State of the art and progress made in high throughput testing

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EU REACH Definition of UVCB:

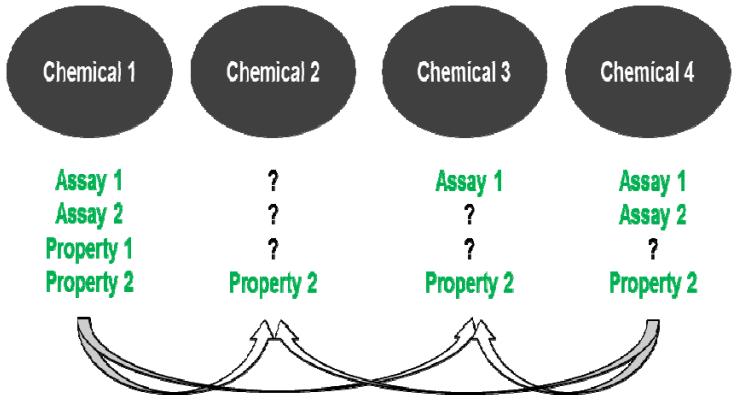
"Substances of Unknown or Variable composition, Complex reaction products or Biological materials"

- A UVCB is **difficult to define by chemical composition** because:
  - It has a large number of constituents
  - Composition is largely unknown, variable, or poorly predictable.
- A UVCB substance **has no definite molecular formula** representation and either a partial structural diagram or no structural diagram.
- UVCB substance **must be identified by other types of information** (e.g., the source of the substance or the process used to obtain the substance)

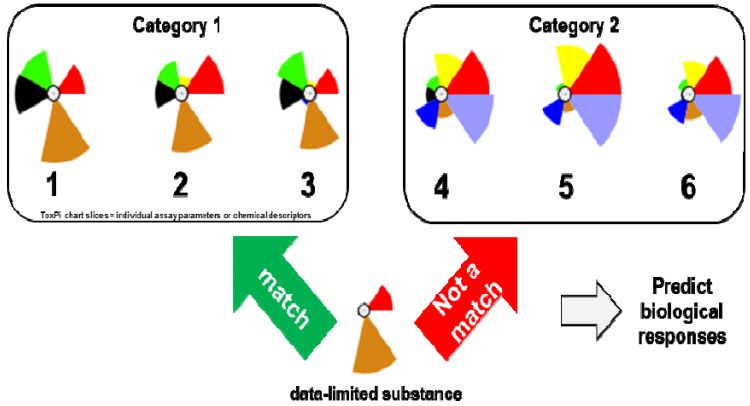
Slide content courtesy of Dr. Graham Whale (Shell)

Hazard characterization through read-across

Traditional Read Across



Toxicological data-based read-across



Structural Categorization/Grouping

Toxicological Categorization/Grouping

“Chemical” Read-Across

“Biological” Read Across

Complex Substances / UVCBs

Not amenable to full chemical characterization	High Production Volume Chemicals	Batch-to-Batch Variability (same CAS)

Opportunities for incorporating *in vitro/in silico* data into read-across

1. Replacing an animal study/endpoint with an "apical endpoint-relevant" non-animal alternative method

- EDSP "Pivot": Replacement of a uterotrophic assay with a battery of 18 *in vitro* tests (FRL-9928-69 06/19/2015, Judson et al. Tox Sci 2015)
- Providing additional "biological" plausibility to the chemical structure-based similarity argument using relevant alternative method(s)

2. Reducing uncertainty in a read-across argument in a regulatory submission:

- Using *in vitro/in silico* data to confirm the similarity in the mechanism of action within a category and/or between the "target" and "source" compounds
- Confirming or refuting a hypothesis that analogues may have "other" effects
- Assessing relative "potency" of the analogues

Analogue Read-Across

with CBRA

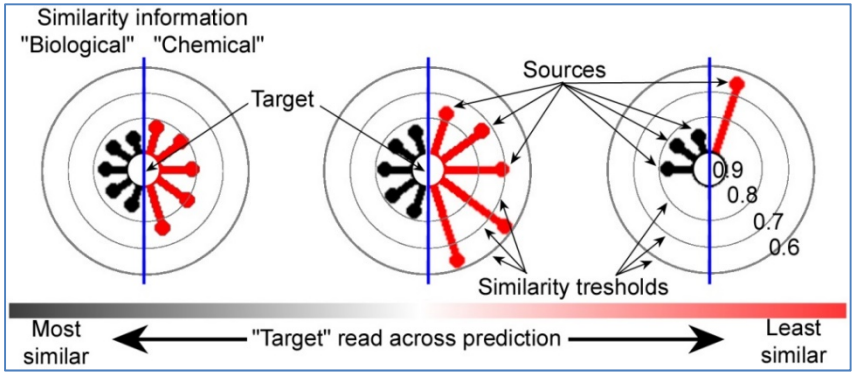
Chemical Research in Toxicology

Article

pubs.acs.org/crt

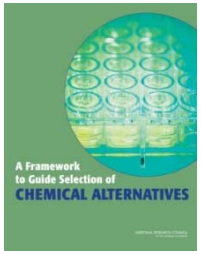
Integrative Chemical–Biological Read-Across Approach for Chemical Hazard Classification

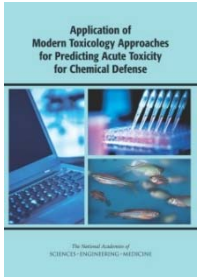
Yen Low,[†,‡] Alexander Sedykh,[†] Denis Fourches,[†] Alexander Golbraikh,[†] Maurice Whelan,[‡] Ivan Rusyn,[*,‡] and Alexander Tropsha[*,†]



Category Grouping

with ToxPi





BIOINFORMATICS APPLICATIONS NOTE Vol. 29 no. 3 2013, pages 402–403
doi:10.1093/bioinformatics/bts686

Systems biology

Advance Access publication November 29, 2012

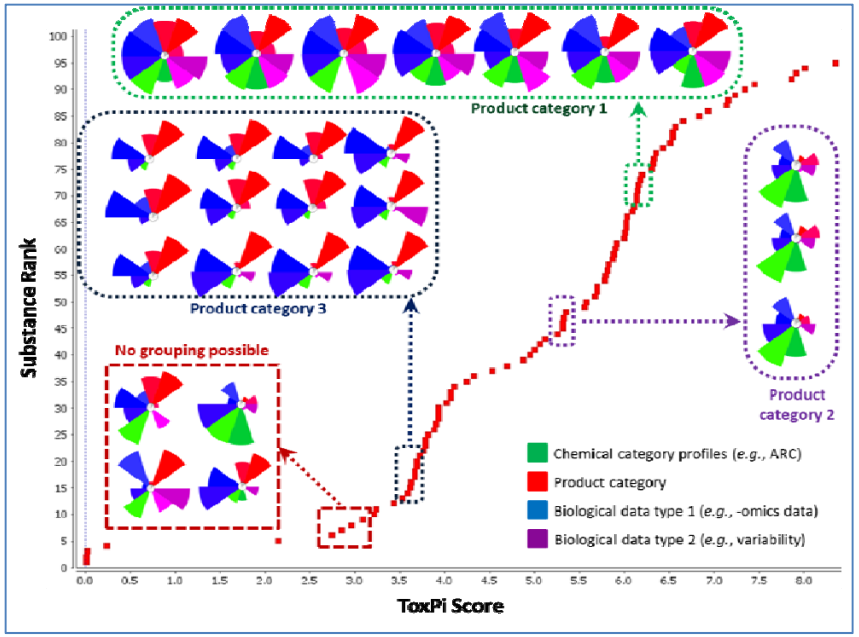
ToxPi GUI: an interactive visualization tool for transparent integration of data from diverse sources of evidence

David M. Reif[1,*], Myroslav Sypa[2], Eric F. Lock[2], Fred A. Wright[3], Ander Wilson[1], Tommy Cathey[4], Richard R. Judson[1] and Ivan Rusyn[2]

Endocrine Profiling and Prioritization of Environmental Chemicals Using ToxCast Data

David M. Reif,[1] Matthew T. Martin,[1] Shirlee W. Tan,[2] Keith A. Houck,[1] Richard S. Judson,[1] Ann M. Richard,[1] Thomas B. Knudsen,[1] David J. Dix,[1] and Robert J. Kavlock[1]

[1]National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA; [2]Office of Science Coordination and Policy, Office of Pollution Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency, Washington, DC, USA



Case Study: Grouping of Petroleum Substances With Toxicity Profiling

Complex, UVCB

C number	Boiling point °C (n-alkanes)	Number of isomers (n-alkanes only!)
3	-42	1
4	-1	2
5	36	3
6	69	5
7	98	9
8	126	18
10	174	75
15	269	4 347
20	343	366 231
25	402	36 777 419
30	450	4 108 221 447
35	490	493 054 243 760
40	525	62 353 826 654 563

High Production Volume Chemicals

C2-100+

Chemically Diverse Product Groups

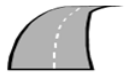
C4-11

C8-15

C10-25

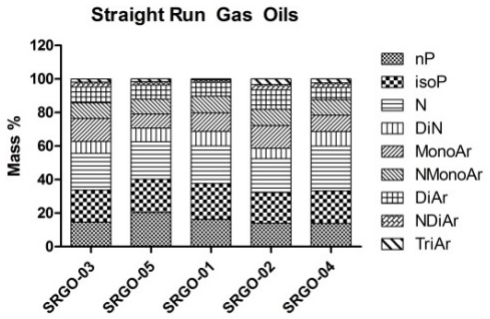
C15-65

C20-100+



C35-100+

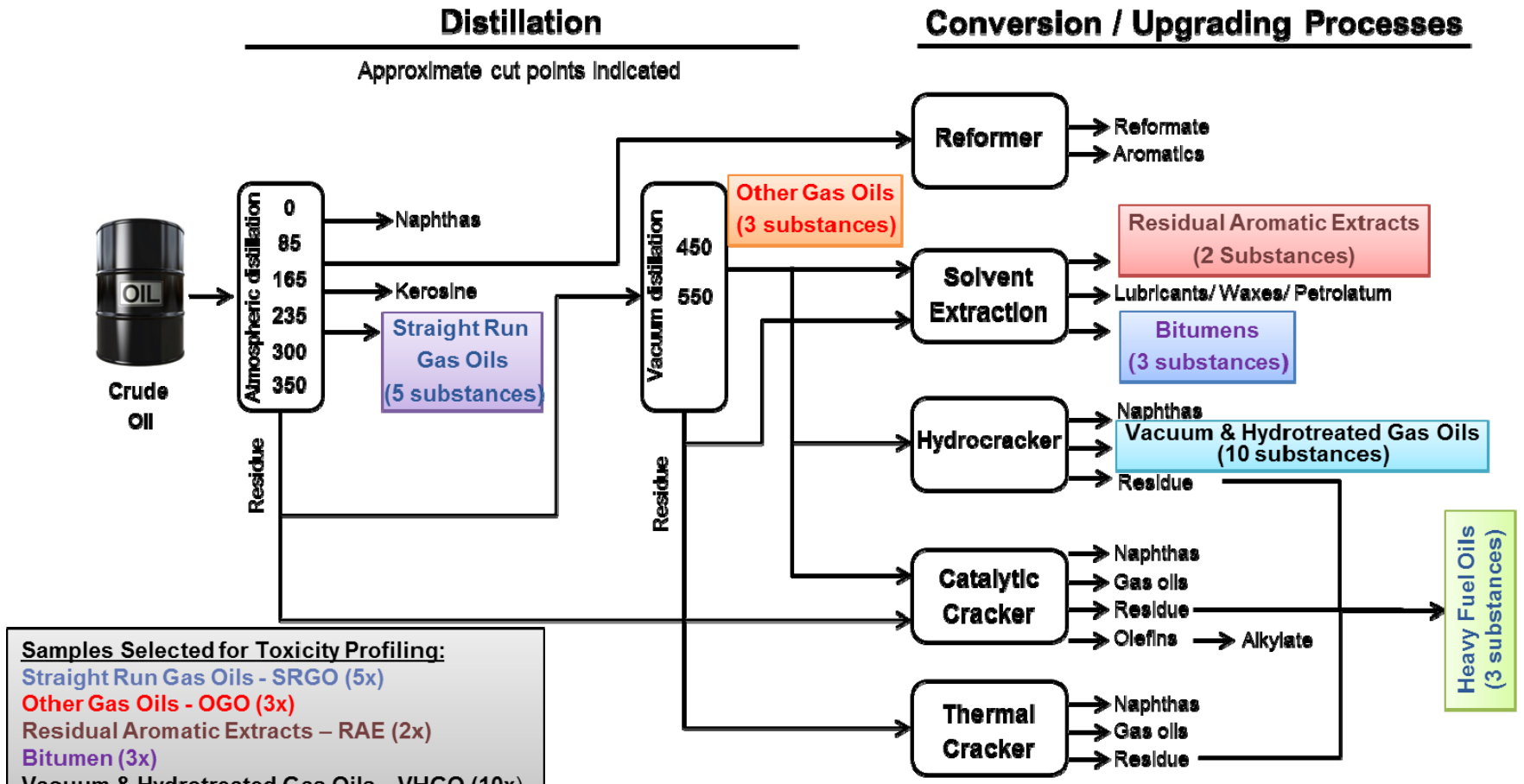
Batch-to-Batch Variability in Composition (same CAS)



Regulators have issues with:

Categories as such (not sufficiently defined) Substance ID (too sketchy) Variability within a category

Sample Selection From Representative Stages of the Oil Refining Process



Modified from Dr. Peter Boogaard (Shell)

Designation: E1687 – 10

An American National Standard

May 2010

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

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/

A1. METHODS FOR ESTIMATION

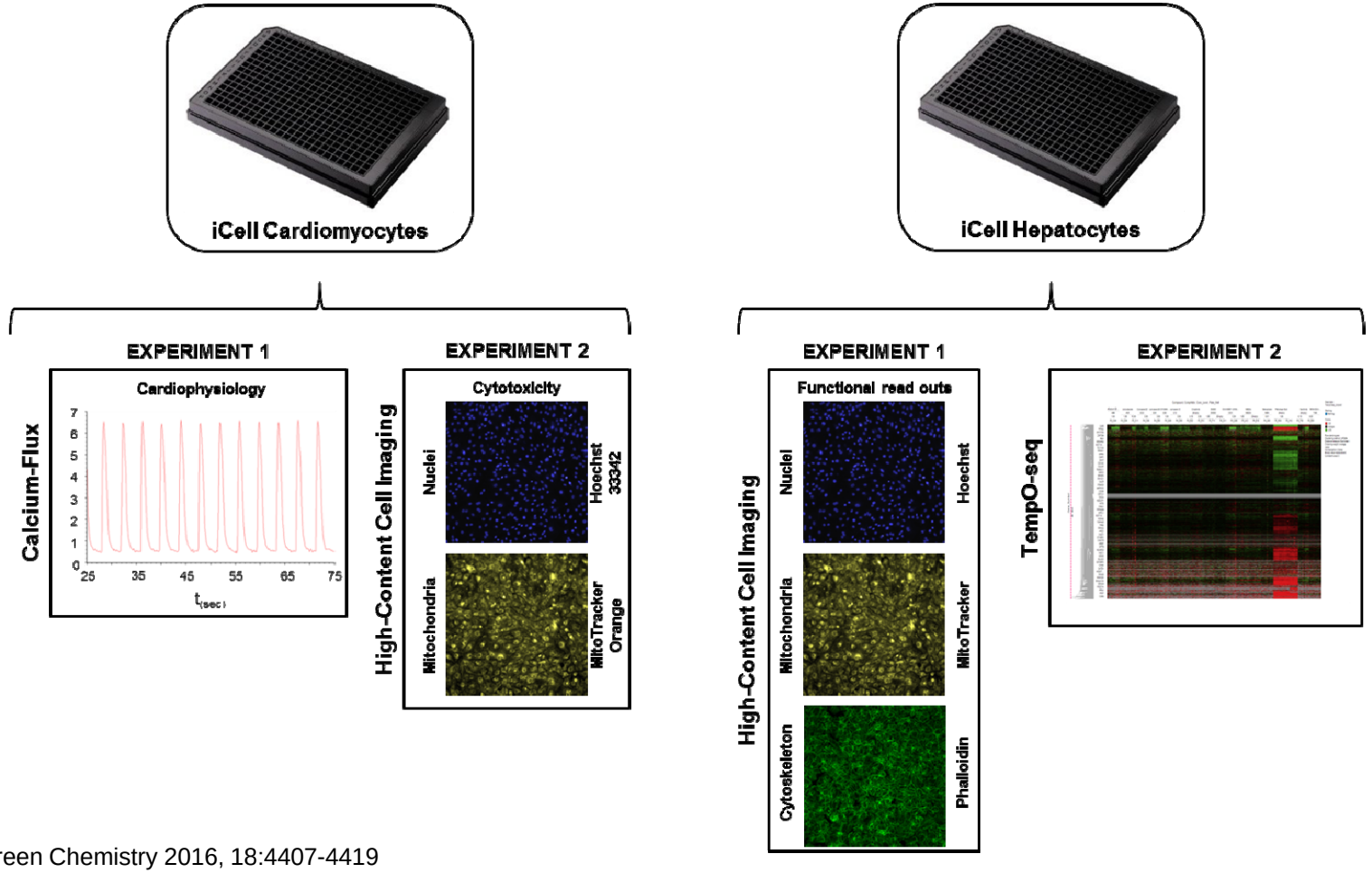
A1.1 Methods for estimation of relative PAC content of oils, or for its correlation with MI in the modified Ames assay, or both, and with dermal carcinogenic potency. These analytical methods do not predict the mutagenicity or dermal carcinogenicity of petroleum fractions in the naphtha, kerosine, low-boiling 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.

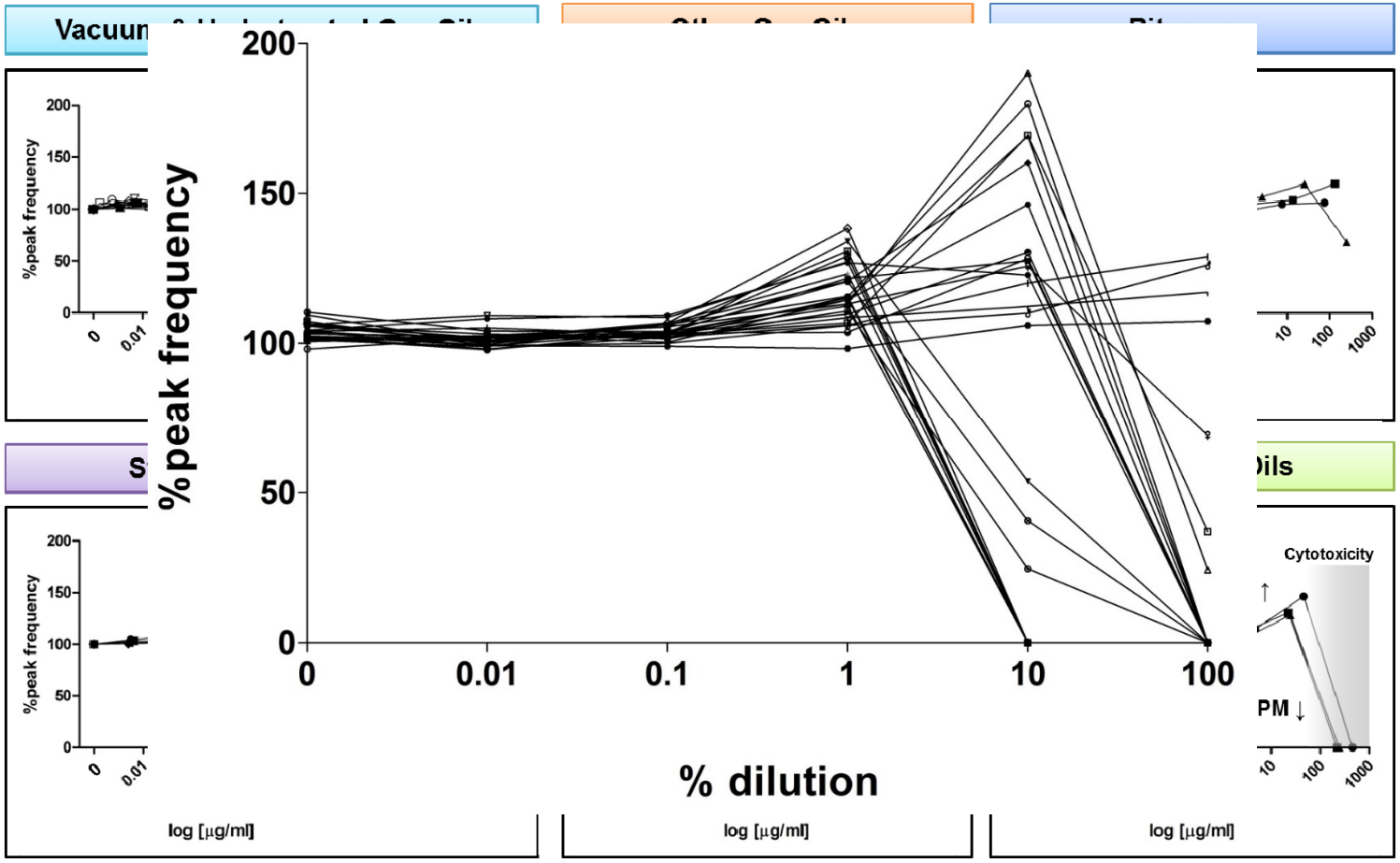
High-Content Bio-profiling of UVCBs (Petroleum Substances)



Grimm *et al*. Green Chemistry 2016, 18:4407-4419

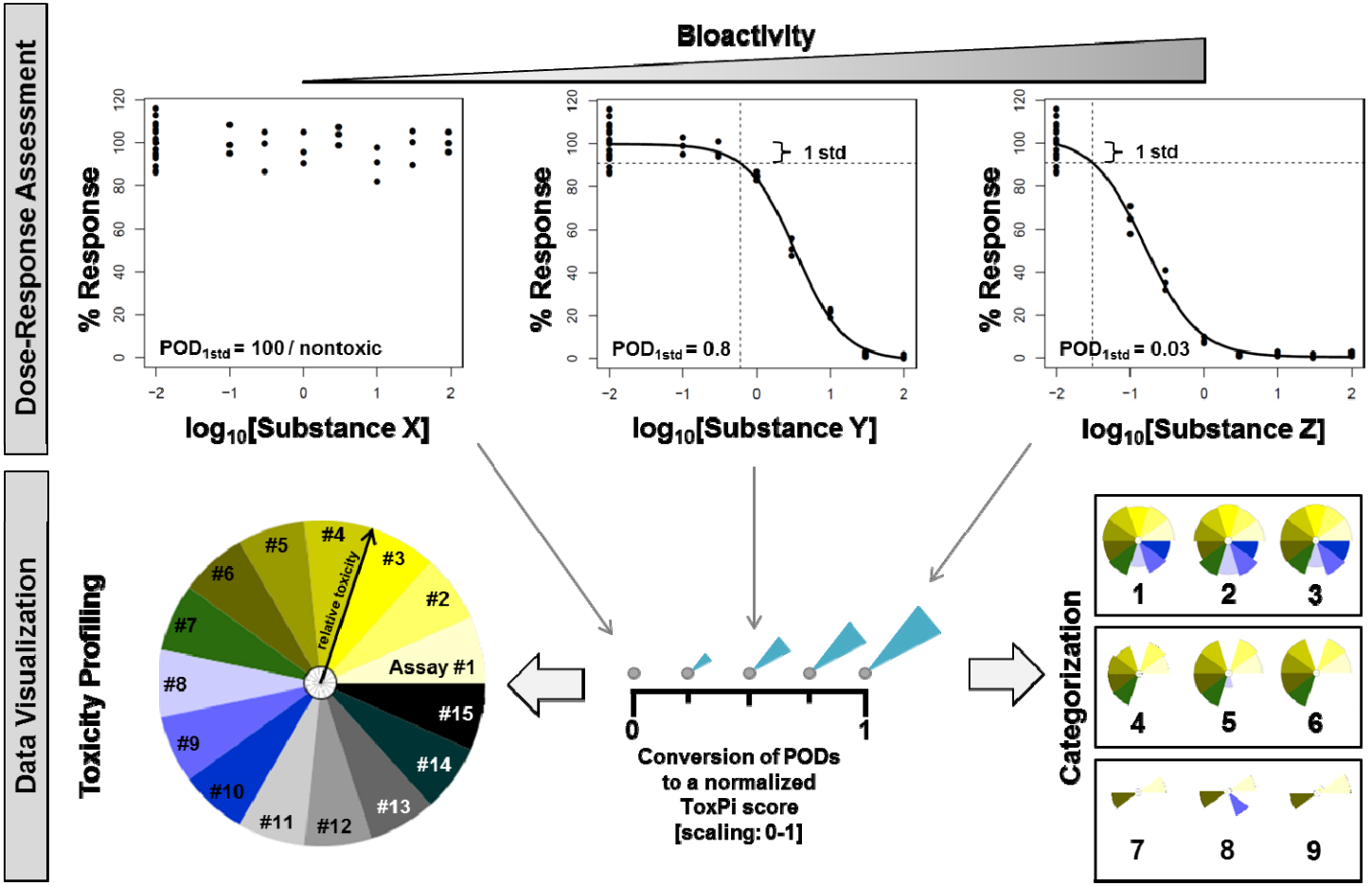
Category-Specific Effects of Petroleum Substances on Cardiomyocytes

[Cardiomyocyte Beating Frequency and Cytotoxicity Phenotypes as an Example]



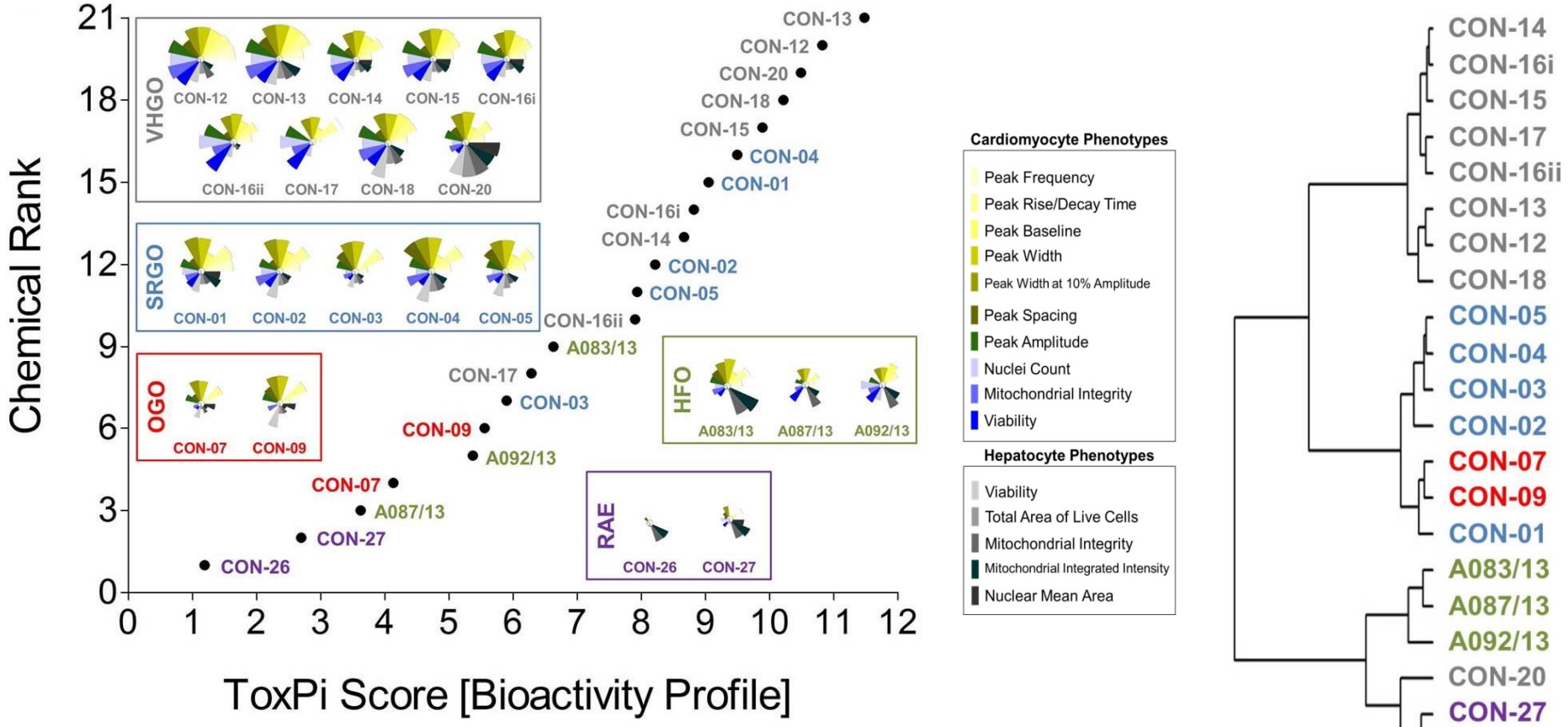
Data Integration using Toxicological Priority Index (ToxPi) Approach

(Converting *In Vitro* Screening Point of Departure Values Into Each Compound's ToxPi "Signature")



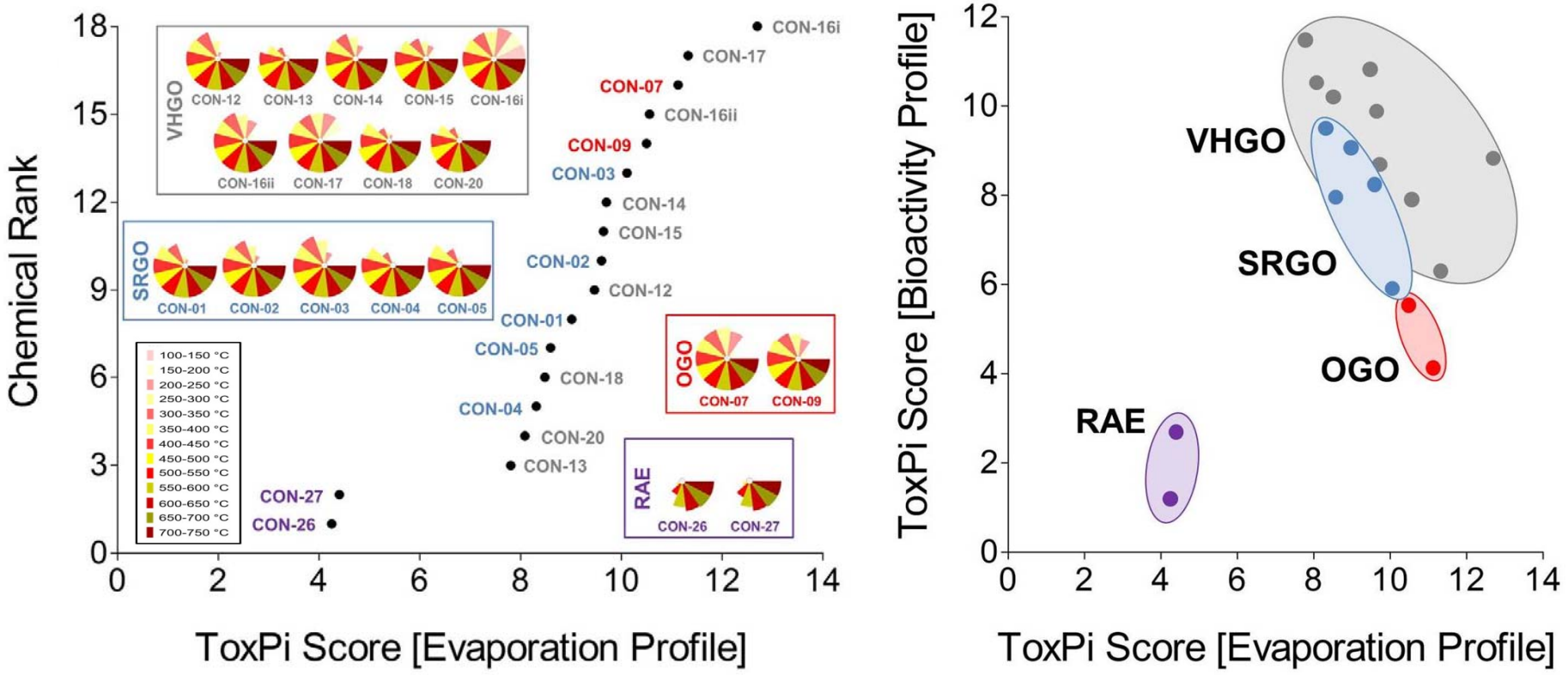
In Vitro Toxicity Data Reveals Similarities Among Petroleum Substances

(Category Grouping Using Toxicity Profiles From Human iCell Cardiomyocytes and Hepatocytes)



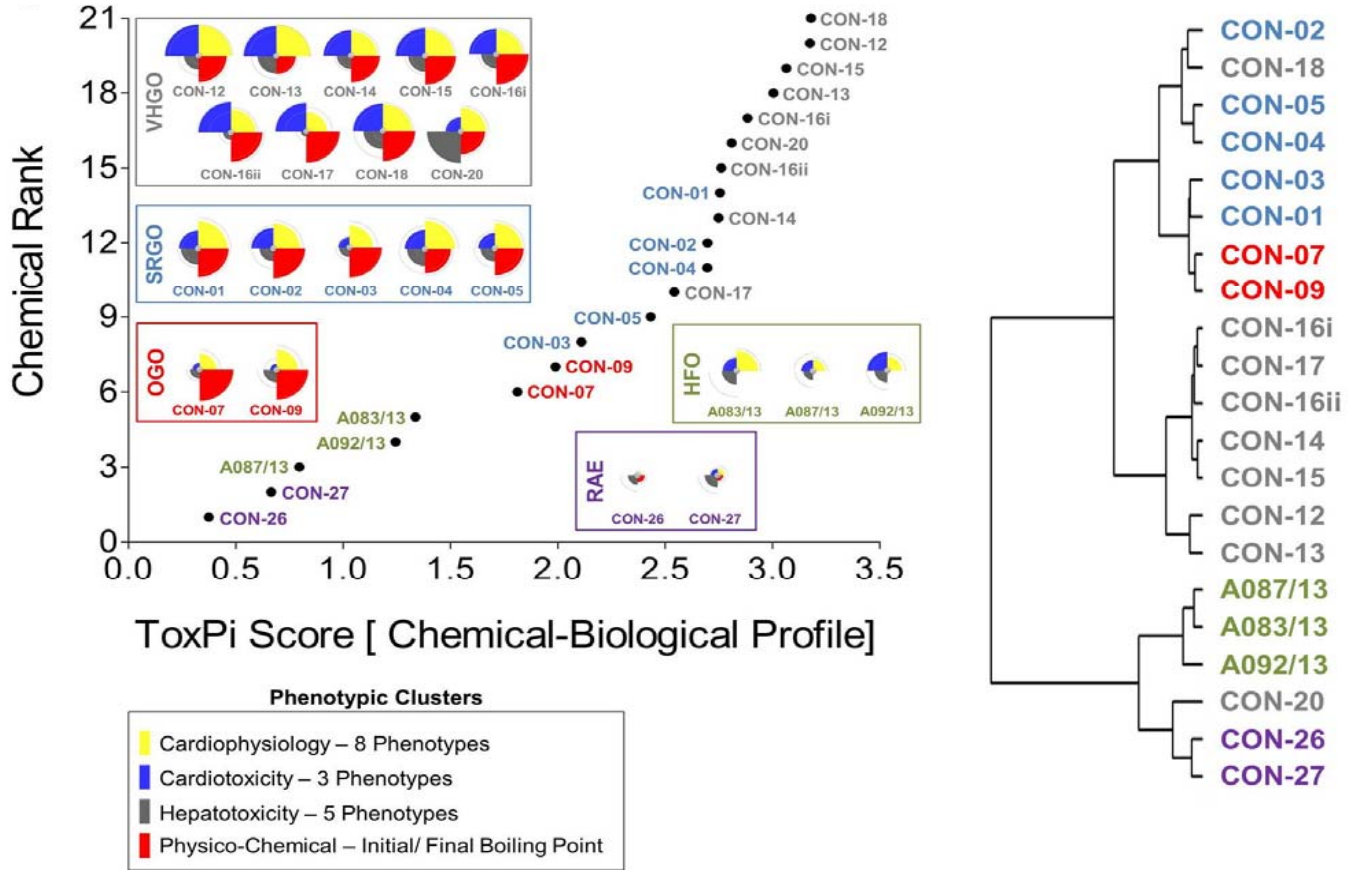
Grimm *et al*. Green Chemistry 2016, 18:4407-4419

Physico-chemical data-integrative grouping of petroleum substances
(Category Grouping Using Evaporation Profiles Available for 18 Petroleum Substances)



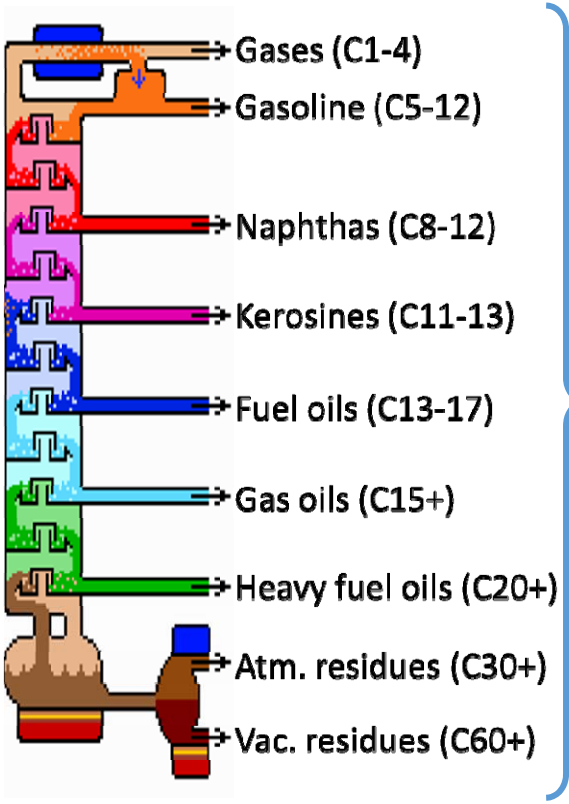
Grimm *et al*. Green Chemistry 2016, 18:4407-4419

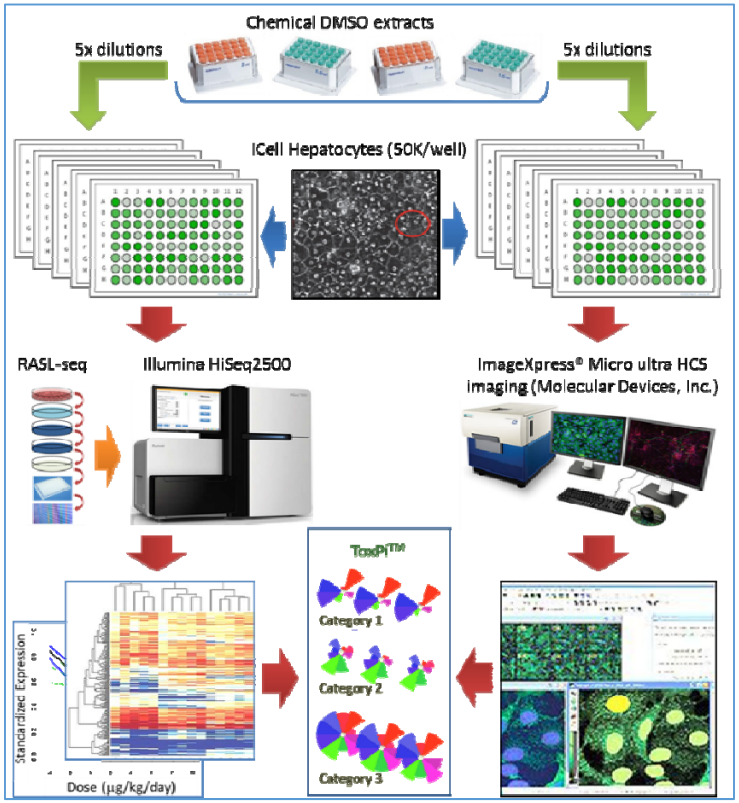
Chemical-biological data-integrative categorization of petroleum substances
(Category Grouping Using Phenotypic Screening Data and Physico-Chemical Descriptors)

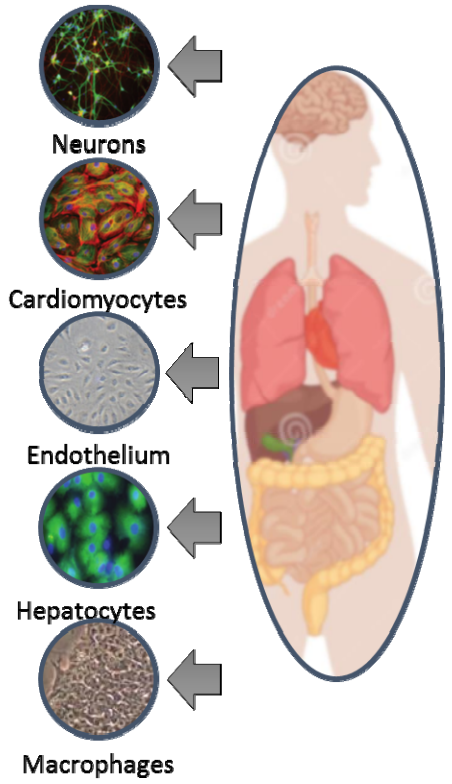


Grimm *et al*. Green Chemistry 2016, 18:4407-4419

Cat-App project: Bioactivity profiling scope

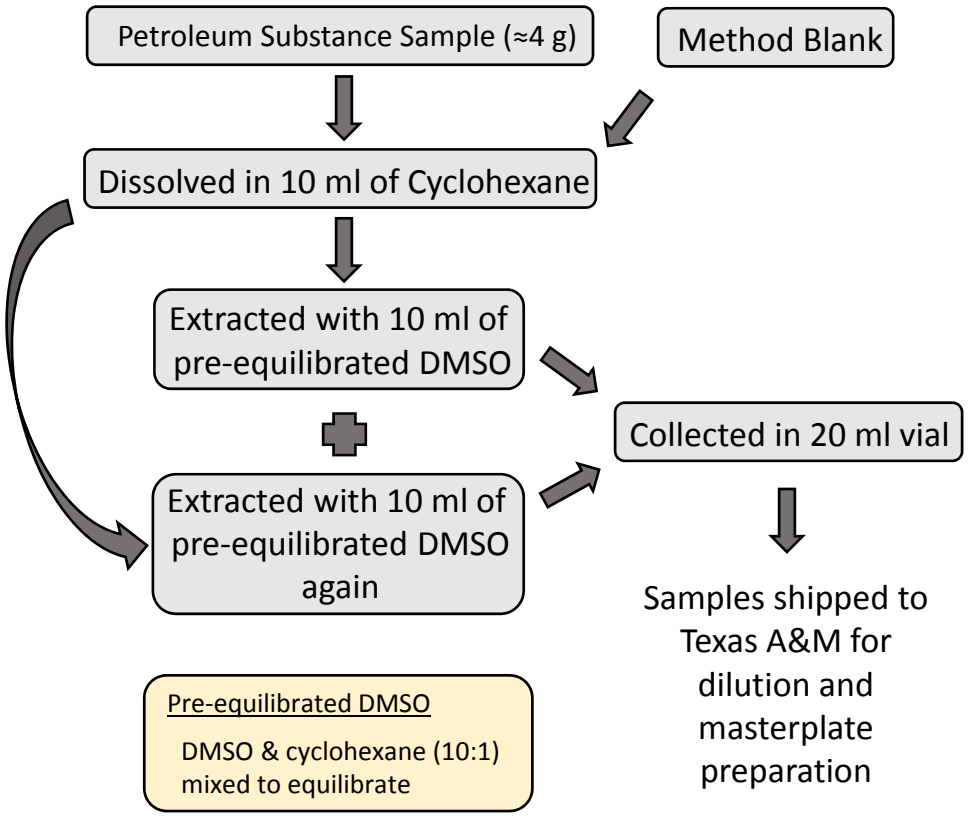






Petroleum UVCBs → Bioactivity data-enabled read-across ← *in vitro* models

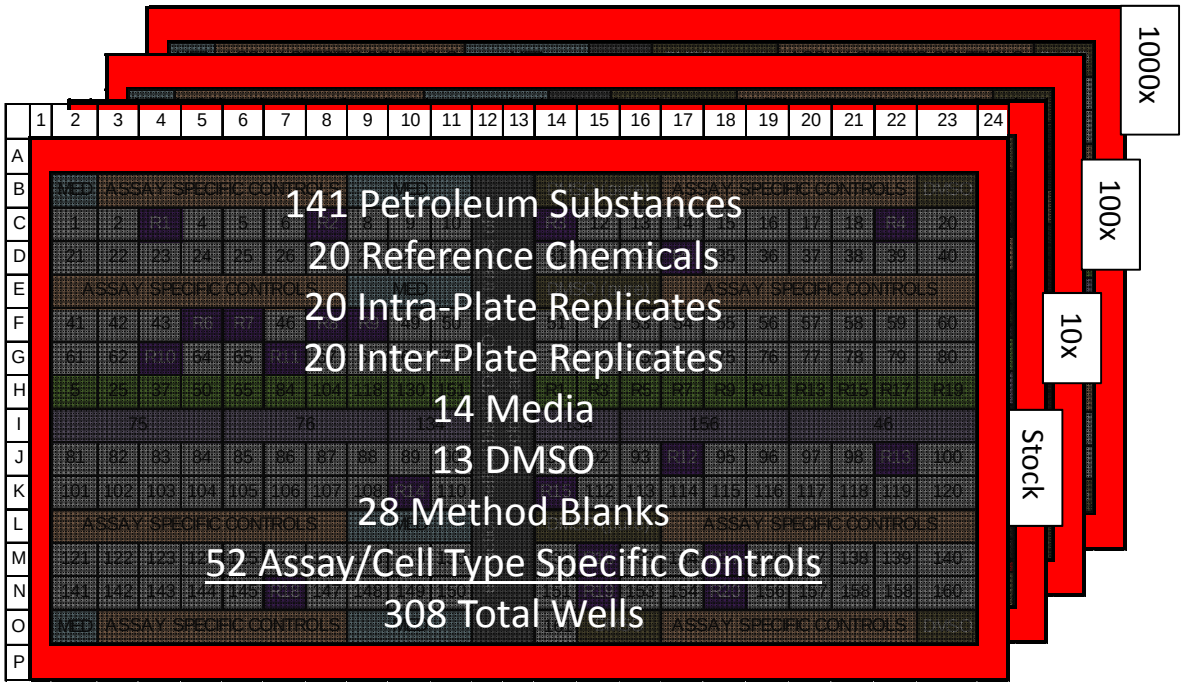
Cat-App project: Bioactivity profiling scope



Substances (141 total):

1. Bitumen (n=4)
2. CGO (n=8)
3. Diesel Fuel (n=1)
4. FO (n=3)
5. Gasoline (n=10)
6. HFO (n=27)
7. HRBO (n=1)
8. Kerosine (n=9)
9. OGO (n=4)
10. OLBO (n=32)
11. Ox Asph (n=1)
12. P&H Wax (n=6)
13. Paraffin Wax (n=1)
14. Petrolatum (n=3)
15. Slack Wax (n=3)
16. SRGO (n=6)
17. TDAE (n=2)
18. UATO (n=4)
19. UDAE (n=4)
20. RAE (n=2)
21. VHGO (n=10)

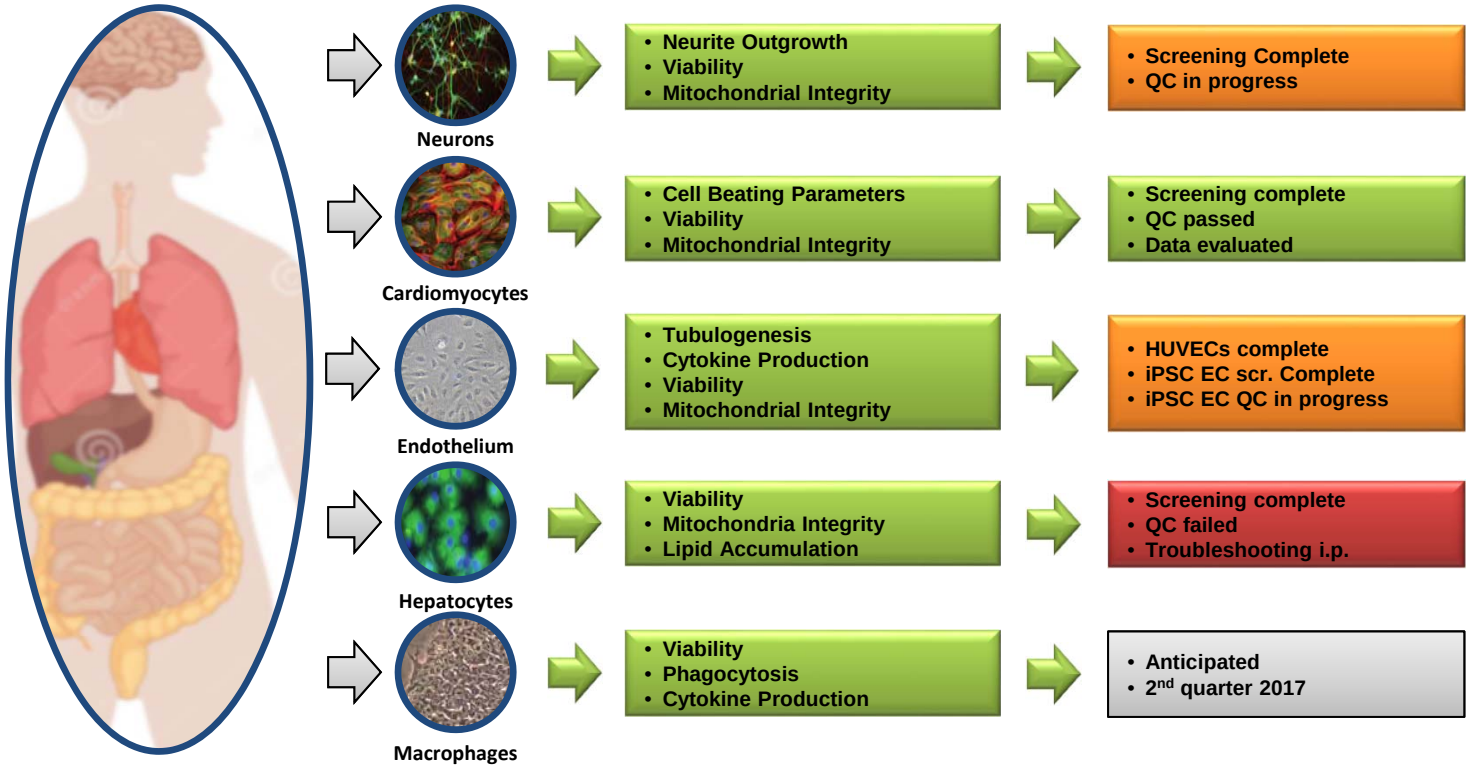
Cat-App project: Bioactivity profiling scope



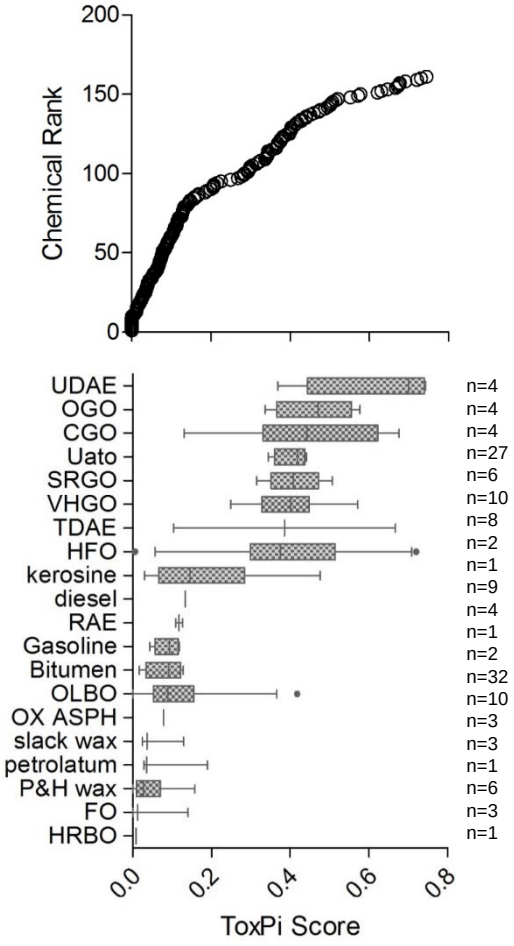
Reference Standards
Inter-Plate Replicates
Intra-Plate Replicates
Assay controls
Media
DMSO (method blank)
DMSO (pure)
Empty Wells

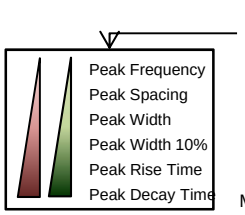
4 total plates (Stock Plate, 10 fold, 100 fold, 1000 fold diluted)
Plates diluted in “Method Blank” (cyclohexane equilibrated DMSO)
4 sets of plates prepared (16 total plates)
Stored at -20°C.
Identical substance plates are used at TAMU/AgriLife and PHE screening

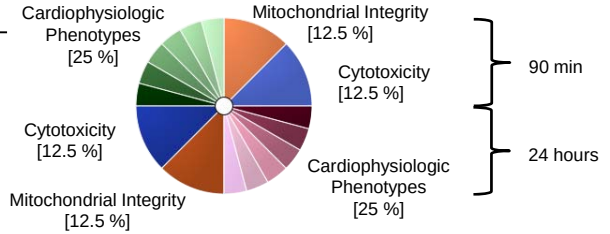
CAT-APP Work Package 2a - Progress Summary
[March 2017]

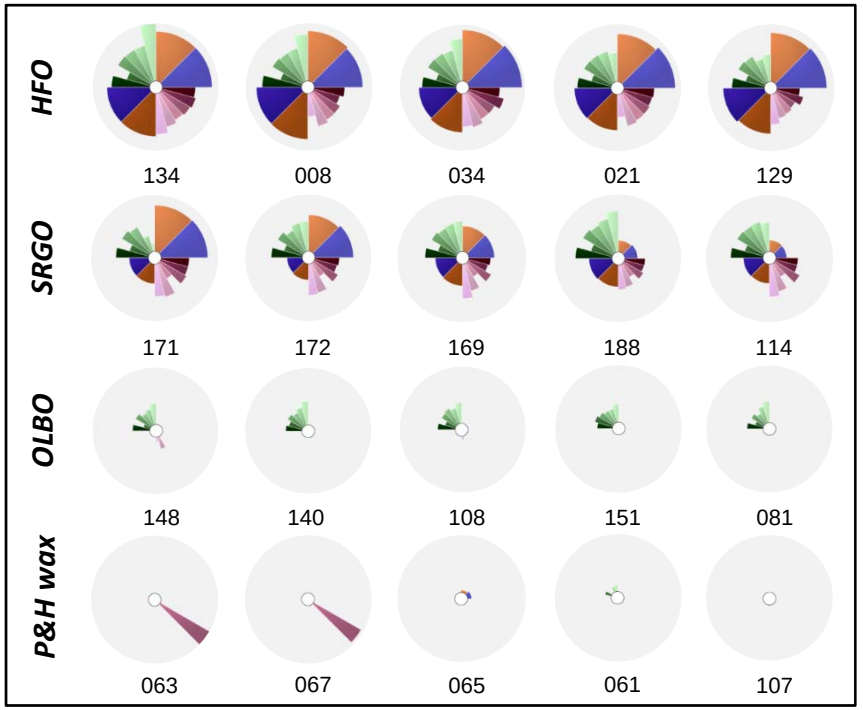


Similarity-Assessment of Petroleum Substances in iCell Cardiomyocytes

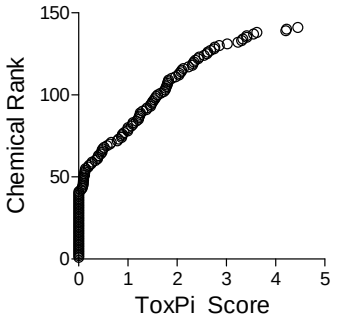


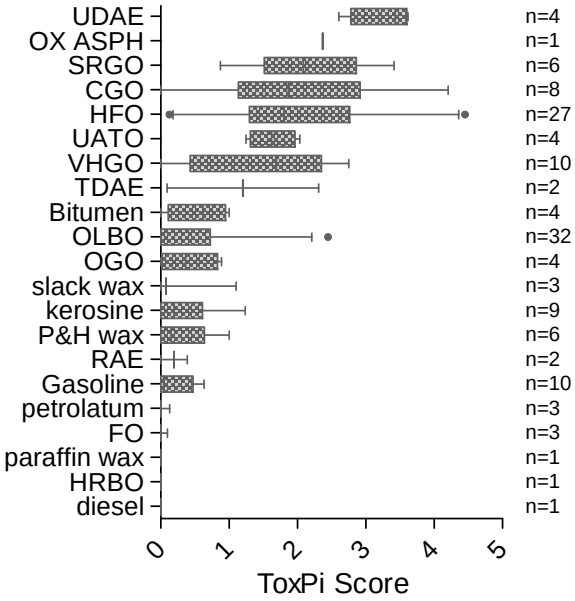


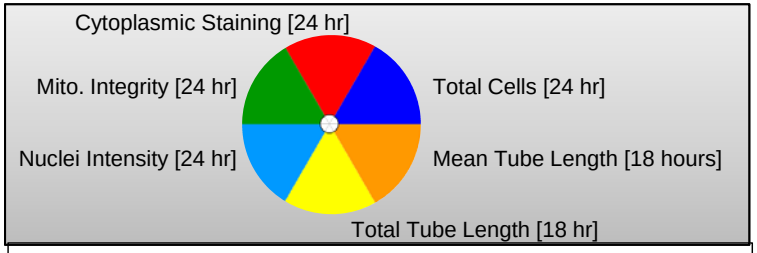


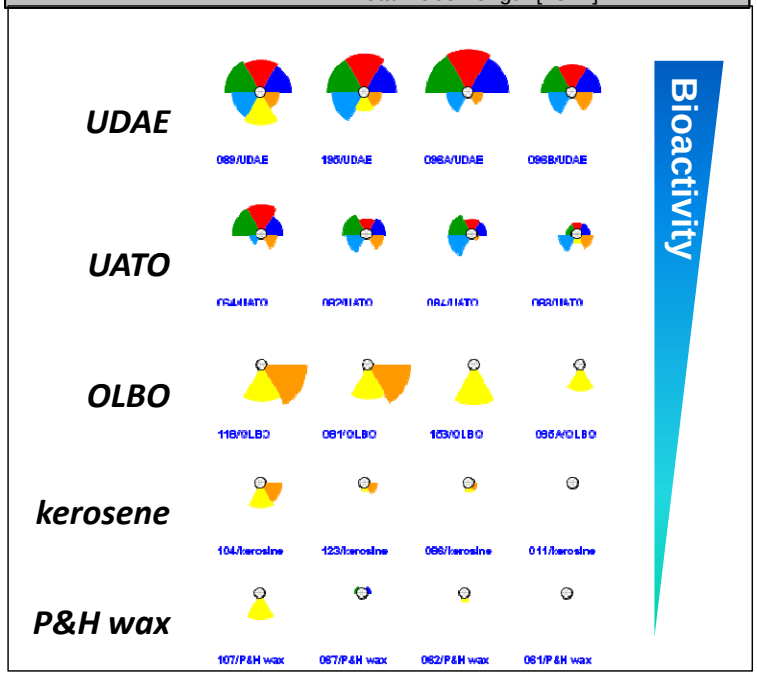


Similarity-Assessment of Petroleum Substances in HUVECs



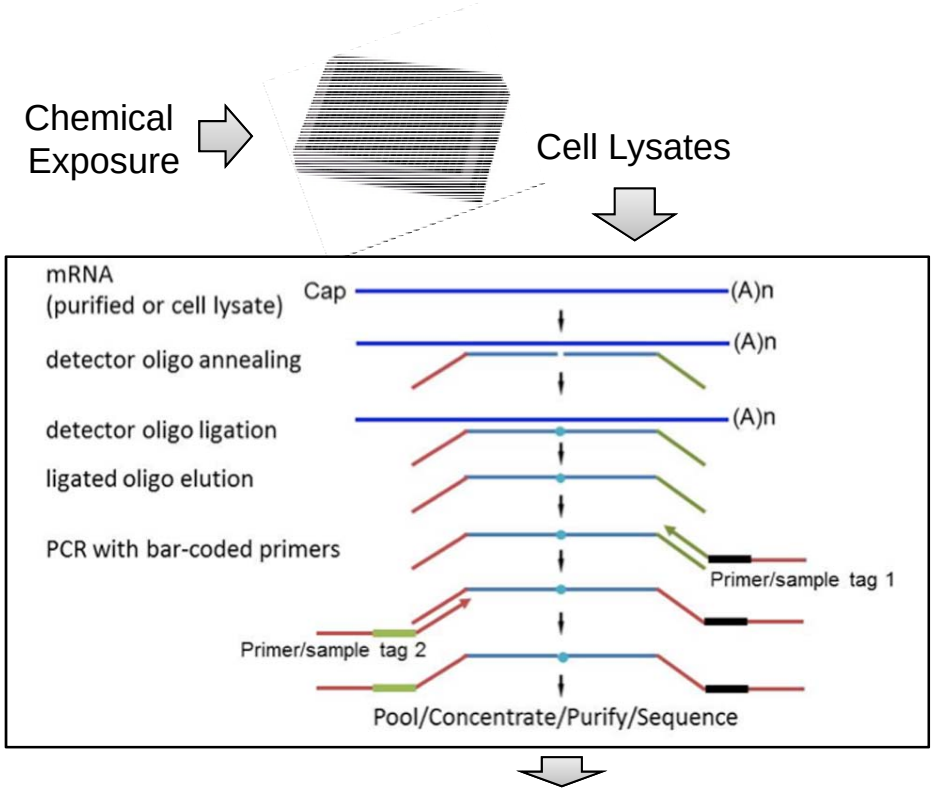






Templated Oligo Detection Sequencing:

Targeted transcriptomic analysis (n~3,000) in high throughput



Remove Media
Add 10 µl 1x Lysis Buffer

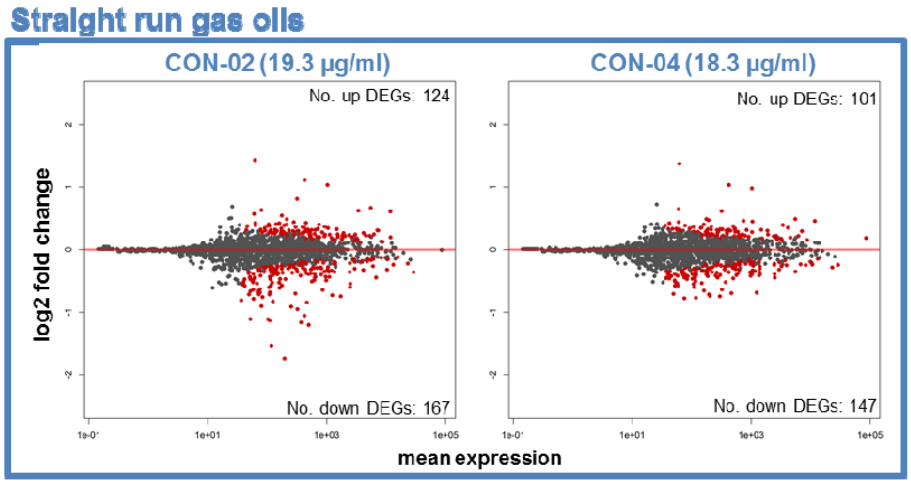
Capture-free assay design
[no RNA cleanup required]

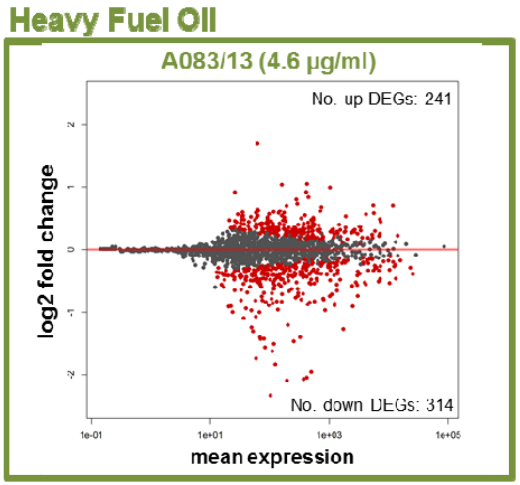
Tox Detector Oligo Panel
[S1500+ gene set]

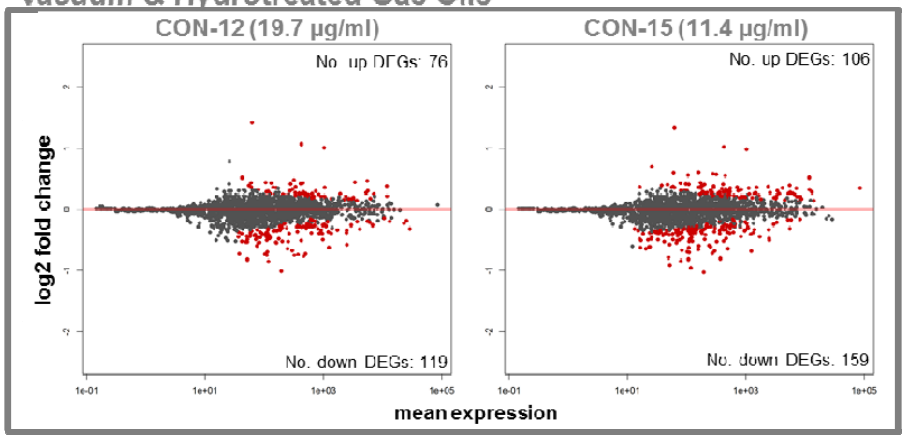
Barcoding for sample IDs
[current capacity: 384/library]

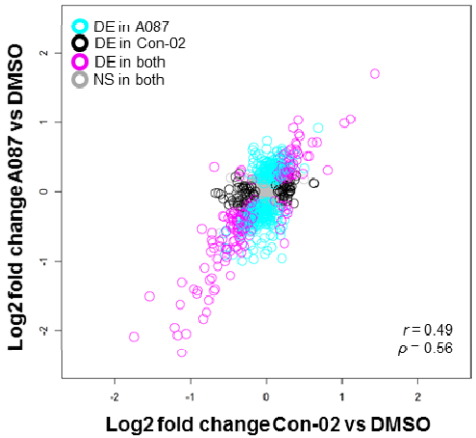
Sequencing (TAMU) and Data Processing (NCSU)

TempO-seq – robust and highly reproducible dataset for biological read-across



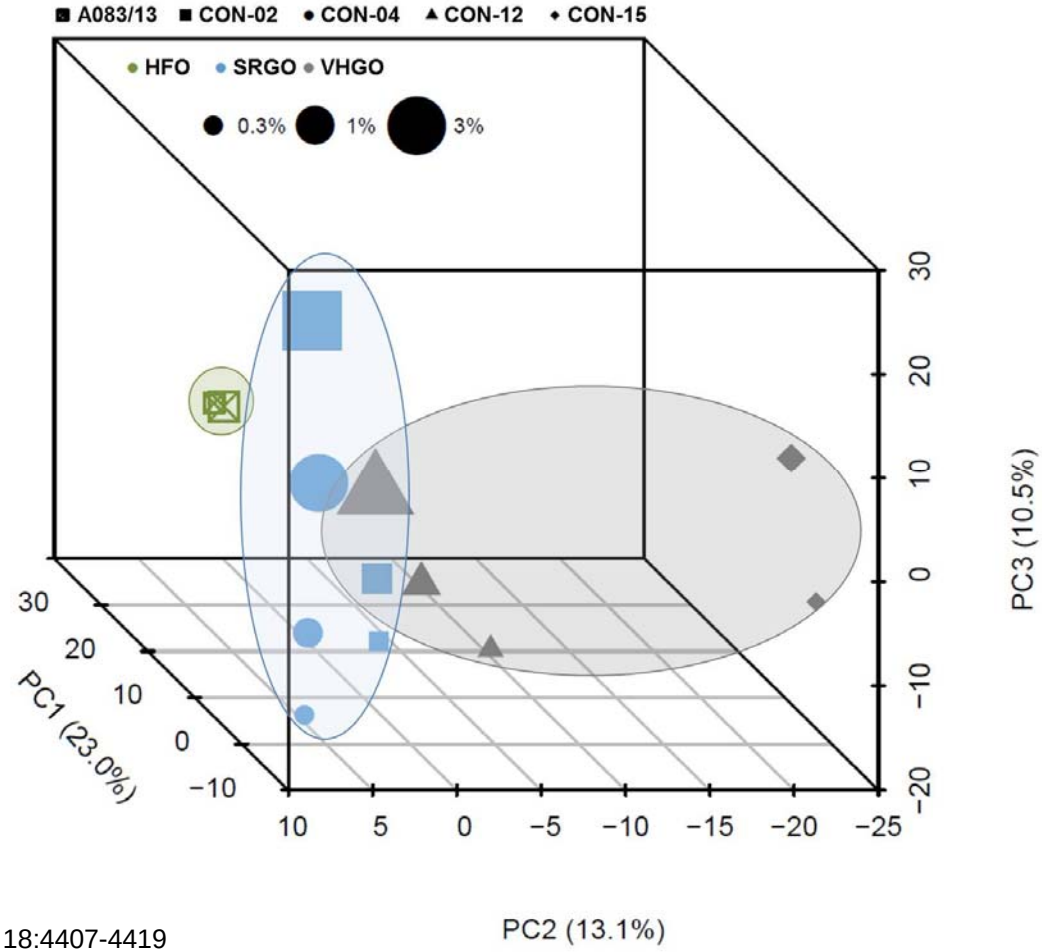






Grimm *et al*. Green Chemistry 2016, 18:4407-4419

Category grouping with concentration-response TempO-seq data



Grimm *et al*. Green Chemistry 2016, 18:4407-4419

Conclusions

1. Petroleum substances are an excellent example of the challenges that exist in evaluating potential health hazards of UVCBs
2. Read-across-based testing proposals for petroleum substances have been ***provisionally*** accepted by ECHA, where the category and read-across are cited with deficiencies and uncertainties with respect to the amount of information available to "***fully characterize*** *the chemical composition of the petroleum substances in question* "
3. When full chemical characterization of a substance, such as petroleum substance UVCB, is unattainable, *in vitro* bioactivity data may be the strongest basis for applying a similarity principle for grouping and read-across
4. Our initial experience with using *in vitro* screening of petroleum substances from 6 production-based groupings demonstrates excellent groupings based on concentration-response hazard indicators from different human cell types
5. Integration of *in vitro* screening information with multi-dimensional chemical characterization should improve confidence in read-across and grouping of complex UVCB