Chen et al.:

Rapid Hazard Characterization of Environmental Chemicals Using a Compendium of Human Cell Lines from Different Organs

Supplementary Data

Text S1: Detailed cell culture procedures

iCell hepatocytes 2.0 (Grimm et al., 2016)

Vials of hepatocytes were thawed for 3 min at 37°C in a water bath and subsequently resuspended in RPMI medium containing 2% (v/v) iCell hepatocyte medium supplement, 0.1 µM dexamethasone, 2% (v/v) B27 supplement, 25 µg/mL gentamicin, and 20 ng/mL Oncostatin-M. Following microscopic evaluation of the cell density, the suspension was further diluted to a final concentration of 6.72×10^5 cells/mL. 25 µL of this suspension was then added to each well on collagen I coated 384-well plates (Corning, Product# 354664), yielding a final cell density of 16,800 cells per well. Plates were initially kept at room temperature (RT) for 30 min and then transferred to an incubator set at 37°C and 5% CO_2. After 4 h of incubation, the plating medium was replaced with 25 µL fresh medium, a step that was repeated daily for 4 days. On day five, the plating medium was exchanged with 25 µL per well maintenance medium, consisting of RPMI containing 2% (v/v) iCell hepatocyte medium supplement, 0.1 µM dexamethasone, 2% (v/v) B27 supplement, and 25 µg/mL gentamicin. Maintenance medium was exchanged daily for the duration of the experiment.

iCell Neurons (Sirenko et al., 2014):

Cryopreserved cells were thawed and plated according to the protocol provided by Cellular Dynamics International. Briefly, cells were plated on poly-d-lysine precoated 384-well plates (Greiner-Bio, Ref#: 781946) with iCell Neural Base Medium (Catelog#: M1010) added with iCell Neural Supplement A (Catalog#: M1032) and 3.3 mg/mL of laminin. Cells were plated at densities of 7,500 cells/well. Plates were initially kept at RT for 30 min before transferring to an incubator set at 37°C and 5% CO_2 for 48 h until assay day.

iCell Cardiomyocytes (Grimm et al., 2016)

384-well microplates were precoated with 25 µL 0.1% (w/v) gelatin solution per well for 2 h at 37°C and 5% CO_2. Cryopreserved cells were thawed according to the manufacturer's instruction using iCell cardiomyocyte plating medium with 1:500 (v/v) penicillin/streptomycin. Cell suspension was diluted in plate medium to provide a final cell concentration of 2×10^5 cells/mL. Subsequently, the gelatin solution was aspirated from the plates and 25 µL cell suspension was added to each well, making the final cell plating density 5000 viable cells/well. Plates were kept at RT for 30 min before they were incubated at 37°C and 5% CO_2. 48 h following cell seeding, the plating medium was exchanged with 40 µL of maintenance medium containing 1:500 penicillin/streptomycin. Maintenance medium was subsequently changed every other day for another 12 days until assay day.

iCell Endothelial cells (Iwata et al., 2017)

Endothelial cells were plated and expanded on T-75 tissue culture flasks coated with human fibronectin solution at 3 µg/cm^2. Cells were cultured with maintenance medium containing the VascuLife VEGF Medium Complete Kit (SKU: LL-0003), with FBS, and iCell Endothelial cells medium supplement. Cell density was determined using Trypan Blue exclusion test and a cell suspension was prepared that resulted in 1.0×10^4 cells/cm^2. The fibronectin solution was aspirated and cells were seeded in a T-75 flask. Cells were incubated at 37°C and 5% CO_2 with media changes every 2 days and passaged every 3-4 days by TrypLE Express. Experiments were conducted with cells between passages 1 and 5. Cells were transferred into 384-well plates with 50 µL maintenance medium at a density of 750 cells/well for cytotoxicity assay and 7,500 cells for angiogenesis assay. Cells were kept in microplates for 2-3 days until a monolayer formed before adding chemicals for cytotoxicity assays.

Human Umbilical Vein Endothelial Cells (Iwata et al., 2017): HUVECs were plated and expanded on T-75 tissue culture flasks coated with 0.1% (w/v) gelatin solution. The culture medium contains Medium 199 with the EGM-2 BulletKit (Lonza, Catalog#: CC-3162). HUVECs were incubated at 37°C and 5% CO_2 and passaged every 2-3 days using TrypLE Express. Cell density was determined by cell counting with Trypan Blue. Experiments were performed with cells between passages 1 and 5. Cells were transferred into 384-well plates with 50 µL maintenance medium at a density of 750

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cells/well for cytotoxicity assay and 3,500 cells for angiogenesis assay. Cells were kept in microplates for 2-3 days until a monolayer formed before adding chemicals for cytotoxicity assays.

Text S2: ATP production of iCell neurons and HUVECs

Production of ATP in iCell neurons and HUVECs was measured using CellTiter-Glo® Luminescent Cell Viability Assay according to the manufacturer's instructions. In detail, after the high content imaging process, equal volumes of pre-equilibrated CellTiter-Glo reagent were added into each well in assay plates. Then contents were mixed for 2 min on an orbital shaker to induce cell lysis and plates were allowed to incubate at RT for 10 min to stabilize the luminescent signal. Luminescence was read using a FLIPR tetra (Molecular Devices) instrument, with a read time interval of 1 s per well. Quantitative data was exported for concentration-response profiling.

Text S3: Calcium flux assay of iCell cardiomyocytes

Intracellular calcium flux in iCell cardiomyocytes exposed to the test solutions for 15 and 90 min was measured using a FLIPR tetra (Molecular Devices) instrument using the EarlyTox™ Cardiotoxicity Kit as described in a previous study (Grimm et al., 2016). Cardiomyocytes were incubated for 2 h at 37°C after the addition of one volume of pre-equilibrated calcium-dye reagent. Prior to exposure to test solutions, baseline calcium flux measurements were recorded at 515-575 nm following excitation at 470-495 nm and at a frequency of 8 Hz for 100 s. The internal instrument temperature was regulated at 37°C. Cells were then simultaneously exposed to test solutions using the internal fluidics handling system. 15- and 90-min post-exposure, the beating of cardiomyocytes was monitored as described above. Between measurements, cells were incubated under cell culture conditions at 37°C and 5% CO_2. Recorded data were further analyzed in Screenworks 4.0 software (Molecular Devices LLC., Sunnyvale, CA) for peak processing, and statistical parameters were exported as Microsoft Excel files for concentration-response assessment.

Text S4: Angiogenesis of iCell endothelial cells and HUVECs

Angiogenic assays were performed using Geltrex LDEV-Free Reduced Growth Factor Basement Membrane for both iCell endothelial cells and HUVECs in 384-well format according to our previous study (Iwata et al., 2017). iCell endothelial cells were incubated with VascuLife® Basal Medium containing 4 nM L-glutamine LifeFactor and 0.1% iCell Endothelial Cells Medium Supplement. HUVECs were incubated with Medium 199 containing the EGM-2 BulletKits at 2X concentration; also the VEGF component was replaced with 12.5 ng/mL VEGF, and this was referred to as "2 X Assay Medium." Geltrex was thawed at 4°C and dispensed to coat the plates (10 µL/well) on ice. The plates were incubated for 1 h at 37°C. Following the incubation, a 2X chemical working solution (25 µL/well), prepared in basal medium, was added to the plate and cells resuspended in 2X assay medium (25 µL/well) were seeded at a density of 7,500 (iCell-ECs) or 3,500 (HUVECs) cells/well. Cells were exposed to chemicals overnight at 37°C at 5% CO_2 and stained with Calcein AM (25 µL/well, 6 µmol/L) for 15 min and processed to live cell high-content imaging.

References

Grimm, F. A., Iwata, Y., Sirenko, O. et al. (2016). A chemical-biological similarity-based grouping of complex substances as a prototype approach for evaluating chemical alternatives. *Green Chem 18*, 4407-4419. doi:10.1039/c6gc01147k

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Sirenko, O., Hesley, J., Rusyn, I. et al. (2014). High-content high-throughput assays for characterizing the viability and morphology of human iPSC-derived neuronal cultures. *Assay Drug Dev Technol 12*, 536-547. doi:10.1089/adt.2014.592

Tab. S1: Summary of the quality control parameters evaluated for each cell type and phenotype

Cell Type	Phenotype	CV% medium	CV% DMSO	t-test p-value	Intra-plate replicates (n = 60)				Inter-plate replicates (n = 210)			
					Pearson (r)	p-value	Spearman (ρ)	p-value	Pearson (r)	p-value	Spearman (ρ)	p-value
iCell hepatocytes	Cell number	5.15	4.34	0.70	0.84	<0.0001	0.52	<0.0001	0.84	<0.0001	0.36	<0.0001
	Nuclei intensity	2.77	2.07	0.05	0.84	<0.0001	0.30	0.02	0.69	<0.0001	0.37	<0.0001
	All cell mean area	8.86	11.58	0.22	0.27	0.03	0.01	0.94	0.42	<0.0001	0.34	<0.0001
	Mitochondrial intensity	10.32	13.31	0.33	0.40	0.00	-0.01	0.94	0.46	<0.0001	0.31	<0.0001
	Mitochondrial integrity	4.64	4.18	0.32	0.79	<0.0001	0.10	0.43	0.83	<0.0001	0.36	<0.0001
iCell neurons	Cell number	8.68	12.42	0.34	-0.15	0.25	-0.33	0.01	0.77	<0.0001	0.44	<0.0001
	Total outgrowth	12.50	17.93	0.80	0.35	0.01	0.20	0.13	0.75	<0.0001	0.52	<0.0001
	Mean outgrowth	12.26	12.33	0.10	0.32	0.01	0.19	0.14	0.73	<0.0001	0.43	<0.0001
	Total process	11.15	12.82	0.78	-0.07	0.60	-0.30	0.02	0.75	<0.0001	0.38	<0.0001
	Total branches	27.44	26.82	0.93	0.18	0.17	0.17	0.20	0.60	<0.0001	0.47	<0.0001
	Total cell body area	8.63	9.46	0.02	-0.08	0.55	-0.21	0.11	0.78	<0.0001	0.37	<0.0001
	Cell with significant growth	8.67	12.64	0.33	-0.15	0.24	-0.34	0.01	0.77	<0.0001	0.44	<0.0001
	Cytoplasmic integrity	11.15	14.59	0.61	-0.08	0.55	-0.32	0.01	0.75	<0.0001	0.42	<0.0001
	Mitochondrial integrity	10.78	15.25	0.22	-0.03	0.84	-0.31	0.02	0.71	<0.0001	0.41	<0.0001
	ATP	15.09	10.75	0.88	0.50	<0.0001	0.14	0.28	0.85	<0.0001	0.68	<0.0001
iCell cardio-myocytes	Beats per minute_15min	18.11	14.44	<0.01	0.75	<0.0001	0.54	<0.0001	0.88	<0.0001	0.67	<0.0001
	Beats per minute_90min	14.68	14.40	0.48	0.83	<0.0001	0.65	<0.0001	0.86	<0.0001	0.70	<0.0001
	Cell number	9.45	8.05	0.05	0.59	<0.0001	0.15	0.25	0.71	<0.0001	0.54	<0.0001
	Mitochondrial integrity	11.36	10.46	0.03	0.57	<0.0001	0.16	0.22	0.71	<0.0001	0.58	<0.0001
	Peak amplitute_15min	17.56	17.05	0.25	0.82	<0.0001	0.31	0.01	0.89	<0.0001	0.67	<0.0001
	Peak amplitute_90min	16.38	15.12	0.29	0.80	<0.0001	0.33	0.01	0.87	<0.0001	0.65	<0.0001
	Peak spacing_15min	13.03	16.37	<0.01	0.76	<0.0001	0.40	0.00	0.86	<0.0001	0.64	<0.0001
	Peak spacing_90min	13.28	11.98	0.68	0.86	<0.0001	0.55	<0.0001	0.60	<0.0001	0.67	<0.0001
	Peak width_15min	15.56	18.51	<0.01	0.70	<0.0001	0.40	0.00	0.82	<0.0001	0.65	<0.0001
	Peak width_90min	16.58	15.80	0.47	0.72	<0.0001	0.53	<0.0001	0.82	<0.0001	0.68	<0.0001
	Peak rise time_15min	8.84	9.16	0.01	0.90	<0.0001	0.23	0.07	0.88	<0.0001	0.51	<0.0001
	Peak rise time_90min	8.57	8.07	0.18	0.85	<0.0001	0.42	0.00	0.88	<0.0001	0.54	<0.0001
	Peak decay time_15min	16.77	19.81	<0.01	0.68	<0.0001	0.40	0.00	0.81	<0.0001	0.66	<0.0001
	Peak decay time_90min	18.66	17.70	0.42	0.68	<0.0001	0.53	<0.0001	0.80	<0.0001	0.69	<0.0001
	Decay to rise ratio_15min	18.61	16.73	<0.01	0.67	<0.0001	0.32	0.01	0.83	<0.0001	0.69	<0.0001
	Decay to rise ratio_90min	19.38	16.74	0.14	0.65	<0.0001	0.45	0.00	0.80	<0.0001	0.71	<0.0001
iCell endothelial cells	Cell number	8.24	8.67	0.20	0.58	<0.0001	0.29	0.02	0.79	<0.0001	0.34	<0.0001
	Mitochondrial integrity	8.02	8.72	0.34	0.77	<0.0001	0.28	0.03	0.86	<0.0001	0.33	<0.0001
	Nuclei mean area	2.72	3.03	0.16	0.07	0.59	0.13	0.31	0.62	<0.0001	0.18	0.01
	Mitochondrial intensity	13.12	8.03	<0.01	0.70	<0.0001	0.37	0.00	0.62	<0.0001	0.36	<0.0001
	Cytoplasmic integrity	11.81	9.76	0.06	0.53	<0.0001	0.33	0.01	0.71	<0.0001	0.30	<0.0001
	Total tube length	19.55	13.51	<0.01	0.63	<0.0001	0.37	0.00	0.60	<0.0001	0.57	<0.0001

	Mean tube length	6.81	5.36	0.42	0.63	<0.0001	0.39	0.00	0.26	0.00	0.32	<0.0001
	Total tube area	20.85	14.06	<0.01	0.62	<0.0001	0.41	0.00	0.61	<0.0001	0.61	<0.0001
HUVECs	Cell number	7.05	6.27	0.19	0.61	<0.0001	0.37	0.00	0.79	<0.0001	0.33	<0.0001
	Mitochondrial integrity	6.95	6.06	0.11	0.66	<0.0001	0.37	0.00	0.82	<0.0001	0.36	<0.0001
	Nuclei mean area	3.15	2.80	<0.01	0.95	<0.0001	0.49	<0.0001	0.98	<0.0001	0.36	<0.0001
	Mitochondrial intensity	12.57	6.38	<0.01	0.83	<0.0001	0.29	0.02	0.78	<0.0001	0.31	<0.0001
	Cytoplasmic integrity	7.04	6.24	0.18	0.63	<0.0001	0.37	0.00	0.85	<0.0001	0.38	<0.0001
	Total tube length	13.36	8.77	0.28	0.63	<0.0001	0.27	0.04	0.61	<0.0001	0.61	<0.0001
	Mean tube length	6.62	5.90	<0.01	0.77	<0.0001	0.10	0.43	0.76	<0.0001	0.39	<0.0001
	Total tube area	10.90	8.51	0.63	0.74	<0.0001	0.29	0.02	0.62	<0.0001	0.56	<0.0001
	ATP	2.88	5.20	0.82	0.88	<0.0001	0.07	0.61	1.00	<0.0001	0.99	<0.0001

Tab. S2: EC_{50} values (µM) of positive controls in five tested cell types

Cell type	Phenotype	TAB[a]	Doxorubicin (10)[b]	Brefeldin A (10)	Mitomycin C (100)	Retinoic acid (250)	Rotenone (50)	Cisapride (10)	Propranolol (50)	Isoproterenol (10)	Nocodazole (20)	Suramin (100)	Chloroquine (1000)	Histamine (400)
iCell hepato-cytes	Cell number	1.58	0.29											
	Nuclei intensity	77.53	2.34											
	All cell mean area	85.13	0.62											
	Mitochondrial intensity	58.40	0.32											
	Mitochondrial integrity	1.67	0.31											
iCell neurons	Cell number	0.00		NA[c]	4.36	NA	8.50							
	Total outgrowth	0.00		0.22	1.93	NA	2.60							
	Mean outgrowth	0.00		0.22	6.50	NA	7.39							
	Total process	0.00		NA	3.57	NA	6.48							
	Total branches	0.00		0.06	1.66	221.30	2.40							
	Total cell body area	0.00		NA	5.31	NA	8.84							
	Cell with significant growth	0.00		NA	3.64	NA	7.86							
	Cytoplasmic integrity	0.00		NA	3.61	NA	6.02							
	Mitochondrial integrity	1.79		NA	6.23	NA	6.01							
	ATP	1.57		NA	0.73	241.80	0.82							
iCell cardiomyocytes	Beats per minute_15min	0.00						0.01	1.29	0.08				
	Beats per minute_90min	0.00						0.73	1.95	0.13				
	Cell number	5.83						NA	NA	NA				
	Mitochondrial integrity	4.48						NA	NA	NA				
	Peak amplitute_15min	0.00						0.00	1.81	0.02				
	Peak amplitute_90min	0.00						0.05	6.56	0.06				
	Peak spacing_15min	0.00						0.00	1.27	0.03				
	Peak spacing_90min	0.00						8.73	2.14	0.55				
	Peak width_15min	0.00						0.00	0.33	0.03				
	Peak width_90min	0.00						NA	43.29	0.69				
	Peak rise time_15min	0.00						0.01	3.02	NA				
	Peak rise time_90min	0.00						1.00	25.25	NA				
	Peak decay time_15min	0.00						NA	0.17	0.03				
	Peak decay time_90min	0.00						NA	44.63	0.47				
	Decay to rise ratio_15min	0.00						0.01	11.56	0.07				
	Decay to rise ratio_90min	0.00						1.26	6.43	0.12				
iCell endothelial cells	Cell number	54.44									0.43	NA	68.96	NA
	Mitochondrial integrity	0.41									0.26	NA	59.39	NA
	Nuclei mean area	66.77									NA	NA	NA	NA
	Mitochondrial intensity	68.61									0.12	NA	83.49	NA
	Cytoplasmic integrity	39.17									NA	NA	NA	NA
	Total tube length	0.00									0.00	NA	5.93	0.20

	Mean tube length	0.00									2.03	NA	NA	86.04
	Total tube area	0.00									0.00	NA	43.33	0.25
HUVECs	Cell number	84.79									5.26	NA	76.52	NA
	Mitochondrial integrity	37.14									4.70	NA	70.35	NA
	Nuclei mean area	44.70									NA	NA	NA	NA
	Mitochondrial intensity	44.38									5.01	NA	61.31	NA
	Cytoplasmic integrity	0.02									NA	NA	NA	NA
	Total tube length	0.00									0.10	4.73	395.90	365.00
	Mean tube length	0.00									0.29	23.20	665.30	67.85
	Total tube area	0.00									0.08	5.28	172.60	187.80
	ATP	8.27									NA	NA	NA	NA

[a] TAB, Tetra-octyl ammonium bromide (50 µM), cytotoxicity control, values are response (%) normalized to vehicle control. [b] Highest concentrations tested in the experiments (µM). [c] EC_{50} value could not be derived.

Tab. S3: Overlap in the chemicals tested in different *in vivo* and *in vitro* datasets
"1" indicates the chemical was present in the dataset; "0" indicates it was not included in the dataset.

Chemical	This study	ToxCast	POD_{RfD}	Paul Friedman et al. (2020)
Dibutyl phthalate	1	1	1	1
Di(2-ethylhexyl) phthalate	1	1	1	0
2-Methyl-4,6-dinitrophenol	1	1	0	0
1,2,3-Trichlorobenzene	1	1	0	0
Pentachlorophenol	1	1	0	0
p-Cresol	1	1	1	1
Benzidine	1	1	0	0
2,4,5-Trichlorophenol	1	1	1	1
2,4,6-Trichlorophenol	1	1	0	0
2,4-Dinitrotoluene	1	1	1	1
Methoxychlor	1	1	1	0
Endosulfan	1	1	1	0
Dieldrin	1	1	1	1
Dicofol	1	1	1	0
Heptachlor	1	1	1	1
Aldrin	1	1	1	1
p,p'-DDD	1	1	1	1
Chlorpyrifos	1	1	1	1
o,p'-DDT	1	1	0	0
Azinphos-methyl	1	1	1	1
Dichlorodiphenyltrichloroethane	1	1	1	1
Trifluralin	1	1	0	0
2,4-Dinitrophenol	1	1	1	1
Diazinon	1	1	0	0
Lindane	1	1	1	0
Parathion	1	1	1	0
Endrin	1	1	1	1
Ethion	1	1	1	1
Disulfoton	1	1	0	0
Heptachlor epoxide	1	1	1	0
Fluoranthene	1	1	1	1
Benzo(b)fluoranthene	1	1	1	0
Acenaphthene	1	1	1	1
Naphthalene	1	1	0	0
Benzo(a)anthracene	1	1	0	0
Cadmium chloride	1	1	0	0
Nickel(II) chloride	1	1	0	0
Cobalt chloride	1	1	0	0
Mercuric chloride	1	1	0	0
Zinc chloride	1	1	0	0
Lead nitrate	1	1	0	0
Potassium chromate(VI)	1	1	0	0

Tab. S4: Detailed descriptions of each phenotype evaluated in each tested cell type

Cell type	Phenotype	Description
iCell hepatocytes	Cell number	Number of cell bodies in the image
	Nuclei intensity	Average area of nucleus for all cells found in the image
	All cell mean area	Average area of the cell (nucleus + cytoplasm) for all cells found in the image
	Mitochondrial intensity	Total pixel intensity of MitoTracker stain over the stained area in positive cells, divided by the number of cells positive for MitoTracker stain
	Mitochondrial integrity	Total number of cells positive for MitoTracker staining
iCell neurons	Cell number	Number of cell bodies in the image
	Total outgrowth	Total length of skeletonized outgrowth
	Mean outgrowth	Average skeletonized outgrowth divided by the number of cells
	Total process	Number of outgrowths in the image that are connected to cell bodies
	Total branches	Total number of branching junctions in the image
	Total cell body area	Total area of the cell bodies in the image (excluding outgrowths)
	Cell with significant growth	Number of cells in the image with outgrowth greater than the threshold length specified in the settings
	Cytoplasmic integrity	Total number of cells positive for Calcein AM staining
	Mitochondria integrity	Total number of cells positive for MitoTracker staining
	ATP	Luminescence readouts from CellTiterGlo assay
iCell cardiomyocytes	Cell number	Number of cell bodies in the image
	Mitochondrial integrity	Total number of cells positive for MitoTracker staining
	Beats per minute	Beats per minute after exposure
	Peak amplitude	Average amplitude of peaks after exposure
	Peak spacing	Average spacing between each peak after exposure
	Peak width	Average width between each peak after exposure
	Peak rise time	Average rise time of each peak after exposure
	Peak decay time	Average decay time of each peak after exposure
	Decay to rise ratio	Average ratio of decay to rise time of each peak after exposure
iCell endothelial cells and **HUVECs**	Cell number	Number of cell bodies in the image
	Mitochondrial integrity	Total number of cells positive for MitoTracker staining
	Nuclei mean area	The average area of nucleus for all cells found in the image
	Mitochondrial intensity	Total pixel intensity of MitoTracker stain over the stained area in positive cells, divided by the number of cells positive for MitoTracker stain
	Cytoplasmic integrity	Total number of cells positive for Calcein AM staining
	Total tube length	Total microns of the tube length (excluding nodes)
	Mean tube length	Total tube length divided by the number of segments
	Total tube area	Total square microns of tube area (excluding nodes)
	ATP	Luminescence readouts from CellTiterGlo assay

Tab. S5: ToxPi score for 42 Superfund priority list chemicals in each cell type

Cell types	iCell hepatocytes		iCell neurons		iCell cardio.		iCell endo.		HUVECs	
Chemicals	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Benzo(a)anthracene	0.00	0.00	0.00	0.60	0.00	0.98	0.00	1.00	0.00	1.00
Naphthalene	0.00	0.00	0.00	0.42	0.00	0.88	0.00	0.16	0.00	0.00
Fluoranthene	0.00	0.30	0.00	0.40	0.00	0.93	0.00	0.14	0.00	0.77
Dichlorodiphenyltrichloroethane	0.00	0.24	0.09	0.44	0.09	0.97	0.00	0.44	0.00	0.49
Dieldrin	0.04	0.25	0.06	0.30	0.00	1.00	0.00	0.74	0.00	0.53
Aldrin	0.09	0.22	0.11	0.46	0.24	1.00	0.00	0.49	0.05	0.73
Heptachlor	0.08	0.29	0.11	0.37	0.32	0.98	0.00	0.54	0.09	0.64
Lindane	0.00	0.78	0.00	0.47	0.00	0.59	0.00	0.73	0.00	0.00
Disulfoton	0.00	0.02	0.00	0.00	0.10	0.51	0.00	0.16	0.00	0.48
Endrin	0.00	0.08	0.00	0.00	0.00	0.97	0.00	0.52	0.00	0.59
Diazinon	0.00	0.19	0.00	0.38	0.36	0.78	0.00	0.63	0.00	0.69
Heptachlor epoxide	0.00	0.00	0.00	0.22	0.00	1.00	0.00	0.00	0.00	0.93
Pentachlorophenol	0.00	0.21	0.04	0.50	0.09	0.49	0.14	0.80	0.22	0.78
Dibutyl phthalate	0.00	0.22	0.00	0.00	0.11	0.97	0.00	0.03	0.00	0.58
Chlorpyrifos	0.00	0.75	0.08	0.30	0.00	0.77	0.00	0.24	0.00	0.81
Di(2-ethylhexyl) phthalate	0.00	0.10	0.28	0.61	0.00	1.00	0.00	0.07	0.00	0.62
2,4,6-Trichlorophenol	0.00	0.06	0.00	0.32	0.00	1.00	0.00	0.40	0.00	0.36
Ethion	0.00	0.03	0.00	0.49	0.00	1.00	0.00	0.36	0.00	0.90
Azinphos-methyl	0.00	1.00	0.00	0.97	0.00	0.95	0.00	0.97	0.00	0.50
2,4,5-Trichlorophenol	0.00	0.10	0.28	0.79	0.00	0.59	0.00	0.40	0.05	0.59
Parathion	0.00	0.49	0.00	0.67	0.09	0.62	0.00	0.16	0.00	0.88
Benzo(b)fluoranthene	0.00	0.25	0.00	0.10	0.00	0.69	0.00	1.00	0.00	0.55
Trifluralin	0.00	0.29	0.06	0.39	0.00	0.84	0.00	0.63	0.00	0.42
Acenaphthene	0.00	0.06	0.00	0.00	0.00	0.30	0.00	1.00	0.00	0.65
p,p'-DDD	0.10	0.17	0.08	0.31	0.09	0.94	0.09	0.24	0.05	0.39
Benzidine	0.00	0.19	0.00	0.00	0.00	0.47	0.00	0.36	0.00	0.56
Endosulfan	0.11	0.38	0.08	0.22	0.31	0.84	0.00	0.56	0.00	0.68
Methoxychlor	0.25	0.28	0.10	0.19	0.00	1.00	0.06	0.44	0.00	0.18
2,4-Dinitrophenol	0.00	0.18	0.00	0.00	0.10	0.35	0.00	1.00	0.00	0.40
2,4-Dinitrotoluene	0.00	0.00	0.00	0.00	0.00	0.43	0.00	0.00	0.00	0.11
Dicofol	0.11	0.25	0.07	0.30	0.09	0.65	0.03	0.49	0.07	0.29
p-Cresol	0.00	0.36	0.00	0.04	0.00	0.05	0.00	1.00	0.00	0.19
o,p'-DDT	0.00	0.24	0.09	0.19	0.08	0.72	0.00	0.32	0.00	0.71
2-Methyl-4,6-dinitrophenol	0.05	0.89	0.00	0.33	0.00	0.42	0.00	0.23	0.00	0.58
1,2,3-Trichlorobenzene	0.00	1.00	0.00	0.00	0.00	0.75	0.00	0.11	0.00	0.00
Lead nitrate	0.00	0.09	0.32	0.64	0.00	0.60	0.00	0.90	0.00	0.72
Cadmium chloride	0.60	1.00	0.08	0.29	0.00	0.89	0.05	0.25	0.00	1.00
Zinc chloride	0.00	0.25	0.00	0.11	0.00	0.81	0.00	0.44	0.00	1.00
Mercuric chloride	0.00	0.84	1.00	1.00	0.00	1.00	0.12	1.00	0.19	1.00
Potassium chromate(VI)	0.00	0.00	0.31	0.87	0.00	0.87	0.42	1.00	0.30	1.00
Cobalt chloride	0.00	0.30	0.00	0.36	0.00	0.37	0.00	0.97	0.00	1.00
Nickel(II) chloride	0.00	0.26	0.00	0.60	0.00	0.00	0.00	0.19	0.00	0.77

Tab. S6: Detailed list of the chemicals shown in the clustering diagrams (Fig. 5)

All cell combined	ToxCast/Tox21	Morgan FP	All cell combined + Morgan FP
Mercuric chloride	Lindane	2,4-Dinitrotoluene	Azinphos-methyl
2,4,5-Trichlorophenol	Azinphos-methyl	2,4-Dinitrophenol	2,4,5-Trichlorophenol
Lead nitrate	Parathion	2-Methyl-4,6-dinitrophenol	Nickel (II) chloride
Potassium chromate (VI)	Diazinon	Trifluralin	Lead nitrate
Chlorpyrifos	Disulfoton	Lead nitrate	Chlorpyrifos
Azinphos-methyl	Trifluralin	Potassium chromate (VI)	Di(2-ethylhexyl) phthalate
Di(2-ethylhexyl) phthalate	Methoxychlor	Diazinon	Cadmium chloride
2,4,6-Trichlorophenol	Dibutyl phthalate	Chlorpyrifos	Methoxychlor
Nickel (II) chloride	Di(2-ethylhexyl) phthalate	Parathion	Heptachlor
Endrin	Endosulfan	Ethion	Fluoranthene
Disulfoton	Dicofol	Disulfoton	Lindane
Acenaphthene	Potassium chromate (VI)	Dibutyl phthalate	Diazinon
Lindane	Cadmium chloride	Di(2-ethylhexyl) phthalate	Endrin
Diazinon	Lead nitrate	Azinphos-methyl	Heptachlor epoxide
Methoxychlor	Cobalt chloride	Fluoranthene	Aldrin
Dieldrin	Zinc chloride	Benzo(b)fluoranthene	Dicofol
Heptachlor	Nickel (II) chloride	Naphthalene	1,2,3-Trichlorobenzene
Fluoranthene	Heptachlor epoxide	Acenaphthene	p,p'-DDD
Endosulfan	Dieldrin	Benz(a)anthracene	Naphthalene
Trifluralin	2-Methyl-4,6-dinitrophenol	p-Cresol	Disulfoton
Ethion	Chlorpyrifos	Benzidine	Acenaphthene
Heptachlor epoxide	2,4,5-Trichlorophenol	2,4,6-Trichlorophenol	2,4-Dinitrotoluene
Aldrin	Heptachlor	2,4,5-Trichlorophenol	Parathion
Naphthalene	Aldrin	Pentachlorophenol	Dibutyl phthalate
Parathion	p,p'-DDD	1,2,3-Trichlorobenzene	2,4-Dinitrophenol
Dibutyl phthalate	o,p'-DDT	o,p'-DDT	Trifluralin
2,4-Dinitrophenol	p,p'-DDT	p,p'-DDT	Cobalt chloride
2,4-Dinitrotoluene	Pentachlorophenol	p,p'-DDD	o,p'-DDT
o,p'-DDT	Mercuric chloride	Dicofol	p,p'-DDT
p,p'-DDT	Fluoranthene	Methoxychlor	Ethion
p,p'-DDD	Endrin	Nickel (II) chloride	Benz(a)anthracene
Dicofol	2,4-Dinitrotoluene	Lindane	Endosulfan
Benz(a)anthracene	1,2,3-Trichlorobenzene	Endrin	Dieldrin
Cobalt chloride	p-Cresol	Dieldrin	Benzo(b)fluoranthene
Cadmium chloride	Naphthalene	Heptachlor epoxide	2-Methyl-4,6-dinitrophenol
Zinc chloride	Acenaphthene	Heptachlor	p-Cresol
Pentachlorophenol	2,4,6-Trichlorophenol	Aldrin	Benzidine
2-Methyl-4,6-dinitrophenol	Benzidine	Endosulfan	2,4,6-Trichlorophenol
1,2,3-Trichlorobenzene	Ethion	Zinc chloride	Zinc chloride
Benzo(b)fluoranthene	Benzo(b)fluoranthene	Cobalt chloride	Pentachlorophenol
p-Cresol	Benz(a)anthracene	Mercuric chloride	Potassium chromate (VI)
Benzidine	2,4-Dinitrophenol	Cadmium chloride	Mercuric chloride

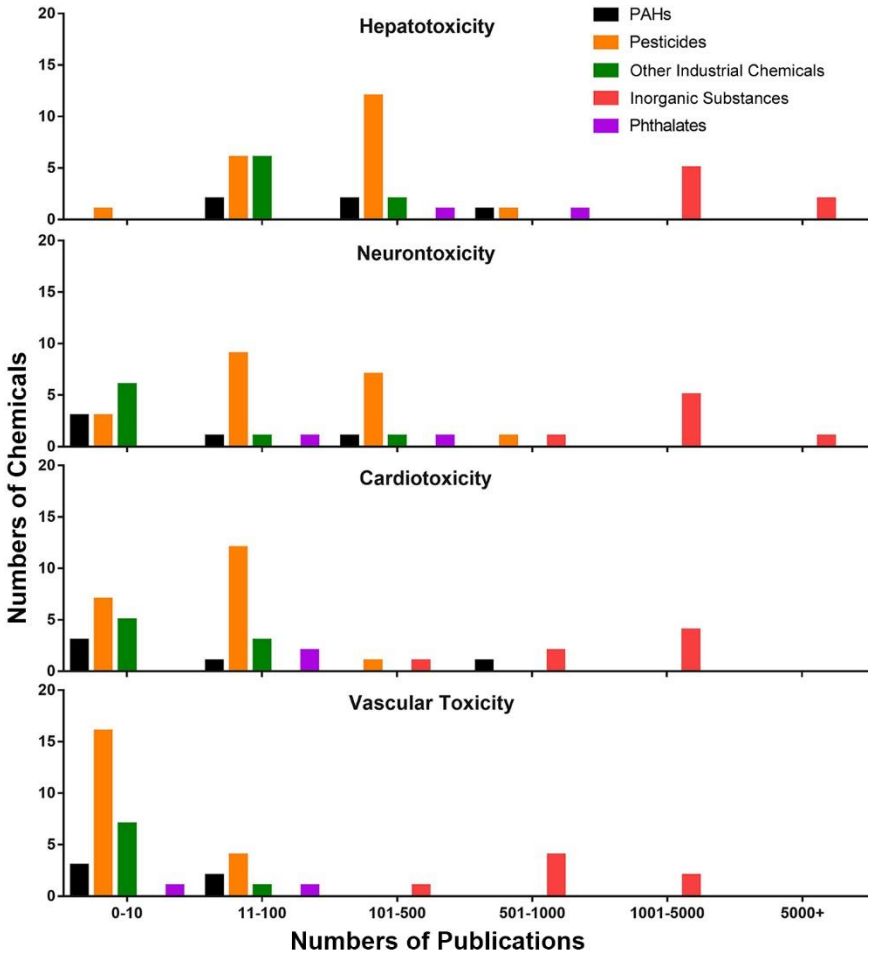

Fig. S1: Summary of the literature review of the published evidence for the effects of the 42 Superfund priority list chemicals on various organs
The literature review is available on the Health Assessment Workspace Collaborative (Shapiro et al., 2018) web portal (https://hawcproject.org/assessment/783; https://hawcproject.org/assessment/784; https://hawcproject.org/assessment/785; https://hawcproject.org/assessment/786).

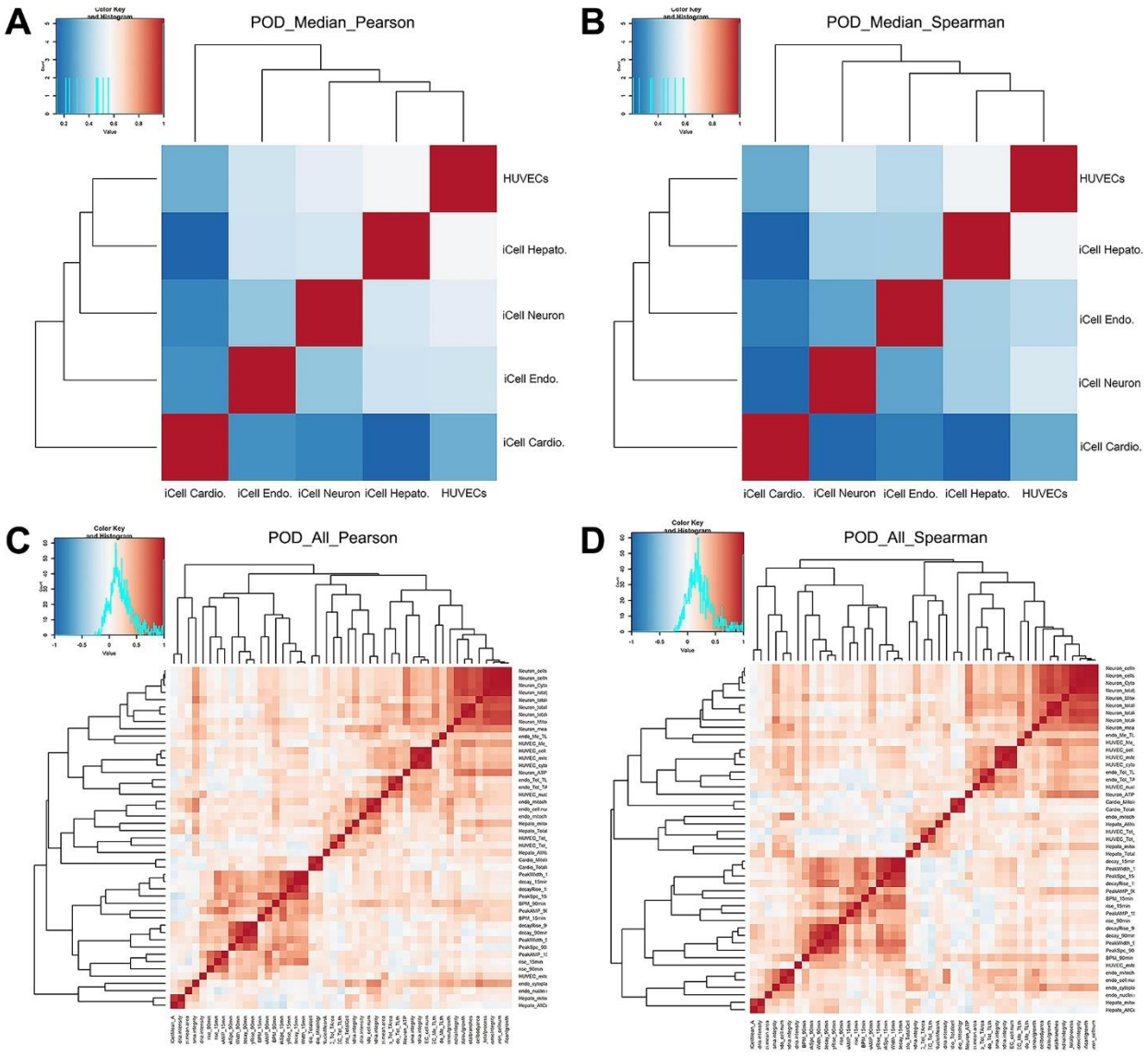

Fig. S2: Correlation of the PODs in different cell types. Pearson (A) and Spearman (B) correlation of the POD median from each cell type are shown

Pearson (C) and Spearman (D) correlation of all PODs generated from all phenotypes of five tested cell types are shown. The color key indicates positive (red) and negative (blue) correlation values.

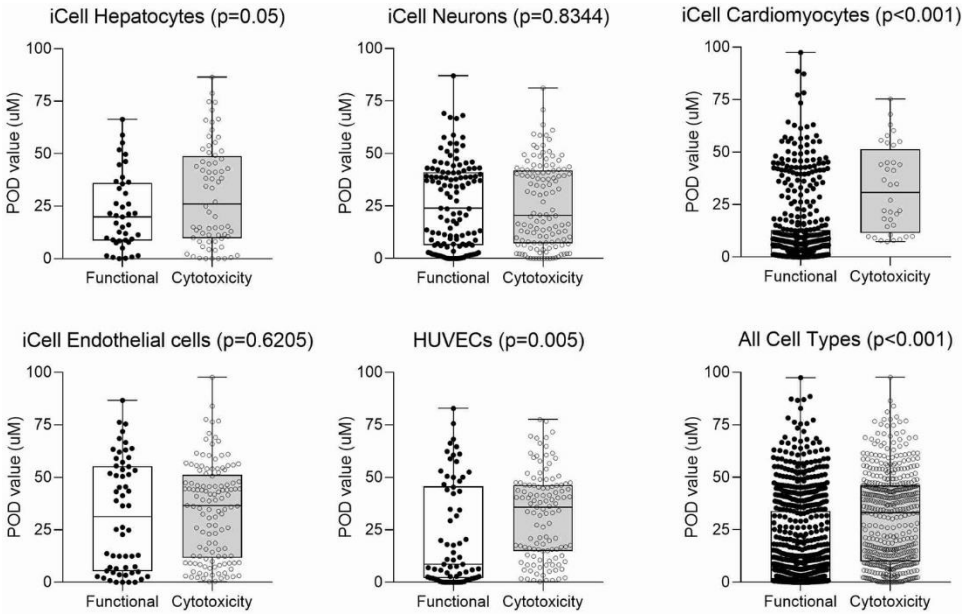

Fig. S3: Statistical comparison of PODs generated from cytotoxicity and functional endpoints in each tested cell type
PODs for chemical/phenotype combinations that were less than the top concentration tested (100 µM) were included in the analysis. P-values shown are from unpaired t-test with Welch's correction.

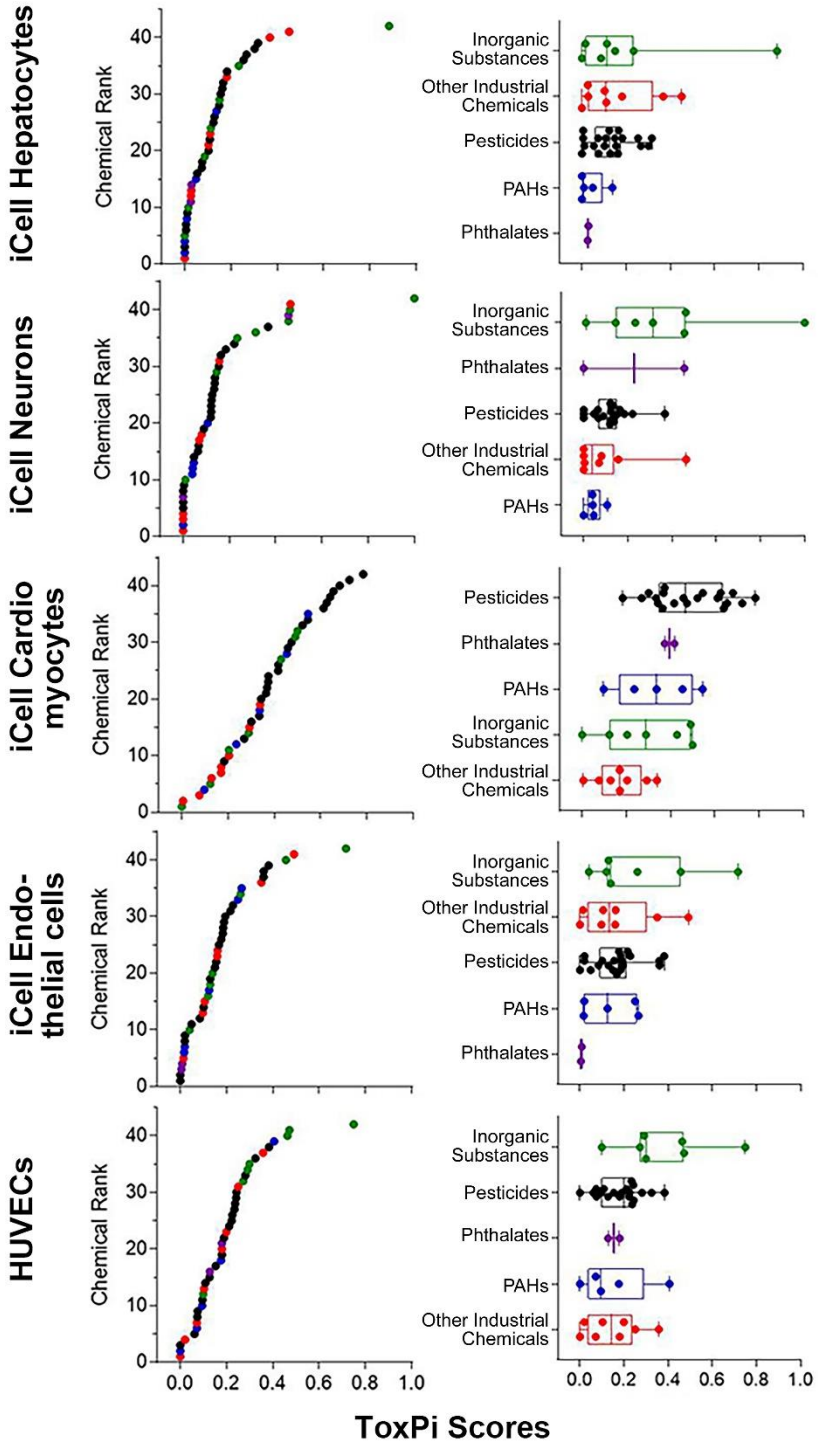

Fig. S4: ToxPi scores of 42 Superfund priority chemicals calculated from each cell type
Each chemical (left panel) and all chemical classes (right panel) were ranked based on each cell type. Each dot represents one chemical and the box (inter-quantile range and median) and whiskers (min to max) plots show the range of ToxPi scores.

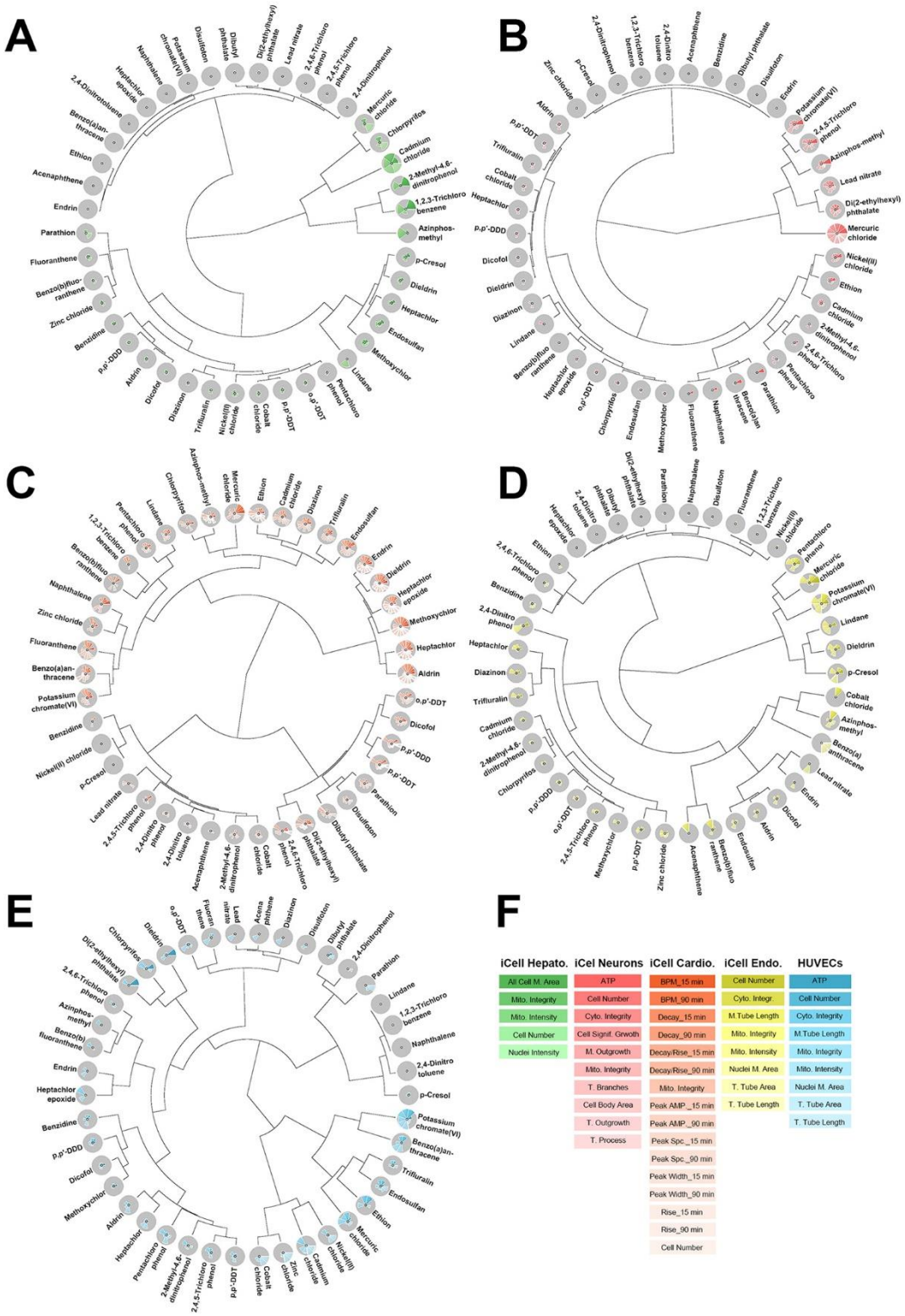

Fig. S5: Clustering (Ward's D method) of 42 Superfund priority list chemicals using ToxPi scores calculated from iCell hepatocytes (A), iCell neurons (B), iCell cardiomyocytes (C), iCell endothelial cells (D) and HUVECs (E)
Color of each slice in ToxPi represents different phenotypes in each cell type (F).

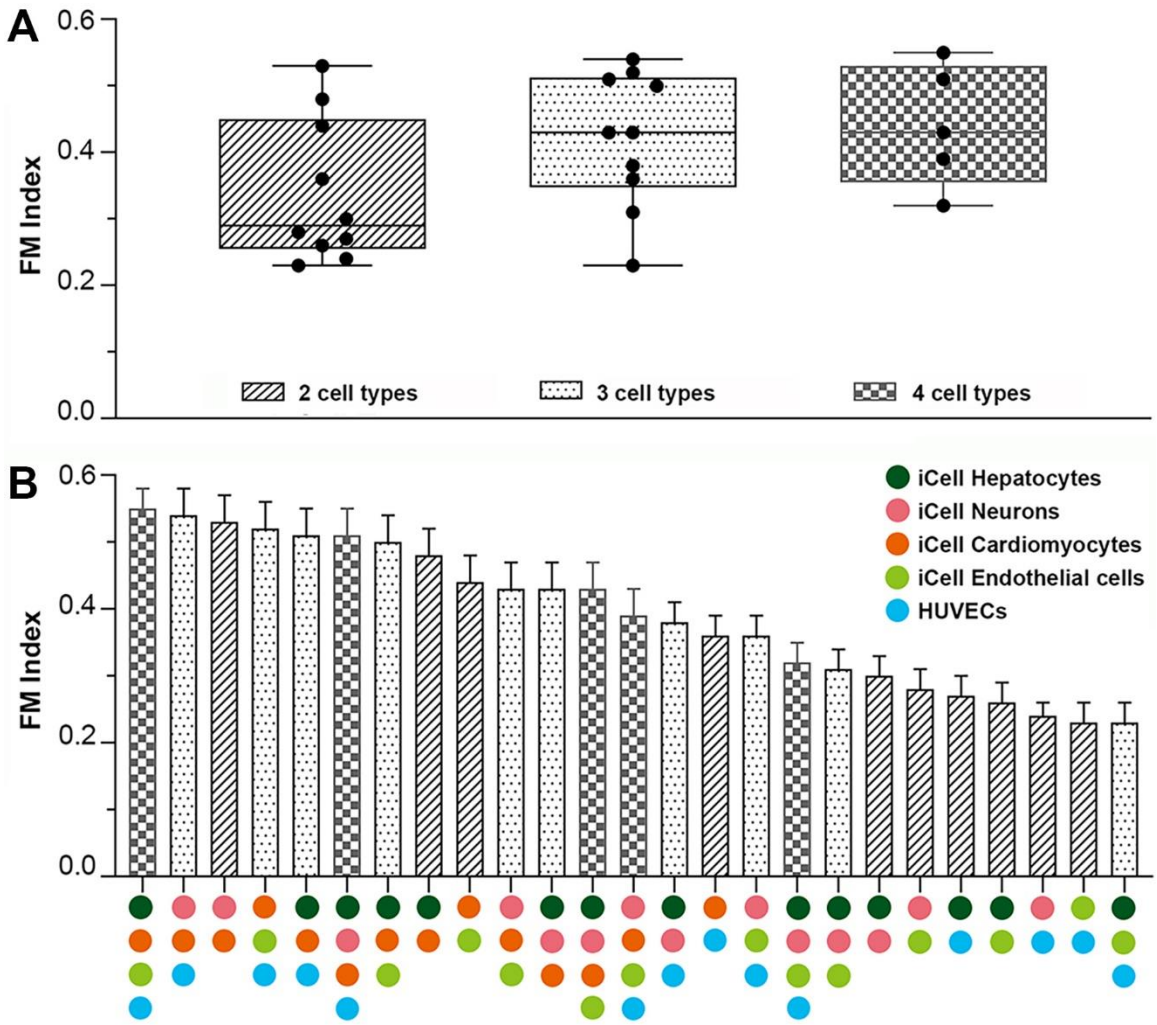

Fig. S6: The Fowlkes-Mallows (FM) index for clustering of chemicals into 5 classes based on different combinations of cell types used in this study

(A) Box (inter-quantile range and median) and whiskers (min to max) plots indicate the overall FM indexes from the combination of 2, 3, and 4 cell types; each dot represents one specific combination, which is detailed in (B), where different combinations are ranked based on the FM index.

A

ToxCast/ Tox21+All Endpoints Combined (71%)	Pesticides	Other Ind. Ch.	PAHs	Phthalates	Inorganic Subst.
Pesticides	19	3	1	2	1
Other Ind. Ch.	1	5	4		
PAHs					
Phthalates					
Inorganic Subst.					6

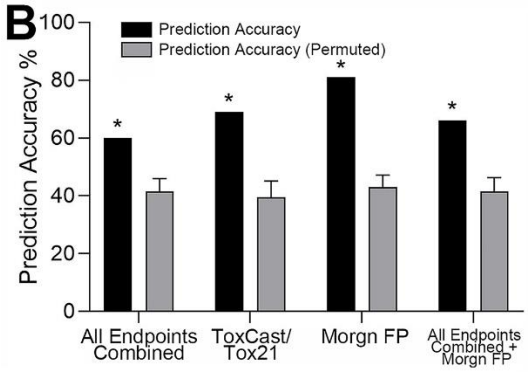

Fig. S7: Accuracy of predicting 42 Superfund priority list chemicals into classes using the combination of *in vitro* datasets from this study and the ToxCast (A) and the comparison of prediction accuracy between biological/chemical database and permutation-based class assignment (B)