotted as connected black points, turning red when it surpasses the empirical significance threshold line (grey).

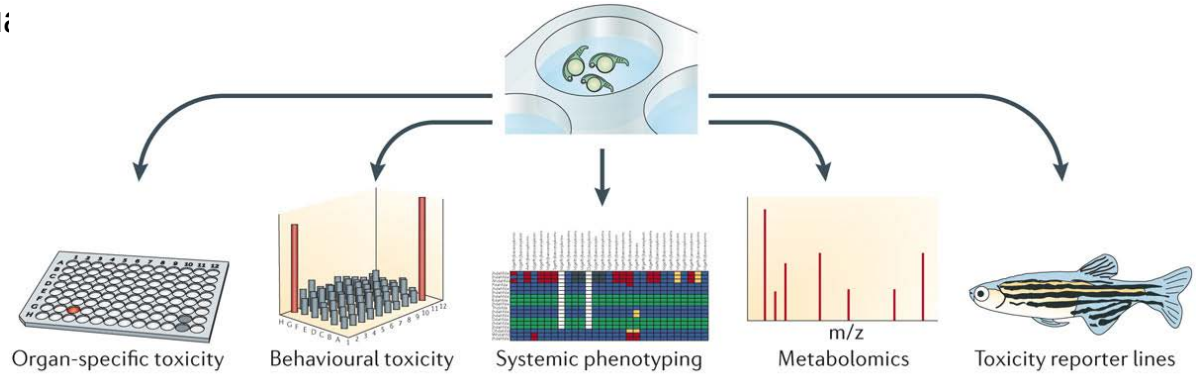


[Zhang et al. (2016) *Reproductive Toxicology*]

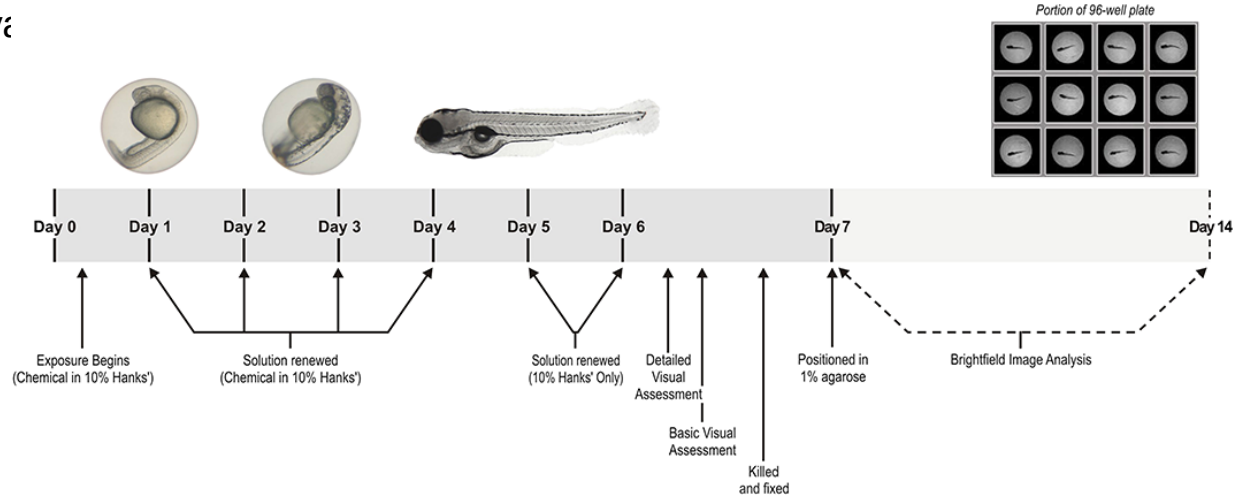
Key elements affecting harmonization

Resolution:

Pooled vs. individua



Time points for eva



[MacRae et al. (2015) *Nature Reviews Drug Discovery;* Deal et al. (2016) *Applied Toxicology*]

Key elements affecting harmonization | Informatics

Resolution:

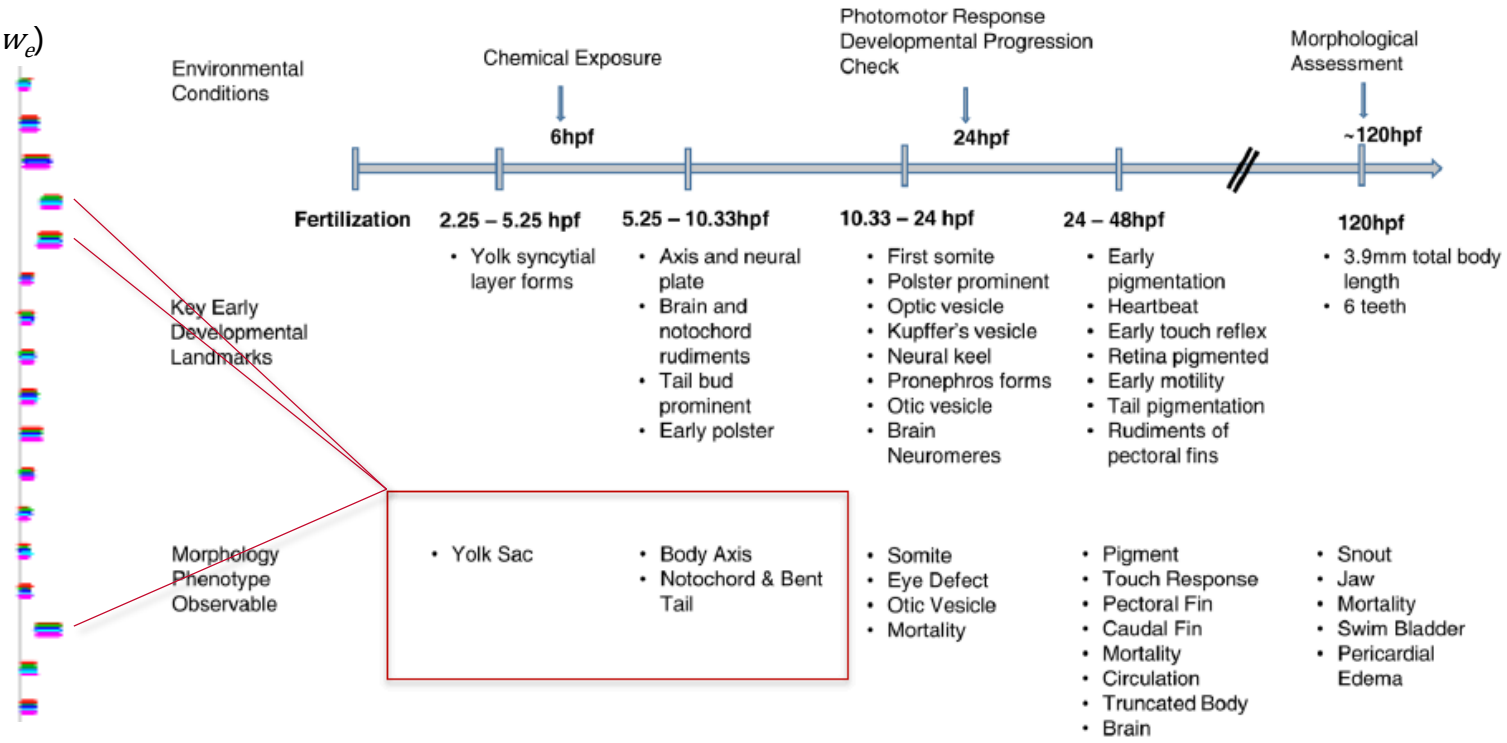
- Pooled vs. individual zebrafish wells
- Time points for evaluation(s)

If scoring is performed at the individual level for multiple endpoints (phenotypes), we can use Bayesian methods to statistically optimize the weighting of relevant endpoints.

These empirical weights (w_e) can recapitulate developmental cascades – even when morphological assessment is only performed at the end (5 dpf) of an experiment.

Endpoint weights (w_e)

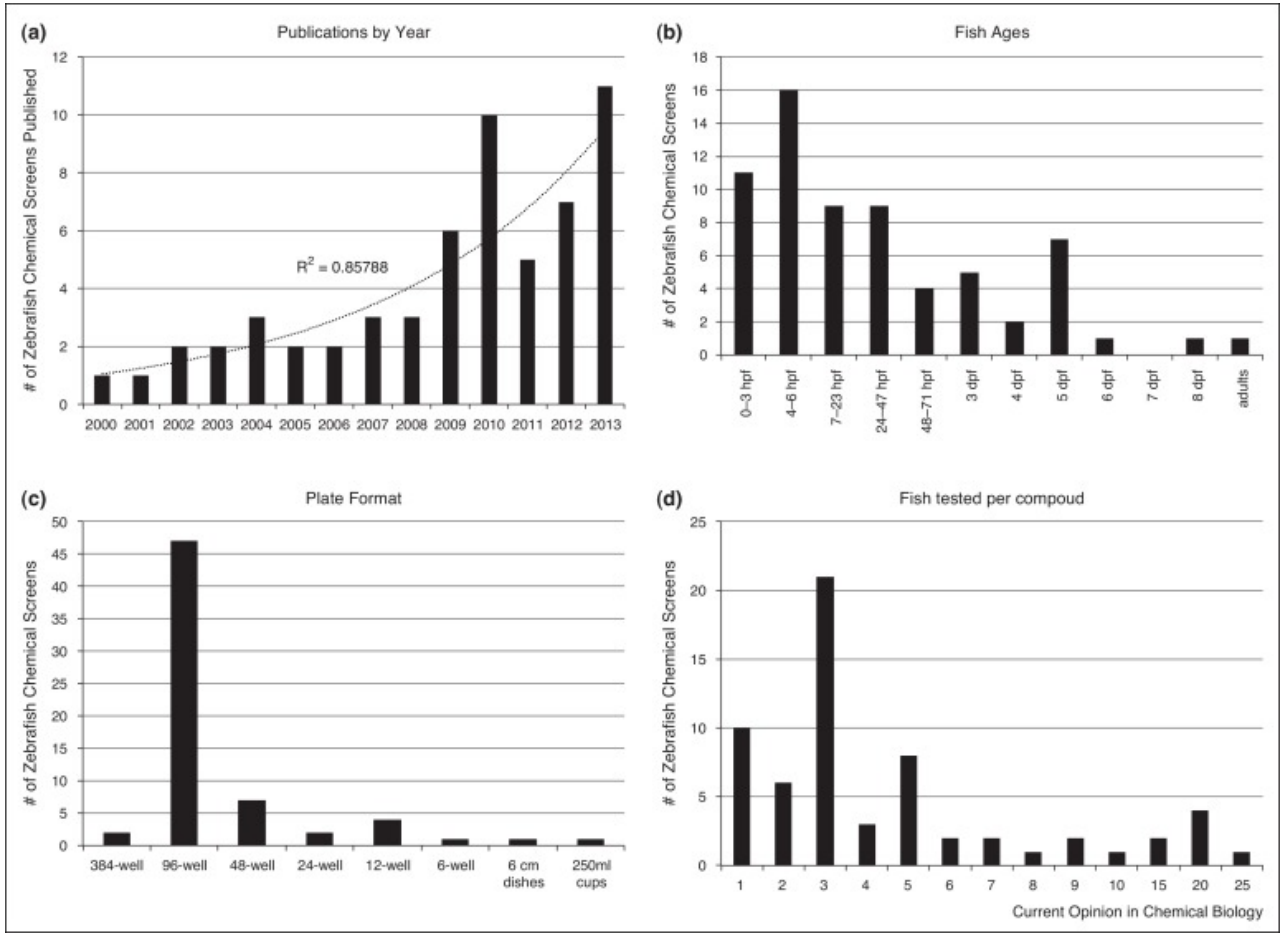
w_1
w_2
w_3
w_4 $AXIS = 0.81$
w_5 $YSE = 0.74$
w_6
w_7
w_8
w_9
w_{10}
w_{11}
w_{12}
w_{13}
w_{14}
w_{15} $NC = 0.73$
w_{16}
w_{17}



[Zhang et al. (2016) *Toxicology and Applied Pharmacology*]

Key elements affecting harmonization

- Reproducibility:
  - Chemical delivery & automation
  - Throughput, replicates, and historical data tracking



[Rennekamp and Peterson (2015) *Current Opinion in Chemical Biology*]

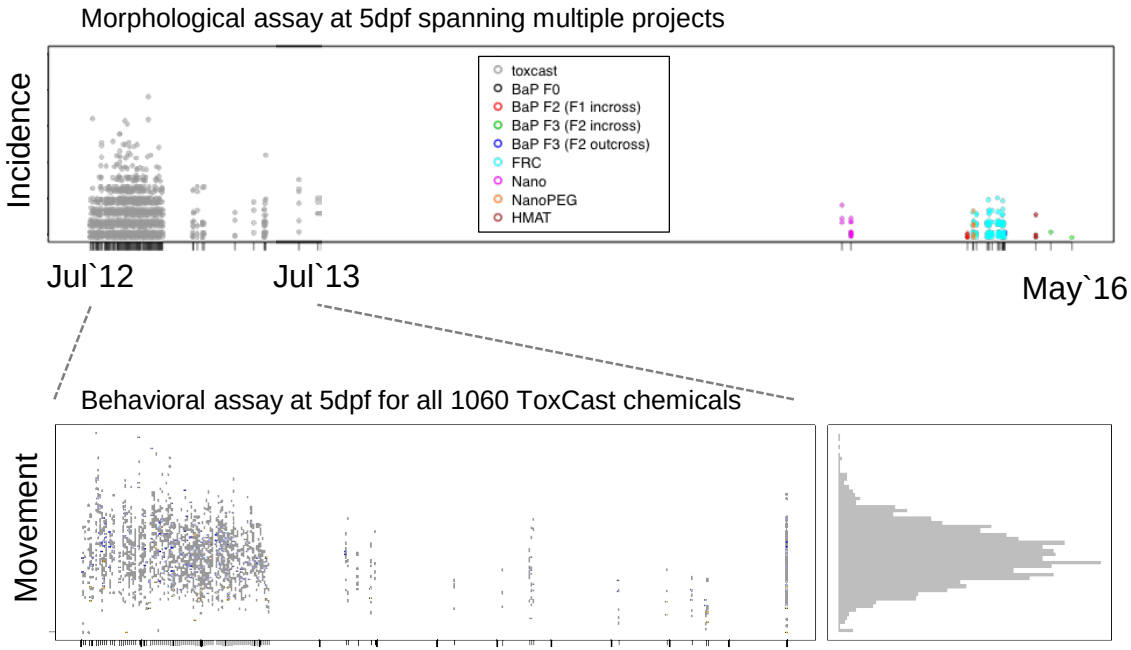
Key elements affecting harmonization | Informatics

Reproducibility:

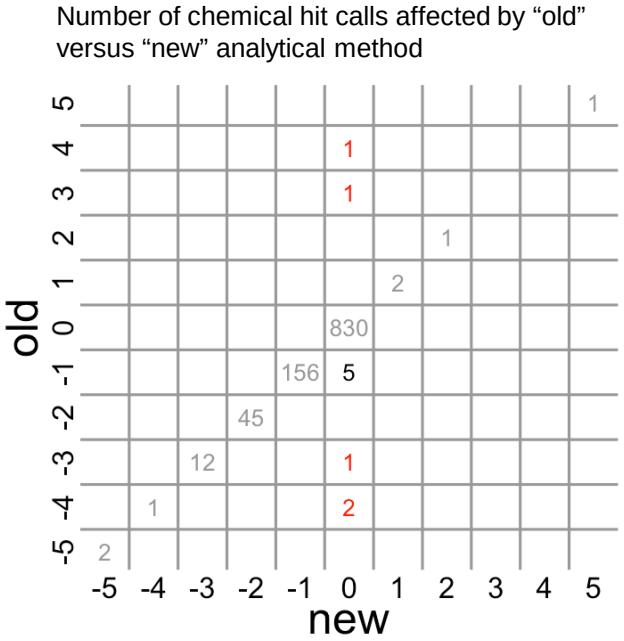
Chemical delivery & automation

Throughput, replicates, and historical data tracking

Historical data tracking:
The distributions of key phenotypes are tracked over multiple years to keep tabs on population health, effects of equipment or personnel changes, reagent fidelity, project tracking, etc.



Change analysis:
Effects of changes in experimental or analytical factors are formally compared to quantify effects.



[Skylar Marvel, Lisa Truong, Robert Tanguay, David Reif]

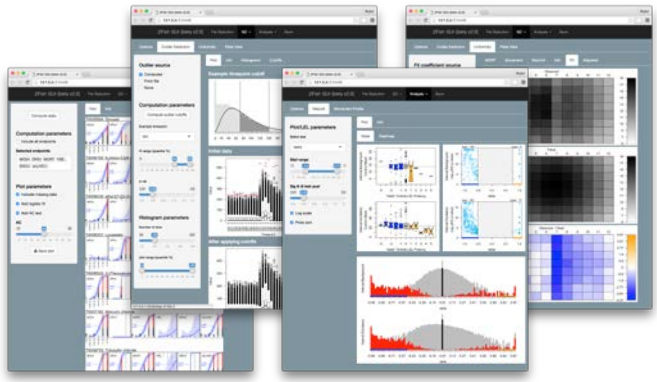
Conclusions and Next Steps

Conclusions

- What might harmonization require, and would it be worth the effort?
  - In the most strict sense, when experimental parameters differ, we should consider each as a different assay
    - It may be difficult and restrictive to experimental innovation to force conformity in lab protocols
    - Given the near-infinite chemical space for which testing must be done, each assay will have advantages and disadvantages for certain purposes
- How can informatics help?
  - Informatics offers an attractive path toward harmonization
    - If data are shared, specific performance characteristics of each assay are quantifiable
    - Integrative methods can account for these specific characteristics

Next Steps

- Shared methods, software, data and consortium efforts
- Behavioral data (preview)
  - ANOVA is the workhorse method, but violations of data assumptions are common….
  - Behavioral data can be integrated with morphological endpoints….



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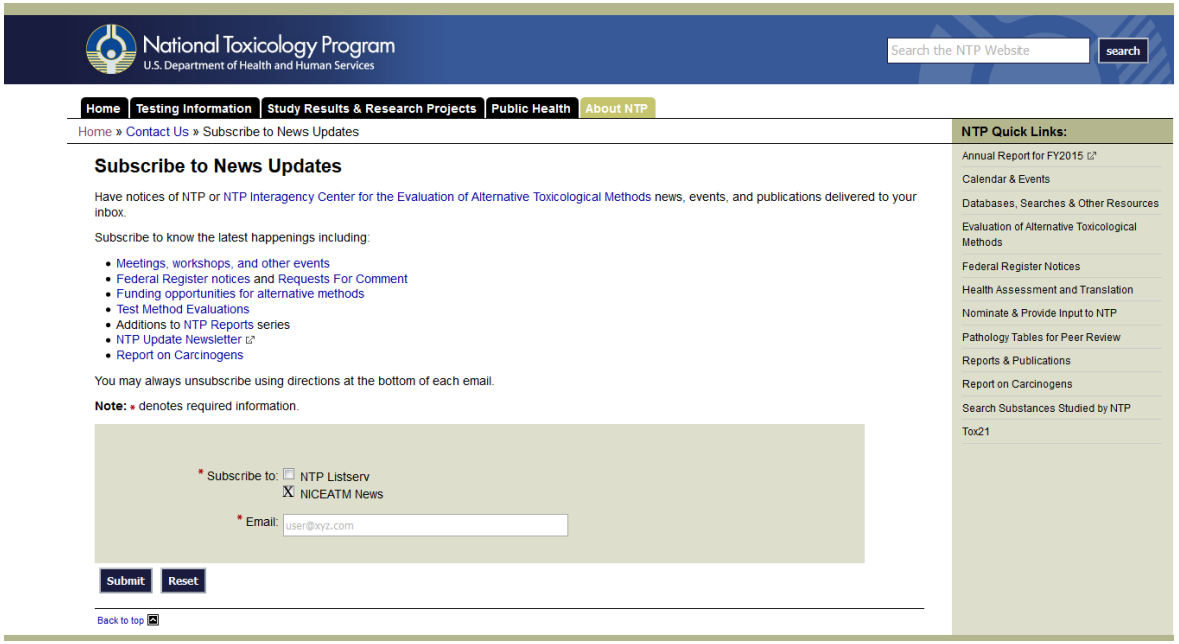

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https://zfin.org/ (ZFIN)

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