High-throughput phenotypic profiling approach for the screening and prioritization of potential developmental neurotoxicity hazard

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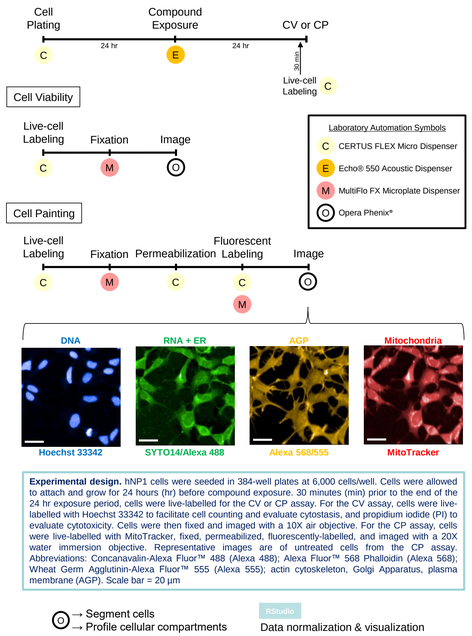
Background

- Numerous environmental chemicals lack data on potential developmental neurotoxicity (DNT) hazard.
- As such, reliable and efficient new approach methods (NAMs) are needed to fill this critical data gap.
- For this purpose, our laboratory adapted a previously established high-throughput phenotypic profiling (HTPP) NAM for use with proliferating hNP1 human neural progenitor cells Culbreth et al., 2022 (https://doi.org/10.3389/ftox.2021.803987).
- HTPP integrates cell viability (CV) and cell painting (CP) assay data to determine a benchmark concentration (BMC) for chemical effects.
- Presently, 282 DNT-relevant compounds have been evaluated in the hNP1 HTPP approach to determine whether this NAM can identify potential DNT hazard.
- These compounds include some with evidence for DNT in humans and *in vivo* (reference "positives"), as well as some with no evidence for DNT (reference "negatives").

Experimental Design

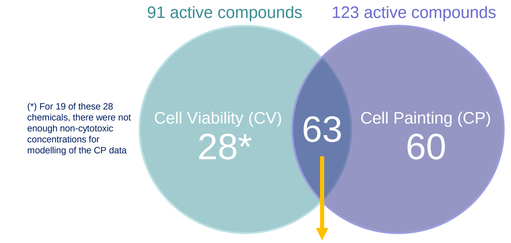


Experimental design. hNP1 cells were seeded in 384-well plates at 6,000 cells/well. Cells were allowed to attach and grow for 24 hours (hr) before compound exposure. 30 minutes (min) prior to the end of the 24 hr exposure period, cells were live-labelled for the CV or CP assay. For the CV assay, cells were live-labelled with Hoechst 33342 to facilitate cell counting and evaluate cytostasis, and propidium iodide (PI) to evaluate cytotoxicity. Cells were then fixed and imaged with a 10X air objective. For the CP assay, cells were live-labelled with MitoTracker, fixed, permeabilized, fluorescently-labelled, and imaged with a 20X water immersion objective. Representative images are of untreated cells from the CP assay. Abbreviations: Concanavalin-Alexa Fluor™ 488 (Alexa 488); Alexa Fluor™ 568 Phalloidin (Alexa 568); Wheat Germ Agglutinin-Alexa Fluor™ 555 (Alexa 555); actin cytoskeleton; Golgi Apparatus, plasma membrane (AGP). Scale bar = 20 µm

O → Segment cells
→ Profile cellular compartments

RStudio
Data normalization & visualization

151 of 282 compounds evaluated were active in the human neural progenitor cell HTPP approach

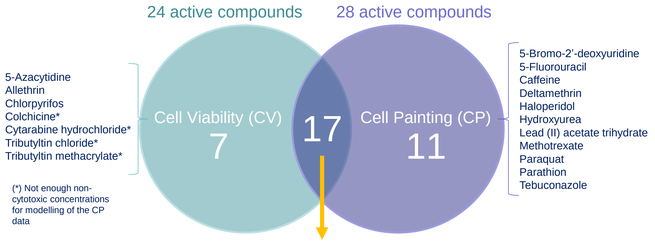


(*) For 19 of these 28 chemicals, there were not enough non-cytotoxic concentrations for modelling of the CP data

		Mean	Median	SD	Min	Max
Selective	70% (44/63)	11.4	1.82	53.9	1.01	360
Non-selective	30% (19/63)	0.703	0.701	0.171	0.416	0.989

Selective: CP BMC < CV BMC; Non-selective: CP BMC > CV BMC; Mean, Median, SD, Min, & Max of the ratio of CV BMC/CP BMC
Abbreviations: standard deviation (SD), minimum (Min), maximum (Max)

Human neural progenitor cell HTPP identified 35 of the 57 DNT reference positive[1] compounds screened

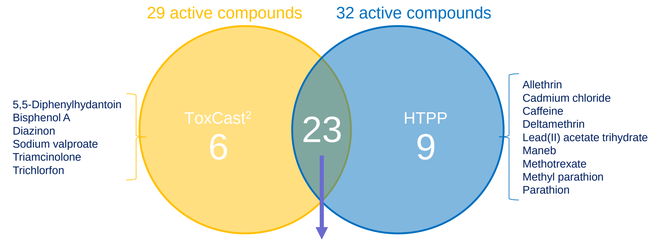


(*) Not enough non-cytotoxic concentrations for modelling of the CP data

		Mean	Median	SD	Min	Max
Selective	71% (12/17)	5.13	2.32	7.23	1.01	26.2
Non-selective	29% (5/17)	0.714	0.747	0.188	0.480	0.956

Selective: CP BMC < CV BMC; Non-selective: CP BMC > CV BMC; Mean, Median, SD, Min, & Max of the ratio of CV BMC/CP BMC
Abbreviations: standard deviation (SD), minimum (Min), maximum (Max)

53 DNT reference positive[1] compounds screened in both hNP1 DNT ToxCast assays[2,3] and the HTPP approach



HTPP BMC < ToxCast AC_{50}: 15 of 23 compounds

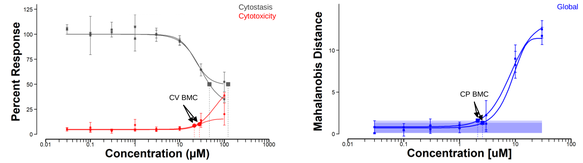
3 of the 35 DNT reference negative[4] compounds were HTPP false-positives

		HTPP BMC (µM)		hNP1 DNT ToxCast AC_{50} (µM)[2]		
Chemical Name	**DTXSID**	**CV[a]**	**CP[b]**	**Viability**	**Proliferation**	**Apoptosis**
Acetaminophen	DTXSID2020006	-	-	NT	-	-
Amoxicillin	DTXSID3037044	-	-	NT	-	-
Ampicillin	DTXSID4022602	-	-	-	-	-
Anthracene	DTXSID0023878	-	-	-	-	-
Aspirin	DTXSID5020108	-	-	-	-	-
Buspirone	DTXSID2022707	-	80.9	-	-	-
Captopril	DTXSID1037197	-	-	-	-	-
Chloramben	DTXSID2020262	-	-	-	-	-
Chlorpheniramine maleate	DTXSID4020321	-	-	-	-	-
Cotinine	DTXSID1047576	-	-	-	-	-
D-Glucitol	DTXSID5023588	-	-	NT	-	-
Diethylene glycol	DTXSID8020462	-	-	-	-	-
Dinotefuran	DTXSID7034549	-	-	-	-	-
D-Mannitol	DTXSID1023235	-	-	-	-	-
Doxylamine succinate	DTXSID7020552	-	-	-	-	-
Erythromycin	DTXSID4022991	-	-	-	-	-
Famotidine	DTXSID5023039	-	-	-	-	-
Fluconazole	DTXSID3020627	-	-	-	-	-
Folic acid	DTXSID0022519	-	-	NT	NT	NT
Galactosamine hydrochloride	DTXSID4031356	-	-	-	-	-
Glycerol	DTXSID9020663	-	-	-	-	-
Ibuprofen	DTXSID5020732	-	-	-	-	-
Isoniazid	DTXSID8020755	-	-	-	-	-
L-Ascorbic acid	DTXSID5020106	-	45.5	-	-	-
Metformin	DTXSID2023270	-	-	-	-	-
Metoprolol	DTXSID2023309	-	-	5.41	-	-
Mifepristone	DTXSID5023322	-	19.9	12.4	16.6	20.3
Penicillin VK	DTXSID7021102	-	-	-	-	-
Phenol	DTXSID5021124	-	-	-	-	-
Selegiline hydrochloride	DTXSID9044584	-	-	-	-	-
Sodium benzoate	DTXSID1020140	-	-	-	-	-
Sodium saccharin hydrate	DTXSID7021992	-	-	NT	-	-
Sulfisoxazole	DTXSID6021292	-	-	-	-	-
Tetracycline	DTXSID7023645	-	-	-	-	-
Warfarin	DTXSID5023742	-	-	-	-	-

[a]cytostatic BMC (BMR = EC_{10}) or cytotoxicity BMC (BMR = 3*nMAD); [b]global Mahalanobis distance approach
Abbreviations: DSSTox substance identifier (DTXSID); micromolar (µM); activity concentration at 50% of the maximal activity (AC_{50}); not active (-), not tested or not reported in ToxCast (NT), benchmark response (BMR), 50% maximal effective concentration (EC_{50}), normalized median absolute deviation (nMAD)

12 compounds screened in technical duplicate[a] elicited a reproducible HTPP response

Hexachlorophene



Hexachlorophene HTPP. Each concentration-response curve represents data from 3 biological replicates (error bars) and 1 technical replicate/biological replicate. Cytostasis (normalized cell count) and cytotoxicity (PI-positive cells) are depicted on the left and phenotypic profile (global Mahalanobis distance) on the right. Enlarged shapes are the BMC for cytostasis (BMR = EC_{10}), cytotoxicity (BMR = 3*nMAD), and phenotypic profiling (BMR = 1*nMAD), respectively. The rectangles on the phenotypic profile graph illustrate the concentration range tested in the horizontal direction and ±1*nMAD of the vehicle control in the vertical direction.

			HTPP BMC (µM)	
Chemical Name	**DTXSID**	**Type**	**CV[b]**	**CP[c]**
5-Fluorouracil	DTXSID2020634	DNT Reference positive[1]	-	6.16
			-	10.9
Colchicine	DTXSID5024845	DNT Reference positive[1]	0.0244	*
			0.0193	*
Cytarabine	DTXSID3022877	DNT Reference positive[1]	0.248	-
			-	0.00947
Di(2-ethylhexyl) phthalate	DTXSID5020607	DNT Reference positive[1]	4.8	-
			6.83	2.49
Dieldrin	DTXSID9020453	DNT Reference positive[1]	37.6	22.6
			28.6	10.2
Hexachlorophene	DTXSID6020690	DNT Reference positive[1]	27.7	2.12
			21.3	2.58
Hydroxyurea	DTXSID6025438	DNT Reference positive[1]	-	97.2
			-	-
Methamidophos	DTXSID6024177	OP-pesticide	-	-
			-	-
Naled	DTXSID1024209	OP-pesticide	20.3	25.6
			24.7	-
Phenol	DTXSID5021124	DNT Reference negative[2]	-	-
			-	-
Tebuconazole	DTXSID9032113	DNT Reference positive[1]	-	88
			-	52.4
Tributyltin methacrylate	DTXSID9035204	DNT Reference positive[1]	0.213	*
			0.0357	*

[a]3 biological replicates and 2 technical replicates/biological replicate; [b]cytotoxicity BMC (BMR = 3*nMAD); [c]global Mahalanobis distance approach; (*) Not enough non-cytotoxic concentrations for modelling of the CP data
Abbreviations: organophosphate (OP)

Conclusions

- hNP1 HTPP is an efficient NAM for the prioritization of potential DNT hazard.
- This approach can identify both known DNT reference positive and negative compounds.
- DNT reference positive and negative compounds are detected at a similar rate to previously established hNP1 DNT ToxCast assays; however, hNP1 HTPP is higher throughput and more cost effective.
- Future analyses will examine potential similarities in phenotypic profiles within compound groups (e.g., organophosphates)

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References: [1]Mundy et al., 2015 (https://doi.org/10.1016/j.ntt.2015.10.001); [2]invitrodb version 3.5 database (https://doi.org/10.23645/epacomptox.6062623.v8), ToxCast pipeline (tcpl) R package (version 2.1.0) (https://cran.r-project.org/web/packages/tcpl/index.html); [3]Harrill et al., 2018 (https://doi.org/10.1016/j.taap.2018.04.001) [4]Martin et al., 2022 (https://doi.org/10.1016/j.ntt.2022.107117)