Population Variability in Neurotoxicity Outcomes Modeled In Vitro with Diversity Outbred Neural Progenitor Cells

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Background

Challenges in Testing Developmental Neurotoxicity (DNT)

- Early life exposures to chemicals have potential to cause developmental neurotoxicity (DNT), and DNT remains one of the most challenging health effects of chemicals to study[1]
- Susceptibility can be affected by both the developmental stage of the fetus/neonate and **the genetic background of the individual**[2,3]
- Improving DNT testing has been identified as **a priority area for the NTP as a new Health Effect Innovation hub and by the OECD**, which recently established a working group focused on DNT testing guidance

Toxicodynamic Variability Factors (TDVFs)

- A toxicodynamic variability factor (TDVF) can be a chemical specific adjustment factor that **quantifies interindividual differences in toxicodynamic responses** based on the chemical-specific data collected across a population of individuals[4]

Utilization of the Diversity Outbred (DO) Mouse Population

- Diversity Outbred (DO) mouse population is a genetically heterogeneous population designed to **mimic human genetic diversity** that provides a unique opportunity for assessing the TDVF and population-based points of departure[5,6]
- DO mice offer an opportunity to identify mode of action and small, refined transcript co-expression networks that underlie interindividual susceptibility

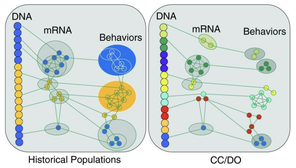

Figure 1. The fine recombination structure of the DO genome allows for precision detection of transcripts that regulate adverse neurologic responses[7]

Study Aims

1. To evaluate the utility of the **DO NPC lines as a population-based assay** to quantify interindividual variability in dose-response effects of DNT agents and to subsequently produce **data-driven uncertainty factors** that better protect sensitive subpopulations
2. To determine the **intracellular mechanisms and pathways** critical for susceptibility of sensitive subpopulations to DNT agents

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References

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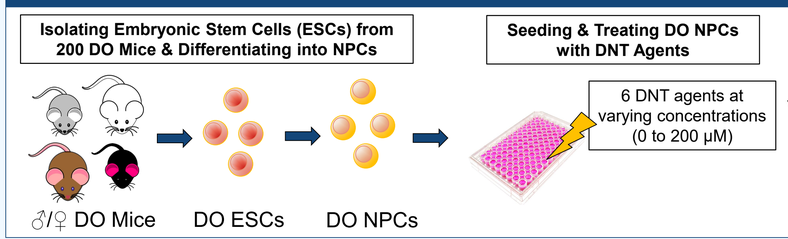
Diversity Outbred genetically diverse mouse model can quantitatively assess interindividual variability of developmental neurotoxicity relevant to human populations and improve the protection of genetically sensitive individuals.

Study Design



Variability in Cytotoxicity to DNT Agents in DO NPCs

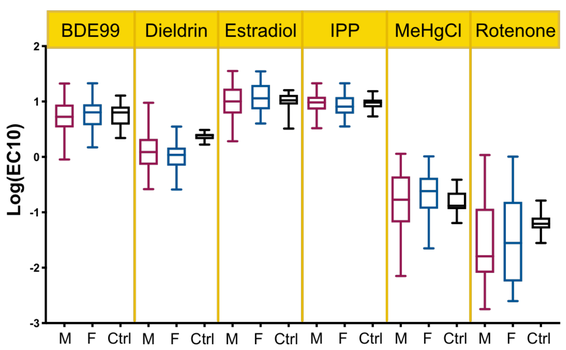


Figure 2. Cytotoxicity of DNT Agents in DO NPCs
Log-transformed EC10 (µM) ranges of the cytotoxicity of DNT agents at 114 h are compared between a single batch control DO NPC (Ctrl, Black, PB361.14, n=12-13) and a diverse panel of male (M, Red, n=43-51) and female (F, Blue, n=31-36) DO NPC lines. Wider distributions are observed for the DO NPC lines, implying the contribution of genetic variants to differential sensitivities to the chemical agents. We observed that rotenone and methyl mercury display wide interindividual toxicodynamic variability.

Chemical	Rationale
2,2',4,4',5-pentabromodiphenyl ether (BDE99)	Flame retardant; NTP programmatic interest
Dieldrin	Known DNT/NT Potential
Ethinyl estradiol (Estradiol)	Other
Phenol, isopropylated, phosphate (3:1) (IPP)	Flame retardant; NTP programmatic interest
Methyl mercuric (II) chloride (MeHgCl)	Known DNT/NT Potential
Rotenone	Known DNT/NT Potential

Table 1. Chemical agents used in cytotoxicity screening in DO NPCs

Chemical	TDVF05 (90% CI)	
	DO Mouse NPCs	Human Lymphoblastoid Cell Lines[1]
IPP	1.71 (1.60, 1.86)	-
Estradiol	1.82 (1.66, 2.05)	-
BDE 99	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

Table 2. DO TDVF and confidence intervals (CIs) for cytotoxicity potency of the DNT agents at 114 h. – indicates no available human data;

Validation of TempO-Seq® Mouse S1500+

Approach

RNA-Seq

- Aligned to mm10 using STAR
- TPM Normalization

TempO-Seq® S1500+

- Aligned using Bowtie
- TPM Normalization after adjusting for attenuation

SNP Assessment in Probes

- Utilized the Giga Mouse Universal Genotyping Array (GigaMUGA)

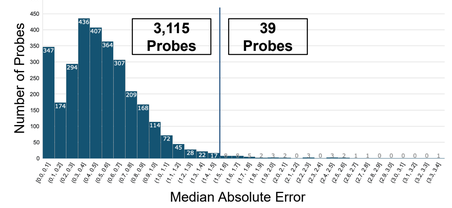


Figure 3. Mean Absolute Error for 36 Pairwise Comparisons of 9 DO NPC lines in Two Sequencing Platforms. 3,115 of total 3,154 probes passed MAE < 1.5 criteria, i.e., at least 18 out of 36 pairwise comparisons resulted in a TempO-Seq vs. RNA-seq fold change ratio less than 2.8).

TempO-seq Probe	Probe Chrom	Probe Start	Probe Stop	GigaMUGA SNP	SNP Position	Ref Base	Alt Base
Myom2_29260	chr8	15132719	15132768	**UNCHS022272**	chr8:15132764	T	C
Csrp1_30002	chr1	135720103	135720152	**UNCHS002491**	chr1:135720131	A	G
Cfd_30145	chr10	79892140	79892189	**UNCrs221128740**	chr10:79892183	G	A
Smc3_30235	chr19	53640872	53640921	**UNC30509226**	chr19:53640883	G	A
Maoa_30665	chrX	16672887	16672936	**JAX00709767**	chrX:16672929	G	A
Itgal_30744	chr7	127328701	127328750	**UNC13812014**	chr7:127328701	C	T
Naa15_31429	chr3	51415237	51415286	**UNC5250424**	chr3:51415249	G	A
Myt1_32190	chr2	181763376	181763425	**UNC4608520**	chr2:181763387	C	T
C8b_32265	chr4	104791835	104791884	**UNCrs28158610**	chr4:104791883	T	G

Table 3. List of Probes with SNPs Found in the Cell Lines in the TempO-Seq Validation Study. 11 of 3,154 mouse S1500+ probes are at the locations overlapping with 12 SNP locations identified by GigaMUGA (of which 10 are present in the study NPC lines). 9 SNPs are present in at least one of the cell lines included in the validation. Only 1 SNP was among the poorly performing probes (yellow).

Cell Painting: High-Content Imaging for Morphometric Assessment

Cell Painting Workflow

-18h	Cell Plating
0h	Dispensing Chemicals
24h	Fixation & labelling
High-content Imaging & Data analysis	

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

Assay Optimization

- Optimal DO NPC seeding density for 24 h treatment window was determined (2,000 cells/well).

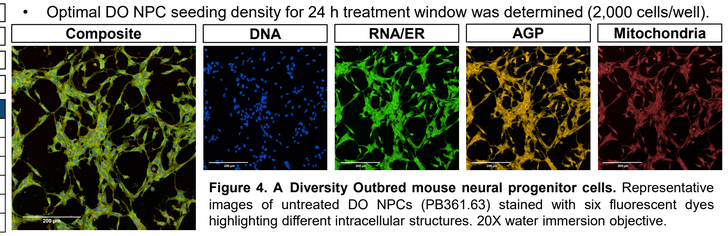


Figure 4. A Diversity Outbred mouse neural progenitor cells. Representative images of untreated DO NPCs (PB361.63) stained with six fluorescent dyes highlighting different intracellular structures. 20X water immersion objective.

Future Directions

- Gene- and pathway-based benchmark concentration analysis
- Calculating TDVF for sensitive molecular endpoints
- Comparing DO NPCs and human NPCs
- Classification and regression tree (CART) machine learning analysis to identify sensitive biomarkers reflective of the toxicity/susceptibility