Review

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Foreword

Following the REACH registration phase, ECHA began the substances evaluation phase in 2012. The proof of similarity and subsequent justification of read-across between Concawe's substances, which is necessary to limit the number of vertebrate animal studies, is especially challenging given their UVCB nature which makes it impossible to determine with precision every constituent or its concentration. This *Review* summarises some important progress that Concawe has achieved to determine the structural and biological similarity of our substances, and to speed up and improve the assessment of their human health and ecotoxicity hazard.

The first article in this *Review* describes a novel approach developed by Concawe to position our gas oils using a hydrocarbon space mapping approach to select a minimum number of representative samples for higher-tier vertebrate testing, which, altogether, are representative of a full set of gas oil substances and can be used to assess their human health hazard. The second article summarises the findings of a ready biodegradability testing programme on six 3-ring polycyclic aromatic hydrocarbons, for which improvement had to be developed to increase the relevance of the tests, as our substances present specificities which make them difficult to test. The third article presents a novel project that Concawe has developed with SINTEF and other researchers to assess the biodegradation of many constituents simultaneously by testing full substances and using advanced analytical methods. The recent update to Petrorisk — the model developed by Concawe to calculate the environmental risk assessments needed for the REACH registration dossiers of our substances — is described in the final article of this *Review*.

With the development of the toxicology and ecotoxicology requirements resulting from the European Green Deal and of the Chemicals Strategy for Sustainability, the science and the knowledge developed by Concawe, as shown by the examples in this *Review*, will become even more important for the activities of fuel manufacturers in the future.

Jean-Marc Sohier
Concawe Director

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Hydrocarbon space mapping to support gas oil read-across for human health hazard assessment 4

As vertebrate animal studies form the basis for assessing certain human health and ecotoxicity hazards under the EU (European Union) REACH regulation, appropriate alternative approaches to fulfil information requirements must be considered in order to avoid unnecessary animal studies in testing each substance. Read-across is a commonly used approach, justifiable only with robust evidence of structural similarity. From their manufacturing process, petroleum substances constitute a continuum of hydrocarbons; however, the precise identity and composition of every constituent is, for most petroleum substances, unknown and the composition may vary across samples of the same substance. Read-across justification in accordance with the REACH requirements for these substances is therefore challenging. This article sets out the development and application of a hydrocarbon space mapping approach for gas oils based on comprehensive two-dimensional gas chromatography, which in combination with biological response and lower-tier hazard data can be used for the selection of a minimum number of representative samples for higher-tier vertebrate testing.

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Generation of biodegradability data on petroleum constituents using enhanced tests (OECD guideline 301 F) 16

To improve the persistence assessment of petroleum substances, Concawe started a testing programme for several hydrocarbons. Ready biodegradability tests were proposed on six 3-ring polycyclic aromatic hydrocarbons (PAHs), in accordance with the OECD 301 F protocol. Since these substances are difficult to test, bioavailability improvement methods were also used to increase their relevance. The results obtained show that half of the constituents tested can be considered not persistent, with three of them requiring further testing to reach a conclusion. The data generated in this exercise have been submitted to the European Chemicals Agency (ECHA) for their consideration, and will be included in future PBT (persistent, bioaccumulative and toxic) assessments of Concawe's substances.

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A novel approach for assessing the biodegradability of constituents in petroleum substances 23

Petroleum substances contain hundreds to thousands of constituents, making it very difficult to generate biodegradation data on all of those constituents individually. Concawe is undertaking a novel project with SINTEF — one of Europe's largest independent research organisations — and other independent researchers to utilise an analytical method (GCxGC-FID) to track the constituents during biodegradation testing, allowing generation of biodegradation data on many constituents simultaneously. This approach develops data that are consistent with Concawe's existing risk assessment framework (e.g. PetroRisk).

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PetroRisk version 8.01: Improved model for performing REACH Environmental Risk Assessments on petroleum substances 28

Concawe has updated the PetroRisk model used to calculate the environmental risk assessments (ERAs) for petroleum substances needed for REACH registration dossiers. The updates result in a model which is (1) faster, (2) more transparent, (3) better aligned with the European Chemicals Agency (ECHA) Guidance, (4) more applicable to hydrocarbons and (5) more user-friendly. Compared to previous PetroRisk versions, version 8.01 generally predicts lower risks for professional and consumer uses, but higher risks for industrial uses. The new version will be publicly released in May 2022. A more detailed description of PetroRisk and the upgrades are provided in this article.

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Abbreviations and terms 31

Hydrocarbon space mapping to support gas oil read-across for human health hazard assessment

Justification for the use of read-across to assess the human health hazards of petroleum substances under the REACH regulation can be challenging. This article describes the development and application of a hydrocarbon space mapping approach for gas oils which, in combination with other data, can be used to provide evidence of structural similarity to support human health hazard assessment read-across.

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Read-across in REACH hazard assessment

The EU (European Union) REACH Regulation (EC) No 1907/2006 aims to ensure a high level of protection of human health and the environment, including the promotion of alternative methods for assessment of hazards of substances. Manufacturers and importers, as registrants, are required to ensure that they place on the market substances that do not adversely affect human health or the environment. As vertebrate animal studies form the basis to assess human health toxicity and some end points in ecotoxicological hazard assessment, registrants must consider appropriate alternative approaches to fulfil information requirements in order to avoid unnecessary animal studies in testing each substance. Read-across is a commonly used alternative approach for data gap filling, and involves the use of relevant information from analogous substances, i.e. the 'source' information, to predict properties of the 'target' substances under consideration. Relevant information requires primarily structural or compositional characterisation, but physical-chemical properties and biological activity profiles are also important. The application of read-across not only reduces the time required to provide compliant information per substance, but also improves the quality of the assessment of hazard by bringing into consideration the weight of evidence of closely related substances.

Because structural similarity is a fundamental aspect of read-across, it poses a number of challenges for UVCB[1] substances and, specifically, for Concawe portfolio substances. The precise identity and composition of every constituent is, for most substances, unknown and the composition may vary across samples of the same substance.

Annex XI, section 1.5 of the Regulation has recently been amended to address UVCB structural similarity stating, *'Structural similarity for UVCB substances shall be established on the basis of similarities in the structures of the constituents, together with the concentration of these constituents and variability in the concentration of these constituents. If it can be demonstrated that the identification of all individual constituents is not technically possible or impractical, the structural similarity may be demonstrated by other means, to enable a quantitative and qualitative comparison of the actual composition between substances.'* The amended requirement is supported by ECHA's *Advice on using read-across for UVCB substances*[1] published in May 2022.

Registrants are therefore required to demonstrate an understanding of the identity, concentration and variability of substance constituents and justify the data provided to enable a quantitative and qualitative approach to read-across. As it may not be technically possible to characterise each constituent, justification is also required when identification/measurement is not feasible. Information on constituents that have been analysed and found not to be present is also to be provided. More compositional data is required for those constituents that drive hazard properties, as compared with constituents that are known to be non-hazardous, since the aim is to support read-across. Likewise, the characterisation of variability may require more compositional data for those constituents that drive the hazard profile, as compared with those that do not.

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[1] Chemical substances of unknown or variable composition, complex reaction products and biological materials

Read-across for petroleum substances

The type of read-across applicable to UVCB substances is based on the hypothesis that different substances have qualitatively similar properties. The properties investigated in studies conducted with different source substances are used to predict the properties that would be observed in a study of the target substance.

The naming and identification of petroleum substances derived from refining crude oil has historically reflected their manufacturing processes, leading to a multitude of overlapping descriptions. Each petroleum stream has a hydrocarbon distribution within the boiling point range defined by the distillation process. This determines the boundaries for the chemical composition and physical properties of the stream, which are reflected in general terms in the EC/CAS[2] description. The initial distillation process from a common source material (crude oil) means that, at its simplest, the resulting substances can be considered a continuum of hydrocarbon substances, separated by boiling point, but with many of the same types of constituents. The higher end of the constituent range of a lower boiling point refining stream will overlap with the lower end of the constituent range of a higher boiling point refining stream in a continuum of hydrocarbon constituents. Subsequent conversion and upgrading steps can then alter the relative quantities of different constituents.

Petroleum substances will therefore vary in their chemical composition, but this variation is limited within the range of the specifications for each specific product. The full analytical characterisation of petroleum substance composition is limited because of the sheer number of constituents and their complexity. These limitations are emphasised for higher boiling point streams with more constituents and with constituents of higher molecular weight and multiple chemical functionalities. Thus, the granularity of analytical characterisation of the composition of a petroleum substance decreases with increasing boiling point.

Compositional information for petroleum substances is typically obtained using industry-standard methodologies such as simulated distillation gas chromatography (SIMDIS-GC) to determine the boiling point/carbon number range, and high-performance liquid chromatography (HPLC) to quantify the different aromatic classes present. These methodologies are the same as those used by registrants when generating substance identity profiles (SIPs) to identify the appropriate substance registration for data sharing in REACH Annex VI. However, they are insufficient to meet the data requirements to justify compositional similarity for read-across.

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[2] European Commission/Chemical Abstract Service

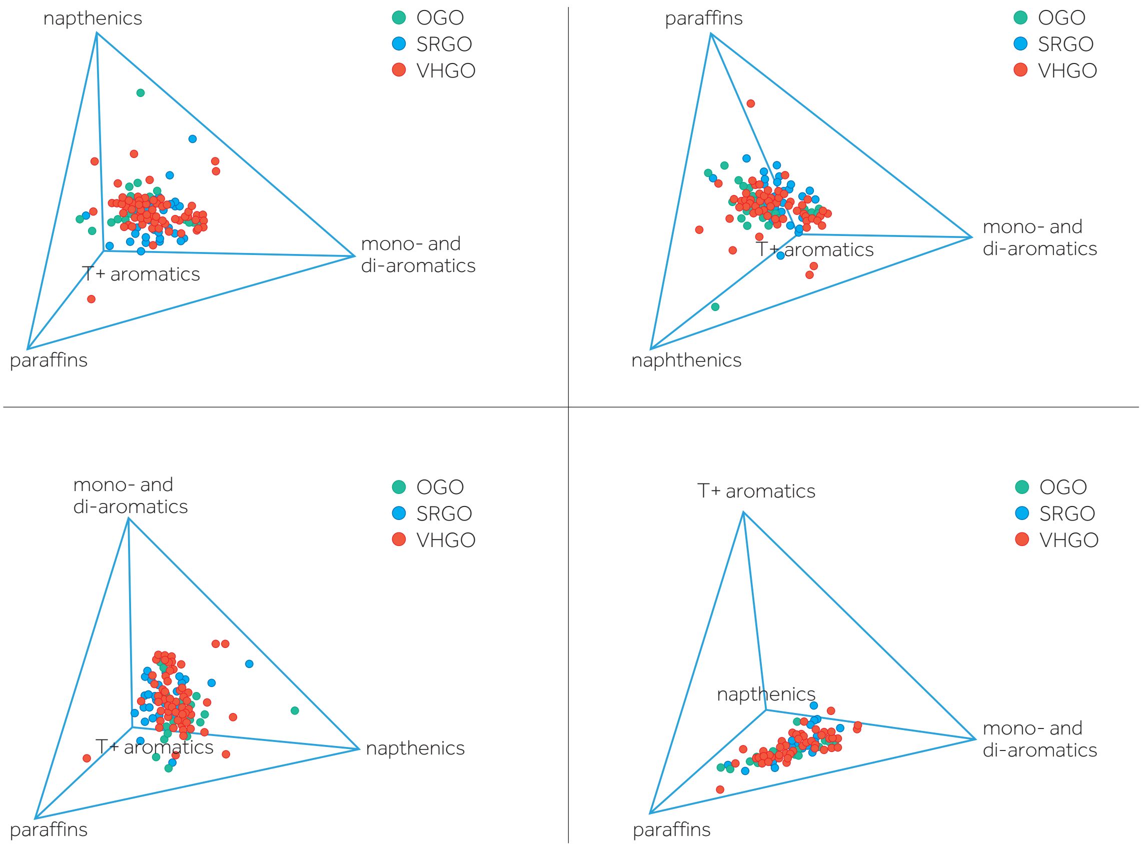
Chemical similarity: hydrocarbon space mapping

To demonstrate an understanding of the identity, concentration and variability of substance constituents to meet the information requirements for read-across, additional non-standard analytical techniques are required to characterise petroleum substances. Substances in the three Concawe gas oils categories—vacuum gas oils, hydrocracked gas oils and distillate fuels (VHGO); straight run gas oils (SRGO); and other gas oils (OtherGO)—will be taken as an example throughout this article as Concawe is currently developing testing proposals. The methodology is intended to be reproduced progressively in all categories where there is a need to generate new toxicological data. For these three categories, more detailed chemical compositional information is generated by techniques such as comprehensive two-dimensional gas chromatography (GCxGC). This technique provides detailed quantitative information on the carbon number range of the constituents and on the types of hydrocarbon classes present for each carbon number, and is applied in the hydrocarbon block approach.[2] Constituents are first separated according to their volatility, and then further separated based on their polarity, to provide detailed compositional information on complex substances such as gas oils. Flame ionisation detection (FID) with response correction for different hydrocarbon functionalities is used to quantify the separated constituents in the approximate carbon number range C6 to C30 for the following 10 hydrocarbon classes: n-paraffins (n-P); iso-paraffins (iso-P); mono-naphthenes (N); di-naphthenes (DN); mono-aromatics (MoAr); naphthenic mono-aromatics (NmoAr); di-aromatics (DiAr); naphthenic di-aromatics (NDiAr); tri-aromatics (TriAr); and tetra-aromatics (TetraAr). A mean of > 98% of the gas oil sample constituents across all analysed gas oil samples completely elute for the GCxGC columns, thereby addressing the > 95% requirements for fingerprinting in ECHA Advice on using read-across for UVCB substances. Petroleum substances with higher boiling points and higher molecular weight constituents (> C30) are less likely to be fully eluted in GCxGC and these substances require alternative non-standard methods to support characterisation, such as field ionisation mass spectrometry (FIMS).

The variability of substance constituents can only be assessed by the above analysis of multiple registrant samples per substance. A multi-year programme at Concawe has permitted the collection of multiple samples from as many registrants as possible in volumes that would allow both analytical characterisation and full animal studies (lower- and higher-tier studies) to be performed on the same sample.

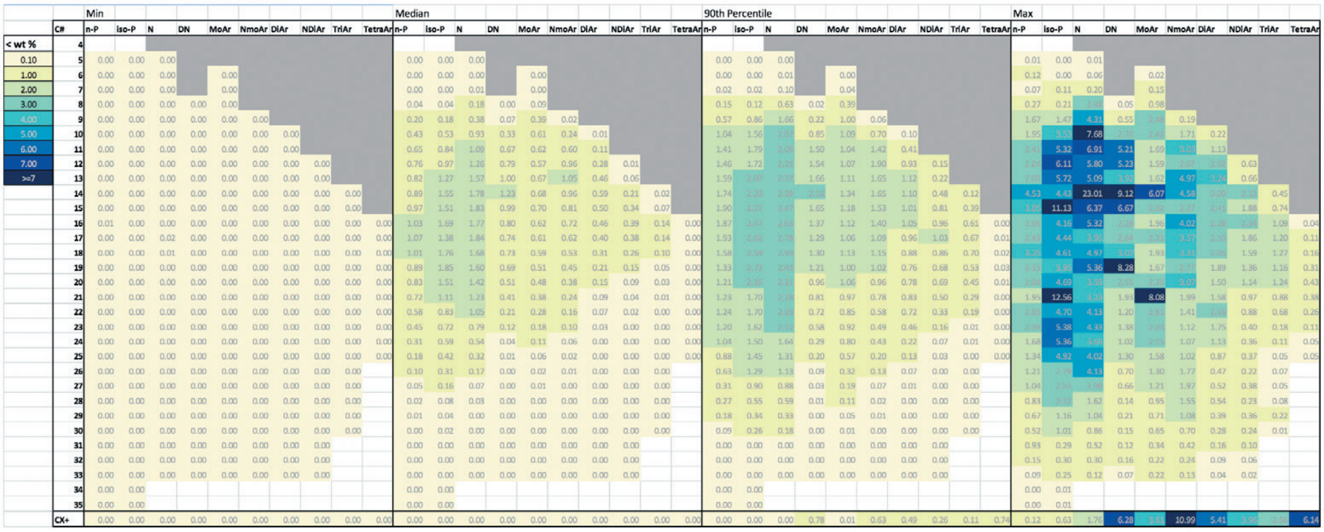
GCxGC analysis of these samples initially revealed a clustering of data for samples from all gas oil categories when GCxGC data are plotted as paraffinics, naphthenics, mono and di-aromatics and tri+ aromatics across all carbon numbers (see Figure 1 on page 7). There are sample outliers in the clustering but these are not associated with any particular category. From this we can see that gas oil substance constituents occupy the same 'hydrocarbon space', supporting the structural similarity of VHGO, SRGO and OtherGO substances.

Figure 1: Rotated tetrahedral plot of measured naphthenic, paraffinic, mono and di-aromatics and tri+ aromatic constituents across all carbon numbers on OtherGO, SRGO and VHGO samples



To improve the granularity of the hydrocarbon space mapping, the concentrations of each individual hydrocarbon class of constituents per carbon number were quantified and compared across multiple samples. To compare concentrations across samples and understand the variation in measured concentrations of each hydrocarbon block, the measured concentrations are normalised by converting to percentiles with 0% assigned to the minimum measured concentration of a specific hydrocarbon block, 50% to the median concentration and 100% to the maximum measured concentration of the same block. An example of such a concentration map of the hydrocarbon space is illustrated in Figure 2 on page 8, with carbon number on the y axis and hydrocarbon class on the x axis, depicting the 0% (min), 50% (median), 95% and 100% (max) percentiles for the VHGO category.

Figure 2: Concentration (weight %) of hydrocarbon blocks across all VHGO samples by GCxGC



< wt %
0.10
1.00
2.00
3.00
4.00
5.00
6.00
7.00
>=7

Min

C#	n-P	iso-P	N	DN	MoAr	NmoAr	DiAr	NDiAr	TriAr	TetraAr
4										
5	0.00	0.00	0.00							
6	0.00	0.00	0.00		0.00					
7	0.00	0.00	0.00		0.00					
8	0.00	0.00	0.00	0.00	0.00					
9	0.00	0.00	0.00	0.00	0.00	0.00				
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
11	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
16	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
34	0.00	0.00								
35	0.00	0.00								
CX+	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Median

C#	n-P	iso-P	N	DN	MoAr	NmoAr	DiAr	NDiAr	TriAr	TetraAr
4										
5	0.00	0.00	0.00							
6	0.00	0.00	0.00		0.00					
7	0.00	0.00	0.01		0.00					
8	0.04	0.04	0.18	0.00	0.09					
9	0.20	0.18	0.38	0.07	0.39	0.02				
10	0.43	0.53	0.93	0.33	0.61	0.24	0.01			
11	0.65	0.84	1.09	0.67	0.62	0.60	0.11			
12	0.76	0.97	1.26	0.79	0.57	0.96	0.28	0.01		
13	0.82	1.27	1.57	1.00	0.67	1.05	0.46	0.06		
14	0.89	1.55	1.78	1.23	0.68	0.96	0.59	0.21	0.02	
15	0.97	1.51	1.83	0.99	0.70	0.81	0.50	0.34	0.07	
16	1.03	1.69	1.77	0.80	0.62	0.72	0.46	0.39	0.14	0.00
17	1.07	1.38	1.84	0.74	0.61	0.62	0.40	0.38	0.14	0.00
18	1.01	1.76	1.68	0.73	0.59	0.53	0.31	0.26	0.10	0.00
19	0.89	1.85	1.60	0.69	0.51	0.45	0.21	0.15	0.05	0.00
20	0.83	1.51	1.42	0.51	0.48	0.38	0.15	0.09	0.03	0.00
21	0.72	1.11	1.23	0.41	0.38	0.24	0.09	0.04	0.01	0.00
22	0.58	0.83	1.05	0.21	0.28	0.16	0.07	0.02	0.00	0.00
23	0.45	0.72	0.79	0.12	0.18	0.10	0.03	0.00	0.00	0.00
24	0.31	0.59	0.54	0.04	0.11	0.06	0.00	0.00	0.00	0.00
25	0.18	0.42	0.32	0.01	0.06	0.02	0.00	0.00	0.00	0.00
26	0.10	0.31	0.17	0.00	0.02	0.01	0.00	0.00	0.00	
27	0.05	0.16	0.07	0.00	0.01	0.00	0.00	0.00	0.00	
28	0.02	0.08	0.03	0.00	0.00	0.00	0.00	0.00	0.00	
29	0.01	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
30	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
34	0.00	0.00								
35	0.00	0.00								
CX+	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

90th Percentile

C#	n-P	iso-P	N	DN	MoAr	NmoAr	DiAr	NDiAr	TriAr	TetraAr
4										
5	0.00	0.00	0.00							
6	0.00	0.00	0.01		0.00					
7	0.02	0.02	0.10		0.04					
8	0.15	0.12	0.63	0.02	0.39					
9	0.57	0.86	1.66	0.22	1.00	0.06				
10	1.04	1.56	2.07	0.85	1.09	0.70	0.10			
11	1.41	1.79	2.09	1.50	1.04	1.42	0.41			
12	1.46	1.72	2.25	1.54	1.07	1.90	0.93	0.15		
13	1.59	2.07	2.37	1.66	1.11	1.65	1.12	0.22		
14	1.74	2.20	2.39	2.10	1.34	1.65	1.10	0.48	0.12	
15	1.90	2.27	2.47	1.65	1.18	1.53	1.01	0.81	0.39	
16	1.87	2.47	2.63	1.37	1.12	1.40	1.05	0.96	0.61	0.00
17	1.93	2.02	2.78	1.29	1.06	1.09	0.96	1.03	0.67	0.01
18	1.58	2.59	2.59	1.30	1.13	1.15	0.88	0.86	0.70	0.02
19	1.33	2.73	2.43	1.21	1.00	1.02	0.76	0.68	0.53	0.03
20	1.21	2.35	2.31	0.96	1.06	0.96	0.78	0.69	0.45	0.01
21	1.23	1.70	2.28	0.81	0.97	0.78	0.83	0.50	0.29	0.00
22	1.24	1.70	2.28	0.72	0.85	0.58	0.72	0.33	0.19	0.00
23	1.20	1.62	2.12	0.58	0.92	0.49	0.46	0.16	0.01	0.00
24	1.04	1.50	1.64	0.29	0.80	0.43	0.22	0.07	0.01	0.00
25	0.88	1.45	1.31	0.20	0.57	0.20	0.13	0.03	0.00	0.00
26	0.63	1.29	1.13	0.09	0.32	0.13	0.07	0.00	0.00	
27	0.31	0.90	0.88	0.03	0.19	0.07	0.01	0.00	0.00	
28	0.27	0.55	0.59	0.01	0.11	0.02	0.00	0.00	0.00	
29	0.18	0.34	0.33	0.00	0.05	0.01	0.00	0.00	0.00	
30	0.09	0.26	0.18	0.00	0.01	0.00	0.00	0.00	0.00	
31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
34	0.00	0.00								
35	0.00	0.00								
CX+	0.00	0.00	0.00	0.78	0.01	0.63	0.49	0.26	0.11	0.74

Max

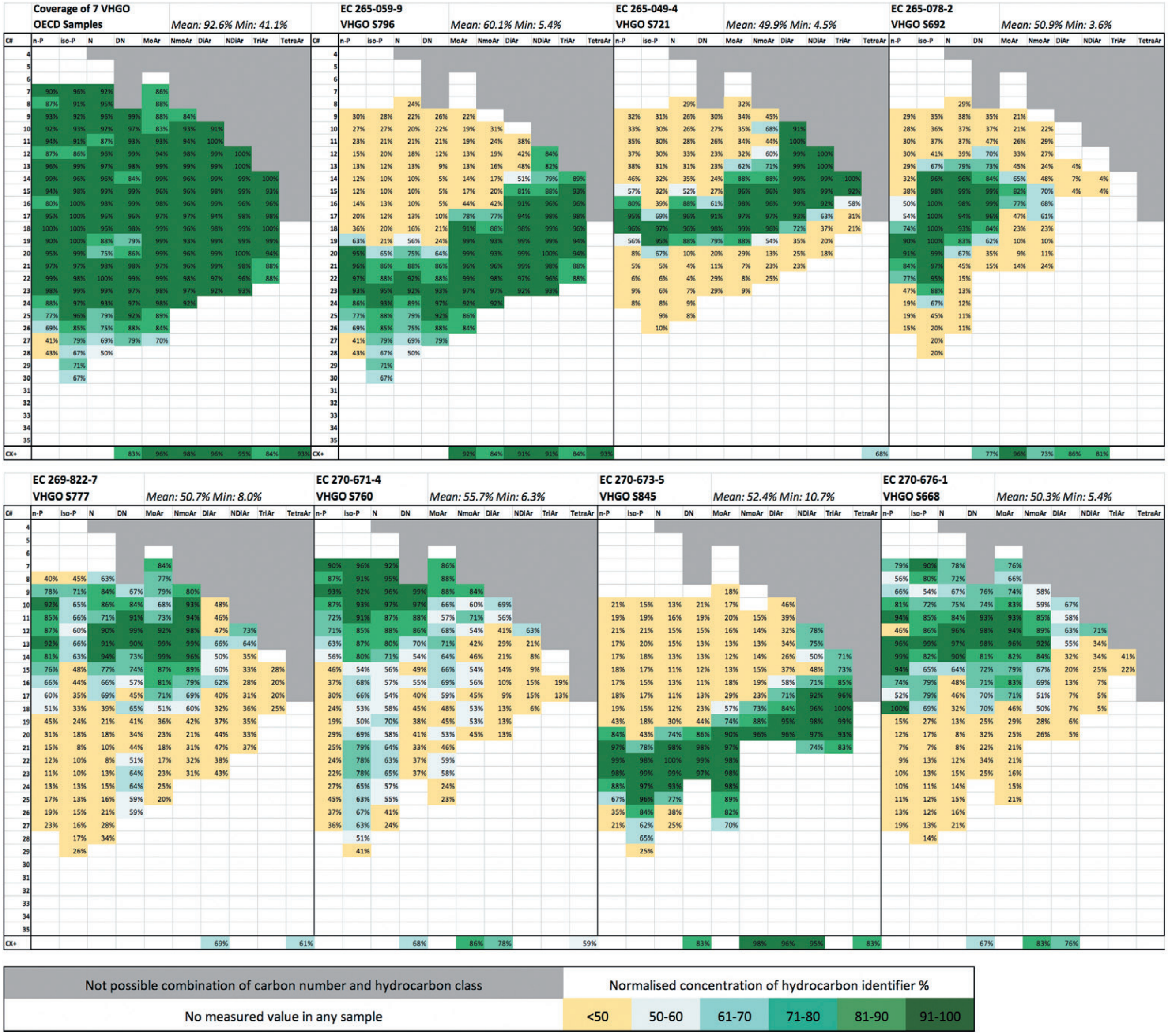
C#	n-P	iso-P	N	DN	MoAr	NmoAr	DiAr	NDiAr	TriAr	TetraAr
4										
5	0.01	0.00	0.01							
6	0.12	0.00	0.06		0.02					
7	0.07	0.11	0.20		0.15					
8	0.27	0.21	2.48	0.05	0.98					
9	1.67	1.47	4.31	0.55	2.48	0.19				
10	1.95	3.53	7.68	2.76	2.42	1.71	0.22			
11	2.41	5.32	6.91	5.21	1.69	3.03	1.13			
12	2.24	6.11	5.80	5.23	1.59	2.07	2.92	0.63		
13	2.03	5.72	5.09	3.92	1.62	4.97	3.24	0.66		
14	4.53	4.43	23.01	9.12	6.07	4.58	3.00	2.12	0.45	
15	3.05	11.13	6.37	6.67	2.02	2.27	2.41	1.88	0.74	
16	2.66	4.16	5.32	2.28	1.96	4.02	2.28	2.34	1.09	0.04
17	2.43	4.44	3.95	2.64	2.21	3.57	2.10	1.86	1.20	0.11
18	3.25	4.61	4.97	3.07	1.93	3.31	2.05	1.59	1.27	0.16
19	2.15	3.95	5.36	8.28	1.67	2.77	1.89	1.36	1.16	0.31
20	2.04	4.69	3.59	2.55	2.35	3.07	1.50	1.14	1.24	0.43
21	1.95	12.56	3.23	1.93	8.08	1.99	1.58	0.97	0.88	0.38
22	2.85	4.70	4.13	1.20	2.81	1.41	2.16	0.88	0.68	0.26
23	2.39	5.38	4.33	1.38	2.24	1.12	1.75	0.40	0.18	0.11
24	1.68	5.36	3.65	1.02	2.15	1.07	1.13	0.36	0.11	0.05
25	1.34	4.92	4.02	1.30	1.58	1.02	0.87	0.37	0.05	0.05
26	1.21	2.79	4.13	0.70	1.30	1.77	0.47	0.22	0.07	
27	1.04	2.55	2.98	0.66	1.21	1.97	0.52	0.38	0.05	
28	0.83	2.12	1.62	0.14	0.95	1.55	0.54	0.23	0.08	
29	0.67	1.16	1.04	0.21	0.71	1.08	0.39	0.36	0.22	
30	0.52	1.01	0.86	0.15	0.65	0.70	0.28	0.24	0.01	
31	0.93	0.29	0.52	0.12	0.34	0.42	0.16	0.10		
32	0.15	0.30	0.30	0.16	0.22	0.24	0.09	0.06		
33	0.09	0.25	0.12	0.07	0.22	0.13	0.04	0.02		
34	0.00	0.01								
35	0.00	0.01								
CX+	0.12	0.63	1.76	6.28	3.61	10.99	5.41	3.96	2.56	6.14

The normalised concentrations for each hydrocarbon block at 0% and 100% data define the applicability domain or boundary of substances within a category. It is within this compositional scope that read-across can be justified, providing that test samples, or combinations of samples, are representative of this space in terms of constituent type and concentration. Test samples that are best representative of this applicability domain or the hydrocarbon space of a category are selected on the basis that, alone or in combination with other samples, they represent the highest measured concentrations of the most hydrocarbon blocks amongst the sample population within that category.

Computational analysis is used to identify samples and combinations of samples that best represent the hydrocarbon space of a category. To identify the group of samples to be proposed for further animal testing, samples and combinations of samples are ranked in function of their hydrocarbon block with the lowest percentile. Highest ranked samples have the maximal lowest percentile coverage of all hydrocarbon blocks.

The coverage of the hydrocarbon space by selected test samples is visualised in a heat map (see Figure 3 on page 9). Unlike the concentration map in Figure 2, the hydrocarbon space in these heat maps is represented by percentile concentrations per hydrocarbon block expressing the percentage of the highest measured concentration within the category sample population measured in the test sample. The combined test sample heat map (top left in Figure 3) represents the highest coverage of each hydrocarbon block among the individual test samples. A threshold of a mean of 90% highest coverage across all blocks was set as the minimum that was considered to represent the full hydrocarbon space of the category. The set of samples proposed is then considered sufficient to demonstrate the chemical similarity with all the substances of the category.

Figure 3: Coverage of VHGO hydrocarbon space with seven combined and individual selected samples



Coverage of 7 VHGO
OECD Samples *Mean: 92.6% Min: 41.1%*

C#	n-P	iso-P	N	DN	MoAr	NmoAr	DiAr	NDiAr	TriAr	TetraAr
4										
5										
6										
7	90%	96%	92%		86%					
8	87%	91%	95%		88%					
9	93%	92%	96%	99%	88%	84%				
10	92%	93%	97%	97%	83%	93%	91%			
11	94%	91%	87%	93%	93%	94%	100%			
12	87%	86%	96%	99%	94%	98%	99%	100%		
13	96%	99%	97%	98%	99%	99%	99%	100%		
14	99%	96%	96%	84%	99%	96%	99%	99%	100%	
15	94%	98%	99%	99%	96%	96%	98%	99%	93%	
16	80%	100%	98%	99%	98%	96%	99%	96%	96%	
17	95%	100%	96%	96%	97%	97%	94%	98%	98%	
18	100%	100%	96%	98%	99%	96%	98%	99%	100%	
19	90%	100%	88%	79%	99%	93%	99%	99%	99%	
20	95%	99%	75%	86%	99%	96%	99%	100%	94%	
21	97%	97%	98%	98%	97%	96%	99%	98%	88%	
22	99%	98%	100%	99%	99%	98%	97%	96%	88%	
23	98%	99%	99%	97%	98%	97%	92%	93%		
24	88%	97%	93%	97%	98%	92%				
25	77%	96%	79%	92%	89%					
26	69%	85%	75%	88%	84%					
27	41%	79%	69%	79%	70%					
28	43%	67%	50%							
29		71%								
30		67%								
31										
32										
33										
34										
35										
CX+				83%	96%	98%	96%	95%	84%	93%

EC 265-059-9
VHGO S796 *Mean: 60.1% Min: 5.4%*

C#	n-P	iso-P	N	DN	MoAr	NmoAr	DiAr	NDiAr	TriAr	TetraAr
4										
5										
6										
7										
8			24%							
9	30%	28%	22%	26%	22%					
10	27%	27%	20%	22%	19%	31%				
11	23%	21%	21%	21%	19%	24%	38%			
12	15%	20%	18%	12%	13%	19%	42%	84%		
13	13%	12%	13%	9%	13%	16%	48%	82%		
14	12%	10%	10%	5%	14%	17%	51%	79%	89%	
15	12%	10%	10%	5%	17%	20%	81%	88%	93%	
16	14%	13%	10%	5%	44%	42%	91%	96%	96%	
17	20%	12%	13%	10%	78%	77%	94%	98%	98%	
18	36%	20%	16%	21%	91%	88%	98%	99%	96%	
19	63%	21%	56%	24%	99%	93%	99%	99%	94%	
20	95%	65%	75%	64%	99%	93%	99%	100%	94%	
21	96%	86%	88%	86%	96%	96%	99%	98%	88%	
22	97%	88%	92%	88%	99%	98%	97%	96%	88%	
23	93%	95%	92%	93%	97%	97%	92%	93%		
24	86%	93%	89%	97%	92%	92%				
25	77%	88%	79%	92%	86%					
26	69%	85%	75%	88%	84%					
27	41%	79%	69%	79%						
28	43%	67%	50%							
29		71%								
30		67%								
31										
32										
33										
34										
35										
CX+					92%	84%	91%	91%	84%	93%

EC 265-049-4
VHGO S721 *Mean: 49.9% Min: 4.5%*

C#	n-P	iso-P	N	DN	MoAr	NmoAr	DiAr	NDiAr	TriAr	TetraAr
4										
5										
6										
7										
8			29%		32%					
9	32%	31%	26%	30%	34%	45%				
10	33%	30%	26%	27%	35%	68%	91%			
11	35%	30%	28%	26%	34%	44%	100%			
12	37%	30%	33%	23%	32%	60%	99%	100%		
13	38%	31%	31%	23%	62%	71%	99%	100%		
14	46%	32%	35%	24%	88%	88%	99%	99%	100%	
15	57%	32%	52%	27%	96%	96%	98%	99%	92%	
16	80%	39%	88%	61%	98%	96%	99%	92%	58%	
17	95%	69%	96%	91%	97%	97%	93%	63%	31%	
18	96%	97%	96%	98%	99%	96%	72%	37%	21%	
19	56%	95%	88%	79%	88%	54%	35%	20%		
20	8%	67%	10%	20%	29%	13%	25%	18%		
21	5%	5%	4%	11%	7%	23%	23%			
22	6%	6%	4%	29%	8%	25%				
23	9%	6%	7%	29%	9%					
24	8%	8%	9%							
25		9%	8%							
26		10%								
27										
28										
29										
30										
31										
32										
33										
34										
35										
CX+										68%

EC 265-078-2
VHGO S692 *Mean: 50.9% Min: 3.6%*

C#	n-P	iso-P	N	DN	MoAr	NmoAr	DiAr	NDiAr	TriAr	TetraAr
4										
5										
6										
7										
8			29%							
9	29%	35%	38%	35%	21%					
10	28%	36%	37%	37%	21%	22%				
11	30%	37%	37%	47%	26%	29%				
12	30%	41%	39%	70%	33%	27%				
13	29%	67%	79%	73%	45%	24%	4%			
14	32%	96%	96%	84%	65%	48%	7%	4%		
15	38%	98%	99%	99%	82%	70%	4%	4%		
16	50%	100%	98%	99%	77%	68%				
17	54%	100%	94%	96%	47%	61%				
18	74%	100%	93%	84%	23%	23%				
19	90%	100%	83%	62%	10%	10%				
20	91%	99%	67%	35%	9%	11%				
21	84%	97%	45%	15%	14%	24%				
22	77%	95%	15%							
23	47%	88%	13%							
24	19%	67%	12%							
25	19%	45%	11%							
26	15%	20%	11%							
27		20%								
28		20%								
29										
30										
31										
32										
33										
34										
35										
CX+				77%	96%	73%	86%	81%		

EC 269-822-7
VHGO S777 *Mean: 50.7% Min: 8.0%*

C#	n-P	Iso-P	N	DN	MoAr	NmoAr	DiAr	NDiAr	TriAr	TetraAr
4										
5										
6										
7					84%					
8	40%	45%	63%		77%					
9	78%	71%	84%	67%	79%	80%				
10	92%	65%	86%	84%	68%	93%	48%			
11	85%	66%	71%	91%	73%	94%	46%			
12	87%	60%	90%	99%	92%	98%	47%	73%		
13	92%	66%	91%	90%	99%	99%	66%	64%		
14	81%	63%	94%	73%	99%	96%	50%	35%		
15	76%	48%	77%	74%	87%	89%	60%	33%	28%	
16	66%	44%	66%	57%	81%	79%	62%	28%	20%	
17	60%	35%	69%	45%	71%	69%	40%	31%	20%	
18	51%	33%	39%	65%	51%	60%	32%	36%	25%	
19	45%	24%	21%	41%	36%	42%	37%	35%		
20	31%	18%	18%	34%	23%	21%	44%	33%		
21	15%	8%	10%	44%	18%	31%	47%	37%		
22	12%	10%	8%	51%	17%	32%	38%			
23	11%	10%	13%	64%	23%	31%	43%			
24	13%	13%	15%	64%	25%					
25	17%	13%	16%	59%	20%					
26	19%	15%	21%	59%						
27	23%	16%	28%							
28		17%	34%							
29		26%								
30										
31										
32										
33										
34										
35										
CX+							69%			61%

EC 270-671-4
VHGO S760 *Mean: 55.7% Min: 6.3%*

C#	n-P	Iso-P	N	DN	MoAr	NmoAr	DiAr	NDiAr	TriAr	TetraAr
4										
5										
6										
7	90%	96%	92%		86%					
8	87%	91%	95%		88%					
9	93%	92%	96%	99%	88%	84%				
10	87%	93%	97%	97%	66%	60%	69%			
11	72%	91%	87%	88%	57%	71%	56%			
12	71%	85%	88%	86%	68%	54%	41%	63%		
13	63%	87%	80%	70%	71%	42%	29%	21%		
14	56%	80%	71%	54%	64%	46%	21%	8%		
15	46%	54%	56%	49%	66%	54%	14%	9%		
16	37%	68%	57%	55%	69%	56%	10%	15%	19%	
17	30%	66%	54%	40%	59%	45%	9%	15%	13%	
18	24%	53%	58%	45%	48%	53%	13%	6%		
19	19%	50%	70%	38%	45%	53%	13%			
20	29%	69%	58%	41%	53%	45%	13%			
21	25%	79%	64%	33%	46%					
22	24%	78%	63%	37%	59%					
23	22%	78%	65%	37%	58%					
24	27%	65%	57%		24%					
25	45%	63%	55%		23%					
26	37%	67%	41%							
27	36%	63%	24%							
28		51%								
29		41%								
30										
31										
32										
33										
34										
35										
CX+				68%		86%	78%			59%

EC 270-673-5
VHGO S845 *Mean: 52.4% Min: 10.7%*

C#	n-P	Iso-P	N	DN	MoAr	NmoAr	DiAr	NDiAr	TriAr	TetraAr
4										
5										
6										
7										
8										
9					18%					
10	21%	15%	13%	21%	17%		46%			
11	19%	19%	16%	19%	20%	15%	39%			
12	21%	21%	15%	15%	16%	14%	32%	78%		
13	17%	20%	15%	13%	13%	13%	34%	75%		
14	17%	18%	13%	13%	12%	14%	26%	50%	71%	
15	18%	17%	11%	12%	13%	15%	37%	48%	73%	
16	17%	15%	13%	11%	18%	19%	58%	71%	85%	
17	18%	17%	11%	13%	29%	23%	71%	92%	96%	
18	19%	15%	12%	23%	57%	73%	84%	96%	100%	
19	43%	18%	30%	44%	74%	88%	95%	98%	99%	
20	84%	43%	74%	86%	90%	96%	96%	97%	93%	
21	97%	78%	98%	98%	97%			74%	83%	
22	99%	98%	100%	99%	98%					
23	98%	99%	99%	97%	98%					
24	88%	97%	93%		98%					
25	67%	96%	77%		89%					
26	35%	84%	38%		82%					
27	21%	62%	25%		70%					
28		65%								
29		25%								
30										
31										
32										
33										
34										
35										
CX+				83%		98%	96%	95%		83%

EC 270-676-1
VHGO S668 *Mean: 50.3% Min: 5.4%*

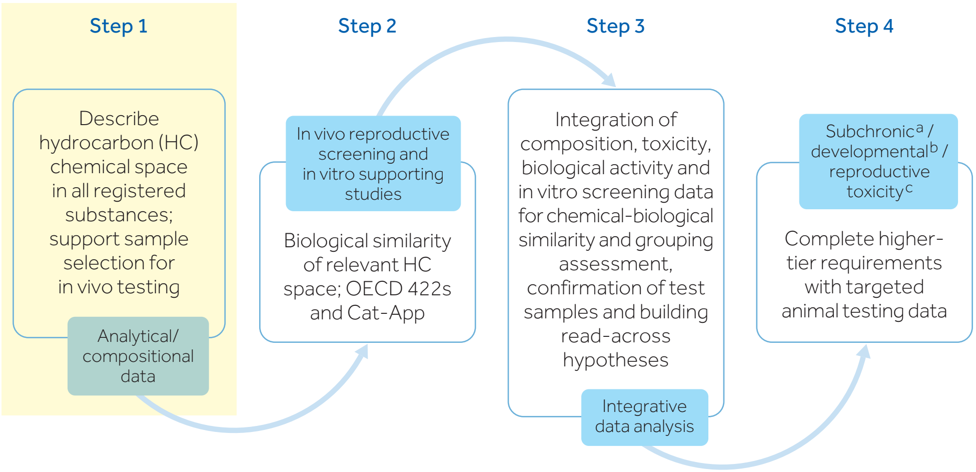
C#	n-P	Iso-P	N	DN	MoAr	NmoAr	DiAr	NDiAr	TriAr	TetraAr
4										
5										
6										
7	79%	90%	78%		76%					
8	56%	80%	72%		66%					
9	66%	54%	67%	76%	74%	58%				
10	81%	72%	75%	74%	83%	59%	67%			
11	94%	85%	84%	93%	93%	85%	58%			
12	46%	86%	96%	98%	94%	89%	63%	71%		
13	96%	99%	97%	98%	96%	92%	55%	34%		
14	99%	82%	90%	81%	82%	84%	32%	34%	41%	
15	94%	65%	64%	72%	79%	67%	20%	25%	22%	
16	74%	79%	48%	71%	83%	69%	13%	7%		
17	52%	79%	46%	70%	71%	51%	7%	5%		
18	100%	69%	32%	70%	46%	50%	7%	5%		
19	15%	27%	13%	25%	29%	28%	6%			
20	12%	17%	8%	32%	25%	26%	5%			
21	7%	7%	8%	22%	21%					
22	9%	13%	12%	34%	21%					
23	10%	13%	15%	25%	16%					
24	10%	11%	14%		15%					
25	11%	12%	15%		21%					
26	13%	12%	16%							
27	19%	13%	21%							
28		14%								
29										
30										
31										
32										
33										
34										
35										
CX+				67%		83%	76%			

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

Biological similarity: in vivo and in vitro screening tests

The hydrocarbon space mapping approach has been applied in the selection of samples for lower-tier (OECD Test Guideline 422) animal studies of VHGO substances. Such studies, which are performed voluntarily, alongside information about chemical similarity, are fundamental to the read-across strategy in providing information about human health hazard properties. The above analytical characterisation and lower-tier animal studies form the first two critical steps in the human health hazard testing strategy, as described in Figure 4.

Figure 4: Human health hazard testing strategy for petroleum substances



Notes: [a] OECD 408 (rat); [b] OECD 414 (rat and rabbit); [c] OECD 443

The OECD 422 studies are considered as 'bridging studies', meaning that results from studies sharing the same protocol — in this case a screening study over 28 days using the oral route — can be directly compared across test substances. Similar results across such studies justifies bridging the higher-tier study results of the source or test substance(s) to the target substance(s).

The OECD 422 standard provides the widest possible toxicological screening for a substance over a 28-day period, and can be performed without a decision from the European Chemicals Agency (ECHA) and member states according to the REACH Regulation.

Most hydrocarbon constituents found in petroleum substances can be assigned to aromatics, aliphatics, those consisting of saturates, or resins and asphaltenes. Although the ratio of constituent types varies between petroleum stream and category, the information from each type can provide a useful insight into which are responsible for the observed toxicity or lack thereof in different petroleum substances.

Polycyclic aromatic hydrocarbons (PAH) have a conjugated hydrocarbon ring structure. They are of particular concern because, historically, certain PAH are considered to be associated with a number of health and environmental toxicities of which benzo[a]pyrene is the best-known example. Cancer related to exposure to PAH was one of the first occupational cancers identified back in the 18th century among London chimney sweepers exposed to soot, which is known to contain very high PAH concentrations compared to what is typically found in petroleum substances today.

In addition to their carcinogenic hazard, the reprotoxic properties (developmental toxicity) of PAH are hypothesised to be attributed to their interaction with the aryl hydrocarbon (Ah) receptor. This protein acts as a gene regulator. Not all PAH interact with this receptor and the precise mechanisms are not completely understood. Different PAH express different toxicity potencies.

The available data on kerosene with carbon number ranging between C9 and C16 indicated no toxicity to reproduction. These substances are predominantly made of aliphatics (80%) and single-ring aromatics (20%). Polycyclic aromatics are higher in molecular weight with boiling point above the typical boiling point range of kerosenes. It can be concluded from the studies available that no specific toxicity is expected from this range of constituents, namely aliphatics and monoaromatics, with the obvious exception of benzene that is not present in kerosene.

Considering higher boiling range constituents, no toxicity has been found in substances with carbon atom number range above C20, such as those present in highly refined base oils made of aliphatics without aromatics.

Substances from non-petroleum-source materials, such as diesel from gas-to-liquid processing, can also be used as supporting information. This substance is made of more than 99% aliphatics constituents with carbon atom range between C8 and C26. The available studies with this substance show no toxicity.

The available data indicate that the aliphatic constituents of petroleum substances are not developmental toxicants, do not affect fertility, and do not produce reproductive organ toxicity.[3,4] In addition, the heavier well-refined petroleum substances such as highly refined base oils and synthetic petroleum products in which the PAH levels are negligible do not show any systemic toxicological effect[5,6] Furthermore, the data indicate that the observed toxicity is related to 3–7 ring PAH specifically.

Resins and asphaltenes are polar components with high molecular weights containing small amounts of oxygen, sulphur and nitrogen. They have carbon ranges well above C30 and, due to their high molecular weight and polar nature, both resins and asphaltenes are not biologically available. As a consequence, they are irrelevant for toxicological hazard assessment.

With these toxicological considerations in mind, a sample for each of the 7 actively registered VHGO substances was selected from a total of 61 analysed VHGO samples taking into the consideration both the PAH hypothesis and the full hydrocarbon space (all constituents) based on the hydrocarbon mapping approach.

Hydrocarbon space mapping to support gas oil read-across for human health hazard assessment

The selection of one substance in the VHGO category for lower-tier testing was based on the maximum quantitative assessment of > 3-ring PAH concentration by dimethyl sulphoxide (DMSO) extraction of samples followed by gas chromatography mass spectrometry (GC-MS) of the extract (a non-standard method known as PAC2).

For the remaining six substances in the VHGO category, one sample per substance was identified using the hydrocarbon space mapping approach. The sample per substance that best represented the full VHGO category hydrocarbon space was selected for OECD 422 study. These studies are ongoing and the outcome will inform the final selection of samples for higher-tier studies (90-day repeat dose, pre-natal development and extended one-generation reproductive toxicity). Information about structural similarity and biological response data are also influential in the selection of samples for higher-tier testing.

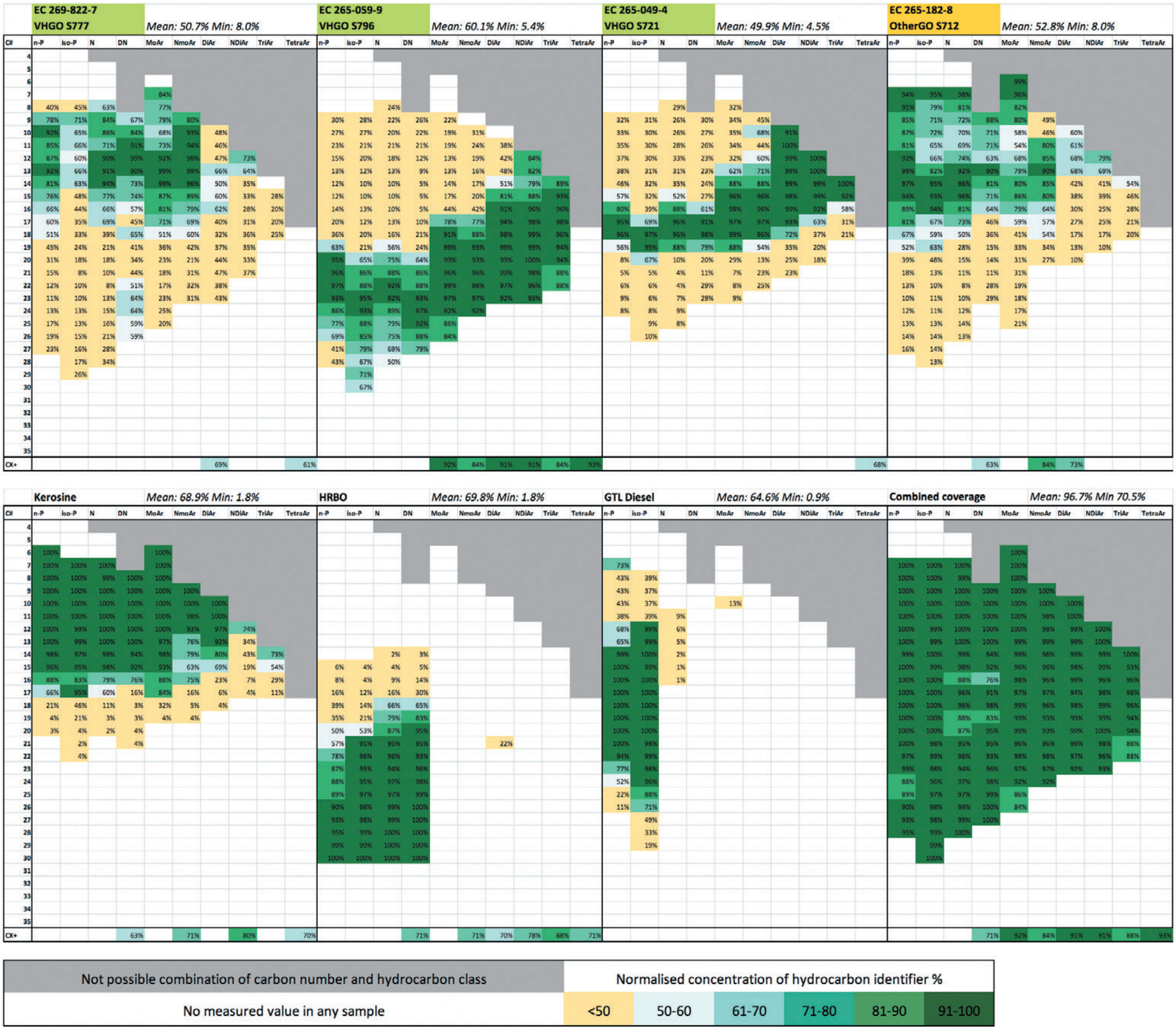
Based on structural similarity to support read-across to all VHGO substances, Concawe has proposed to ECHA in the testing proposal submitted in December 2021 to select three VHGO substances for higher-tier animal studies (see Appendix 1 on page 15 for a list of substance EC numbers, names and CAS numbers). This selection is to be reviewed for similarity of human health hazard properties as a function of the results of the OECD 422 tests expected by year-end. The VHGO hydrocarbon space coverage of the three selected samples (one from each of the three VHGO substances), when combined with a sample of the OtherGO EC 265-182-8 (already identified by ECHA for higher-tier testing) and neighbouring petroleum substances is at a mean of 97% and a minimum of 76% (see Figure 5 on page 13). These neighbouring substances (kerosene, highly refined base oil and GTL diesel) have already been tested for reproductive toxicity and determined to be without human health hazard effects, and act as a weight of evidence for the VHGO category.

The carbon-specific block with minimum coverage of 76% represents C16 di-naphthenics. Neighbouring hydrocarbon blocks of C15 di-naphthenics and C17 di-naphthenics are however, more representative of the VHGO hydrocarbon space with coverages exceeding 91% of the maximum concentration recorded in any VHGO sample for those hydrocarbon blocks. Therefore, C15–C17 di-naphthenics are well represented in the proposed selection of samples and study data covering the VHGO hydrocarbon space. Paraffinic and naphthenic hydrocarbons are expected to be less hazardous than the PAH constituents hypothesised to drive toxicity in the VHGO category.

The inherent complexity and variability of UVCBs present considerable challenges for establishing sufficient substance similarity based on chemical characteristics or other data. In addition to the 28-days rat toxicity studies (OECD 422) used as bridging studies, Concawe hypothesised that new approach methodologies (NAMs) for animal testing, including in vitro test-derived biological activity signatures to characterise substance similarity, can be used to demonstrate similarity of UVCBs.

This has been the main aim of the Cat-App project led by Concawe since 2015, with 141 petroleum substances having been tested as representative UVCBs in a compendium of 15 human cell types representing a variety of tissues. Petroleum substances were assayed in dilution series to identify the concentration at which an effect could be identified for each cell type.

Figure 5: Coverage of VHGO hydrocarbon space with three selected VHGO samples, Other Gas Oil EC 265-182-8 and neighbouring petroleum substances



Because such assays generate a large amount of data, extensive quality control measures were taken to ensure that only high-confidence in vitro data are used to determine whether current groupings of these petroleum substances in categories are justifiable. Overall, it was found that bioactivity-data-based groupings of petroleum substances were generally consistent with the current categories grouping. Concawe also showed that these data, especially bioactivity from human induced pluripotent stem cell (iPSC)-derived and primary cells, can be used to rank substances in a manner that is highly concordant with their expected in vivo hazard potential based on their chemical compositional profile. Overall, this study demonstrates that NAMs can be used to inform groupings of UVCBs and to identify representative substances of each category for further read-across to fill data gaps and inform further testing, where needed.

The intention, therefore, is to apply these in vitro biological techniques to the set of seven selected VHGO samples, to enable a specific comparison of all results available for these samples on hydrocarbon composition together with in vivo and in vitro data.

Discussion

Wider application of hydrocarbon mapping in read-across

The hydrocarbon mapping approach to provide evidence for compositional similarity to support read-across is now being applied to the SRGO category of substances. One substance, EC 272-817-2, is likely to be proposed for testing in higher-tier studies for read-across to the other three SRGO substances. As with VHGO, the final selection of test sample for higher-tier testing will depend on the outcome of the OECD 422 lower-tier study. The testing proposal will again be informed by the coverage of the SRGO hydrocarbon space by the proposed test substance in addition to test substances in the VHGO and OtherGO categories, and neighbouring tested petroleum substances as described above. This strategy of testing in series across categories is aimed at reducing unnecessary replicate testing while ensuring that the full range of types and concentrations of constituents within each category is represented.

Information regarding the biological response of gas oils informs the selection of samples for final higher-tier vertebrate studies.

While the concept of hydrocarbon mapping of petroleum substance constituents remains valid beyond the gas oil categories, the selection of appropriate analytical methodology to quantitate constituent groups requires consideration. GCxGC is limited in its ability to separate and elute constituents above C30; therefore, alternative technologies need to be applied to heavier streams such as some lubricant base oils. FIMS provides quantitative information on the different classes of saturated hydrocarbons and aromatic hydrocarbons present for each carbon number. Analysis involves an initial HPLC separation of samples into saturate and aromatic fractions followed by FIMS analysis of each fraction. The saturate fraction may also be examined by gas chromatography to determine the quantities of normal and branched acyclic alkanes present.

The application of the hydrocarbon matching approach to provide evidence of structural similarity was developed to support human health hazard assessment read-across. The potential to apply the same analytical data and/or the approach to ecotoxicology hazard assessment is currently in progress. Since the objective for the environment has less to do with showing hydrocarbon space coverage and more with showing similarity between substances in a category, the choice of sample for comparison may differ, perhaps being the most conservative sample in terms of toxicity, or a sample for which testing data is available.

Appendix 1

Substance EC and CAS identifiers

EC number	CAS number	EC name	Concawe category
269-822-7	68334-30-5	Fuels, diesel	Vacuum gas oils, hydrocracked gas oils and distillate fuels (VHGO)
265-059-9	64741-58-8	Gas oils (petroleum), light vacuum	
265-049-4	64741-49-7	Condensates (petroleum), vacuum tower	
265-182-8	64742-79-6	Gas oils (petroleum), hydrodesulphurised	Other gas oils (OtherGO)
272-817-2	68915-96-8	Distillates (petroleum), heavy, straight run	Straight run gas oils (SRGO)

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Generation of biodegradability data on petroleum constituents using enhanced tests (OECD Guideline 301 F)

In 2020, Concawe began an experimental programme to assess the ability of petroleum constituents to degrade in the environment. This article provides an overview of the results of the programme, which will aid in the development of new strategies for overcoming the difficulties of testing hydrocarbons for biodegradability in the environment.

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Biodegradation assessment

The assessment of biodegradation (the breakdown of chemicals by microbes in the environment) is a major step in the environmental risk assessment of chemicals. Indeed, substances that are eliminated quickly from the environment pose very low risk, while those that can remain for longer periods (days, months or even decades in the most extreme cases) have a much higher potential to build up in the environment, reach living organisms and cause toxic effects. For this reason, persistence assessment is of great importance under European Union chemicals legislation, and is the first step for identifying a substance as PBT/vPvB (Persistent, Bioaccumulative and Toxic/very Persistent and very Bioaccumulative), a category of Substances of Very High Concern under REACH.

In general, the assessment of persistence starts with cheap, fast and stringent screening tests, and gradually progresses to more complex and time-consuming analysis, if needed. The most frequent starting point for testing biodegradability is the ready biodegradability test (RBT), such as the OECD 301 series.[1] In an RBT, a test substance is mixed with a microbial inoculum, typically samples from a wastewater treatment plant, and the breakdown of that substance is monitored over time. Briefly, a substance is considered readily biodegradable if it reaches 60–70% removal in 28 days. These tests do not provide an exact estimation of how long a substance will remain in the environment, but are so stringent that a 'pass' level in a reliable RBT test is normally considered sufficient to conclude that a substance is 'not persistent' in any environmental compartment. The reverse, however, does not apply, i.e. if a substance is found to be 'not readily biodegradable', this does not allow us to conclude that it will persist in the environment, and would warrant further, more complex testing.

For PBT assessment, petroleum substances, which are comprised of hundreds to thousands of different chemicals, are not assessed at the level of the whole substance but by its chemical constituents. Although a lot of data exist on the environmental biodegradation of petroleum substances, tests on individual hydrocarbon constituents following one of the 301 Guidelines are less frequent, and any conclusions based on testing data generated according to these Guidelines are easier to accept for regulatory bodies. Based on the European regulator's stated intention to evaluate triaromatic PAHs for PBT, Concawe started a project in 2020 aimed at generating ready biodegradability data on a number of triaromatic PAHs. These new data can inform the environmental assessment of petroleum substances.

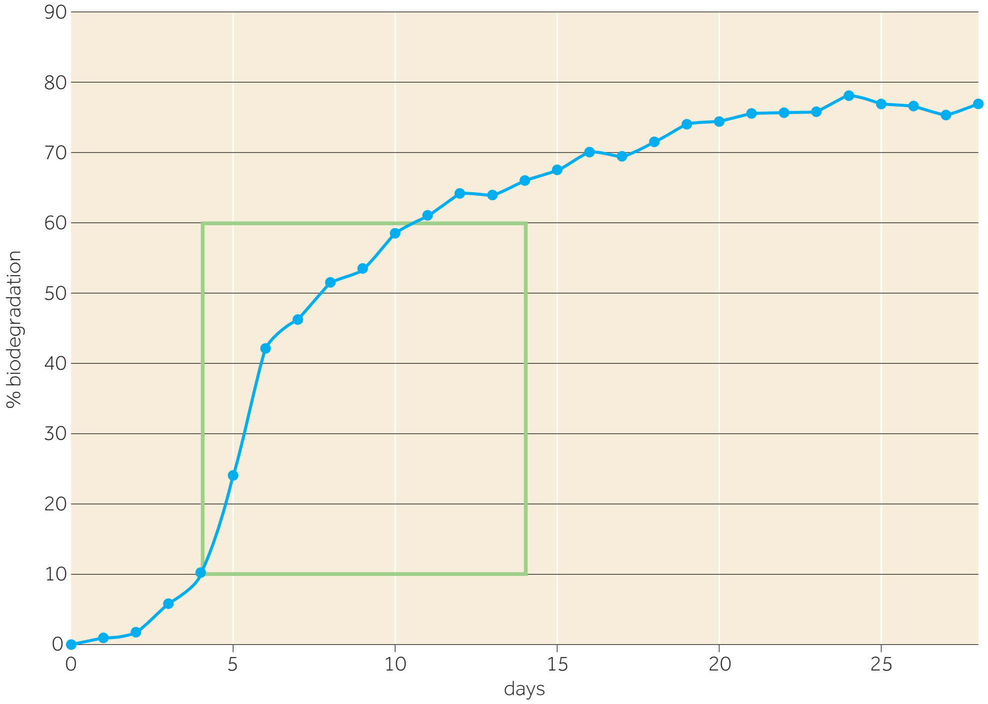
Ready biodegradability tests

RBTs involve incubation of a test substance with a microbial inoculum that is expected to be biodiverse, frequently from waste-water treatment plants, while monitoring the mineralisation of the test substance, which is the complete breakdown of the substance to water and CO_2.

RBTs are thus used to identify those substances that will mineralise quickly and rapidly in the environment (i.e. will not persist). Specifically, a chemical which achieves '≥ 70% biodegradation measured as DOC[1] removal (OECD Test Guidelines 301 A, 301 E and 306) or ≥ 60% biodegradation measured as $ThCO_2$[2] (OECD Test Guideline 301 B) or ThOD[3] (OECD Test Guidelines 301 C, 301 D, 301 F, 306 and 310)' within a 10-day window are designated as readily biodegradable.

Figure 1: Example of biodegradation with time in a 301 F test for a readily biodegradable substance

Note: the green box is the 10-day window within which 60% biodegradation must take place in order for the substance to be considered readily biodegradable.



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[1] DOC = dissolved organic carbon

[2] $ThCO_2$ = theoretical amount of carbon dioxide

[3] ThOD = theoretical oxygen demand

Generation of biodegradability data on petroleum constituents using enhanced tests (OECD Guideline 301 F)

The OECD Guidelines state that a readily biodegradable chemical can be assumed to undergo rapid and ultimate biodegradation under most environmental conditions (revised introduction to the *OECD Guidelines for the Testing of Chemicals*, Section 3, 2006). Within the context of the REACH standard information requirements, for mono-constituent substances, higher tier fate information on degradation in water, sediment and soil, such as the degradation rate of the substance and its degradation products, can be waived if the substance is readily biodegradable.[2] Furthermore, for persistence in the context of PBT/vPvB under REACH, additional information can be utilised from ready biodegradation tests, to reach a conclusion of 'not persistent'. Such additional information can be obtained through the waiving of the 10-day window, and extension of the test to 60 days.

Bioavailability improvement methods

Apart from the design of the test used to determine ready biodegradability, it also has to be born in mind that the determination of the biodegradability of a substance can be more challenging due to the inherent properties of the substance. Chemicals displaying high values in their physicochemical properties are harder to test in any kind of setting. For example, a highly volatile chemical will tend to escape from the test system, and a particularly adsorptive chemical may attach itself to the walls of a test vessel, etc. In the case of PAHs, one of the issues is that they are highly insoluble in water. These properties make it likely that, in a test performed in aqueous media, the microbial population that is supposed to biodegrade the test substance will be unable to access the substance from the media (the water). This is what is commonly known as a 'bioavailablity limitation', and in practice means that a chemical which would not persist in the environment appears as failing the ready biodegradability test.

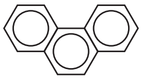
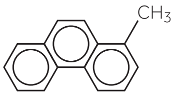
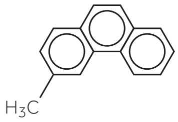
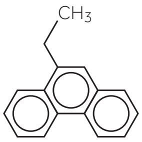
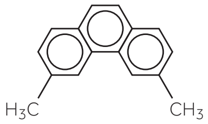
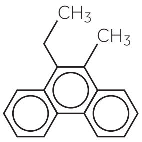
To correct this experimental artefact, the 301 Guideline was modified to include the possibility of applying a 'bioavailability improvement method' (BIM). BIMs increase the possibility that a microbe will be able to access the chemical in the test media, and therefore degrade it, thus allowing a better assessment of its intrinsic biodegradability. Concawe has already applied several such methods for PAHs in the past.[3]

A further possibility allowed for the regulatory assessment of persistence is to extend the incubation time of an RBT, which is normally 28 days, to 60 days.

Constituents tested

Six hydrocarbons were tested in this study. All of them can be obtained commercially, can appear in petroleum substances, and have physico-chemical properties that make them difficult to test (see Table 1).

Table 1: Identities of the hydrocarbons tested

Substance name	CAS number[a]	Structure
Phenanthrene	85-01-8	
1-methylphenanthrene	832-69-9	
3-methylphenanthrene	832-71-3	
9-ethylphenanthrene	3674-75-7	
3,6-dimethylphenanthrene	1576-67-6	
9-ethyl-10-methylphenanthrene	17024-02-1	

[a] Chemical Abstract Service registry number

The six substances were tested according to an OECD protocol, specifically the 301 F test. This Guideline is suitable for poorly soluble and adsorptive substances, and Concawe has obtained good results in the past when applying it to different cyclic hydrocarbons. Briefly, the substances were incubated in aqueous media with sewage from an urban wastewater treatment plant as inoculum (i.e. a source of microorganisms that will act as degraders of the chemicals present in the water). The sewage was sampled at several different days, mixed, and then blended to provide a homogeneous inoculum, with a variety of different microorganisms. The consumption of oxygen was evaluated with a manometric respirometer, and the pH was controlled daily throughout the test. The degradation of the test substance was calculated daily from the oxygen consumption using the equation provided in the 301 Guideline.

As mentioned above, since bioavailability limitations were very likely for these hydrocarbons, two of the BIMs allowed by the 301 Guideline were used: silicone oil and silicon dioxide (SiO_2). Thus, each hydrocarbon was tested:

- with no extra BIM;
- with SiO_2, in the form of silica gel; and
- with silicone oil.

Each of these treatments was applied to three different bottles, and incubated as indicated above. The overall biodegradation was calculated as the average value for the three bottles.

The tests would normally run for 28 days; however, in some cases it was decided to extend the duration to 60 days. The REACH guidance allows the extension of the test duration for the assessment of persistence in case a substance suffers from a bioavailability constraint, and in this case the time extension is considered as a type of BIM.

Any substance reaching a biodegradation level > 60% within 28 days, and with the degradation happening in a 10-day period (the so called '10 day window') can be considered as readily biodegradable, and thus disappearing very quickly from the environment. If the biodegradation reaches > 60% in 60 days, but does not meet the 10-day window, the substance is not readily biodegradable, but can still safely be considered as not persistent.

Results and discussion

For most of the hydrocarbons tested, significant biodegradation was observed during the first 28 days, which is the standard duration of the test. The results indicate that phenanthrene, 1-methylphenanthrene and 3,6-dimethylphenanthrene can clearly be considered as not persistent, since the level of biodegradation obtained was > 60% during the 28-day period with at least one of the BIMs, as displayed in Table 2.

Table 2: results of the OECD 301 tests according to the BIM used and incubation time (28 or 60 days)

Note: not all the tests were run up to 60 days.

	% biodegradation (average)					
	Direct addition (no BIM)		**Silicone oil**		**SiO_2**	
Substance name	**28 days**	**60 days**	**28 days**	**60 days**	**28 days**	**60 days**
Phenanthrene	60	N/A	77	N/A	75	N/A
1-methylphenanthrene	48	N/A	64	100	69	76
3-methylphenanthrene	56	58	46	51	46	41
3,6-dimethylphenanthrene	13	N/A	10	N/A	64	75
9-ethylphenanthrene	7	N/A	0	N/A	7	N/A
9-ethyl-10-methylphenanthrene	6	N/A	0	N/A	0	N/A

The degradation of 3-methylphenanthrene shows a less clear conclusion. Although the average biodegradation values are less than the required 60%, significant biodegradation is observed. Regarding the interpretation of the results, the 301 Guideline states that, *'Because of the stringency of the methods, low values do not necessarily mean that the test substance is not biodegradable under environmental conditions, but indicates that more work will be necessary to establish biodegradability.'* Thus, even if 3-methylphenanthrene cannot be flagged as readily biodegradable based on these results, it seems likely that further, more complex testing would show a result of non-persistence.

Two of the hydrocarbons—9-ethylphenanthrene and 9-ethyl-10-methylphenanthrene—showed hardly any degradation, even with the use of a BIM and the prolonged test duration. As mentioned above, the OECD 301 Guideline states that this is not a definitive indication of non-biodegradability under environmental conditions. These two constituents are the heaviest and most lipophilic tested in this programme, so it is not surprising that they show the lowest level of biodegradation. However, it is not possible to ascertain from these data whether this is due to actual persistence, or to very high bioavailability limitations which could not be overcome even with the use of BIMs.

It is worth mentioning that the use of BIMs enables a significant improvement in the meaningfulness of the 301 tests. As observed from the table above, the biodegradation improves when silicone oil or SiO_2 are used with the incubation, thanks to the enhanced bioavailability provided by these substances. In this case, overcoming the bioavailability limitations (i.e. ensuring that the microbes can access the test substance in order to degrade it) leads to a clear conclusion that 1-methylphenanthrene and 3,6-dimethylphenanthrene are not persistent (> 60 % in 28 days). Without the BIMs, these two substances would artificially appear as being more persistent than they would actually be in the environment.

A further conclusion is that the most suitable BIM seems to depend on the actual substance tested, which was not expected considering how closely related all of them are. For instance, both silicone oil and SiO_2 were effective when used with phenanthrene and 1-methylphenanthrene. However, in the case of 3,6-dimethylphenanthrene a noticeable difference is observed between BIMs, in that this substance is readily biodegradable when tested with SiO_2 but degrades very little if tested with silicone oil. Finally, one can observe how the BIMs had barely any effect on the degradation of 3-methylphenanthrene.

With regard to the extension of the test duration, although some differences are observed between the degradation values at 28 and 60 days, this does not change the conclusion of the tests in any case, so it is perhaps a less effective technique for overcoming bioavailability problems.

Conclusion

Concawe's experimental programme has enabled the generation of reliable results concerning the ability of PAHs to degrade in the environment, and the development of new strategies for overcoming the difficulties of testing hydrocarbons. The results of the 301 F tests described above have been submitted to ECHA's Petroleum and Coal stream Substances (PetCo) working group (in charge of the regulatory approach for petroleum substances), and will enable support for the lack of environmental concern for some hydrocarbon blocks commonly appearing in Concawe's substances. The learnings of this project will be applied in future environmental testing strategies.

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A novel approach for assessing the biodegradability of constituents in petroleum substances

Difficulty in generating biodegradation data on all petroleum substance constituents

Petroleum substances (PS) are derived from crude oil and contain hundreds to thousands of individual chemicals or constituents. They are considered UVCBs, i.e. substances of unknown or variable composition, complex reaction products or biological materials. PS must undergo environmental assessment as part of the REACH registration process to be marketed in the EU (European Union). This assessment includes information on the biodegradability of the chemical constituents in a substance, which determines whether a substance is considered persistent in the environment.

Regulatory-accepted biodegradation tests are typically performed on single chemicals in multiple environmental media, and the half-lives are compared against regulatory criteria for persistence determination.[1] However, the complex nature of PS UVCBs makes it challenging to evaluate the biodegradation of all the constituents. Performing a biodegradation test on the whole PS is possible; however, unless detailed analytical techniques are applied, the result would generally be the average biodegradation rate of all of the constituents. In such a case, a very persistent constituent could possibly be overlooked, which is not ideal from a regulatory perspective. Still, there are so many constituents in most PS UVCBs that testing them individually or even testing representative constituents would be time- and resource-intensive, especially as most of the constituents are not commercially available. However, as explained below, combining analytical techniques such as two-dimensional gas chromatography (GCxGC) with biodegradation testing on whole petroleum substances can generate persistence screening data for the constituents of PS more efficiently than relying on single constituent biodegradation testing.

Petroleum substance constituents can be separated using GCxGC

Petroleum substances that are refined or processed from crude oil are composed of hundreds to thousands of hydrocarbon constituents. Petroleum analytical chemists use GCxGC to separate the different constituents, which are then gauged by an appropriate detector for petroleum molecules, such as a flame ionization detector (FID).[2] The GCxGC technique separates constituents based on polarity in the first dimension and volatility in the second dimension; a conceptual diagram of the output is shown in Figure 1 on page 24. For PS, like the vacuum and hydrotreated gas oil (VHGO) and straight run gas oil (SRGO) investigated in this work, there are thousands of peaks on the chromatogram, indicating the presence of thousands of constituents.

Concawe is undertaking a novel project with SINTEF and other researchers to explore the use of analytical techniques to generate biodegradation data on multiple petroleum substance constituents simultaneously.

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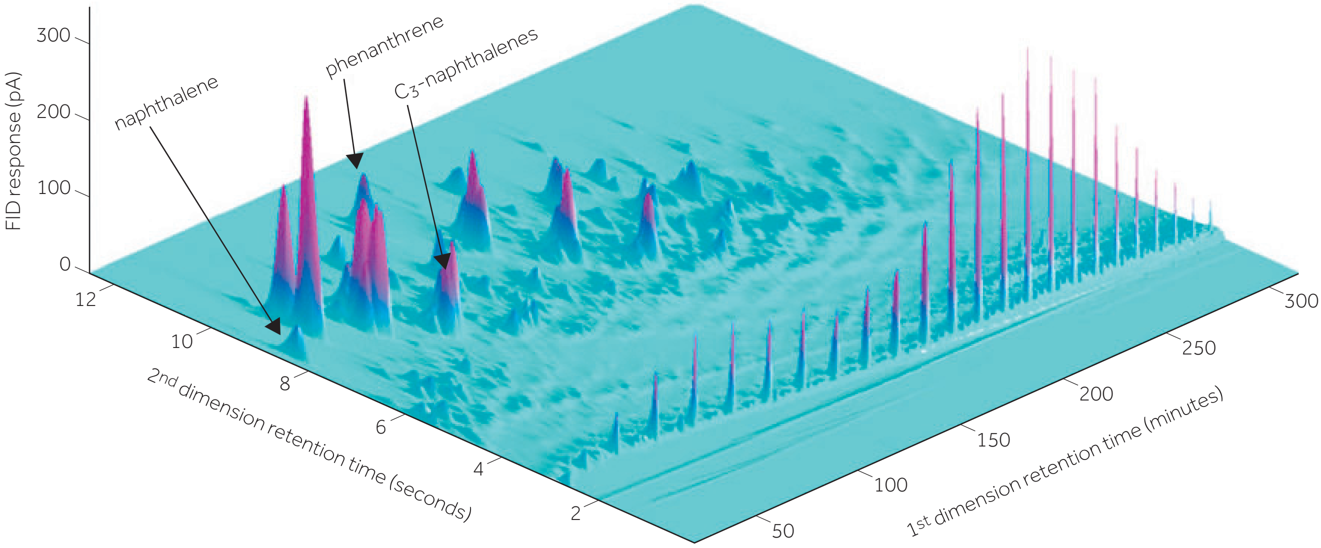
Figure 1: A visual diagram of a GCxGC-FID chromatogram[3]

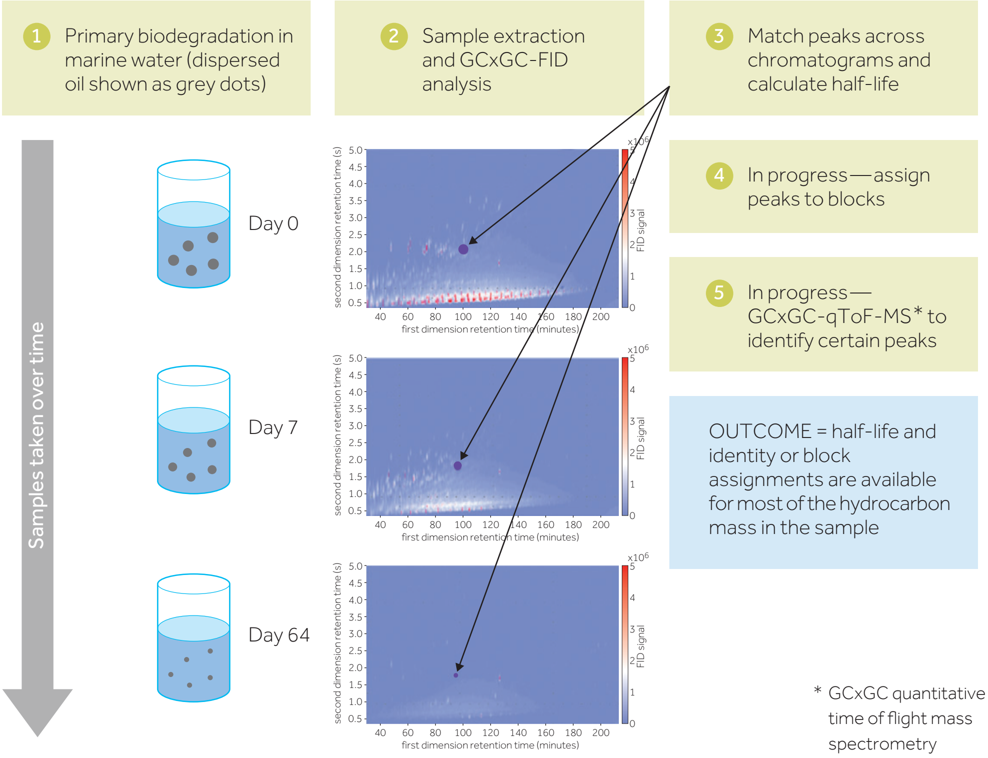


Pairing a whole PS biodegradation test with GCxGC analysis can give biodegradation information on PS constituents

To assess the biodegradability of the numerous constituents in PS, Concawe is working with several scientific research contractors (SINTEF, Jonas Gros and Oleolytics) to combine the biodegradation testing methodology with the capabilities of the GCxGC analytical technique plus data analysis, as summarised in Figure 2 on page 25. The objective of this study is to generate biodegradation information for the constituents in PS during whole petroleum substance biodegradation testing.

For simplicity, related constituents, which would come out close together on the chromatogram, can be grouped into 'hydrocarbon blocks'.[4] Samples of the test medium are taken at different times over the course of the biodegradation test (64 days) and analysed by GCxGC-FID to see which peaks and blocks are present and at what concentration.

Figure 2: Flow diagram for the SINTEF biodegradation peak tracking project



1 Primary biodegradation in marine water (dispersed oil shown as grey dots)

2 Sample extraction and GCxGC-FID analysis

3 Match peaks across chromatograms and calculate half-life

4 In progress—assign peaks to blocks

5 In progress—GCxGC-qToF-MS* to identify certain peaks

OUTCOME = half-life and identity or block assignments are available for most of the hydrocarbon mass in the sample

* GCxGC quantitative time of flight mass spectrometry

By tracking the peaks and blocks over time, as shown in Figure 2, it is possible to generate primary biodegradation half-life data for all of the trackable peaks and blocks. Only *primary* biodegradation half-lives are obtained and not complete mineralisation, as the GCxGC-FID is mostly capable of detecting PS but not its degradation products. This limits the regulatory application of these primary biodegradation half-lives; they can be used as screening data rather than full persistence assessment data. However, this process can identify constituents that show a long primary biodegradation half-life and which may need further assessment.

So far in the project, SINTEF Ocean has performed biodegradation testing similar to an OECD 306 Biodegradability in Seawater protocol,[5] which is a regulatory-accepted biodegradation screening test. SINTEF used natural seawater as the microbial inoculum in rotated closed bottles containing VHGO and SRGO at 13°C for a 64-day biodegradation test.

Samples were taken weekly or bi-weekly, and hydrocarbon material in the samples was extracted from the water samples and analysed by GCxGC-FID. At the end of the experiment (day 64), 73–81% of the overall VHGO mass and 84–88% of the overall SRGO mass had been degraded. The GCxGC chromatograms from the different sampling time points were then aligned using an alignment algorithm,[6] so that a peak representing the same constituent is able to be compared over the different chromatograms over time (see Figure 2). The alignment algorithm only works on peaks that are unequivocally the same constituent; thus not all the peaks on the chromatogram can be tracked. A little over half the mass of the VHGO or SRGO is made up of trackable peaks in this exercise.

A half-life for the tracked peak is calculated using the data from the GCxGC-FID, where peak size on the chromatogram is reflective of the relative concentration of that constituent(s) versus a non-biodegraded control peak (e.g. steranes). The half-lives were calculated by fitting to an exponential decay curve.

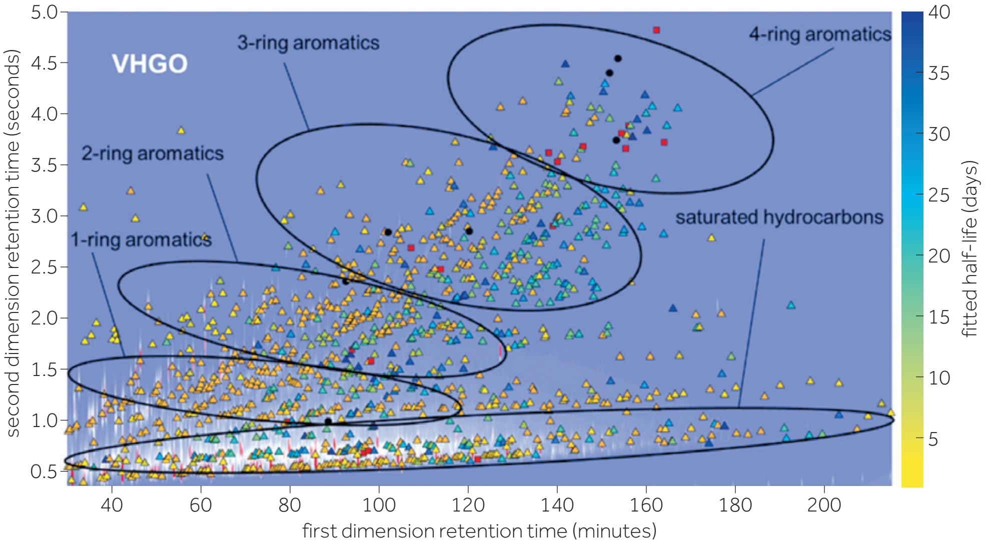
Ongoing and future work

In the first phase of this work, the focus was on individual tracked peaks representing constituents comprising half of the total mass of the VHGO or SRGO. Future and ongoing work is now focusing on two open questions:

1. How will we assess the biodegradation potential of the remaining mass not represented in the peak assessment; and
2. How can the peaks that require further assessment be identified?

For the first question, while about half of the mass is not trackable as individual peaks, the remaining mass is still detectable on the chromatogram (Figure 3).

Figure 3: Half-lives for the tracked peaks overlaid on the chromatogram of the fresh, non-biodegraded oil for VHGO



Notes:
The locations of general hydrocarbon classes are indicated on the chromatogram. Darker colours indicate longer half-lives (less biodegradable).

These non-tracked peaks can be grouped into hydrocarbon blocks, and a half-life for a block could be calculated. This is the same idea as the hydrocarbon blocking approach which has historically been used by Concawe to describe the composition of petroleum substances by grouping together similar constituents in a 'block'. This process is formalised in the Concawe PetroRisk tool,[1] which is widely used by the petrochemical industry to support REACH registrations. Concawe is now working with Oleolytics to determine a hydrocarbon blocking template to derive half-lives for the blocks.

For the second question, Concawe is also working with SINTEF-Ocean to perform GCxGC-quantitative time of flight mass spectrometry (GCxGC-qToF-MS) on the VHGO and SRGO samples to more confidently identify peaks. The use of the mass spectrometer detector will give information on the molecular structure of the constituents in certain peaks. The peak identification will be used to confirm which constituents need further assessment, and to verify that the hydrocarbon blocking approach is as accurate as possible.

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[1] https://www.concawe.eu/reach/petrorisk/

PetroRisk version 8.01: Improved model for performing REACH environmental risk assessments on petroleum substances

This article provides an overview of the improved PetroRisk model, which was released on 27 July.

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The REACH Regulation requires registrants to perform an environmental risk assessment (ERA) on each hazardous substance manufactured or imported above a total annual EU tonnage of 10 tonnes. An ERA compares the estimated concentration of the substance in the environment (based on its volume and emissions) with the toxicity of the substance; if the concentration in the environment exceeds the concentration at which it becomes toxic, then the registration is disallowed.[1]

Due to their UVCB[1] nature, petroleum substances (PS) necessitate a complex and tailored ERA approach: PS contain an indeterminably large and variable number of different hydrocarbon constituents, which all partition, degrade and exert toxicity differently once released to the environment. Initially, the ERA requires (a prediction of) the identity and concentrations of the constituents released to the environment (to air, water and soil) during each use of the PS. Subsequently, the fate of each emitted constituent in the environment is modelled separately, based on predicted constituent properties and 'generic' environmental conditions. Finally, the predicted concentrations in the different environmental compartments (such as marine sediment, or terrestrial worms) are compared to compartment- and constituent-specific toxicity thresholds (the predicted no-effect concentration), in the form of a 'risk characterisation ratio' (RCR, see equation below). Due to the additive nature of hydrocarbon toxicity (narcosis), the compartment-specific RCRs of the *n* constituents can be summed up to compartment-specific RCRs representing the entire PS:

$$\text{Risk characterisation ratio (RCR)} = \sum_{i=1}^{n} \frac{\text{Predicted environmental concentration (PEC)}_i}{\text{Predicted no-effect concentration (PNEC)}_i}$$

If all compartment-specific RCRs are equal to, or lower than, a value of 1, the risks for adverse environmental effects resulting from the use of the substance are considered adequately managed, and the use can be considered 'safe'. If one or more RCRs have a value above 1, then risk management measures need to be applied to reduce the emissions from use to 'safe' levels.

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[1] Chemical substances of unknown or variable composition, complex reaction products and biological materials

PetroRisk version 8.01: Improved model for performing REACH Environmental Risk Assessments on petroleum substances

To enable the execution of the ERAs for its 2010 product registrations, Concawe commissioned an Excel™-based tool, called PetroRisk. Major innovations compared to existing ERA tools include the following:

- The use of the so-called 'hydrocarbon block method', which extrapolates analytical data of a PS (for example two-dimensional gas chromatography (GCxGC)) to representative concentrations of hydrocarbon constituents.
- The use of a 'constituent library', which contains the required (predicted) physicochemical and degradation properties for 1,560 representative constituents.
- The use of a 'fate-factor' (FF) library. Each FF linearly extrapolates a specific emission (e.g. waste water) of a specific constituent (e.g. naphthalene) to the predicted concentration in a specific environmental compartment (e.g. marine sediment). The FF approach allows the user to 'shortcut' many calculations which are independent of PS composition, use conditions and emission levels.

For other stakeholders to benefit from PetroRisk, the model was made freely available on Concawe's website.

Since 2017, an increasing number of improvements to PetroRisk have been identified by the Concawe Secretariat, culminating in a complete reorganisation and optimisation of the model using a more transparent interface during 2021–2022.

Major innovations in the updated model are described below:

- A visual representation of the data flow (see Figure 1 on page 30), and the possibility to review the data after each calculation step.
- Full alignment of the basic ('Tier 1') model with the ECHA Guidance.
- Update of the embedded fate models (sewage treatment plant, regional environment and human exposure) and implementation of hydrocarbon-specific parameters.
- The application of a minimalistic FF concept, covering only the complex and extensive calculations of the fate in a sewage treatment plant and in the regional environment. All other calculations embedded in the previous FF concept have now been implemented as individual calculations in PetroRisk, thereby increasing the transparency and flexibility of the model.
- Robust calculation and facultative implementation of a minimal set of air and wastewater RMM[2] efficiencies required to achieve safe emission levels.
- Implementation of a batch mode, allowing the tool to consecutively execute ERAs for many substances. While previously two working weeks were required, the updated model requires about three hours to perform ERAs on all Concawe substances.

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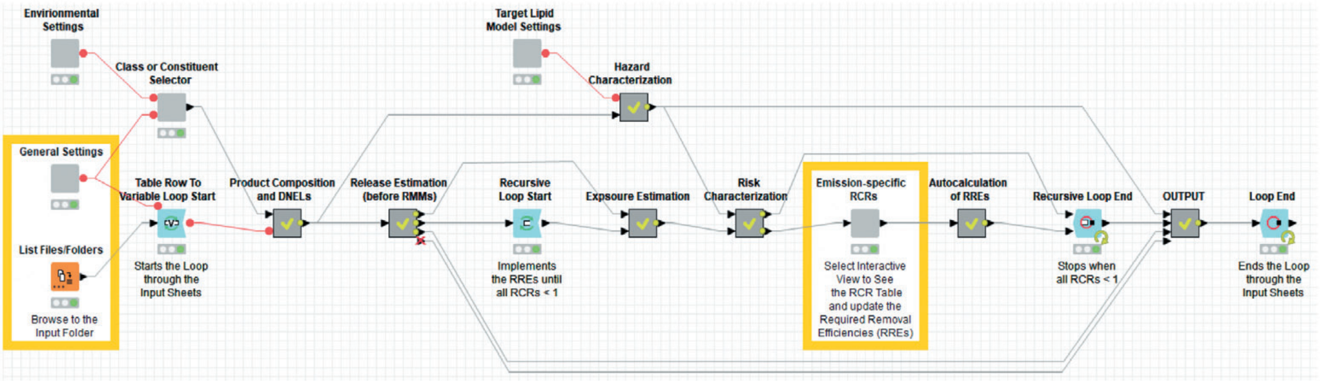
[2] Risk Management Measures

PetroRisk version 8.01: Improved model for performing REACH Environmental Risk Assessments on petroleum substances

Figure 1: The KNIME PetroRisk v8.01 workflow



PetroRisk has been programmed as a workflow in the Konstanz Information Miner (KNIME), which is an open source data analytics, reporting and integration platform based on Eclipse and written in Java. An intuitive drag and drop style graphical user interface allows visual assembly, execution and analysis of complex workflows without the extensive need for programming skills.

The updates result in a model which is (1) faster, (2) more transparent, (3) better aligned with ECHA Guidance, (4) more applicable to hydrocarbons and (5) more user-friendly. Compared to previous PetroRisk versions, version 8.01 generally predicts lower risks for professional and consumer uses, but higher risks for industrial uses.

PetroRisk version 8.01 was released on 27 July and is available free-of-charge from the Concawe website.[3] There have already been two Concawe training sessions on the general REACH ERA concept[4] and on the PetroRisk tool.[5] The tool will be accompanied by a detailed manual and a dedicated scaling tool. The scaling tool is a simple Excel™ workbook designed to aid registrants and downstream users in assessing whether their specific use conditions are covered by a generic exposure scenario generated with PetroRisk.

Reference

1. ECHA (2016). *Guidance on information requirements and Chemical Safety Assessment. Chapter R.16: Environmental exposure assessment.* Version 3.
https://echa.europa.eu/documents/10162/17224/information_requirements_r16_en.pdf/b9f0f406-ff5f-4315-908e-e5f83115d6af?t=1455553705739

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[3] https://www.concawe.eu/reach/petrorisk

[4] https://youtu.be/Icg3ANNiz2M

[5] https://youtu.be/nnP5tOtMSm8

Abbreviations and terms

BIM	Bioavailability Improvement Method
CAS	Chemical Abstract Service
DiAr	Di-Aromatics
DMSO	Dimethyl Sulphoxide
DN	Di-Naphthenes
DOC	Dissolved Organic Carbon
EC	European Commission
ECHA	European Chemicals Agency
ERA	Environmental Risk Assessment
EU	European Union
FF	Fate Factor
FID	Flame Ionisation Detector
FIMS	Field Ionisation Mass Spectrometry
GCxGC	Two-dimensional Gas Chromatography
GC-MS	Gas Chromatography Mass Spectometry
GTL	Gas-To-Liquids
HC	Hydrocarbon
HPLC	High-Performance Liquid Chromatography
iso-P	iso-Paraffins
KNIME	Konstanz Information Miner
MoAr	Mono-Aromatics
N	mono-Naphthenes
n-P	n-Paraffins
NAM	New Approach Methodology
NDiAr	Naphthenic Di-Aromatics
NmoAr	Naphthenic Mono-Aromatics
OECD	Organisation for Economic Co-operation and Development
OtherGO	Other Gas Oils
PAH	Polycyclic Aromatic Hydrocarbon
PBT	Persistent, Bioaccumulative and Toxic
PEC	Predicted Environmental Concentration
PetCo	Petroleum and Coal stream substances (working group)
PNEC	Predicted No-Effect Concentration
PS	Petroleum Substances
qToF-MS	Quantitative Time Of Flight Mass Spectometry
RBT	Ready Biodegradability Test
RCR	Risk Characterisation Ratio
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RMM	Risk Management Measures
SIMDIS-GC	Simulated Distillation Gas Chromatography
SiO_2	Silicone Dioxide
SIP	Substance Identity Profile
SRGO	Straight Run Gas Oil
TetraAr	Tetra-Aromatics
$ThCO_2$	Theoretical amount of Carbon Dioxide
ThOD	Theoretical Oxygen Demand
TriAr	Tri-Aromatics
UVCB	(Substances of) Unknown or Variable composition, Complex reaction products or Biological materials
VHGO	Vacuum gas oils, Hydrocracked Gas Oils and Distillate Fuels
vPvB	very Persistent and very Bioaccumulative

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