Challenge 1:

Human Health Hazard Assessment

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Jean-Philippe Gennart
Carol Banner
Hans Ketelslegers

Concawe
Environmental Science for European Refining

Challenge

How to overcome the practical challenges for petroleum UVCB substances in delivering a testing program which takes into account animal welfare considerations and delivers required information within an acceptable timeframe while avoiding underestimation of human health hazards?

To tackle this challenge, a combination of animal testing with alternative methods underpinning similarity and read-across is needed;

Concawe 4 step approach:

1. Analytical data to support chemical similarity
2. Toxicological data to support biological similarity (both in vitro and in vivo)
3. Integrative analysis of data to support
   (3a) chemical-biological similarity
   and build (3b) read across hypotheses
4. Fulfilling higher tier in vivo data requirements

Human Health testing Strategy

Step 1

Analytical/ Compositional Data

- Describe hydrocarbon (HC) chemical space in all registered substances; support sample selection for in-vivo testing

Step 2

In-vivo reproductive screening and in-vitro supporting studies

- Biological similarity of relevant HC space; OECD 422s and Cat-App

Step 3

Integrative data analysis

- Integration of composition, toxicity, biological activity and in-vitro screening data for chemical-biological similarity and grouping assessment, confirmation of test samples and building read-across hypotheses

Step 4

Subchronic* / Developmental **/ Reproductive Toxicity***

- Complete higher-tier requirements with targeted animal testing data

** OECD 408 (rat) **OECD 414 (rat and Rabbit) *** OECD 443*

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Example: Gas Oil hydrocarbon space mapping

Characterisation of 15 substances in 3 categories

Concawe category	EC Name	EC	Number of samples
Other Gas Oils	Gas oils (petroleum), hydrodesulfurized	265-182-8	11
	Distillates (petroleum), hydrotreated middle	265-148-2	10
	Distillates (petroleum), hydrodesulfurized middle	265-183-3	8
Vacuum, Hydrocracked & Distillate Gas Oils	Condensates (petroleum), vacuum tower	265-049-4	5
	Distillates (petroleum), light hydrocracked	265-078-2	11
	Fuel oil, no. 2	270-671-4	6
	Fuel oil, no. 4	270-673-5	3
	Fuels, diesel	269-822-7	18
	Fuels, diesel, no. 2	270-676-1	5
	Gas oils (petroleum), hydrodesulfurized light vacuum	265-190-1	2
	Gas oils (petroleum), light vacuum	265-059-9	13
Straight Run Gas Oils	Distillates (petroleum), full-range straight-run middle	272-341-5	12
	Distillates (petroleum), heavy straight-run	272-817-2	9
	Distillates (petroleum), straight-run middle	265-044-7	2
	Gas oils (petroleum), straight-run	265-043-1	14
Total			129

Analytical Technique	Quantitative information
HPLC	Total mono-, di- and tri+ aromatics
PAC2	Total of 3, 4, 5, 6, 7+ ring PACs
PAH	EPA and Grimmer PAHs
GC x GC	Hydrocarbon classes per carbon number

Concawe

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	Distillates (petroleum), light hydrocracked	265-078-2	11
	Fuel oil, no. 2	270-671-4	6
	Fuel oil, no. 4	270-673-5	3
	Fuels, diesel	269-822-7	18
	Fuels, diesel, no. 2	270-676-1	5
	Gas oils (petroleum), hydrodesulfurized light vacuum	265-190-1	2
	Gas oils (petroleum), light vacuum	265-059-9	13
Straight Run Gas Oils	Distillates (petroleum), full-range straight-run middle	272-341-5	12
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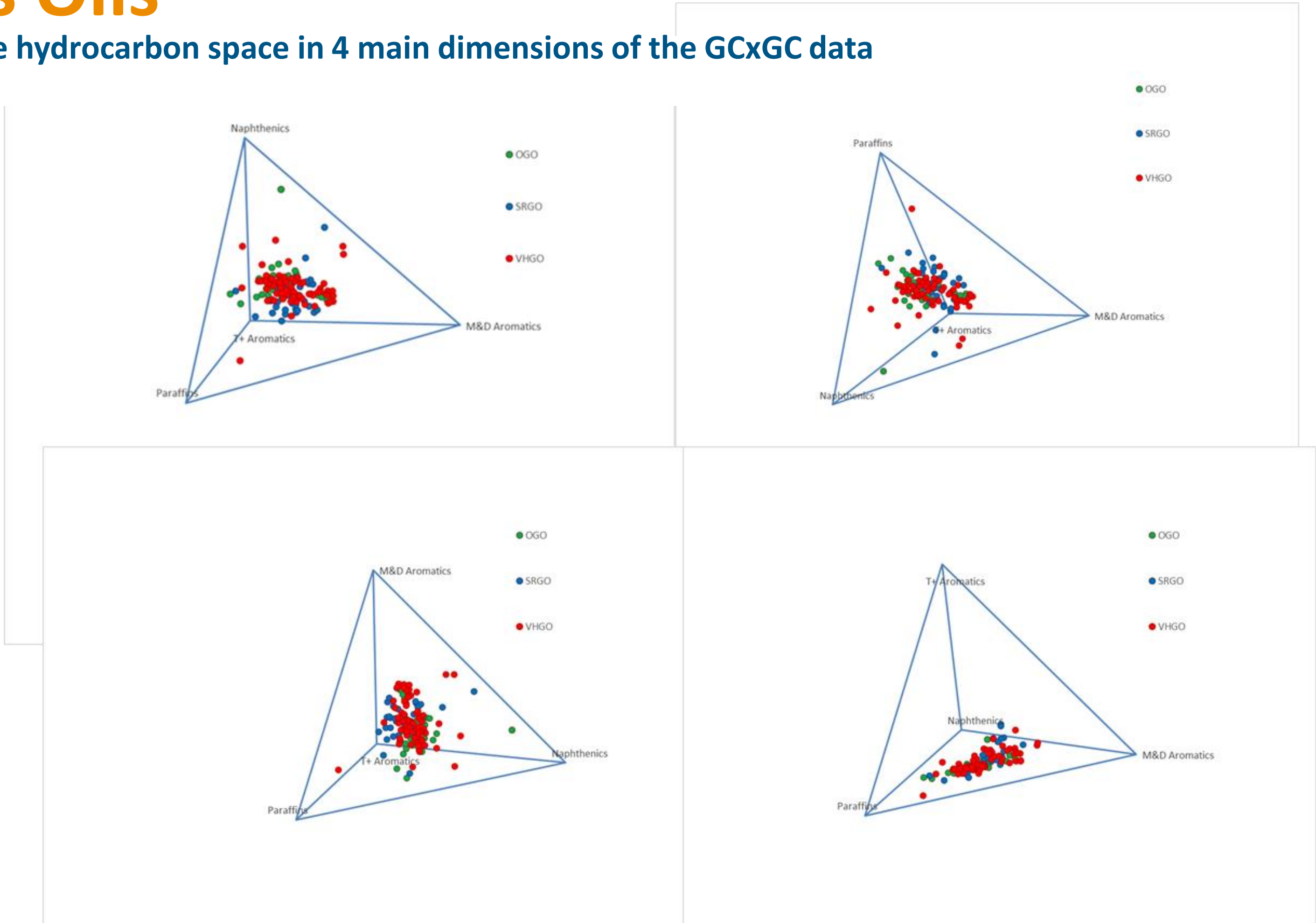
PAH	EPA and Grimmer PAHs
GC x GC	Hydrocarbon classes per carbon number

Gas Oils

A single hydrocarbon space in 4 main dimensions of the GCxGC data



Hydrocarbon space coverage – Other GO example

Combined OGO coverage

Mean: 86.6% Min: 57.1%

C#	n-P	iso-P	N	DN	MoAr	NmoAr	DiAr	NDiAr	TriAr	TetraAr
5										
6					96%					
7	74%	85%	82%		89%					
8	89%	70%	82%		79%					
9	86%	82%	79%	86%	79%	75%				
10	93%	82%	89%	82%	86%	64%	100%			
11	93%	89%	93%	71%	71%	61%	100%			
12	89%	86%	89%	75%	64%	71%	100%			
13	93%	82%	89%	93%	64%	71%	100%			
14	96%	93%	93%	93%	75%	68%	100%		100%	
15	86%	96%	93%	93%	89%	61%	96%		100%	
16	79%	86%	86%	89%	75%	61%	93%		100%	
17	71%	79%	86%	82%	71%	64%	89%		93%	
18	64%	79%	93%	89%	79%	75%	89%		92%	
19	57%	86%	82%	92%	79%	86%	88%		92%	
20	64%	75%	75%	92%	82%	86%	92%		96%	
21	82%	68%	75%	92%	82%	100%			96%	
22	86%	75%	82%	80%	89%				97%	
23	89%	75%	89%	88%	93%					
24	89%	82%	92%		93%					
25	96%	85%	92%		92%					
26	96%	89%	96%		96%					
27	96%	89%	96%		96%					
28	96%	93%	100%		96%					
29	100%	100%	100%		100%					
30	100%	100%	100%		100%					
CX+			96%	93%	93%	96%	96%	93%	100%	90%

Individual Other GO samples

S726 *Mean: 52.0% Min: 14.3%*

C#	n-P	iso-P	N	DN	MoAr	NmoAr	DiAr	NDiAr	TriAr	TetraAr
5										
6					64%					
7	63%	69%	43%		56%					
8	56%	52%	39%		39%					
9	36%	36%	25%	25%	32%	21%				
10	39%	32%	32%	25%	21%	14%	18%			
11	36%	29%	25%	43%	18%	32%	32%			
12	64%	32%	54%	75%	46%	50%	36%			
13	82%	79%	89%	93%	64%	61%	32%			
14	75%	93%	93%	93%	75%	68%	14%		27%	
15	61%	96%	93%	93%	89%	61%	14%		26%	
16	43%	86%	86%	89%	75%	61%	37%		14%	
17	32%	79%	86%	82%	71%	64%	33%		21%	
18	18%	79%	93%	89%	68%	71%	44%		27%	
19	14%	86%	82%	92%	61%	79%	31%		36%	
20	14%	75%	75%	92%	50%	46%	36%		58%	
21	18%	68%	71%	92%	46%	100%			81%	
22	21%	36%	50%	76%	30%				97%	
23	22%	36%	41%	72%	30%					
24	22%	36%	40%		22%					
25	22%	30%	40%		36%					
26	22%	22%	40%		44%					
27	23%	26%	40%		48%					
28	36%	33%	56%		60%					
29	40%	42%	56%		83%					
30	44%	44%	71%		92%					
CX+			96%	30%	93%	41%	28%	22%	96%	40%

S712 *Mean: 55.7% Min: 14.3%*

C#	n-P	iso-P	N	DN	MoAr	NmoAr	DiAr	NDiAr	TriAr	TetraAr
5										
6					96%					
7	70%	73%	82%		89%					
8	89%	70%	82%		79%					
9	86%	82%	79%	86%	79%	50%				
10	93%	82%	89%	82%	86%	54%	86%			
11	93%	89%	93%	71%	71%	61%	79%			
12	89%	86%	89%	50%	64%	71%	79%			
13	93%	82%	86%	79%	57%	71%	71%			
14	96%	86%	82%	79%	64%	61%	50%		62%	
15	86%	82%	79%	61%	61%	43%	36%		63%	
16	79%	68%	61%	50%	50%	32%	33%		61%	
17	71%	57%	36%	46%	25%	18%	41%		57%	
18	64%	50%	29%	25%	25%	29%	30%		50%	
19	57%	57%	14%	36%	25%	18%	50%		40%	
20	36%	39%	18%	32%	29%	25%	52%		58%	
21	25%	21%	18%	32%	29%	100%			81%	
22	25%	21%	21%	36%	26%				97%	
23	26%	21%	22%	40%	26%					
24	26%	21%	36%		33%					
25	30%	22%	36%		44%					
26	33%	26%	44%		48%					
27	38%	30%	48%		48%					
28	44%	37%	56%		60%					
29	44%	38%	56%		83%					
30	44%	40%	71%		92%					
CX+			96%	19%	93%	37%	44%	56%	96%	40%

S809 *Mean: 62.0% Min: 7.1%*

C#	n-P	iso-P	N	DN	MoAr	NmoAr	DiAr	NDiAr	TriAr	TetraAr
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7	74%	85%	64%		78%					
8	52%	67%	50%		57%					
9	39%	46%	39%	29%	46%	75%				
10	29%	39%	25%	14%	50%	64%	100%			
11	21%	25%	14%	11%	46%	57%	100%			
12	18%	18%	11%	11%	36%	61%	100%			
13	18%	18%	11%	11%	18%	46%	100%			
14	14%	18%	7%	11%	18%	25%	100%		100%	
15	18%	14%	7%	7%	18%	18%	96%		100%	
16	18%	18%	7%	7%	14%	18%	93%		100%	
17	18%	18%	7%	7%	39%	57%	89%		93%	
18	21%	11%	11%	11%	79%	75%	89%		92%	
19	46%	14%	25%	40%	79%	86%	88%		92%	
20	64%	32%	46%	68%	82%	86%	92%		96%	
21	82%	54%	75%	76%	82%	100%			96%	
22	86%	75%	82%	80%	89%				97%	
23	89%	75%	89%	88%	93%					
24	89%	82%	92%		93%					
25	96%	85%	92%		92%					
26	96%	89%	96%		96%					
27	96%	89%	96%		96%					
28	96%	93%	100%		96%					
29	100%	100%	100%		100%					
30	100%	100%	100%		100%					
CX+			96%	93%	93%	96%	96%	93%	100%	90%

Concentration (wt %) of a single hydrocarbon identifier measured in samples

Not possible carbon number hydrocarbon class combination

No measured value in any sample

Normalised concentration of hydrocarbon identifier (%)					
≤50	51-60	61-70	71-80	81-90	91-100

Hydrocarbon space coverage – Other GO example

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11	93%	89%	93%	71%	71%	61%	100%			
12	89%	86%	89%	75%	64%	71%	100%			
13	93%	82%	89%	93%	64%	71%	100%			
14	96%	93%	93%	93%	75%	68%	100%		100%	
15	86%	96%	93%	93%	89%	61%	96%		100%	
16	79%	86%	86%	89%	75%	61%	93%		100%	
17	71%	79%	86%	82%	71%	64%	89%		93%	
18	[illegible]	79%	93%	89%	79%	75%	89%		92%	
19	57%	[illegible]	82%	92%	79%	86%	88%		92%	
20	64%	75%	75%	92%	[illegible]	86%	92%		96%	
21	82%	68%	75%	92%	82%	100%			96%	
22	86%	75%	82%	80%	89%				97%	
23	89%	75%	89%	88%	93%					
24	89%	82%	92%		93%					
25	96%	85%	92%		92%					
26	96%	89%	96%		96%					
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13	82%	79%	89%	93%	64%	61%	32%			
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23	22%	36%	[illegible]	72%	30%					
24	22%	36%	40%		22%					
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11	93%	89%	93%	71%	71%	61%	79%			
12	89%	86%	89%	50%	64%	71%	79%			
13	93%	82%	86%	79%	57%	71%	71%			
14	96%	86%	82%	79%	64%	61%	50%		62%	
15	86%	82%	79%	61%	61%	43%	36%		63%	
16	79%	68%	61%	50%	50%	32%	33%		61%	
17	71%	57%	36%	46%	25%	18%	41%		57%	
18	64%	50%	29%	25%	25%	29%	30%		50%	
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22	25%	21%	21%	36%	26%				97%	
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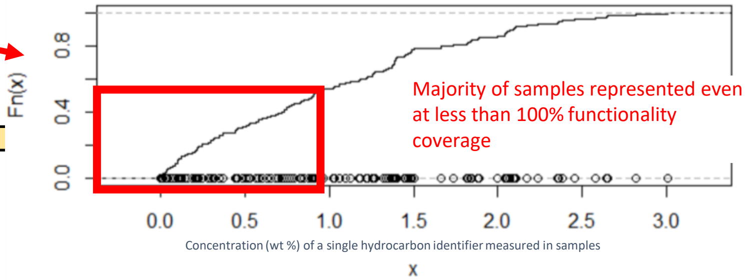
11	21%	25%	14%	11%	46%	57%	100%			
12	18%	18%	11%	11%	36%	61%	100%			
13	18%	18%	11%	11%	18%	46%	100%			
14	14%	18%	7%	11%	18%	25%	100%		100%	
15	18%	14%	7%	7%	18%	18%	96%		100%	
16	18%	18%	7%	7%	14%	18%	93%		100%	
17	18%	18%	7%	7%	39%	57%	89%		93%	
18	21%	11%	11%	11%	79%	75%	89%		92%	
19	46%	14%	25%	40%	79%	86%	88%		92%	
20	64%	32%	46%	68%	82%	86%	92%		96%	
21	82%	54%	75%	76%	82%	100%			96%	
22	86%	75%	82%	80%	89%				97%	
23	89%	75%	89%	88%	93%					
24	89%	82%	92%		93%					



Not possible carbon number hydrocarbon class combination	Normalised concentration of hydrocarbon identifier (%)					
No measured value in any sample	≤50	51-60	61-70	71-80	81-90	91-100

Sample selection for in vivo testing

OECD 422: one sample per registered substance = 15 samples

	VHGO Coverage Mean: 95.6% Min: 78.9%								SRGO Coverage Mean: 85.8% Min: 55.6%				OtherGO Coverage Mean: 86.6% Min: 57.1%		
Category	VHGO	VHGO	VHGO	VHGO	VHGO	VHGO	VHGO	VHGO	SRGO	SRGO	SRGO	SRGO	OtherGO	OtherGO	OtherGO
EC	265-059-9	265-049-4	265-078-2	265-190-1	269-822-7	270-671-4	270-673-5	270-676-1	272-817-2	265-043-1	265-044-7	272-341-5	265-183-3	265-148-2	265-182-8
Sample	S796	S721	S692	S682	S777	S760	S845	S668	S715	S686	S795	S836	S809	S726	S712
3-7+ring PAC wt%	5.18	2.50	0.06	2.24	0.47	0.24	3.65	0.22	6.97	0.14	3.57	1.85	3.61	0.29	0.51
Wt % ≤C30	99.93	99.90	99.92	86.20	99.98	99.99	100.00	99.99	91.60	99.19	100.00	100.00	99.44	100.00	100.00

Cells highlighted in orange represent samples selected for higher tier testing (see below)

Higher tier testing: 5 samples proposed for testing providing the best coverage[1]

3 Category Coverage Mean: 89.5% Min: 57.8%				
OtherGO	VHGO	SRGO	VHGO	SRGO
265-183-3	265-059-9	272-817-2	269-822-7	272-341-5
S809	S796	S715	S777	S836
Highest [PAC] sample OGO/ Highest tonnage OGO	Highest [PAC] sample VHGO	Highest [PAC] sample SRGO	Highest tonnage VHGO	Highest tonnage SRGO

[1] Sample selection to be confirmed in step 3

Human Health testing Strategy

Step 1

Analytical/ Compositional Data

- Describe hydrocarbon (HC) chemical space in all registered substances; support sample selection for in-vivo testing

Step 2

In-vivo reproductive screening and in-vitro supporting studies

- Biological similarity of relevant HC space; OECD 422s and Cat-App

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Integrative data analysis

- Integration of composition, toxicity, biological activity and in-vitro screening data for chemical-biological similarity and grouping assessment, confirmation of test samples and building read-across hypotheses

Step 4

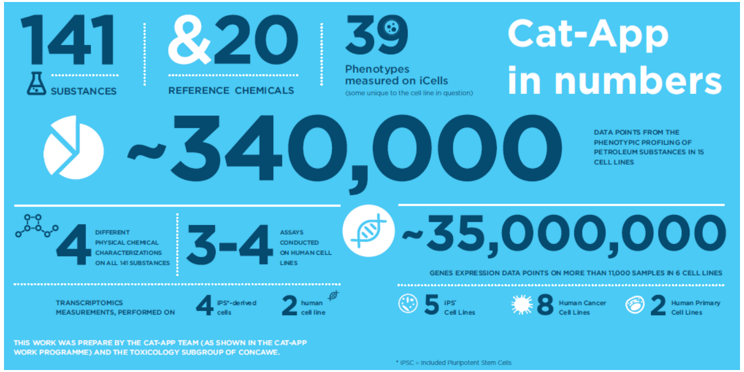
Subchronic* / Developmental **/ Reproductive Toxicity***

- Complete higher-tier requirements with targeted animal testing data

** OECD 408 (rat) **OECD 414 (rat and Rabbit) *** OECD 443*

Going beyond chemical similarity to address the challenges with grouping & read across for UVCB substances (1/2)

In vitro: Cat-App[1], adding a biological element to similarity assessment of the hydrocarbon space



https://www.concawe.eu/cat-app

Cat-App work programme

Cat-App: New technologies to underpin the category approaches and read across in regulatory programmes
Project Management: Hans Ketelslegers, Concawe
Steering: Concawe's scientific committee and toxicology subgroup

WP1

Organisation of data available on PS (Ivan Rusyn/Texas A&M University)

1.1 Obtain, process and share chemical samples
1.2 Collect available records (manufacturing process info., phys./chem. properties, analytical chemistry, existing toxicity data on mammalian, ecotox)
1.3 Digitise records into flexible and inter-operable databaseformat

WP2

Bioactivity screening (Ivan Rusyn/Texas A&M University

WP2.a
(Ivan Rusyn/Texas A&M University)
- High content screening of iPS*-derived cells
- Hepatocytes, neurons, cardiomyocytes, macrophages, endothelial

WP2.b
(Tim Gant/PHE)
- Toxicity phenotyping in 10 diverse cell lines

WP3

High throughput genomics (Ivan Rusyn/Texas A&M University)
3.1 High-throughput transcriptomics profiling of ~11,000 samples for TempO-seq

WP4

Perform data integration and chemical biological read across (Fred Wright/NCSU)

WP 4.a
(Fred Wright/NCSU)
4a.1 Coordinate data management and workflow
4a.2 Perform uncertainty and variability analyses
4a.3 Process and analyse omics data
4a.4 Perform ToxPi analysis

WP4.b (Shu-Dong Zhang/Ulster)

4b.1 Perform connectivity mapping
4b.2 Develop and apply analysis algorithms to robustness testing, investigate grouping accuracy and profiling cost

WP5

Dissemination, project administration and Outreach (Klaus Lenz/SYNCOM)

5.1 Project Dissemination and website
5.2 Project Administration
5.3 Outreach

Advisory Board

George Daston
Procter & Gamble

Shirley Price
University of Surrey

Chris Rowat
Health Canada

Xiaowei Zhang
Nanjing University

Institute abbreviations:
Texas A&M University Research - NCSU: North Carolina State University - PHE: Public Health England
Ulster: Ulster University - SYNCOM: SYNCOM R&D consulting GmbH

* induced Pluripotent Stemcells

[1] *New Technologies to Underpin Category Approaches and Read-across in Regulatory Programmes*

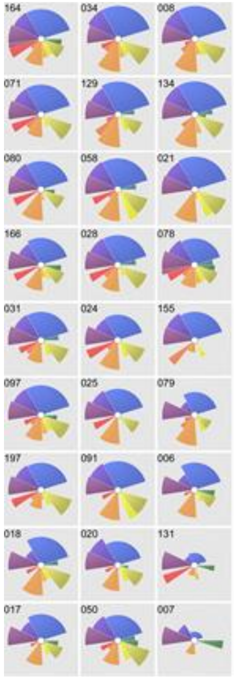
Similarity of bioactivity patterns: ToxPi approach

ToxPi Legend: cell types

CM (28.6%) Heart
ENDO (9.5%) Endothelium
HEP (9.5%) Liver
HEPARG (2.4%) Liver
HEPG2 (4.8%) Liver
HLMVEC (4.8%)
HUVEC (11.9%)
LN229 (4.8%)
MCF7 (4.8%)
NEUR (9.5%)
SH-SY5Y (4.8%)
A375 (4.8%)

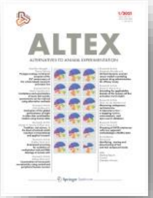
ToxPi: bioactivity scoring

Heavy Fuel Oils



Waxes

Concawe report 24/20:
https://www.concawe.eu/wp-content/uploads/Rpt_20-24.pdf



House et al (2021). Grouping of UVCB substances with new approach methodologies (NAMs) data. ALTEX 38 (1)

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Going beyond chemical similarity to address the challenges with grouping & read across for UVCB substances (2/2)

In vivo: OECD 422 bridging studies

Each CAS number in the OGO, SRGO and VHGO categories tested in a series of 15 dietary and 6 additional dermal OECD 422 studies, with the aim of obtaining the following information:

- Compare toxicity across the hydrocarbon chemical space (supporting biological similarity)
- Screen for repeat dose and reproductive toxicity endpoints
- Provide additional comparative oral & dermal data to the existing data base which is predominantly dermal
    - *Exposure route – to – route extrapolation to support risk assessment*
- Facilitate anchoring between in vivo and in vitro to grow confidence in the in vitro data and further develop these
- Provide information to confirm sample selection for further higher tier in vivo testing
    - *For these selected samples, OECD 422 study will be extended to act as range-finder for the EOGRTS to minimize animal use*

Human Health testing Strategy

Step 1

Analytical/ Compositional Data

- Describe hydrocarbon (HC) chemical space in all registered substances; support sample selection for in-vivo testing

Step 2

In-vivo reproductive screening and in-vitro supporting studies

- Biological similarity of relevant HC space; OECD 422s and Cat-App

Step 3

Integrative data analysis

- Integration of composition, toxicity, biological activity and in-vitro screening data for chemical-biological similarity and grouping assessment, confirmation of test samples and building read-across hypotheses

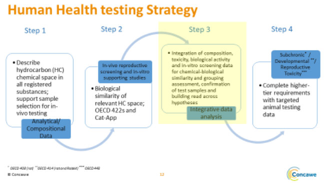
Step 4

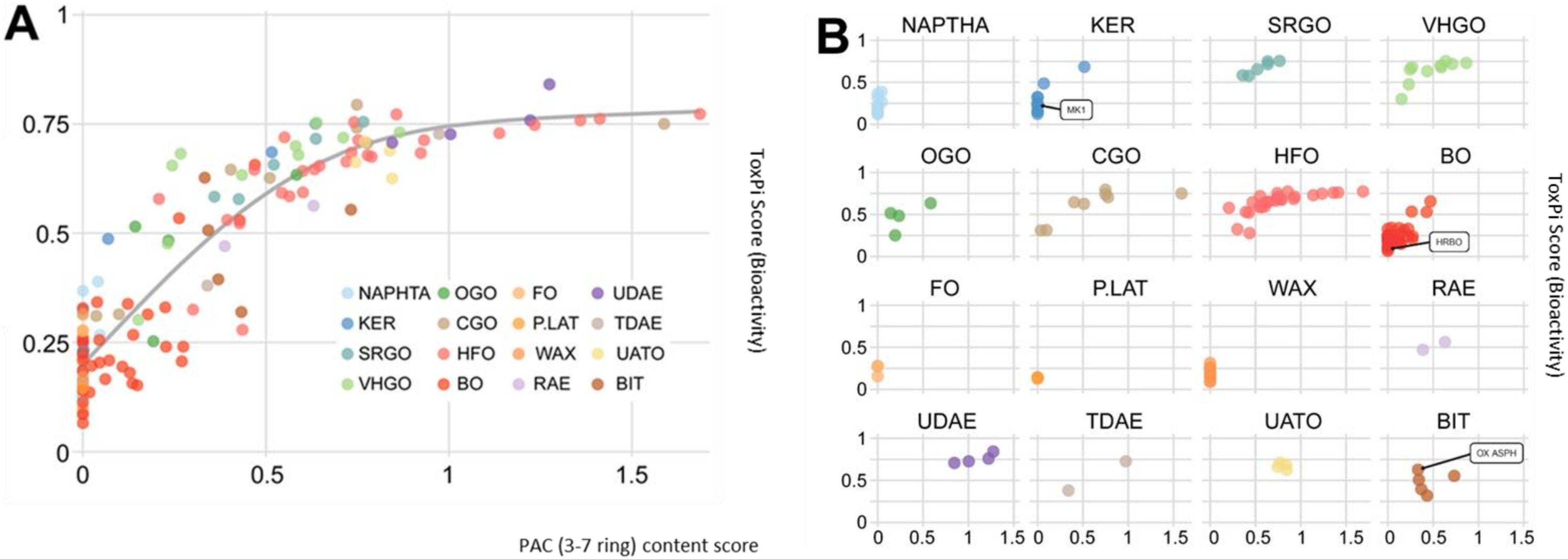
Subchronic* / Developmental **/ Reproductive Toxicity***

- Complete higher-tier requirements with targeted animal testing data

** OECD 408 (rat) **OECD 414 (rat and Rabbit) *** OECD 443*

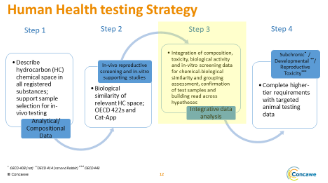
Trends in bioactivity explained by polycyclic aromatic hydrocarbon content?

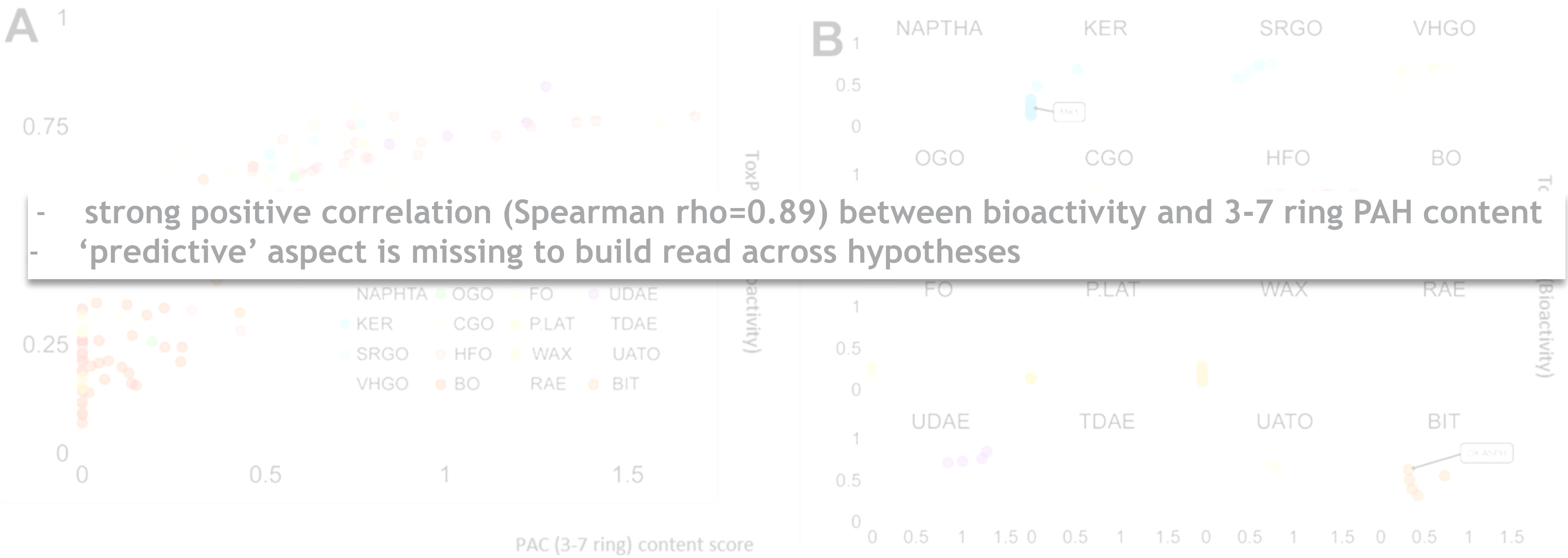




www.concawe.eu/cat-app

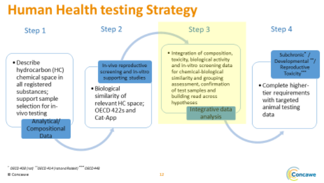
Trends in bioactivity explained by polycyclic aromatic hydrocarbon content?





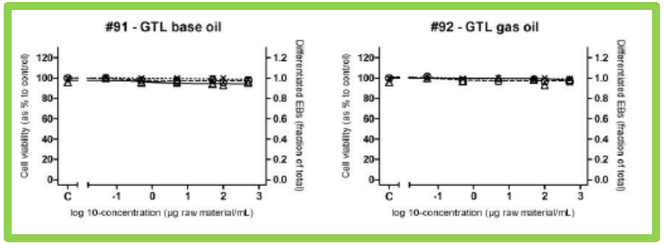
- strong positive correlation (Spearman rho=0.89) between bioactivity and 3-7 ring PAH content
- ‘predictive’ aspect is missing to build read across hypotheses

Adding a predictive aspect to the in-vitro data to support hazard assessments and build read across hypotheses

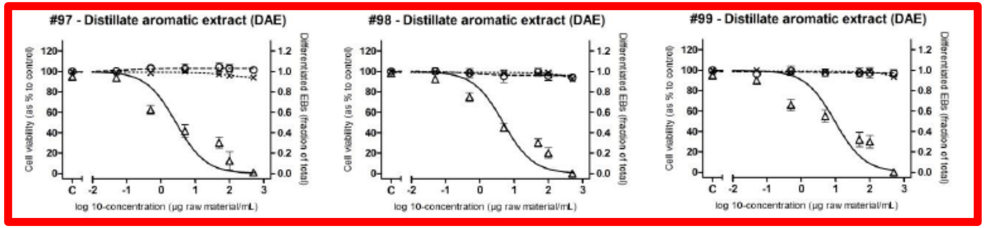


Kamelia et al., Toxicology in Vitro (2017)

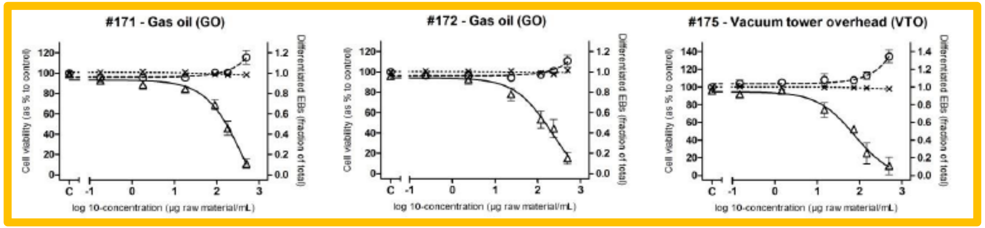
Base Oil (similar to Concawe category of Highly Refined Base Oil):
no aromatics / (3-7 ring) PAH



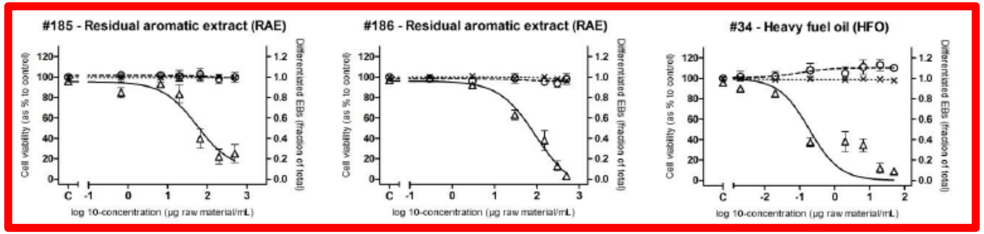
DAE (extracts):
high (3-7 ring) PAH



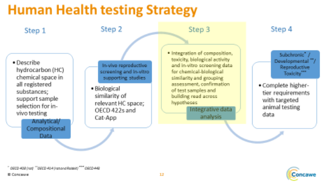
Gas Oils (e.g., Diesel):
low to moderate (3-7 ring) PAH



RAE (aromatic extracts) & heavy fuel oil:
low to high (3-7 ring) PAH

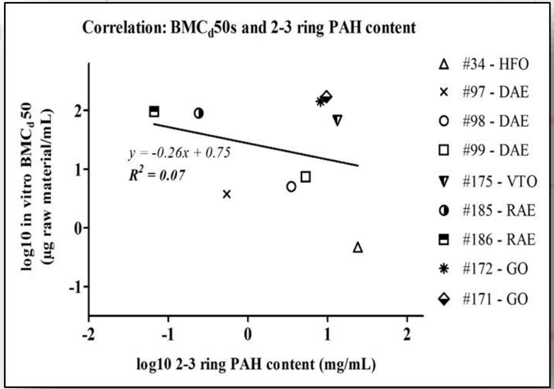


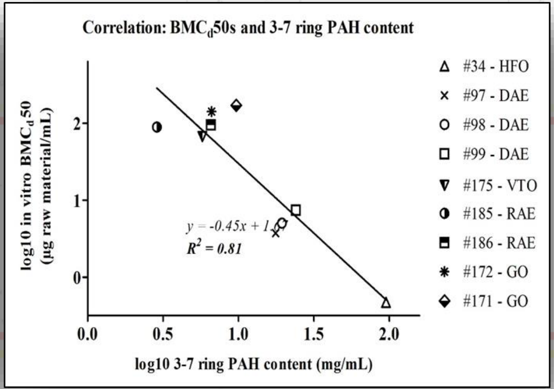
Adding a predictive aspect to the in-vitro data to support hazard assessments and build read across hypotheses



Base Oil (simi... category of H... Base Oil): n...

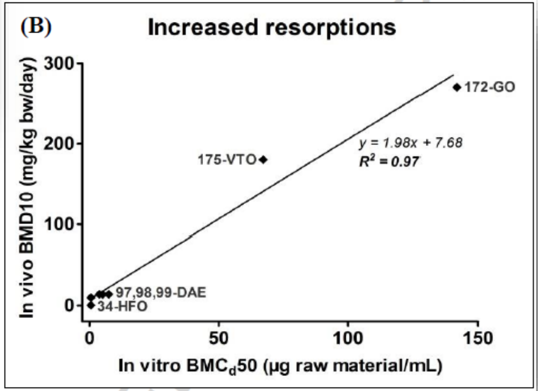
In-vitro results correlate
with 3-7 ring PAH content, not 2-3 ring PAH…





Kamelia et al., Toxicology in Vitro (2017)

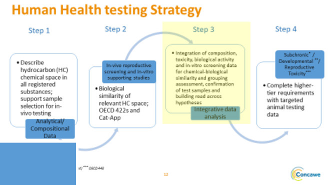
… and with in-vivo data



Gas Oils (e.g., Diesel):
low to moderate (3-7 ring) PAH

RAE (aromatic extracts) & heavy fuel oil:
low to high (3-7 ring) PAH

Integrative data analysis (Gas Oils example)



Assess all available information from 15 CAS numbers to confirm the choice of samples for higher tier testing
e.g. chemical space, in vitro profile (Cat-App), OECD 422 toxicity and in vitro screening (hazard endpoint support)

↓

Confirm representative samples across Gas Oil categories				
Sample 1	Sample 2	Sample 3	Sample 4	Sample 5

↓

Perform higher tier studies (in vitro screening data to support read-across hypotheses)		
Rat PNDT	Rabbit PNDT	Extended-One-Generation-Reproductive-Toxicity-Study

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Discussion

How to overcome the practical challenges for petroleum UVCB substances in delivering a testing program which takes into account animal welfare considerations and delivers required information within an acceptable timeframe while avoiding underestimation of human health hazards?

Two statements to start brainstorming:

- ***A holistic approach addressing the full hydrocarbon space overcomes the practical challenges of a substance by substance approach for UVCBs***
    - *Which improvements could increase the confidence (and decrease the remaining uncertainties) in the presented chemical-biological similarity assessment?*
    - *Does the holistic approach facilitate inter-category read across?*
- ***UVCB tailored guidance on information requirements regarding similarity, grouping and read across assessments would help progress the regulatory assessment of these substances under REACH***

Concawe
Environmental Science
for European Refining

www.concawe.eu

Thank you for your attention

jean-philippe.gennart@concawe.eu

- Jean-Philippe Gennart
- Carol Banner
- Hans Ketelslegers