CONSIDERATIONS OF ALTERNATIVE METHODS ON TESTING PROPOSALS IN YOUR REGISTRATION

Please complete this form and provide information for each of the points below.

If you have more than one testing proposal, please copy and paste the three bullet points within the same document and complete the details as appropriate for each testing proposal.

This document will be published on ECHA website along with the third party consultation on the testing proposal(s).

Public substance name: Linked to CONCAWE cases, status to be checked in 2018.
EC Number (omit if confidential): 941-364-9
CAS Number (omit if confidential): NS

Date of considerations: 8 June 2018

- **Hazard endpoint for which vertebrate testing was proposed:**

  Reproductive toxicity (extended one-generation reproductive toxicity study) with the [~~registered~~ / analogue] substance: Gas oils (petroleum), light vacuum, Gas oils (petroleum), light vacuum (CAS 64741-58-8, EC 265-059-9)

- **Considerations that the general adaptation possibilities of Annex XI of the REACH Regulation were not adequate to generate the necessary information** (instruction: please address all points below):

  - available GLP studies

    There are no available GLP studies within the vacuum gas oils, hydrocracked gas oils, and distillate fuels (VHGO) group,that fully address the reproductive toxicity endpoints needed for classification.

  - available non-GLP studies

    There are no non-GLP studies available for the endpoint toxicity to reproduction.

  - historical human data

    No relevant human health data are available.

  - (Q)SAR

    There are no QSAR's which are adequate to fully evaluate a UVCB substance (for more detailed explanation on [the complexity of] petroleum UVCBs see Section 2 of the document "VHGO EOGRTs study proposal ~~v9~~" attached below).

  - *in vitro* methods

    There are no validated in vitro methods that fulfil this endpoint.

Comentario [GCMJ1]: This substance forms part of the overall Concawe testing strategy for petroleum substances and the VHGO category (see attached below "VHGO EOGRTs study proposal").

- weight of evidence

  Not available according to ECHA guidance document (Chapter R.7a: Endpoint specific guidance Version 4.1 – October 2015).

- grouping and read-across

This EOGRT will be conducted on one representative worst-case sample from the CONCAWE's VHGO group to which belongs the CAS Number 64741-58-8 (based on PAC content, see Section 2 of the document "VHGO EOGRTs study proposal" for the testing hypothesis and Section 4 for selection of the worst-case test sample).

Historically data for toxicity assessment has been generated via the dermal and inhalation route to replicate the route of human exposure to petroleum substances. However, to comply with regulatory requirements for hazard identification, oral exposure is required. Therefore, part of informed testing strategy "VHGO EOGRTs study proposal" is to supplement these historical data with targeted oral studies: the range-finding work to be conducted for this EOGRTS will be conducted as an OECD 422 guideline study (oral) which, together with OECD 422 data from other category members, will be used to inform repeated dose toxicity endpoints via the oral route (also underpinning the exposure route / dermal database) and further support grouping & read-across in the group.

- substance-tailored exposure driven testing [if applicable]

  Not applicable

- [approaches in addition to above [if applicable]

  Not applicable

- other reasons [if applicable]

  Not applicable

- **Considerations that the specific adaptation possibilities of Annexes VI to X (and column 2 thereof) were not applicable** (instruction: free text):

Adaptation options as defined in Annexes VI to X were not applicable for this group and this endpoint. Regarding the column 2 rules for adaption, the data base was fully evaluated according to 'Guidance on information requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance'.

FURTHER INFORMATION ON TESTING PROPOSAL IN ADDITION TO INFORMATION PROVIDED IN THE MATERIALS AND METHODS SECTION:
VHGO's are widely used as fuels, but do not have significant nonfuel widespread use. Some VHGO's contain 4 – 7 ring PACs which are believed to interact with the Aryl Hydrocarbon (Ah) receptor which may then lead to modifications of the estrogen receptor pathway; the rationale for this hypothesis and the data to support it are described in detail in section 2 of

the document "VHGO EOGRTs study proposal" . As the available data base is limited and because the potential role of PAC's in effects on fertility is not fully understood it is proposed that the study includes cohort 1B (second generation) to fully evaluate the effects on the developing offspring.

There are no pathological changes in the nervous system or CNS type clinical signs in the current database of dermal studies. However, in one inhalation study on a similar petroleum substance a minor effect on startle response was observed which may be indicative of neurotoxic effects. Due to uncertainties in the data base it is proposed that cohort 2 is included.

VHGOs are classified as H373: May cause damage to thymus, liver, and bone marrow through prolonged or repeated exposure, suggesting organs associated with the immune system are a target for toxicity. It is therefore proposed that cohort 3 for immunotoxicity is also included.

-Overall
With the aim to minimize -and avoid unnecessary- animal testing while not underestimating the potential human health risks, a worst case testing approach and overall informed tiered testing strategy is proposed holistically across the entire portfolio of Concawe petroleum substances. Targeted CAS numbers will be subject to in-vivo testing covering all Concawe petroleum categories.

The sample selected for this test to cover the Concawe's VHGO category is the CAS number with the highest % (w/w) of 4 – 7 ring PAC's, as it is considered that this has the greatest potential for reproductive and developmental toxicity (see grouping of VHGO substances and testing hypothesis in section 2 of the document "VHGO EOGRTs study proposal" attached below). The results of this test, supported with other targeted oral studies, historical data and mechanistic data from in-vitro assays, will be used as read-across for the remaining Concawe's VHGO group members therefore avoiding additional high animal consuming in vivo studies.

The proposed EOGRTS on VHGOs is part of the overall informed testing strategy (see the document "VHGO EOGRTs study proposal" attached below). The results of this study is expected to help inform the decision making on potential further testing needs and design of similar studies which are required for other petroleum categories. The basic types of hydrocarbon molecules found in this substance and related petroleum category (ie straight and branched alkanes and alkenes, cycloalkanes and cycloalkenes, aromatic and aromatic cycloalkanes) are found in other petroleum categories and, although petroleum substances are UVCB substances of limited variability within product specifications, their similarities can help define a targeted approach to minimize animal use in further testing.

VACUUM GAS OILS, HYDROCRACKED GAS OILS, AND DISTILLATE FUELS (VHGO) TESTING PROPOSAL FOR THE EXTENDED ONE GENERATION REPRODUCTIVE TOXICITY STUDY (EOGRTS)

Contents

1. Consideration of non-animal methods

For all testing proposals submitted since 11 September 2015 ECHA has sent a request asking registrants to inform ECHA of 'their considerations of alternative methods to support their testing proposal involving vertebrate animals'. This information will be published together with the testing proposal on ECHA' s website. The following section follows the format of ECHA's questions and addresses each of these for this study.

Hazard endpoint for which vertebrate testing was proposed:

Reproductive toxicity (extended one-generation reproductive toxicity study) with the registered substance

Considerations that the general adaptation possibilities of Annex XI of the REACH Regulation were not adequate to generate the necessary information (instruction: please address all points below):

· available GLP studies
There are no available GLP studies within the vacuum gas oils, hydrocracked gas oils, and distillate fuels (VHGO) group that fully address the reproductive toxicity endpoints needed for classification.

· available non-GLP studies
There are no non-GLP studies available for the endpoint toxicity to reproduction.

· historical human data
No relevant human health data are available.

· (Q)SAR
There are no QSAR's which are adequate to fully evaluate a UVCB substance (for more detailed explanation on [the complexity of] petroleum UVCBs is referred to section 2).

· *in vitro* methods
There are no validated *in vitro* methods that fulfil this endpoint. An academic research programme applying an *in vitro* battery of developmental toxicity screening assays has been launched to underpin the reprotoxicity testing hypothesis that 4-7 ring Poly Aromatic Compounds (PAC) are the only constituents in petroleum substances that are associated with prenatal developmental toxicity (see section 2 for the testing hypothesis, a summary of this in-vitro project and some first published data). These data will be used in combination with *in vivo* data to further underpin grouping of these substances, in order to minimize animal testing as it supports the selection of the most representative worst case group member for testing and read-across of this test outcome to the other group members.

· weight of evidence
Not available according to ECHA guidance document (Chapter R.7a: Endpoint specific guidance Version 4.1 – October 2015). Concawe are undertaking a multi-year research project to support the grouping of petroleum substances for human health risk assessment (see below) and the data being generated from the various assays applied in this- and other ongoing projects may allow more substantial weight of evidence arguments to be developed in the future.

· grouping and read-across
This EOGRT will be conducted on one representative worst-case sample from the VHGO group (based on PAC content, see Section 2 of this testing proposal for the testing hypothesis and Section 4 for selection of the worst-case test sample). An ongoing research programme, in which high-content in-vitro screening assays and transcriptomic data in combination with phys/chem, analytical chemistry and already available in-vivo data are applied in an integrative analysis to further underpin grouping (www.concawe.eu/cat-app-project), will strengthen the read across assessment from the tested worst-

case substance to the other group members. This is expected to significantly reduce the number of tests in vertebrate animals compared to conducting Individual substance evaluations.

There are 9 CAS numbers in the VHGO group. Historically data for toxicity assessment has been generated via the dermal and inhalation route to replicate the route of human exposure to petroleum substances. However, to comply with regulatory requirements for hazard identification, oral exposure is required. Therefore, part of Concawe's informed testing strategy is to supplement these historical data with targeted oral studies: the range-finding work to be conducted for this EOGRTS will be conducted as an OECD 422 guideline study (oral) which, together with OECD 422 data from other category members, will be used to inform repeated dose toxicity endpoints via the oral route (also underpinning the exposure route / dermal database) and further support grouping & read-across in the group.

· substance-tailored exposure driven testing [if applicable]
Not applicable

· [approaches in addition to above [if applicable]
Not applicable

· other reasons [if applicable]
Not applicable

· **Considerations that the specific adaptation possibilities of Annexes VI to X (and column 2 thereof) were not applicable:**

Adaptation options as defined in Annexes VI to X were not applicable for this group and this endpoint. Regarding the column 2 rules for adaption, the data base was fully evaluated according to 'Guidance on information requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance'.

VHGO's are widely used as fuels, but do not have significant nonfuel widespread use. Some VHGO's contain 4 – 7 ring PACs which are believed to interact with the Aryl Hydrocarbon (Ah) receptor which may then lead to modifications of the estrogen receptor pathway; the rationale for this hypothesis and the data to support it are described in detail in section 2 of this testing proposal. As the available data base is limited and because the potential role of PAC's in effects on fertility is not fully understood it is proposed that the study includes cohort 1B (second generation) to fully evaluate the effects on the developing offspring.

There are no pathological changes in the nervous system or CNS type clinical signs in the current database of dermal studies. However, in one inhalation study on a similar petroleum substance a minor effect on startle response was observed which may be indicative of neurotoxic effects. Due to uncertainties in the data base it is proposed that cohort 2 is included.

VHGOs are classified as H373: May cause damage to thymus, liver, and bone marrow through prolonged or repeated exposure, suggesting organs associated with the immune system are a target for toxicity. It is therefore proposed that cohort 3 for immunotoxicity is also included.

- **Overall**

With the aim to minimize -and avoid unnecessary- animal testing (on all 192 petroleum substances) while not underestimating the potential human health risks, a worst case testing approach and overall informed tiered testing strategy is proposed holistically across the entire portfolio of petroleum substances. Targeted CAS numbers will be subject to in-vivo testing covering all Concawe petroleum categories.

The sample selected for this test to cover the VHGO category is the CAS number with the highest % (w/w) of 4 – 7 ring PAC's, as it is considered that this has the greatest potential for reproductive and developmental toxicity (see grouping of VHGO substances and testing hypothesis in section 2). The results of this test, supported with other targeted oral studies, historical data and mechanistic data from

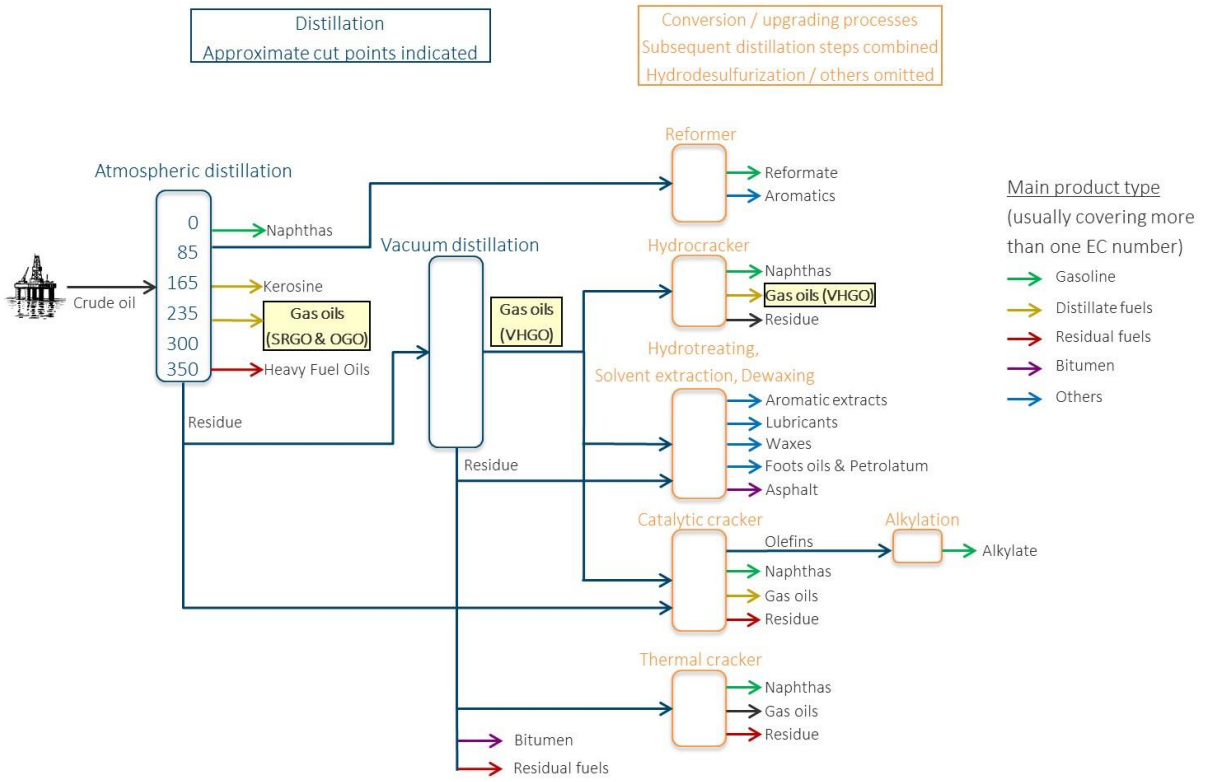
in-vitro assays, will be used as read-across for the remaining VHGO group members therefore avoiding additional high animal consuming in vivo studies.

The proposed EOGRTS on VHGOs is part of the overall informed testing strategy (see section 13 of the dossier). The results of this study is expected to help inform the decision making on potential further testing needs and design of similar studies which are required for other petroleum categories. The basic types of hydrocarbon molecules found in this substance and related petroleum category (ie straight and branched alkanes and alkenes, cycloalkanes and cycloalkenes, aromatic and aromatic cycloalkanes) are found in other petroleum categories and, although petroleum substances are UVCB substances of limited variability within product specifications, their similarities can help define a targeted approach to minimize animal use in further testing.

2. Background on the category of VHGO and the known reproductive toxicity of the components

Crude oil (Petroleum, CAS 8002-05-9) is a complex combination of hydrocarbons, a so called UVCB (unknown variable composition, complex reaction products, biological materials) extracted in its natural state from the ground. It consists predominantly of aliphatic, alicyclic and aromatic hydrocarbons. It is used as a feedstock for petroleum refining operations, which separate and convert it into UVCB fractions (streams) as shown in the diagram below. Gas Oils (SRGO, OGO and VHGO – the latter produced by vacuum distillation; all gas oils have an approximate carbon range of C9-C30 with SRGO at the lighter- and VHGO at the heavier end) are one of these fractions and are consistently distilled between the lighter stream (lower boiling pt) of Kerosine and the heavier stream (higher boiling pt) of Fuel Oil. Therefore only the hydrocarbon constituents present in crude oil that come off at this specific boiling point range will be present in VHGO with some overlap between neighbouring streams. They will share some common molecule types, which can give us some indication to the reproductive toxicity potential of the VHGO's when this information is available for these streams.

Simplified refining scheme – Relevant Gas oils are highlighted



The components found in other petroleum streams will be further discussed below in the light of endpoints relevant for reproductive toxicity. However, it is important to note that VHGO are not intentional mixtures of chemicals but are *substances* comprising of complex combinations of hydrocarbon species (i.e., UVCB substances), produced to meet physical-chemical and technical performance specifications. As explained above the domain of this category is established by the refining processes by which the category members are produced and therefore described by the corresponding boiling point / carbon number ranges. See Category Justification Document for more details, and the section "Testing Proposal Hypothesis" for more information about the justification for read across to the other CAS numbers in the VHGO category.

VHGO substances are used to manufacture distillate fuels (automotive diesel fuels, home heating oils, marine gas oils), as petrochemical intermediates or as components of formulated lubricants and additives (see Concawe identified uses of petroleum substances document: https://www.concawe.eu/wp-content/uploads/2017/05/Uses_Map_for_Website-21_December_2016.pdf). The only relevant routes of exposure for VHGO, as with most petroleum products especially those which are mainly used as fuels, are considered to be dermal and inhalation.

Hydrocarbon Classes and Relevance for Reproductive Toxicity

The registered members of the VHGO category comprise complex combinations of hydrocarbon constituents. Based on boiling point distribution, the hydrocarbons present will be predominantly in the range of C9-C30 (boiling in the range of 160-450^{0}C) and can be broadly characterized into a number of distinct hydrocarbon classes, namely

- **Aliphatics**, consisting of paraffinics (n-alkanes), iso-paraffinics (branched alkanes), naphthenics (cycloalkanes), and
- **Aromatics**, consisting of mono-aromatics, di-aromatics and poly-aromatics.

In general, characterization of the toxicity of hydrocarbon UVCBs, including VHGO, by summing the contributions of the individual constituents is not feasible because of the very large number of individual hydrocarbons and their isomers present (thousands to millions), which cannot be identified at the individual constituent level with available analytical technology.

Gas Oils share some of the hydrocarbon characteristics of other neighbouring petroleum streams, and consideration of the hydrocarbon types and toxicity of other streams can provide some information as to the expected reproductive toxicity of vacuum gas oils, hydrocracked gas oils, and distillate fuels (VHGO).

Aliphatics *(paraffinics, iso-paraffinics and naphthenics)*

The aliphatic constituents (saturates) found in VHGOs **paraffinics, iso-paraffinics** and **naphthenics** predominantly in the range of C9-C30. The studies on hydrocarbon UVCBs summarised below show that these three classes of aliphatic hydrocarbons in this carbon range can be considered together as they do not cause any reproductive toxicity effects based on the studies below:

- C8-C26 GTL (gas-to-liquid) gas oil (CAS 848301-67-7) does not originate from crude oil but is a synthetic gas oil prepared from natural gas by Fischer-Tropsch synthesis, consisting primarily of **(iso)paraffinics** (99+%) with small amounts (<1%) of **naphthenics**, but totally devoid of aromatics [PAC]): There was no reproductive toxicity in rats following oral administration in a two-generation reproductive toxicity study [OECD 416] (Shell, 2011a) and a prenatal development study [OECD 414] (Boogaard 2017). Essentially this is a gas oil with similar aliphatic components to the 'other gas oils' group, but without the polyaromatic hydrocarbons that are found in crude oil; this allows us to propose that the aliphatic components of VHGO's are not associated with reproductive toxicity.
- C9-C12 (predominantly) aliphatics (CAS 64742-81-0); **80% aliphatics**/20% aromatics of which the majority of the aromatics were alkylated single ring compounds (in round numbers 17-18%) with the **naphthenics** comprising the remaining 2-3%: No treatment-related effects on any reproductive or developmental parameters in a reproductive/developmental toxicity screening study [OECD 421] (Schreiner et al., 1997)*. This substance is a Kerosene. Kerosenes do contain some PAH's but these are limited because 3 to 7 fused-ring PAH's have a boiling point which is above the boiling point range of straight-run kerosene streams. Kerosenes do contain

1 – 3 ring PAH's, and in this screening study these were not associated with any indication of reproductive or developmental toxicity.

- C9-C16 (predominantly) aliphatics (CAS 8008-20-6 MIL-T-83133A); **80% aliphatics**/20% aromatics of which the majority of the aromatics were alkylated single ring compounds (approximately 17-18%) with the naphthenics comprising the remaining 2-3%: No reproductive toxicity in rats following oral administration in one generation reproductive toxicity tests in which male and female rats were assessed separately [similar to OECD 415] (Mattie et al., 2000)*. This substance is another Kerosene which again demonstrates a lack of effect of aliphatics and 1 – 3 ring PAH's.
- C20-C30 Highly refined base oils have no detectable aromatics (CAS 8042-47-5 and 8012-95-1): No target organ [OECD 408] (McKee et al, 2012a) or developmental effects [OECD 414 and 415] (McKee et al, 1987a, b; Mobil 1987a, b)*. Highly refined base oils have a very low/no PAH content, they are subject to a number of additional refining procedures and comprise mainly of saturated hydrocarbons (**paraffinics**), branched alkanes (**isoparaffinics**), cycloalkanes (**napthenics**)), providing additional proof that aliphatics are not associated with developmental effects.
- C18-C50 GTL base oil (CAS 848301-69-9; is a synthetic base oil prepared by Fischer-Tropsch synthesis, consisting primarily of **(iso)paraffinics** with small amounts (<5%) of **naphthenics**, but totally devoid of aromatics): No reproductive toxicity was observed in rats following oral administration in a two-generation reproductive toxicity study [OECD 416] (Shell, 2011b) and a prenatal development study [OECD 414] (Boogaard 2017). This also demonstrates the absence of reproductive toxicity in the absence of PAHs.

In conclusion, the available data indicate that the aliphatic constituents of gas oils are not developmental toxicants, do not affect fertility, and do not produce reproductive organ toxicity. (Shell, 2011a; McKee et al., 2012b).

Aromatics

Aromatics consist of mono-aromatics, di-aromatics and poly-aromatics. They occur naturally in crude oil and their distribution in petroleum streams depends on their boiling point and the down-stream processes applied. Poly-aromatic hydrocarbons (PAHs) have a conjugated hydrocarbon ring structure and can include other groups such as alkyl, nitro and amino groups and other elements such as nitrogen, sulphur or oxygen. PAHs are of particular concern as historically certain PAH'sare considered to be associated with a number of health and environmental toxicities of which benzo[a]pyrene is the best known example.

The reproductive and developmental toxicity of PAHs has been reviewed by IARC (1983), ATSDR (1995), IPCS (1998) and most recently by the EU Scientific Committee on Food (2002). PAHs are lipid soluble and are absorbed through biological membranes. Some PAHs, eg., benzo[a]pyrene, have been shown to cross the placenta, are found in foetal tissue and can be metabolically activated by the foetus (Autrup et al., 1996).

Experimental reproductive and developmental toxicity data are available for benzo[a]pyrene and naphthalene.

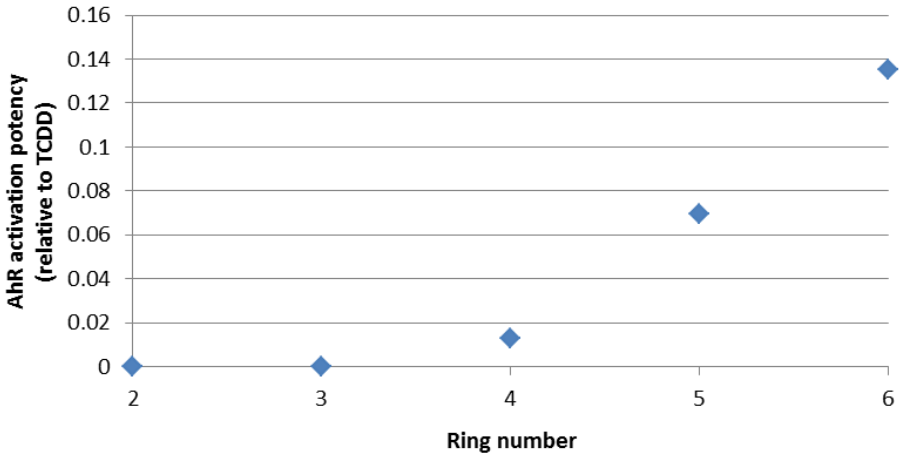
- At relatively high oral doses of benzo[a]pyrene, foetal effects have been reported in a number of studies (Hoshino et al., 1981; Bui et al., 1986; Mackenzie et al., 1981). Benzo[a]pyrene is recognized as a transplacental carcinogen (IARC 1993) and is also classified by the EU as a reproductive and developmental toxicant.

- In developmental toxicity studies (Plasterer et al. 1985, Hardin 1987, NTP 1991, NTP 1992, Hardin et al., 1981) naphthalene, a C10 aromatic hydrocarbon, was not a developmental toxicant.

The reprotoxic properties (developmental toxicity) of PACs are hypothesized to be attributed to their interaction with the Aryl Hydrocarbon (Ah) receptor. While the precise mechanisms are not completely understood, the Ah receptor activation may mediate reprotoxicity via (1) inhibitory cross talk with the estrogen receptor pathway (ER) or (2) the induction of specific isozymes of the CYP450 family which may lead to increased oxidative metabolism of polycyclic compounds and subsequent formation of DNA or protein adducts or (3) redirecting the Ah Receptor transcriptional machinery from its endogenous functions, which remain poorly understood (Chaloupka et al., 1994; Machala et al., 2001; Kizu et al., 2003; Kummer et al., 2008, 2013; Balabanic et al., 2011).

However, not all PACs interact with the Ah receptor, nor does oxidative metabolism of all PACs lead to adduct formation. A closer look into the available scientific data indicates that the smaller, 2-ring (naphthalene, Ziccardi et al., 2002), 3-ring polycyclic hydrocarbons and -heterocyclic hydrocarbons (acenaphthylene, acenaphthene, fluorene, phenanthrene, dibenzofuran) do not bind to the Ah receptor (Chaloupka et al., 1994) as summarized in Figure 1.

Figure 1. Potency of PACs with different ring numbers to activate the Ah-receptor (Figure is based on data obtained from Machala et al., 2001 and Ziccardi et al., 2002).



Further support for the PAC reprotox hypothesis from petroleum substances

As indicated earlier, information from other petroleum streams can be helpful to further support the testing hypotheses based on the common molecules present across petroleum substances. Analysis of data on 13 petroleum refinery streams indicated that, regarding developmental toxicity, increased fetal death and resorption along with reduced fetal weights were associated with content of poly-aromatics with ≥4 rings (mainly 4-7 ring PAC's), (Feuston et al., 1994). Further building on the Feuston study, the American Petroleum Institute (API 2008, White 2012) developed a statistical regression model for pre- and post-natal developmental toxicity and systemic toxicity of higher boiling point petroleum substances, based on the profile of 1- to 7-ring aromatic compounds, present in a number of high boiling point UVCB petroleum substances (crude oil, gas oils, heavy fuel oils, lubricating oils and aromatic extracts) that were also tested in rat studies. Predicted dose response curves with an accuracy > 95 % were derived for maternal thymus weight, fetal body weight, live foetuses/litter and percentage resorptions, based on the 1 – 7 PAC profile. The model was subject to vigorous peer review (Patterson et al 2013) and as a result some changes were made and additional data incorporated. Refinement of the developmental toxicity relationship was made and work published by Murray et al

(2013), found a strong correlation between the predicted vs. observed values for specific sensitive endpoints (percent resorptions, r = 0.99; live fetuses per litter, r = 0.98; fetal body weight, r = 0.94). The model was based on 21 rat dermal developmental toxicity studies and results were compared to PAC content (only 1 – 7 ring without specifying individual PACs).

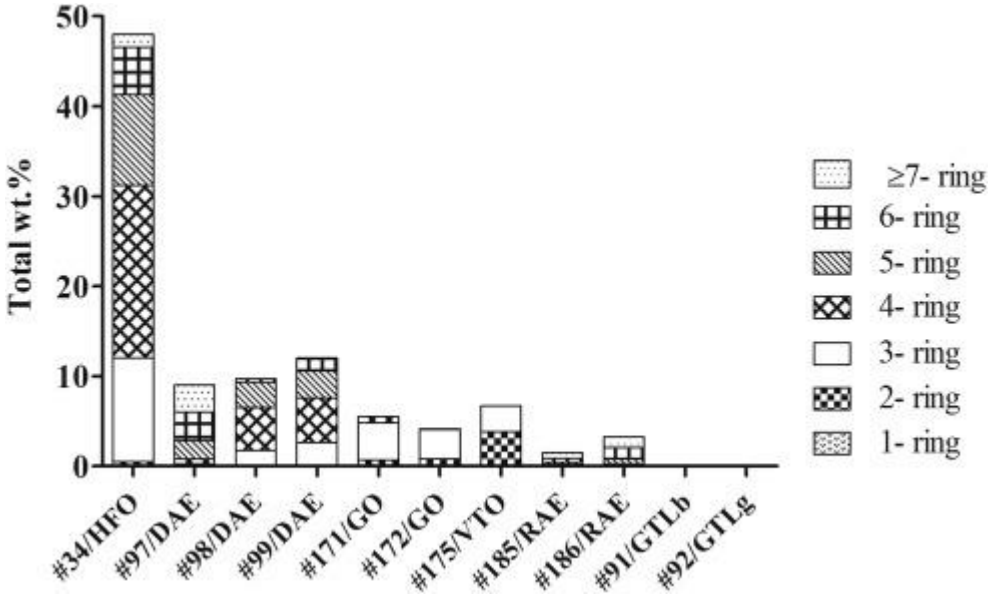
In addition, two of Concawe's ongoing research projects are relevant in this context:

One research project further investigates the potential role of the AhR in reprotoxicity of PACs as well as petroleum products in general. In this project, wild-type and AhR knock-out Sprague-Dawley rats are exposed orally to known developmental toxicants (Benzo(a)Pyrene and distillate aromatic extracts), and evaluated for developmental toxicity parameters. In addition, selected tissues are saved for further toxicogenomics analyses to obtain additional mechanistic insights into the role of the AhR in reprotoxicity. This work is in progress and results are expected in the course of 2018.

The other academic research programme aims at applying an in vitro battery of developmental toxicity screening assays to underpin the hypothesis that 3-7 ring PAH are the only constituents in petroleum substances that are associated with prenatal developmental toxicity (PDT). The first part of this work has recently been published (Kamelia et al 2017) and involved the evaluation of in vitro embryotoxicity with a number of petroleum substances. Substances from different petroleum streams were evaluated in the embryonic stem cell test (EST) which is a validated in-vitro test for embryotoxicity. In order to assess the applicability of the EST to predict in vivo PDT of petroleum substances, the BMCd50 values from ES-D3 cell differentiation assay were compared with BMD10 values derived from the in vivo PDT data.

The study investigated the petroleum streams shown in Figure 2 (HFO – heavy fuel oil; DAE - distillate aromatic extracts; GO – Gas oil; VTO- vacuum tower overheads; RAE; residual aromatic extracts and GTL – gas-to-liquid gas oil). The GTL products are synthetic analogues of petroleum substances, which are devoid of aromatic compounds (i.e. PAH's) and they do not induce any effect in PDT studies in vivo. Tests were done on a DMSO-soluble extract.

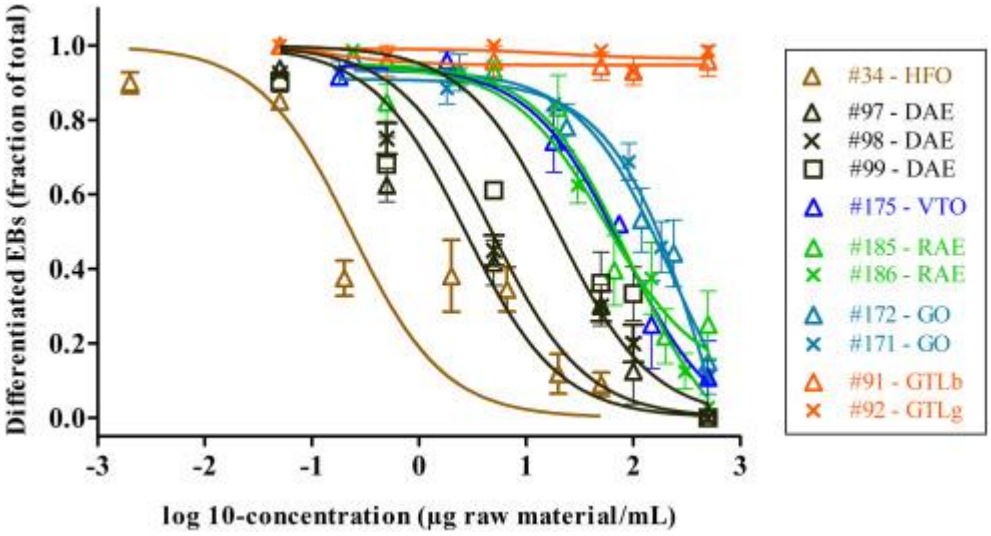
Figure 2: Aromatic Ring Class (ARC) profiles of petroleum substances and GTL products (DMSO extract)



In the first experiment, the DMSO-extract of the petroleum substances and the GTL products had no effect on the viability of ES-D3 cells over 1 – 5 days; the highest concentration tested was 500 µg raw material/mL except for HFO where it was 50 µg raw material/mL (due to solubility).

However, despite the absence of cytotoxicity the petroleum substance extracts did effect the differentiation of ES-D3 cells into contracting cardiomyocytes. A review of the concentration response curves (fig 3) shows that among all samples tested, HFO was the most potent in inhibiting the differentiation of ES-D3 cells, followed by DAE, VTO, RAE, and GO. It can be seen in figure 3 that without the presence of PAH (i.e. the GTL products) there is no inhibition of differentiation.

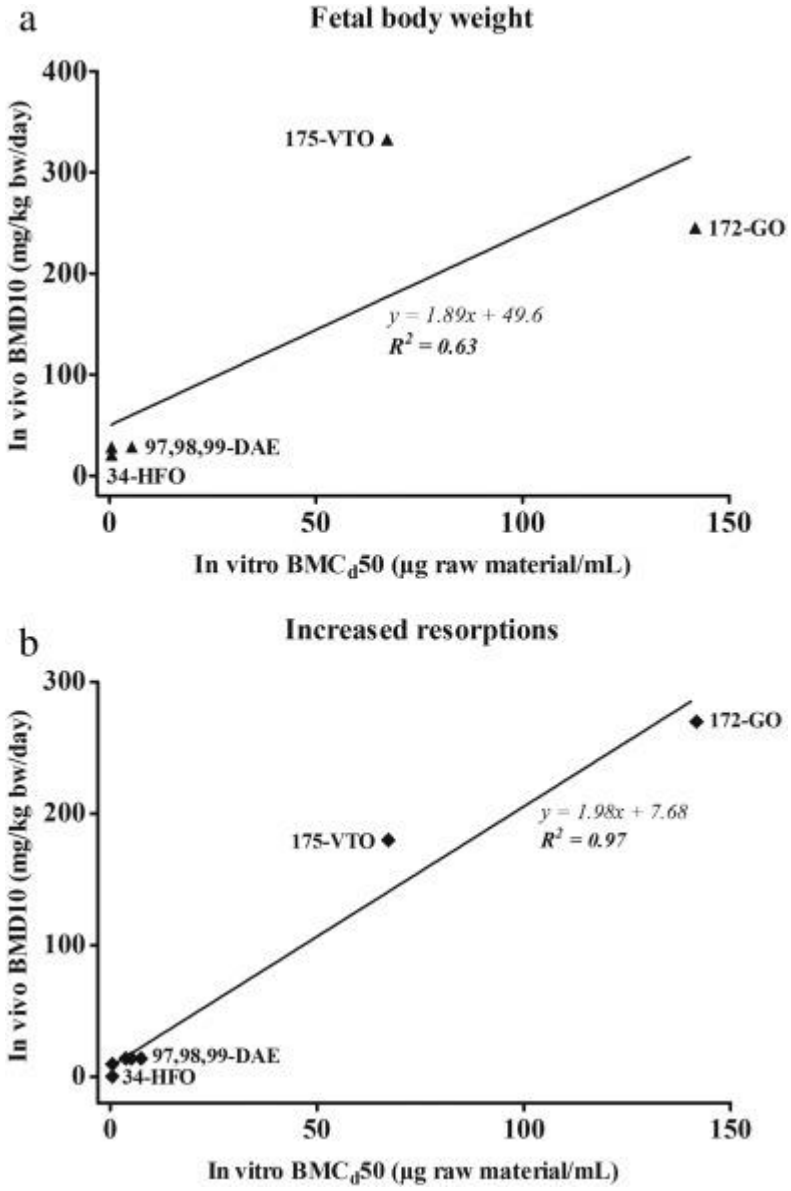
Fig. 3. Comparison of the concentration-dependent inhibition of ES-D3 cell differentiation upon exposure to DMSO-extracts of petroleum substances and GTL products. Results represent data from at least three independent experiments and are presented as mean ± standard error of the mean (SEM).



A comparison of the in vitro data to in vivo results was made. In vivo data was available for one GO sample, one VTO sample, 3 DAE samples and one HFO sample: for each of these substances the BM 10 for the endpoints foetal body weight and increased resorptions was calculated (BMD10 values were calculated from a dose-response curve using a dichotomous model (EPA BMD software), and data were taken from cited studies.)

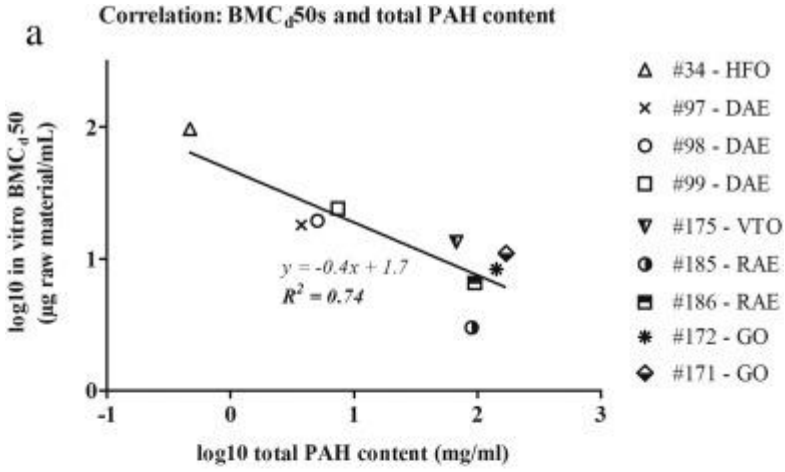
To compare in vitro and in vivo developmental toxicity potencies, the BMC_d50 values from the ES-D3 cell differentiation assay were plotted against the BMD10 values for decrease in fetal body weight and increase in resorptions. The results show that there is a highly significant correlation between the obtained in vitro BMC_d50s for inhibition of ES-D3 cell differentiation and the in vivo BMD10s for increase in resorptions, showing an R^2 of 0.97. However, a rather poor correlation was observed when plotting the BMC_d50s against the BMD10s for decrease in foetal body weight, showing an $R^2$of 0.63 (figure 4).

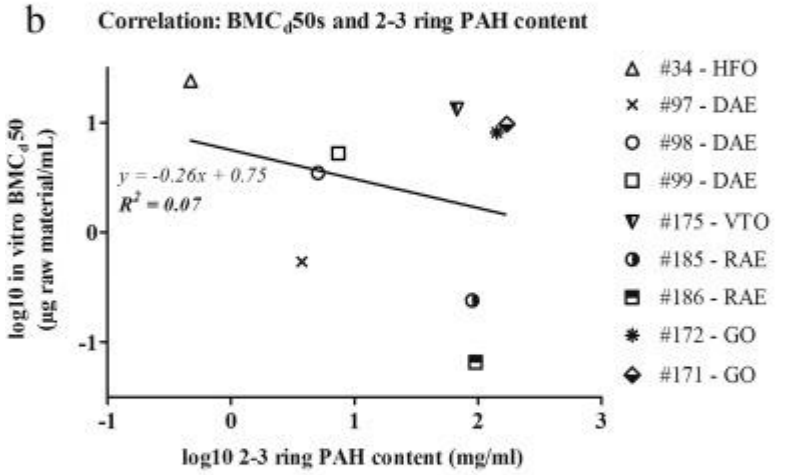
Fig. 4. Correlation between in vitro BMC_d50 values, obtained from the ES-D3 cell differentiation assay of the EST, and in vivo BMD10 values, based on (A) the decrease in foetal body weight and (B) the increased incidence in resorptions, of four different classes of petroleum substances: #34-heavy fuel oil (HFO), #97, 98, 99-distillate aromatic extract (DAE), #175-vacuum tower overhead (VTO), and #172-gas oil (GO).

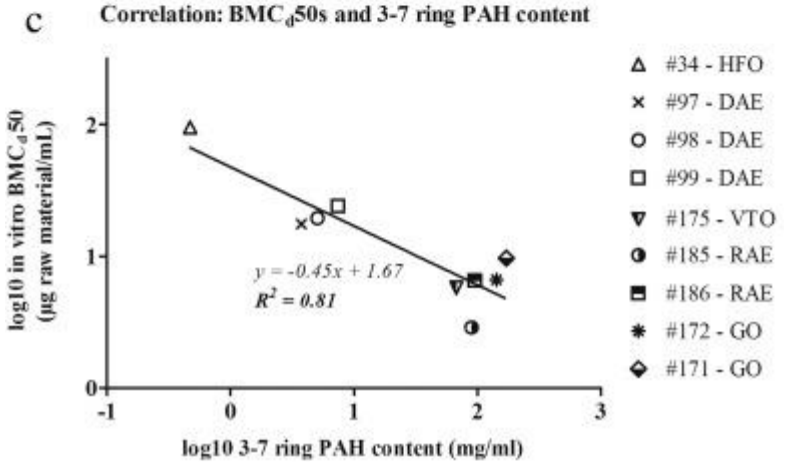


The BMC_d50 values from the ES-D3 cell differentiation assay were also compared to the PAH content present in each sample, to see if there is any association between the observed effects in vitro and the quantity of either 2–3 ring or 3–7 ring PAHs present. The in vitro BMC_d50s did correlate to some extent to the total PAH content, generating an R^2 of 0.74 (Fig. 5). Interestingly, a better correlation was obtained when the BMC_d50s were compared to only 3–7 ring PAH content, generating an R^2 of 0.81, and no correlation was seen when BMC_d50s were compared to 2–3 PAH content (R^2 = 0.07).

Fig. 5. Correlation between in vitro BMC_d50 values, obtained from the ES-D3 cell differentiation assay of the EST, with (A) total PAH content, (B) 2–3 ring PAH content, (C) 3–7 ring PAH content present in each sample.







This work is part of a number of investigations being conducted by Concawe to support hazard identification and read-across within petroleum streams and using in vitro work to target appropriate substances for further in vivo tests. Other planned work includes introducing a metabolism phase to the EST test, so that the results can be applied to risk assessment. Results are expected in the course of 2018 and 2019 for the next phases of the project.

PAC hypothesis for reprotoxicity

The in vitro EST work described above found a correlation between 3 – 7 ring PAC content and a reduction in embryo differentiation. The work discussed earlier in this section has suggested that it is the 4 – 7 PAC's that are mostly, if not solely, responsible for embryotoxicity and Ah-receptor binding. The mode of action for PAC embryotoxicity is not fully understood and additional work is ongoing to fully understand the role of individual PACs or specific types of PACs.

However, for the purposes of hazard identification, based on the available data, the following statements can be made about the reproductive toxicity potential of aromatic constituents (API 2008; EMBSI 1992; Feuston et al., 1994; McKee et al., 1990; McKee et al., 2012b; Schreiner et al., 1997; White 2012; Kamelia et al., 2017):

- **<3 ring polycyclic aromatics:** no effects on reproductive organs and no selective developmental effects.
- **≥4 ring polycyclic aromatics** (mainly 4-7 ring PACs) with specific structures (not necessarily present in gas oils) are associated with systemic toxicity (effects on liver, thymus and blood forming organs but not reproductive organs) and are potentially mutagenic and dermal carcinogens. In developmental studies they produce foetal death and resorption. Recent in vitro work suggests that ≥3 ring polycyclic aromatics can alter embryo development.

Based on this information, it is hypothesised that the reproductive toxicity of VHGO will be related to the types and levels of aromatics present, and will generally follow a pattern of increasing severity with increasing ring number. Any trend for the developmental toxicity of gas oils would thus be hypothetically described in terms of increasing aromatic content and number of fused aromatic rings.

The predominant PACs in VHGOs are 2- and 3-ring aromatic compounds but some VHGOs may contain very low levels of PACs with four and more aromatic rings. It is hypothesised therefore that there is low potential for adverse effects in developmental reproductive toxicity tests from exposure to VHGOs.

In conclusion, there is a hypothetical case to suggest that any developmental reproductive effects observed in petroleum substances are associated with 4 – 7 ring (possible 3 – 7 ring) PACs, and there is in-vitro and in-vivo toxicity data to support this hypothesis. However, there is no comprehensive investigation of fertility and the implications of interaction with the AhR receptor are not yet fully evaluated (work in progress, as explained above). It is therefore proposed to complement the ongoing work through fully investigating the potential reproductive effects in this study by including cohort 1B.

3. The need for an EOGRT's

As described in 'commission regulation (EU) 2015/282 amending Annexes VII, IX and X to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards the Extended One-Generation Reproductive Toxicity Study' to address the endpoint 8.7.3 an EOGRT's is required; this has replaced the original requirement for a 2-generation reproductive study. The design of this study will be based on the regulation requirements and the ECHA guidance document; the appraisal of available relevant information is given in section 5.

As described in section 2, it is hypothesized that the only putative reprotoxic constituents in VHGO are PACs with 4 or more rings which may cause developmental toxicity; their effects on fertility have not been fully evaluated. In addition, the predominant aliphatic constituents in VHGO are not developmental- or reproductive toxicants and produce minimal systemic toxicity. Although reproductive effects are considered unlikely to be observed with exposure to (the mostly aliphatic) VHGOs, it is hypothesized that a sample containing the highest concentration of aromatics with 4 or more rings would serve as a reasonable worst case to test for the reproductive toxicity endpoints.

It is proposed to conduct an Extended One-Generation Reproductive Toxicity Test (EOGRTS, according to OECD guideline 443) on a sample of VHGO which represents a reasonable worst case. Following the hypothesis outlined above, this will be a VHGO sample containing high levels of aromatic constituents with four or more rings, as these represent the most potentially hazardous compounds likely to be present (see section on toxicity of aromatics above).

4. Selection of VHGO for the EOGRT's

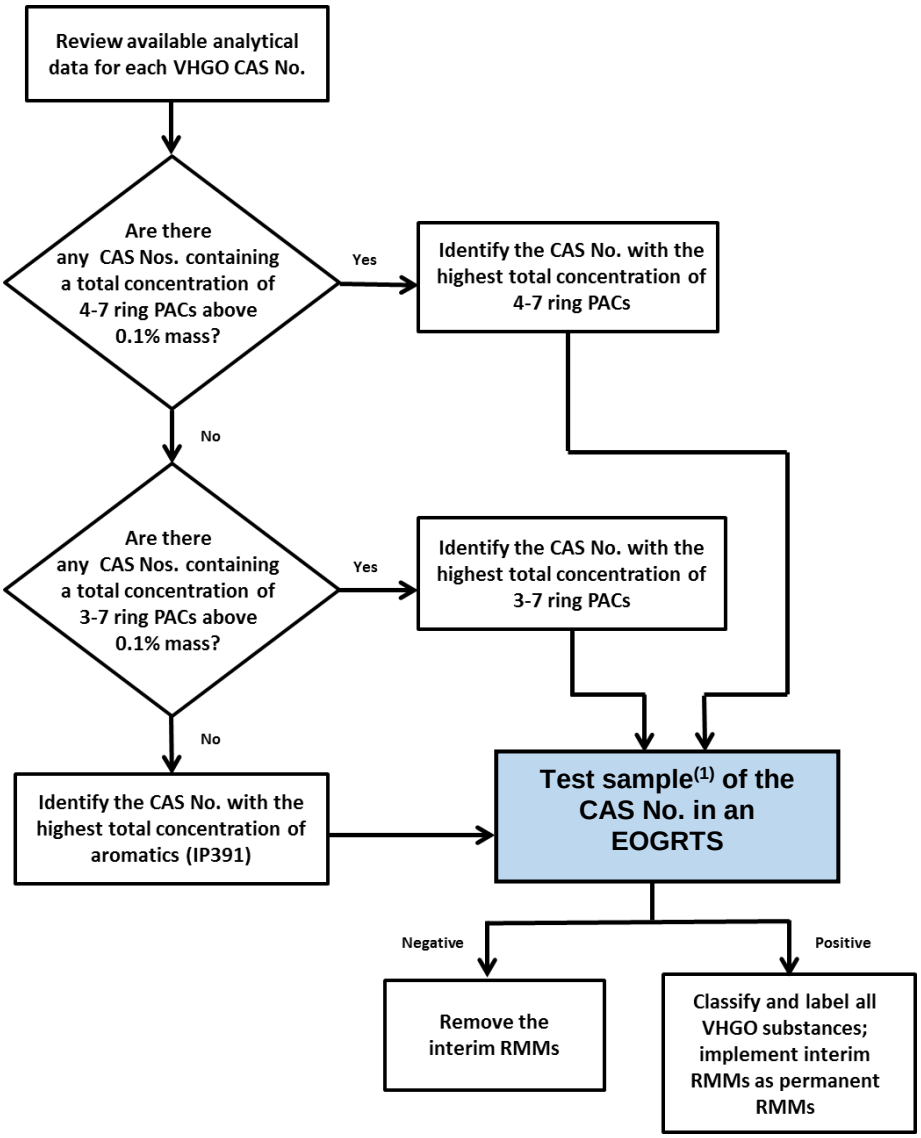
There are nine CAS numbers in the VHGO category, as shown in table 1

Table 1: VHGO category members

Name	EINECS definition	CAS	EINECS
Condensates (petroleum), vacuum tower	A complex combination of hydrocarbons produced as the lowest boiling stream in the vacuum distillation of the residuum from atmospheric distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C11 through C25 and boiling in the range of approximately 205°C to 400°C (401°F to 752°F).	**64741-49-7**	265-049-4
Gas oils (petroleum), light vacuum	A complex combination of hydrocarbons produced by the vacuum distillation of the residuum from atmospheric distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C13 through C30 and boiling in the range of approximately 230°C to 450°C (446°F to 842°F).	**64741-58-8**	265-059-9
Distillates (petroleum), light hydrocracked	A complex combination of hydrocarbons from distillation of the products from a hydrocracking process. It consists predominantly of saturated hydrocarbons having carbon numbers predominantly in the range of C10 through C18, and boiling in the range of approximately 160°C to 320°C (320°F to 608°F).	**64741-77-1**	265-078-2
Gas oils (petroleum), hydrodesulfurized light vacuum	A complex combination of hydrocarbons obtained from a catalytic hydrodesulfurization process. It consists of hydrocarbons having carbon numbers predominantly in the range of C13 through C30 and boiling in the range of approximately 230°C to 450°C (446°F to 842°F).	**64742-87-6**	265-190-1
Fuels, diesel	A complex combination of hydrocarbons produced by the distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C9 through C20 and boiling in the range of approximately 163°C to 357°C (325°F to 675°F).	**68334-30-5**	269-822-7
Fuel oil, no. 2	A distillate oil having a minimum viscosity of 32.6 SUS at 37.7°C (100°F) to a maximum of 37.9 SUS at 37.7°C (100°F).	**68476-30-2**	270-671-4
Fuel oil, no. 4	A distillate oil having a minimum viscosity of 45 SUS at 37.7°C (100°F) to a maximum of 125 SUS at 37.7°C (100°F).	**68476-31-3**	270-673-5
Fuels, diesel, no. 2	A distillate oil having a minimum viscosity of 32.6 SUS at 37.7°C (100°F) to a maximum of 40.1 SUS at 37.7°C (100°F).	**68476-34-6**	270-676-1
Gas oils (petroleum), hydrotreated light vacuum	A complex combination of hydrocarbons that is obtained by treatment of light vacuum petroleum gas oils with hydrogen in the presence of a catalyst. It consists of hydrocarbons having carbon numbers predominantly in the range of C13 through C30 and boiling in the range of approximately 230°C to 450°C (446°F to 842°F).	**92045-24-4**	295-407-5

Following the decision tree given in Figure 2, sample selection is based on identifying the test sample with the highest concentration of PACs with mainly 4-7 rings, within the boundaries of the VHGO category (first decision point in Figure 2).

Figure 2: Decision tree for selection of a VHGO CAS number representing a reasonable worst-case test sample based on the level of PAH.



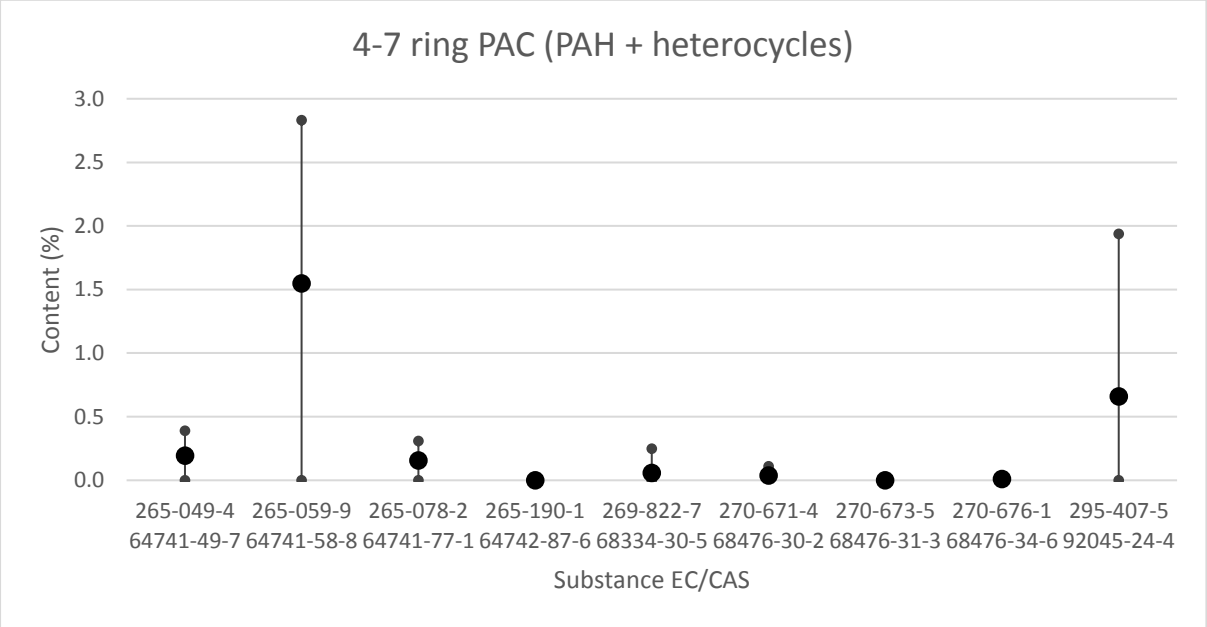
(1) Sample selected using the same criteria applied to identify the CAS No.

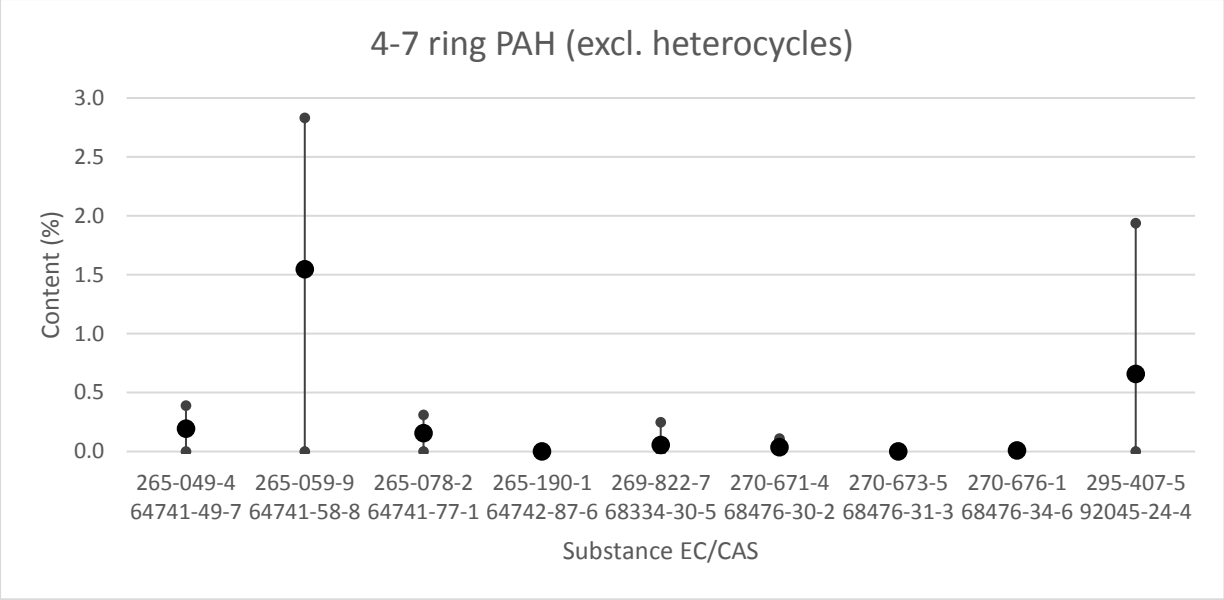
Following a recent GC x GC analysis program of gas oils the number of 3 – 7 and 4 – 7 ring PAH's were identified, both including and excluding heterocyclic molecules. Analysis of the available analytical data on VHGOs showed that some VHGOs contain low levels of 4-7 ring PACs (table 2).

Table 2: Average, maximum and minimum number of 3 – 7 and 4 – 7 PAH's in VHGO from GC x GC analysis.

	EC/ CAS	**265-049-4 64741-49-7**	**265-059-9 64741-58-8**	**265-078-2 64741-77-1**	**265-190-1 64742-87-6**	**269-822-7 68334-30-5**	**270-671-4 68476-30-2**	**270-673-5 68476-31-3**	**270-676-1 68476-34-6**	**295-407-5 92045-24-4**
Number of samples		3	3	2	1	32	8	1	3	3
3-7 PAH (no heterocycles)	Min	1.3	2.9	0.2	0.6	0.1	0.1	2.4	0.1	0.7
	Avg	**2.8**	**6.1**	**0.3**	**0.6**	**0.9**	**1.0**	**2.4**	**0.6**	**3.1**
	Max	3.7	8.3	0.5	0.6	2.6	2.5	2.4	1.7	6.3
4-7 PAH (no heterocycles)	Min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Avg	**0.2**	**1.5**	**0.2**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.7**
	Max	0.4	2.8	0.3	0.0	0.2	0.1	0.0	0.0	1.9
3-7 PAC (PAH + heterocycles)	Min	1.3	2.9	0.2	0.6	0.1	0.1	2.4	0.1	0.8
	Avg	**3.3**	**6.9**	**0.5**	**0.6**	**1.0**	**1.1**	**2.4**	**0.6**	**3.2**
	Max	4.7	10.7	0.8	0.6	3.2	2.8	2.4	1.7	6.3
4-7 PAC (PAH + heterocycles)	Min	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Avg	**0.2**	**1.5**	**0.2**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.7**
	Max	0.4	2.8	0.3	0.0	0.2	0.1	0.0	0.0	1.9

Figure 3: 4 – 7 ring PAH content (%) in VHGO's

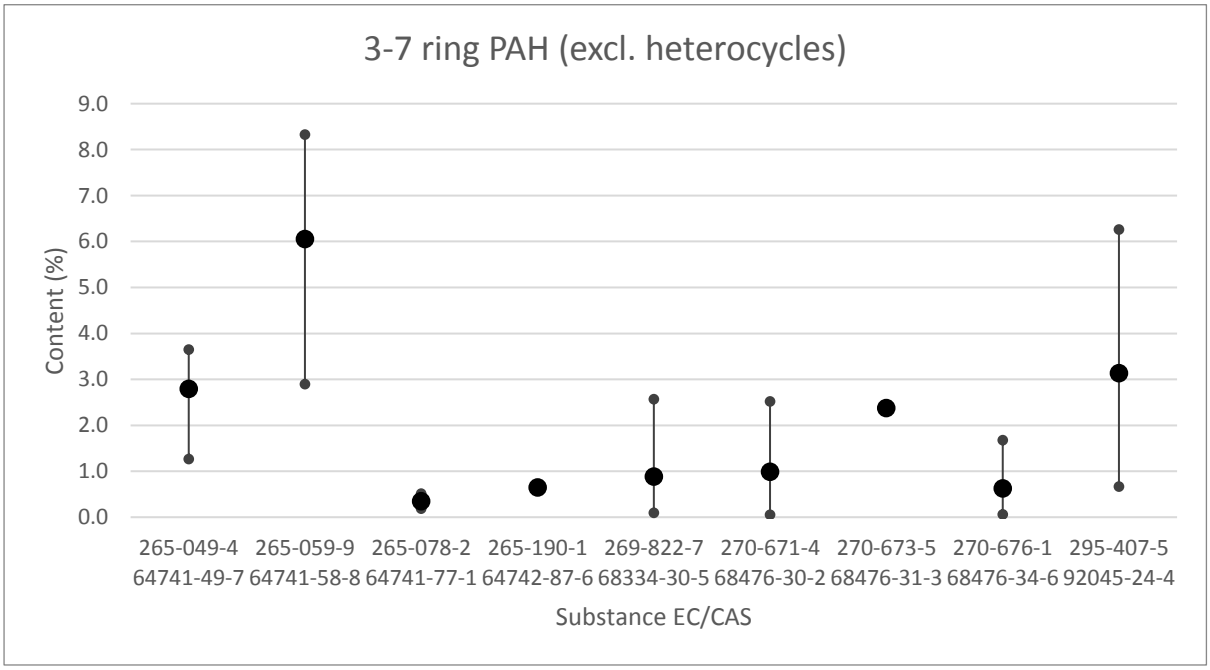


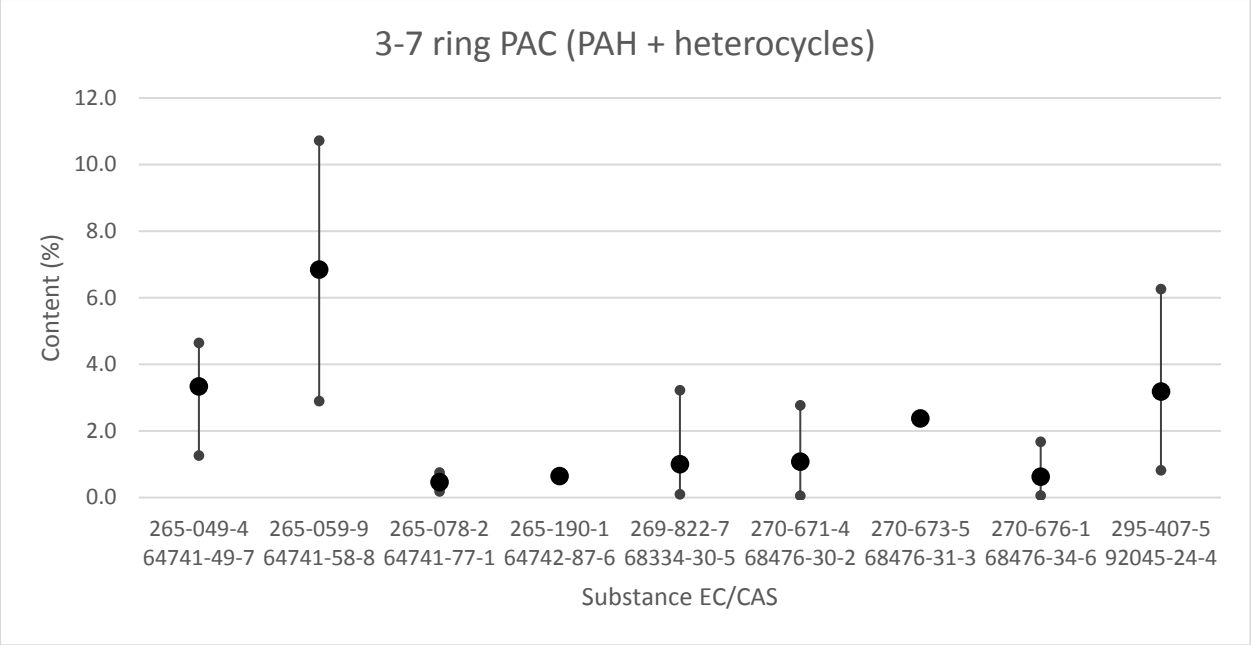


These data show that CAS nos 64741-49-7, 64741-58-8, 64741-77-1, 68334-30-5 and 92045-24-4 have a mean value of PAC > 0.1 % and that the highest levels are found in CAS nos 64741-58-8 and 92045-24-4.

If the criteria is expanded to include 3 – 7 ring PACs then these two CAS numbers still have the highest level.

Figure 4: 3 – 7 ring PAH content (%) in VHGO's





Based on the evaluation of the data in Table 2 and following the process outlined in the decision tree, a substance described by CAS No. 64741-58-8 will be selected for testing as a representative worst-case sample for testing based on the PAH hypothesis for read-across for the VHGO category of petroleum substances. The other eight substances within the group will not be tested and will read-across to the result for this substance.

5. Review of existing data for alerts or triggers relevant to the design of the extended one generation toxicity study (EOGRT's).

As described in the Guidance on information requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance.

The following evaluation of data is based on Table R.7.6-2 (annex 1) of
Draft version x.0 at
http://echa.europa.eu/documents/10162/13643/ir_csa_r7a_r76_reprotox_draft_en.pdf

This information gives only a brief overview of the studies reviewed for relevant information, for more detail on the studies please refer to the full CSR for VHGOs.

5.1 End points for cohort 1B (second generation)

REACH specifies that the extension of cohort 1B to include the F2 generation shall be proposed by the registrant or may be required by the Agency if:
a) "the substance has uses leading to significant exposure of consumers or professionals, taking into account, inter alia, consumer exposure from articles, and
b) any of the following conditions are met:
- *the substance displays genotoxic effects in somatic cell mutagenicity tests in vivo which could lead to classifying it as Mutagen Category 2, or*
- *there are indications that the internal dose for the substance and/or any of its metabolites will reach a steady state in the test animals only after an extended exposure, or*
- *there are indications of one or more relevant modes of action related to endocrine disruption from available in vivo studies or non-animal approaches."*

Uses leading to significant exposure of consumers or professional, taking into account inter alia consumer exposure from articles

Vacuum Gas Oils, Hydrocracked Gas Oils, and Distillate Fuels have a range of industrial, professional and consumer uses and are manufactured/imported at over 10,000 tonnes/year. Please refer to PROCs in the REACH CSR. The criteria for significant use is as follows:

Criteria for uses leading to significant exposure:
- *If the substance is used in the EU by consumers (i.e. members of the public) or professionals (i.e. workers in trades), either neat or in a chemical mixture (above the criteria for Art. 57(f) of 0.1% for endocrine disrupters), and there is one very wide use or several limited uses potentially affecting major part of consumers and/or professionals, then this is considered as meeting the criterion.*
- *If the substance is in an article used by consumers or professionals in the EU the criterion would be met if the substance is intended to be released from the article during use of the article by the consumers or professionals (e.g. ink jet printers or photocopy toners) and there is one very wide use or several limited uses potentially affecting major part of consumers and/or professionals.*

CONCLUSION: There is significant exposure to VHGOs when used as fuel, exposure is predominantly dermal and inhalation. There is not widespread non-fuel use.

Genotoxicity potentially meeting classification criteria to Mutagen Category 2

The following is from the CSR.

The genotoxicity of members of the vacuum gas oil, hydrocracked gas oil and distillate fuel category has been investigated in a number of in-vitro and in-vivo studies. In-vitro studies include 13 bacterial mutation assays, a mouse lymphoma assay and an SCE assay in Chinese hamster ovary cells. Genotoxicity in-vivo has been investigated in 3 bone marrow cytogenicity studies and a male dominant lethal assay.

Although some ambiguous results were seen the majority of in-vitro studies showed no, or little mutagenic activity. In the in vivo micronucleus test home heating oil showed no evidence of genotoxic activity. This lack of activity was supported by further in-vivo studies including a male dominant lethal assay.

Value used for CSA: *Genetic toxicity: No adverse effect observed (negative)*

CONCLUSION: VHGO are not classified as mutagenic

Extended exposure is needed to reach the steady state kinetics

Substances in the VHGO category are UVCBs; hence it is difficult to apply standard methodology for assessing absorption, distribution, and metabolism.

There is no toxicokinetic data of VHGO's *in vivo*, so assumptions are made based on other sources:

The assessment in the CSR concluded the following:

Results of experimental studies in animals provide qualitative evidence of absorption by the lung, as indicated by a modest increase in startle reflex in rats inhaling respirable aerosols of diesel fuel. Physico-chemical considerations also suggest that highly respirable aerosols of poorly water soluble substances with a log Pow greater than zero will be absorbed to some extent from the respiratory tract. In the absence of further guidance, it will be assumed that 50% of an inhaled dose of aerosolised gas oil will be absorbed by animals and humans.

No measured data are available on the dermal absorption of gas oils, however the occurrence of systemic tissue changes in repeated dose toxicity studies (in the absence of dermal irritation, and after controlling for incidental ingestion during grooming) indicates that some absorption across the skin is possible. Results from the SKINPERM model indicate that uptake of gas oil across the skin is likely to be low, with an estimated dermal flux of 0.0001058 mg cm^{-2}. hour for human skin. However the reliability of this value is not known, and therefore complete absorption of gas oil by human skin has been assumed (conservative default) as recommended by the TGD (ECB, 2003). This is probably highly conservative given that the log Pow of the majority (>98.5%) of gas oil components falls outside the 1-4 range that favours dermal uptake (ECB, 2003). Since experimental studies demonstrate greater absorption of lipophilic substances by animal skin compared to human skin, it will be therefore be assumed during risk characterisation that animal skin is 2-fold more permeable to topically applied gas oils than is human skin.

CONCLUSION: Systemic exposure is limited and there is no evidence that extended exposure is required to reach steady state kinetics

Indications of modes of action related to endocrine disruption from in vivo or non-animal approaches

The available data (studies conducted with substances from this category, or appropriate read-across substances) was reviewed for information on the following endpoints: increased foetal weight, prolonged gestation, anogenital distance/nipple retention, changes in oestrous cycle, increased parental bodyweight, measurement of hormone levels, thyroid weight, thyroid pathology, changes in reproductive organ weight, changes in reproductive organ pathology and sperm morphology.

There are four prenatal developmental toxicity studies conducted with substances from this category; the studies do not comply with the current protocol for testing but no adverse effects on development were noted. A fifth study maintained pups and did not find any effect on bodyweight on day 4.

There are 11 repeat dose toxicity studies. Examination of reproductive organs was generally limited to testes and ovary and no effects were observed. Where the thyroid was examined this was also found to be unremarkable. There is no data on sperm morphology or analysis.

There are no obvious alerts in the data base for toxicity caused by endocrine disruption, but it is acknowledge that the data base is limited.

Reference can also be made to studies conducted on SRGOs and OGOs – these show similar results (with no specific triggers) and can be reviewed in the EOGRTS testing proposals for these two groups.

As noted in the discussion on aromatics, polycyclic compounds can interact with the Aryl Hydrocarbon (Ah) receptor and (1) the subsequent inhibitory cross talk to the estrogen receptor (ER) or (2) the induction of specific isozymes of the CYP450 family which may lead to increased oxidative metabolism of polycyclic compounds and subsequent interaction of the metabolites with steroid hormone receptors (Chaloupka et al., 1994; Machala et al., 2001; Kizu et al., 2003; Kummer et al., 2008, 2013; Balabanic et al., 2011). On its own this in vitro data is not full confirmation of an endocrine disrupting effect however, $\geq$ 4 ring polycyclic aromatics are associated with foetal death and resorption in developmental studies and this is a cause for concern.

CONCLUSION: some of the 4 – 7 ring aromatic substances present in the VHGO may lead to interactions with the estrogen receptor and may be associated with foetal death and resorption. There are no indications of developmental effects in the absence of maternal toxicity nor robust evidence of endocrine disruptor activity. However, it is recognized that the data base is limited and the potential role of PACs is not fully understood and hence it is requested that the study includes a cohort to fully evaluate the effects on the developing offspring.

CONCLUSION on the need for the second generation cohort:
REACH specifies that the extension of cohort 1B to include the F2 generation shall be proposed by the registrant or may required by the Agency if:
a) "the substance has uses leading to significant exposure of consumers or professionals, taking into account, inter alia, consumer exposure from articles, and
b) any of the following conditions are met:
- *the substance displays genotoxic effects in somatic cell mutagenicity tests in vivo which could lead to classifying it as Mutagen Category 2, or*
- *there are indications that the internal dose for the substance and/or any of its metabolites will reach a steady state in the test animals only after an extended exposure, or*
- *there are indications of one or more relevant modes of action related to endocrine disruption from available in vivo studies or non-animal approaches."*

VHGO's result in significant exposure, fertility has not been assessed and the questionable role of PAH's on development mean that this cohort is required.

Table 2 – Information on endocrine disruption endpoints - VHGO studies

CAS number	**68334-30-5**	**64741-49-7**	**64741-49-7**	**64742-44-2/64741-59-9**	**68334-30-5**
Category	**VHGO**	**VHGO**	**VHGO**	**fuel oil**	**VHGO**
Reference	**ACRO 1994 d**	**Mobil 1989b**	**Mobil 1989b**	**API 1979 a (c infile)**	**API 1979 b (b infile)**
Study type	**rat dermal PNDT (GD 0 – 20) Pups maintained until PND 4**	**rat dermal PNDT (GD 0 – 19)**	**rat dermal DT (GD 0 – 15) Pups maintained until PND 4**	**rat inhalation PNDT (GD 6 – 15)**	**rat inhalation PNDT (GD 6 – 15)**
Dose levels	**0, 125, 250 or 1000 mg/kg/day**	**0, 30, 125, 500 and 1000 mg/kg/day**	**0 and 500 mg/kg/day**	**0, 86.9 and 408.4 ppm**	**0, 101.8, 401.5 ppm**
increased foetal weight	no	no decreased	similar to controls on PND 4	No	No
prolonged gestation	Not measured	Not measured	No	Not measured	Not measured
changes in oestrous cycle	Not measured	Not measured	Not measured	Not measured	Not measured
increased body weight	no	no decreased	no decreased	no	no
changes in reproductive organ weights	Not measured	Not measured	Not measured	Not measured	Not measured
changes in reproductive organ pathology	Not measured	Not measured	Not measured	Not measured	Not measured

No changes in parameter evaluated	Changes that require further consideration

Not measured=not an endpoint in this type of study, or not included in particular study design

Table 2 – Information on endocrine disruption endpoints - VHGO studies continued

CAS number	68334-30-5	64741-49-7	68334-30-5	64741-77-1	64741-58-8	68476-34-6	68334-30-5	68334-30-5	68334-30-5	68476-34-6	68476-34-6
category	**diesel fuel**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**
Reference	**Lock et al (1984)**	**Mobil 1989a**	**ARCO, 1994a**	**ARCO 1992f**	**ARCO 1993j (a in file)**	**ARCO 1988h**	**ARCO 1992e (a in file)**	**ARCO, 1994b**	**ARCO 1986k (b in file)**	**ARCO 1986j**	**ARCO 1986l (c in file)**
Study type	**rat inhalation 13 weeks**	**rat dermal 13 weeks**	**rat dermal 13 weeks (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**
Dose levels	**0, 0.25, 0.75, 1.50 mg/L**	**0, 30, 125 and 500 mg/kg/day**	**0, 0.01 0.1 and 1 ml/kg/day**	**0, 0.05, 0.25 and 1.0 ml/kg/day**	**0, 0.05, 0.25 and 1.0 ml/kg/day**	**0, 0.1, 0.5 and 1.0 ml/kg/day**	**sham, 0(acetone), 0.0001, 0.005 and 0.5 ml/Kg**	**0, 0.5 ml/kg/day**	**0, 0.25, 2.0, 5.0 mg/kg/day**	**0, 0.5, 2.0, 5.0 ml/kg/day**	**0, 0.5, 2.0, 5.0 ml/kg/day**
increased body weight	decreased bodyweight	decreased bodyweight	decreased bodyweight.	no change	decreased bodyweight	decreased bodyweight	no change	no change	no change	decreased bodyweight	decreased bodyweight
thyroid pathology	not measured	No change	no change	no change	no change	not measured	no change	not measured	not measured	not measured	not measured
changes in reproductive organ weights	no change in testes	no change in epididymides, testes, ovaries, uterus	no change in testes or ovary	no change in testes or ovary	no change in testes or ovary	no change in testes or ovary	no change in testes or ovary	no change in testes or ovary	no change in testes or ovary	no change in testes or ovary	no change in testes or ovary
changes in reproductive organ pathology	no change in testes	no change	no change in testes, ovaries	no change in testes, ovaries	no change in testes, ovaries	not measured	no change in testes, ovaries	not measured	no change in testes or ovary	no change in testes or ovary	no change in testes or ovary
Sperm analysis	not measured	no change	not measured	not measured	not measured	not measured	not measured	not measured	not measured	not measured	not measured

No changes in parameter evaluated | Changes that require further consideration

Not measured=not an endpoint in this type of study, or not included in particular study design

5.2 Endpoints for cohort 2 (developmental neurotoxicity)

Information on neurotoxicity from in vivo studies or non-animal approaches.

The available data (studies conducted with substances from this category, or appropriate read-across substances) was reviewed for information on the following endpoints: clinical observation associated with neurotoxicity (ie not general toxicity) in adults and pups, Functional Observation Battery (FOB), brain weight, brain histopathology, spinal cord histopathology, peripheral nerve histopathology, Serial brain section and spinal cord of the foetus.

In the identified studies there were no clinical observation associated with neurological effects in the adults or pups (two studies had pups up to day 4 only). There was not a complete FOB for any study. In the repeat dose studies there were no histopathological changes in the brain, spinal cord or peripheral nerves and no brain or spinal cord abnormalities following macroscopic examination of fetuses.

Reference can also be made to studies conducted on SRGOs and OGOs – these show similar results (with no specific triggers) and can be reviewed in the EOGRTS testing proposals for these two groups.

In an inhalation study using diesel fuel (Lock et al 1984), there was a statistically significant increase in auditory startle of 1 – 2 mSecs. The significance of this change is difficult to interpret as other studies yielded differences of 17 – 20 mSec. This was the only parameter examined and has not been observed in any oral or dermal studies.

No validated in vitro tests for neurotoxicity were identified in the data set.

CONCLUSION: Neurotoxicity endpoints are not fully addressed in the existing data base, a slight delay in startle response was observed in one inhalation study which may be indicative of a neurotoxic effect

Specific mechanism/modes of action with association to (developmental) neurotoxicity.

VHGO's are multi component substances. None of the molecule classes have been associated specifically with neurotoxic modes of action, other than following inhaltion. In vitro investigation have identified other target receptors, but not those associated with neurotoxicity

CONCLUSION: There are no specific mechanisms/modes of action indicative of neurotoxicity

In vivo information on (developmental) neurotoxicity from structurally analogues substance

VHGOs are UVCB substances. Evaluation of the predominant molecule types does not reveal any indication of neurotoxicity

CONCLUSION: Although there does not appear to be any pathological effects on the nervous system, in animal studies a slight delay in startle response was apparent.

CONLCUSION on the need for the developmental neurotoxicity cohort
REACH specifies that cohorts 2A/2B (developmental neurotoxicity) and/or cohort 3 (developmental immunotoxicity) shall be proposed by the registrant or may be required by the Agency in accordance with Article 40 or 41, in case of particular concerns on (developmental) neurotoxicity or (developmental) immuno toxicity justified by any of the following:
— existing information on the substance itself derived from relevant available in vivo or non-animal approaches (e.g. abnormalities of the CNS, evidence of adverse effects on the nervous or immune system in studies on adult animals or animals exposed prenatally), or
— specific mechanisms/modes of action of the substance with an association to (developmental) neurotoxicity and/or (developmental) immunotoxicity (e.g. cholinesterase inhibition or relevant changes in thyroidal hormone levels associated to adverse effects), or

— existing information on effects caused by substances structurally analogous to the substance being studied, suggesting such effects or mechanisms/modes of action
Based on a minor effect on startle response, cohort 2 is required.

Table 3 – Information on neurotoxicity

CAS number	**68334-30-5**	**64741-49-7**	**64741-49-7**	**64742-44-2/64741-59-9 read across**	**68334-30-5**
Category	**VHGO**	**VHGO**	**VHGO**	**/cracked gas oil**	**VHGO**
Reference	**ACRO 1994 d**	**Mobil 1989b**	**Mobil 1989b**	**API 1979 b (c infile)**	**API 1979 a (b infile)**
Study type	**rat dermal PNDT (GD 0 – 20) Pups maintained until PND 4**	**rat dermal PNDT (GD 0 – 19)**	**rat dermal DT (GD 0 – 15) Pups maintained until PND 4**	**rat inhalation PNDT (GD 6 – 15)**	**rat inhalation PNDT (GD 6 – 15)**
Dose levels	**0, 125, 250 or 1000 mg/kg/day**	**0, 30, 125, 500 and 1000 mg/kg/day**	**0 and 500 mg/kg/day**	**0, 86.9 and 408.4 ppm**	**0, 101.8, 401.5 ppm**
clinical observation	pups normal (up to PND 4), no maternal findings (other than irritation)	none	pups normal (up to PND 4), no maternal findings (other than irritation)	none	none
foetal serial brain section	not measured	not measured	not measured	one 86.9 ppm female with hydrocephaly (not considered treatment related)	no change
foetal spinal cord	not measured	not measured	not measured	no findings reported	no findings reported

No changes in parameter evaluated	Changes that require further consideration

Not measured=not an endpoint in this type of study, or not included in particular study design

Table 3 – Information on neurotoxicity, continued

CAS number	68334-30-5	64741-49-7	68334-30-5	64741-77-1	64741-58-8	68476-34-6	68334-30-5	68334-30-5	68334-30-5	68476-34-6	68476-34-6
category	**diesel fuel**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**
Reference	**Lock et al (1984)**	**Mobil 1989a**	**ARCO, 1994b**	**ARCO 1992b**	**ARCO 1993j (a in file)**	**ARCO 1988h**	**ARCO 1992e (a in file)**	**ARCO, 1994a**	**ARCO 1986k (b in file)**	**ARCO 1986a**	**ARCO 1986l (c in file)**
Study type	**rat inhalation 13 weeks**	**rat dermal 13 weeks**	**rat dermal 13 weeks (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**
Dose levels	**0, 0.25, 0.75, 1.50 mg/L**	**0, 30, 125 and 500 mg/kg/day**	**0, 0.01 0.1 and 1 ml/kg/day**	**0, 0.05, 0.25 and 1.0 ml/kg/day**	**0, 0.05, 0.25 and 1.0 ml/kg/day**	**0, 0.1, 0.5 and 1.0 ml/kg/day**	**sham, 0(acetone), 0.0001, 0.005 and 0.5 ml/Kg**	**0, 0.5 ml/kg/day**	**0, 0.25, 2.0, 5.0 mg/kg/day**	**0, 0.5, 2.0, 5.0 ml/kg/day**	**0, 0.5, 2.0, 5.0 ml/kg/day**
Brain weight											
Brain histopathology (3 sections)	no change	no change	no change	no change	no change	no change	no change	not measured	no change	no change	no change
Spinal cord histopathology	no change	not measured	not measured	not measured	not measured	not measured	not measured	not measured	not measured	not measured	not measured
Peripheral nerve histopathology	no change	no change	no change	not measured	not measured	not measured	not measured	not measured	not measured	not measured	not measured
Clinical observation indicative of neurotoxicity	none	none	none	none	none	none	none	none	none	none	none
FOB battery	only startle reflex tested: increase in reaction time at 1.5, 0.75 and 0.25 mg/L, after exposure, but recovered after 24 hours. Diff in the range 1 - 2 mSec. Questionable	not measured	not measured	not measured	not measured	not measured	not measured	not measured	not measured	not measured	not measured

	significance See A										

No changes in parameter evaluated	Changes that require further consideration		

Not measured=not an endpoint in this type of study, or not included in particular study design

A
Auditory startle was measured by an automated system following a 10 mSec pulse (note this study was conducted in 1984 so this is not equivalent to current systems); each animal was used as its own control, based on pre-treatment measurements. There were some statistically significant changes in reaction time which was increased by 1 – 2 mSecs in 1.50 mg/L males; this was not considered significant as other studies had yielded differences of 17 – 20 mSec. Other statistically significant changes in peak height and time were noted before treatment and periodically; these were not considered to be of biological significance. It is difficult to assess the quality and relevance of this data.

5.3 Endpoints for cohort 3 (developmental immunotoxicity)

Information on immunotoxicity from in vivo studies or non-animal approaches.

The available data (studies conducted with substances from this category, or appropriate read-across substances) was reviewed for information on the following endpoints: white blood cell count and differential, albumin/globulin ratio, weight of the thymus, spleen and lymph nodes, histopathology of the thymus, spleen, lymph nodes and bone marrow.

A number of repeat dose rat and rabbit studies were reviewed for endpoints related to immunotoxicity. The endpoints assessed for each of these studies are given in table 4. There were changes in the WBC differential in a number of studies, mostly an increase neutrophils and a decrease in lymphocytes, the effected dose levels were also associated with notable skin irritation. There were also some incidences of changes in thymus and spleen weight but no pathological changes. Enlarged lymph node was noted in one study.

Reference can also be made to studies conducted on SRGOs and OGOs – these show similar results (with no specific triggers) and can be reviewed in the EOGRTS testing proposals for these two groups.

This group carries the classification 'H373: May cause damage to thymus, liver, and bone marrow through prolonged or repeated exposure' and there is a concern that the effects on the developing foetus and neonate have not been fully assessed.

No validated in vitro tests for immunotoxicity were identified in the data set.

CONCLUSION: Thymus, liver and bone marrow are target organs of VHGO toxicity and hence immunotoxicity investigations are required.

Specific mechanism/modes of action with association to (developmental) immunotoxicity.

VHGO's are multi component substances. PAH's may beassociated specifically with immunotoxic modes of action.

CONCLUSION: There are specific mechanisms/modes of action indicative of immunotoxicity

In vivo Information on (developmental) immunotoxicity from structurally analogues substance

VHGO are UVCB substances that contain poly aromatic hydrocarbons which may be associated with toxicity of the bone marrow and thymus.

CONCLUSION:VHGO's are classified as H373: May cause damage to thymus, liver, and bone marrow through prolonged or repeated exposure and these organs are associated with the immune system.

CONCLUSION on the need for the developmental immunotoxicity cohort
REACH specifies that cohorts 2A/2B (developmental neurotoxicity) and/or cohort 3 (developmental immunotoxicity) shall be proposed by the registrant or may be required by the Agency in accordance with Article 40 or 41, in case of particular concerns on (developmental) neurotoxicity or (developmental) immuno toxicity justified by any of the following:
— existing information on the substance itself derived from relevant available in vivo or non-animal approaches (e.g. abnormalities of the CNS, evidence of adverse effects on the nervous or immune system in studies on adult animals or animals exposed prenatally), or

— specific mechanisms/modes of action of the substance with an association to (developmental) neurotoxicity and/or (developmental) immunotoxicity (e.g. cholinesterase inhibition or relevant changes in thyroidal hormone levels associated to adverse effects), or
— existing information on effects caused by substances structurally analogous to the substance being studied, suggesting such effects or mechanisms/modes of action
VHGO's have a category 2 classification for repeated dose toxicity of the liver, thymus and bone marrow and therefore there is potentially risk to the developing immune system. Cohort 3 for the EOGRTS is requested.

Table 4 – Information on immunotoxicity

CAS number	**68334-30-5 diesel fuel**	**64741-49-7**	**68334-30-5**	**64741-77-1**	**64741-58-8**	**68476-34-6**	**68334-30-5**	**68334-30-5**	**68334-30-5**	**68476-34-6**	**68476-34-6**
category	**diesel fuel**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**
Reference	**Lock et al (1984)**	**Mobil 1989a**	**ARCO, 1994b**	**ARCO 1992b**	**ARCO 1993j (a in file)**	**ARCO 1988h**	**ARCO 1992e (a in file)**	**ARCO, 1994a**	**ARCO 1986k (b in file)**	**ARCO 1986a**	**ARCO 1986l (c in file)**
Study type	**rat inhalation 13 weeks**	**rat dermal 13 weeks**	**rat dermal 13 weeks (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**
Dose levels	**0, 0.25, 0.75, 1.50 mg/L**	**0, 30, 125 and 500 mg/kg/day**	**0, 0.01 0.1 and 1 ml/kg/day**	**0, 0.05, 0.25 and 1.0 ml/kg/day**	**0, 0.05, 0.25 and 1.0 ml/kg/day**	**0, 0.1, 0.5 and 1.0 ml/kg/day**	**sham, 0(acetone), 0.0001, 0.005 and 0.5 ml/Kg**	**0, 0.5 ml/kg/day**	**0, 0.25, 2.0, 5.0 mg/kg/day**	**0, 0.5, 2.0, 5.0 ml/kg/day**	**0, 0.5, 2.0, 5.0 ml/kg/day**
Haematology: white blood cell count (WBC) and differential	no change in WBC differential not measured	no change in WBC differential not measured	at 1 ml/kg/day increase in neutrophils & esoinophils & decrease in lymphocytes (C)	no change	no change	no change	WBC increased at 0.5 ml/kg (M), neutrophils increased and lymphocytes decreased at 0.5 ml/kg (M) (E)	no change (stat sig but within background range)	neutrophils increased and lymphocytes decreased at 5.0 ml/kg (F)	neutrophils increased and lymphocytes decreased at 2.0 and 5.0 ml/kg (G)	neutrophils increased and lymphocytes decreased at 2.0 and 5.0 ml/kg (H)
Albumin/globulin ratio	not measured	no change	decreased ratio at 1 ml/kg/day (C)	decreased ratio at 0.25 and 1.0 ml/kg/day (D)	no change	not measured	no change	not measured	not measured	not measured	not measured
Weight of the thymus	not measured	decreased at 500 mg/kg/day (B)	not measured	not measured	not measured	not measured	not measured	not measured	not measured	not measured	not measured
Weight of the spleen	no change	no change	no change	not measured	not measured	no change	not measured	no change	no change	relative increased at 5.0 ml/kg (G)	5.0 mL/kg females increased)

Table 4 – Information on immunotoxicity (cont/…)

CAS number	**68334-30-5**	**64741-49-7**	**68334-30-5**	**64741-77-1**	**64741-58-8**	**68476-34-6**	**68334-30-5**	**68334-30-5**	**68334-30-5**	**68476-34-6**	**68476-34-6**
category	**diesel fuel**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**	**VHGO**
Reference	**Lock et al (1984)**	**Mobil 1989a**	**ARCO, 1994b**	**ARCO 1992b**	**ARCO 1993j (a in file)**	**ARCO 1988h**	**ARCO 1992e (a in file)**	**ARCO, 1994a**	**ARCO 1986k (b in file)**	**ARCO 1986a**	**ARCO 1986l (c in file)**
Study type	**rat inhalation 13 weeks**	**rat dermal 13 weeks**	**rat dermal 13 weeks (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**	**rat dermal 28 days (5/7)**
Dose levels	**0, 0.25, 0.75, 1.50 mg/L**	**0, 30, 125 and 500 mg/kg/day**	**0, 0.01 0.1 and 1 ml/kg/day**	**0, 0.05, 0.25 and 1.0 ml/kg/day**	**0, 0.05, 0.25 and 1.0 ml/kg/day**	**0, 0.1, 0.5 and 1.0 ml/kg/day**	**sham, 0(acetone), 0.0001, 0.005 and 0.5 ml/Kg**	**0, 0.5 ml/kg/day**	**0, 0.25, 2.0, 5.0 mg/kg/day**	**0, 0.5, 2.0, 5.0 ml/kg/day**	**0, 0.5, 2.0, 5.0 ml/kg/day**
Histopathology of thymus	not measured	lymphoid reduction at 500 mg/kg/day (B)	no change	no change	no change	not measured	not measured	not measured	not measured	not measured	not measured
Histopathology of lymph nodes	not measured	not reported	enlarged axillary lymph nodes at 1 ml/kg/day (C)	no change	no change	not measured	no change	not measured	not measured	not measured	not measured
Histopathology of spleen	no change	not reported	no change (C)	no change	no change	no change	no change	no measured	no change	no change	no change
histology of bone marrow	not measured	no change	no change	no change	no change	no change	no change	not measured	no change	not measured	not measured

No changes in parameter evaluated	Changes that require further consideration

Not measured=not an endpoint in this type of study, or not included in particular study design

(B) At 500 mg/kg/day there was also weight loss, notable skin irritation, effects on hematology, blood chemistry and increased liver weight. Thymus weight was reduced at necropsy and histopathological examination also revealed a reduction in lymphoid tissue.
(C) There was notable skin irritation including epidermal abscess, ascanthosis, crusting, erosion, fibrosis and hyperkeratosis; the changes in the immune system observed are considered to be in response to irritation. In the spleen 1 animal with lymphoid depletion was considered a background finding
(D) no other changes were observed

(E) There was notable skin irritation including acanthosis, epidermal crusting, erosion, fibrosis, hyperkeratosis and ulceration
(F) There was notable skin irritation including acanthosis, hyperkeratosis, crusting, inflammation and ulceration
(G) The lymphocyte changes are considered to be related to dermal irritation, which included acanthosis, fibrosis, hyperkeratosis, crusting, inflammation and ulceration. The increase in relative weight of the spleen was small and not considered to be biologically significant and there was no histopathological changes.
(H)There was notable skin irritation which included acanthosis, hyperkeratosis, crusting, inflammation and ulceration. In females the spleen weight was increased at 5.0 mL/kg/day, but this was due to the high value of a single female.

..

6. Proposed design of the EOGRTs

The VHGO selected from the category for this test is the CAS number with the highest % (w/w) of 4 – 7 ring PAH's as it is considered that this has the greatest potential for reproductive and developmental toxicity. The results of this test, supported with other targeted oral studies and a battery of in vitro tests, will be used as read-across for the remaining VHGO group members therefore avoiding additional in vivo studies.

The results of this study may also help inform the design of similar studies which are required for other petroleum categories. The basic types of hydrocarbon molecules found in this substance and category (ie straight and branched alkanes and alkenes, cycloalkanes and cycloalkenes, aromatic and aromatic cycloalkanes) are found in other petroleum categories and although petroleum substances are UVBC substances their similarities can help define a targeted approach to further testing.

It is considered that the most likely routes of exposure to VHGO are dermal and inhalation however, ECHA have previous indicated to Concawe that the route of administration for the study addressing this endpoint in Annex X would be oral. It should be noted that the oral route may result in a pattern and degree of exposure that is different to that observed following dermal application.

Concawe propose to conduct the range-finding and study and EOGRT's via the dietary route. An OECD 422 will be conducted as the range-finding study.

This substance is an aspiration hazard and this is of concern, especially regarding the dose administration to neonates. In addition, VHGO's are classified as irritating to the skin and petroleum substances are associated with the de-fatting of dermal tissue. Therefore the dietary route may ameliorate any possible gastrointestinal disturbances and irritancy.

Dietary administration is less invasive for the animals, therefore promoting animal welfare. In addition, the procedure of gavage administration may result in mild stress, which may influence some of the sensitive outcomes in this type of study (Vandanberg, 2014).

The diet will be administered to achieve a constant mg/kg/day dose, the dietary concentration being adjusted to the weekly bodyweight. However, it is necessary to first ensure that homogeneous mixing with the diet and adequate concentration analysis and stability can be achieved and that the treated diet is palatable at sufficiently high concentrations; if this is not possible then the route of administration will revert to gavage. The following approach is suggested to the final design of the EOGRT's.

Use the CAS number selected as described in section 4:

Use the dietary route of exposure.

It is proposed that cohort 1B (breeding for 2nd generation) is required

It is proposed that cohort 2 (developmental neurotoxicity) is included based on the CNS delayed startle response.

It is proposed that cohort 3 (developmental immunotoxicity) is included because organs associated with the immune system are targets for toxicity.

7. References

ARCO (1986a). Acute Dermal Toxicity Study in Rabbits Administered with Test Article F-91-01. Testing laboratory: Utah Biomedical Test Laboratory, Salt Lake City, Utah. Report no.: 62769. Owner company: ARCO, Los Angeles, CA. Study number: ATX-86-0056. Report date: 1986-11-03.

ARCO (1986b). Primary dermal irritation study in rabbits administered cherry point diesel fuel number two. Testing laboratory: UBTL, Salt Lake City, Utah. Report no.: 60579. Owner company: ARCO, Los Angeles, CA. Study number: ATX-85-0161. Report date: 1986-12-18.

ARCO (1986c). Primary dermal irritation study in rabbits administered naval distillate. Testing laboratory: UBTL, Salt Lake City, Utah. Report no.: 60576. Owner company: ARCO, Los Angeles, CA. Study number: ATX-85-0146. Report date: 1986-12-18.

ARCO. (1992). 28-Day dermal toxicity study in rats. Testing laboratory: Utah Biomedical Test Laboratory, Salt Lake City, Utah, USA. Report No. 65863. Owner company: Atlantic Richfield Company (ARCO). Study Number: ATX 90-0050. Report date: 1992-09-15.

ARCO (1992a). Approximate lethal dose study in rats administered test article F-102-01 (Naval Distillate). Testing laboratory: UBTL, Salt Lake City, Utah. Owner company: ARCO, Los Angeles, CA. Study number: 89-0013. Report date: 1992-10-16.

ARCO (1992b). Acute inhalation toxicity study in rats administered test article F-188 (sweet distillates). Testing laboratory: UBTL, Salt Lake City, Utah. Report no.: 66366. Owner company: ARCO, Los Angeles, CA. Study number: ATX-91-0093. Report date: 1992-09-21.

ARCO (1993). A photoirritation study in rabbits administered test article F-233. Testing laboratory: UBTL, Inc., Salt Lake City, UT. Owner company: Atlantic Richfield Company (ARCO). Study number: ATX-91-0231. Report date: 1993-01-21.

API. (2008). The Relationship Between Aromatic Ring Class Content and selected endpoints of repeat-dose and developmental toxicity of high boiling point petroleum streams. American Petroleum Institute, Washington D.C.

ATSDR. Agency for Toxic Substances Disease Registry. (1995). Toxicological profile for polycyclic aromatic hydrocarbons.

Autrup, H and Vestergaard, (1996). Transplacental transfer of environmental genotoxins – polycyclic aromatic hydrocarbon-albumin in nonsmoking women. Environ Health Perspect. May; 104(Suppl 3): 625–627. (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1469656/)

Balabanič D, Rupnik M, Klemenčič AK. Negative impact of endocrine-disrupting compounds on human reproductive health (2011). Reprod Fertil Dev. 23(3):403-16.

Boogaard PJ, Carrillo J-C, Roberts LG and Whale GF. (2017) Toxicological and ecotoxicological properties of gas-to-liquid (GTL) products. 1. Mammalian toxicology. Critical Reviews in Toxicology Vol. 47 , Iss. 2,

Bui, Q.Q (1986). A comparative study of the reproductive effects of methadone and benzo[a]pyrene in the pregnant and pseudopregnant rat. Toxicol 42: 195-204.

Chaloupka K, Santostefano M, Goldfarb IS, Liu G, Myers MJ, Tsyrolv IB, Gelboin HV, Krishnan V, Safe S (1994). Aryl hydrocarbon (Ah) receptor-independent induction of Cyp1a2 gene expression by acenaphthylene and related compounds in B6C3F1 mice. Carcinogenesis 15(12):2835-40.

European Commission (2002). Opinion of the Scientific Committee on Food on the risks to human health of Polycyclic Aromatic Hydrocarbons in food. European Commission Health and Consumer Protection Directorate-General. SCF/CS/CNTM/PAH/29 Final.

Feuston, M.H., Low, L.K., Hamilton, C.E., Mackerer, C.R. (1994). Correlation of systemic and developmental toxicities with chemical component classes of refinery streams. Fundamental and Applied Toxicology 22:622-630.

Hardin, B.D. et al. (1981). Testing of selected workplace chemicals for teratogenic potential. Scand J Work Environ Health 7(Suppl 4):66-75.

Hardin BD, Schuler RL, Burg JR, Booth GM, Hazelden KP, MacKenzie KM, Piccirillo VJ, Smith KN. (1987). Evaluation of 60 chemicals in a preliminary developmental toxicity test. Teratog Carcinog Mutagen. 7(1):29-48.

Hoberman, A., Christian, M., Roth, R., Lovre, S. and Koschier, F. (1995a). Reproductive toxicity study of clarified slurry oil in the rat. J Amer Coll Toxicol 14:119-128.

Hoberman, A., Christian, M., Lovre, S. Roth, R., and Koschier, F. (1995b). Developmental toxicity study of clarified slurry oil (CSO) in the rat. Fund Appl Toxicol 28:34-40.

Hoshino, K. et al., (1981). Influences of genetic factors on the teratogenicity of environmental pollutants: Teratogenic susceptibiolity of benzo[a]pyrene and Ah locus in mice. Congenital Anomalies, 97-103.

IARC. (International Agency for Research on Cancer (1993). Polynuclear Aromatic Compounds, Part 1,Chemical, Environmental and Experimental Data Vol 32.

IARC (International Agency for Research on Cancer) (1983) Benzo[a]pyrene Part 1, Chemical Environmental and Experimental Data. Vol 32.

IPCS (International Programme on Chemical Safety) (1998). Selected non0heterocyclic polycyclic aromatic hydrocarbons. Environmental Health Criteria 202.

Kamelia L., Louisse J., de Haan L., Rietjens IMCM., PJ. Prenatal developmental toxicity testing of petroleum substances: Application of the mouse embryonic stem cell test (EST) to compare in vitro potencies with potencies observed in vivo. Toxicology in Vitro Vol 44, October 2017, Pages 303-312

Kizu R, Okamura K, Toriba A, Kakishima H, Mizokami A, Burnstein KL, Hayakawa K (2003). A role of aryl hydrocarbon receptor in the antiandrogenic effects of polycyclic aromatic hydrocarbons in LNCaP human prostate carcinoma cells. Arch Toxicol 77(6):335-43. Epub 2003 Mar 12.

Kummer V, Masková J, Zralý Z, Neca J, Simecková P, Vondrácek J, Machala M (2008). Estrogenic activity of environmental polycyclic aromatic hydrocarbons in uterus of immature Wistar rats. Toxicol Lett. 80(3):212-21..

Lock, S., Dalbey, W., Schmoyer, R., Griesemer, K. (1984). Inhalation toxicology of diesel fuel obscurant in Sprague-Dawley rats, phase 3, subchronic exposures. Testing laboratory: Oak Ridge

National Laboratory, Oak Ridge, Tennessee. Report no.: TM-9403. Owner company: U. S. Army Medical Research and Development.

Machala M, Ciganek M, Bláha L, Minksová K, Vondrácк J. (2001). Aryl hydrocarbon receptor-mediated and estrogenic activities of oxygenated polycyclic aromatic hydrocarbons and azaarenes originally identified in extracts of river sediments. Environ Toxicol Chem. 20(12):2736-43.

MacKenzie, K.M. and D.M. Angevine. 1981. Infertility in mice exposed in utero to benzo[a]pyrene. Biol. Reprod. 24: 183-191.

Mattie et al., (2000). Reproductive effects of jp-8 - jet fuel on male and female Sprague-Dawley rats after exposure by oral gavage. United States Air Force Research Laboratory. 20060630299.

McKee, R.H., Pasternak, S.J., Traul, K.A. (1987a). Developmental toxicity of EDS recycle solvent and fuel oil. Toxicology 46: 205-215.

McKee, R., Wong, Z., Schmitt, S., Beatty, P., Swanson, M., Schreiner, C., and Schardein, J. (1990). The reproductive and developmental toxicity of high flash aromatic naphtha. Toxicology and Industrial Health 6:441-460.

McKee, R.H., Drummond, J.G., Freeman, J.J., Letinski, D.J., and Miller, M.J. (2012a). Light white oils exhibit low tissue accumulation potential and minimal toxicity in F344 rats. International Journal of Toxicology. 31(2): 175-183.

McKee, R., Schreiner, C., White, R., Saperstein, M., Charlap, J., O'Neil, T., and Goyak, K. (2012b). Characterization of the non-cancer hazards of gas oils. International Journal of Toxicology. Manuscript submitted, Nov 30, 2012.

Mobil (1987a). Interim report: Stock 461 rat reproduction study. Testing laboratory: Mobil Environmental and Health Science Laboratory. Owner company: Mobil. Study number: 40921-IA. Report date: 1987-04-20.

Mobil (1987b). Stock 461 rat teratology study. Testing laboratory: Mobil Environmental and Health Science Laboratory. Owner company: Mobil. Study number: 40922. Report date: 1987-02-06.

Mobil (1989a). Thirteen Week Dermal Administration of Vacuum Tower Overheads to Rats. Testing laboratory: Mobil Environmental and Health Science Laboratory, Princeton, New Jersey. Report no.: 62326. Study number: 62326. Report date: 1989-11-03.

Mobil (1989b). Developmental toxicity study in rats exposed dermally to vacuum tower overheads (VTO). Report no.: 62328. Owner company: Concawe, Brussels, Belgium. Study number: 7059

Murray FJ, Roth RN, Nicolich MJ, Gray TM, Simpson BJ. The relationship between developmental toxicity and aromatic-ring class profile of high-boiling petroleum substances Regulatory Toxicology and Pharmacology 67 (2013) S46–S59

NTP. National Toxicology Program (1991). Toxicological and carcinogenicity studies on naphthalene.

NTP. National Toxicology Program (1992). Report on carcinogens.

Patterson J, Maier A, Kohrman-Vincent M, Dourson ML. Peer consultation on relationship between PAC profile and toxicity of petroleum substances. Regulatory Toxicology and Pharmacology 67 (2013) S86–S93

Plasterer M.R., et al. (1985). Developmental toxicity of nine selected compounds following prenatal exposure in the mouse: Naphthalene, p-nitrophenol, sodium selenite, dimethyl phthalate, ethylenethiourea and four glycol ether derivatives. Toxicol Environ Health 15:25-38.

Schreiner C, Bui Q, Breglia R, Burnett D, Koschier F, Podhasky P, Lapadula L, White R, Feuston M, Krueger A, Rodriquez S. (1997). Toxicity evaluation of petroleum blending streams: reproductive and developmental effects of hydrodesulfurized kerosene. *Journal of Toxicology and Environmental Health,* 52:211-229.

Shell (2011a). Two-Generation Reproductive Toxicity Evaluation of Shell GTL Diesel Administered Via Oral Gavage to CD® (Sprague-Dawley) Rats. Research Triangle Institute - RTI 1071 - Report 0212131.000.002, Research Triangle Park, NC, USA; Owner Company: Shell International bv.

Shell (2011b). Two-Generation Reproductive Toxicity Evaluation of Shell GTL Base Oil Administered Via Oral Gavage to CD® (Sprague-Dawley) Rats. Research Triangle Institute - RTI 1070 - Report 0212131.000.001, Research Triangle Park, NC, USA; Owner Company: Shell International bv.

White, R. (2012). The Relationship Between Aromatic Ring Class Profile and the Toxicity of High-Boiling Petroleum Substances. SOT abstract, March 2012.

Vandenberg LN**,** Welshons WV**,** vom Saal FS**,** Toutain P-L **and** Peterson Myers P (2014) Should oral gavage be abandoned in toxicity testing of endocrine disruptors? Environmental Health 2014, **13**:46 doi:10.1186/1476-069X-13-46 at http://www.ehjournal.net/content/13/1/46

Ziccardi, M.H., Gardner, I.A., Denison, M.S (2002). Application of the luciferase recombinant cell culture bioassay system for the analysis of polycyclic aromatic hydrocarbons. Environ Toxicol Chem. 21(10):2027-33.