# 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.

[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.

[2] European Commission/Chemical Abstract Service

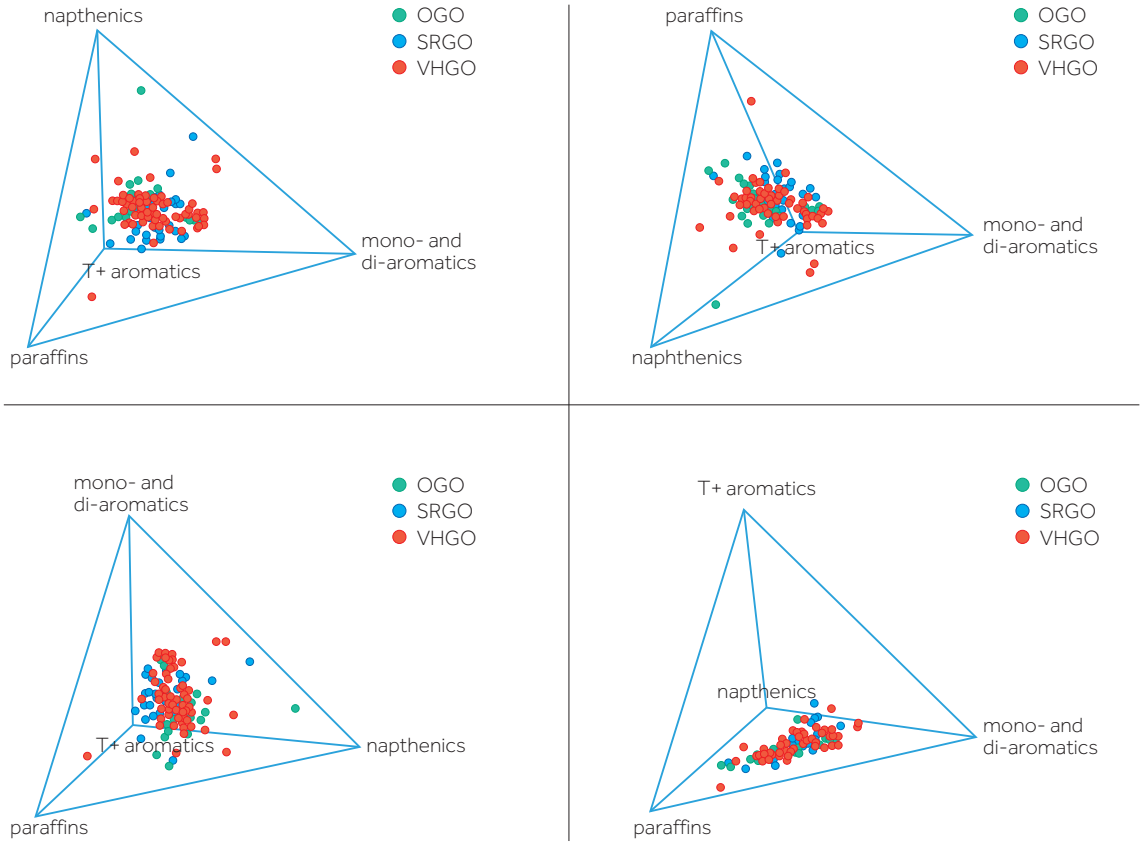
## Chemical similarity: hydrocarbon space mapping

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.

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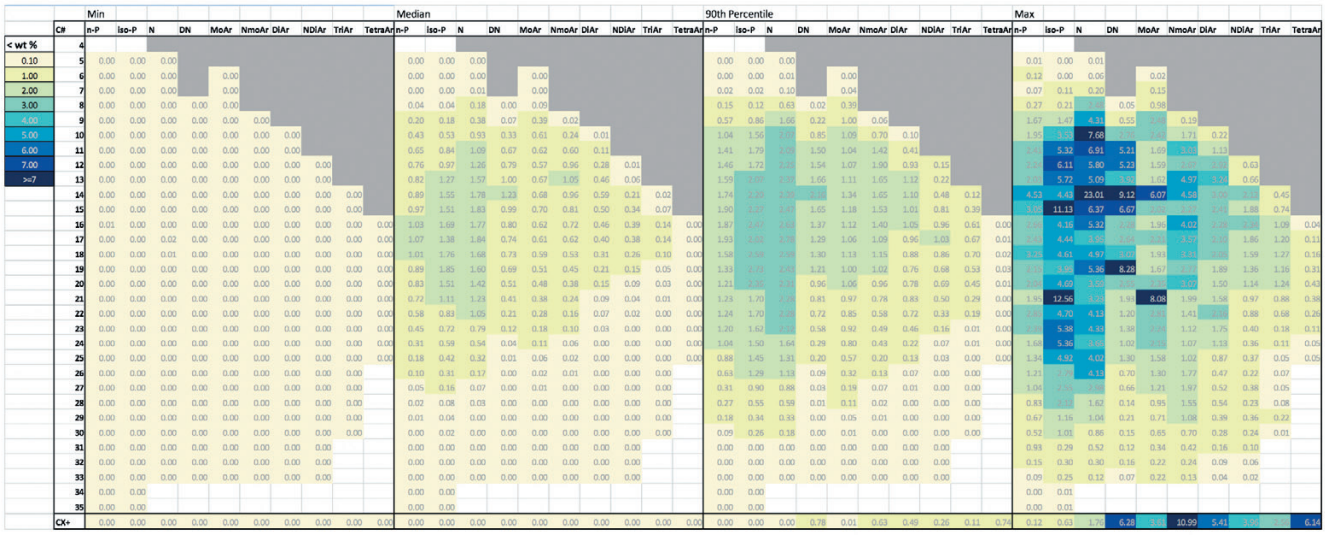
Figure 2: Concentration (weight %) of hydrocarbon blocks across all VHGO samples by GCxGC

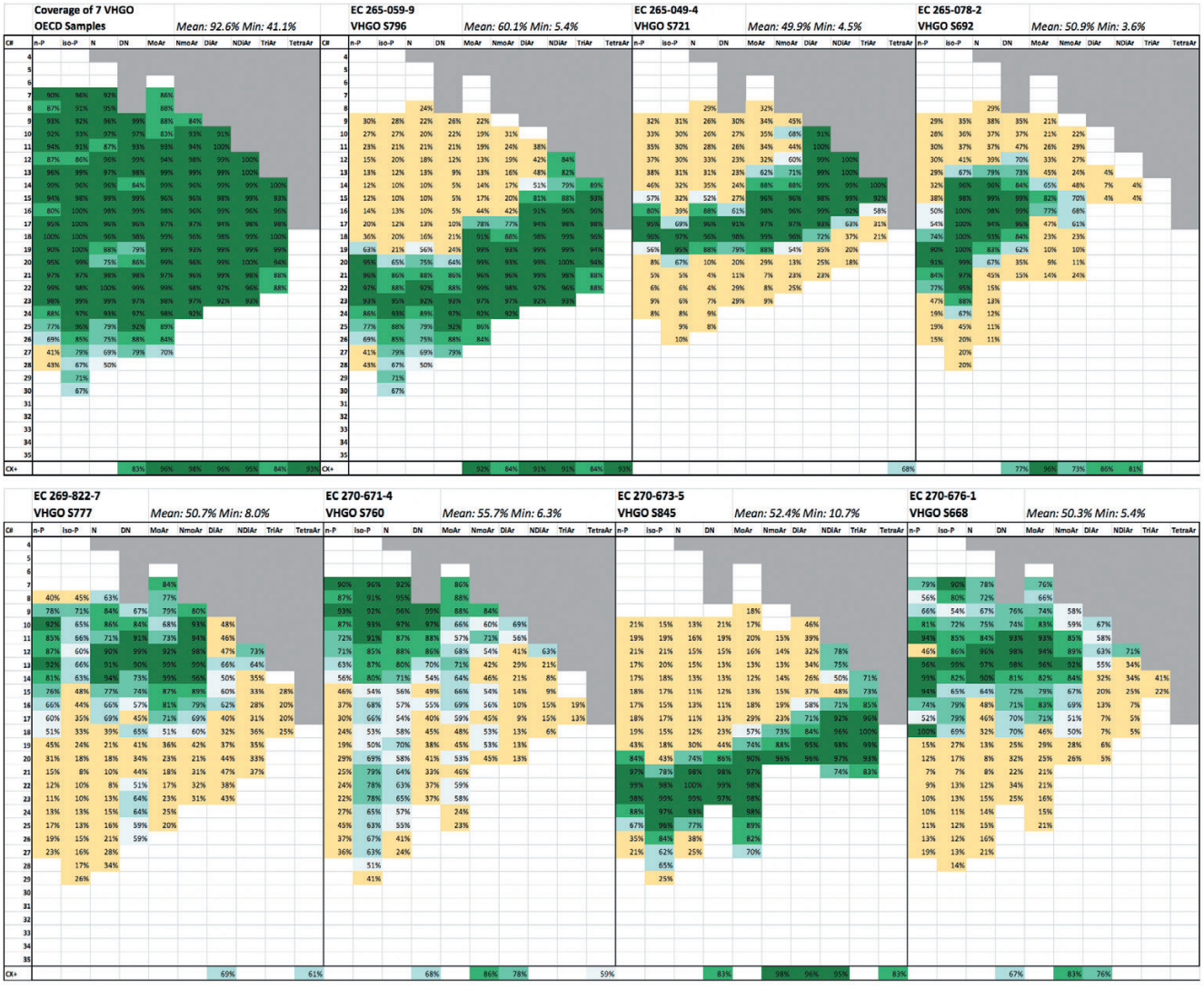



The normalised concentrations for each hydrocarbon block at 0% and 100% data define the applicability domain or boundary of substances within a category. It is within this compositional scope that read-across can be justified, providing that test samples, or combinations of samples, are representative 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.

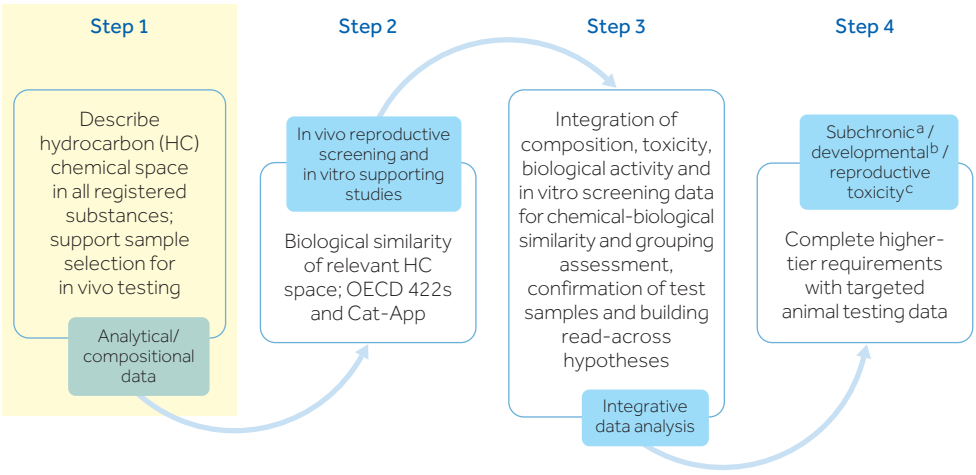
Figure 3: Coverage of VHGO hydrocarbon space with seven combined and individual selected samples



## 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.

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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.

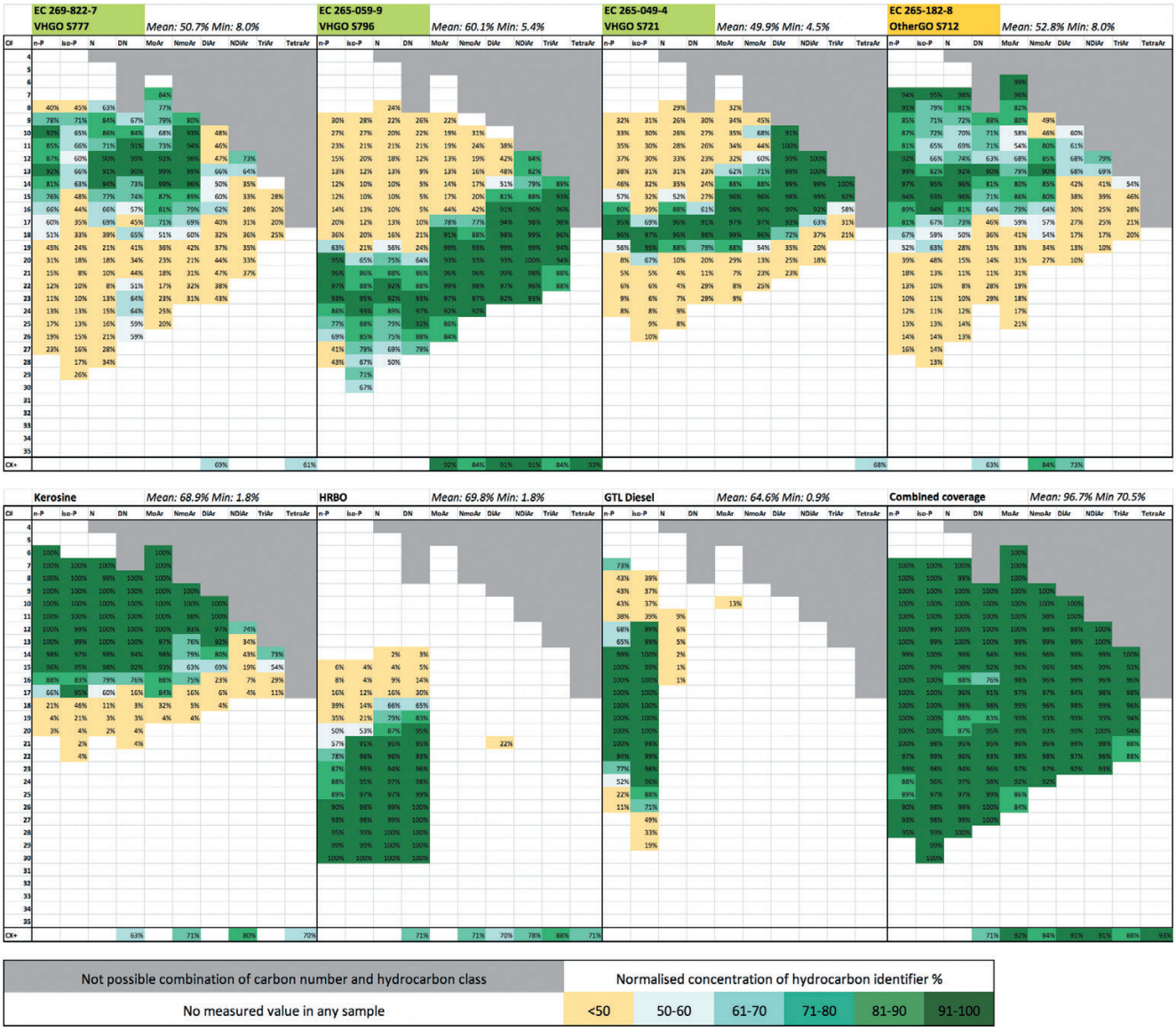
Based on structural similarity to support read-across to all VHGO substances, Concawe has proposed to ECHA in the testing proposal submitted in December 2021 to select three VHGO substances for higher-tier animal studies (see Appendix 1 on page 15 for a list of substance EC numbers, names and CAS numbers). 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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