Toxicity Screening of the ToxCast Phase II Chemical Library Using a Zebrafish Developmental Assay

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Abstract

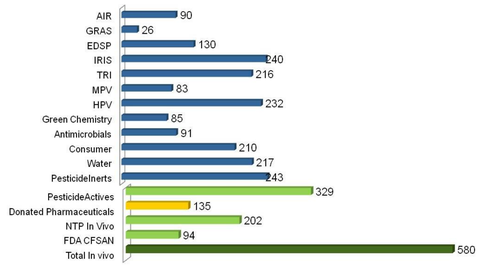
As part of the chemical screening and prioritization research program of the US EPA, the ToxCast Phase II chemicals were assessed using a vertebrate screen for developmental toxicity. Zebrafish embryos (*Danio rerio*) were exposed in 96-well plates from late-blastula stage (6hr post fertilization, pf) through day 5pf (1-2 days post-hatch). All exposures were by immersion and renewed daily. The 685 chemicals included food additives, consumer use product ingredients, pesticides, failed pharmaceuticals, and "green" plasticizers (http://epa.gov/ncct/toxcast/chemicals.html). Intra- and inter-plate replicates were included for quality control. Developmental toxicity was initially assessed using a single nominal concentration of 80 µM: positives and a selection of negatives were confirmed by concentration-response determinations. On day 5pf, larvae were moved from exposure solution to a control solution without chemical, and on day 6pf were assessed for overt toxicity (*i.e.,* death, non-hatching and dysmorphology; n=4 embryos per chemical). Dysmorphology was a combined score using both in-life observation and Brightfield, high-content image analysis. Overt toxicity was noted with 46% of the chemicals tested, compared to 62% positive chemicals when the ToxCast Phase I library, consisting of mostly pesticide active ingredients, was previously tested. As with the Phase I library, the octanol-water partition coefficient (log_{kow}) of the Phase II library chemicals was positively correlated with overt toxicity: there were 18% positive chemicals with log_{kow} <0; 41% positive chemicals with log_{kow} of 0 to 4; and 67% positive chemicals with a log_{kow} >4. All chemicals positive at the single concentration were further assessed for potency using a Dose-Response Study (8-point, semi-log concentration curve: n=3 embryos per concentration). These data demonstrate the utility of zebrafish in medium-throughput chemical testing programs for detection of adverse developmental outcomes. *This abstract may not necessarily reflect official Agency policy*.

Objective

- Screen the ToxCast Phase II chemicals using the zebrafish developmental assay as part of the US EPA's chemical prioritization research program.

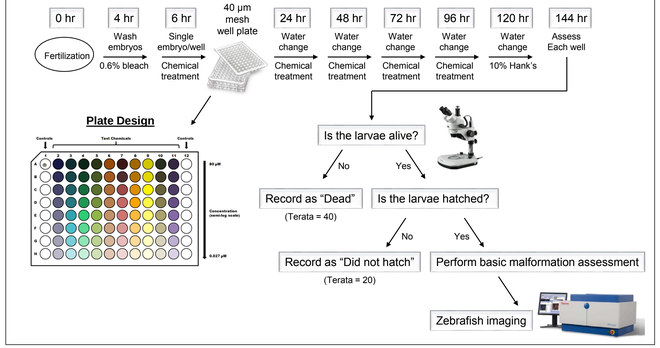
Chemicals

The ToxCast Phase II chemical library consisted of a total of 685 unique chemical structures. Tested compounds were selected predominately from EPA chemical inventories and included data-rich (*green*) and data-poor (blue) chemicals. Also included were 135 failed pharmaceuticals donated by industry partners.



Methods

Zebrafish Developmental Assay

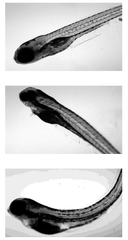


Malformation Assessment

(Based on two forms of assessment)

Basic Visual Assessment

- Performed at 6 days post-fertilization
- Overall classification given: normal(N), abnormal(A), or severely abnormal(SA)
- Fins, swim bladder, cranial-facial development, organ development, and position assessed: N or A

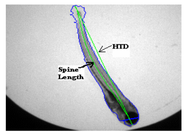


Overall:	Normal
Fins	N
Cranial-facial	N
Position	N
Swim Bladder	N
Organ Development	N

Overall:	Abnormal
Fins	N
Cranial-facial	A
Position	A
Swim Bladder	A
Organ Development	N

Overall:	Severely Abnormal
Fins	A
Cranial-facial	A
Position	A
Swim Bladder	A
Organ Development	A

Arrayscan® Image Analysis



- Automated image analysis of euthanized larvae placed in 1% agarose on their side
- Several parameters taken including:
  1. Area
  2. Perimeter-to-area
  3. Length-width-ratio
  4. Head-tail-distance
  5. Spine length
  6. Width
  7. Straightness
  8. Convexity

Total Terata Score Assigned

Positive if > Control Terata Mean+(3*SE)

Results

Controls

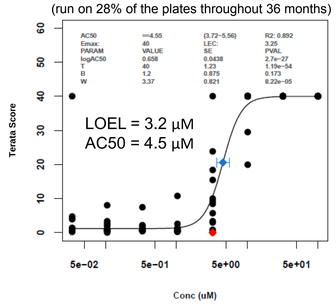
Controls >96% normal (n=1668)
Mean Terata score = 1.38 (± 2.38)

Mortality incidence rate in controls = 2.4%

Did-Not-Hatch incidence rate in controls = 0%

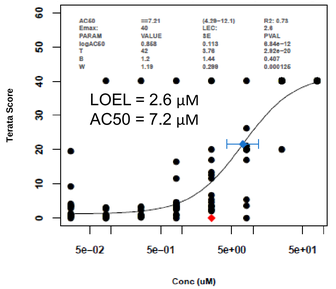
Positive Control: Chlorpyrifos

(run on 28% of the plates throughout 36 months)

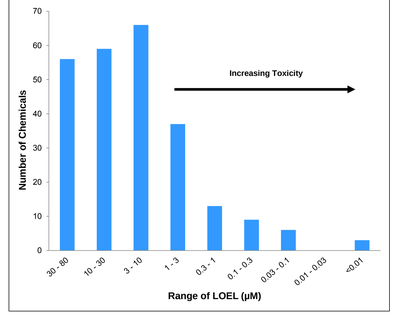


Blinded Internal Replicate: Nitrofen

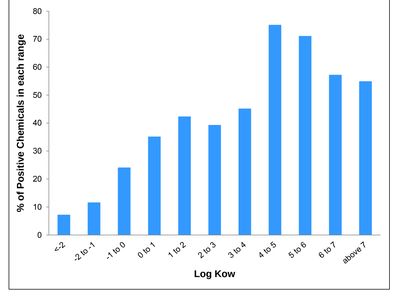
One of 6 chemicals that were run 5 times as blinded replicates to determine reproducibility of findings



Distribution of the Activities of the 260 Positive Chemicals



Log Kow as it relates to the number of Positive Chemicals



Comparison to Phase I Results

(Run 3 years apart)

Of 8 chemicals run in both Phases, all were similar

Chemical	Phase II	Phase I
Azoxystrobin	AC50= 2.9	AC50= 3.6
Oryzalin	AC50= 16.0	AC50= 11.7
Triclosan	AC50= 4.6	AC50= 2.7

Conclusions

- Approximately 40% of the 685 chemicals were positive in the assay.
- High repeatability with internal and positive controls.
- Consistent with previous findings, Log_kow affects number of positive chemicals.

Acknowledgments

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