Public Health England

The application of reference chemicals in Cat-App?

WP2b – Tim Gant and Abigail Dalzell

Cat-App

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- John House
- Ivan Rusyn
- Fred Wright
- Fabian Grimm
- William Klaren

Reference materials in CAT-APP

Positive controls

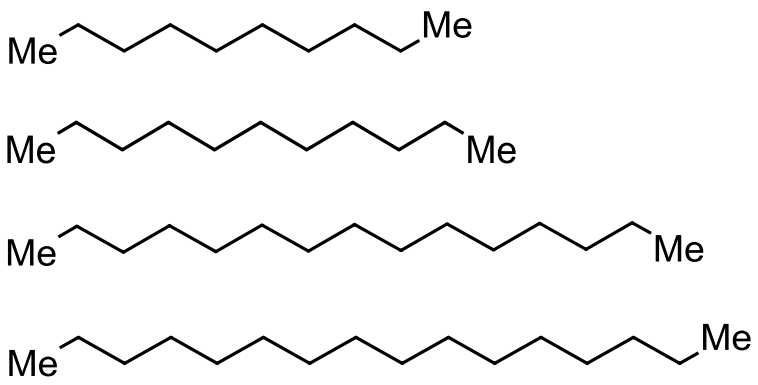
- Assay specific reference materials. Uses as dose responses in each plate
- Acted as positive controls for the apical assays
- Different positive controls used in PHE & TAMU

- Reference materials (20) in duplicate in each plate, four concentrations on a log order basis.
- These reference materials represented the classes and most common materials in the UVCBs
- Common between PHE and TAMU

R20 bioactivity chemicals

‘20 “reference substances” representing the major known structural classes of chemistries in petroleum substances: n-paraffins, monoaromatics, diaromatics, triaromatics, 4-ring aromatics and heteroatoms. Specific substances selected from chemicals screened in ToxCast by US EPA; data on these agents from >800 assay readouts publicly available through EPA’s iCSS dashboard’

R20 - n-paraffins



Decane (R18)

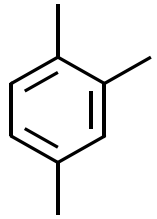
Udecane (R12)

Pentadecane (R2)

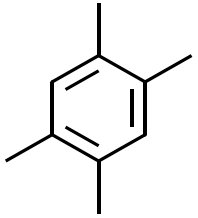
Hexadecane (R14)

5,0.5,0.05, and 0.005 nM

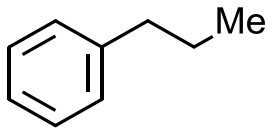
R20 Monoaromatics



1,2,4-trimethylbenzene (R16)

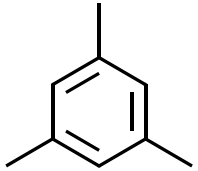


1,2,4,5-tetramethylbenzene (R5)

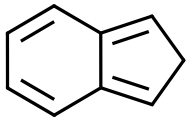


Propylbenzene (R11)

500,50,5, 0.5 nM



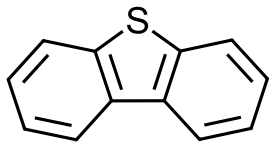
P-Cymene (R17)



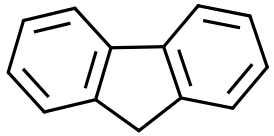
Indene (R20)

50,5,0.5, 0.05 nM

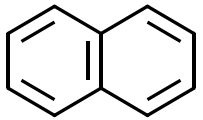
R20-Di-Aromatics



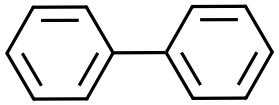
Dibezothiophene (R4)



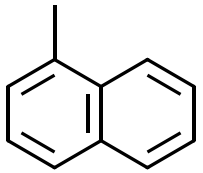
Fluorene (R19)



Naphthalene (R10)



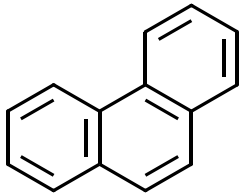
Biphenyl (R15)



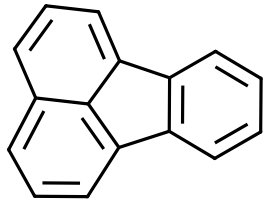
1-methylnaphthene (R7)

50,5,0.5, 0.05 nM

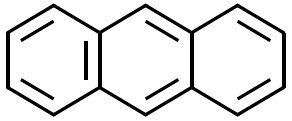
R20 - Tri-Aromatics



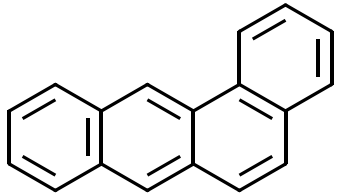
Phenanthrene (R6)



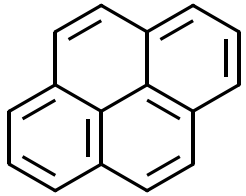
Fluoranthrene (R9)



Anthracene (R13)



Benz(a)anthracene (R3)



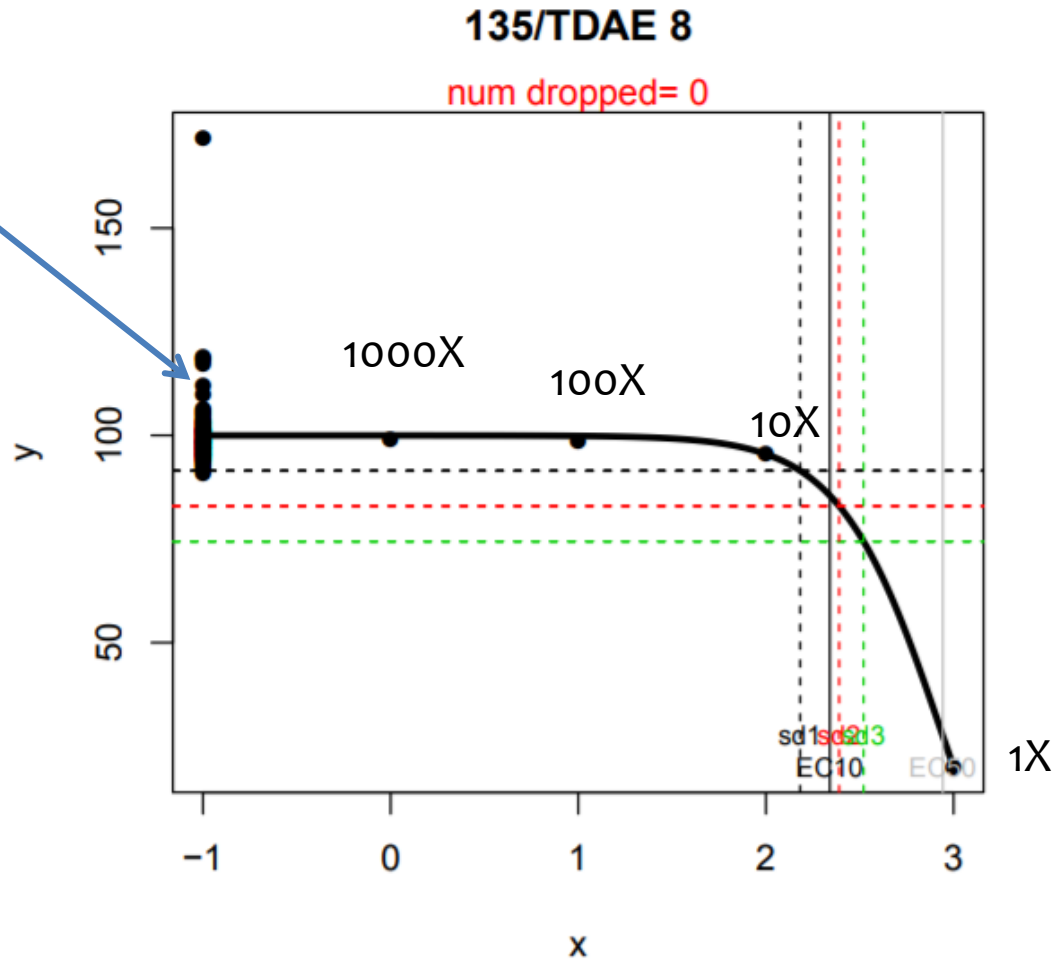
Pyrene (R8)

Concentrations (nM)	AHR activity as percentage of TCDD at 24 hour exposure	Carcinogenic activity as relative to B(a)P =1
50,5,0.5,0.05	<5%	0.001
50,5,0.5,0.05	<5%	0.001
50,5,0.5,0.05	<5%	0.01
50,5,0.5,0.05	49%	0.1
50,5,0.5,0.05	<5%	0.001

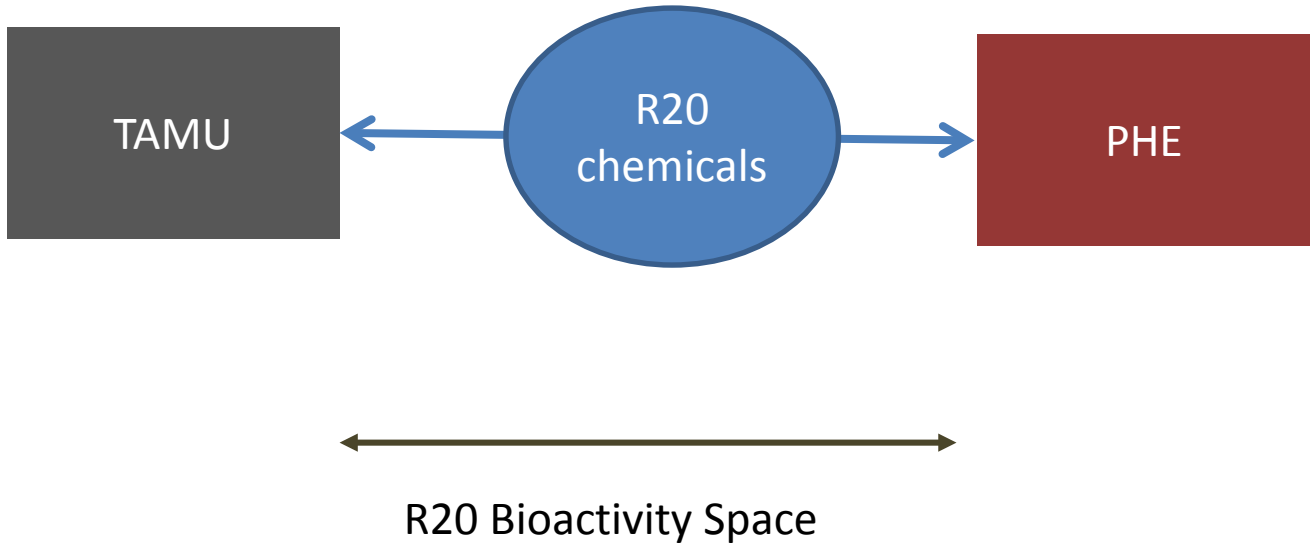
Bioactivity Space

POD calculation

Method blanks



Assays consolidated by reference to R20 bioactivity space



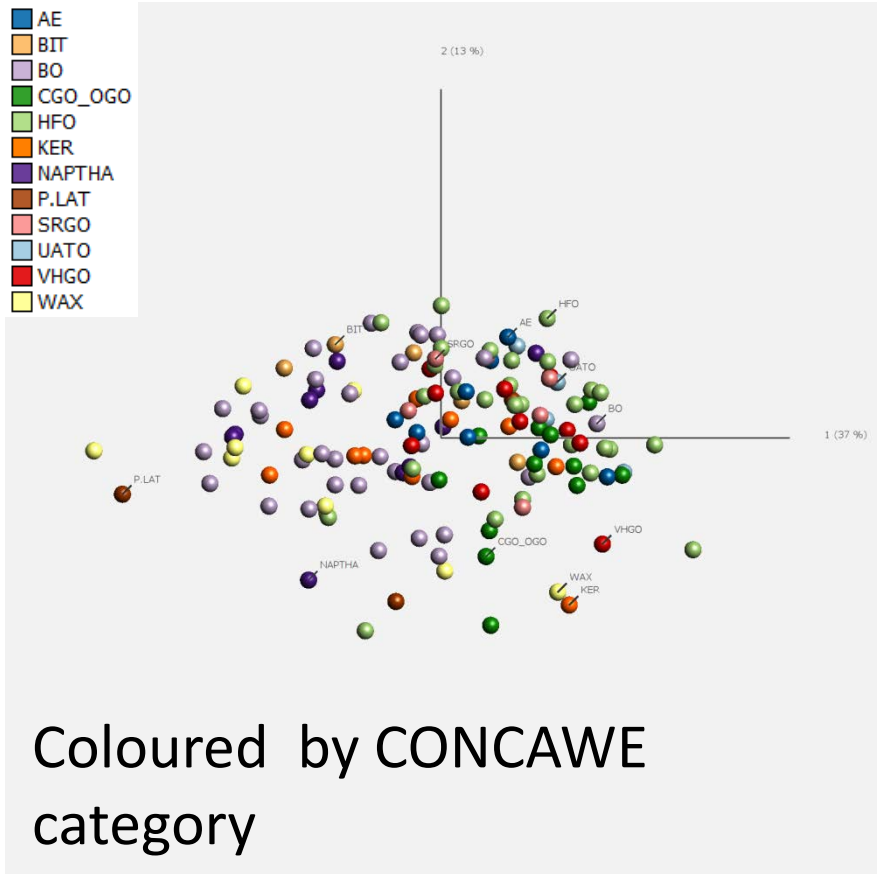
Each UVCB correlated to each of the 20 reference chemicals using the 68 assays available.

Calculate the Spearmann's Rank Correlation for each UVCB against all each of the R20 chemicals across the 68 used assay space using PODs

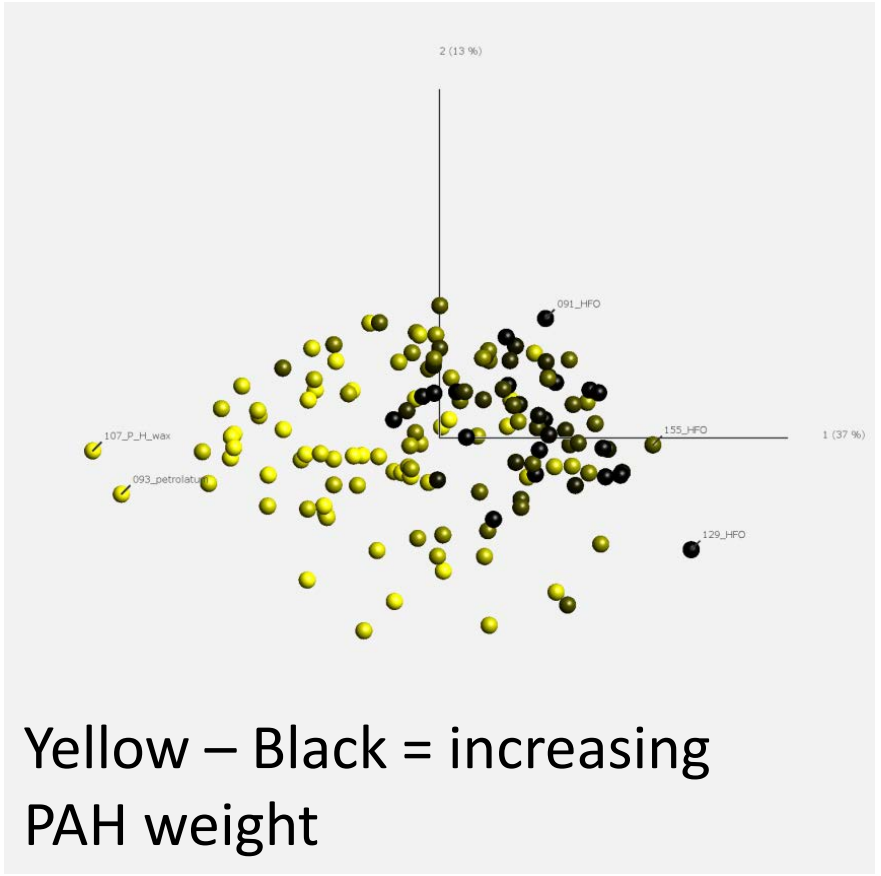
	R_1	R_2	R_3	R_4	>>>	R_{20}
$UVCB_1$	Corr (1-68)	Corr (1-68)	Corr (1-68)	Corr (1-68)		
$UVCB_2$	Corr (1-68)	>>				
$UVCB_3$		Corr (1-68)	Corr (1-68)			
>>>						
$UVCB_{141}$						

			R02	R12	R14	R18	R01	R05	R16	R11	R17	R20	R04	R07	R10	R15	R19	R06	R09	R13	R03	R08
UVCBs	Category_Class	PAH weight	Pentadecane	Undecane	Hexadecane	Decane	1_2_4_Trimethylbenzene	1_2_4_5_Tetramethylbenzene	1_3_5_Trimethylbenzene	Propylbenzene	p_Cymene	Indene	Dibenzothiophene	1_Methylnaphthalene	Naphthalene	Biphenyl	Fluorene	Phenanthrene	Fluoranthene	Anthracene	Benz_a_anthracene	Pyrene
003_CGO	CGO_OGO	0.404	0.037007	0.25464	-0.05937	0.132437	0.147813	0.002295	0.121851	-0.009	0.024693	-0.00801	-0.16307	0.109992	0.150185	0.135758	0.235992	0.164533	0.168788	0.139061	0.069224	0.058416
006_HFO	HFO	0.564	0.070079	0.222342	-0.03782	0.115323	-0.10615	0.008373	0.049621	-0.1596	-0.05597	0.18375	-0.03821	0.046361	0.152388	0.090442	0.044553	0.394368	0.371353	0.104532	0.213888	0.14509
007_HFO	HFO	0.436	-0.00069	0.053415	0.216224	0.043528	-0.0727	0.003682	0.15753	-0.08561	0.207225	0.408611	0.204828	0.084625	0.12349	0.200125	0.150479	0.329754	0.479888	0.281204	0.259653	0.264373
008_HFO	HFO	1.412	0.052179	-0.0133	-0.02955	0.077082	-0.13366	-0.2138	-0.04839	-0.18479	0.066833	0.164406	-0.13831	-0.04478	-0.00883	0.075373	-0.03718	0.205639	0.369974	0.172558	0.057782	0.216327
009_gasoline	NAPTHA	0.000	0.13036	0.233106	0.154112	0.096049	0.032288	0.148086	0.405256	0.124061	0.072619	0.37441	0.181928	0.370436	0.317832	0.307857	0.235365	0.583263	0.186303	0.187746	0.181099	0.299403
011_kerosine	KER	0.000	0.060218	0.177282	0.110959	0.199978	0.230888	0.175475	0.259313	0.166186	0.216774	0.233418	0.176053	0.264908	0.31801	0.230572	0.24114	0.426939	0.208272	0.117081	-0.05069	0.289444

UVCB categories (samples) in R20 bioactivity space

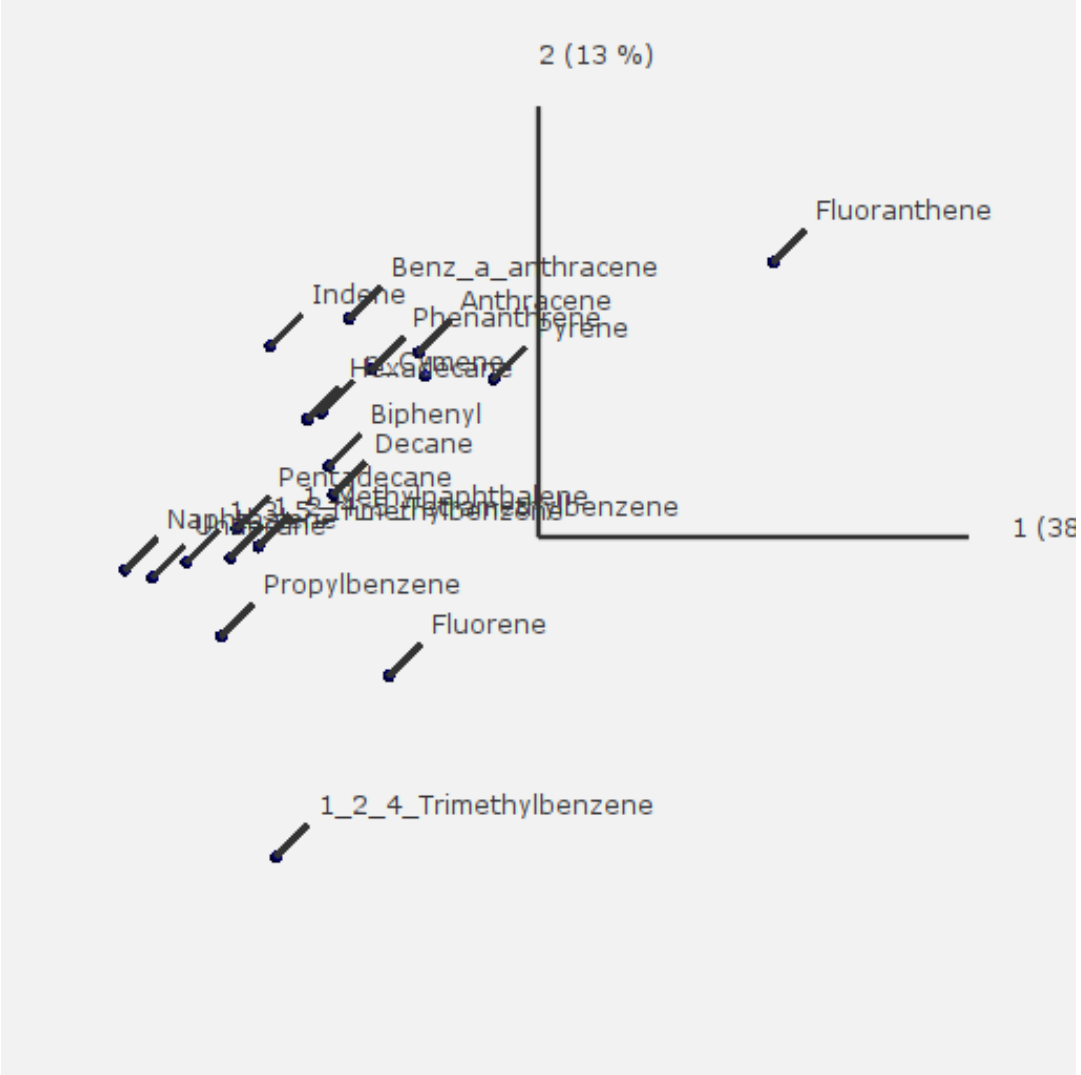


Coloured by CONCAWE category

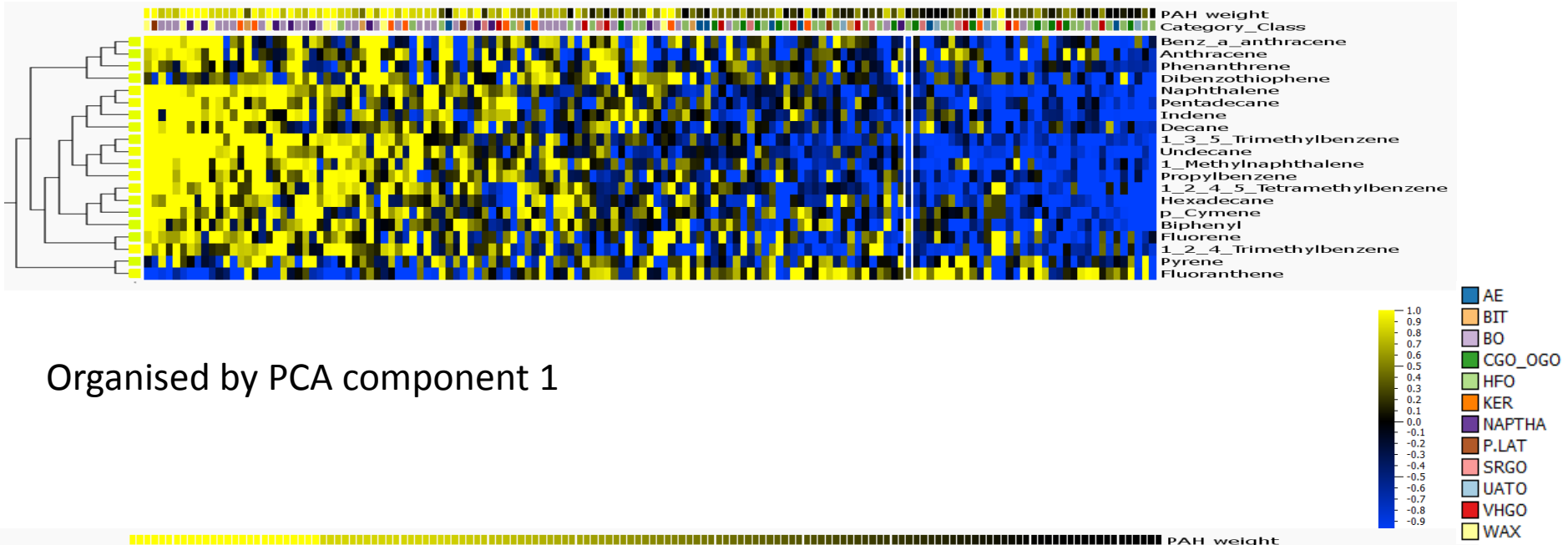


Yellow – Black = increasing PAH weight

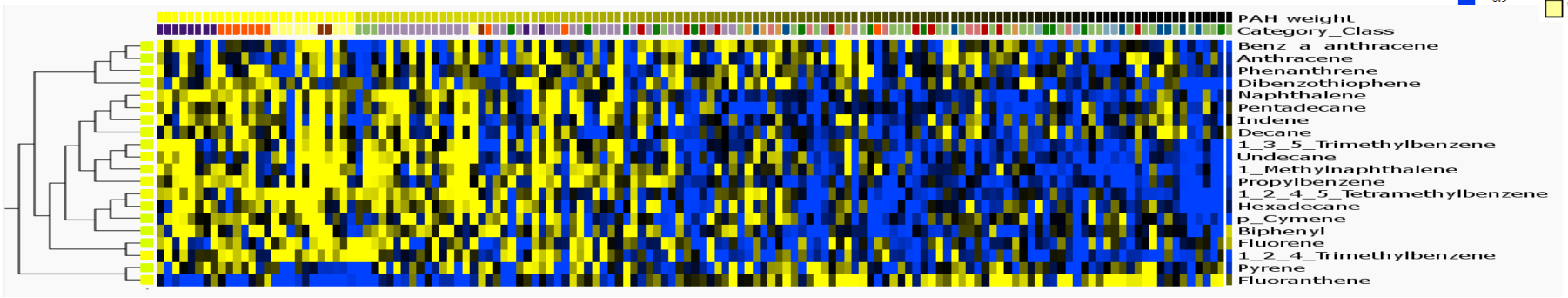
R20 variables



PCA component 1 Clustering in Bioactivity R20 space

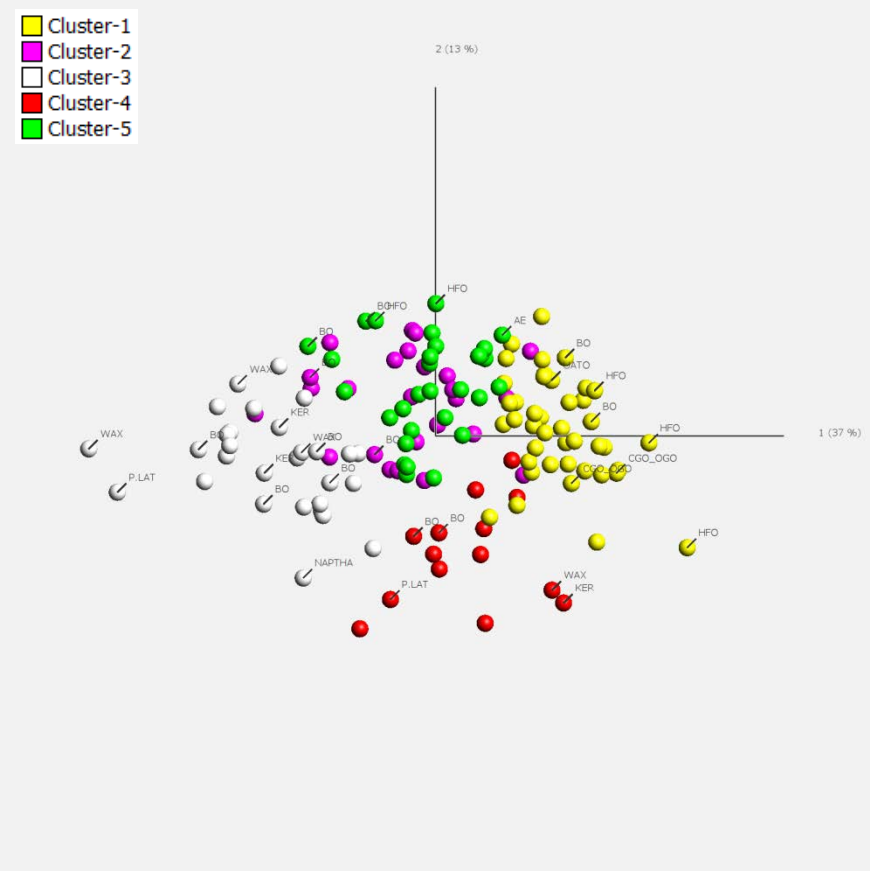


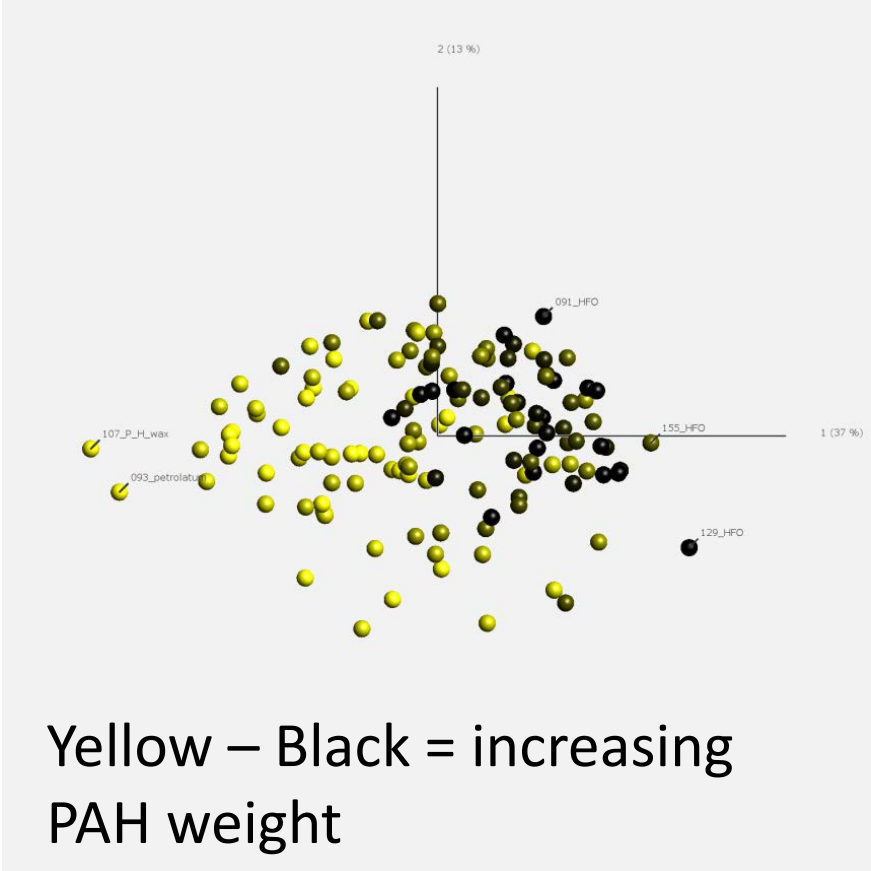
Organised by PCA component 1



Organised by PAH weight

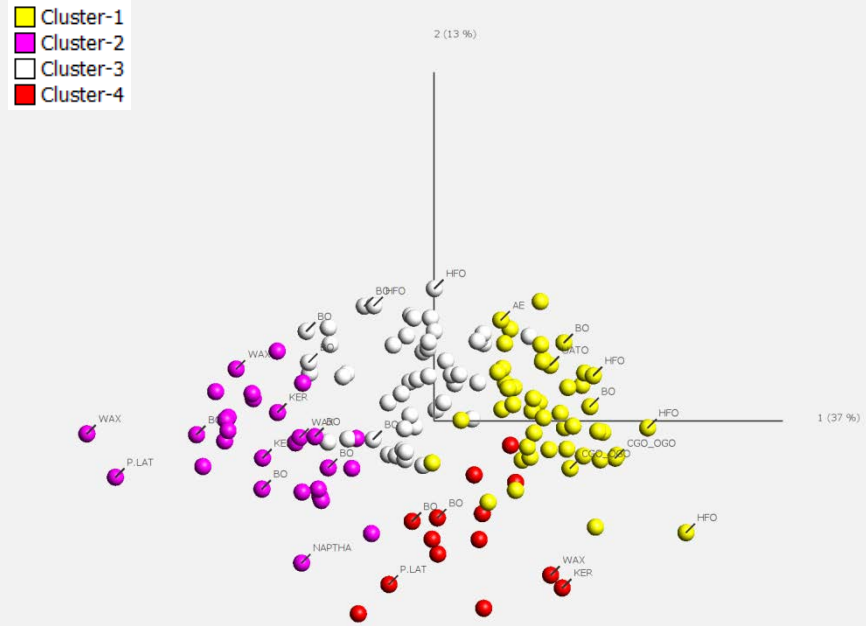
K means 5 cluster in R20 bioactivity space

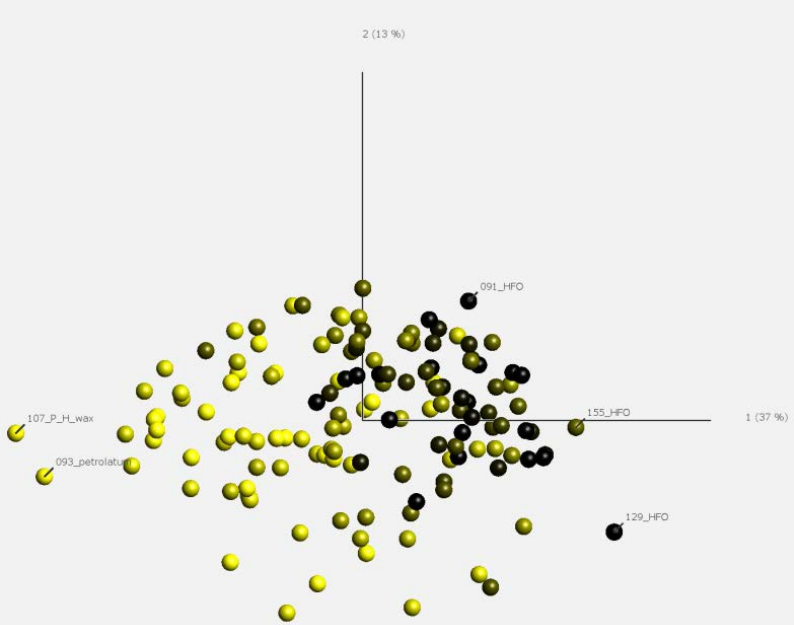




Yellow – Black = increasing PAH weight

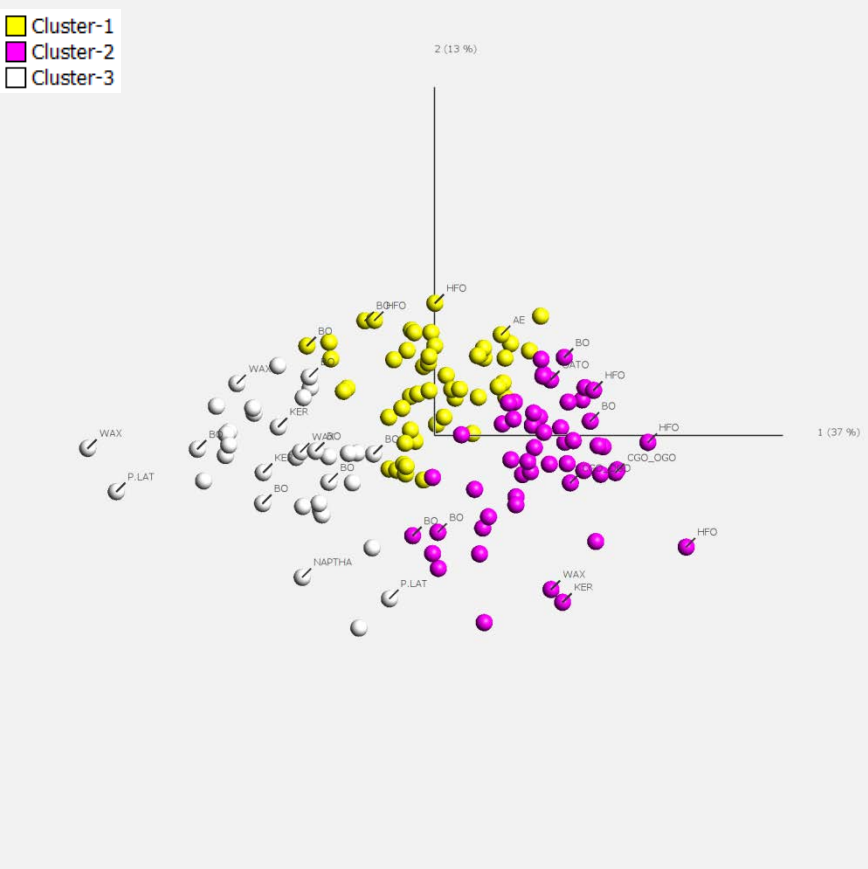
K means 4 cluster in R20 bioactivity space

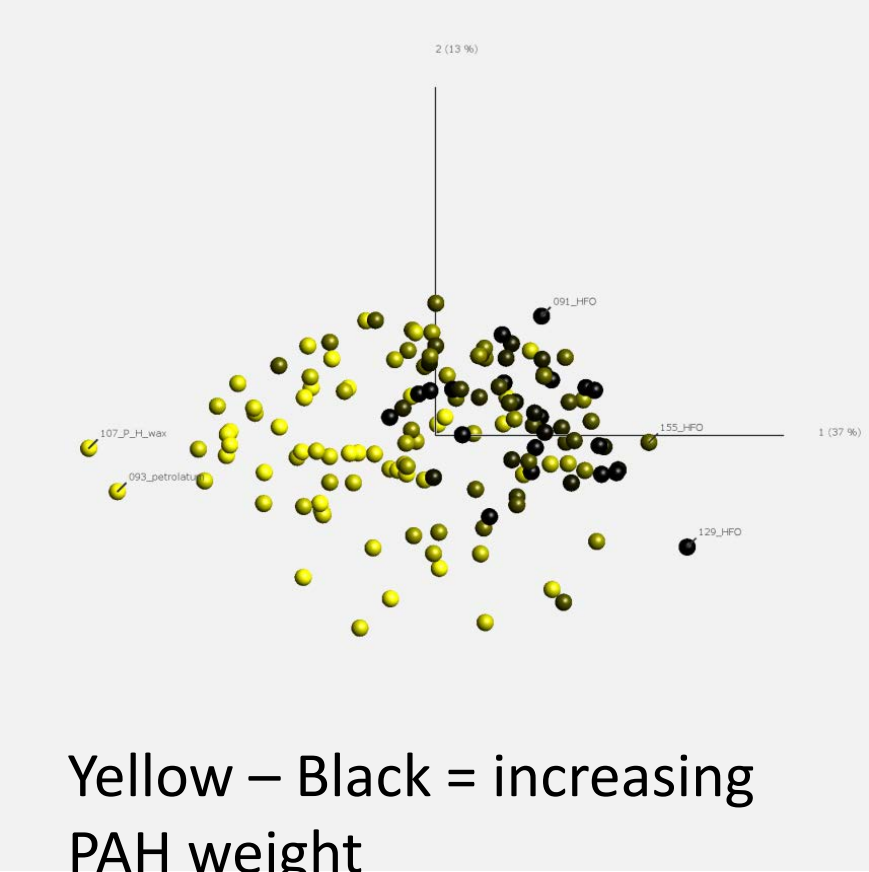




Yellow – Black = increasing PAH weight

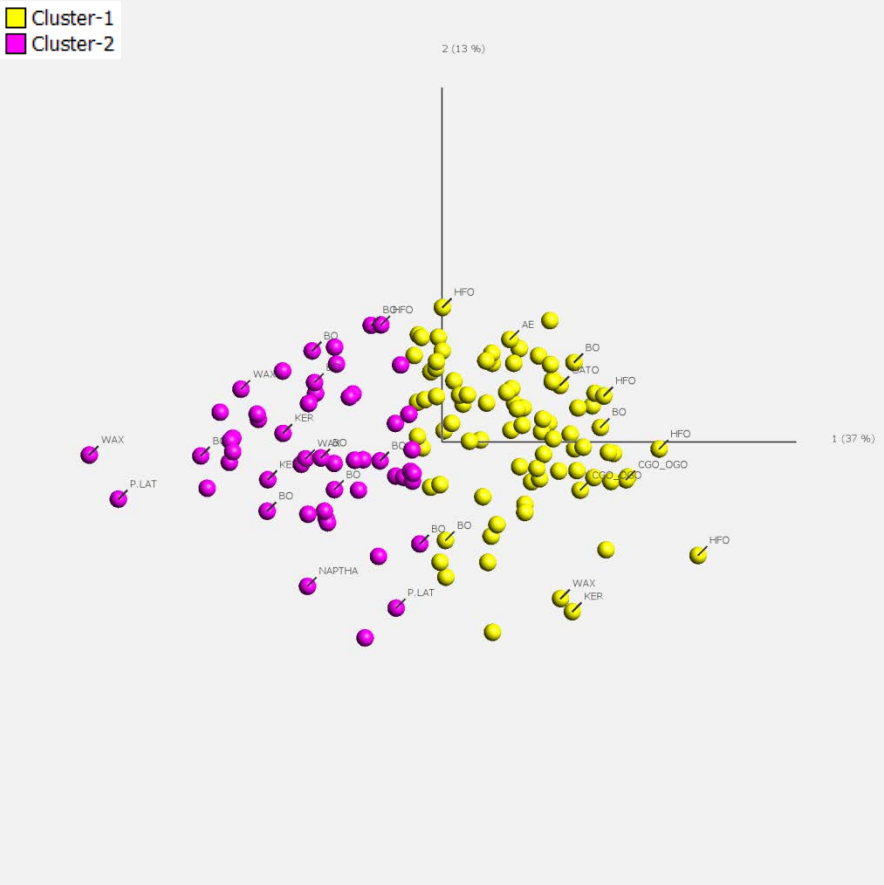
K means 3 cluster in R20 bioactivity space

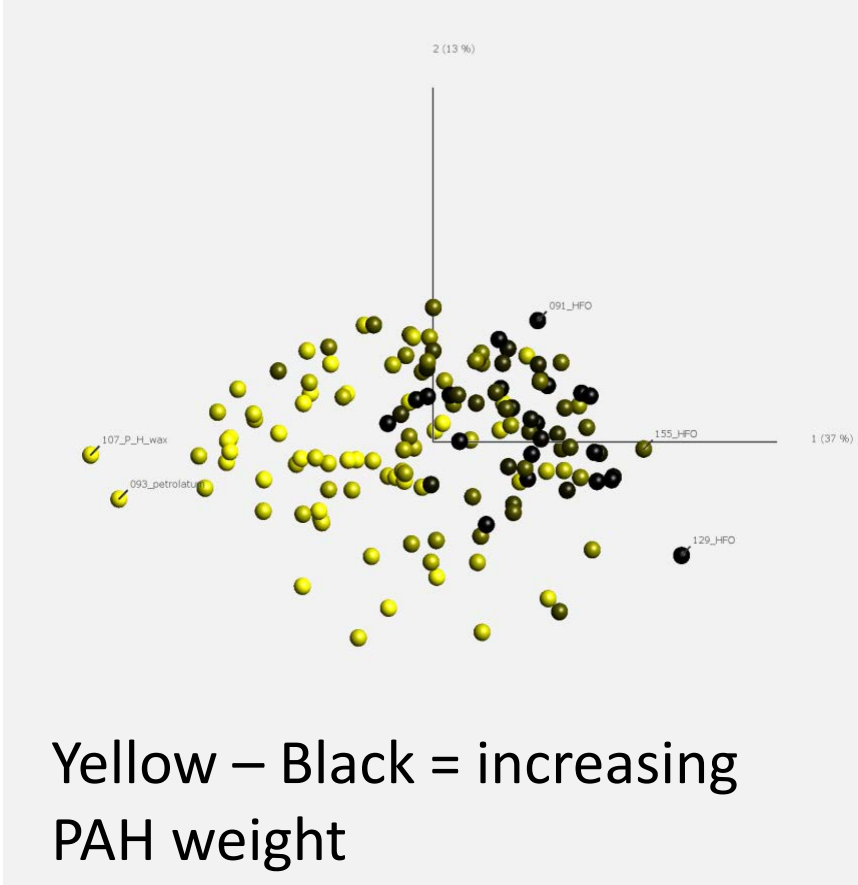




Yellow – Black = increasing PAH weight

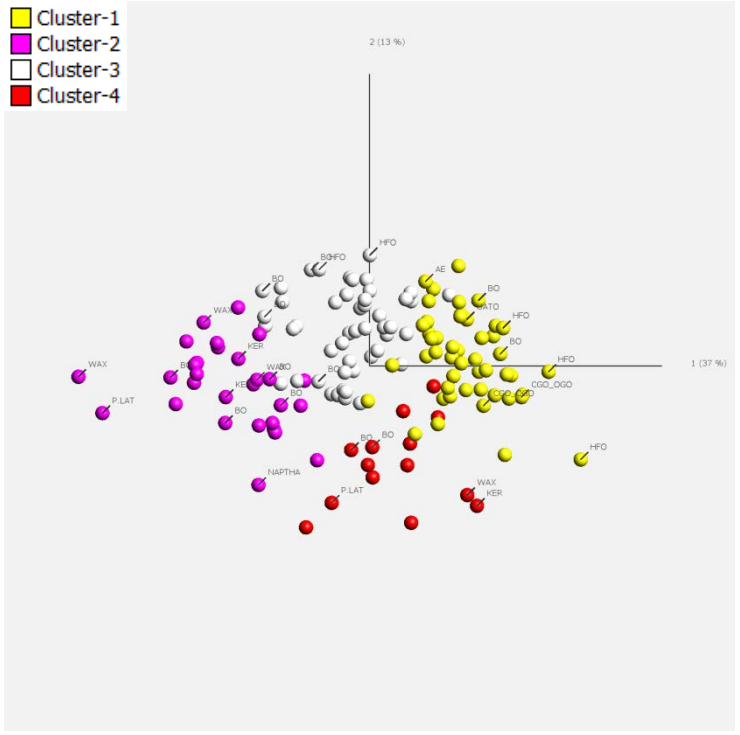
K means 2 cluster in R20 bioactivity space

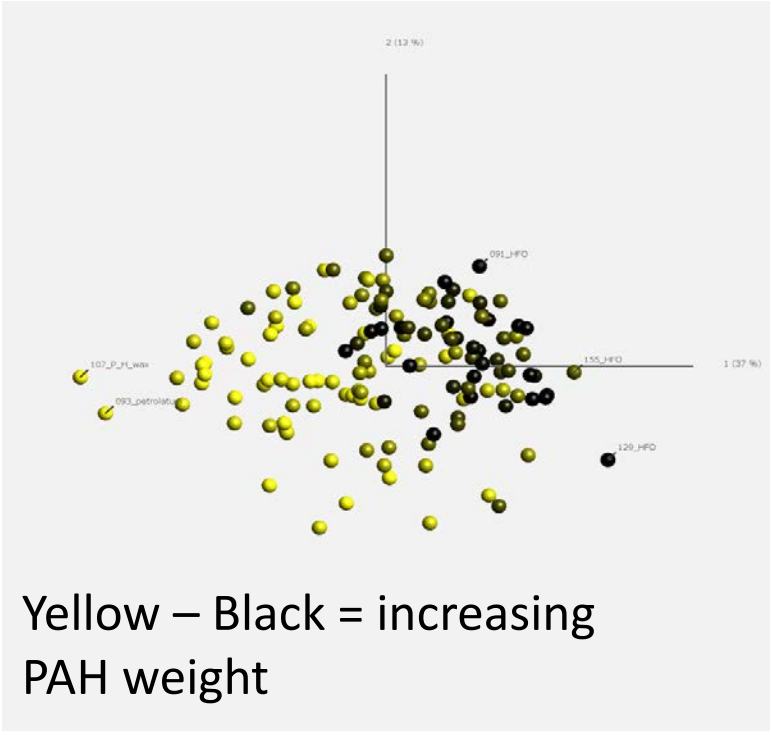




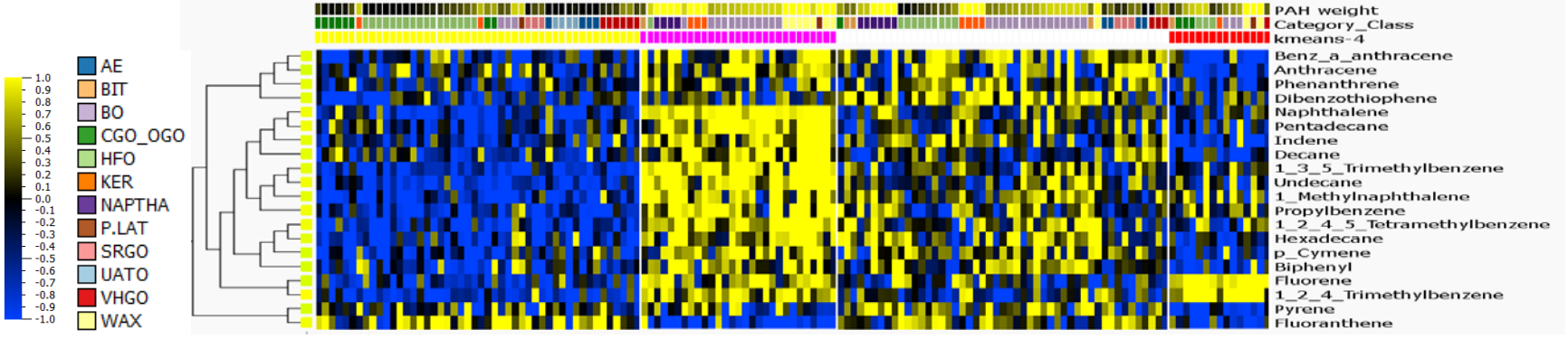
Yellow – Black = increasing PAH weight

4 categories about optimal for R20 bioactivity – also indicated by other methods

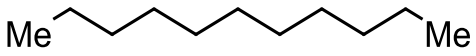




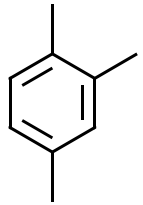
Yellow – Black = increasing PAH weight



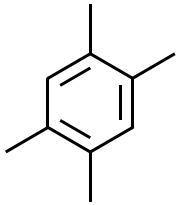
R6 space – the most discerning variables to UVCB sample separation.



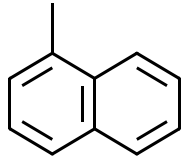
Udecane (R12)



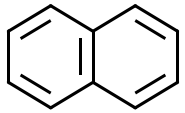
1,2,4-trimethylbenzene (R16)



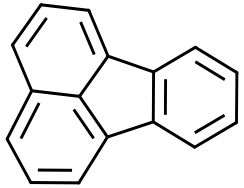
1,2,4,5-tetramethylbenzene (R5)



1-methylnaphthene (R7)



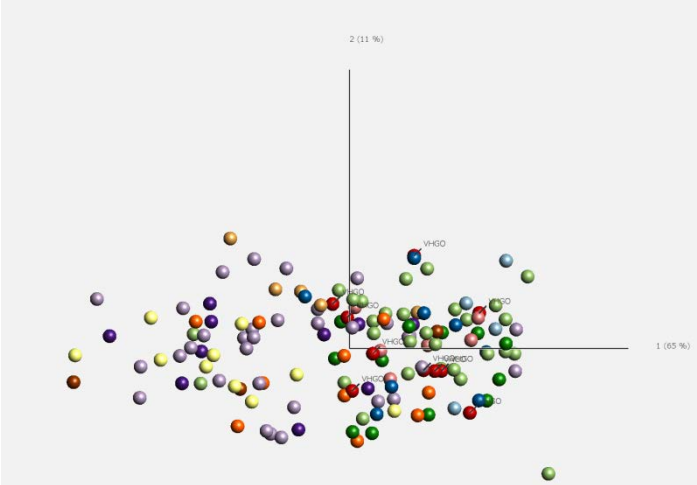
Naphthalene (R10)



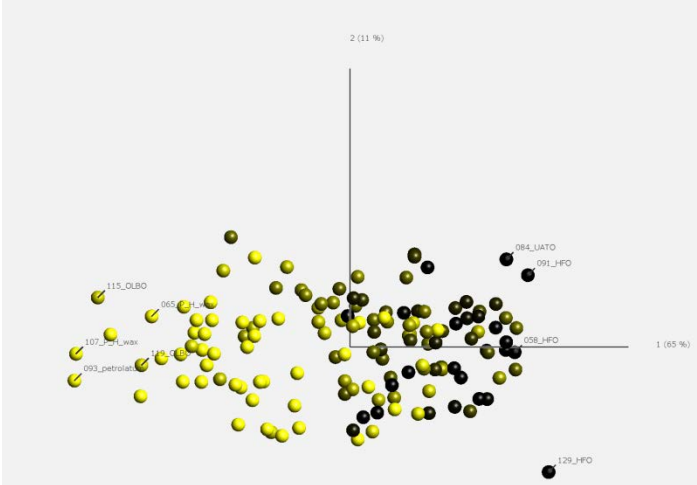
Fluoranthrene (R9)

PCA in R6 bioactivity space

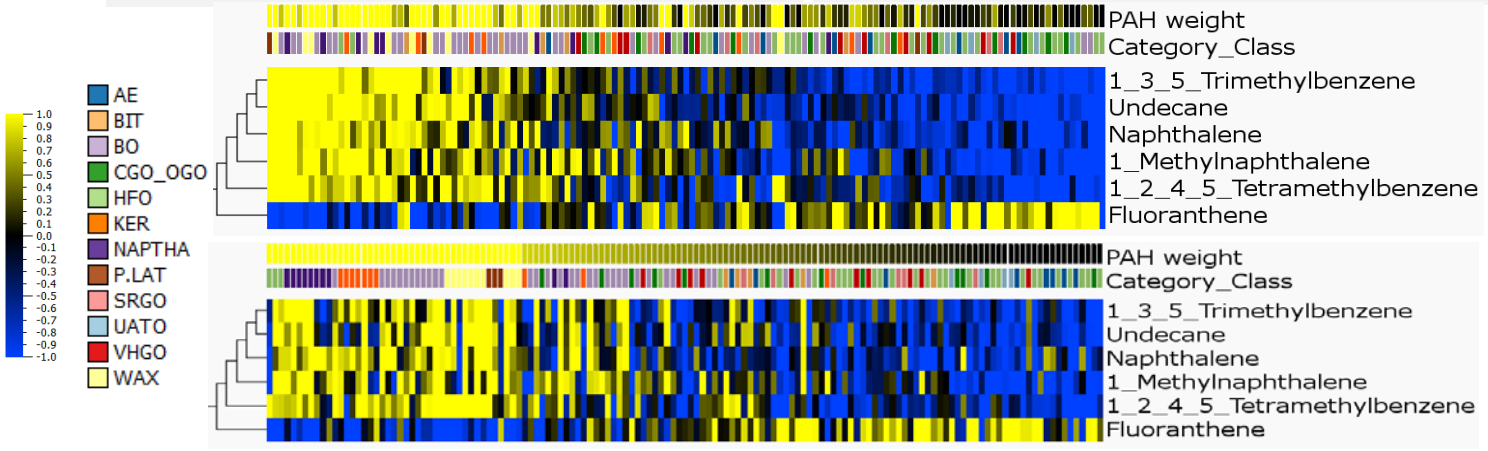
CONCAWE category



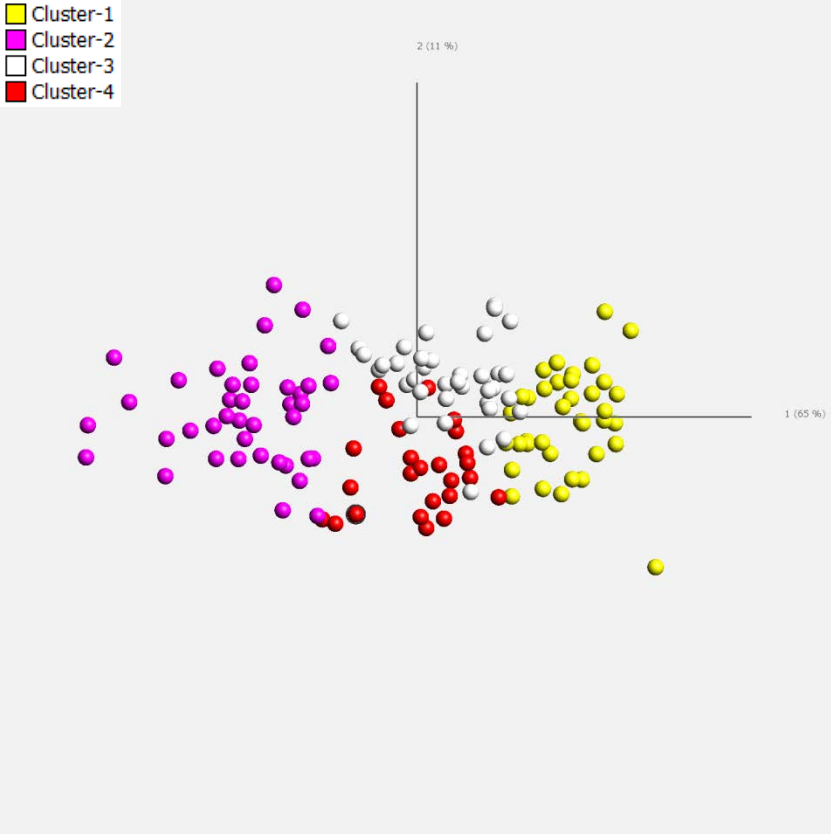
Coloured by CONCAWE category

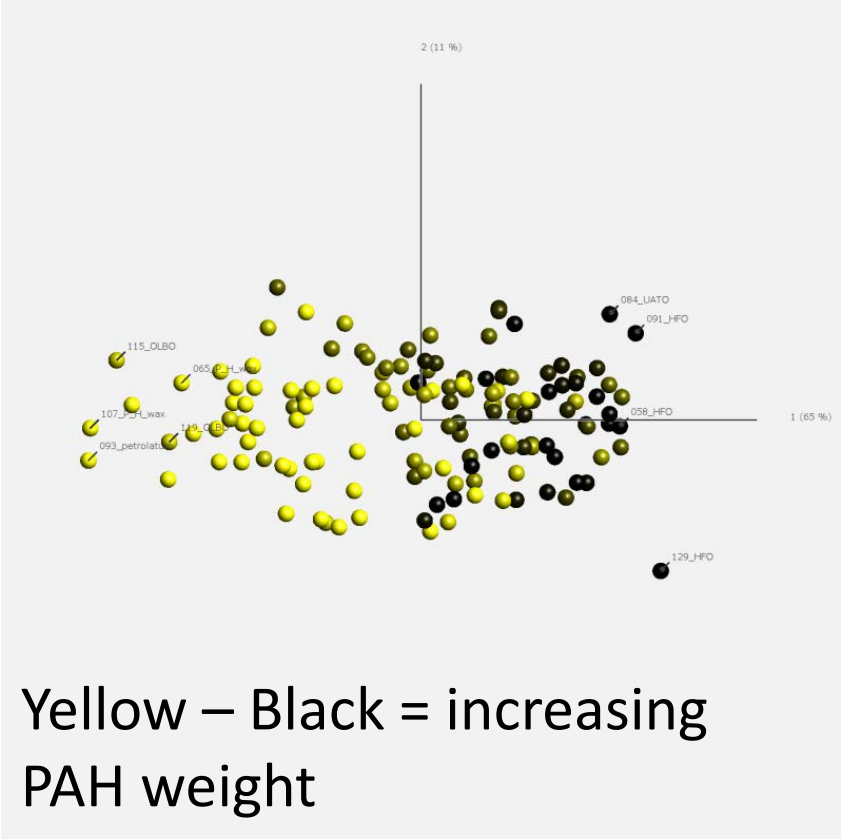


Yellow – Black = increasing PAH weight



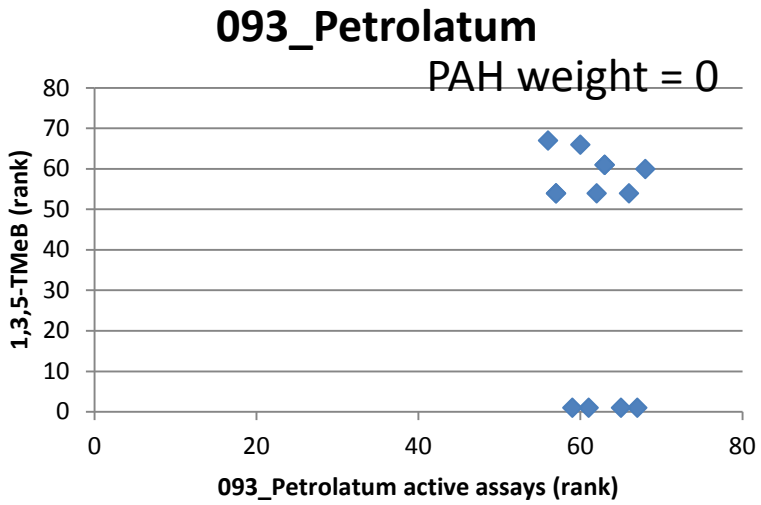
K means 4 cluster in R6 bioactivity space

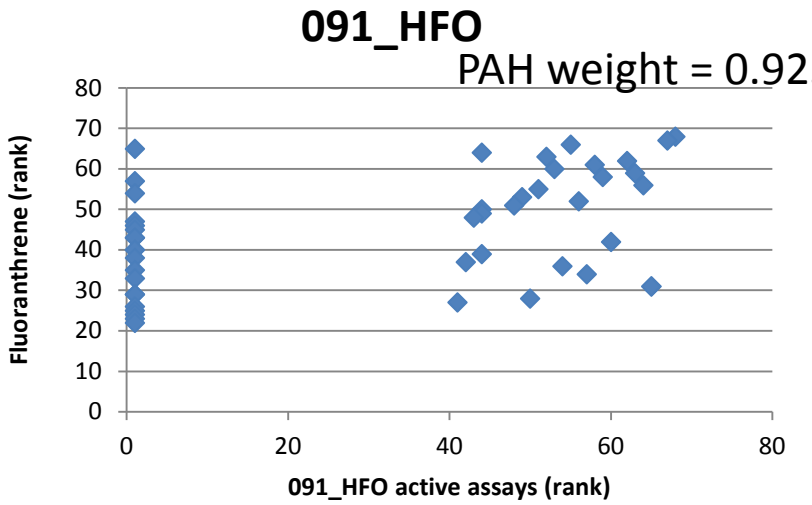




Yellow – Black = increasing PAH weight

093_Petrolaum/091 HFO bioactivity assay rank correlations with 1,3,5 trimethylbenzene/Fluoranthrene

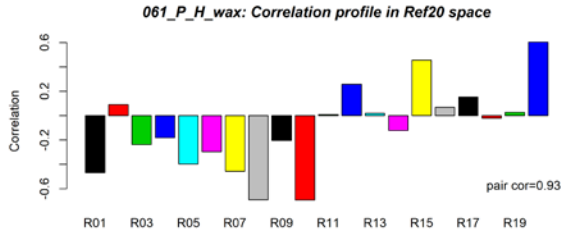


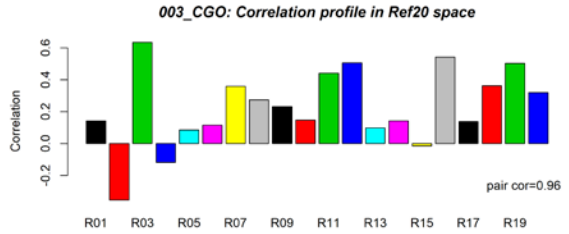


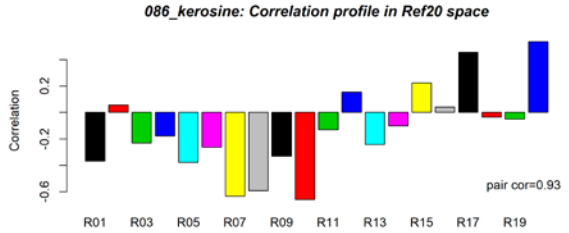
- 091_HFO is more biologically active over all than 093_petrolatum
- 093_Petrolatum is active in more assays than 1,2,5-Trimethylbenzene
- 091_HFO is active in more assays than fluoranthrene
- In assays where both the UVCB and R20 chemical are active there are good rank correlations

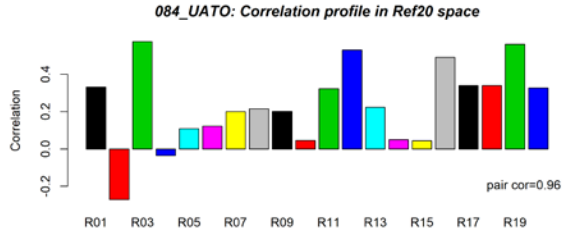
Combined Bioactivity and Transcriptome space

Mapping UVCBs to R20 space



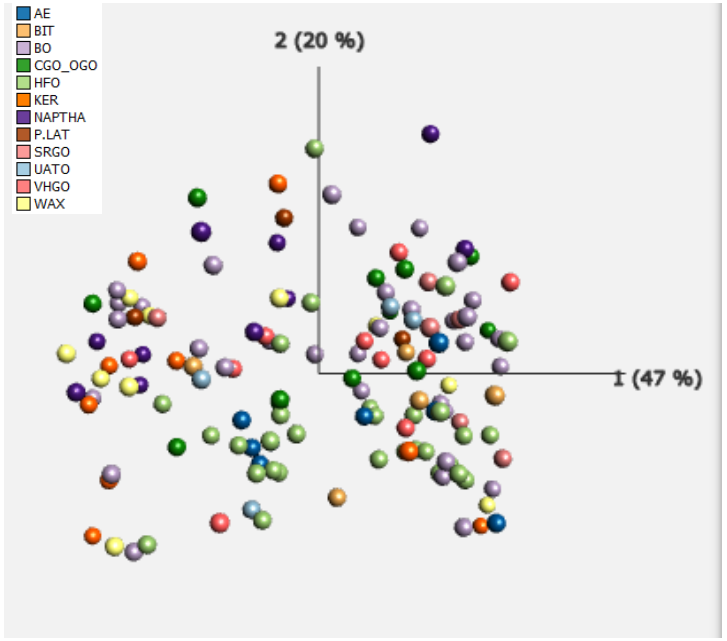


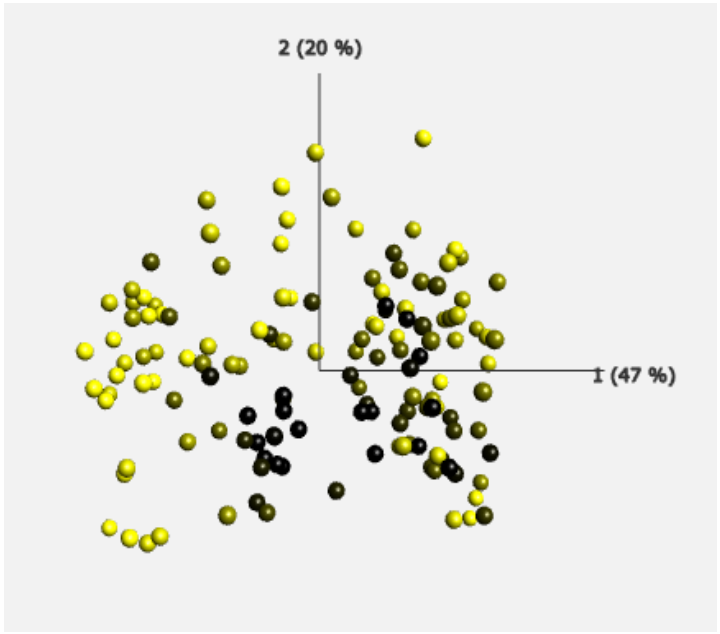


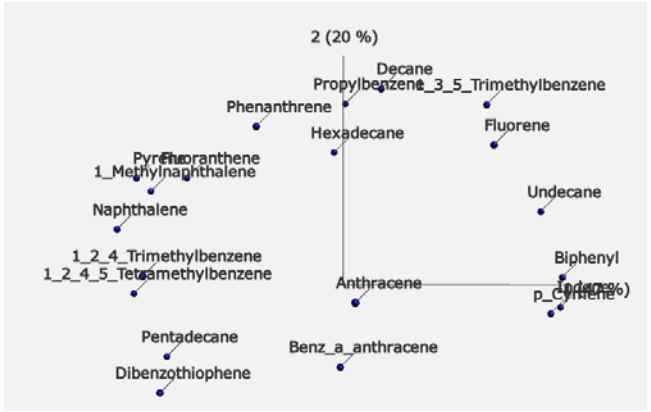


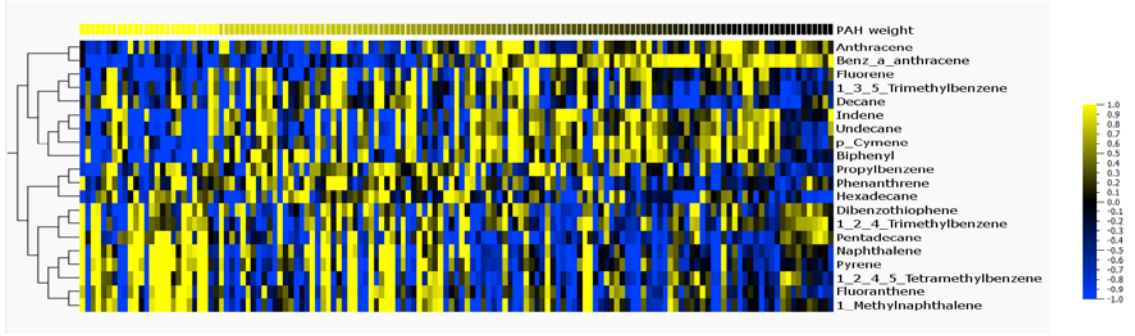
UVCBs	Category_Class	PAH weight	1_2_4_Trimethylbenzene	Pentadecane	Benz_a_anthracene	Dibenzothiophene	1_2_4_5_Tetramethylbenzene	Phenanthrene	1_Methylnaphthalene	Pyrene	Fluoranthene	Naphthalene	Propylbenzene	Undecane	Anthracene	Hexadecane	Biphenyl	1_3_5_Trimethylbenzene	p_Cymene	Decane	Fluorene	Indene
003_CGO	CGO_OGO	0.404	0.141373655	-0.35493	0.636035	-0.11831	0.084825	0.115288	0.359785	0.274148	0.232207	0.146366	0.441784	0.505963	0.097417	0.142271	-0.0147	0.542324	0.137998	0.362853	0.502679	0.31981
006_HFO	HFO	0.564	0.420844012	0.192944	0.752257	0.315488	0.447408	-0.03505	0.102377	0.091753	0.033279	0.162688	-0.10619	0.123356	0.233936	-0.14974	-0.18468	-0.07237	0.193631	-0.17904	0.070077	0.051691
007_HFO	HFO	0.436	-0.039089356	-0.25029	0.456503	-0.18382	-0.25452	-0.10231	-0.30767	-0.29915	-0.28009	-0.2425	-0.08795	0.392139	0.067531	0.122259	0.303028	0.411319	0.44536	0.11592	0.318577	0.508625
008_HFO	HFO	1.412	0.45579361	0.245218	0.57913	0.234317	0.010188	0.103584	0.175378	0.04205	-0.12808	0.145254	-0.01646	0.042544	0.166204	-0.17764	-0.08866	0.021833	-0.04614	0.170111	0.052305	0.115691
009_gasoline	NAPTHA	0.000	0.741805911	0.309817	0.35573	0.448905	0.517107	0.424367	0.614247	0.808631	0.369399	0.798544	0.006844	-0.07943	0.170478	-0.12898	-0.3943	-0.08761	-0.29014	0.076779	0.082014	-0.46772

SSC-map correlations of UVCBs in R20 transcriptome space

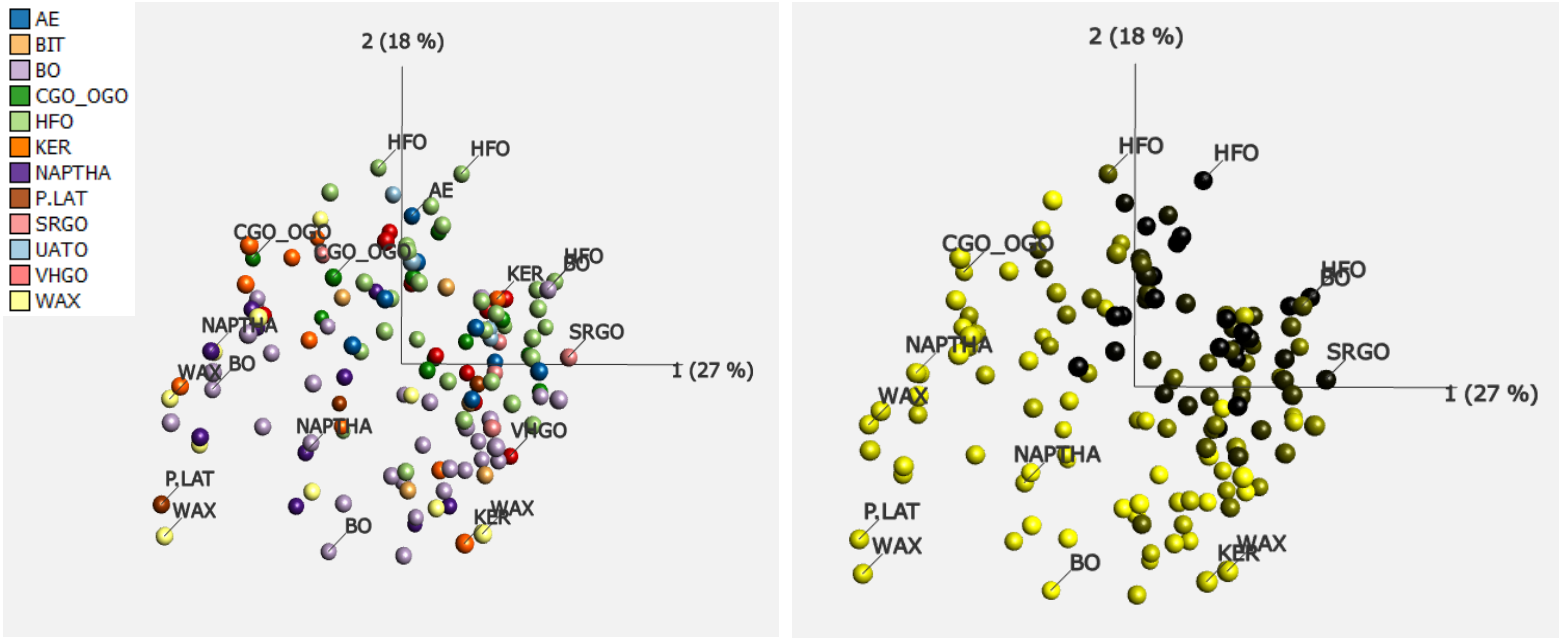


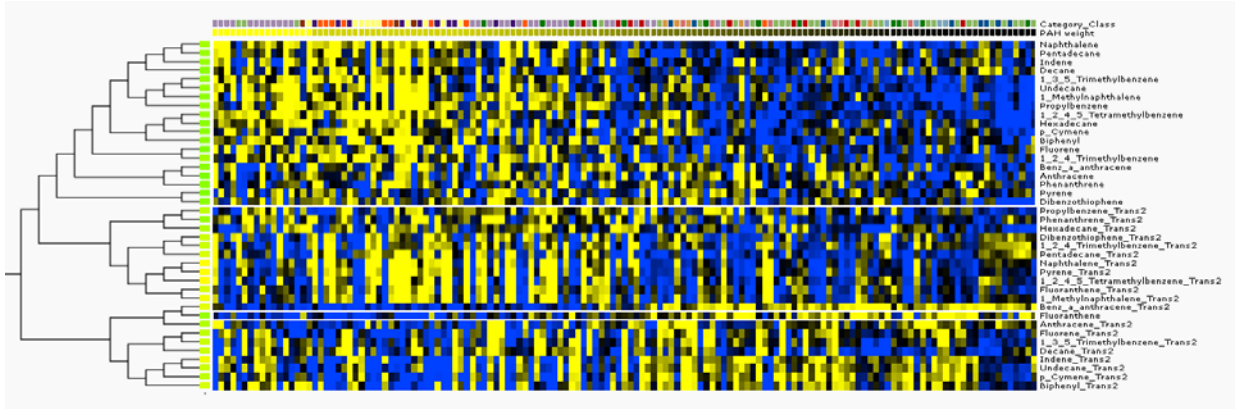






Combined bioactivity and transcription (ssMap) in R20 space



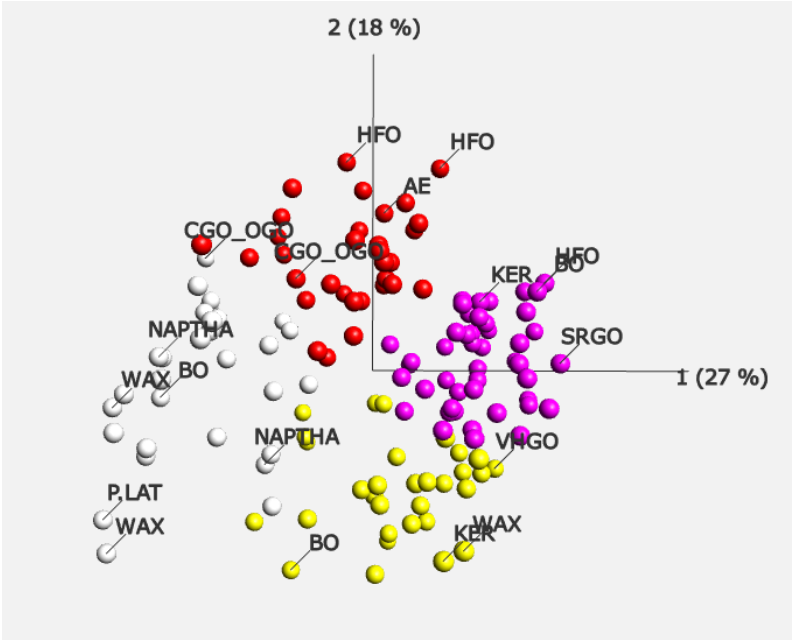


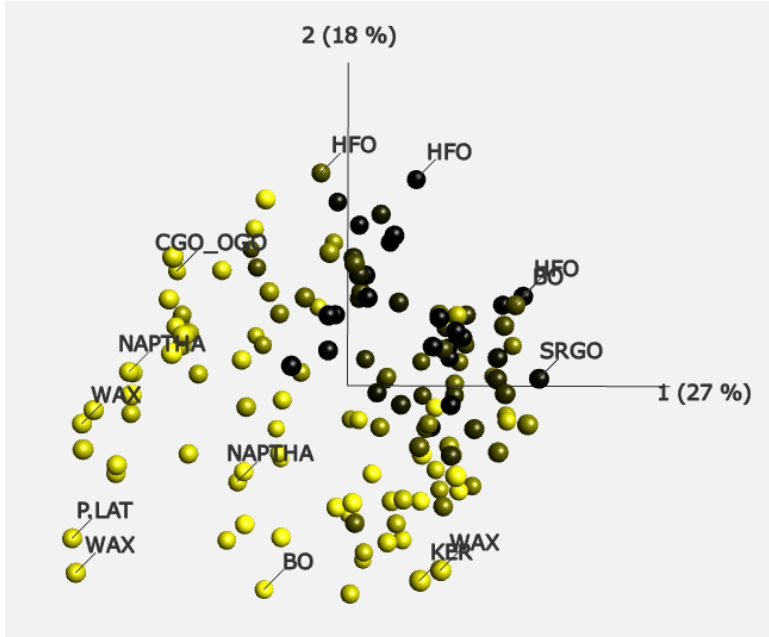
Bioactivity

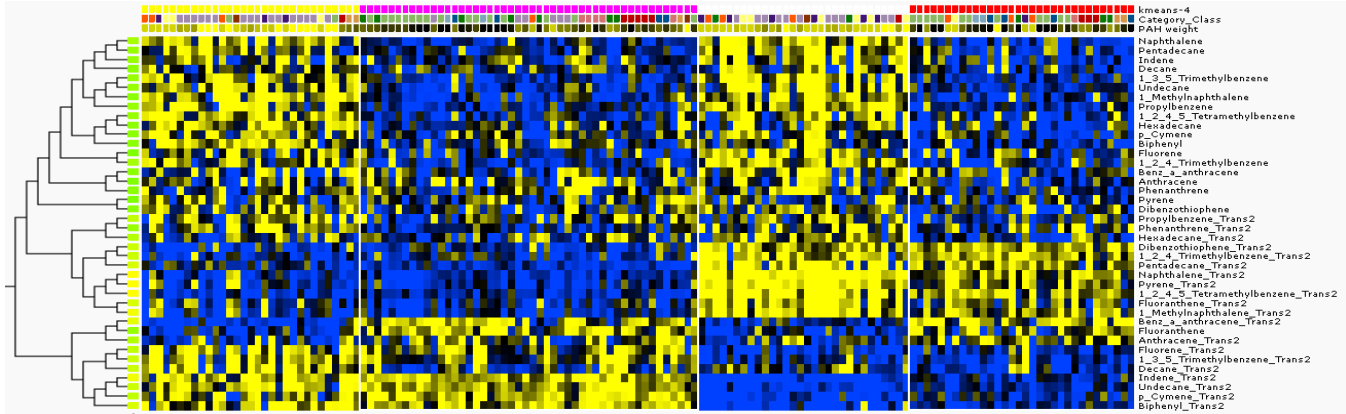
Transcription

Fluoranthrene - bioactivity
Transcription

Combined bioactivity and transcription (ssMap) in R20 space – K means 4 cluster







Bioactivity

Transcription

Fluoranthrene - bioactivity

Transcription

Conclusions

- In R20 Space the UVCBs with the higher percentage 3-7 ring aromatics are more biologically active, but there is biological activity across the spectrum associating with some of the non-carcinogenic and non AhR active chemicals.
- A smaller list of positive control chemicals can provide the same results as the R20 set.
- Transcriptome space shows more variability but there is some lack of correlation between transcriptome activity and biological activity
- Bioactivity space is somewhat more effective than transcriptome space for discerning biological grouping and distinguishing UVCB type.
- A positive control reference space is a useful place to operate providing an anchor for the analysis removing some of the variability associated with individual assays and could provide a basis for read across to product categories.