**Introduction**

- Chemical lipophilicity contributes to bioconcentration in aquatic species. Lipophilicity correlates with developmental toxicity in various aquatic models.
- Zebrafish is being used as a model organism to screen thousands of chemicals in the ToxCast and Tox21 research programs for potential to induce developmental defects or overt toxicity.
- The partition coefficient (log P) is an indicator of lipophilicity.
- We examined the relationship between log P, estimated body burden, and developmental toxicity in zebrafish for 209 environmental chemicals from the ToxCast Phase I library.
- We then used hepatic clearance, protein binding data, and reverse toxicokinetic models to compare zebrafish toxicity and ToxCast high-throughput screening (HTS) activity in vivo rat data.

**Chemical Potency Shifts**

- Table 1 shows the ten most toxic ToxCast Phase I chemicals to zebrafish ranked by estimated maximum activity concentration (EC₅₀) or estimated maximum activity concentration (EC₅₀) in rat prenatal toxicity assays.
- For most chemicals tested, zebrafish assays provide a conservative estimate of developmental toxicity at low effect levels. However, the developmental toxicity of certain chemical classes, such as azoxides, may be underestimated by zebrafish studies.
- For all chemicals tested, the ToxCast HTS assays were more sensitive than zebrafish or rat prenatal studies.
- ToxCast in vitro assay targets may provide insight into the biological relevance of zebrafish assays for predicting mammalian developmental toxicity.

**Comparison to In Vivo and HTS Data**

- ToxCast Phase I compounds were also screened in -600 HTS assays (Kavlock et al., 2012), including:
  - Human primary cells measuring protein signaling
  - Cell-free biochemical assays measuring enzymatic activity and receptor binding
  - Assays for nuclear receptor activity
  - Transcription factor activation assays
  - Assay measuring photocytotoxicity
- Most of these chemicals have in vivo rodent toxicity data (genotoxic, multigenerational, chronic, and/or nontoxicological) available in ToxRefDB (http://toxrefdb.epa.gov/zebrafish/).
- A subset of 27 compounds active in the zebrafish and in vivo prenatal ToxRefDB rat assays also had hepatic clearance and protein binding data (Weissman et al., 2012).
- We computed the oral equivalent values from the zebrafish data and the most sensitive ToxCast LOAELs for each chemical. We compared these values to the LOAELs from ToxRefDB (Table 3).
- Seven chemicals highlighted in Table 3 were developmentally toxic to zebrafish but not rats (i.e., these chemicals had no rat prenatal assays in ToxRefDB but no rat LOAELs).
- From the remaining 20 with rat prenatal LOAELs:
  - Thirteen chemicals had oral and oral equivalent values from the zebrafish data (EC₅₀ vs. LOAEL) that were lower than the prenatal LOAEL.
  - Three chemicals (histoconutamide, permethrin, and nomifensine) highlighted in green in Table 3 had oral prenatal LOAELs that fell between the oral and oral equivalent estimated from the zebrafish data.

**Chemical Classification (Log P) Values for 215 Tox21 Chemicals**

- Table 2 lists the chemicals sorted by rat prenatal LOAEL in ascending order, with chemicals with no rat oral equivalent values from the ToxRefDB listed last. Chemicals highlighted in green had rat prenatal LOAELs that were higher than the predicted LOAELs in rats.

**Estimating Log P**

- We computed experimental Log P values for 2225 ToxCast chemicals to predict oral equivalent Log P values based on chemical structures from EPIsuite (Wetmore et al., 2013).
- The correlation between experimental and predicted Log P values is high (R² = 0.68), with a root mean square deviation of 0.29 log units.
- We used the best-fit equation to estimate Log P values for the chemicals that were not experimentally measured.

**Experimental Methods**

- Chemicals were screened by immobilizing zebrafish embryos in media containing chemical concentrations from 0.001 to 80 µM and determining the nominal maximum activity concentration (AC₅₀) for toxicity or protein binding or transcription activity (Figure 1; Padilla et al., 2012).

**Figure 1. Zebrafish Developmental Assessment Exposure and Evaluation Scheme**

- There was a clearer relationship between log P and incidence of developmental toxicity among the ToxCast Phase I chemicals (Figure 2).

**Table 2. Chemical Class Bioconcentration Example: Pyrethroids**

- Table 2 exemplifies the differences in bioconcentration of chemicals with similar chemical structures. The lipophily of the chemical is likely to determine the degree to which these chemicals are absorbed into the body tissues and accumulate within the body tissues.

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The views expressed in this document are those of the authors and do not necessarily represent the official positions of any federal agency, since the paper was written as part of the scientific tasks of the authors.

**References**

- A summary of EPIsuite and ToxRefDB activities at the National Toxicology Program website is http://toxrefdb.epa.gov/zebrafish/.