Effects of the Contaminant Candidate List – 4 (CCL4) on Differentiation and Cytotoxicity in Mouse Embryonic Stem Cells

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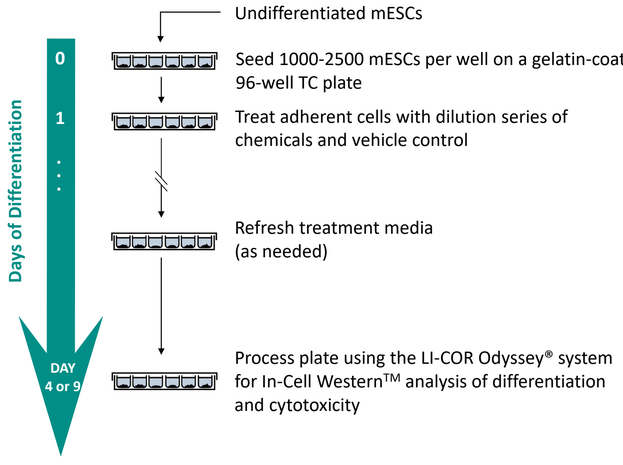
Introduction

Pluripotent stem cells are a model of embryonic development and used to profile biological consequences of chemical exposure. As embryonic development advances, differences are expected in the sensitivity to chemical-perturbation and morphological specificity. The potential for environmental chemicals to produce birth defects is largely unknown. This study was designed using mouse embryonic stem cells to profile the bioactivity of chemicals on the EPA Contaminant Candidate List 4 (CCL4).

Methods: Adherent Cell Differentiation/Cytotoxicity (ACDC) Assay

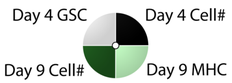
J1 Pluripotent mouse embryonic stem cells (mESCs) (ATCC-SCRC-1010™) are maintained on a mouse embryonic fibroblast (MEF) feeder layer in the presence of murine leukemia Inhibitory factor. To assess chemical effects on mESCs, MEF-depleted mESCs are seeded in gelatin-coated 96 well plates in differentiation medium. After overnight attachment of the mESCs , they are exposed to a series of four chemical concentrations and vehicle control for the appropriate exposure time course. The cells are processed for In-Cell Western™ analysis of differentiation and cytotoxicity and analyzed using the LI-COR Odyssey® system. Differentiation was determined using an antibody to goosecoid (a gastrulation biomarker) on day 4, and an antibody to alpha-myosin heavy chain (a cardiomyocyte protein biomarker) on day 9. Cell number was determined using Red Dot™ at both time points. A 25% change in differentiation or cell number was used as the point of departure from media controls.
Seventy Eight (78) CCL4 chemicals were evaluated. Only commercially available non-volatile, non-explosive CCL4 chemicals were included in this assay.

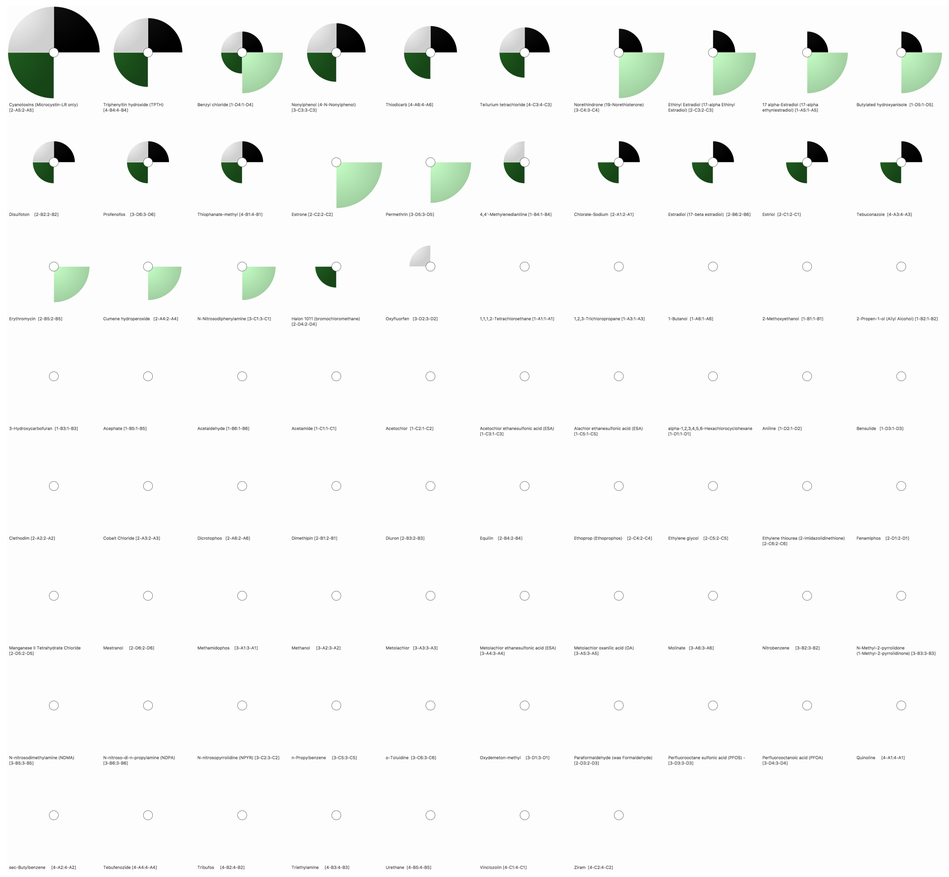


Results

Chemical Effects on mESC

The concentration that produced a 25% change in a stem cell endpoint was used to construct a ToxPI.





Beta version of the new ToxPi software provided by David Reif (DMReif@ncsu.edu) as part of EPA STAR #R835802

Results

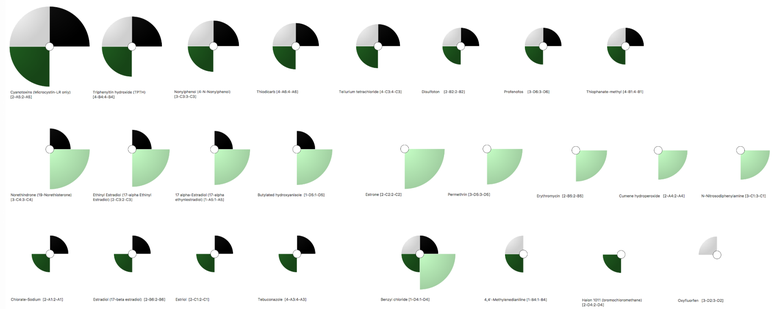
Effects of CCL4 Chemicals on mouse Embryonic Stem Cells

The lowest concentration (µM) that affected a stem cell endpoint

CHEMICAL	D9_MHC/Cell	D9_Cell#	D4_GSC/Cell	D4_Cell#
Triphenyltin hydroxide (TPTH)	-	0.0588	0.0588	0.0588
Tellurium tetrachloride	-	10	10	10
17 alpha-Estradiol (17-alpha ethynlestradiol)	10	-	-	100
Benzyl chloride	10	100	100	100
Butylated hydroxyanisole	10	-	-	100
Chlorate-Sodium	-	100	-	100
Cyanotoxins (Microcystin-LR only)	-	0.0001	0.0001	0.0001
Disulfoton	-	100	100	100
Estradiol (17-beta estradiol)	-	100	-	100
Estriol	-	100	-	100
Ethinyl Estradiol (17-alpha Ethinyl Estradiol)	5	-	-	50
Nonylphenol (4-N-Nonylphenol)	-	1	1	1
Norethindrone (19-Norethisterone)	2.0833	-	-	20.833
Profenofos	-	100	100	100
Tebuconazole	-	100	-	100
Thiodicarb	-	4.77	4.77	4.77
Thiophanate-methyl	-	100	100	100
4,4'-Methylenedianiline	-	100	100	-
Cumene hydroperoxide	100	-	-	-
Erythromycin	50	-	-	-
Estrone	2	-	-	-
Bromochloromethane	-	100	-	-
N-Nitrosodiphenylamine	100	-	-	-
Oxyfluorfen	-	-	100	-
Permethrin	10.2	-	-	-

- indicates no effects produced

Clusters of ToxPIs are based on the endpoints affected by Chemicals



Conclusions

- These studies provide important information of the bioactivity of chemicals on the CCL4 list in a developmental system.
- Similarities in mESC responses may aid in identifying the molecular initiating events of the adverse outcome pathways for certain chemicals.
- Predictive models for developmental toxicity may use mESC results as part of a comprehensive assessment of *in vitro* effects of chemicals

Reference

Barrier M, et al. 2011. Reprod Toxicol 31 (4): 383-391.