

Predictive Modeling of Developmental and Reproductive Toxicity Pathways

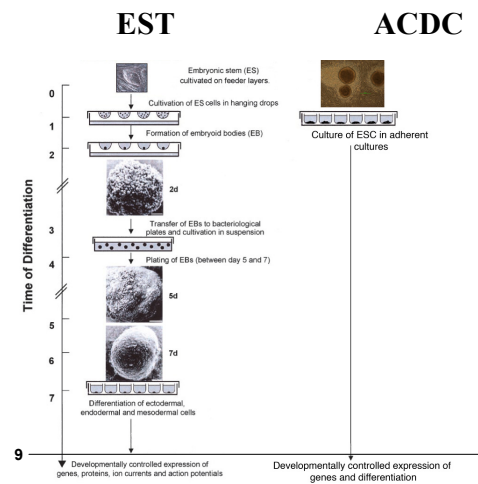
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research & development

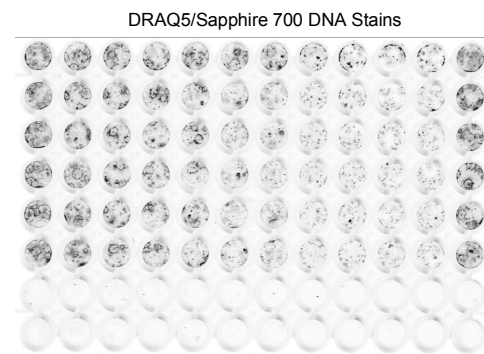
U.S EPA, ORD, Computational Toxicology Research Program

Methods/Approach

Embryonic Stem Cell Differentiation Assay



Scan of ESC plate after chemical exposure.



Fluoroacetic acid
1 - 10,000µM

The Adherent Cell Differentiation/Cytotoxicity method was used to evaluate the effects of the ToxCast 320 chemicals at a 12.5µM concentration

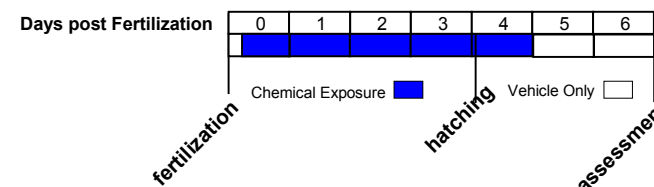
Evaluation of endpoints for Stem Cell Assay

- Nuclei are stained to measure effects of exposure on proliferation and induction of excess cell death (image above)
- Myosin Heavy Chain (MHC) marker protein is measured using in-cell Western technique to assess differentiation of pluripotent ESC to cardiomyocytes
- TacMan low density arrays (PCR) is used to characterize expression of 22 marker genes of differentiation

Zebrafish Morphogenesis Assay

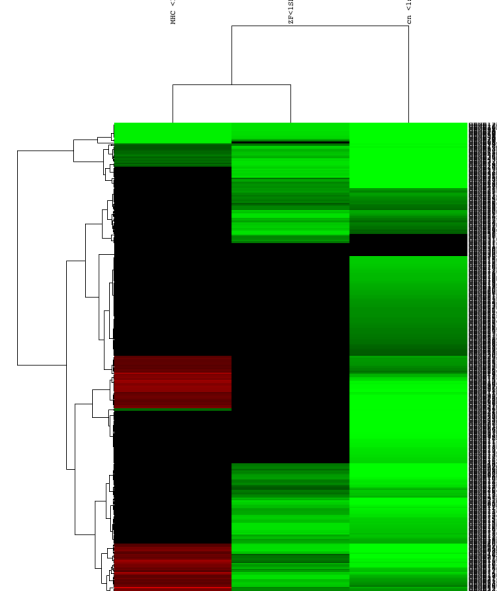


6-8 Hr post fertilization 6 days post fertilization



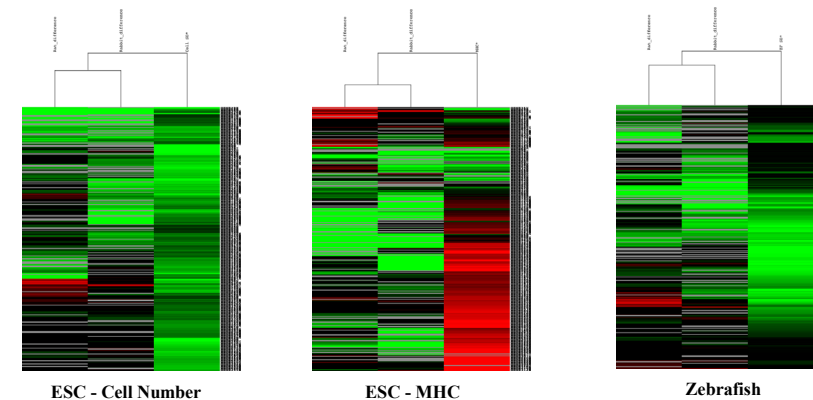
Outbred, wildtype, zebrafish eggs were placed into individual wells of a microtiter plate at approximately 8 hours after fertilization. The eggs were exposed by immersion to each of the ToxCast 320 chemicals in the water (10% Hanks balanced salt solution; 0.4% DMSO final concentration) at a final concentration of 80 µM for the first 4 days of development. On the fifth day, the larvae were removed from the chemical and placed in 10% Hanks solution. On Day 6, each embryo was assessed for malformations and lethality by someone blinded to the treatment group of each animal. Positives were defined as a dead animal or an animal with frank malformations.

Comparison of zebrafish (ZF), Embryonic stem cell differentiation (MHC) and embryonic stem cell number (CN) response to ToxCast chemical exposure

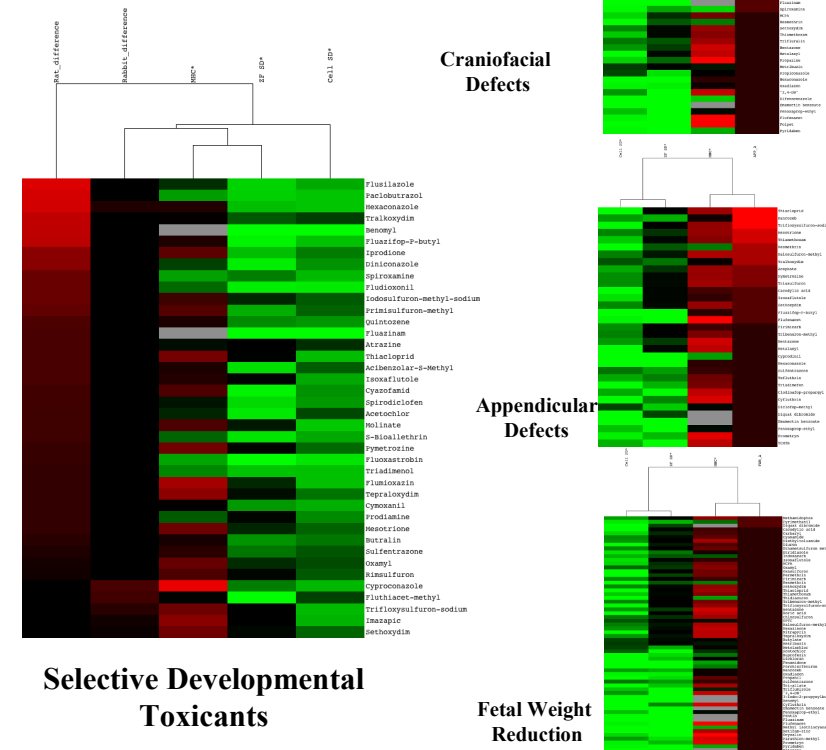


Each chemical was evaluated at a single concentration (80µM Zebrafish; 12.5µM ESC). Criteria for inclusion was set as greater than 1 standard deviation from control response. Clusters show 6 groupings of chemical effects

Comparison of in vitro response to developmental sensitivity in vivo



Comparison of in vivo effects of compound administration and in vitro response to developmental toxicants



Selective Developmental Toxicants

The adverse developmental effects of chemical exposure in vivo are compared to the effects of these compounds in vitro. CRN = Craniofacial defects; APP = Appendicular defects; FWR = Fetal Weight Reduction.

Results/Conclusions

Both zebrafish and ESC model systems respond to chemical exposure, and display chemical-specific effects on patterns of growth, differentiation and development.

Inclusion criteria were established based on the chemical response that shows 6 groups. This analysis demonstrates the combined power of zebrafish with cell number CN-ESC assessment in assessing developmental toxicants.

Impact and Outcomes

Analysis of the effects of the ToxCast 320 demonstrate the reproducibility of each system by replicates in the chemical library.

Both zebrafish and ESC offer complementary model systems and indicates that the fish and mammalian response may correlate well. Differences in "chemical activity" may be related to physical properties such as logP or biotransformation in the fish liver.

Future Directions

Dose response analysis for the ToxCast 320 are underway in both systems. Once a clearer link to potency is established we will compare the results across systems and with the prenatal developmental toxicity studies in ToxRefDB for rats and rabbits.

Computational models are beginning to be developed for ESC that will allow for cell interactions and toxicity pathways to be included.

Science Question

In vitro models have been used to evaluate the biological processes involved in development and evaluate the effects of xenobiotic exposure. These models have included whole embryos and isolated embryonic organs and cells. Two systems that hold great promise are zebrafish embryos and embryonic stem cells (ESC). Each system has unique strengths.

The science questions to be addressed in this project are focused on identifying which toxicity pathways are present and the relationship between perturbation of these pathways with alterations in growth, differentiation and development. The construction of computational models of these pathways linked to phenotypic alterations will be used in development of virtual models.

Research Goals

Develop computational models of development in zebrafish and embryonic stem cell model systems to assess the impact of xenobiotic exposure.

Each system will be used to evaluate the effects of the ToxCast 320 chemicals. Based on the proposed toxicity pathways associated with each chemical we can determine the linkage of pathway perturbation and phenotypic alterations.

Computational models of cell growth, interaction and differentiation will be developed that will correspond to the phenotypic outcomes of ESC culture. Once toxicity pathways are linked to alterations in phenotype, These can be included in the computational models.

