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A 21ST CENTURY STRATEGY FOR THE DESIGN OF SAFER PRODUCTS

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THINKING OUTSIDE THE TANK

OSU Oregon State University

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Challenges and Opportunities

- Traditional laboratory models – slow, costly, not scalable
- Opportunities to integrate recent advances in toxicology to promote green chemistry
- We need to more rapidly identify hazards and mechanisms of toxicity
- Develop predictive models to proactively design high performing and inherently safer products

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Constraints of Cell-based (in vitro) Models

- Cell by definition differentiated cells have limited potential targets
- Limited metabolism
- Experimental problems:
  - What cell type? Answer…do many, What assays? Answer… do many.
  - Assays become rather focused on what we already know
- High throughput is not high content, usually the exact opposite
- Is the data collected informative and predictive? unclear
- Simple cell based systems inherently **have blind spots**

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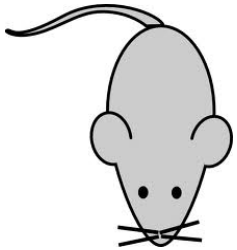
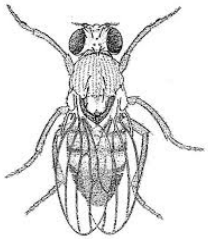
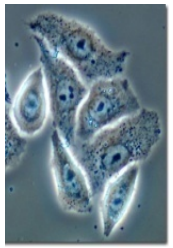
What are the Options?

Humans	Mammals	Vertebrates	Invertebrates	Cells	Biochemical
					
1-3/year	10's/year	1000's/day	10,000's/day	100,000's/day	

Immediate Human Relevance

High Throughput
Molecular mechanism

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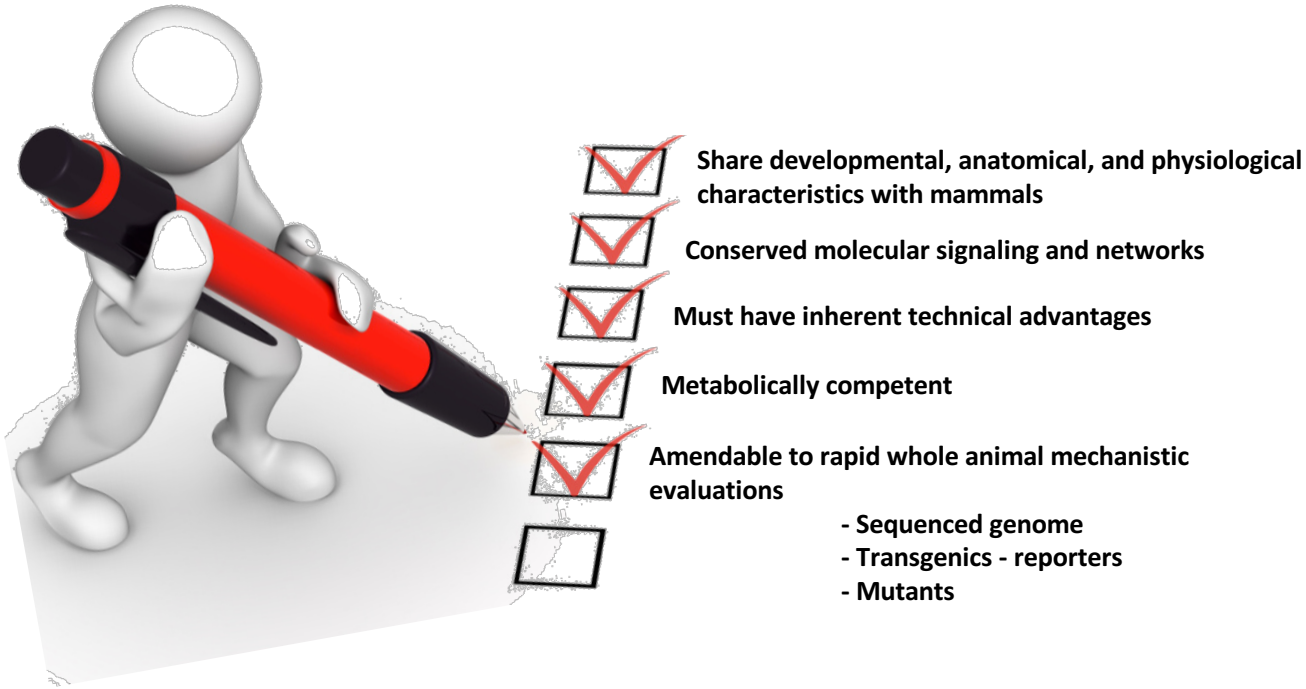
Criteria to Use Whole Animal



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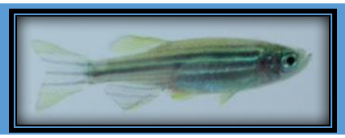
Which Models Fit Basic Criteria?

			
Time to Maturity	3 days	12 days	60 days
Genome Sequenced	☑	☑	☑
Phenotypic Screens	☑	☑	☑
Behavioral Screens	☑	☑	☑
Automation Implemented	☑	☑	☑

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Systems Toxicological Approach using Zebrafish

- Molecular signaling is conserved with humans
- ~80% homology with humans, nearly all human diseases are modeled in zebrafish
- Fully metabolically competent by 72 hpf. Why is this important?

Chemical Structure
Mixture Compositions

mRNA | miRNA | protein Expression
Metabolomics

Morphology
Behavior

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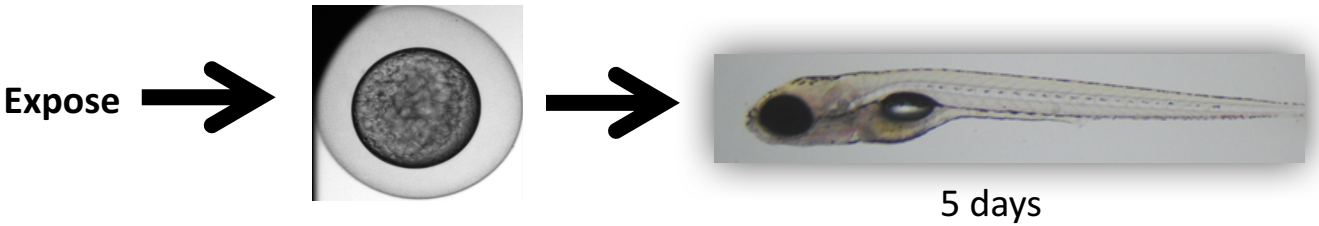
Example: Exposures
- Early Responses in Zebrafish -



Why is this powerful?

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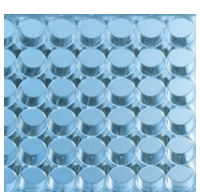
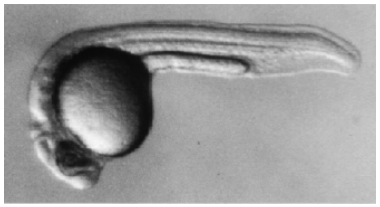
Assessing Biological - Interactions and responses

Tier 1: Toxicity Screening

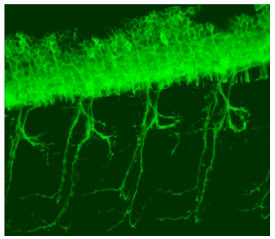
- Toxicity testing whole organisms





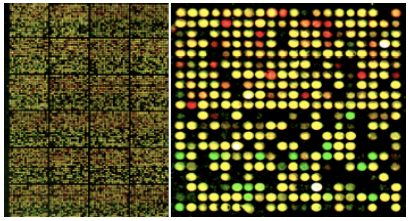
Tier 2: Cellular Targets and Distribution

- Defined *in vivo*
  - Fluorescent nanomateials
  - Targeted assays – i.e. Cell death, proliferation, etc.



Tier 3: Molecular Expression

- Genomic Responses
  - Whole animal gene expression profiles



Structure Activity Relationships

Feed data back into design scheme

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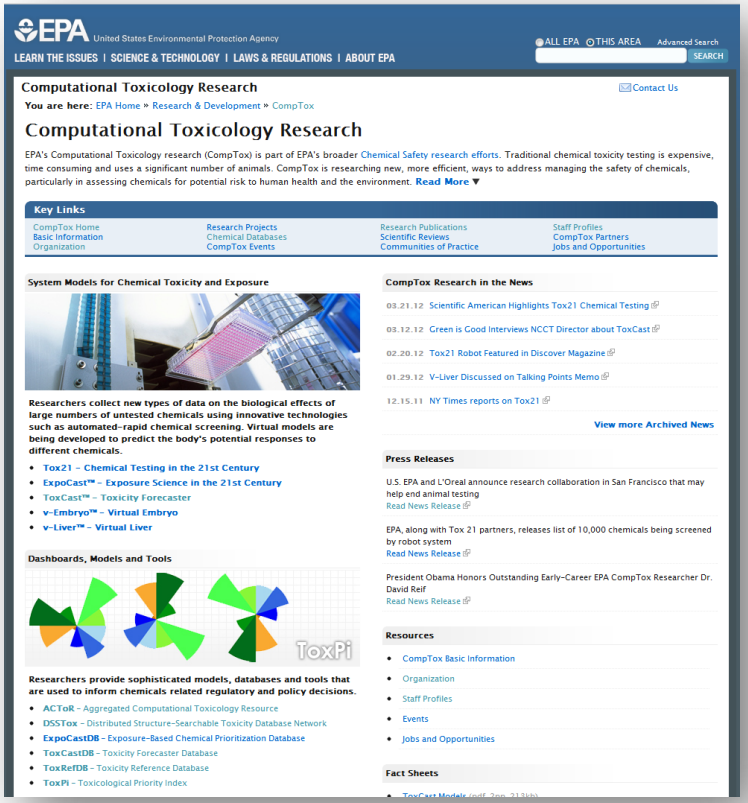
EPA ToxCast Project

- ToxCast Phase I & II (1,060 unique chemicals)
  - Phase 1 - ~300 chemicals
    - Mainly pesticides
    - Traditional toxicity data
  - Phase 2 - ~700 chemicals
    - Broad range of industrial & consumer products, food additives, “green” products, cosmetic related chemicals
    - Lack traditional toxicity data



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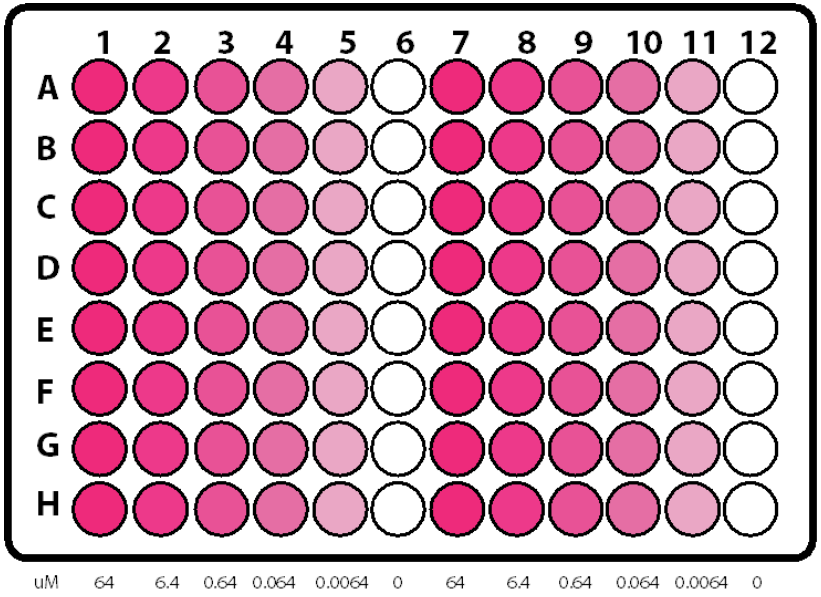
Experimental Design



Truong et al. (2014) Toxicol Sci 137: 212-233.

- Tested 6 concentrations (0 uM, 6.4nm, 64nm, 640nm, 6.4uM, 64 uM)
- Negative control – 0.64% DMSO
- Positive Control – 5 uM Trimethyltin Chloride
- 2 replicate plates (n=16/pp) N=32 animals/conc
- 22 endpoints
- 2 behavioral assays

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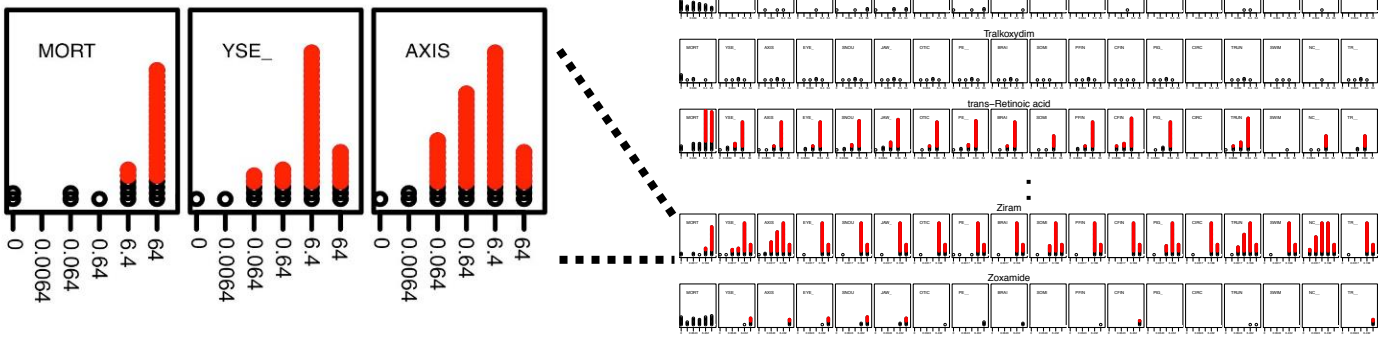
Estimation of Lowest Effect Levels

- Estimated using binomial test
- Maximized statistical power of the model
- Significance threshold determined for each chemical:endpoint pair
- 487 chemical hits



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Flame Retardants

Many types – halogenated, phosphorous and inorganic metal salts

Public safety

- Many uses – electronics, building and construction, furnishings, and transportation
- Primary benefit is to prevent ignition – REDUCE RISK
- Other benefits slow fire spread and heat release – REDUCE HAZARD
- Fire hardening combustible materials (plastics) is an important long standing engineering philosophy

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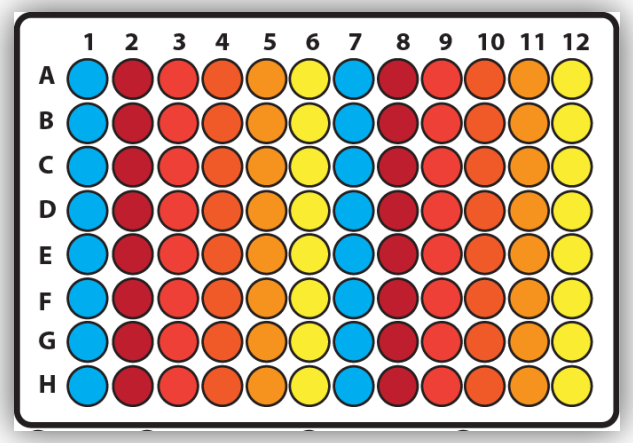
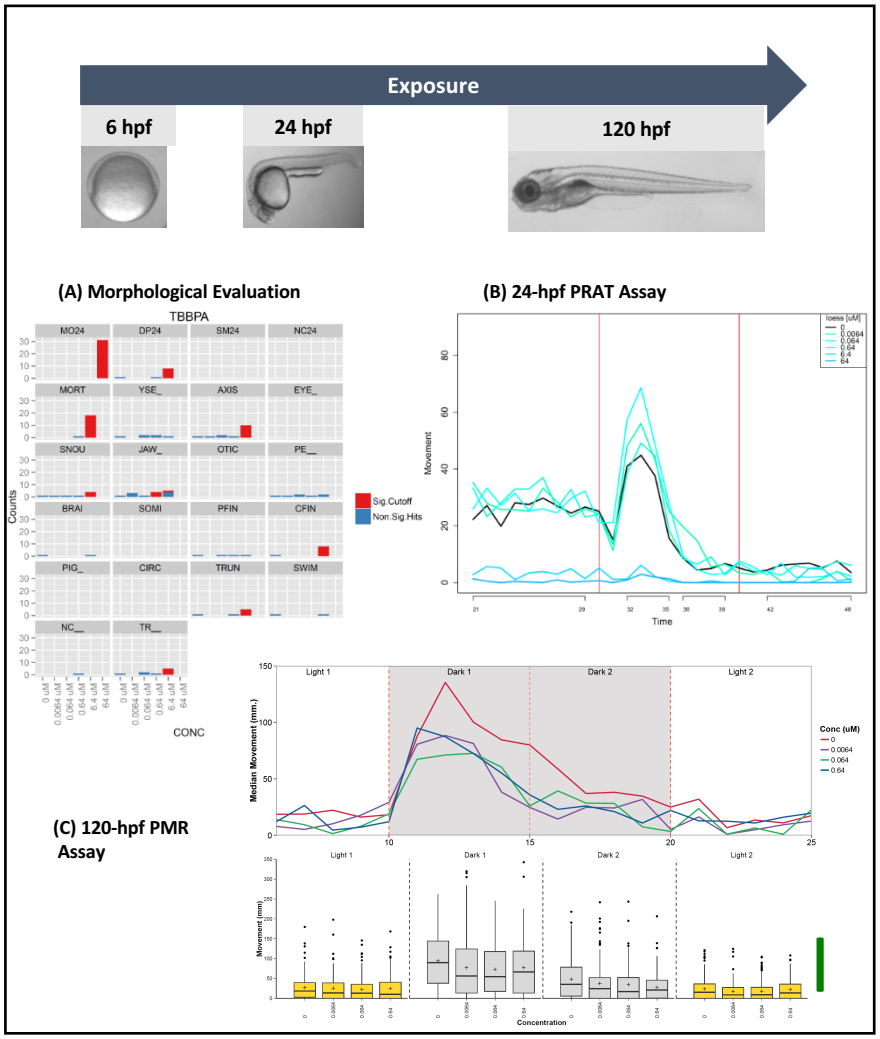
Evaluation of Flame Retardants (44)



N=32
6 concentrations



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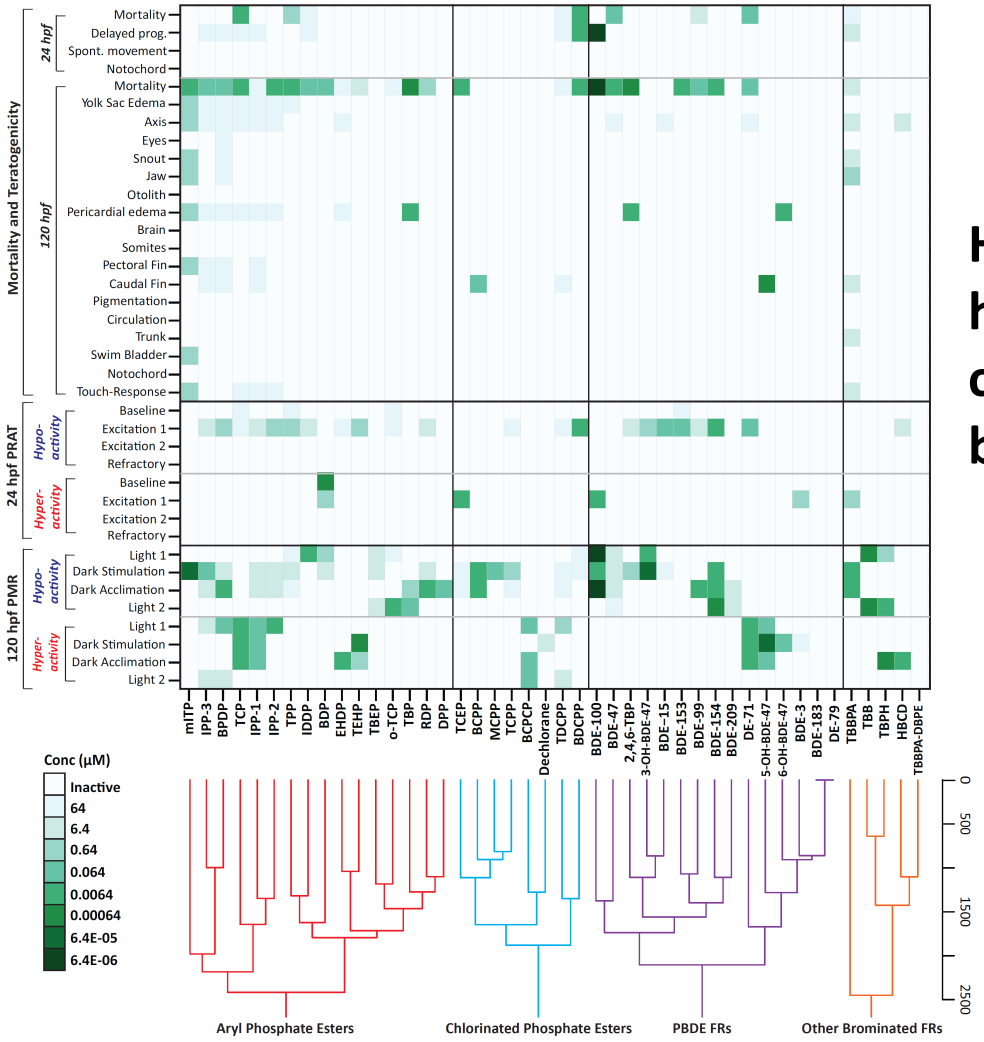
Morphology Mortality

Embryonic Behavior

Larval Behavior



Heatmap and hierarchical clustering of bioactivities

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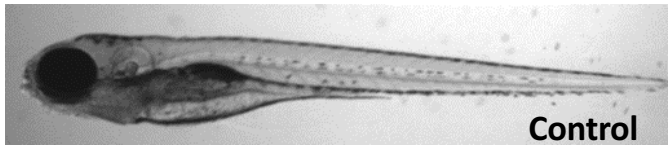
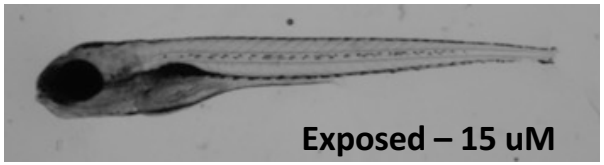
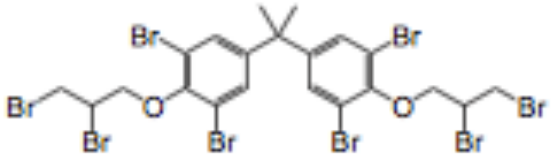
TBBPA-DBPE

Non-Toxic - Not bioactive







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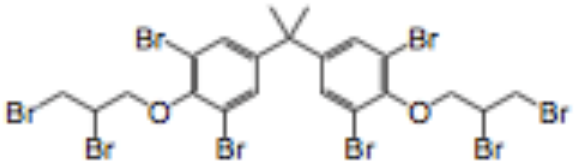
How Does TBBPA-DBPE Zebrafish Responses Compare to Available Toxicological Data?

Mammalian Data: Acute toxicity **Not toxic**

Rat oral LD50 > 2,000 mg/kg

Rabbit dermal LD50 > 2,000 mg/kg

Rat inhalation LC50 >24.4 mg/m3/2 hour



Sub-chronic toxicity: **Not Toxic**

NOAEL 2000 mg/kg/day (13 weeks oral, rat)

Aquatic toxicity: **Not Highly Toxic**

96 Hour-LC50 >100 mg/l (Rainbow trout)

> 500 mg/l in Carp

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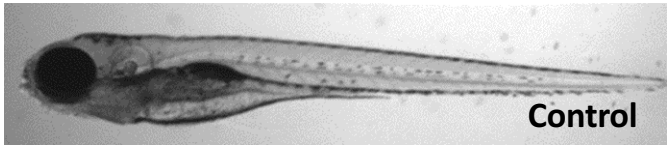
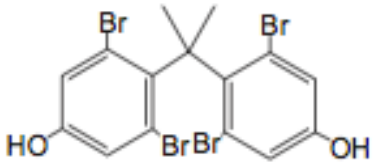
TBBPA

Highly Toxic – Very bioactive





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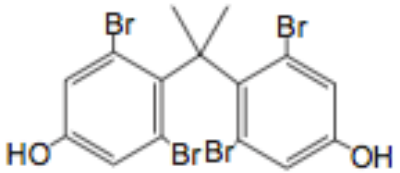
How Does TBBPA Zebrafish Responses Compare to Available Toxicological Data?

Mammalian Data

*Acute toxicity **Not toxic**

Rat oral LD50 > 5,000 mg/kg
Rabbit dermal LD50 > 2,000 mg/kg
Rat inhalation LC50 >2550 mg/m3/2 hour



*Sub-acute toxicity: **Not Toxic**

NOEL >18 mg/l/4 hour (2 weeks , inhalation, rat)
>1000 ppm (4 weeks oral rat)
>2500 mg/kg (3 weeks, dermal, rabbit)

*Sub-chronic toxicity: **Not Toxic**

NOAEL 1000 mg/kg/day (13 weeks oral, rat)

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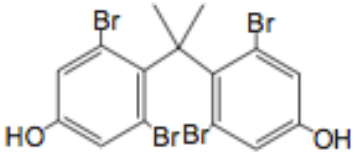
How Does TBBPA Zebrafish Responses Compare to Available Toxicological Data?

Mammalian Data Continued



Reproductive toxicity (2-generation, rat): **Not Toxic**
NOEL 100 mg/kg/day for parental toxicity
NOEL 1000 mg/kg/day for reproduction performance

Teratogenicity(rat, gavage): **Not Teratogenic**
NOEL = 3000 mg/kg

Aquatic Toxicity - **Very toxic** to aquatic life
96 Hour-LC50 1.1 mg a.i./L (Rainbow Trout, flow-through)
0.4 mg/L (Rainbow Trout, static)

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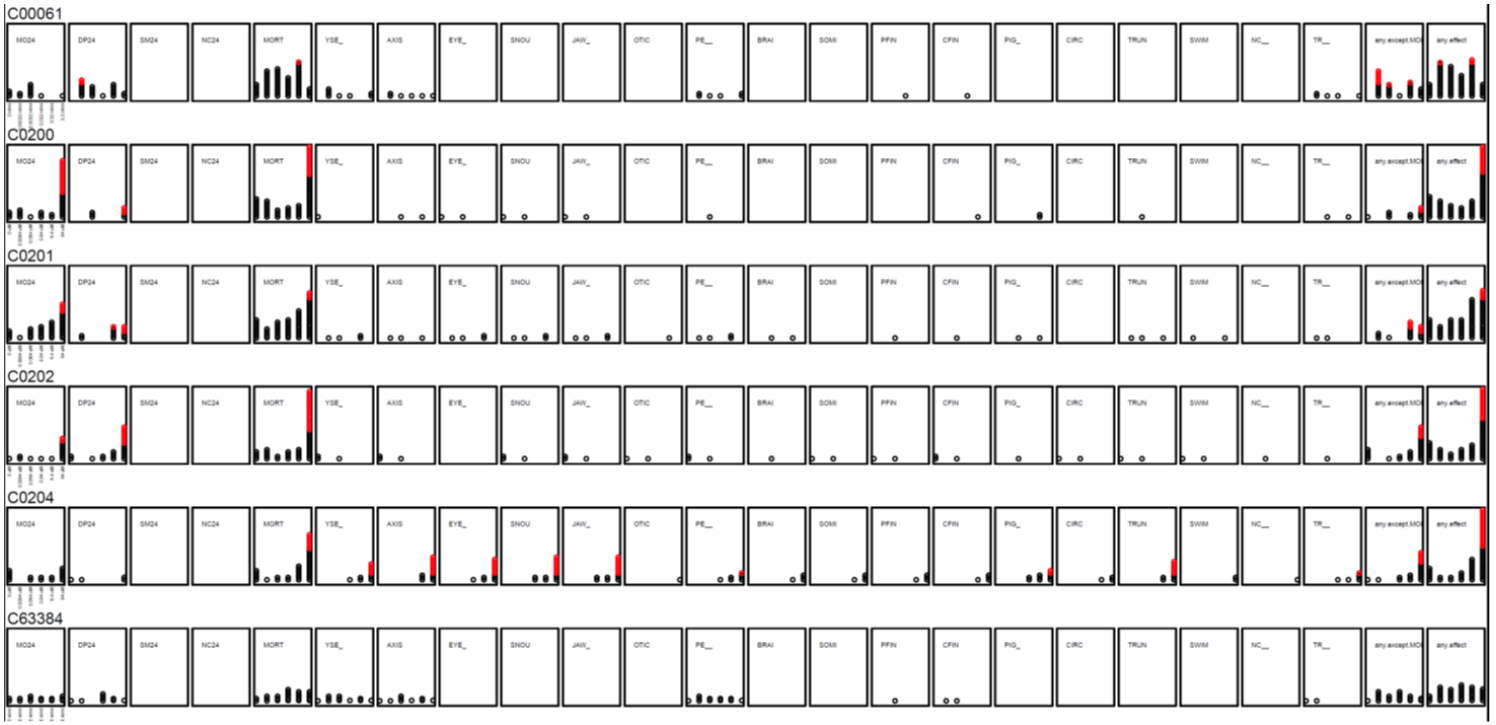
Example Phase 1 FRC Screening

Identified bioactive compounds



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Example Phase 2 FRC Screening

Fewer bioactive compounds

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Emerging Approaches – HTS Adult Assessments

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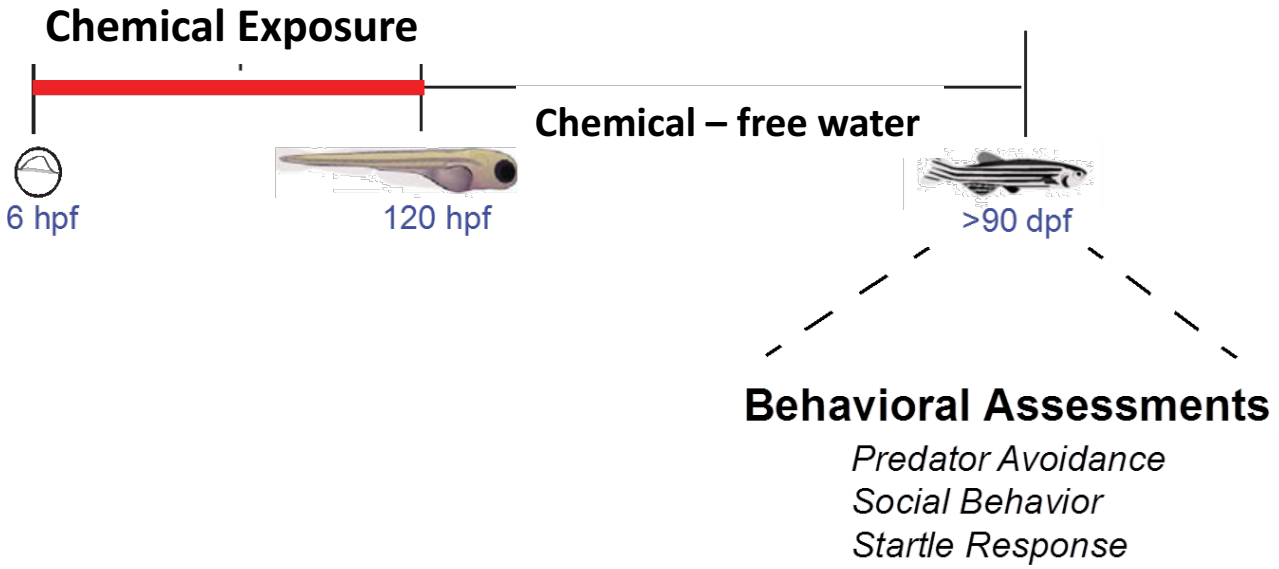
Adult Behavioral Assessments



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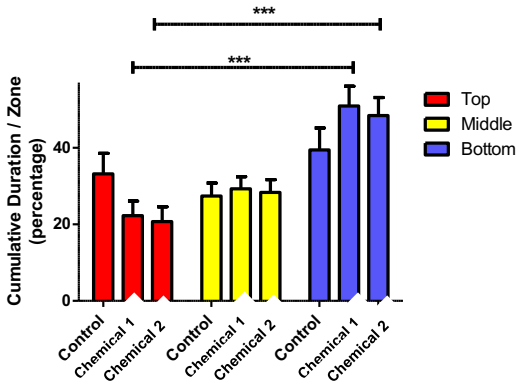
Swimming Activity Over Time



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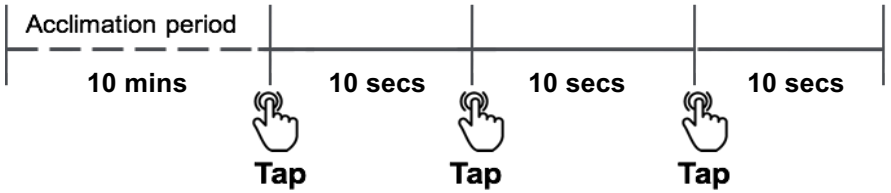
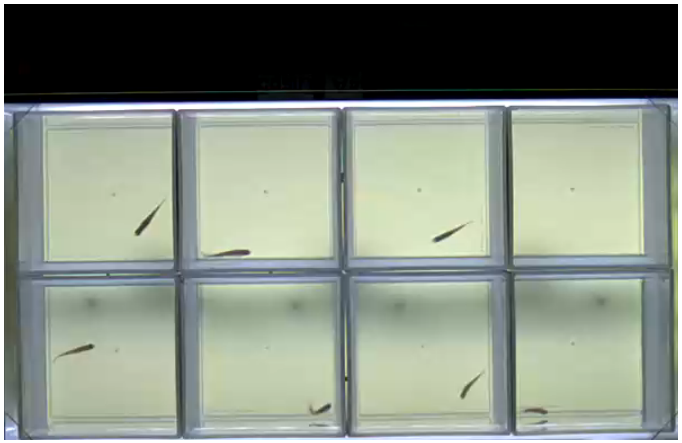
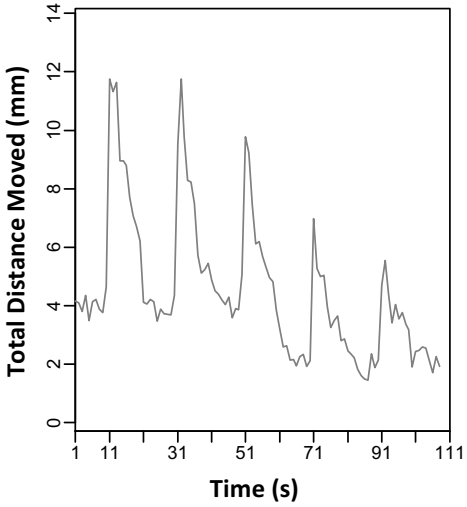
Anxiety Tests







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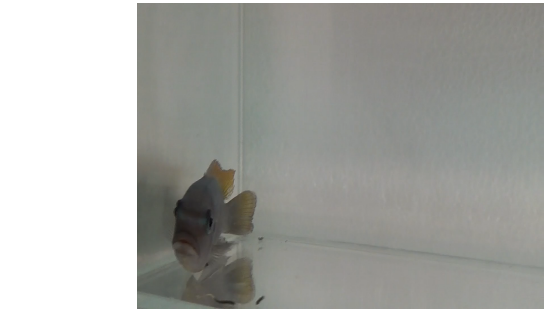
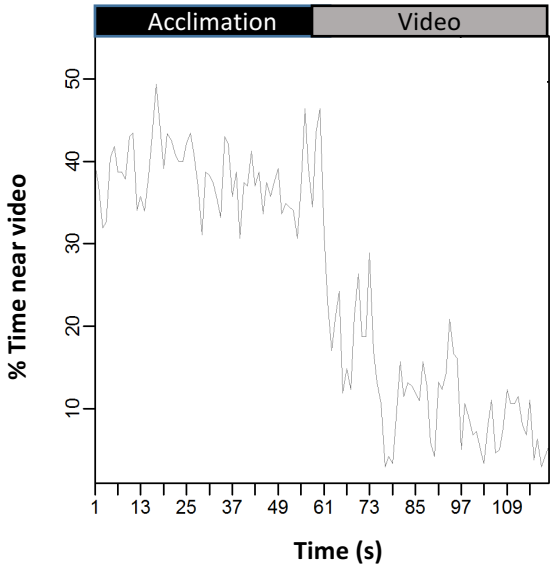
Fear Response





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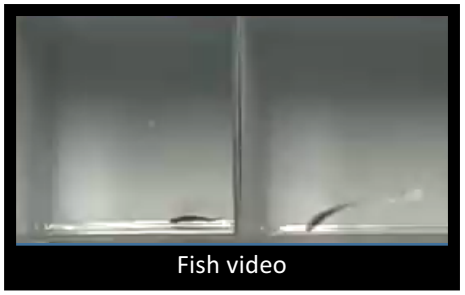
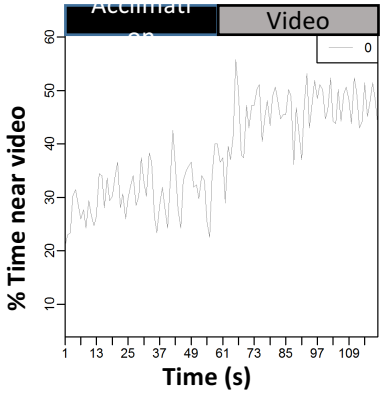
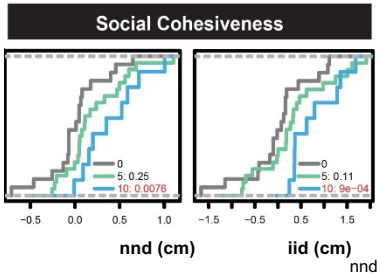
Social Interactions







nnd = nearest neighbor distance
iid = inter-individual distance

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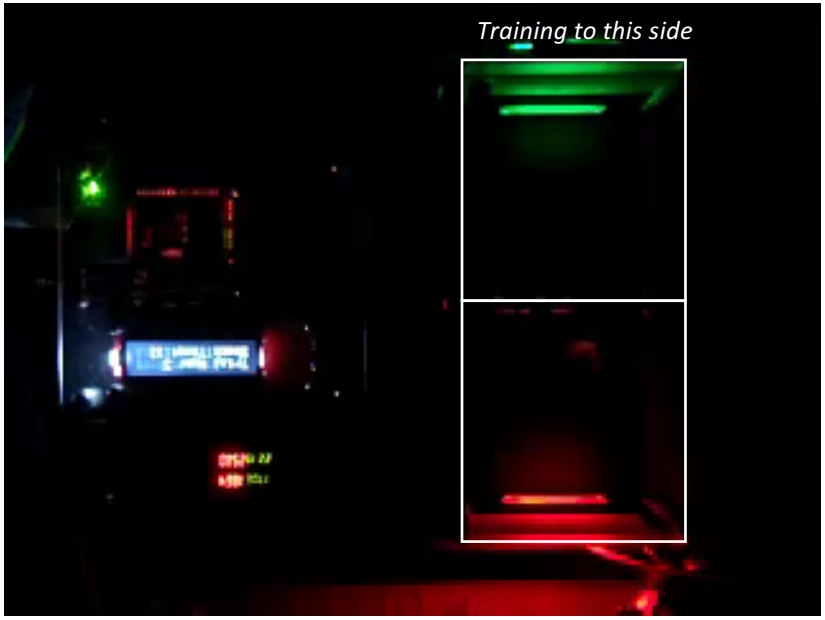
Learning



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