Role of Cat-App in Concawe REACH strategy for human health

Cat-App final event, Brussels, 6 September 2018

Hans Ketelslegers, Science Executive Health, Concawe

Agenda

01 Petroleum UVCBs

02 Mammalian toxicology of petroleum substances

03 Datagaps, Cat-App and the Concawe REACH strategy for human health

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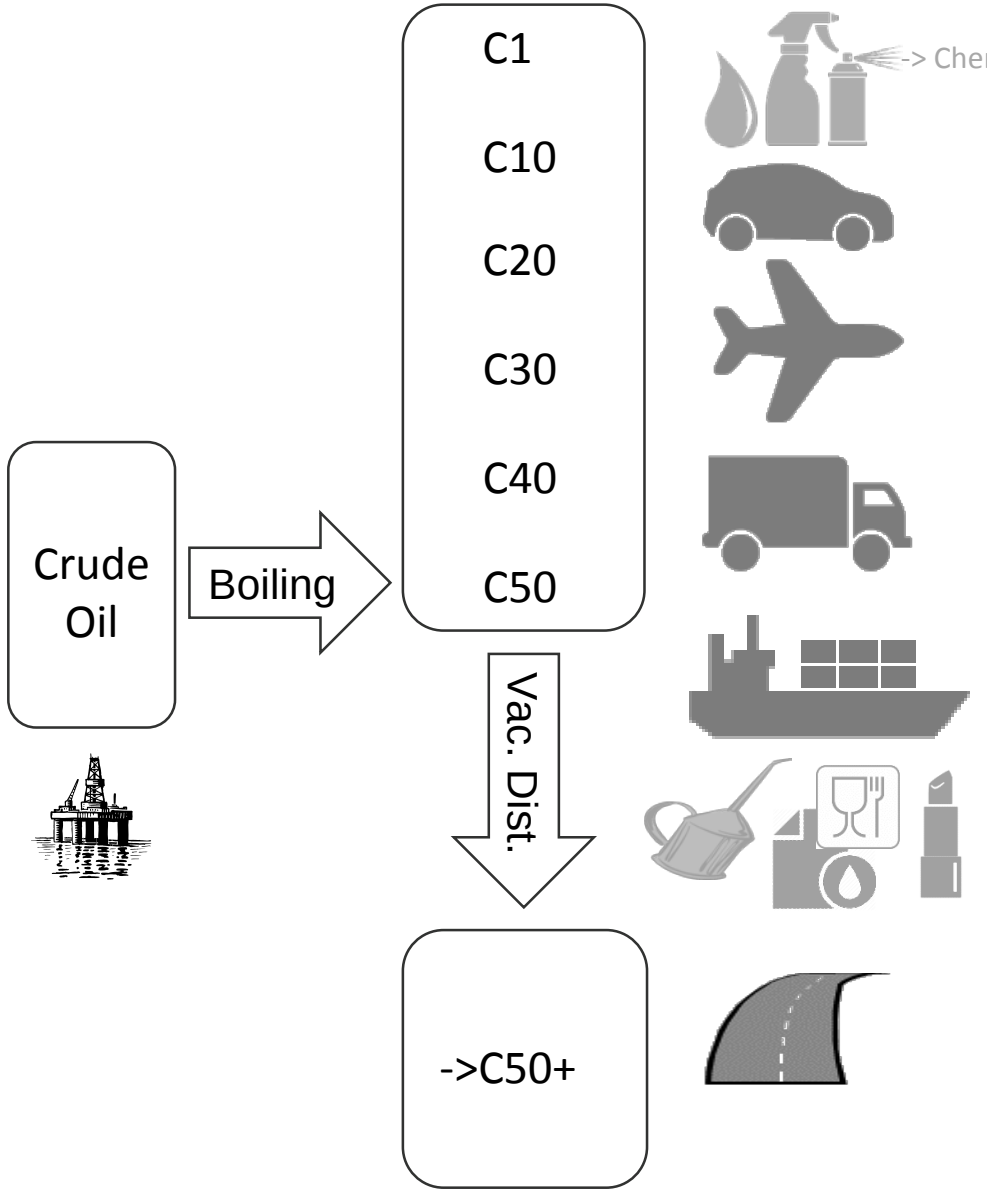
1 Petroleum UVCBs

A regulatory toxicology challenge

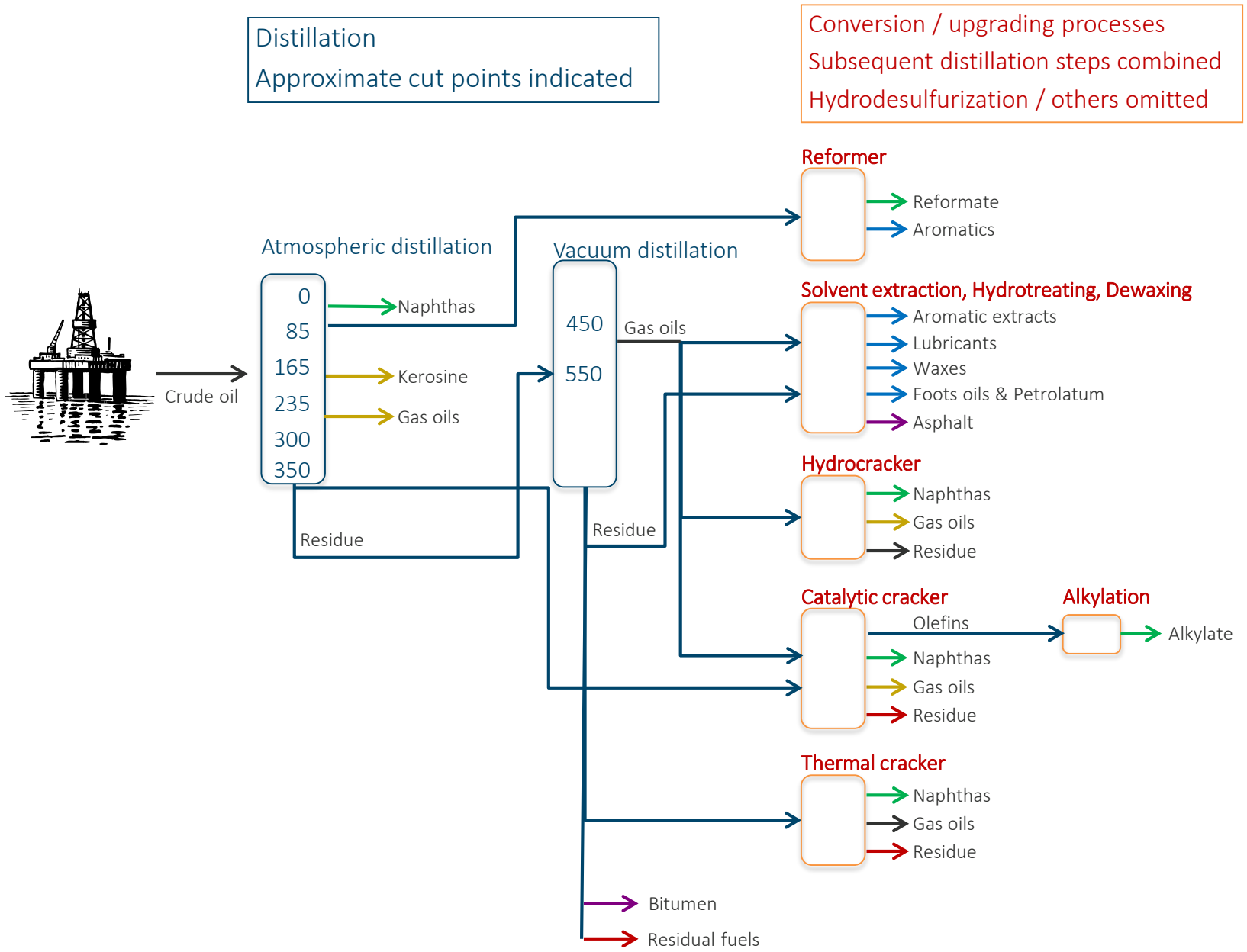
Petroleum substances: a regulatory (toxicology) challenge



- 207 Petroleum Substances (PS)
- **Thousands to millions of molecules** (isomers) per PS
- UVCB
  - **U**nknown or
  - **V**ariable composition,
  - **C**omplex reaction products,
  - **B**iological materials

https://www.fuelseurope.eu/knowledge/how-refining-works

Fractionation and Processing of Crude Oil into Petroleum Substances (**C**omplex [reaction] products of **B**iological origin)



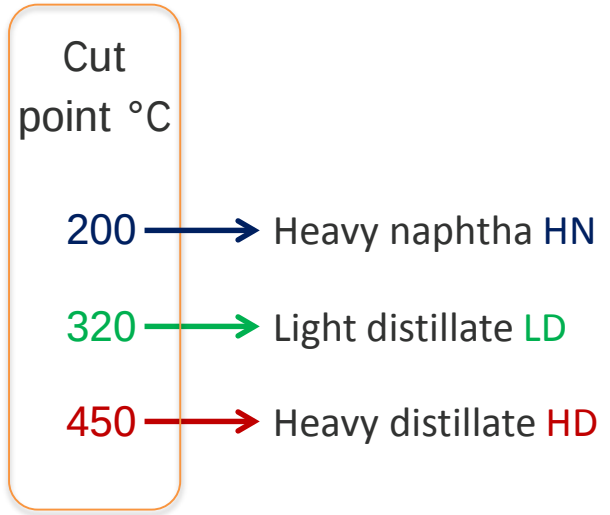
Petroleum Substances are UVCBs

- **U**nknown or
- **V**ariable composition,
- **C**omplex reaction products,
- **B**iological materials

Fractionation Process Yields Complex Reaction Products, with "neighboring" streams overlapping

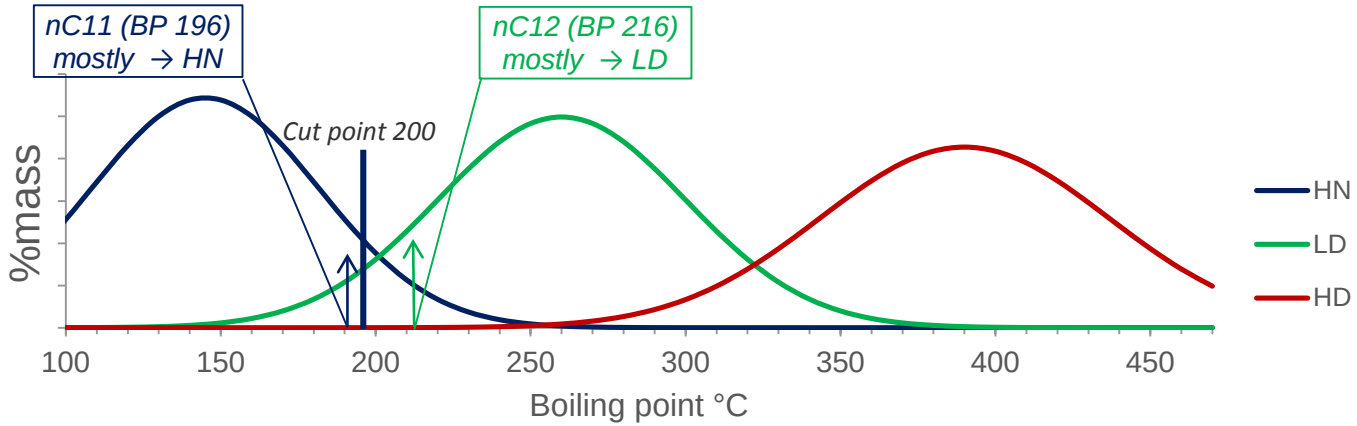
- Fractionated distillation splits a feed stream into product streams with constituents preferentially distilling according to their boiling points.

- Due to imperfect separation each product stream includes compounds with true boiling points outside the intended boiling point range.



Example

- The fractionation scheme for the fictional unit illustrated above could result in product constituent distributions as below, indicated by boiling point ranges



Petroleum Substances are UVCBs

- Unknown or
- Variable composition,
- Complex reaction products,
- Biological materials

Petroleum Substances are Variable in Composition

Stream composition varies continuously over time due to several factors

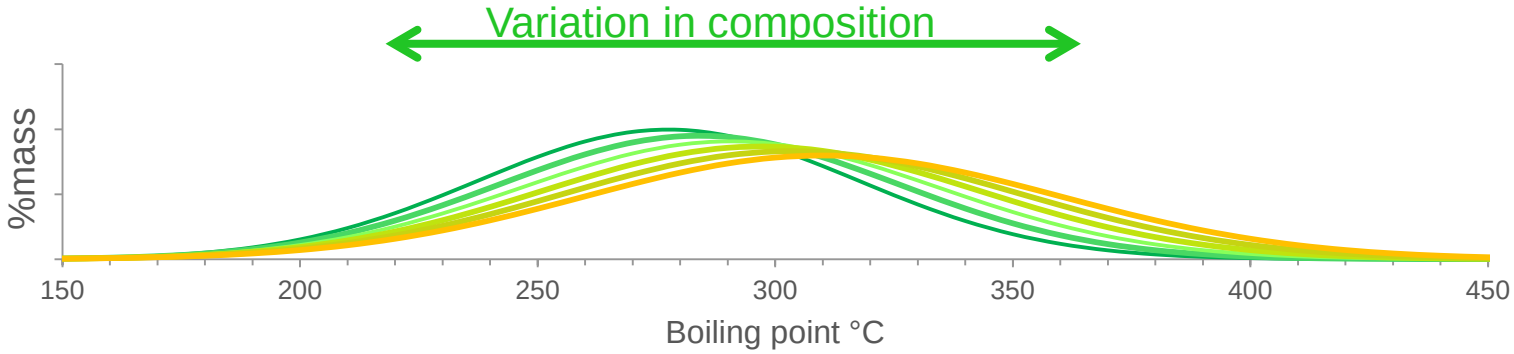
- Feedstock composition
- Processing severity
- Separation temperatures, sharpness
- Catalyst / equipment performance

Variation is limited due to performance specifications of the final products

- Specified by substance identity

Example

- Illustration below based on light distillate fraction (previous slide)



Petroleum Substances are UVCBs

- Unknown or
- Variable composition,
- Complex reaction products,
- Biological materials

Petroleum Substances have Complex Chemical Composition of which a large part is **U**nknown

- The number of individual constituents increases rapidly with carbon number
  - *(*) Including aromatics would increase the number of isomer molecules dramatically*
- The predominant constituents are described by carbon number/boiling point ranges and hydrocarbon types
- Carbon number/boiling point ranges are influenced by fractionation
- Hydrocarbon types (n-/i-alkanes, aromatics, olefins, etc.) are influenced by chemical processing
- Petroleum UVCB can never be fully characterized analytically

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

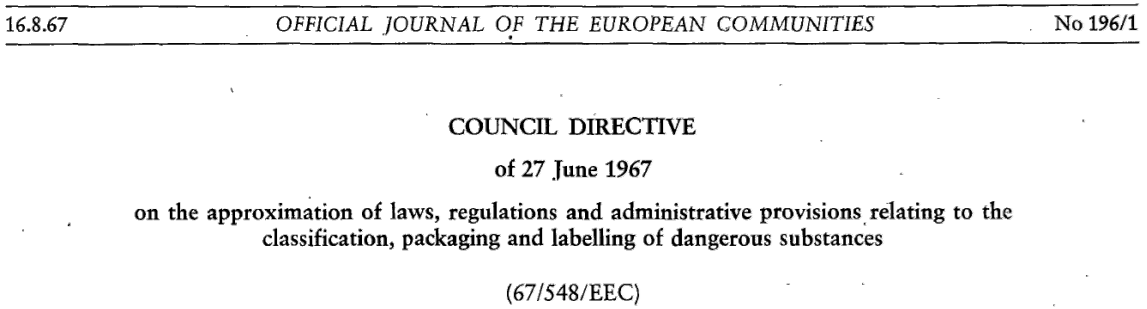
This table is for illustrative purposes only

Petroleum Substances are UVCBs
- **U**nknown or
- **V**ariable composition,
- **C**omplex reaction products,
- **B**iological materials

2 Mammalian toxicology of petroleum products

Toxicological hazards and PAH based testing hypothesis for grouping and read across assessments

Petroleum substances: a regulatory (toxicology) challenge



16.8.67 OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES No 196/1

COUNCIL DIRECTIVE

of 27 June 1967

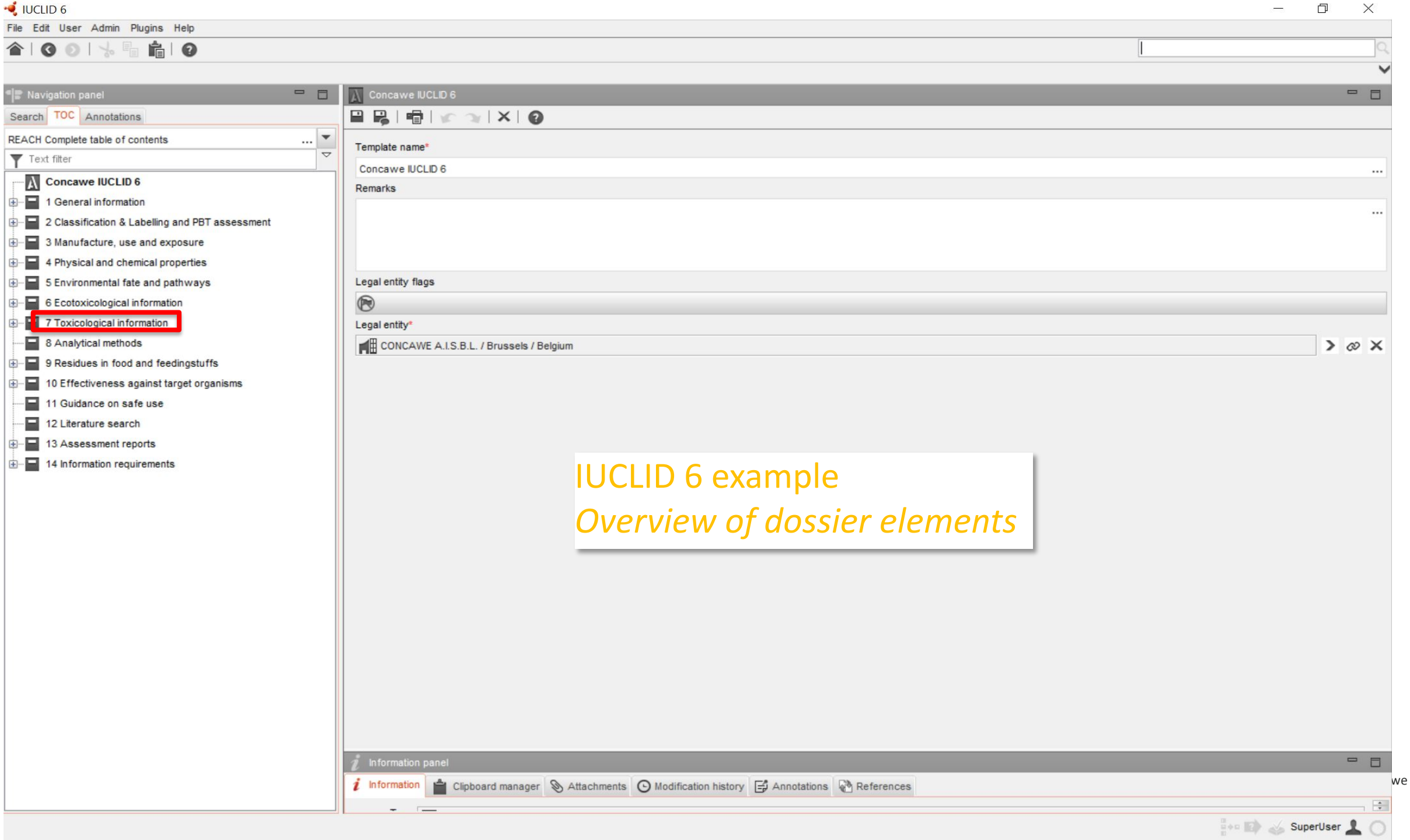
on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances

(67/548/EEC)

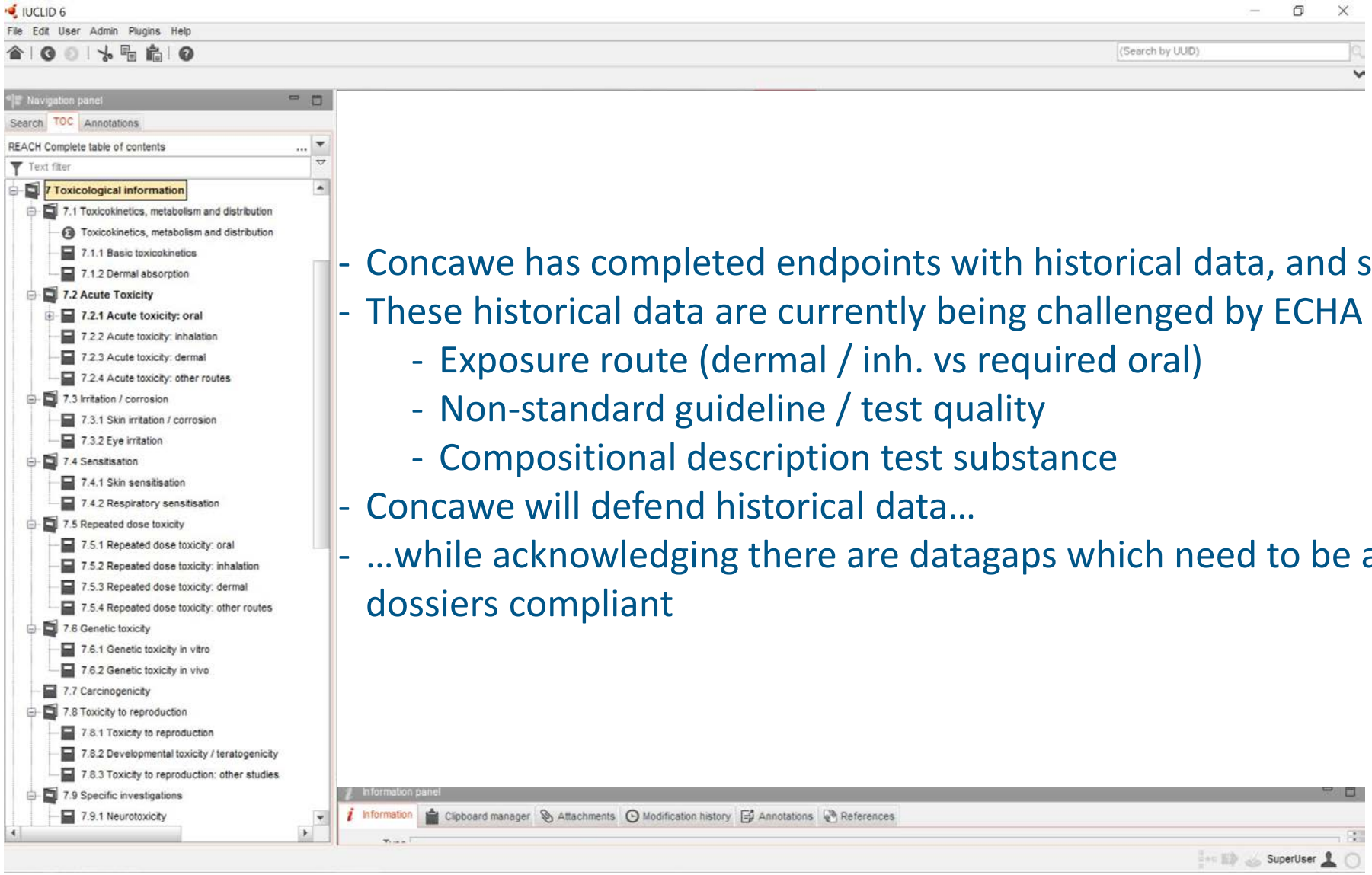
REACH COMPLIANCE

ECHA EUROPEAN CHEMICALS AGENCY

- 207 Petroleum Substances (PS)
- **Thousands to millions of molecules** (isomers) per PS
- UVCB
  - **U**nknown or
  - **V**ariable composition,
  - **C**omplex reaction products,
  - **B**iological materials


IUCLID 6
File Edit User Admin Plugins Help
Navigation panel
Search TOC Annotations
REACH Complete table of contents
Text filter
Concawe IUCLID 6
1 General information
2 Classification & Labelling and PBT assessment
3 Manufacture, use and exposure
4 Physical and chemical properties
5 Environmental fate and pathways
6 Ecotoxicological information
7 Toxicological information
8 Analytical methods
9 Residues in food and feedingstuffs
10 Effectiveness against target organisms
11 Guidance on safe use
12 Literature search
13 Assessment reports
14 Information requirements
Concawe IUCLID 6
Template name*
Concawe IUCLID 6
Remarks
Legal entity flags
Legal entity*
CONCAWE A.I.S.B.L. / Brussels / Belgium
IUCLID 6 example
Overview of dossier elements
Information panel
Information
Clipboard manager
Attachments
Modification history
Annotations
References
SuperUser

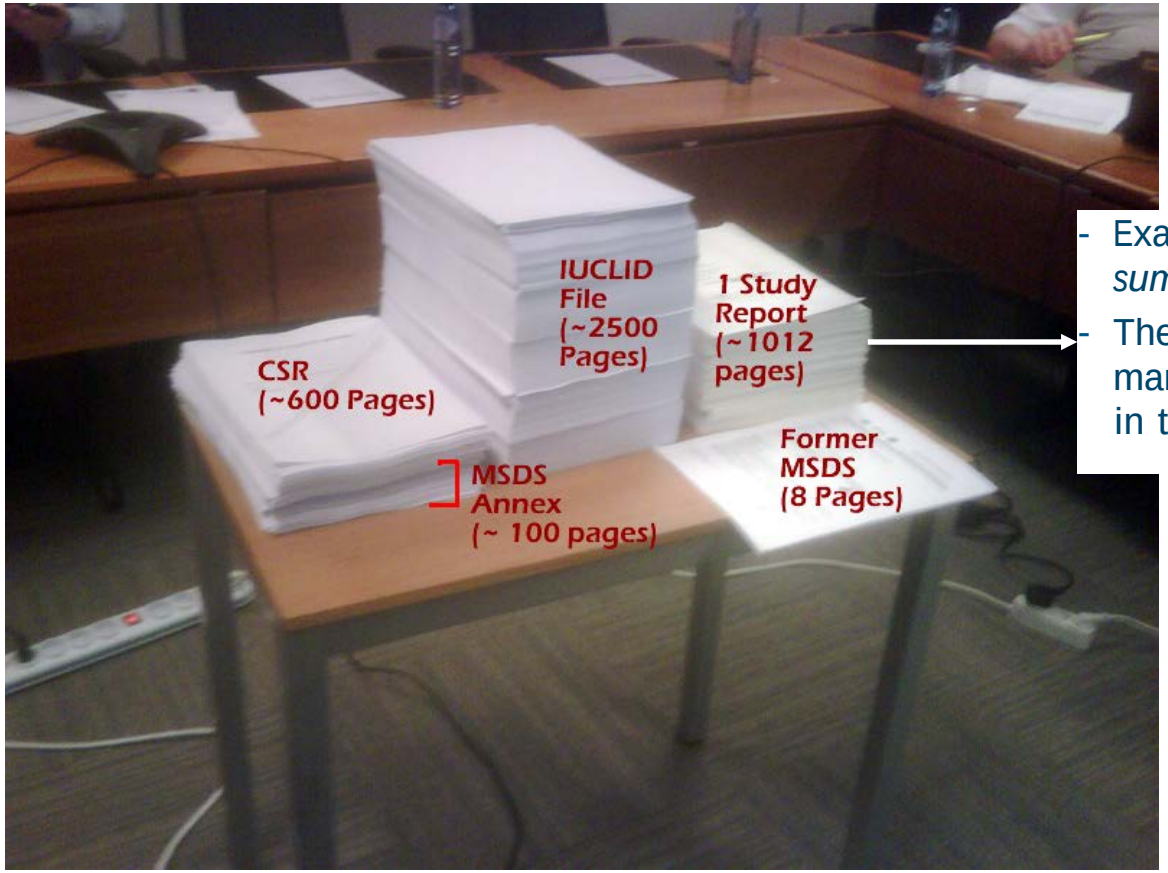
HH endpoints in REACH dossier



- Concawe has completed endpoints with historical data, and some newly generated
- These historical data are currently being challenged by ECHA
  - Exposure route (dermal / inh. vs required oral)
  - Non-standard guideline / test quality
  - Compositional description test substance
- Concawe will defend historical data…
- …while acknowledging there are datagaps which need to be addressed to keep dossiers compliant

All (hazard) data on PS available in Concawe REACH dossiers

Example of Low Boiling Point Naphthas (Gasolines)



- Example of **ONE** tox study *summarized* in IUCLID and the CSR.
- There are >550 studies (phys/chem, mammalian toxicity, environmental) in total summarized in IUCLID/CSR

The IUCLID stack of papers is a mock-up. The others are real, printed previously for other reasons.

We did not really print the IUCLID file (and don't recommend anyone to print it…)

Mammalian toxicological hazards of PS

Crude Oil

Boiling

C1
C10
C20
C30
C40
C50

Vac. Dist.

->C50+

Gases, Naphthas, Kero's

1,3-Butadiene
Toluene,
Benzene,
n-Hexane

Presence of these molecules above regulatory thresholds drives classification of these substances, **not intrinsic hazard of PS**

PAHs

Heavier PS

(Gas Oils and higher boiling PS potential for 3-7 ring PAH-related toxicity)

Observed toxicity of the full UVCB drives classification

PAHs

Some lighter substances, potential for:

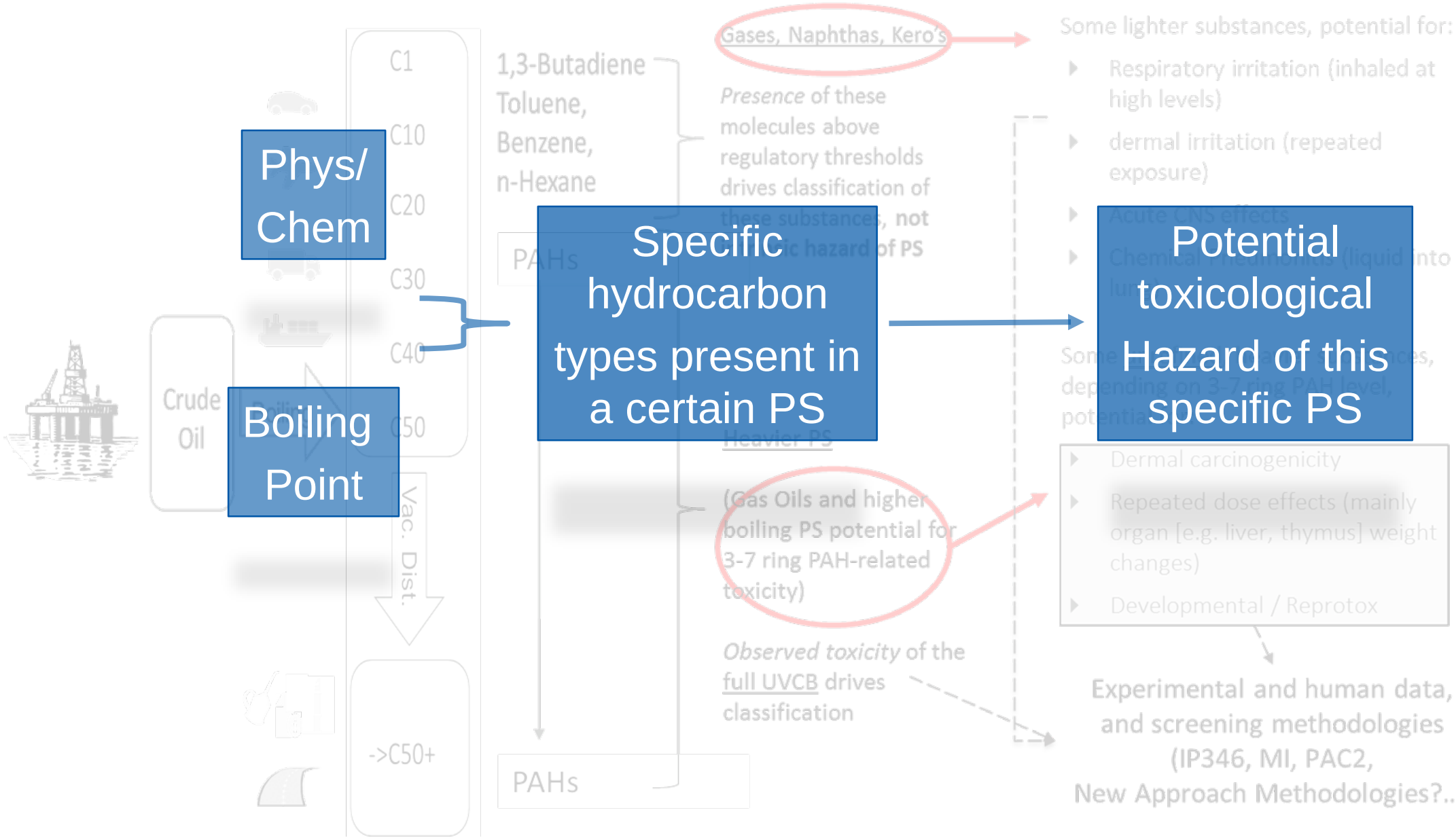
- Respiratory irritation (inhaled at high levels)
- dermal irritation (repeated exposure)
- Acute CNS effects
- Chemical Pneumonitis (liquid into lung)

Some unrefined, heavier substances, depending on 3-7 ring PAH level, potential for:

- Dermal carcinogenicity
- Repeated dose effects (mainly organ [e.g. liver, thymus] weight changes)
- Developmental / Reprotox

Experimental and human data, and screening methodologies (IP346, MI, PAC2, New Approach Methodologies?…)

Mammalian toxicological hazards of PS



Concawe PS “Categories”, historically based on refining history

1. Low Boiling Point Naphthas (Gasolines)
2. Kerosines
3. **Straight-run Gas Oils**
4. Cracked Gas Oils
5. **Vacuum Gas Oils, Hydrocracked Gas Oils& Distillate Fuels**
6. **Other Gas Oils**
7. Heavy Fuel Oil Components
8. Unrefined / Acid Treated Oils
9. Other Lubricant Base Oils
10. Highly Refined Base Oils
11. Foots Oils
12. Paraffin and Hydrocarbon Waxes
13. Slack Wax
14. Petrolatum
15. Untreated Distillate Aromatic Extracts
16. Treated Distillate Aromatic Extracts
17. **Residual Aromatic Extracts**
18. **Bitumen**

In addition CONCAWE has prepared the joint parts of the Registration Dossier for the following stand-alone substances:

- MK1 diesel fuel (EC number 931-250-7),
- **Oxidised Asphalt (EC number 265-196-4)**
- Sulfur (EC number 231-722-6)

Name	EINECS definition	CAS
Asphalt	A very complex combination of high molecular weight organic compounds containing a relatively high proportion of hydrocarbons having carbon numbers predominantly greater than C25 with high carbon-to-hydrogen ratios. It also contains small amounts of various metals such as nickel, iron, or vanadium. It is obtained as the non-volatile residue from distillation of crude oil or by separation as the raffinate from a residual oil in a deasphalting or decarbonization process.	**8052-42-4**
Residues (petroleum), vacuum	A complex residuum from the vacuum distillation of the residuum from atmospheric distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly greater than C34 and boiling above approximately 495°C (923°F)	**64741-56-6**
Residues (petroleum), hydrodesulfurized vacuum	A co- resi prim- havi appr	
Residues (petroleum), thermal cracked vacuum	A co distil pred grea	

CAS numbers relate to (last) refining step(s)

2010 REACH deadline (>1k tonnes):

- large numbers of CAS registered (600+)

to

- “secure” company specific refining operations,

but

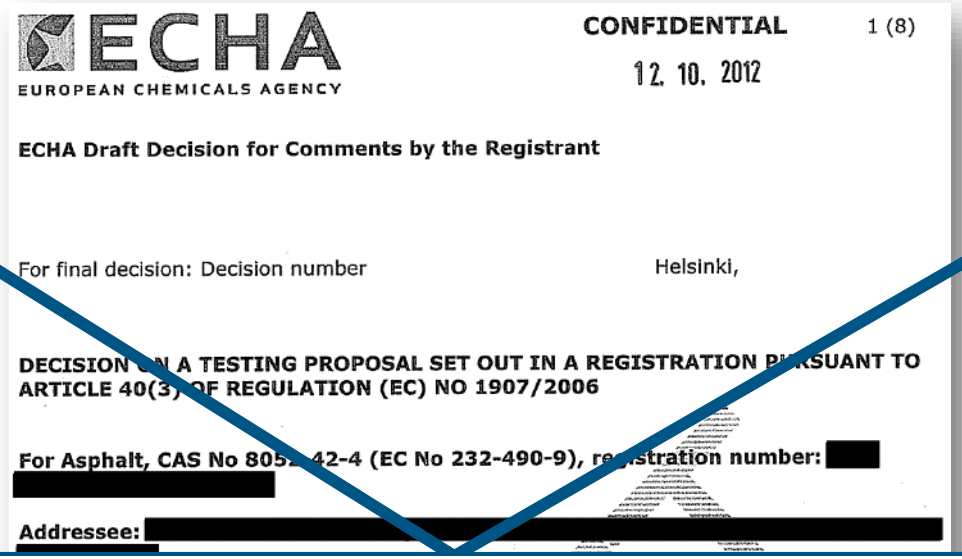
- CAS within a category are eventually describe the “same” petroleum product! (UVCB nature, i.e., “variable” in composition, but variable within product specifications)

-> in general terms: within one petroleum category, CAS numbers describe different ways of making the same substance

Concawe PS “Categories”, historically based on refining history

Need for improved analytical chemical descriptors on *substance level*

Need for improved biological descriptors (e.g., mechanistic data) on *substance level*



ECHA
EUROPEAN CHEMICALS AGENCY

CONFIDENTIAL 1 (8)

12. 10. 2012

ECHA Draft Decision for Comments by the Registrant

For final decision: Decision number Helsinki,

DECISION ON A TESTING PROPOSAL SET OUT IN A REGISTRATION PURSUANT TO ARTICLE 40(3) OF REGULATION (EC) NO 1907/2006

For Asphalt, CAS No 805[redacted]42-4 (EC No 232-490-9), registration number: [redacted]

Addressee: [redacted]

Category or grouping not accepted

…but worst case read-across OK, providing testing hypothesis is proven

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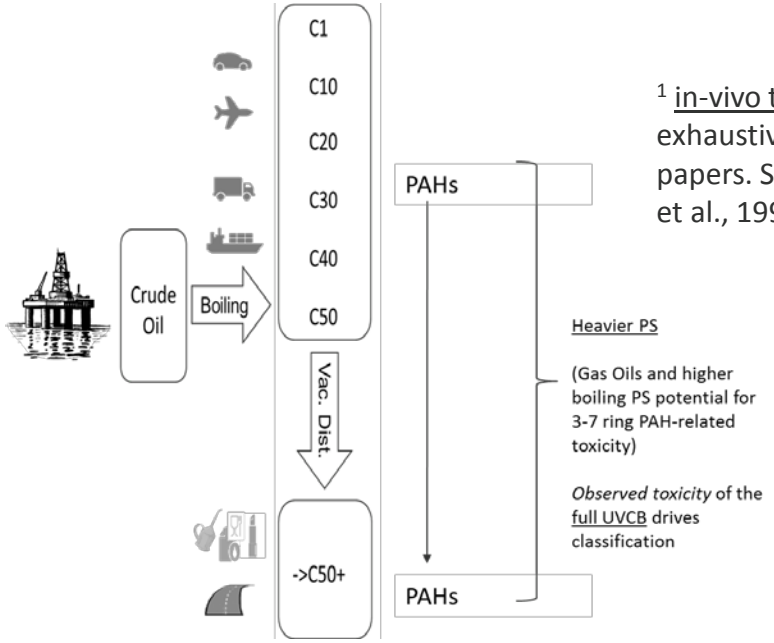
3-7 ring PAH hypothesis for Petroleum Substances

Based on historical toxicological data[1] it can be stated that
the **higher tier mammalian toxicological effects of petroleum substances are associated with the level of 3-7 ring PAH**
in poorly refined high boiling petroleum substances



[1] in-vivo toxicological data: see http://www.petroleumhpv.org/polycyclic-aromatic-compounds for an exhaustive overview of PAC related toxicity of petroleum substances, including public access to relevant papers. Some selected references are Feuston et al., 1994; McKee et al., 1990; McKee et al., 2012; Schreiner et al., 1997; White 2012

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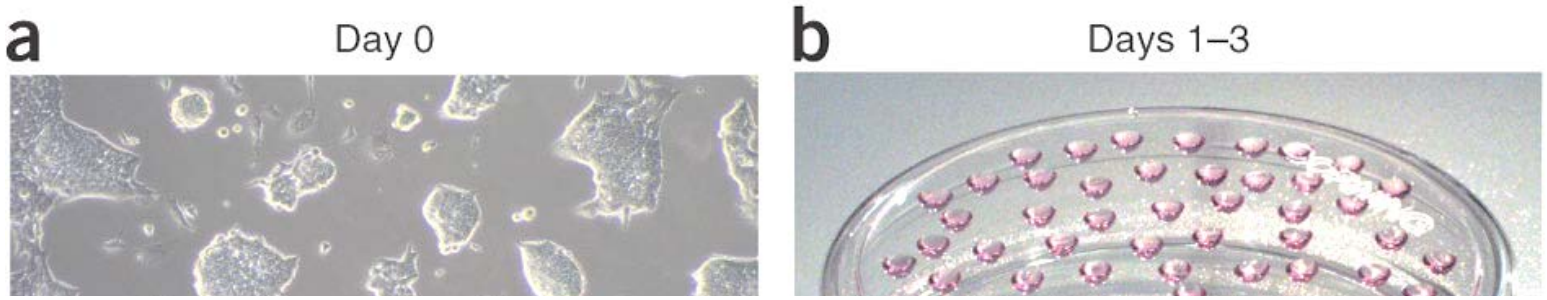
Testing hypothesis that 3-7 ring PAH cause developmental toxicity



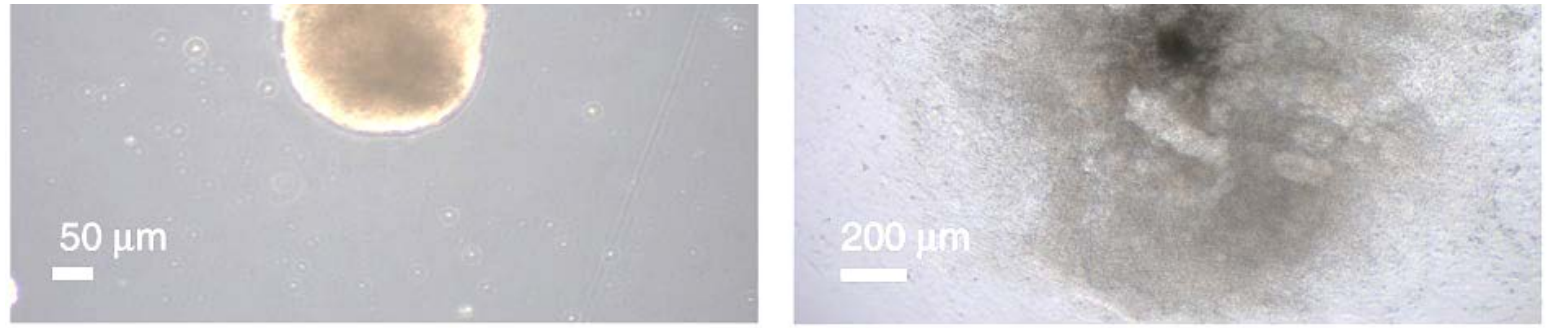
Battery of in-vitro tests in combination with toxicogenomics…

…to support the 3-7 ring PAH hypothesis

…justifying the selection of the worst-case representative for testing

…justifying read-across of this test sample to the other group members

…eventually underpinning prenatal developmental toxicity of PS with mechanistic data



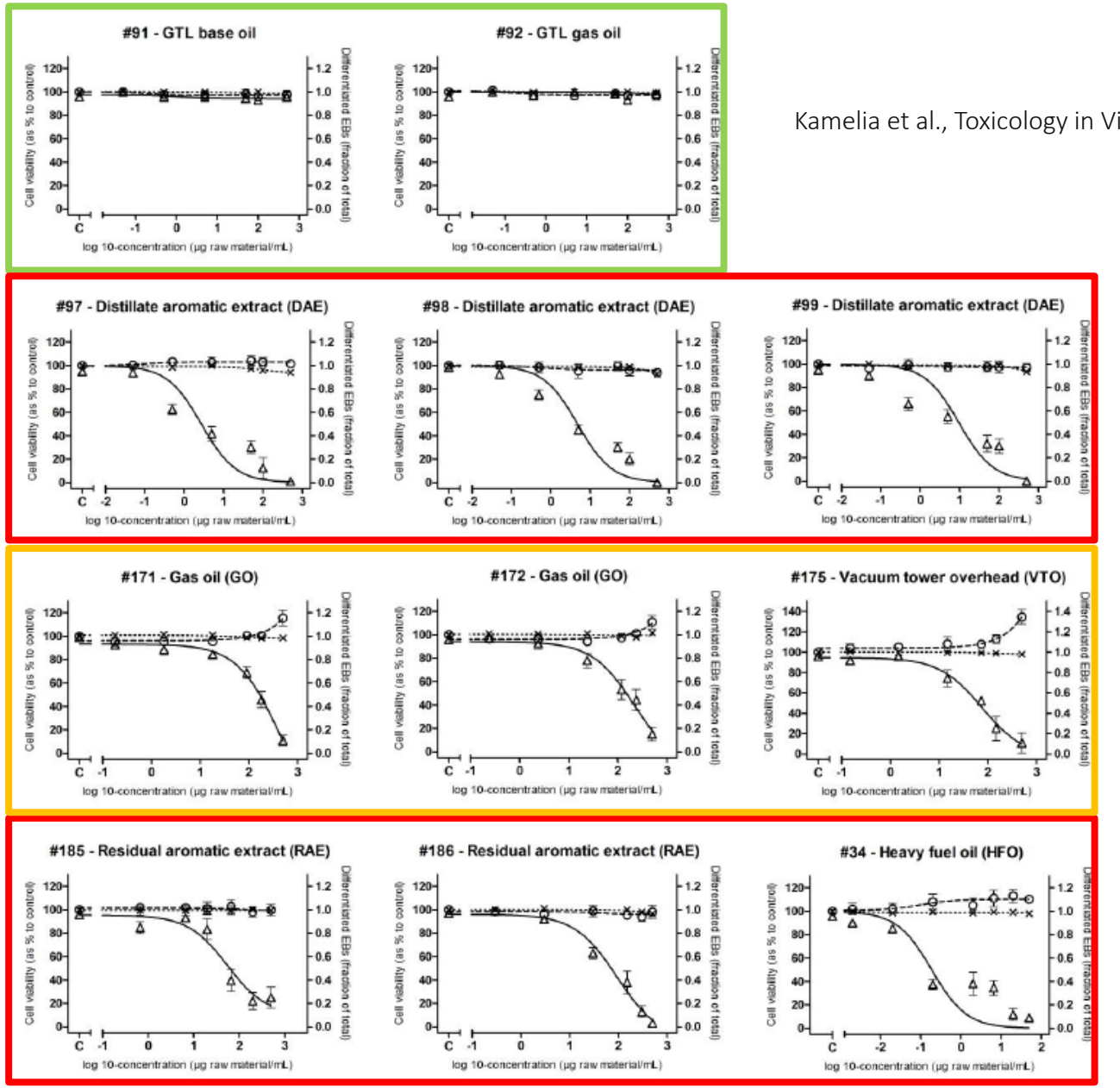
mES assay pilot to support devtox-PAH hypothesis

GTL Reference Oil:
no aromatics

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

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

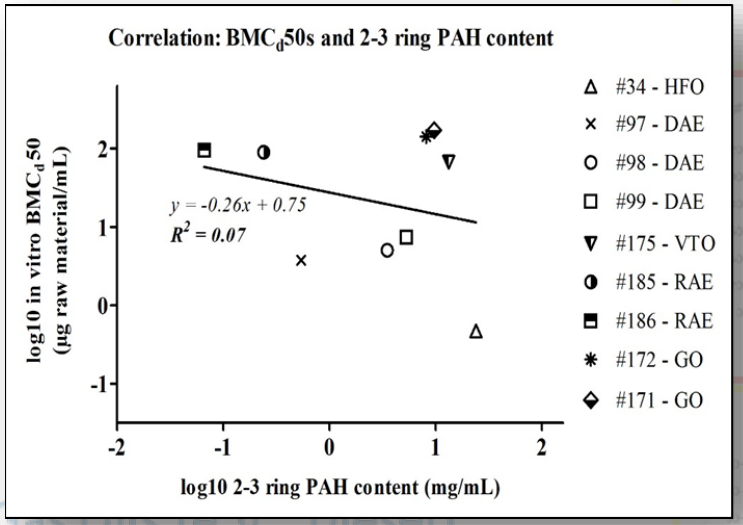
RAE (aromatic extracts) &
heavy fuel oil:
low to high (3-7 ring) PAH

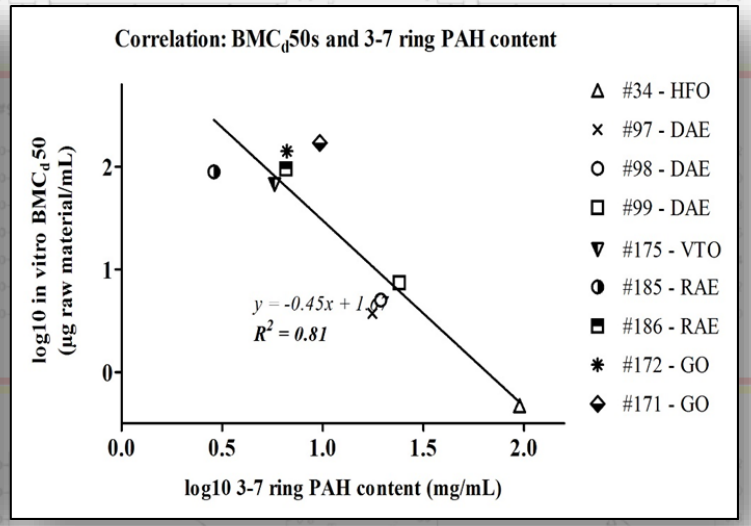


Kamelia et al., Toxicology in Vitro (2017)

mES assay pilot to support devtox-PAH hypothesis

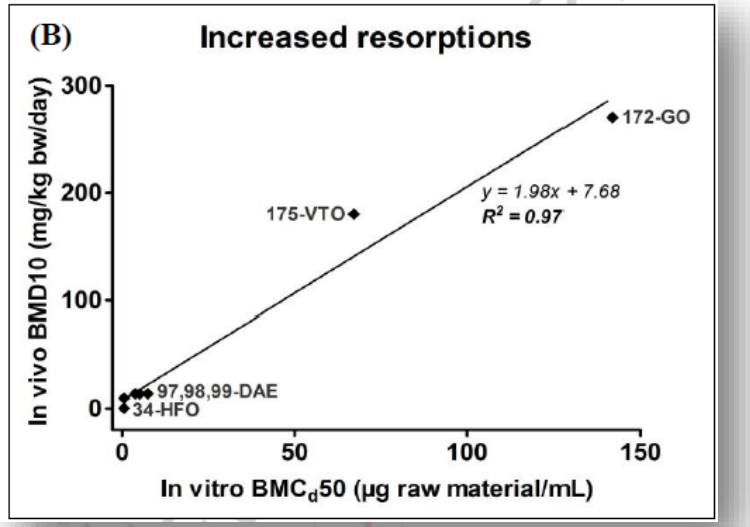
In-vitro results correlate
with 3-7 ring PAH content, not 2-3 ring PAH…





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

AhR CALUX reporter gene assay as further mechanistic support to devtox PAH hypothesis

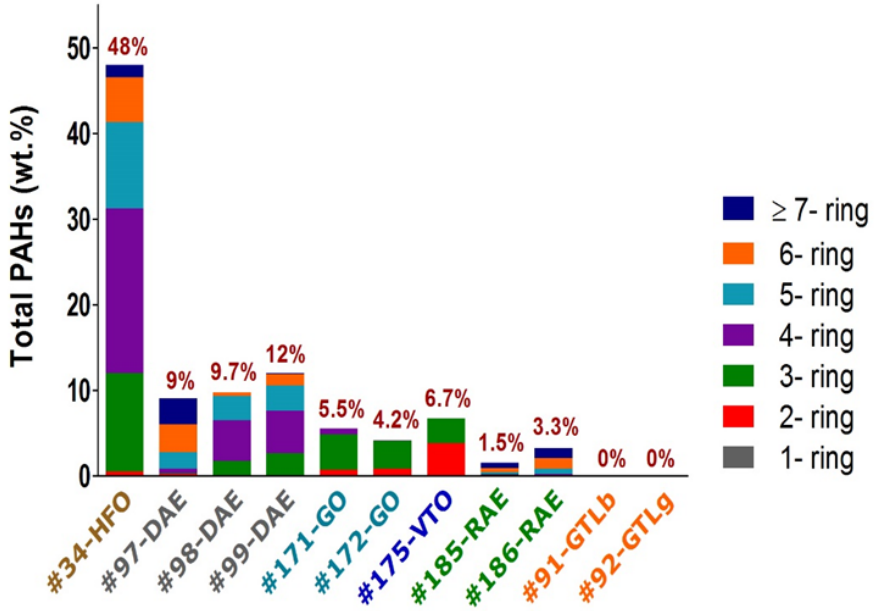
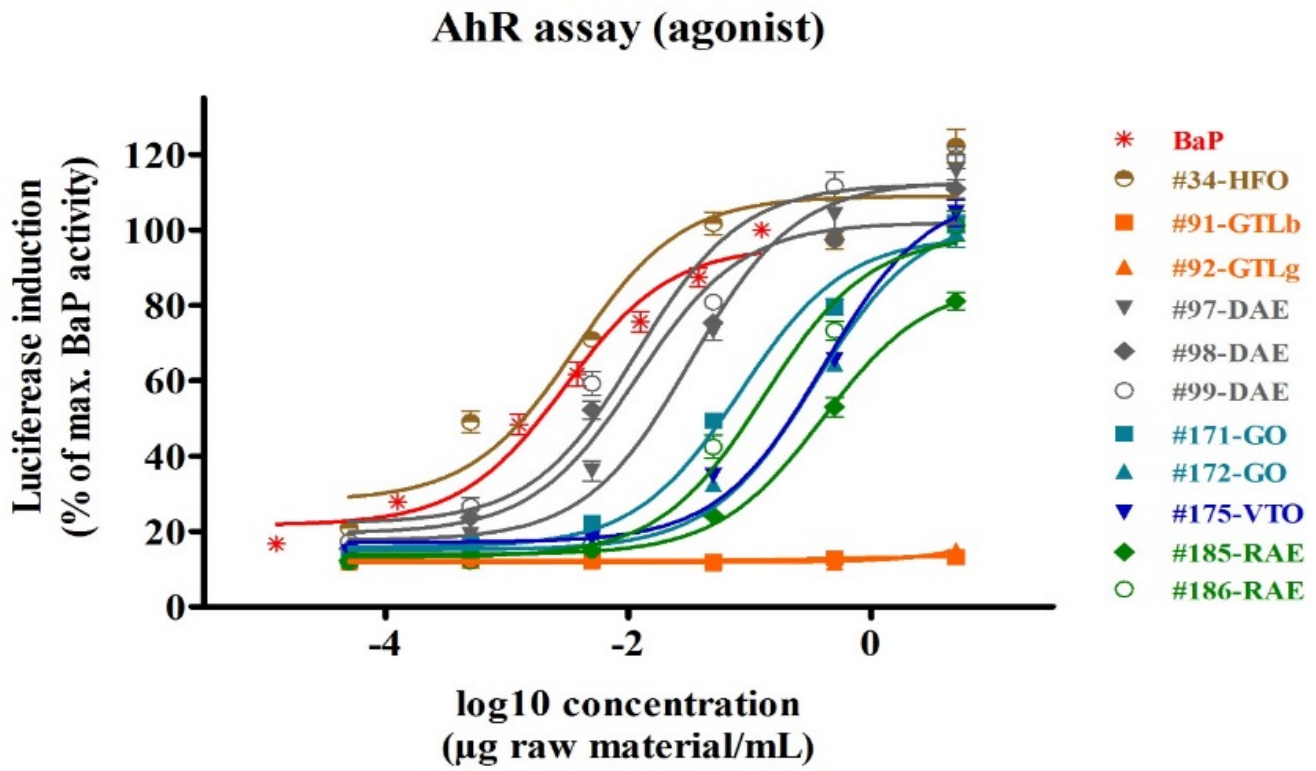


Figure 1. Aromatic Ring Class (ARC) profiles[a] of the DMSO-extracts of PS and GTL tested in the present study.

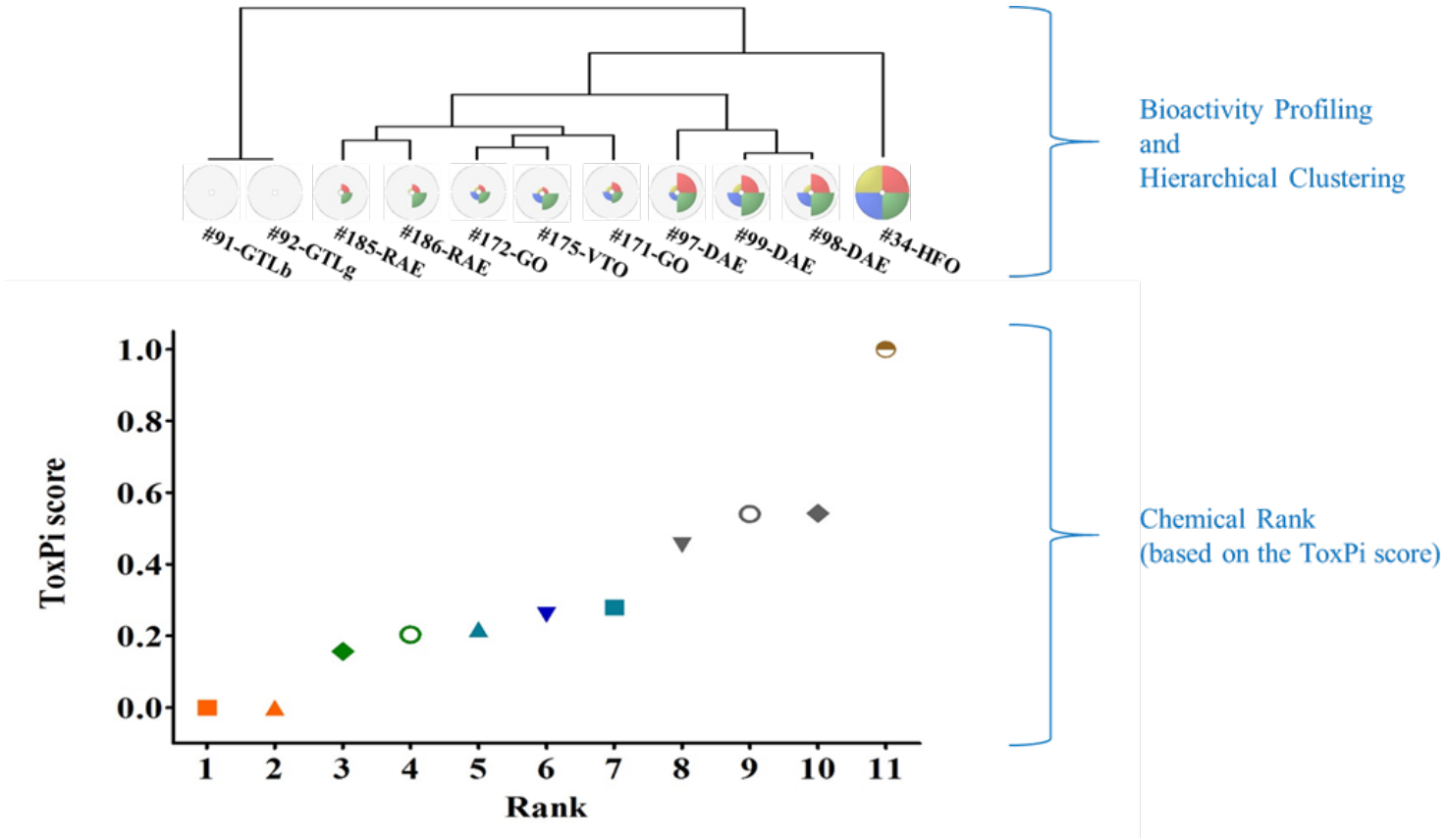
[a]The weight percent of the DMSO-soluble 1- to ≥7 aromatic-ring compounds present in each sample, from the starting material of 4.0 gram, as determined by Method II chemical characterization procedure[4].



Kamelia et al., Toxicologal Sciences (2018)

PAH reprotox hypothesis project: putting it all together…

- Bioactivity profiling, hierarchical clustering, and chemical ranking using ToxPi GUI 2.0.



- Follow up work will include transcriptomics profiling, and then integrating all data into the overall toxicological WoE to support the Concawe intelligent testing strategy

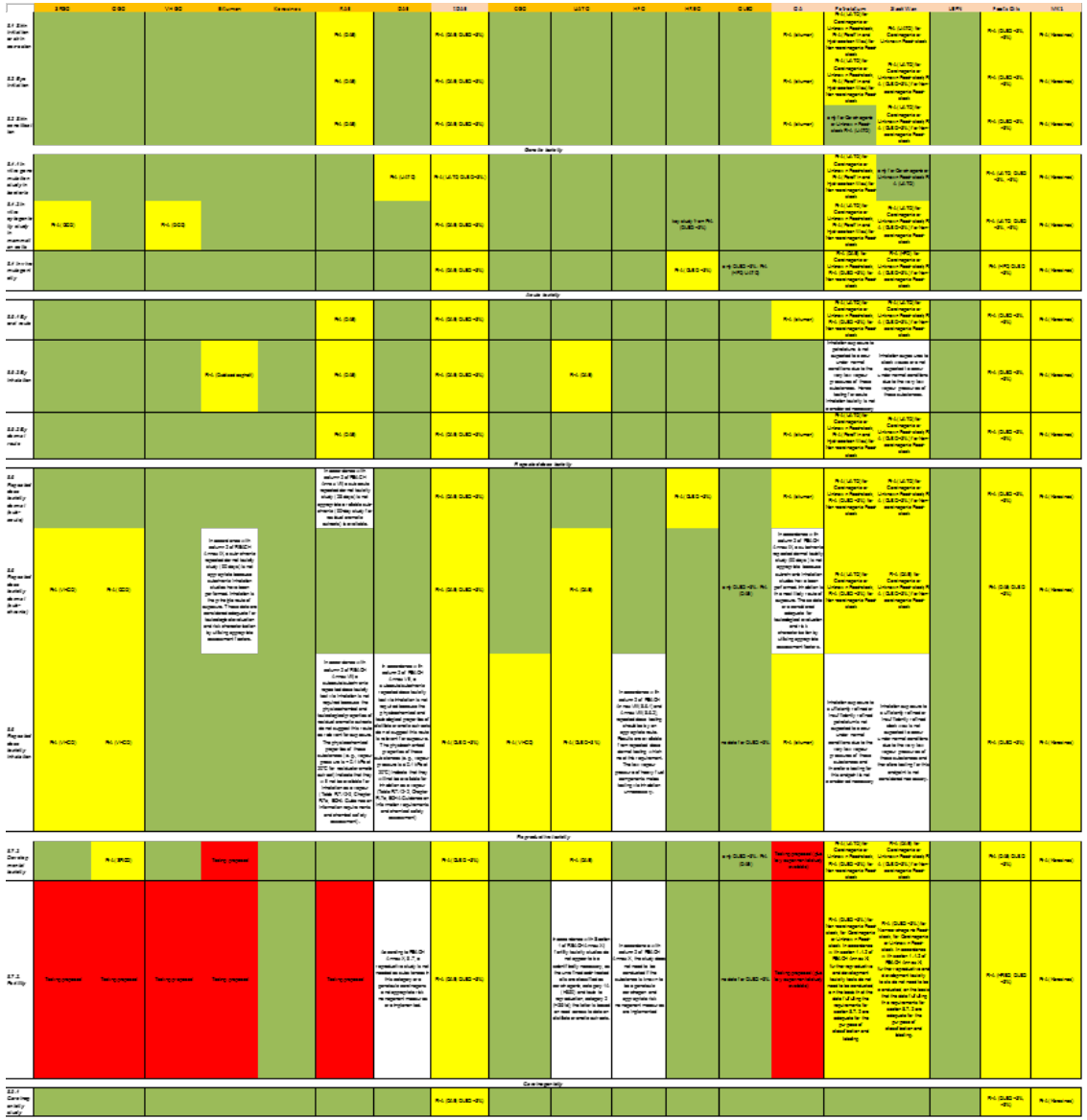
3 Datagaps and Cat-App

Addressing grouping and read across challenges for (petroleum) UVCBs

Datagap Analysis – overall groups

207 Petroleum Products into ~20 groups

Human Health Hazard Endpoint



Datagap Analysis – within group

- SRGOs as example -> comprising 4 out of 207 substances (CAS #s)

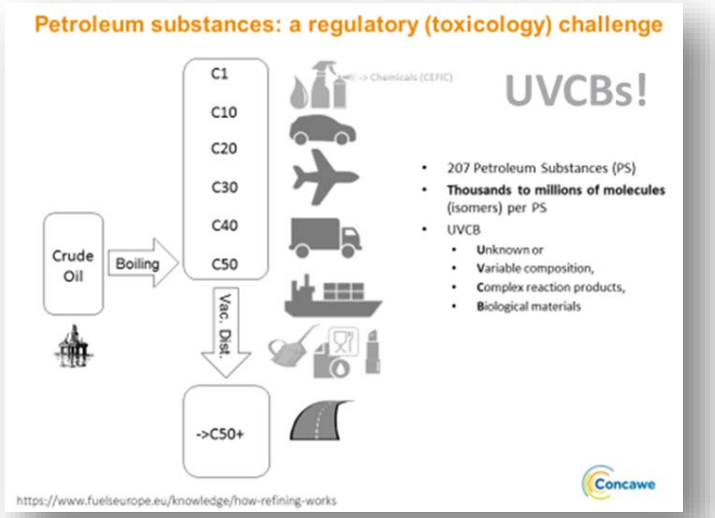
Human Health Hazard Endpoint

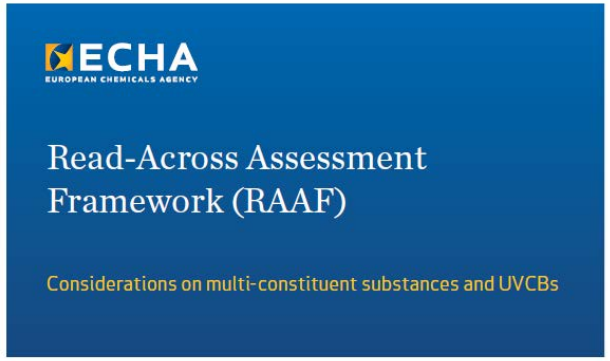
CAS No.	64741-43-1	64741-44-2	68814-87-9	68915-96-8
Name	Gas oils (petroleum), straight-run	Distillates (petroleum), straight-run middle	Distillates (petroleum), full-range straight-run middle	Distillates (petroleum), heavy straight-run
Concawe Registered substance (Y/N)	Y	Y	Y	Y
8.1 Skin irritation or skin corrosion	Not irritating	**Not irritating**	*Not irritating*	*Not irritating*
8.2 Eye irritation	Not irritating	**Not irritating**	*Not irritating*	*Not irritating*
8.3 Skin sensitisation	*Not sensitising*	**Not sensitising**	*Not sensitising*	*Not sensitising*
Genetic toxicity				
8.4.1 In vitro gene mutation study in bacteria	**Negative TA98 only (Modified Ames Test)**	Weak positive TA98 only	Negative TA98 only (Modified Ames Test)	Negative TA98 only (Modified Ames Test)
8.4.2 In vitro cytogenicity study in mammalian cells	*Sister chromatid exchange (CHO) negative –S9, ambiguous +S9 [R-A from OGO - CAS No.64742-80-9]*	*Sister chromatid exchange (CHO) negative –S9, ambiguous +S9 [R-A from OGO - CAS No.64742-80-9]*	*Sister chromatid exchange (CHO) negative –S9, ambiguous +S9 [R-A from OGO - CAS No.64742-80-9]*	*Sister chromatid exchange (CHO) negative –S9, ambiguous +S9 [R-A from OGO - CAS No.64742-80-9]*
8.4 In vivo mutagenicity	*Negative*	**Negative rat cytogenetic tests**	*Negative*	*Negative*
Acute toxicity				
8.5.1 By oral route	LD_{50} > 5000 mg/kg	**LD_{50} > 5000 mg/kg**	*LD_{50} > 5000 mg/kg*	*LD_{50} > 5000 mg/kg*
8.5.2 By inhalation	*LC_{50} > 2530 mg/m^3*	**LC_{50} > 2530 mg/m^3**	*LC_{50} > 2530 mg/m^3*	*LC_{50} > 2530 mg/m^3*
8.5.3 By dermal route	LD_{50} > 2000 mg/kg	**LD_{50} > 2000 mg/kg**	*LD_{50} > 2000 mg/kg*	*LD_{50} > 2000 mg/kg*
Repeated dose toxicity				
8.6 Repeated dose toxicity dermal (sub-acute)	28d rat systemic NOAEL > 0.5 mL/kg [dose levels tested: 0, 0.01, 0.10, 0.50 mL/kg] *28d rat and rabbit systemic NOAEL >2000 mg/kg/day*	28d rabbit systemic NOAEL >2000 mg/kg/day [dose levels tested: 0, 200, 1000, 2000 mg/kg/day]	*28d rat and rabbit systemic NOAEL >2000 mg/kg/day*	*28d rat and rabbit systemic NOAEL >2000 mg/kg/day*
8.6 Repeated dose toxicity dermal (sub-chronic)	*90d NOAEL 30 mg/kg/day [R-A from VHGO - CAS No.64741-49-7; dose levels tested: 30, 125, 500 mg/kg/day]*	*90d NOAEL 30 mg/kg/day [R-A from VHGO - CAS No.64741-49-7; dose levels tested: 30, 125, 500 mg/kg/day]*	*90d NOAEL 30 mg/kg/day [R-A from VHGO - CAS No.64741-49-7; dose levels tested: 30, 125, 500 mg/kg/day]*	*90d NOAEL 30 mg/kg/day [R-A from VHGO - CAS No.64741-49-7; dose levels tested: 30, 125, 500 mg/kg/day]*
8.6 Repeated dose toxicity inhalation	*90d NOAEC ≥ 1710 mg/m3 (systemic); 880 mg/m3 (local, lung) [R-A from VHGO - CAS No. 68334-30-5 (most likely); dose levels tested: 0, 0.35, 0.88, 1.71 mg/L]*	*90d NOAEC ≥ 1710 mg/m3 (systemic); 880 mg/m3 (local, lung) [R-A from VHGO - CAS No. 68334-30-5 (most likely); dose levels tested: 0, 0.35, 0.88, 1.71 mg/L]*	*90d NOAEC ≥ 1710 mg/m3 (systemic); 880 mg/m3 (local, lung) [R-A from VHGO - CAS No. 68334-30-5 (most likely); dose levels tested: 0, 0.35, 0.88, 1.71 mg/L]*	*90d NOAEC ≥ 1710 mg/m3 (systemic); 880 mg/m3 (local, lung) [R-A from VHGO - CAS No. 68334-30-5 (most likely); dose levels tested: 0, 0.35, 0.88, 1.71 mg/L]*
Reproductive toxicity				
8.7.2 Developmental toxicity	**NOAEL 50 mg/kg/day** [dose levels tested: 0, 50, 250, 500 mg/kg/day]	*NOAEL 50 mg/kg/day*	*NOAEL 50 mg/kg/day*	*NOAEL 50 mg/kg/day*
8.7.3. Fertility	Testing proposed *	Testing proposed *	Testing proposed *	Testing proposed *
Carcinogenicity				
8.9.1 Carcinogenicity study	*Not considered a carcinogenic hazard*	**Weakly tumorigenic on the skin secondary to skin irritation; long latent period. Not considered a carcinogenic hazard**	Weak initiator and promoter with borderline effects *Not considered a carcinogenic hazard*	*Not considered a carcinogenic hazard*

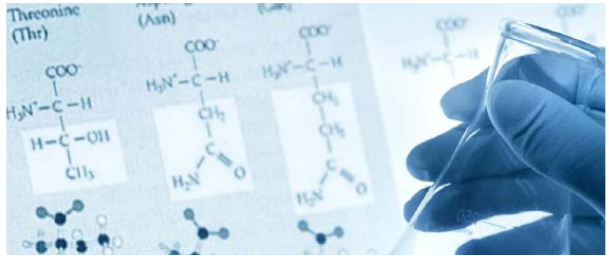
- Where we have data over multiple CAS in a group: alignment – indicating (biological) similarity
- Datagaps: cannot test all endpoints for each and every CAS across all petroleum streams based on practical (time and testing cost) and animal welfare constraints

Need more pragmatic and informed approach

Current alternative approaches not always applicable to (petroleum) UVCBs







- Learn from other sectors
  - Approaches available for lower tier endpoints (e.g., irritation, acute tox) and well defined chemicals
- However:
  - Real challenge is with higher tier endpoints and complex, multi-constituent substances

From ECHA RAAF report (7 March 2017):

All chemical structures involved need to be considered; grouping of substances on the basis of structural similarity must take account of all constituents, and the predictions within proposed groups must likewise consider the impact of all constituents.

The analysis described in this document confirmed the complexity of read-across approaches for multi-constituent substances and UVCBs.

More work is needed to further develop the RAAF based on the findings described in this document.

New Technologies to Underpin **Cat**egory **App**roaches and Read-across in Regulatory Programmes

Cat-App Workflow

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

*induced Pluripotent Stemcells

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
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Shirley Price
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Chris Rowat
Health Canada

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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

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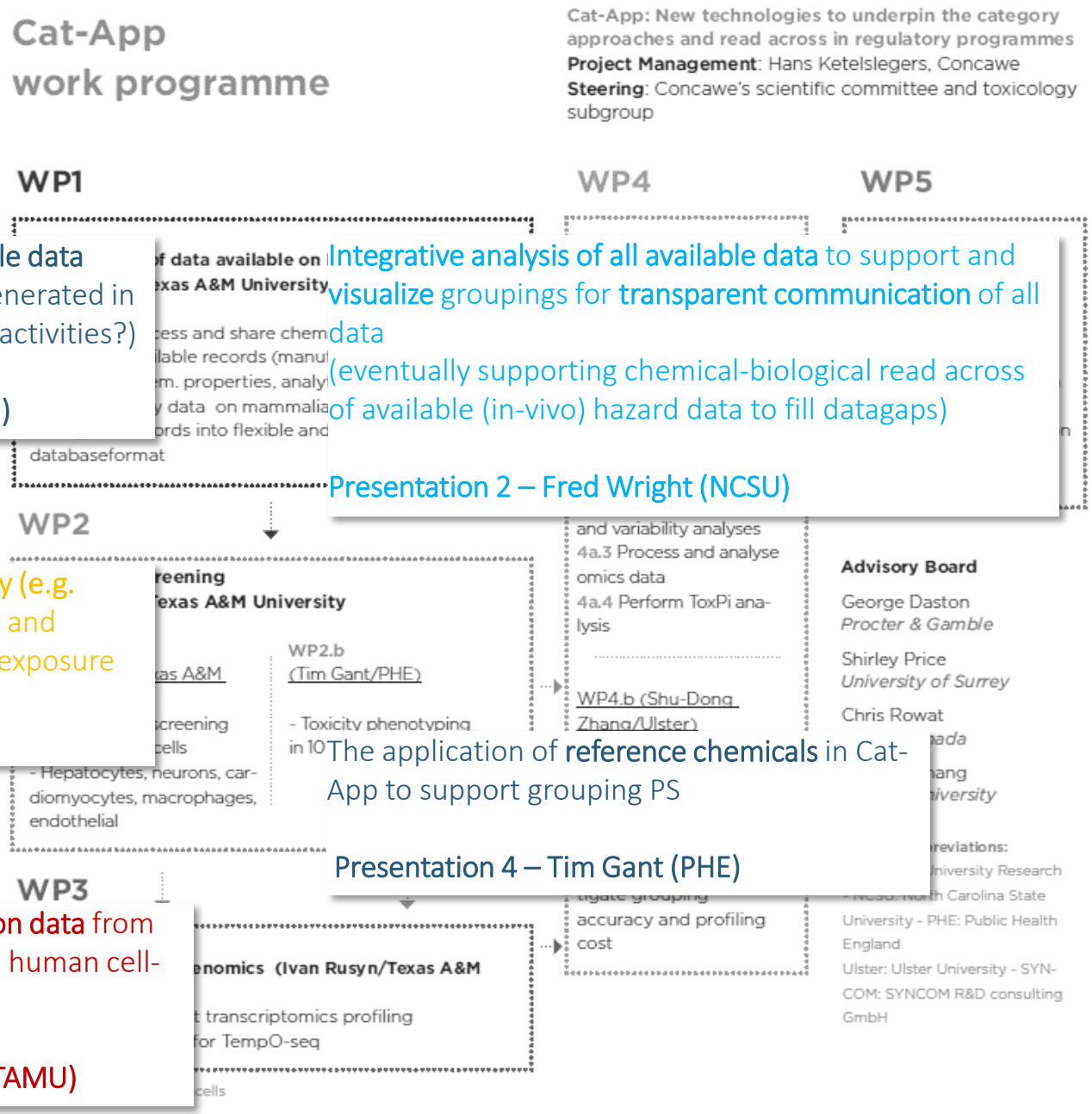
Cat-App Workflow



Setting up **database with all available data** and infrastructure for data to be generated in the project (and in other Concawe activities?)

Presentation 1 – Ivan Rusyn (TAMU)

Integrative analysis of all available data to support and **visualize** groupings for **transparent communication** of all data
(eventually supporting chemical-biological read across of available (in-vivo) hazard data to fill datagaps)

Presentation 2 – Fred Wright (NCSU)

Evaluation of **mechanistic toxicology (e.g. phenotypical) changes** in stem cells and human cell-lines in response to PS exposure

Presentation 1 - Ivan Rusyn (TAMU)

The application of **reference chemicals** in Cat-App to support grouping PS

Presentation 4 – Tim Gant (PHE)

Presentation of how **gene expression data** from the most responsive stem cells and human cell-line models add value to Cat-App

Presentation 3 – Shu-Dong Zhang (TAMU)

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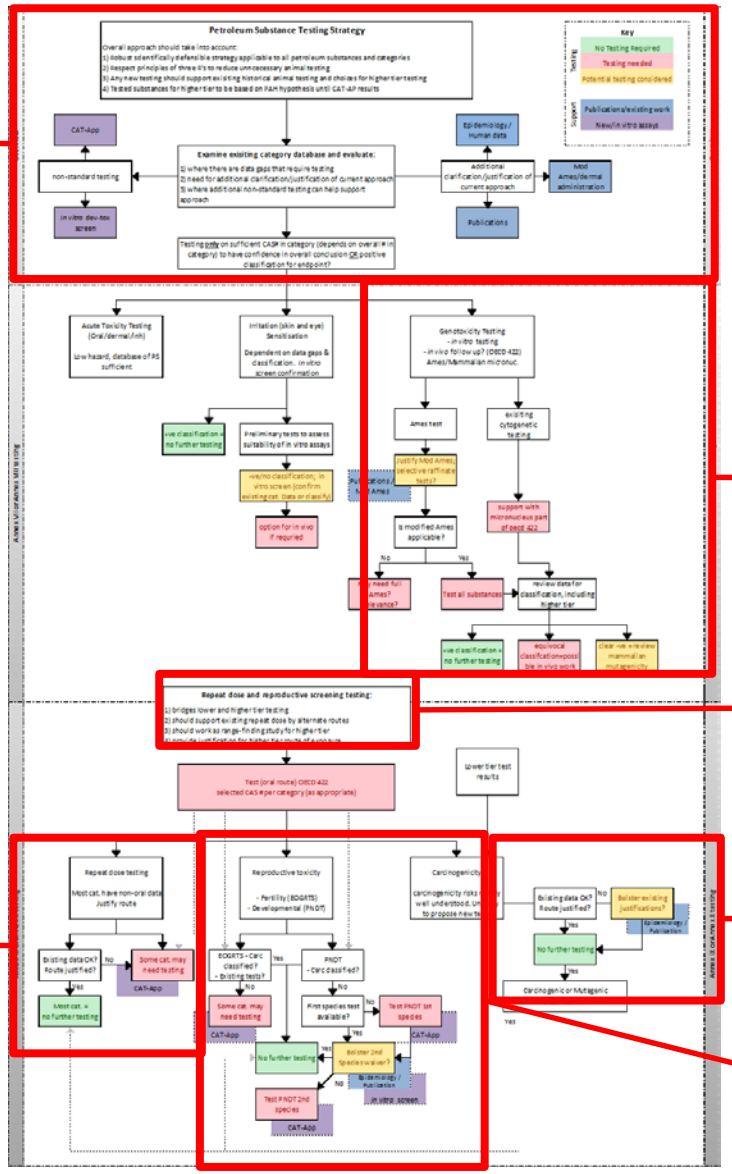
Overview of informed and tiered intelligent testing strategy

Target animal testing where needed as a last resort, based on a worst case approach rather than targeting all substances

Basis for testing strategy:

- Historical (in-vivo) data
- Mechanistic data
- Available human data
- **Cat-App**



Mutagenicity / Genetox **including screening assays**

"optimized" OECD 422 screening studies will "connect the dots" between lower and higher tier testing needs,

Repeated Dose toxicity

Carc WoE, **including screening assays**

Reprotoxicity, including DevTox **in-vitro screening battery**

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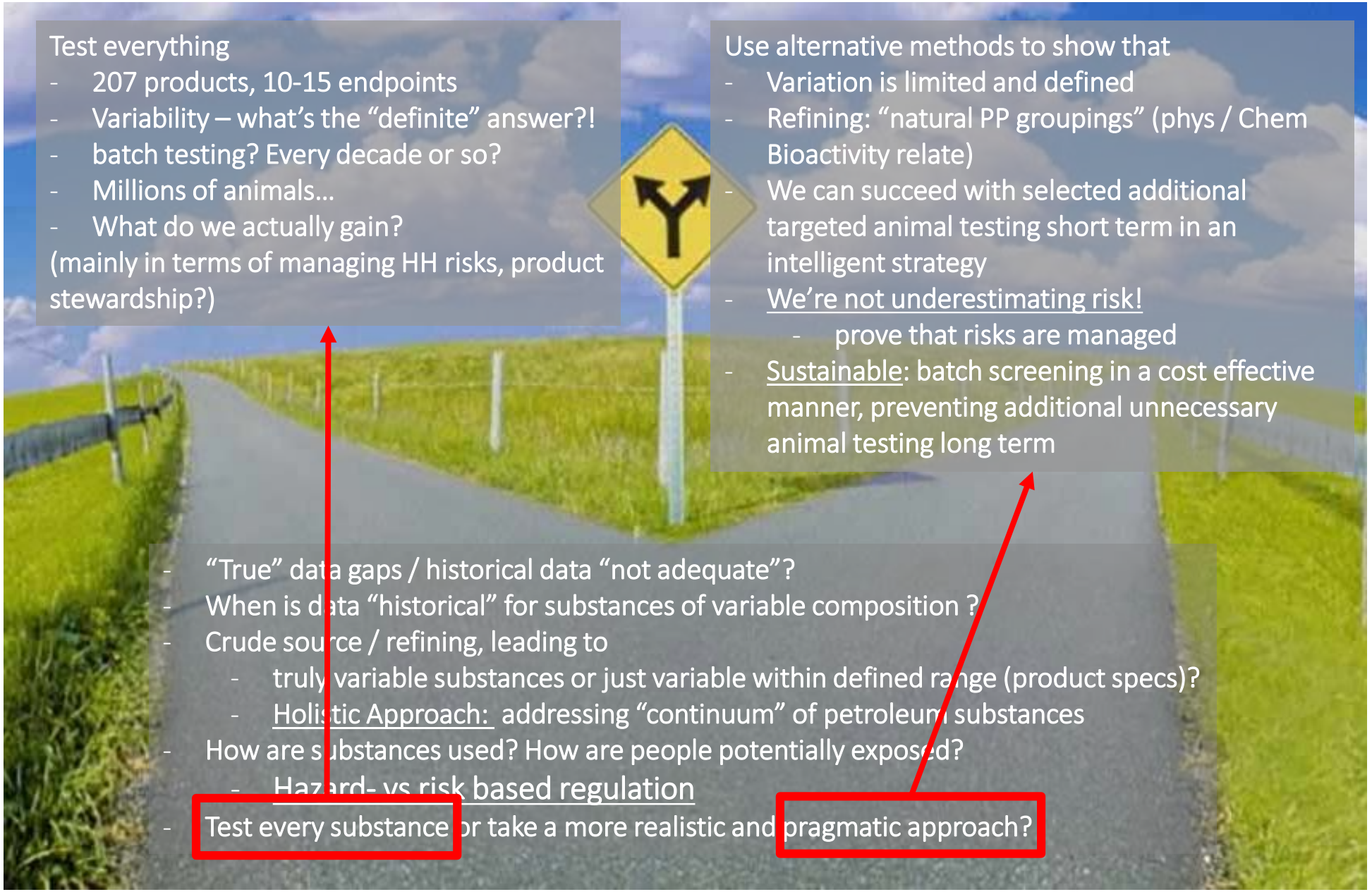
Thought starter for today to "set the scene":
Concawe Health REACH: stop at the crossroad and think before we go…



Thought starter for today to “set the scene”:
Concawe Health REACH: stop at the crossroad and think before we go…

Risk of getting lost in an endless detour?

Taking “preliminary” regulatory action
would again ignore the possibility

to make a significant step towards application of NAM data
in view of “the 3Rs” in a regulatory context

Take away messages for today

- **Petroleum UVCBs are substances (PS)**, not mixtures, and are highly complex – cannot be fully characterized analytically
- **PS form a continuum** of substances, based on their refining history; **neighboring streams overlap**
- Although they therefore are a regulatory challenge, there is a **wealth of historical in-vivo data available**
- Some of these data and **our grouping and read across approach are being challenged**, which could lead to high numbers of (unnecessary) animal testing
- **Read across and testing hypotheses** can be build to address that issue, which are based on the **3-7 ring PAH content in PS**
- Cat-App and other efforts ongoing at Concawe take the opportunities that new approach methodologies provide, **to further support this hypothesis, the grouping of PS and eventually read across of in-vivo data**
- These data will therefore be applied as an integral part of the targeted and **informed testing strategy** for HH REACH endpoints, which is **aimed at minimizing animal testing**

Thank you for your attention

Hans Ketelslegers

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Cat-App

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