High-content bioactivity profiling of 141 petroleum substances

Cat-App final event, Brussels, 6 September 2018

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Acknowledgements:

Texas A&M University:

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Concawe Toxicology Subgroup members

Cat-App

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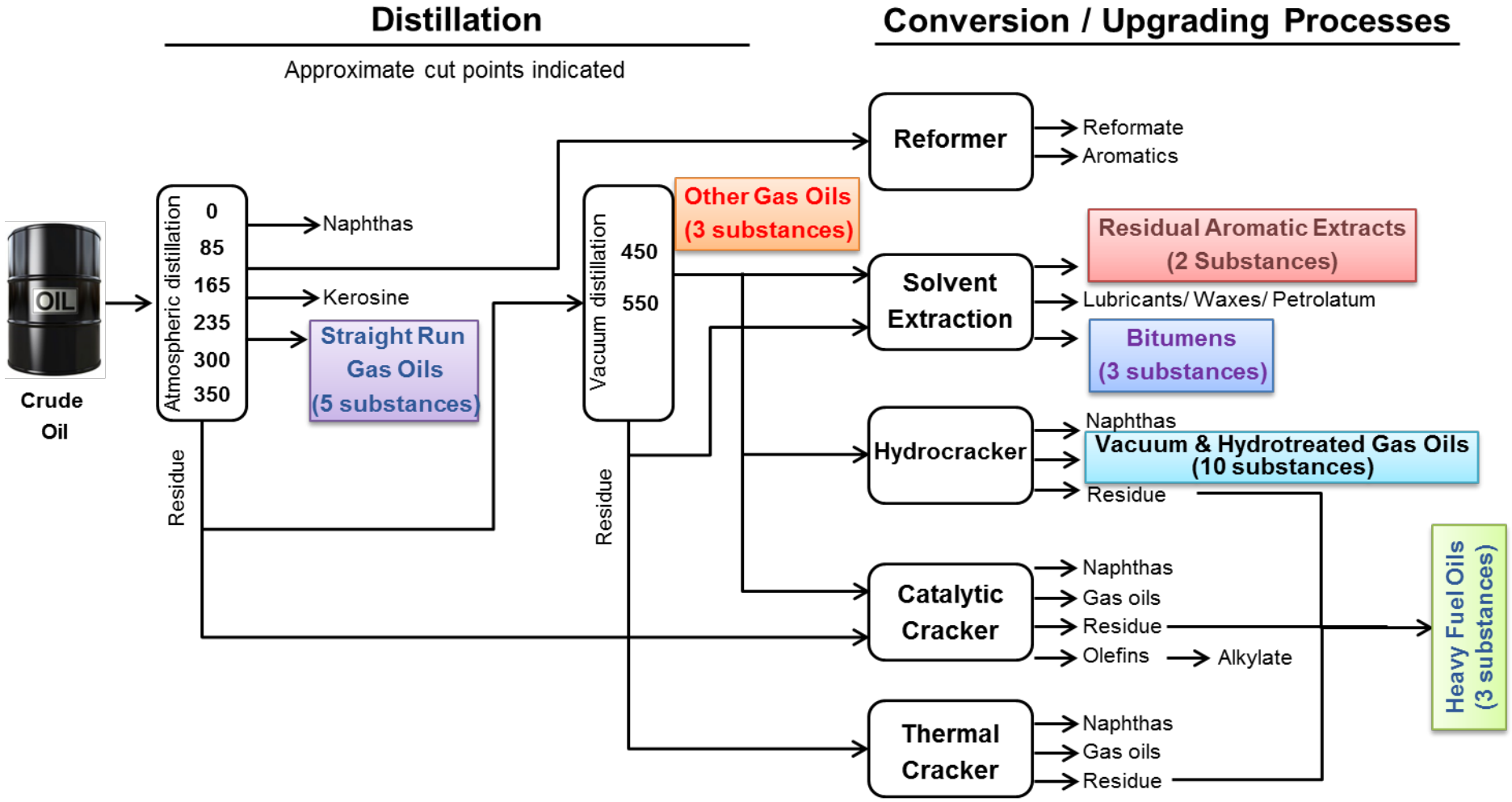
Using bioactivity profiling for grouping petroleum substances

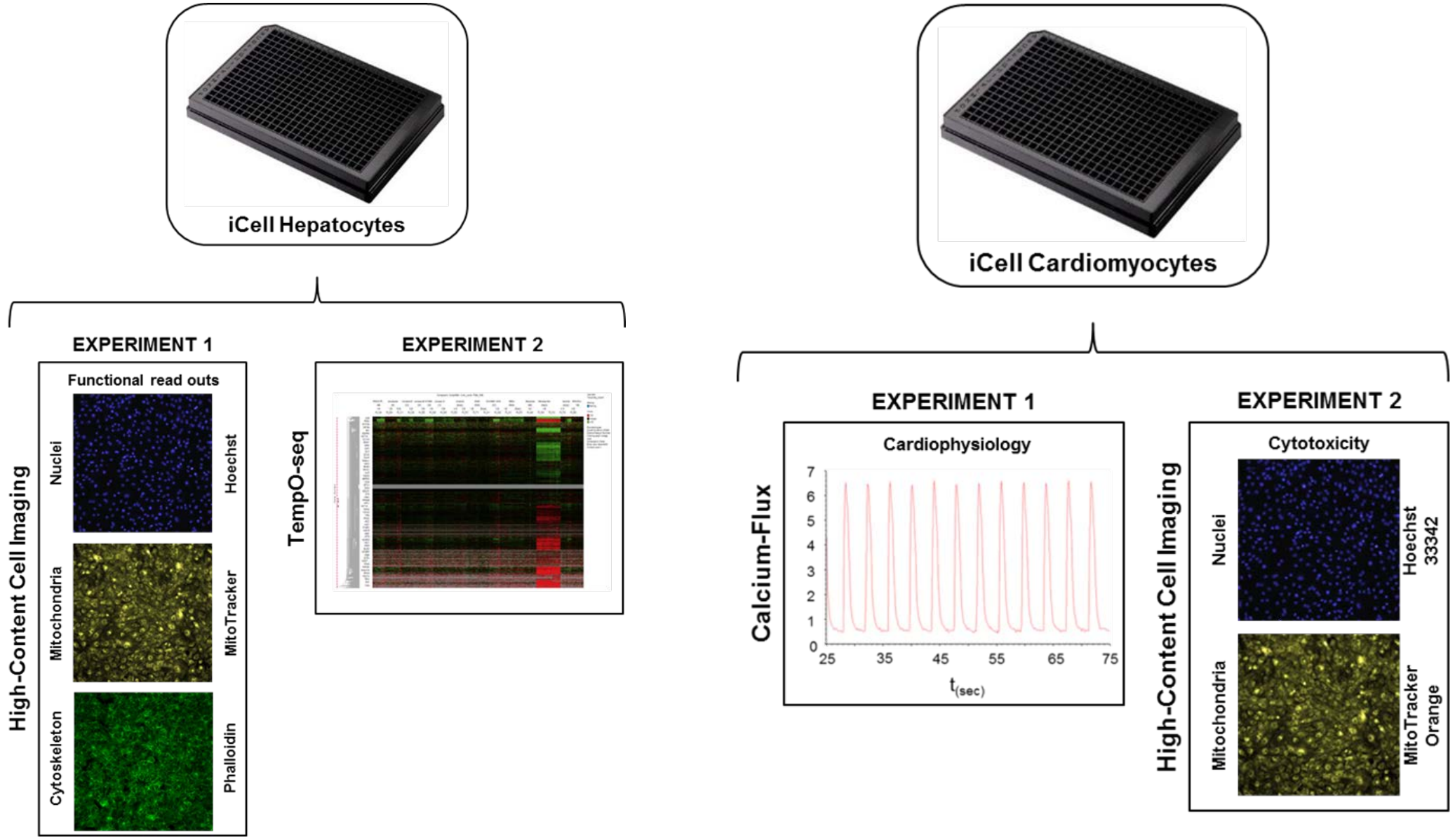


Proof of concept study

Samples Selected for Toxicity Profiling:
- Straight Run Gas Oils - SRGO (5x)
- Other Gas Oils - OGO (3x)
- Residual Aromatic Extracts – RAE (2x)
- Bitumen (3x)
- Vacuum & Hydrotreated Gas Oils – VHGO (10x)
- Heavy Fuel Oils – HFO (3x)

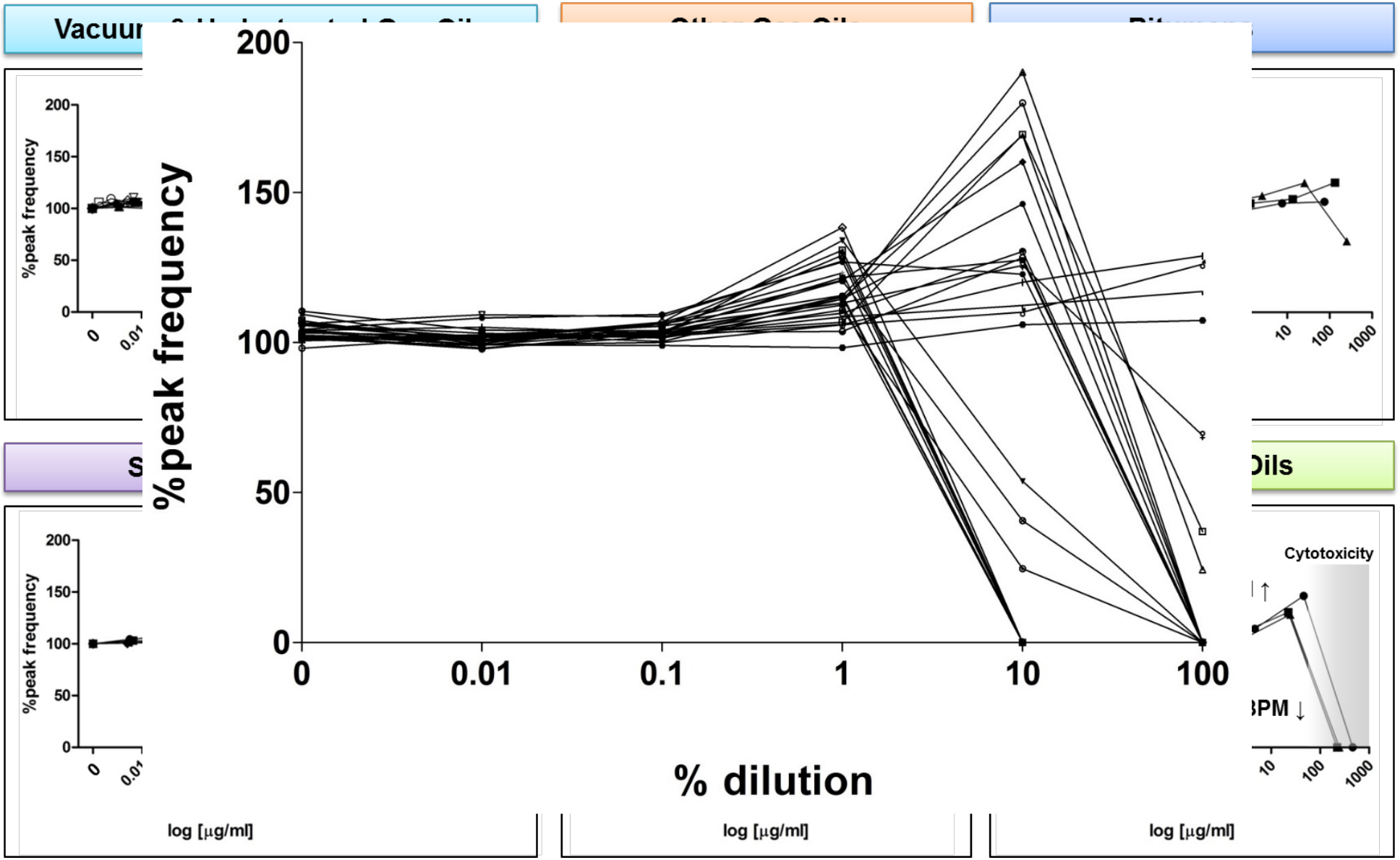
→ Prepare DMSO-based extracts for bioactivity profiling → Concentration-response bioactivity profiling in two human iPS-derived cell lines → Compare substance groupings using "manufacturing class" *vs* bioactivity profiling data

Using bioactivity profiling for grouping petroleum substances



Grimm et al. *A chemical-biological similarity-based grouping of complex substances as a prototype approach for evaluating chemical alternatives.* ***Green Chem*** (2016) 18(16):4407-4419 [PMID: 28035192; PMC5179981]

Using bioactivity profiling for grouping petroleum substances



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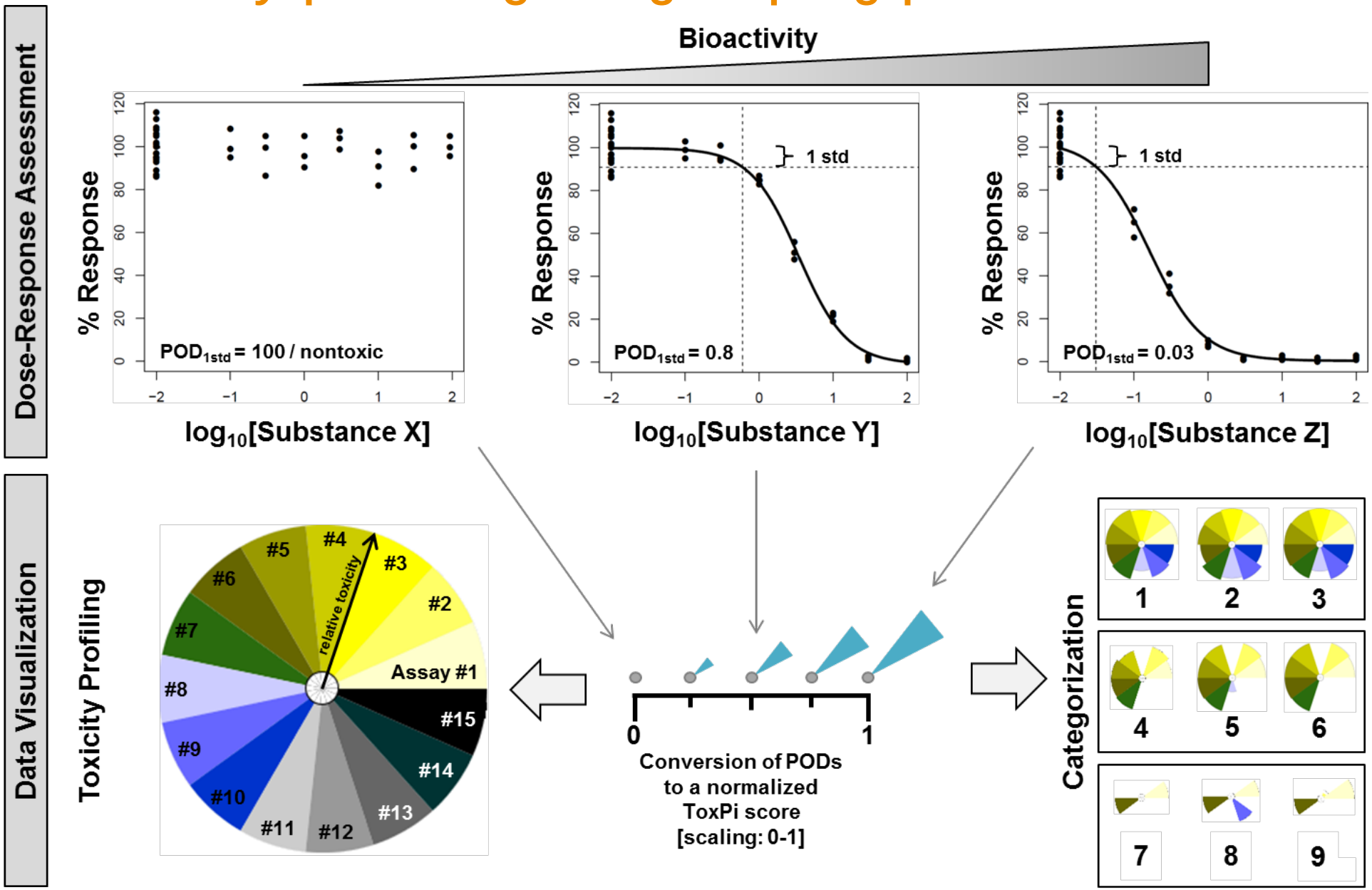

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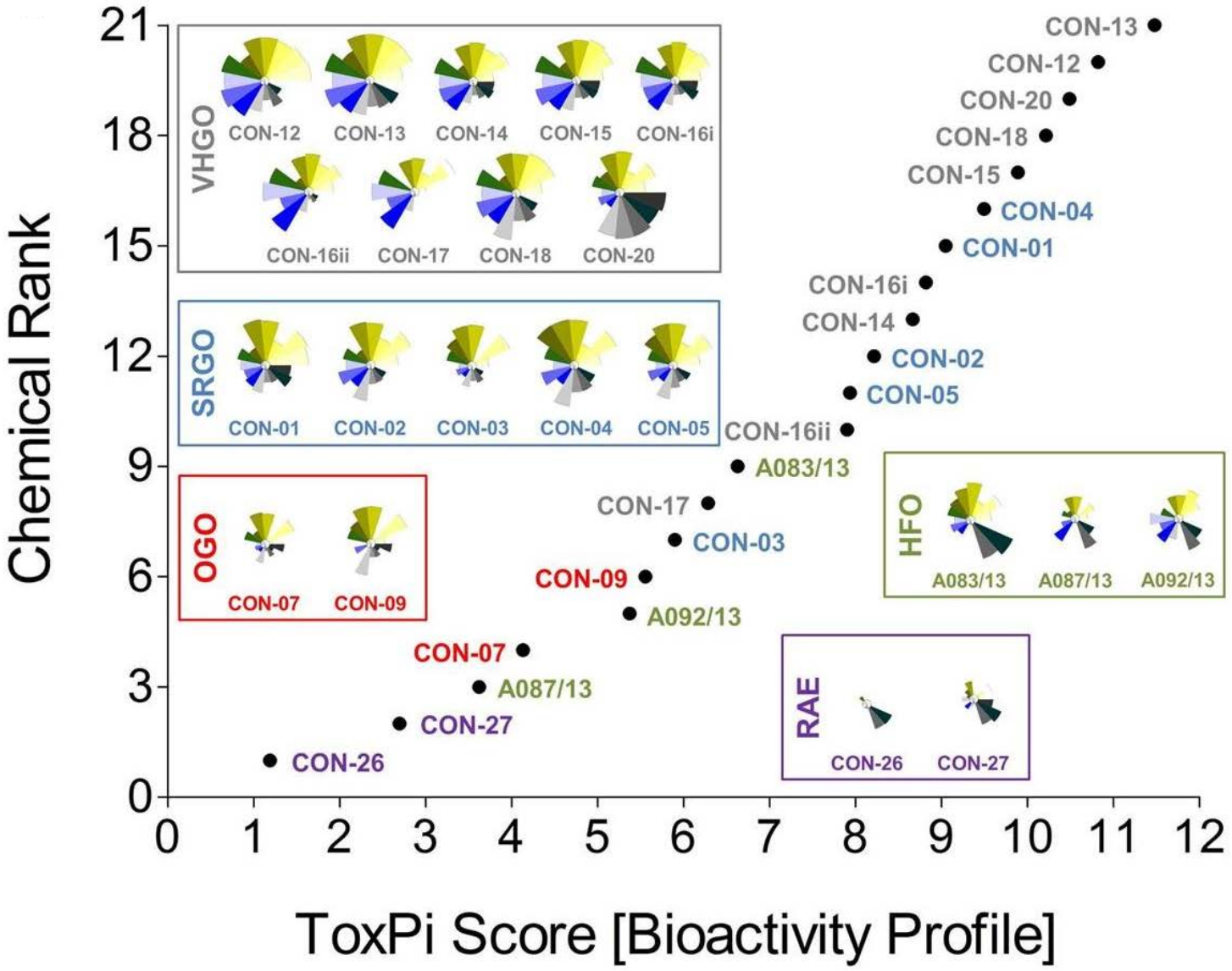

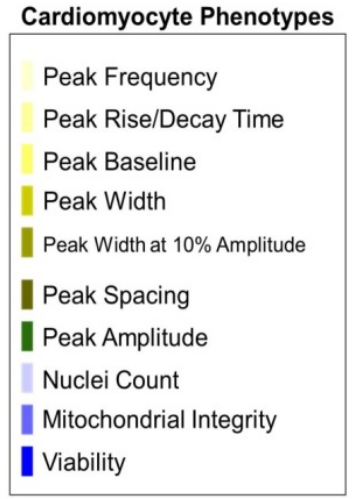

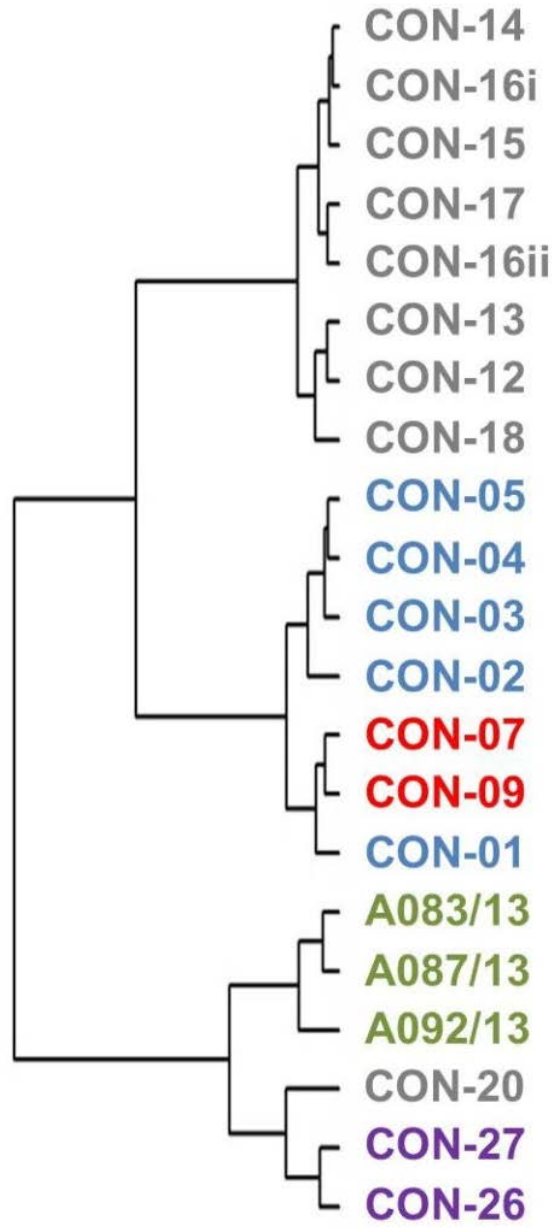

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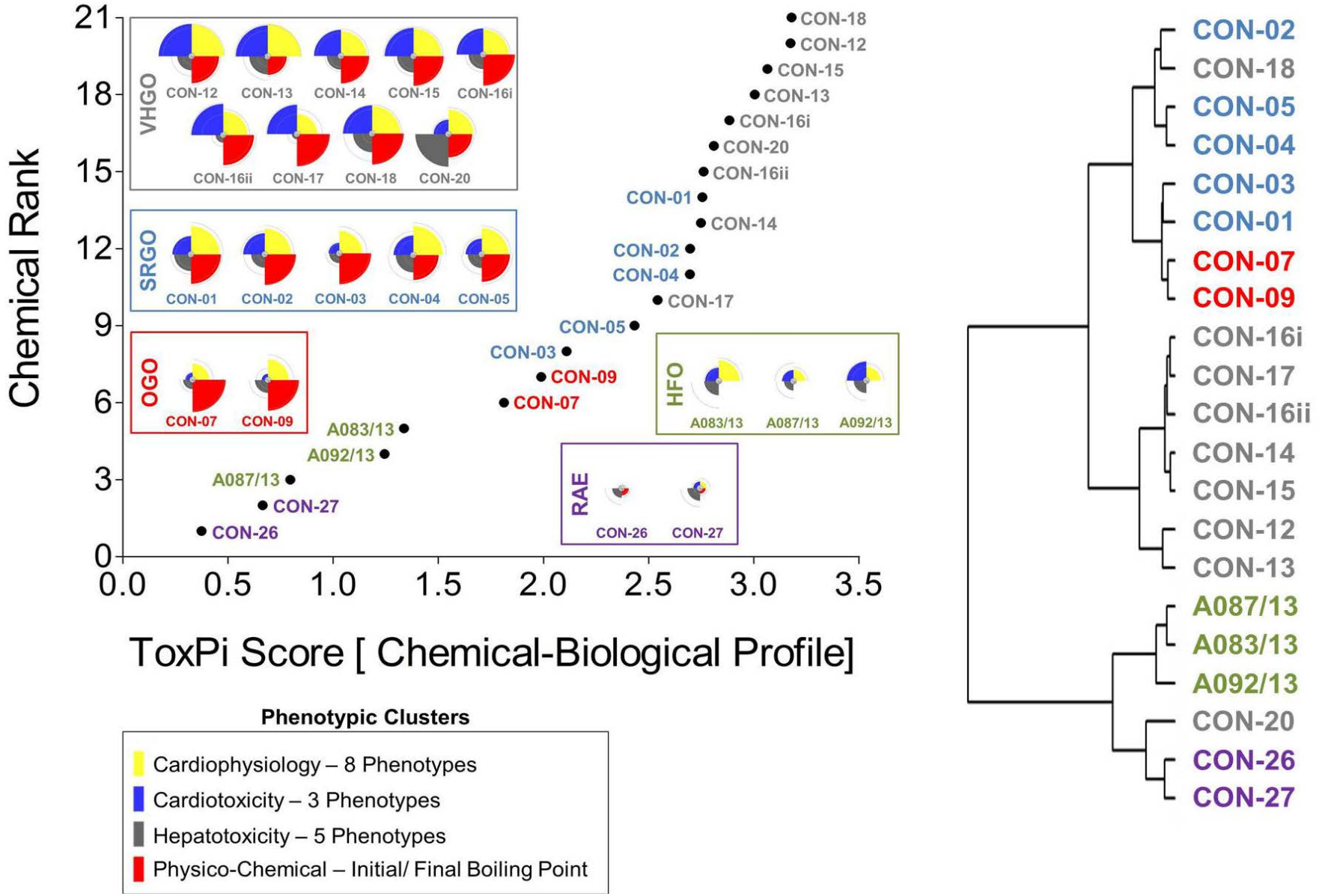

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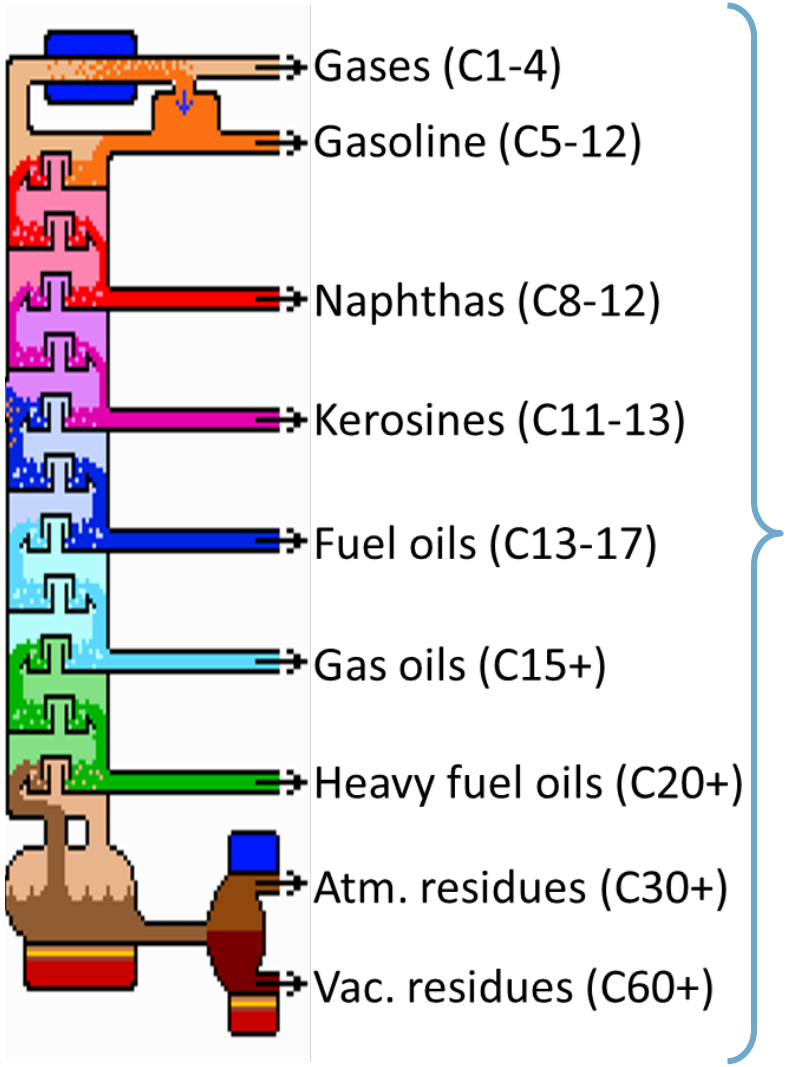
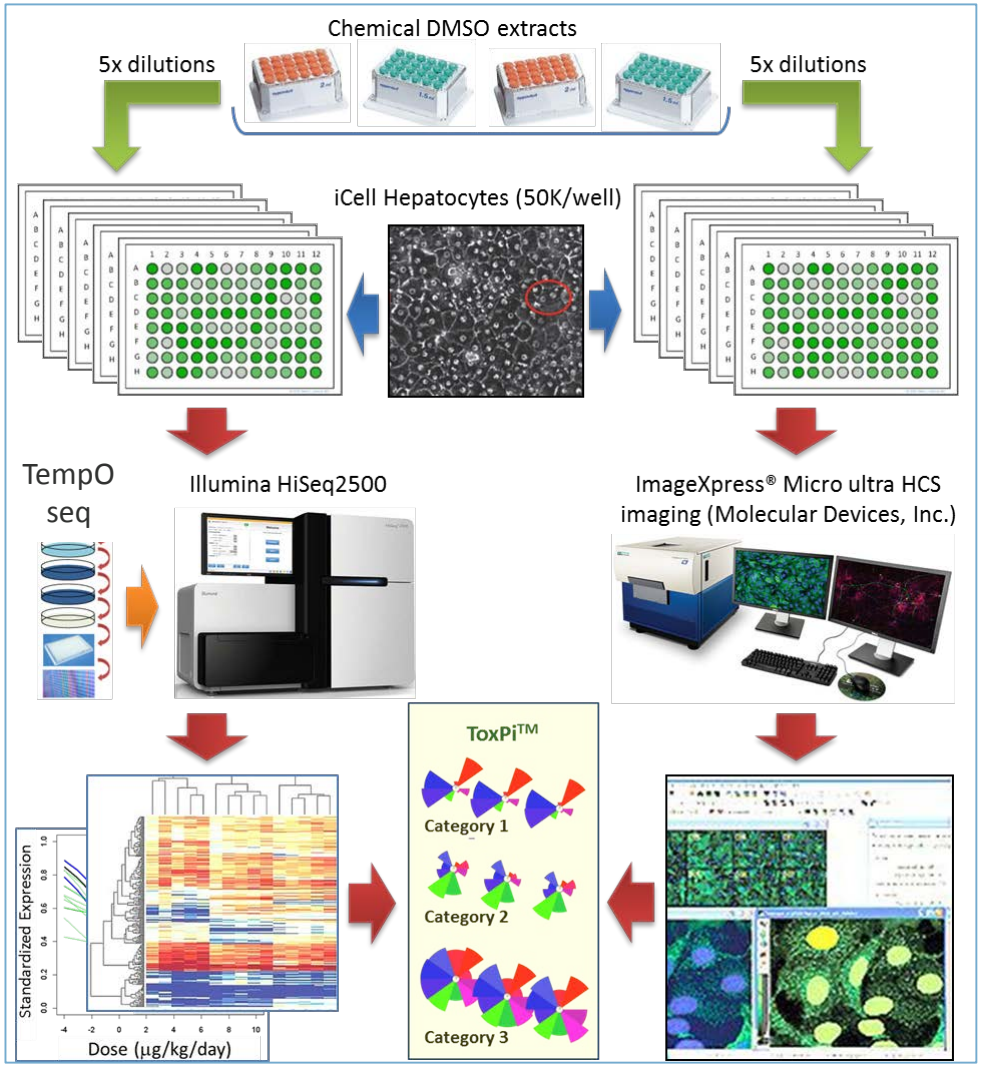
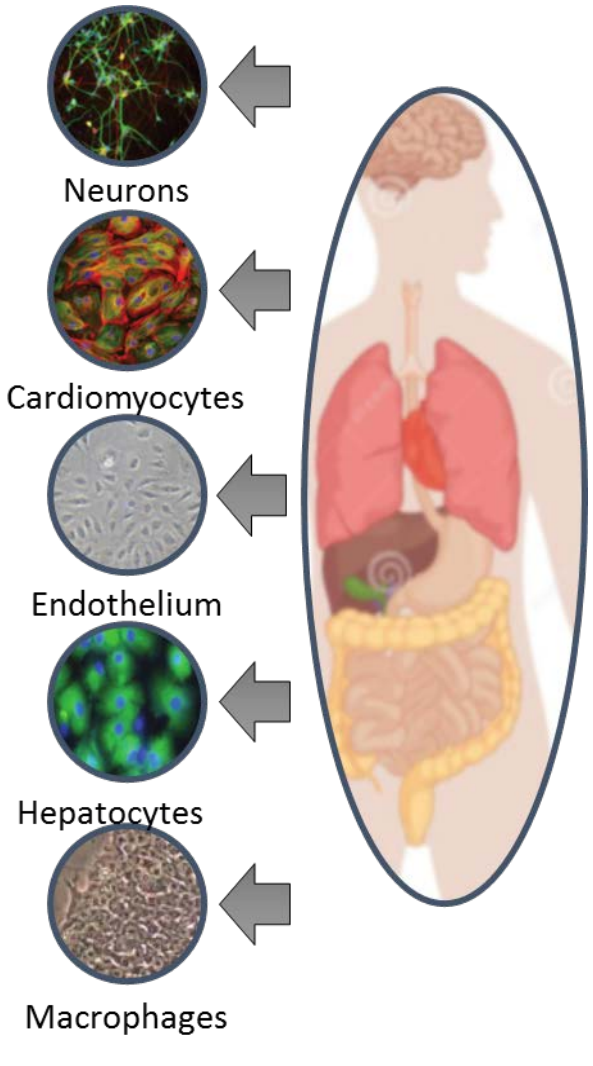
Assessment of new and complex chemistries: **Cat-App concept**



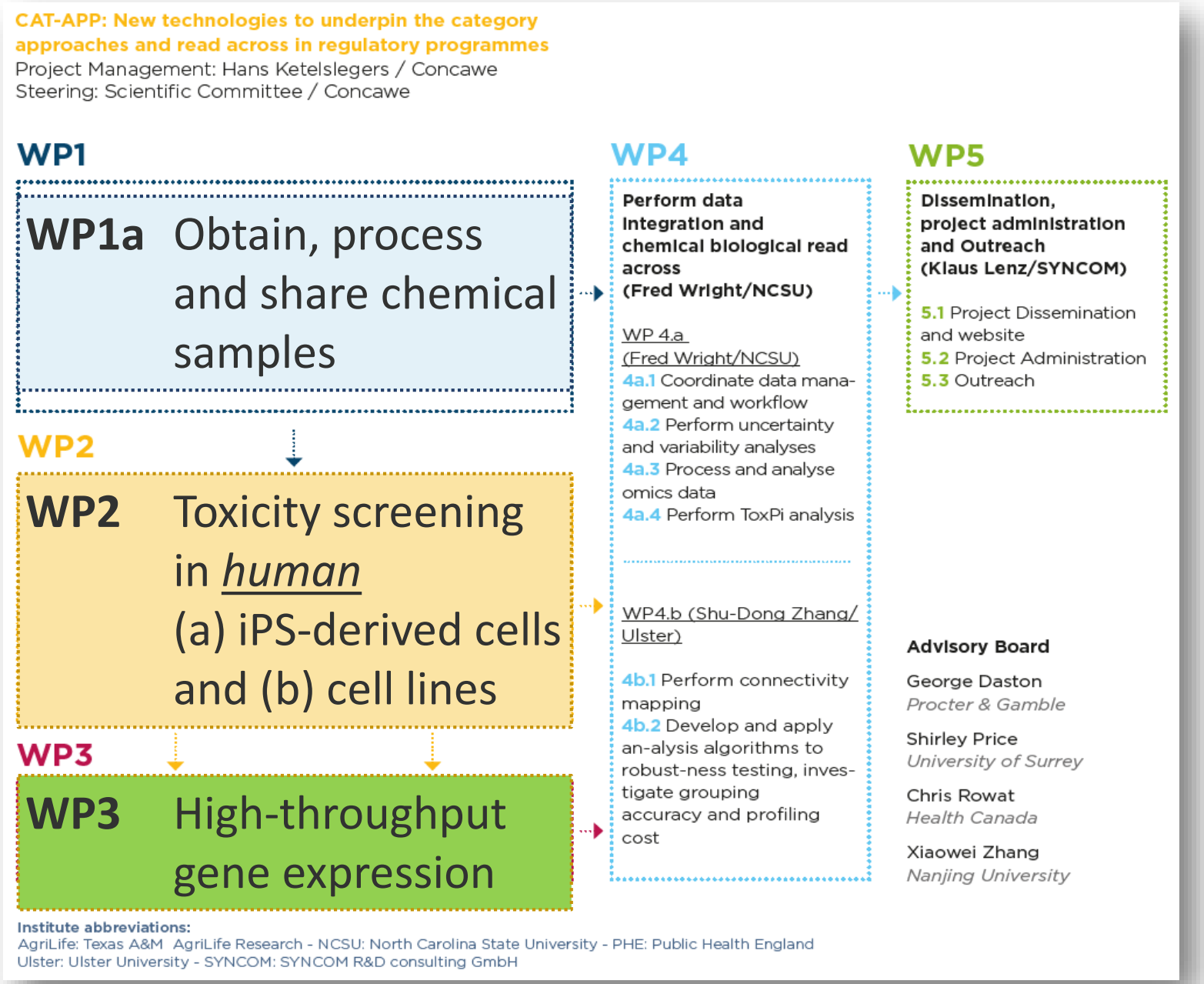




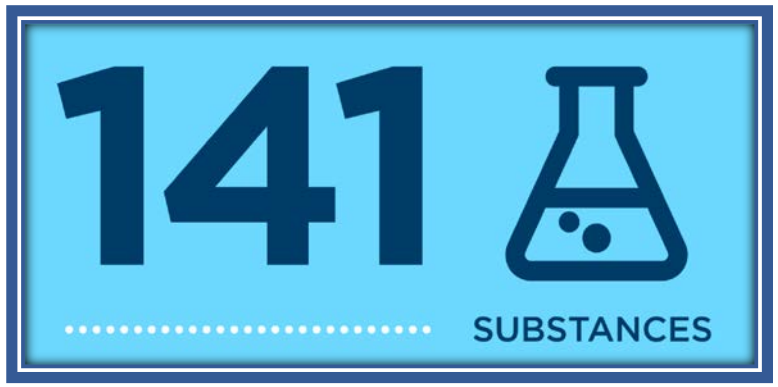
Petroleum UVCBs → **Bioactivity data-enabled read-across** ← ***in vitro* models**

Cat-App Workflow

www.concawe.eu/cat-app



WP1a: Preparing extracts of petroleum substances for screening



Category	# substances
Base Oils	33
Fuel Oils and Heavy Fuel Oils	30
Cracked and Other Gas Oils	12
Vacuum and Hydrocracked Gas Oils	10
Kerosines	10
Naphthas (gasolines)	10
Waxes	10
Untreated/Treated/Residual Aromatic Extracts	8
Straight Run Gas Oils	6
Bitumens	5
Unrefined/Acid Treated Oils	4
Petrolatums	3

Total=141

Designation: E1687 – 10

An American National Standard

May 2010

Standard Test Method for Determining Carcinogenic Potential of Virgin Base Oils in Metalworking Fluids[1]

A1. METHODS FOR ESTIMATION

A1.1 Methods for estimation of relative PAC content of oils, or for its correlation with MI in the modified Ames assay, or both, and with dermal carcinogenic potency. These analytical methods do not predict the mutagenicity or dermal carcinogenicity of petroleum fractions in the naphtha, kerosine, low-boiling atmospheric gas oil (<250°C), or vacuum residuum ranges.

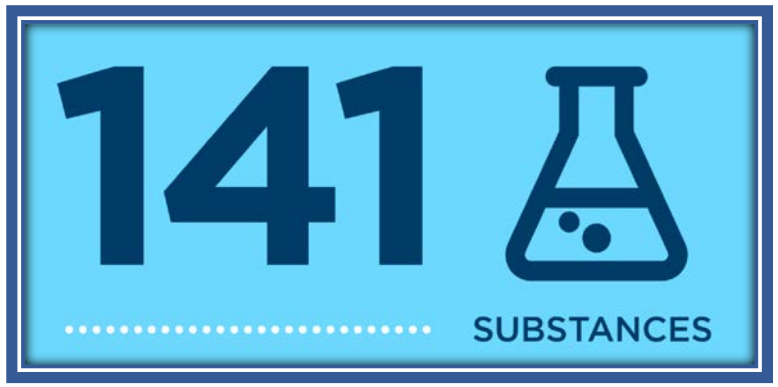
A1.1.1 Haas, J. M., Dimeler, G. R., Basil, E. W., Wilkins, G. W., and Nutter, J. S., "A Simple Analytical Test and a Formula to Predict the Potential for Dermal Carcinogenicity for Petroleum Oils," American Industrial Hygiene Association Journal 48(11), 1987, pp. 935–940.

A1.1.2 "Polycyclic Aromatics in Petroleum Fractions by Dimethyl Sulphoxide—Refractive Index Method," *IP Standards for Petroleum and Its Products, Part I, Methods for Analysis and Testing*, Vol 2, Methods IP262-372, John Wiley and Sons, New York, 1985 (and subsequent issues), pp. 346.1–346.6.

A1.1.3 Roy, T. A., Johnson, S. W., Blackburn, G. R., and Mackerer, C. R., "Correlation of Mutagenic and Dermal Carcinogenic Activities of Mineral Oils with Polycyclic Aromatic Compound Content," Fund. Appl. Toxicol. Vol 10, 1988, pp. 466–476.

Polycyclic Aromatic Compounds (PAC) include "***PAHs, alkylated PAHs,*** *and those* ***multi-ringed aromatic molecules*** *in which one or more atoms of a heteroatom such as nitrogen, oxygen or sulfur replaces a corresponding number of carbon atoms in a ring system. The majority of the PACs found in crude oil and petroleum streams have alkyl-substituents, with from one to twenty carbons, or even higher, depending on the boiling range of the petroleum stream.*" http://petroleumhpv.org/

WP1a: Preparing extracts of petroleum substances for screening



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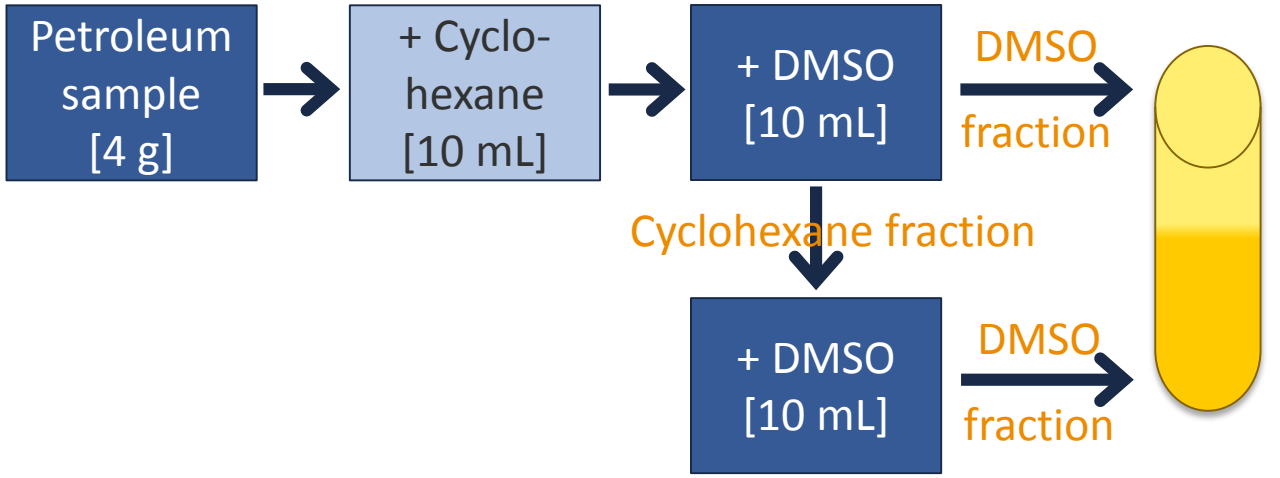
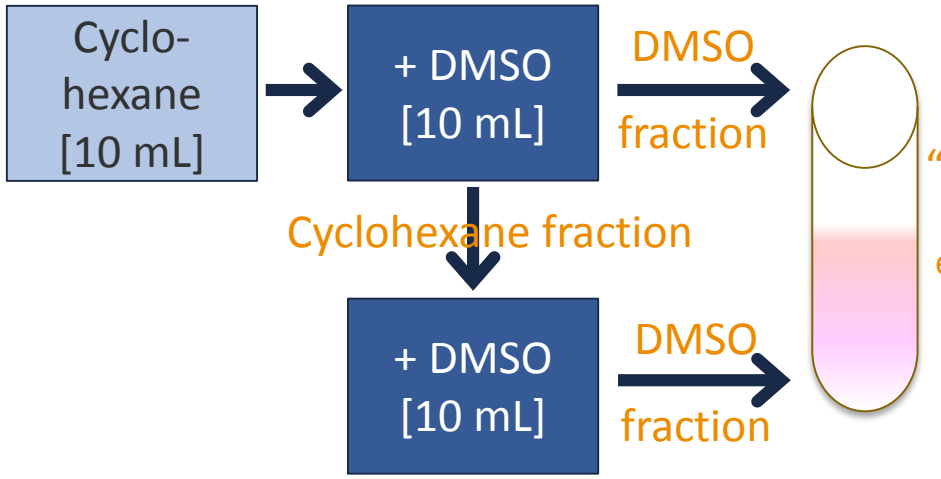
Total=141

Extraction Procedure:

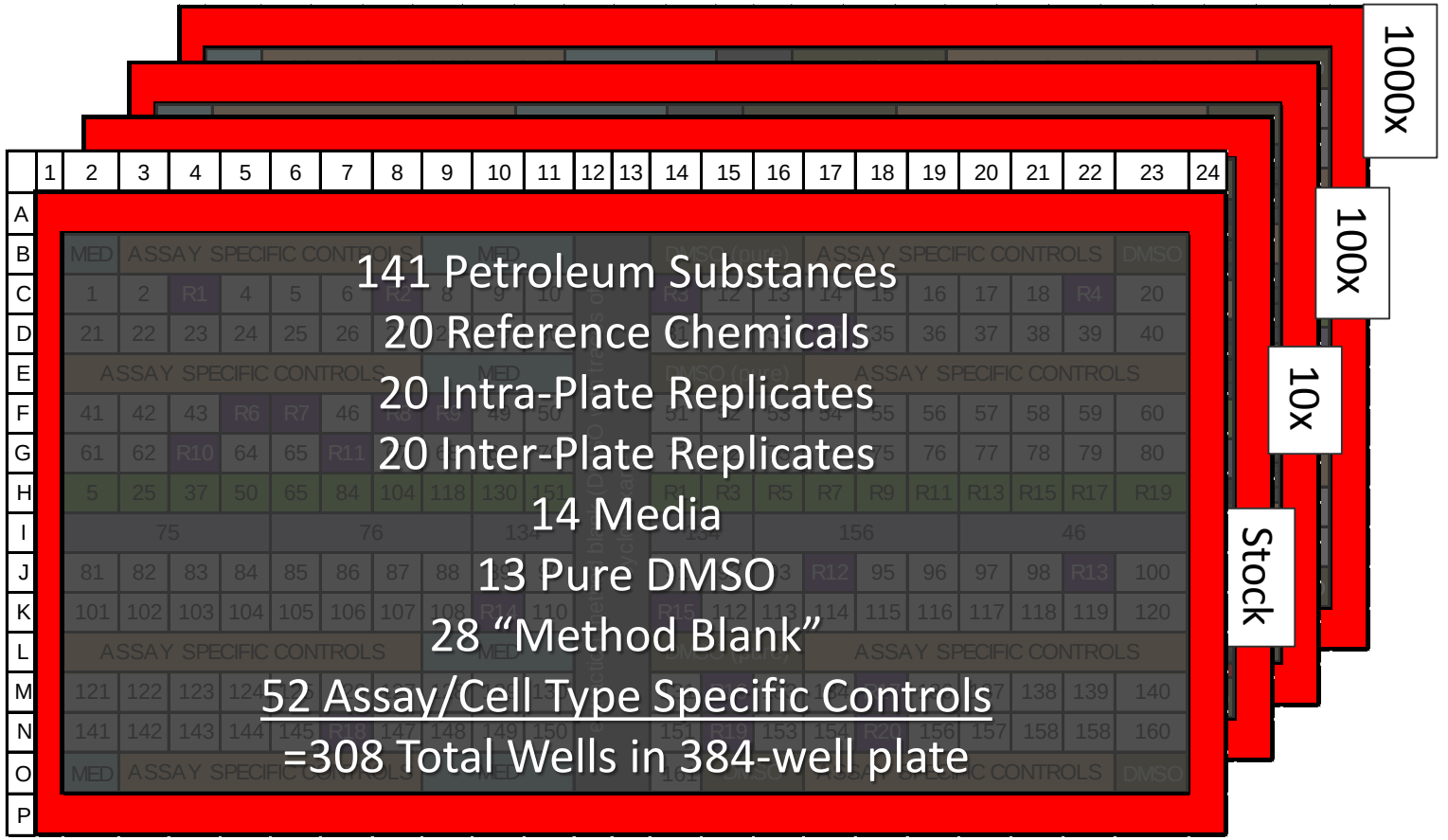


Stock Extract for *in vitro* experiments [in 100% DMSO]



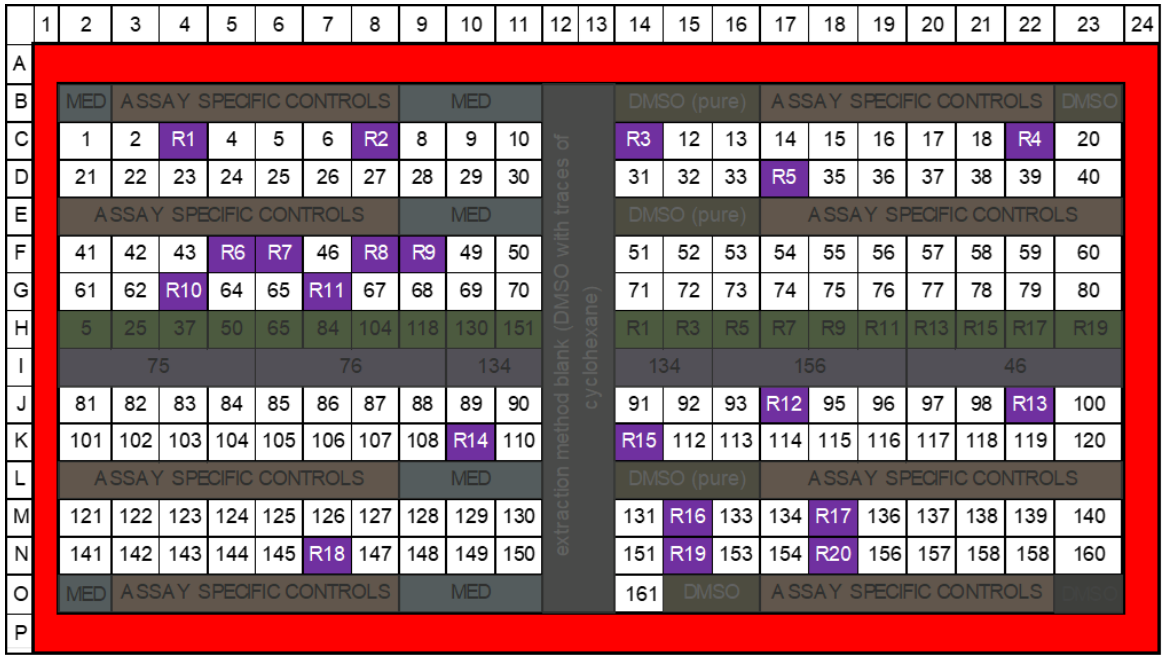
"Method blank" [cyclohexane-equilibrated DMSO]

WP1a: Designing a screening plate (384-well)



- 4 plates (Stock Plate, 1:10, 1:100, 1:1,000 diluted) were prepared (5-point concentration-response)
- Extracts were diluted in "Method Blank" (cyclohexane-equilibrated DMSO)
- 4 sets of plates were prepared and sealed
- Plates were stored at -20°C before use
- Identical "master plates" were used at Texas A&M University and Public Health England laboratories

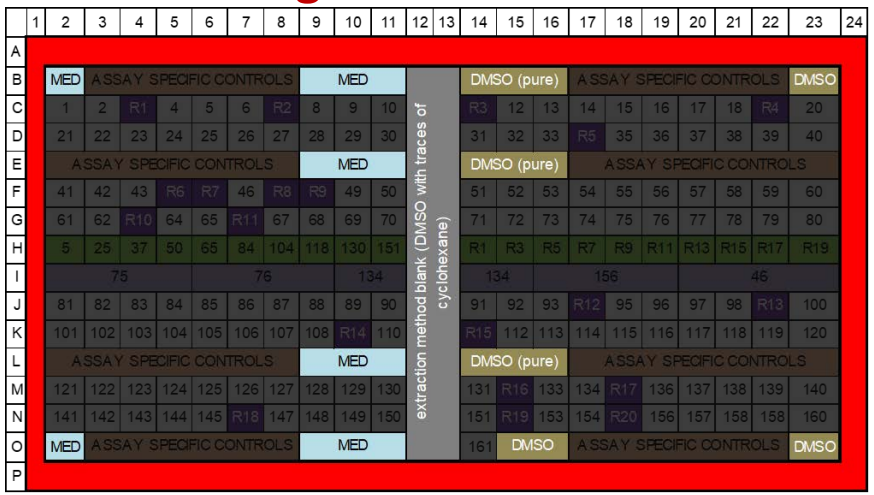
Placement of the substances and positive/negative controls



161 “screened” substances in one dilution/plate:

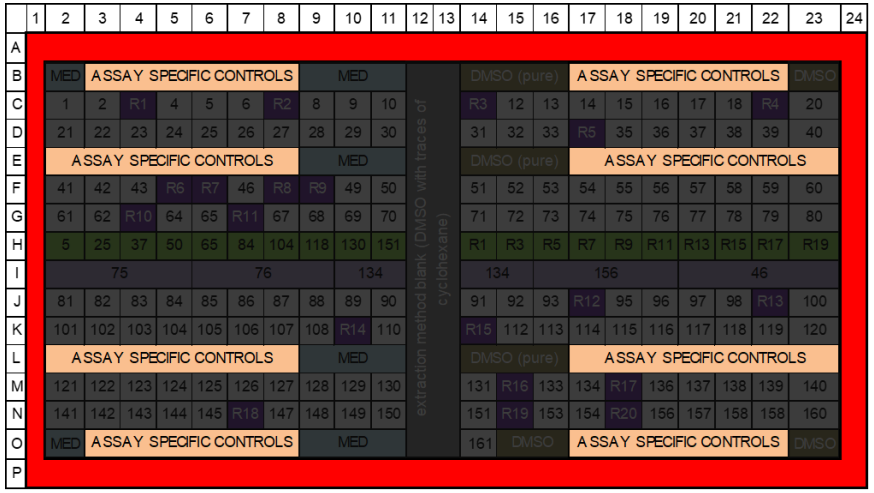
- 141 petroleum substances
- 20 reference chemicals (R20 substances)

Negative Controls



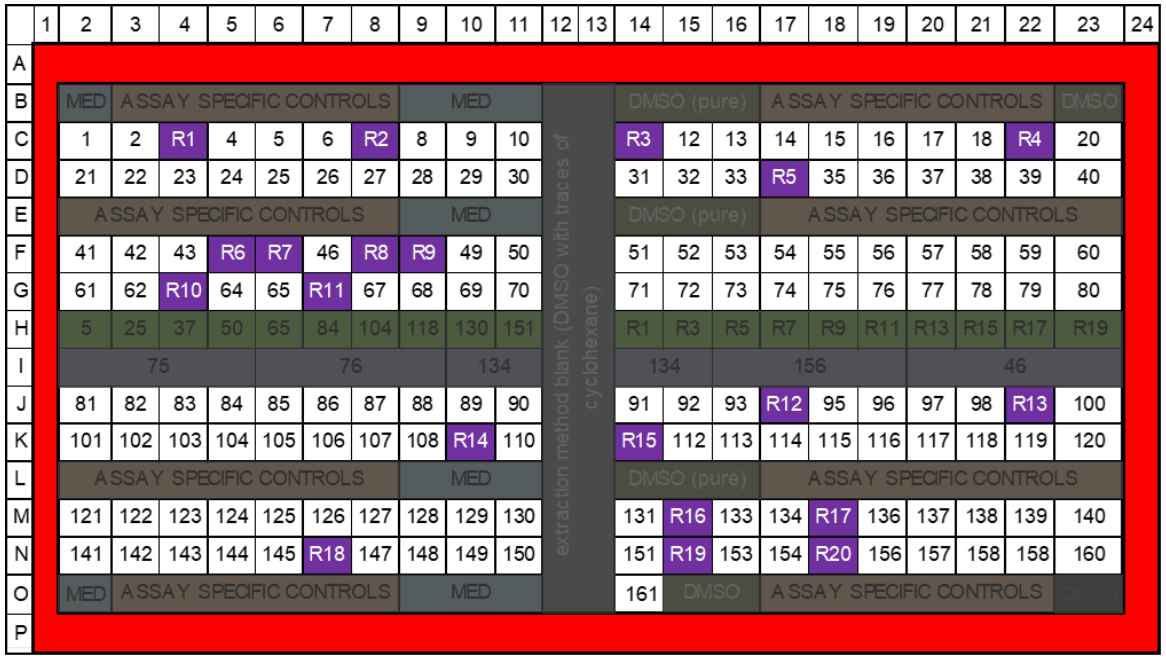
Media (14) Pure DMSO (13) “Method Blanks” (28)

Cell/Assay-Specific Positive Controls



52 wells for cell-specific positive controls

Intra- and inter-plate replicates



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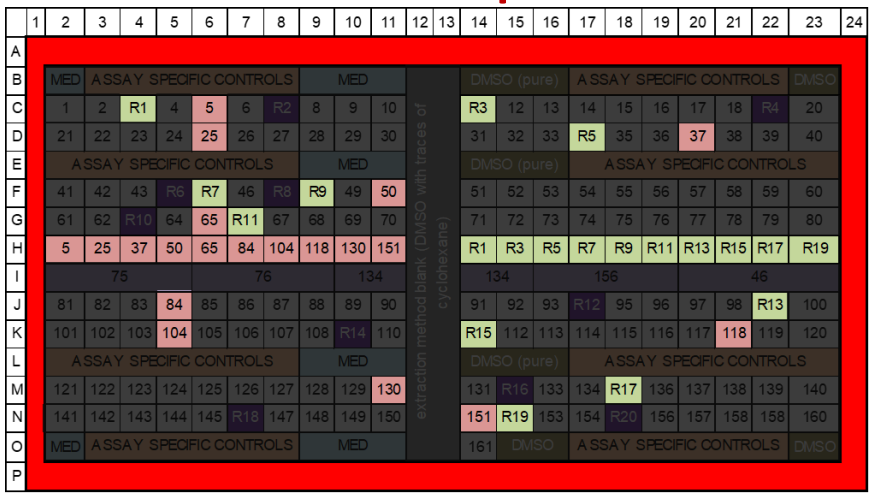
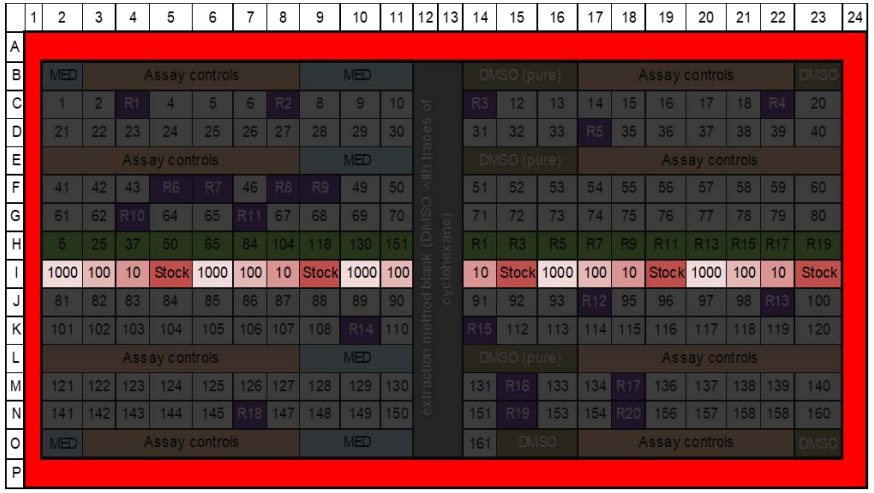
Intra-Plate Replicates



20 duplicates: (10 petroleum subst., 10 ref. chem.)

Inter-Plate Replicates



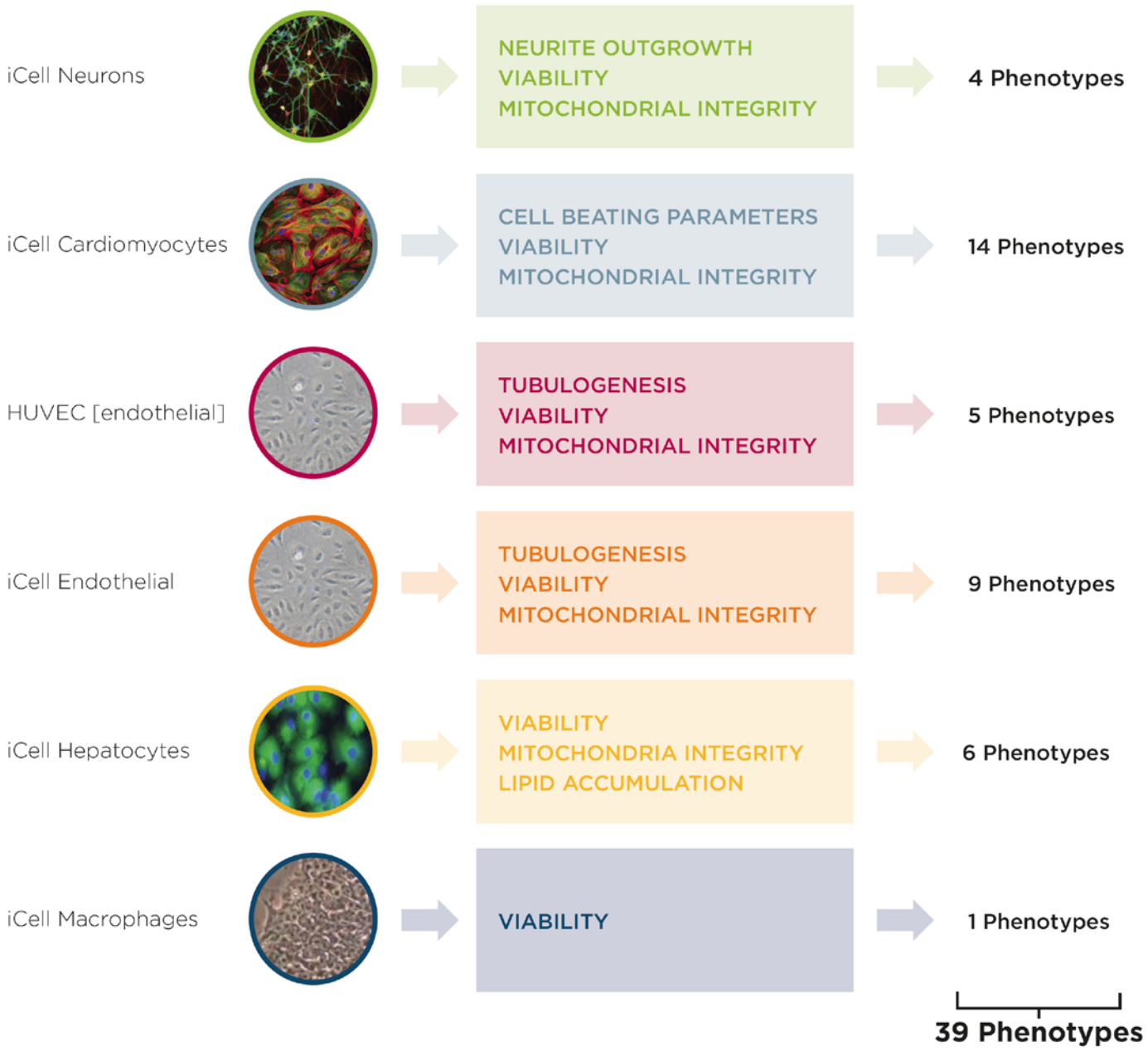
Full concentration-response (5 petroleum subst.)

WP2: Screening of petroleum substances in human cells

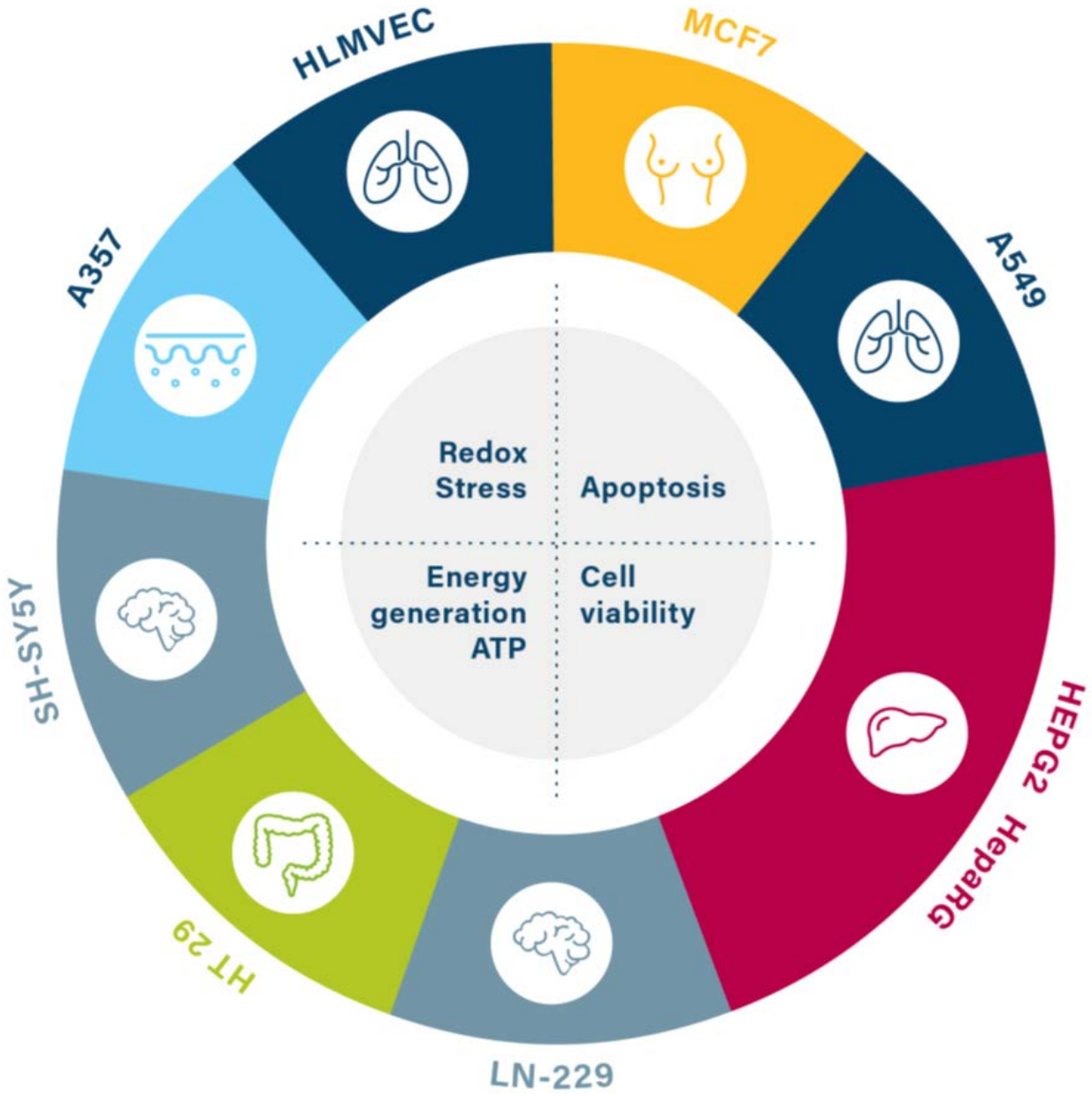
Cell line selection considerations:

- Human origin for the cells
- Represent diversity of organs/tissues
- Include both “primary” (including iPS cells) and “cell line” models
- Models are reproducible (i.e., same cell/donor can be obtained from a commercial source)
- Ability to assess both “functional” and “cytotoxicity” endpoints – to evaluate the specificity of bioactivity
- Cell-based models that are well-accepted in toxicology (i.e., methods published)
- Ability to compare results from Cat-App with other databases (e.g., data from LINCS and other *in vitro* screening)

WP2a: Screening of human iPSC-derived cell lines



WP2b: Screening of human cell lines

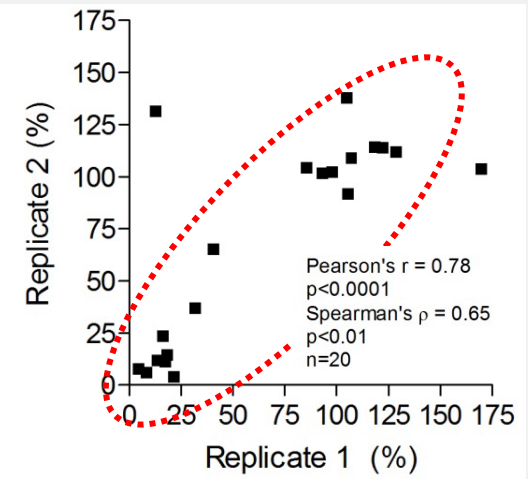


ASSAY	Tag	Control chemical
CELL MEMBRANE INTEGRITY	CMFDA	Triton X100
REACTIVE OXYGEN SPECIES	ROS	DMNQ
APOPTOSIS	CASP	Mitomycin C/ Etopiside
PROTEIN SYNTHESIS INHIBITION	PROT	Hygromycin B
ATP QUANTITATION	APT	Rotenone

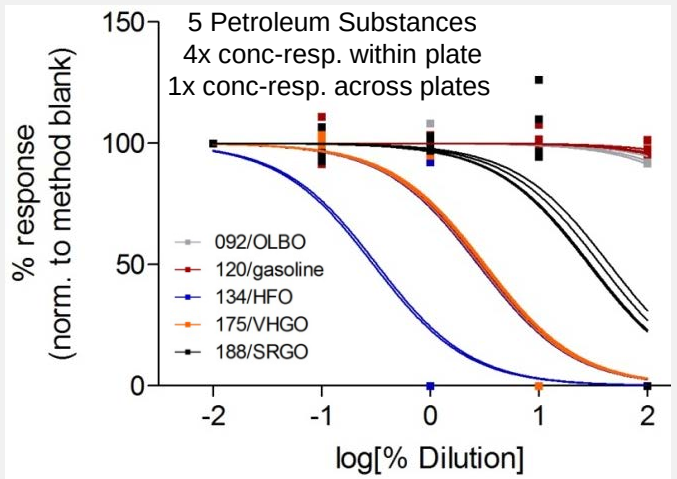
In vitro assay quality assessment

MED	ASSAY SPECIFIC CONTROLS						MED			extraction method blank (DMSO with traces of cyclohexane)	DMSO (pure)	ASSAY SPECIFIC CONTROLS							DMSO
1	2	R1	4	5	6	R2	8	9	10		R3	12	13	14	15	16	17	18	R4	20
21	22	23	24	25	26	27	28	29	30		31	32	33	R5	35	36	37	38	39	40
ASSAY SPECIFIC CONTROLS							MED				DMSO (pure)	ASSAY SPECIFIC CONTROLS								
41	42	43	R6	R7	46	R8	R9	49	50		51	52	53	54	55	56	57	58	59	60
61	62	R10	64	65	R11	67	68	69	70		71	72	73	74	75	76	77	78	79	80
5	25	37	50	65	84	104	118	130	151		R1	R3	R5	R7	R9	R11	R13	R15	R17	R19
75				76				134			134			156			46			
81	82	83	84	85	86	87	88	89	90		91	92	93	R12	95	96	97	98	R13	100
101	102	103	104	105	106	107	108	R14	110		R15	112	113	114	115	116	117	118	119	120
ASSAY SPECIFIC CONTROLS							MED				DMSO (pure)	ASSAY SPECIFIC CONTROLS								
121	122	123	124	125	126	127	128	129	130		131	R16	133	134	R17	136	137	138	139	140
141	142	143	144	145	R18	147	148	149	150		151	R19	153	154	R20	156	157	158	158	160
MED	ASSAY SPECIFIC CONTROLS						MED				161	DMSO	ASSAY SPECIFIC CONTROLS							DMSO

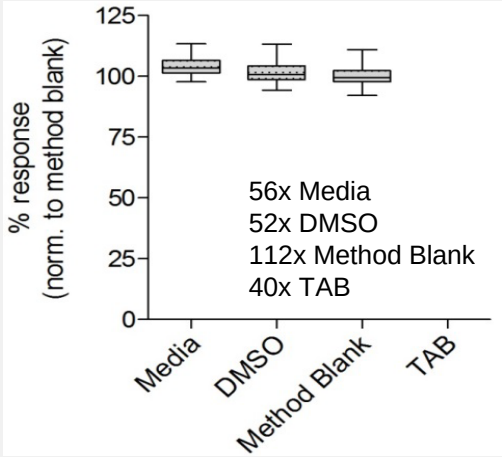
Intra-Plate Replicates (20×)



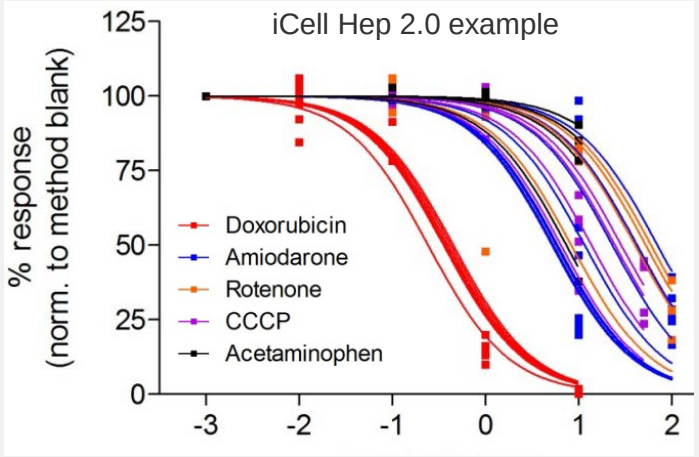
Inter-Plate Replicates (5 substances)



Negative Controls

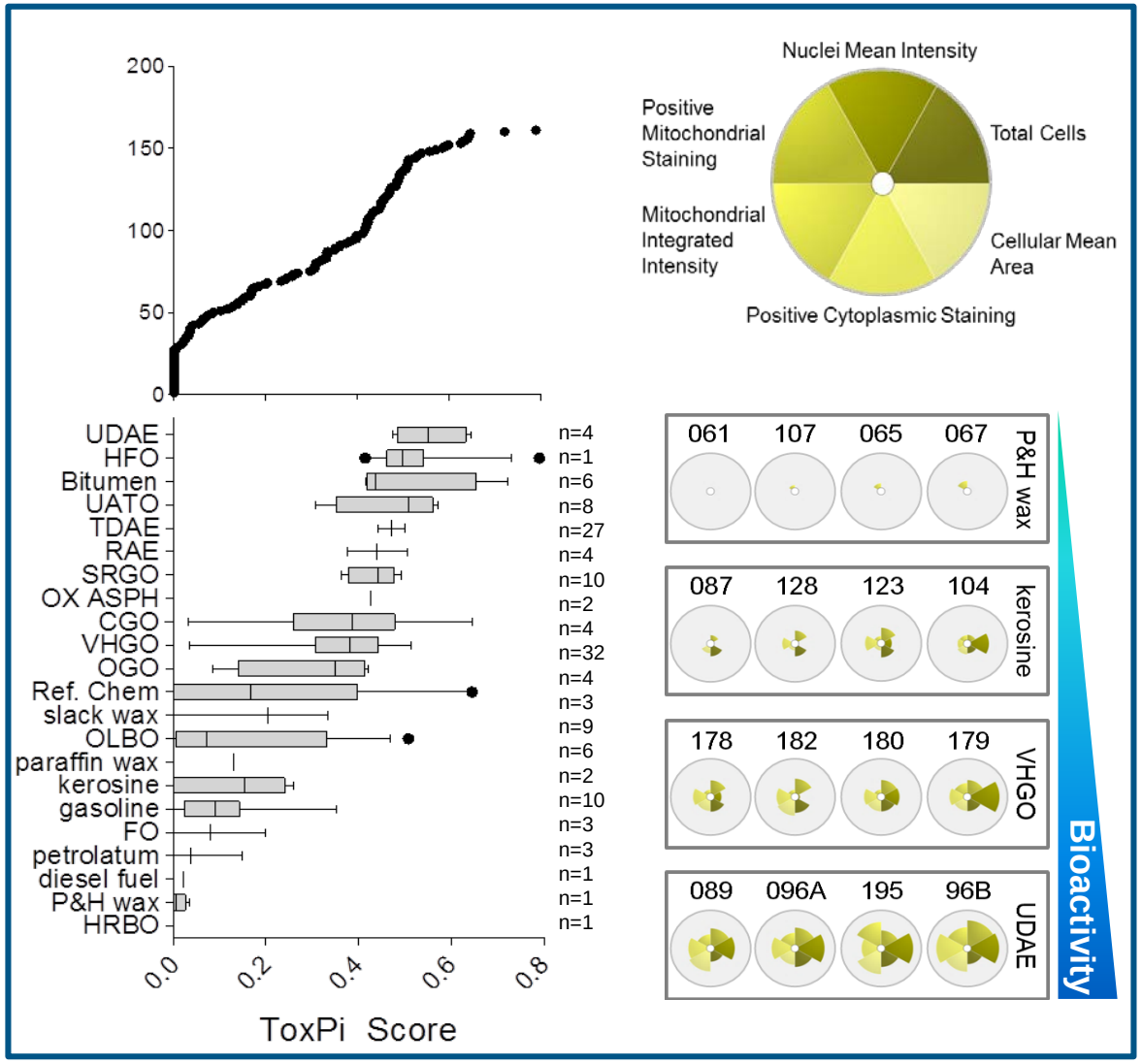


Cell/Assay-Specific Positive Controls

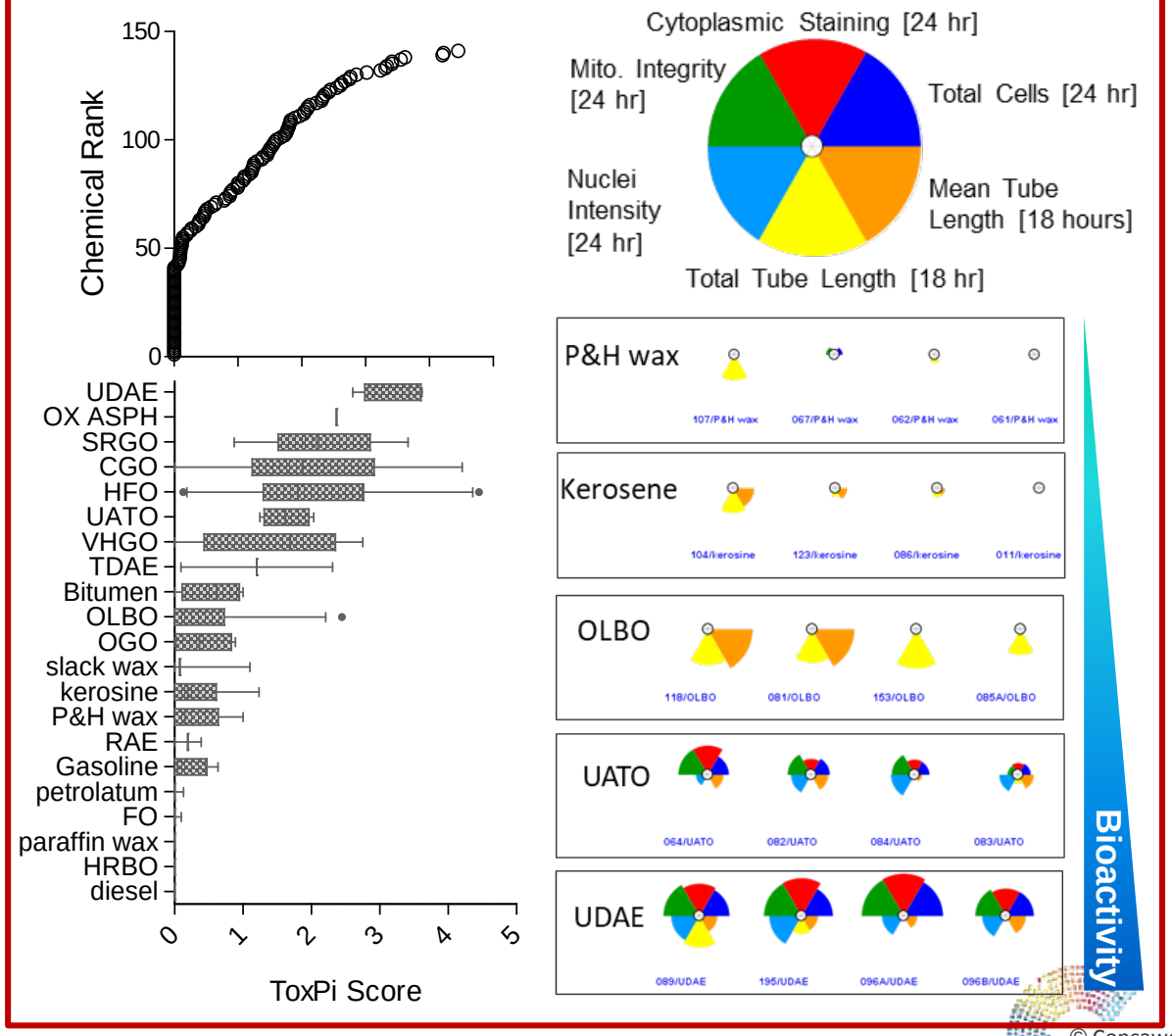


Example biological groupings of petroleum substances

iCell Hepatocytes

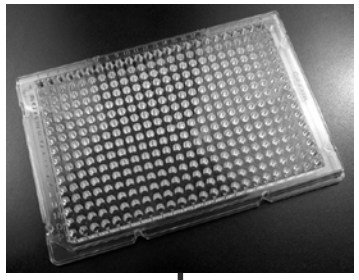


HUVECs (endothelial cells)

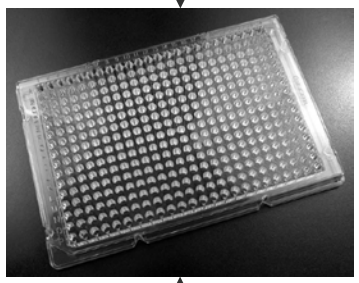


TempO-seq Technology – High-Throughput Targeted Sequencing

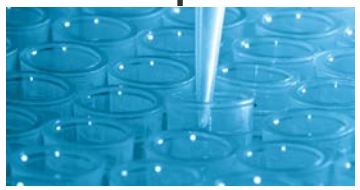
Screening plate (384-well) with cells

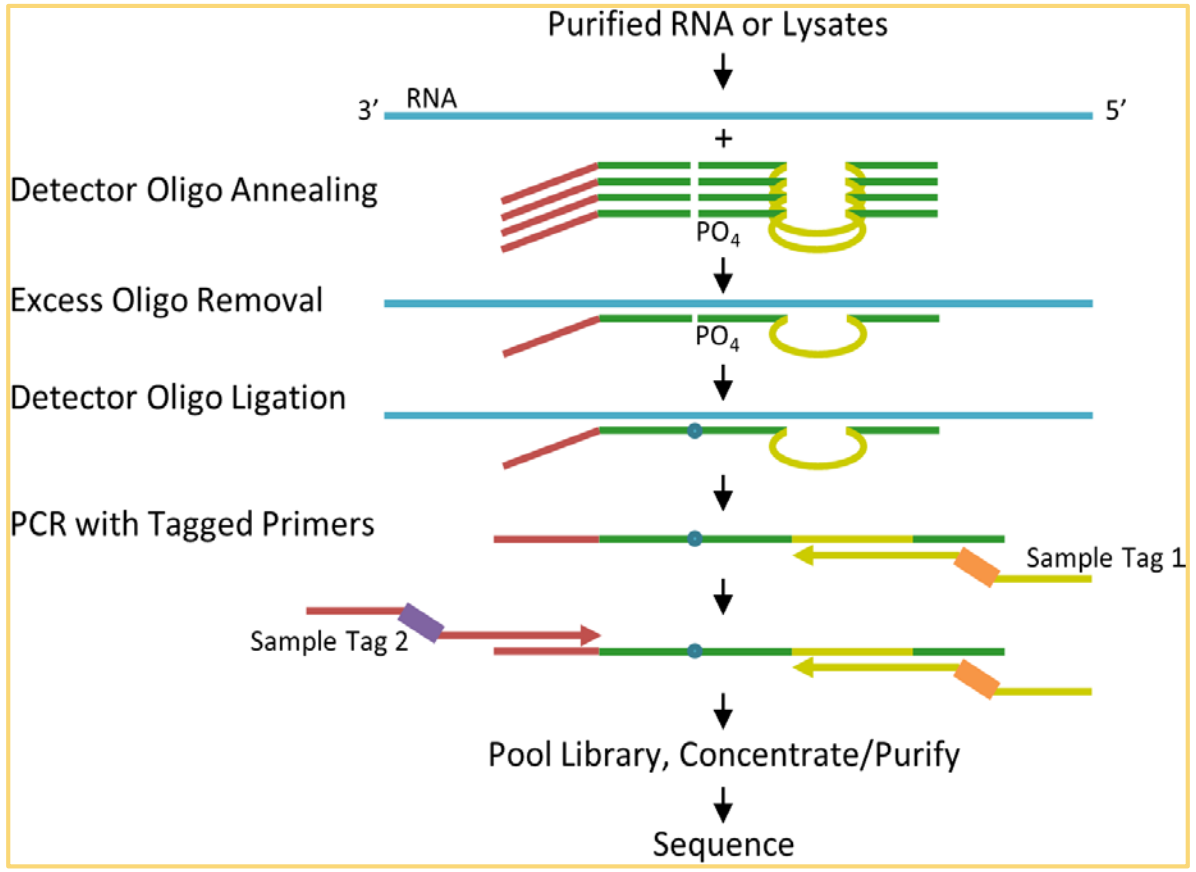


Lysis buffer



Pool of ~3,000 detector oligos [NTP S1500+]





Yeakley et al. PLoS ONE 12(5): e0178302, 2017

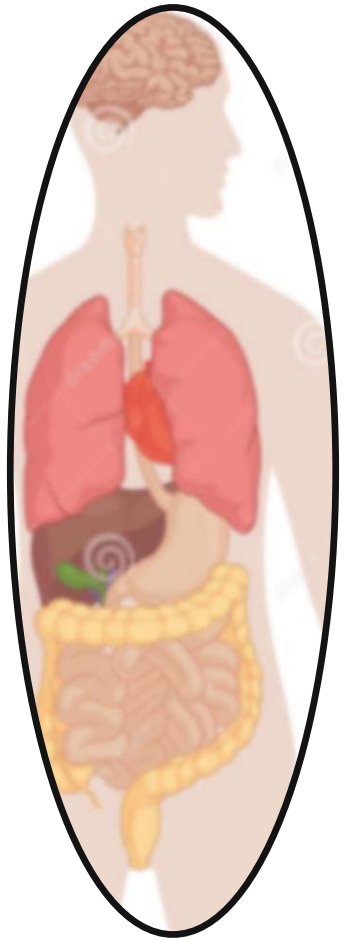
Assay advantages:

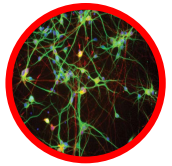
- Works in 384-wells
- No cDNA library prep
- 1000's genes/sample
- High Specificity (probe seq + ligation)

Assay considerations:

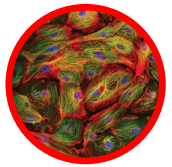
- Sequencing depth (per gene per sample)
- Gene selection (targeted set vs whole genome)
- New technology (no large database for comparisons)

Cat-App Transcriptomics Data – 6 Human Cell Types



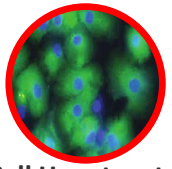


iCell Neurons

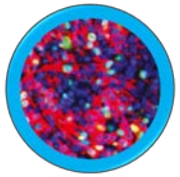


iCell Cardiomyocytes

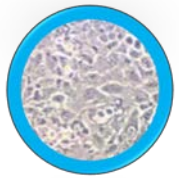
iCell Endothelial



iCell Hepatocytes



A375



MCF7

TempO|Seq™ Transcriptional Profiling

- 11,000+ samples from 6 cell types
- 4-point concentration response data
- Differential gene expression for ~3,000 transcripts (targeted analysis)
- Over 35,000,000 data points
- Novel data processing pipeline
- Concentration-response modeling pipeline
- Transcriptomics data can be combined with other data streams

Conclusions:

- Manufacturing stream/phys-chem properties may be insufficient alone to support “similarity” of complex UVCBs
- Bioactivity profiling of complex UVCBs is a feasible path towards mechanistic toxicology-based characterization of “sufficient similarity”
- Cat-App is the largest to date “case study” that was aimed at testing whether and how *in vitro* bioactivity [including transcriptomics] can be used to inform grouping of UVCBs
- Ambitious goals of Cat-App [in terms of the number of substances, cell types, endpoints and data analysis questions] have been met with respect to both scientific output and timelines