A population screen of chemical toxicity using high-throughput phenotypic profiling (HTPP) in Diversity Outbred neural progenitor cells

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Background

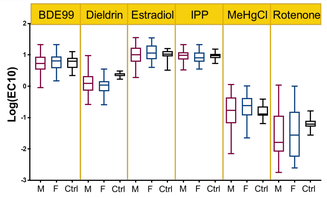
Individuals differ in susceptibility to developmental neurotoxicity

- Early life exposures to chemicals have potential to cause developmental neurotoxicity (DNT).
- Genetics can play a role in susceptibility. Genetically diverse mouse populations, such as the Diversity Outbred (DO), provide a platform to mimic human genetic heterogeneity.
- A panel of 200 DO mouse neural progenitor cells (NPCs; Predictive Biology) has recently become available to test inter-individual susceptibilities in DNT potential of environmental chemicals and drugs.

Toxicodynamic Variability Factors (TDVFs)

- A toxicodynamic variability factor (TDVF) is a chemical specific adjustment factor that quantifies differences in toxicodynamic responses. Data from DO NPCs can be leveraged to calculate a chemical-specific uncertainty factor using a Bayesian approach as in our pilot study (below).

Pilot Data: Cytotoxicity (Alamar Blue) in DO NPC Lines



M: Male lines, F: Female lines, Ctrl: Reference line

Chemical-specific uncertainty factors

Chemical	TDVF05 (90% CI)	
	DO Mouse NPCs	Human LCLs[1]
IPP	1.71 (1.60, 1.86)	-
Estradiol	1.82 (1.66, 2.05)	-
BDE99	2.39 (2.00, 2.96)	-
Dieldrin	2.80 (2.42, 3.33)	3.76
Default factor = 3.16		
Rotenone	11.2 (7.51, 19.1)	-
MeHgCl	26.9 (10.3, 109)	16.03

[1] Chiu WA, et al. ALTEX, 2017. 34(3): 377-388

LCL: lymphoblastoid cell lines

Chemical Exposures and Analysis

Test Chemical	Concentration (uM)	
	Lowest	Highest
BDE99	0.0002	20
Dieldrin	0.00025	25
IPP	0.0005	50
MeHgCl	0.00002	2
Rotenone	0.0002	20
5-Fluorouracil	0.0002	20
Hexachlorophene	0.0002	20
Captan	0.0002	20
Tebuconazole	0.0002	20
p-nitrosodiphenylamine	0.0002	20
Bisphenol A	0.0002	20
Saccharin	0.001	100

Fluorescent Labels
DNA: H-33342
RNA: SYTO14
ER: Concanavalin A-488
Actin: Phalloidin-568
Golgi + Membrane: Wheat Germ Agglutinin - 555
Mitochondria: MitoTracker

Cell Lines: 98 Diversity Outbred neural progenitor cell lines (male and female). Reference cell line included on every test plate. All conditions in triplicate wells.

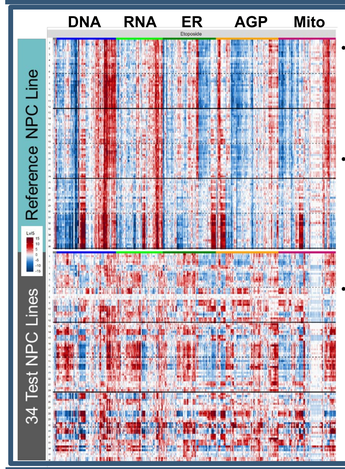
Exposure: 12 chemicals were tested across cell lines, with concentration ranges empirically determined in pilot experiments. These included priority compounds for the NTP and EPA for developmental neurotoxicity testing and putative negative control saccharin. Vehicle: DMSO 0.1%

Assay Control Chemicals: Etoposide, berberine chloride, and rapamycin were included on each plate.

Cell Painting: Cells were fixed and labeled 24 h post-exposure according to Bray et al. 2016 and updated in Nyffeler et al. 2020. Images were acquired using the Opera Phenix. Cells were segmented and cell compartments were profiled (1300 features).

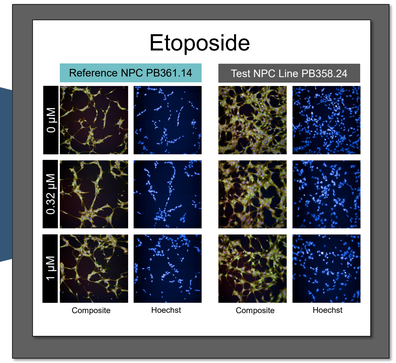
Analysis: Global Mahalanobis distance and concentration-response modeling for potency estimates.

Inter-individual Variation in Chemical-Affected Cellular Compartments



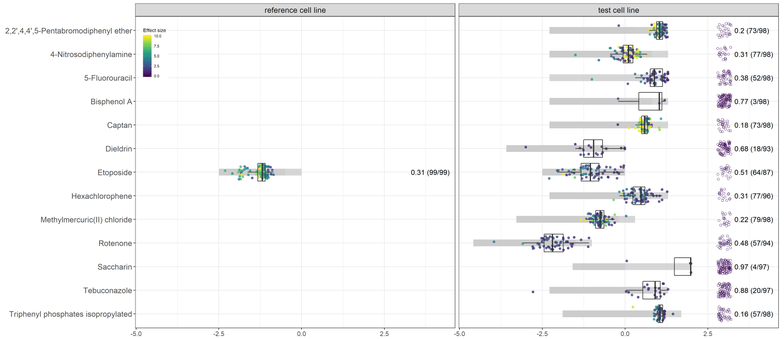
- Heatmap indicates the biological effect size at 1 µM etoposide, with row numbers corresponding to test plates.
- Reference cell line (PB361.14) is included on every test cell plate as an experimental control. Figure displays a subset of 34 DO NPC lines.
- Affected intracellular compartments are consistent for reference cell line, but differ across test cell lines. **This suggests that test cell lines have differential responses associated with etoposide.**

Etoposide heatmap & photomicrographs

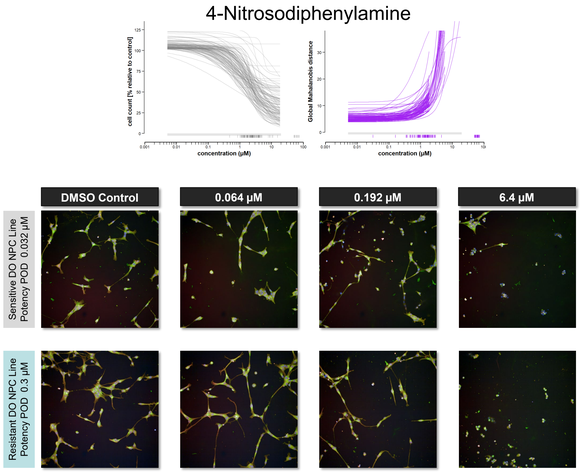


- Etoposide inhibits Topoisomerase II, an enzyme that keeps DNA in the proper shape during cell division.
- Shown side-by-side are the same visual field including all channels (composite) or the Hoechst channel only (20X).
- In highly proliferative NPC line PB358.24, changes in nuclear morphology are readily apparent at 0.32 µM as compared to the reference cell line, which proliferates more slowly.
- Nuclear effects are apparent in both cell lines at 1 µM.

Variability in Biological Potency



- Above: Biological potency POD for each cell line (dots) is shown for all tested chemicals as compared to the tested concentration range (gray bar). Color corresponds to the magnitude of biological effect from low (purple) to high (yellow).
- Right: Selected data for p-nitrosodiphenylamine (4-NDA) and methylmercuric(II) chloride (MeHgCl). [Top] Concentration response curves are shown for all tested DO NPC lines for cell viability and Global Mahalanobis distance (*i.e.* phenotypic effects). [Bottom] Representative photomicrographs are shown for a sensitive and resistant cell line using the 20X objective.



Conclusions

- A panel of Diversity Outbred mouse NPC lines allows for detection of chemicals with a high degree of variability in potency across a genetically diverse population.
- Chemicals with limited biological activity in a small number of lines included saccharin (putative negative control) and bisphenol A.
- Across chemicals tested, a wide degree of inter-individual variability in biological potency was observed for some chemicals (*e.g.* hexachlorophene), while others exhibited a narrower potency range across lines (*e.g.* captan), providing support for a derivation of chemical-specific uncertainty factors.
- Future work: Ongoing analysis to identify chemical- and individual-specific modes of action and calculation of data-driven uncertainty factors that describe inter-individual variability for each chemical.

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