Helsinki, 29 April 2020

Addressees
Registrants of 265-110-5/64742-10-5 listed in the last Appendix of this decision

Date of submission for the jointly submitted dossier subject of a decision
11/04/2019

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: Extracts (petroleum), residual oil solvent
EC number: 265-110-5
CAS number: 64742-10-5

Decision number: [Please refer to the REACH-IT message which delivered this communication (in format TPE-D-XXXXXXXXXX-XX-XX/F)]

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation (EC) No 1907/2006 (REACH), ECHA requests that you submit the information listed below.

A. Requirements applicable to all the Registrants subject to Annex VIII of REACH

1. In vivo mammalian alkaline comet assay (Annex VIII, Section 8.4, Column 2; test method OECD TG 489) with the Substance– see request B.1 for details.

B. Requirements applicable to all the Registrants subject to Annex IX of REACH

1. In vivo mammalian alkaline comet assay (Annex IX, Section 8.4; test method OECD TG 489) with the Substance;

2. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method OECD TG 408) in rats with the Substance;

3. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method OECD TG 414) in a first species (rat or rabbit), oral route, with the Substance.

C. Requirements applicable to all the Registrants subject to Annex X of REACH

1. Pre-natal developmental toxicity study (Annex X, Section 8.7.2.; test method OECD TG 414) in a second species (rabbit or rat), oral route, with the Substance.

Your originally proposed test using the Substance is rejected, according to Article 40(3)(d):
- In vivo mammalian erythrocyte micronucleus test (EU B.12./OECD TG 474) combined with a combined repeated dose toxicity study with reproductive toxicity screening test, (OECD TG 422);

Your originally proposed test using substance extracts (petroleum), deasphalted vacuum residue solvent, EC No 295-332-8, CAS RN 91995-70-9), later referred to as Substance 2 in this decision, is rejected, according to Article 40(3)(d):

- Sub-chronic toxicity study (90-day), dermal route (OECD TG 411).

Conditions to comply with the requests

Each addressee of this decision is bound by the requests for information corresponding to the REACH Annexes applicable to their own registered tonnage of the Substance at the time of evaluation of the jointly submitted dossier.

To identify your legal obligations, please refer to the following:

- you have to comply with the requirements of Annexes VII and VIII of REACH, if you have registered a substance at 10-100 tpa;
- you have to comply with the requirements of Annexes VII to IX of REACH, if you have registered a substance at 100-1000 tpa;
- you have to comply with the requirements of Annexes VII to X of REACH, if you have registered a substance at above 1000 tpa.

Registrants are only required to share the costs of information they are required to submit to fulfil the information requirements for their registration.

When a study is required under several Annexes of REACH, the reasons are provided in the corresponding appendices of this decision. The registrants concerned must perform only one study and make every effort to reach an agreement as to who is to carry out the study on behalf of the other registrants in accordance with Article 53 of REACH.

The Appendix on general considerations addresses issues relevant for several requests while the other Appendices state the reasons for the requests for information to fulfil the requirements set out in the respective Annexes of REACH.

The Appendix entitled Observations and technical guidance addresses the generic approach and the specific requirements for the selection and reporting of the test material used to perform the required studies and provides generic recommendations and references to ECHA guidance and other reference documents.

Based on Article 40(3)(b) and (c) of REACH, you must submit the information requested in points A.1, B.1-3 above in an updated registration dossier by ***4 August 2021***, and the information requested in point C.1 above by ***4 August 2022***.

You must also update the chemical safety report, where relevant, including any changes to classification and labelling based on the newly generated information. The timeline has been set to allow for sequential testing where relevant.

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, has to be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under: http://echa.europa.eu/regulations/appeals.

Approved[1] under the authority of Christel Schilliger-Musset, Director of Hazard Assessment

[1] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Appendix on general considerations

(i) Assessment of the Grouping of substances and read-across approach under Annex XI, Section 1.5.

You seek to adapt the following standard information requirements by grouping substances in the category and applying a read-across approach in accordance with Annex XI, Section 1.5:

- Sub-chronic toxicity study (90-day), (Annex IX, Section 8.6.2.)
- Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)
- Pre-natal developmental toxicity study in a second species (Annex X, Section 8.7.2.)

ECHA has considered the scientific and regulatory validity of your grouping and read-across approach in general before assessing the specific standard information requirements in the following appendices.

Grouping of substances and read-across approach

Legal Background on ECHA's assessment of the grouping of substances and read-across hypothesis

The evaluation by ECHA of testing proposals submitted by registrants aims at ensuring that generation of information is tailored to real information needs. To this end, it is necessary to consider whether programmes of testing proposed by you are appropriate to fulfil the relevant information requirements and to guarantee the identification of health and environmental hazards of substances. In that respect, the REACH Regulation aims at promoting wherever possible the use of alternative means, where equivalent results to the prescribed test are provided on health and environmental hazards.

Article 13(1) of the REACH Regulation provides that information on intrinsic properties of substances may be generated whenever possible by means other than vertebrate animal tests, including information from structurally related substances (grouping of substances and read-across), "*provided that the conditions set out in Annex XI are met*".

The first Recital and the first Article of the REACH Regulation establish the "*promotion of alternative methods for assessment of hazards of substances*" as an objective pursued by the Regulation. In accordance with that objective, ECHA considers whether a prediction of the relevant properties of the substance subject to the present decision by using the results of the proposed tests is plausible based on the information currently available.

General considerations

Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group (addressed under 'Assessment of prediction(s)').

Additional information on what is necessary when justifying a read-across approach can be

found in the ECHA Guidance[2] and related documents[3, 4].

A. Predictions for toxicological properties

You have provided read-across justification documents in IUCLID Section 13 and under the relevant endpoints in IUCLID Section 7.8. and 7.5. These documents are: [redacted]. ECHA notes that these documents have overlapping scope, and that the same topics may be argued differently in the various documents. Based on the totality of your documents, ECHA understands that your arguments are as follows.

You have formed a category of "*Residual Aromatic Extracts (RAE)*' within a wider grouping of petroleum substances. You read-across between the structurally similar substance, Extracts (petroleum), deasphalted vacuum residue solvent (EC No. 295-332-8) as source substance and the Substance as target substance. You have provided a testing strategy, and you plan to perform tests on the source substance and to read-across to the Substance. You also propose to undertake testing to support the hypothesis, including analytical data, *in-vitro* developmental toxicity battery, Cat-App and dermal and oral *in vivo* data.

You provide the following reasoning for the grouping of the substances: "*The RAE substances are aromatic extracts manufactured by* [redacted] *<...>. RAE substances are not intentional mixtures of chemicals but are complex combinations of hydrocarbon species, produced to meet physical -chemical and technical performance specifications. The RAE category is formed on the principle that RAE substances have similar physical - chemical properties, broadly similar composition and present similar health, safety and environmental hazards".* You define the applicability domain of the category as follows: "*The domain of this category is established by the refining processes by which the category members are produced and the minimum carbon numbers".* You further add that "*the RAE category consists of UVCB substances which are sufficiently similar and therefore a category order is not relevant*". You provided a generic compilation of compositional information of these two substances from measurements using chromatographic techniques (i.e. average carbon number distribution and average relative mass (%) of four major hydrocarbon classes named saturates, aromatics, resins and asphaltenes). Furthermore, in your category justification you claimed that "*comprehensive two-dimensional gas chromatography (GCxGC) cannot be used to obtain detailed information on the hydrocarbon composition of RAE substances since the upper temperature limit of GCxGC samples restricts its application to substances with carbon numbers below approximately C 30 (*[redacted] *2006).*"

You have provided the following reasoning for the prediction of toxicological properties: (1) broadly similar [chemical] composition, (2) there are similar physico-chemical, environmental and human health properties, (3) the biological activity profile of the substances in the 'Cat-App' project and other *in vitro* tests provides a basis for predicting the properties in relevant *in vivo* tests, (4) the worst case or PAH hypothesis, that toxicity is determined by the presence and relative abundance of condensed polycyclic aromatic hydrocarbons (PAH) with three or more rings, and that testing the substance with the highest 3+ ring PAH content will be the

[2] ECHA Guidance R.6
[3] ECHA Read-across assessment framework (RAAF, March 2017)
[4] ECHA Read-across assessment framework (RAAF, March 2017) - considerations on multi-constituent substances and UVCBs.

worst case.

ECHA understands that you predict the properties of the Substance using a read-across hypothesis which assumes that different compounds have the same type of effects. The properties of your Substance are predicted to be quantitatively equal to those of the source substance.

ECHA notes the following shortcomings with regards to prediction of toxicological properties, for each of your four reasons for predicting the properties of the registered substance.

a) Basis to predict the properties of the Substance

1. Broadly similar [chemical] composition

Annex XI, Section 1.5 of the REACH Regulation provides that *"substances whose physicochemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of chemical similarity may be considered as group."*

According to the ECHA Guidance, *"the purity and impurity profiles of the substance and the structural analogue need to be assessed"*, and *"the extent to which differences in the purity and impurities are likely to influence the overall toxicity needs to be addressed, and where technically possible, excluded"*. The purity profile and composition can influence the overall toxicity/properties of the Substance and of the source substance(s).[5] Therefore, qualitative and quantitative information on the compositions of the Substance and of the source substance(s) should be provided to allow assessment whether the attempted predictions are compromised by the composition and/or impurities.

Furthermore, whenever the Substance and/or the source substance(s) are UVCB (Unknown or Variable composition, Complex reaction products or of Biological materials) substances qualitative compositional information of the individual constituents of the category members needs to be provided; as well as quantitative characterisation in the form of information on the concentration of the individual constituents of these substances; to the extent that this is measurable.[6]

You have defined the applicability domain of the category as explained above. Your read-across justification document contains compositional information for the members of your category. As set out in the [redacted] 2.2 and 2.3, the category members are UVCBs and their chemical composition is resolved into saturates, aromatics, resins and asphaltenes. You provide average total concentrations of these four hydrocarbon classes measured by two different chromatography methods (thin layer and liquid chromatography). ECHA notes that you report different average composition as outcome of two different analytical methods. You further explain that there is no clear distinction between the hydrocarbon classes of these substances due to their intrinsic variability. There is further limited chemical analysis of PAC-2 in [redacted] which gives the PAC (PAH) content as weight percentage, and the percentage of PAC (PAH) which has ≥3 rings. The individual chemical constituents, their structural features and quantitative variation for the Substance are not further resolved by analytical methods.

In your comments to the draft decision, you argue that these two RAE category members are sufficiently similar so as to validate structural similarity for the purpose of read-across

[5] ECHA Guidance R.6, Section R.6.2.3.1
[6] ECHA Guidance R.6, Section R.6.2.5.5

according to Annex XI, 1.5.

You support these arguments with the following:

a. reference to existing physico-chemical data for characterisation of these two RAE category members;
b. reference to a published report, and specifically analytical data including GC-MS data on specific EPA and Grimmer PAHs, elemental analysis, TLC-FID, LCC, PAH content on the basis of DMSO extraction and gravimetry, SIMDIS-GC, and PAC 2 analysis;
c. that it is practically impossible and serves no purpose to identify all of the components in RAEs;
d. that the standard proposed by ECHA is unrealistic and excludes petroleum substances from using read-across;
e. hypothesising about the potential number of components and their possible concentration, and thus concluding that only simple compositional breakdown has been provided; and
f. arguing that the constituent pools that have been measured are representative of the pools referred to in the multi-constituent/ UVCB RAAF considerations, and thus are compliant with ECHA's Guidance.
g. arguing that the Extracts, (petroleum), residual oil solvent (EC No. 265-110-5) "is manufactured in versions with both MI > 0.4 and MI < 0.4 and in far greater volumes than CAS 91995-70-9", concluding that this is the most representative substance for this category based on production volume and current self-classification for carcinogenicity cat 2.

Without description beyond the broad categories provided (saturates, aromatics, resins and asphaltenes), no qualitative or quantitative comparative assessment of the compositions of the different category members can be completed. ECHA considers that there is insufficient information on (the identity of) the constituents and their quantitative characterisation to be able to determine what the individual constituents of the category members are or their commonality between the substances, and the similarity in terms of concentration of individual constituents. In the absence of this information, it is not possible to come to a view on what the key chemical constituents of the substances are which determine the human health properties of the substances, and whether these are present in a quantitatively similar manner between the substances. In respect of the analysis of PAH content (i.e. the results from PAC-2 analysis), ECHA considers that this information is not sufficient for the reasons set out under "Worst case or PAH hypothesis" below. Therefore you have not established chemical similarity between the substances, which is a prerequisite for prediction of the properties of the Substance.

Your comments do not remedy these deficiencies:

a. physico-chemical characterisation of these two RAE category members does not demonstrate similarity of chemical constituents of these substances.
b. The referenced report is not provided, and ECHA cannot further examine it. The provided elemental analysis, TLC-FID, LCC, DMSO extraction and gravimetry, SIMDIS-GC and PAC2 analysis do not provide information on the individual chemical constituents of the two abovementioned substances which are RAE category members, but rather provide physico-chemical characterisation of the substance as a whole, or provide low resolution analysis on large groups of constituents. ECHA considers that there is insufficient information on (the identity of) the constituents and their quantitative characterisation to be able to determine what the individual constituents of the category members are or their commonality between the substances, and the similarity in terms of concentration of individual constituents. The GC-MS data on specific EPA and Grimmer

PAHs is not provided with a methodological description, nor any characterisation of Limit of Detection, analytical variability or variability within the registration/substance; in the absence of documentation of these key parameters, the information cannot be considered as reliable. In any case, documented GC-MS data account for approximately 8 or 19 mg of every kg of substance; and so it follows that the vast majority of the composition of the substance is uncharacterised at an adequate level.

c. Your claim that it is practically impossible to measure constituents is not substantiated. You have not demonstrated structural similarity in accordance with Annex XI, Section 1.5.
d. You have not (a) shown either the identity of individual constituents OR their commonality between the substances; and (b) characterised the concentration of these constituents, including a measure of variability of the constituents, between the substances. It is not required to provide detailed structural information on all constituents of a UVCB substance, but there must be sufficient characterisation of constituents so as to demonstrate structural similarity, and subsequently provide a basis for predicting the properties of the substance for read-across. ECHA has not expressed an opinion that it is not possible to perform read-across for UVCB substances.
e. The comment on composition of these two substances in the RAE category is based on theoretical considerations without substantiation; in the absence of adequate information on the composition of the substance, structural similarity is not demonstrated for the purpose of read-across.
f. In [redacted] Figure 1 on page 15 refers to pools of substances, saying "*Each individual pool consists of structurally similar substances, but the two different pools are not similar to each other. The graphical representations only relate to the chemical structural similarity, they are not implying that they are similar also in terms of properties.*" Thus this document states that there must be constituents of known structural similarity in order to group into a '*pool*', in line with this decision. You have not demonstrated the identity or commonality, plus the abundance, of constituents, and thus you have not demonstrated structural similarity.

Summarising the above arguments, you did not provide qualitative and quantitative information on the compositions of the Substance and of the source substance(s) to allow assessment whether the attempted predictions are compromised by the composition and/or impurities and you have not demonstrated that there is structural similarity for the purpose of justifying read-across according to Annex XI, 1.5

2. Similar physico-chemical, environmental and human health properties

According to Annex XI, Section 1.5., two conditions shall be necessarily fulfilled. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group (read-across approach).

A read-across hypothesis needs to be provided, establishing why a prediction for a toxicological or ecotoxicological property is reliable. This hypothesis should be based on recognition of the structural similarities and differences between the source substance(s) and your Substance[2]. It should explain why the differences in the chemical structures should not influence the toxicological/ecotoxicological properties or should do so in a regular pattern.

Your read-across hypothesis is that the similarity in chemical structure and in some of the physicochemical/ecotoxicological/toxicological properties between the source substance(s) and your Substance is a sufficient basis for predicting the properties of your Substance for other endpoints. You also propose to undertake testing in future to support the hypothesis, including analytical data, *in-vitro* developmental toxicity battery, Cat-App and dermal and oral *in vivo* data.

In your comments to the draft decision, you claim that these two RAE category members are the same and do share similar physico-chemical properties (boiling point ranges, density, flash point). Furthermore, you refer to your plan to conduct OECD 422 studies on both substances, in order to provide further support to the substance sameness.

Similarity in chemical structure and similarity of some of the physicochemical/ecotoxicological/toxicological properties does not necessarily lead to predictable or similar human health properties in other endpoints. As described above, a well-founded hypothesis is needed to establish a reliable prediction for a toxicological property, based on recognition of the structural similarities and differences between the source substance(s) and your Substance. Further, ECHA cannot take into account the results of future testing in establishing the similarity of the substances at this stage.

The same considerations apply to your comments to the draft decision. Regarding your intention to conduct new studies, future studies cannot be taken into account for this testing proposal examination.

3. Biological activity profile of the substances in the 'Cat-App' project and other *in vitro* tests as a basis for predicting the properties in relevant *in vivo* tests

According to Annex XI, Section 1.5., two conditions shall be necessarily fulfilled. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group (read-across approach).

A read-across hypothesis needs to be provided, establishing why a prediction for a toxicological or ecotoxicological property is reliable. This hypothesis should be based on recognition of the structural similarities and differences between the source substance(s) and your Substance[2]. It should explain why the differences in the chemical structures should not influence the toxicological/ecotoxicological properties or should do so in a regular pattern.
You have provided information about the biological activity profile of the substances in the 'Cat-App' project and other *in vitro* tests, as described below.

In your comments to the draft decision, you have provided comments on this argument (see also b.3 below), and you argue that:

i. The CAT-APP approach should be considered with other information provided and does not replace standard information on its own.
ii. Cat-App's only purpose is to show that a similar biological response or fingerprint can be generated by similar substance.
iii. In respect of the use of DMSO extracts, you contend that the DMSO extractable fraction represents the biologically available and active fraction of the substances, including by reference to information from the modified Ames test. You add that it is possible to compare the results of DMSO extracts from different substances.

The method does not provide a reliable basis to support read-across because:

a) the consequence of testing DMSO extracts of the substances, as opposed to the substances themselves, on the outcome of *in vivo* and *in vitro* testing is not explained. Thus testing DMSO extracts does not provide a basis for reliably predicting the properties of the Substance.
b) In line with the tests of Annex XI, 1.4, ECHA agrees with your statement that the *in vitro* tests 'cannot and should not be interpreted as screening assays for toxicity' and considers that the individual *in vitro* results have no clear relation to *in vivo* toxicity assays. Consequently, any relationship between substances in *in vitro* test results has an unclear relationship to any *in vivo* toxicity assays on the test substances. There is thus no adequate basis for using this data to predict that the results of *in vivo* tests can be read-across from one substance to another.
c) The data analysis methods, by reducing the data from multiple tests to a single dimension (the ToxPi Bioactivity), does not allow examination of the differences between test substances.

For all the above reasons, ECHA considers that the Cat-App and other *in vitro* data does not provide a basis to reliably predict the properties of the Substance.

Your comments do not remedy these deficiencies for the following reasons:

i. ECHA agrees with you that the provided Cat-App data can only be considered as a part of supporting evidence on read-across and grouping approach and that these data are not intended to replace standard information requirements.
ii. ECHA has outlined above a variety of methodological and scientific concerns which mean that it is not possible to independently assess the methods and results of Cat-App, and that the Cat-App data does not provide a basis to reliably predict the properties of the Substance. You have, for example, not addressed point c above. According to ECHA's understanding, Cat-App's only purpose is to show that a similar biological response or fingerprint can be generated by a similar substance. Therefore, it seems that it may only achieve a pre-determined outcome which is considered biased.
iii. There is not a robust justification for extrapolating from the results obtained with DMSO extracts *in vitro* compared with testing the whole substance *in vivo*. ECHA accepts that DMSO extracts 3-7 ring PAHs preferentially; but this is a different proposition to demonstrating that the material which is not extracted has known properties, and this latter aspect is not adequately addressed. For example, you have not provided results from testing on the material which is left after DMSO extraction of the Substance for all human health endpoints.

Therefore, the Cat-App and other *in vitro* data does not provide a basis to reliably predict the properties of the Substance.

4. Worst case or PAH hypothesis

Annex XI, Section 1.5 requires that whenever read-across is used adequate and reliable documentation of the applied method must be provided. Such documentation must provide a justification for the read-across including a hypothesis, and explanation of the rationale for the prediction of properties providing sufficient information to make an independent assessment of the hypothesis and explanation.

You have proposed that reproductive toxicity is primarily associated with the presence of condensed PAH of three or more rings, and that the substance with the highest content of PAH of three or more rings will be worst case. Your hypothesis is based on analysis of (a) *in vivo* data (inter alia) [redacted] 1994, [redacted] 2012, [redacted] 2013 and [redacted] 2013 (b) analysis of *in vitro* data (e.g. [redacted] 2017) and (c) chemical analysis of PAC-2 in [redacted] which gives the PAC (PAH) content as weight percentage, and the percentage of PAC (PAH) which has ≥3 rings.

In your comments to the draft decision, you argue that:

(i) ECHA rejects that PAHs are the causative moieties because historically tests have been done by the dermal route.
(ii) *in vitro* studies: the use of DMSO as a solvent is widely accepted in *in vitro* studies.
(iii) information on the non-DMSO-extractable portion of the substance can be obtained from other petroleum stream substances.

We have evaluated the information in your dossier and found the following deficiencies:

a) *in vivo* studies. ECHA notes that the analyses appear to be based upon principally dermal studies, and ECHA considers that these dermal studies do not provide adequate information about the hazardous properties of the substance, since the oral route of exposure is the most appropriate route. (Repeated dose and reproductive toxicity studies must be performed by the "most appropriate route of administration, having regard to the likely route of human exposure." Please also see the sections on repeated-dose and pre-natal developmental toxicity.) Since the systemic availability of chemical components of a substance after oral and dermal administration would be expected to be significantly different, the toxicological properties of a substance could be significantly different when comparing between oral and dermal administration. As your hypothesis is based principally on dermal studies, and ECHA considers that these do not provide adequate information for oral administration, ECHA considers that the hypothesis is not a reliable basis for predicting the properties of the registered substance after oral administration.

b) *in vitro* studies. As set out in A.a.3 and A.b.3, ECHA considers that the testing of DMSO extracts does not provide a reliable basis for predicting the properties of the Substance.

c) PAH analysis. In view of the inadequate characterisation of the chemical composition of the substances (see issue 1 above), it is not possible to conclude on which chemical components of the source substance or Substance are most hazardous and determine the toxicity of the substance. ECHA agrees that the DMSO-extractable ≥3-ring PAHs in the Substance are capable of being toxic, but notes that there is not a demonstration that other components of the substances are non-toxic when adequately tested.

For the above reasons, your worst case hypothesis does not provide a basis for reliably predicting the properties of the Substance.

Your comments do not remedy these deficiencies for the following reasons:

(i) ECHA does not reject that PAHs are the causative moieties because historically tests have been done by the dermal route. Rather, the studies by the dermal route do not provide adequate information on toxicological effects after oral administration (for the reasons given in A.4.a above), and for this reason a hypothesis based primarily upon dermal route studies cannot reliably predict the

results of oral route studies required under REACH.

(ii) The use of DMSO as a solvent *in vitro* is a distinct issue to the use of DMSO extracts for testing to predict the results of testing the Substance. In particular, the extrapolation from *in vitro* testing using DMSO extracts to *in vivo* testing using whole substance is not adequately justified (see sections a.3 and b.3). One particular aspect is the failure to identify the *in vivo* human health properties of the material which is not extracted by DMSO.

(iii) You have not provided *in vivo* test results on the material left behind after DMSO extraction, nor a substance-specific justification for what these results should be. In the absence of this information, it is not possible to reliably predict the properties of the non-DMSO extractable portion of the Substance.

In summary, ECHA concludes that in your comments to the draft decision, you did not address ECHA's concerns and your worst case hypothesis does not provide a basis for reliably predicting the properties of the Substance.

b) Adequate and reliable documentation

Annex XI, Section 1.5 requires that whenever read-across is used adequate and reliable documentation of the applied method must be provided. Such documentation must provide a justification for the read-across including a hypothesis, and explanation of the rationale for the prediction of properties providing sufficient information to make an independent assessment of the hypothesis and explanation.

3. Biological activity profile of the substances in the 'Cat-App' project and other in vitro tests as a basis for predicting the properties in relevant in vivo tests

You have provided information about the biological activity profile of the substances in the 'Cat-App' project and other *in vitro* tests. You argue that this provides a basis for supporting the chemical-biological grouping and read-across assessment of petroleum substances, i.e. predicting the properties of the Substance from relevant *in vivo* tests on the source substance. The Cat-App project uses DMSO extracts of petroleum substances, and tests these on a number of *in vitro* assays with a number of outputs. The data are subject to complex data-processing (which are not described fully) and the results are described as preliminary and subject to change. The experimental methods are not according to an OECD Test Guideline, and are not clearly described in terms of experimental treatment of cell systems, output parameters, data analysis and processing, and positive and negative controls. The results, including controls, are not provided for all assays and substances.

In your comments to the draft decision, you have provided comments on this argument (see also a.3 above), and you argue that:

(i) the read-across is between these two RAE category members, but that this is part of a much wider category of substances. These category members have limited characterisation, but you believe they are adequately characterised to the extent that this is measurable with today's available analytical methods.

(ii) in respect of the use of DMSO extracts, you contend that the DMSO extractable fraction represents the biologically available and active fraction of the substances. You refer to information from the modified Ames test, and claim that it is possible to compare the results of DMSO extracts from different substances.

(iii) in respect of questions regarding the quality control issues with the studies, several aspects of quality control are discussed, and some details of methodology and quality control are provided.

(iv) that the absence of an OECD test guideline has no bearing.

In view of the very limited information on the methods and results, and the caveat that the results are preliminary and subject to change, it is not possible to make an independent assessment of the information provided, and whether it supports the hypothesis and explanation. Additionally and specifically, there is no specific justification including comparison between the source substance and the Substance which can be used to justify read-across between the source substance and the Substance.

Your comments do not remedy these deficiencies for the following reasons:

i. To the extent that Grouping and read-across is proposed for other substances outside the RAE category, this must be adequately documented according to Annex XI, 1.5. There is no specific justification for read-across between the substances in the Cat-App project and the registered substance (apart from the read-across between the two members of the RAE category).

ii. There is not a robust justification for extrapolating from the results obtained with DMSO extracts *in vitro* compared with testing the whole substance *in vivo*. ECHA accepts that DMSO extracts 3-7 ring PAHs preferentially, but this is a different proposition than to demonstrate that the material which is not extracted has known properties, and this latter aspect is not adequately addressed. For example, you have not provided the results of *in vivo* human health testing for the material which is left behind after DMSO extraction.

iii. While some methodological information is provided, the material provided falls short of that which is needed to conduct an independent assessment of the methodology and the results. For example, you have not provided the details of the 43 assays, including test material preparation and identity, amount of DMSO vehicle added, treatment time, read-out for each assay and the positive and negative controls for each assay. There are inconsistencies in your description of negative controls (e.g. "concordance of three types of negative controls (media, pure DMSO, and "method blank" vehicle)" versus "each negative control well (DMSO or Media) for each assay"), undefined processes (e.g. "normalized to the method control mean", "a variance stabilization was applied") and some confusion between negative controls and experimental results (e.g. "Excessive variation within or across plates would be potential evidence of undesirable plate effects or differential effects of DMSO and media").

iv. Regardless of the absence of OECD test guideline, you have not provided a detailed description of the methodology and results in order to allow an independent assessment of the tests.

Therefore, the above documentation is not adequate and reliable.

4. Worst case or PAH hypothesis

You have proposed that reproductive toxicity is primarily associated with the presence of condensed PAH of three or more rings, and that the substance with the highest content of PAH of three or more rings will be worst case. Your hypothesis is based on referenced papers (inter alia) [redacted] 1994, [redacted] 2012, [redacted] 2013, [redacted] 2013 and [redacted] 2017. The referenced papers are not provided. The general approach is described (assessing a correlation between PAH content and toxicity of a series of petroleum substances), some key conclusions and outputs are listed but the majority of the detail of the studies used, analysis methods and results are not provided in the dossier.

In your comments to the draft decision, you argue that:

(i) information on the non-DMSO-extractable portion of the proposed test substance can be obtained from other petroleum stream substances (without further explanation or details).

In view of the very limited information on the methods and results for key underlying studies which support your hypothesis, it is not possible to make an independent assessment of the information provided, and whether it supports the hypothesis and explanation.

Your comments do not remedy these deficiencies for the following reasons:

(i) You have not provided *in vivo* test results on the material left behind after DMSO extraction, nor a detailed substance-specific justification for what these results should be; you have only provided a reference to data on unspecified other petroleum stream substances. In the absence of this information, there is not adequate and reliable documentation of your hypothesis.

Therefore, the above documentation is not adequate and reliable.

B. Conclusions on the grouping of substances and read-across approach

Based on the above considerations ECHA concludes that you have not provided adequate and reliable information to demonstrate that the proposed read-across approach is plausible for the endpoints in consideration. ECHA therefore concludes that the criteria of Annex XI, Section 1.5, are not met, and consequently the testing proposed on the source substance is not appropriate to fulfil the information requirement of the substance subject to the present decision.

(ii) Assessment of your argument that category substances are the same

The test material in a study must be representative for the Substance (Article 10 and Recital 19 of REACH; ECHA Guidance R.4.1).

In your comments to the draft decision, you propose for all tests covered by this decision to test Extracts (petroleum), residual oil solvent (EC No 265-110-5; CAS 64742-10-5) because you argue that this substance and the Substance are, on the basis of chemical composition, the same substance and should be considered as a single substance for REACH purposes.

You support these arguments with the following:

a. reference to existing physico-chemical data for characterisation of the substances.
b. reference to a published report, and specifically analytical data including GC-MS data on specific EPA and Grimmer PAHs, elemental analysis, TLC-FID, LCC, PAH content on the basis of DMSO extraction and gravimetry, SIMDIS-GC, and PAC 2 analysis.
c. that it is practically impossible and serves no purpose to identify all of the components in RAEs.
d. that the standard proposed by ECHA is unrealistic and excludes petroleum substances from using read-across.
e. hypothesising about the potential number of components and their possible concentration, and thus concluding that only simple compositional breakdown has been provided.
f. arguing that the constituent pools that have been measured are representative of the pools referred to in the multi-constituent/ UVCB RAAF considerations, and thus are

compliant with ECHA's Guidance.

g. arguing that the Extracts, (petroleum), residual oil solvent (EC No. 265-110-5) "is manufactured in versions with both MI > 0.4 and MI < 0.4 and in far greater volumes than CAS 91995-70-9", concluding that this is the most representative substance for this category based on production volume and current self-classification for carcinogenicity cat 2.

However, the information provided is not sufficient to characterise (see Section (i)(A)(1) above) the two substances and thus you have not demonstrated that Extracts (petroleum), deasphalted vacuum residue solvent (EC No. 295-332-8) is the same, or is representative for, the Substance. Therefore, your proposal to test only one category member, the Extracts, (petroleum), residual oil solvent (EC No. 265-110-5), is rejected.

In any case, you are reminded that, when a substance is manufactured or imported by multiple manufacturers/importers, there must be one joint submission (REACH, Article 11); not two joint submissions for the same substance.

Appendix A: Reasons for the requirements applicable to all the Registrants subject to Annex VIII of REACH

This decision is based on the examination of the testing proposals you submitted.

1. **In vivo mammalian alkaline comet assay (Annex VIII, Section 8.4, Column 2; test method OECD TG 489)**

Mutagenicity is an information requirement as laid down in Section 8.4. of Annexes VII to X to REACH. Column 2 of Annex VII, Section 8.4. provides that "appropriate in vivo mutagenicity shall be considered in case of a positive result in any of the genotoxicity studies in Annex VII or VIII."

The technical dossier contains two *in vitro* Ames studies performed according to modified OECD TG 471 with the registered substance that show positive results.

More specifically, you have provided:

- Key study: Modified Ames test, 2012, GLP not specified, ambiguous results (negligible mutagenic activity observed, 3/7 samples positive);
- Supporting study: Modified Ames test, 2014, GLP, positive (mutagenicity index (MI)= 0.47).

The positive results indicate that the Substance is inducing gene mutations under the conditions of the tests.

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations is not available for the Substance (see section B.1) but shall be considered. Consequently, there is an information gap and you considered it necessary to generate information for this endpoint.

Therefore, pursuant to Article 40(3)(c) of the REACH Regulation, you are requested to carry out an additional study with the Substance.

Please consult request B.1 below on the selection and design of the requested additional study.

Appendix B: Reasons for the requirements applicable to all the Registrants subject to Annex IX of REACH

This decision is based on the examination of the testing proposal you submitted.

1. In vivo mammalian alkaline comet assay (Annex IX, Section 8.4; test method OECD TG 489)

An *in vivo* somatic cell genotoxicity study is a standard information requirement as laid down in Section 8.4., Column 2 in Annex IX to REACH. This study must be considered if there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and there are no results available from an *in vivo* study already.

Trigger: concern on gene mutation
The technical dossier contains two *in vitro* Ames studies performed according to modified OECD TG 471 with the registered substance that show positive results.

More specifically, you have provided:
- Key study: Modified Ames test, 2012, GLP not specified, ambiguous results (negligible mutagenic activity observed, 3/7 samples positive);
- Supporting study: Modified Ames test, 2014, GLP, positive (mutagenicity index (MI)= 0.47).

The positive results indicate that the Substance is inducing gene mutations under the conditions of the tests.

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations is not available for the Substance but shall be considered. Consequently, there is an information gap and you considered it necessary to generate information for this endpoint.

Test selection

In vitro cytogenicity data and in vivo data on chromosomal aberration

You did not provide an *in vitro* cytogenicity study. You did provide a negative *in vivo* micronucleus study. To be considered adequate, the *in vivo* study has to meet the requirements of OECD TG 474, and the key parameters of this test guideline include:

- The study must include a minimum of three doses/groups of treated animals, as well as a negative control group and a positive control group.
- The highest dose studied must be the maximum tolerated dose (MTD), i.e. the highest dose that is tolerated without evidence of toxicity (e.g. body weight depression or hematopoietic system cytotoxicity, but not death or evidence of pain, suffering or distress necessitating humane euthanasia). The highest dose can also be a dose that produces toxicity in the bone marrow (e.g. a reduction in the proportion of immature erythrocytes among total erythrocytes in the bone marrow or peripheral blood).
- The proportion of immature among total (immature + mature) erythrocytes must be determined for each animal (by counting a total of at least 500 erythrocytes for bone marrow and 2000 erythrocytes for peripheral blood).
- At least 4000 immature erythrocytes per animal must be scored for the incidence of micronucleated immature erythrocytes.

- The proportion of immature erythrocytes among total erythrocytes and the mean number of micronucleated immature erythrocytes must be reported for each group of animals.

The reported data for the in vivo study submitted did not include:

- the appropriate number of doses (only two doses were used).
- a maximum studied dose that is a MTD or induces toxicity (no toxicity reported).
- the analysis of the adequate number of cells.
- a negative control with a response inside the historical control range of the laboratory.
- data on the proportion of immature erythrocytes among total erythrocytes and the mean number of micronucleated immature erythrocytes for each group of animals.

Therefore, the provided *in vivo* test is not adequate and consequently, your dossier does not contain any relevant *in vivo* data on chromosomal aberration.

In your comments to the draft decision, you have provided the following arguments:

You acknowledge that there are gaps in the amount of lower-tier genotoxicity data currently available (hence new tests proposed), but do not fully agree with ECHA's arguments for conducting the tests outlined in the draft decision because you state that you submitted several Modified Ames data (positive and negative results), and a GLP OECD 476 assay (negative for gene mutations in mammalian cells), which you consider as acceptable. You further state that your proposed *in vivo* micronucleus test conducted as part of an OECD 422 study has been rejected by ECHA. You further refer to available dermal carcinogenicity data for this category, and point out that skin is a target organ, as it has been clearly demonstrated that some RAE's, those with a mutagenicity index of > 0.4 are associated with positive results in mouse skin painting studies. However, you agree that "...there is a data gap for cytogenicity, and positive in-vitro gene mutation tests require further investigation in an in vivo test."

ECHA acknowledges your agreement on the limitations of provided data and an identified data gap for cytogenicity. ECHA further acknowledges your agreement to conduct an *in vivo* assay to investigate both gene mutation and chromosomal aberration.

Proposed standard and modified Ames tests

You have submitted a sequential testing plan proposing to conduct new standard and modified Ames tests with the Substance.

While ECHA acknowledges your intentions, it further notes that a test covering an endpoint of Annex VII as proposed by you does not fall within the scope of the examination of a testing proposal and that it is at your discretion to conduct such tests.

Proposed combined OECD 422/OECD 474 study

You have also submitted a proposal to test the Substance by conducting a combined OECD 422/OECD 474 study, oral route and provided the following information regarding a genotoxicity endpoint:

- Two modified Ames assays conducted on petroleum substances, RAE category members (CAS 91995-70-9 and CAS 64742-10-5), showing mutagenicity Index (MI)

of 0.47 and 0.29, respectively;
- *In vivo* micronucleus study conducted with "most likely" your Substance (CAS 64742-10-5) demonstrating negative results.

ECHA requested your considerations for alternative methods to fulfil the information requirement for Genetic toxicity in vivo. ECHA notes that you provided your considerations concluding that there were no alternative methods which could be used to adapt the information requirement(s) for which testing is proposed. ECHA has taken these considerations into account.

Your proposal for a combined test is not appropriate and cannot be accepted by ECHA for the following reasons:

Firstly, ECHA notes that testing proposals can be only made for the provision of the information specified in Annexes IX and X of the REACH Regulation. A test covering an endpoint of Annex VIII (OECD TG 422), as proposed by you does not fall within the scope of the examination of a testing proposal under Articles 40 and 10(a)(ix) of the REACH Regulation. Therefore, ECHA must reject this part of the proposal.

Secondly, it is at your discretion to conduct such combined tests without compromising the validity of the requested test. ECHA notes, in this regard, that dose settings in the two studies are different and may require separate studies.

Thirdly, the proposed *in vivo* micronucleus test is not the most appropriate test to follow up the concern on gene mutations. ECHA notes that such study does not investigate this concern.

According to ECHA's Guidance, for evidence of clastogenicity, a micronucleus test, a chromosome aberration test or a comet assay would be the appropriate follow up test; whereas for evidence of gene mutations, a transgenic rodent gene mutation assay or a comet assay would be the appropriate follow up test. As indicated above, you did not provide an *in vitro* cytogenicity study and no valid *in vivo* mutagenicity study. ECHA notes that, among tests listed above, only the comet assay is the assay suitable to investigate gene mutation or chromosomal aberration. Hence, ECHA considers that comet assay (OECD TG 489) is appropriate to investigate mutagenicity *in vivo*.

According to the test method OECD TG 489, the test shall be performed in rats. Having considered the anticipated routes of human exposure and adequate exposure of the target tissue(s), performance of the test by the oral route is appropriate.

In your comments to the draft decision, you have provided the following arguments:

You propose conducting the comet assay via dermal route of administration, as:
i. dermal route is the most likely human exposure route,
ii. the assay has only been fully validated for the liver and GI tract, and these are the tissues requested by ECHA; however, skin is a likely target organ, and
iii. study can be combined with the proposed dermal sub-chronic toxicity test.

In line with the test method OECD TG 489, the test shall be performed by analysing tissues from liver as primary site of xenobiotic metabolism, glandular stomach and duodenum as sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable different local

absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

This conclusion is not affected by your comments on the draft decision.

ECHA notes that:

i) according to the OECD TG 489, the anticipated route of human exposure should be considered when designing an assay. Therefore, routes of exposure such as dietary, drinking water, topical, subcutaneous, intravenous, oral (by gavage), inhalation, intratracheal, or implantation may be chosen as justified. In view of the likely poor exposure by the dermal route (see section on repeated dose toxicity), oral exposure is likely to lead to higher absorption and better estimation of the hazard.

ii) While you comment that skin can be identified as a target tissue, your dossier states that "*RAEs with an MI less than 0.4 are not classified as carcinogenic*", and further that "*RAEs with an MI greater than or equal to 0.4, cause concern for man owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment. There is some evidence from appropriate animal studies, but this is insufficient to place the substance in category 1. Therefore RAEs with an MI greater than or equal to 0.4 are classified as category 2, H351 according to CLP Regulation, (EC)1272/2008.*" Accordingly, there is uncertainty about the identification of skin as a target organ, and so the suggestion of skin as a target organ for mutagenesis is not a determinative factor. After oral exposure, there is likely to be much higher systemic exposure to the Substance (as compared with dermal exposure) and this also suggests that oral exposure will lead to better estimation of hazard. It is also necessary to balance the full validation of liver and GI tract in the comet assay, and therefore oral exposure is more appropriate (see section i) above).

iii) Regarding the proposed combination of dermal comet assay and sub-chronic 90-day toxicity test, ECHA notes that for the dermal 90-day study conditions outlined in the Column 2, Annex IX 8.6.2 are not met and therefore oral OECD TG 408 study must be conducted (see Section B.2 below); hence, a proposed combination of tests is not acceptable.

Therefore, pursuant to Article 40(3)(c) of the REACH Regulation, you are requested to carry out the additional study with the Substance, while your originally proposed test for a combined OECD 422/OECD 474 study, oral route is rejected according to Article 40(3)(d) of the REACH Regulation.

Germ cells:

A subsequent germ cell genotoxicity study (TGR/OECD TG 488, or CA on spermatogonia/OECD TG 483) may still be required under Annex IX of REACH, in case 1) an *in vivo* genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.

Therefore, you may consider to collect the male gonadal cells collected from the seminiferous tubules (as described by e.g. O'Brien et al.[7]) in addition to the other aforementioned tissues, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and

[7] O'Brien, J.M., Beal, M.A., Gingerich, J.D., Soper, L., Douglas, G.R., Yauk, C.L., Marchetti, F. (2014) Transgenic Rodent Assay for Quantifying Male Germ Cell Mutant Frequency. J. Vis. Exp. (90), e51576, doi:10.3791/51576

store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells, in accordance to Annex IX, Section 8.4., column 2, you should consider analysing the slides prepared with gonadal cells. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

2. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method OECD TG 408) in rats;

A sub-chronic toxicity study (90 day) is a standard information requirement in Annex IX, Section 8.6.2. to REACH.

You have submitted a testing proposal for a sub-chronic toxicity study (90 day) in rats by the dermal route according to OECD TG 411 with Substance 2.

ECHA notes that you provided your considerations concluding that there were no alternative methods which could be used to adapt the information requirement(s) for which testing is proposed. ECHA has taken these considerations into account.

As a supporting study, you provided a key dermal 90-day study, GLP not specified, OECD 411, 1990, conducted with another petroleum substance (CAS RN 64742-10-5, EC No) and not fulfilling the requirements of the OECD TG 411 (only two dose levels tested instead of three, a limit test corresponding to a dose level of at least 1000mg/kg-body weight not conducted, only 10 animals per sex/group used instead of 20, lack of coverage of key parameters tested). You noted that "*this study will be replaced as Key study by the 90-day dermal study currently being proposed*."

As outlined in the Appendix on general considerations, the read-across adaptation you proposed is rejected.

In your comments, you argue that:

(i) You acknowledge the deficiencies of the provided 90-day study but do not agree with ECHA's conclusions on read-across (see general comments above). You further propose a change of the test substance, "*as CAS 64742-10-5 is manufactured in versions with both MI > 0.4 and MI < 0.4 and in far greater volumes than CAS 91995-70-9*", concluding that this is the most representative substance for this category based on production volume and current self-classification for carcinogenicity cat 2.

(ii) You highlight that the planned repeated dose and reproductive toxicity testing on both members in the RAE category would provide the necessary reassurance on the read-across approach.

We have assessed this information and identified the following issues:

(i) ECHA acknowledges your agreement on the deficiencies and limitations of available 90-day study and your proposal of conducting new study. ECHA notes that the proposed change of the test substance which has now been re-considered by you as the most representative test substance of the RAE category is based on production volume and current self-classification. However, as outlined above in the section on read-across, ECHA maintains its request to test both substances and therefore the proposed change is rejected for the reasons given in the section on read-across.

(ii) ECHA acknowledges your plans to conduct further tests but stresses that future data cannot be currently taken into account in the context of strengthening your read-across hypothesis.

Route of administration

ECHA considers that the oral route (OECD TG 408) is the most appropriate route of administration.

As outlined in Annex IX, Section 8.6.2., column 2, testing by the dermal route is appropriate if:

1) skin contact in production and/or use is likely; and
2) the physicochemical properties suggest a significant rate of absorption through the skin; and
3) one of the following conditions is met:
   - toxicity is observed in the acute dermal toxicity test at lower doses than in the oral toxicity test; or
   - systemic effects or other evidence of absorption is observed in skin and/or eye irritation studies; or
   - *in vitro* tests indicate significant dermal absorption; or
   - significant dermal toxicity or dermal penetration is recognised for structurally-related substances.

You proposed testing by the dermal route, in rats. You provided the following justification for testing by the dermal route in the chemical safety report:

"Dermal is the most relevant exposure route, and is sufficiently robust, to identify any potential hazards from repeated exposures to petroleum products to be able to adequately manage the potentially associated risks. However, the primary objective of the testing required for REACH is the identification of hazard, for which the default exposure route under the regulation is oral as this is considered to maximise systemic exposure. To address the regulatory exposure route issue, [redacted] *will review the current data base for evidence of systemic toxicity after dermal exposure and will also conduct a number of oral OECD 422 studies on prioritized substances in each relevant petroleum category. The document attached provides a concise overview of the information to further support the dermal route of exposure and proposed additional work, as part of a larger testing strategy (the strategy document can be found in Annex 13)."*

You also provided a [redacted] document, dated November 2018 and available in the IUCLID dossier, section 7.5.3. In this document, you referred to the criteria listed in Annex IX, Section 8.6.2., column 2 and considered that they are met and therefore testing should be performed using dermal route of administration.

You have provided the following arguments in your [redacted] document:

1) as based on reported uses and exposure scenarios, dermal is a predominant route of exposure for workers;
2) you state that " although a PS in its entirety is unlikely to cross the dermal barrier the physical-chemical characteristics of UVCB does not prevent the penetration of smaller molecules" and further refer to the possible dermal penetration of polycyclic aromatic

hydrocarbons (PAH), components of the Substance, considered to be responsible for the toxicity. You did not provide any measured data on water solubility and partition coefficient claiming that "substance is a hydrocarbon UVCB" and that "standard tests for this endpoint are intended for single substances and are not appropriate for this complex substance.";

3) one of the following conditions is met:
   - toxicity is observed in the acute dermal toxicity test at lower doses than in the oral toxicity test; or
   - systemic effects or other evidence of absorption is observed in skin and/or eye irritation studies; or
   - in vitro tests indicate significant dermal absorption; or
   - significant dermal toxicity or dermal penetration is recognised for structurally-related substances.

To substantiate the (3) indent, you referred to a several dermal sub-chronic toxicity and pre-natal developmental toxicity studies (OECD TG 411 and 414) conducted with other petroleum substances containing polycyclic aromatics hydrocarbons. In these studies, the systemic toxicity was correlated with the concentration of those substances and resulted into decreased thymus weight, increased liver weight and aberrant hematology and clinical chemistry. You concluded that "*the existing data base clearly demonstrates that systemic toxicity occurs after dermal exposure with some PS, particularly those containing poly aromatic hydrocarbons. The physical-chemical properties of PS cannot be based on a single measurement, as complex UVBCs they contain long hydrocarbon chains with low potential to penetrate the dermis but also small molecules (like PAHs) which do penetrate the dermis*."

In your comments, you argue that:

(i) You maintain your proposal to conduct the study by the dermal route, having regard to the likely route of human exposure. You recognise the difficulties in extrapolating between toxicological findings after oral and dermal administration.

In general comments on route of administration, you argue that the dermal route is most appropriate:

(ii) For substances where there is no or minimal expected human exposure by the oral route, you consider that the rationale justifying the oral route as the likely route of human exposure is not clearly documented in the draft decision letter or elsewhere.

(iii) You argue that exposure should be performed by the likely route of human exposure (which would obviate the problems of route-to-route extrapolation) and cite ECHA Guidance R.7a p.450, plus other guidance documents.

(iv) You argue that there are specific difficulties with route-to-route extrapolation, and that the absence of appropriate route-specific data to understand if effects observed orally are relevant to man means there is uncertainty.

(v) You argue that the data provided meet the spirit of the conditions of Annex IX, 8.6.2 (column 2), and that there is evidence that specific components of the Substance are absorbed.

ECHA's assessment:
(1) Condition (2) of column 2 is not met:
ECHA notes that the physicochemical properties of the Substance do not suggest a significant rate of penetration, as the Substance is a UVCB, including, among other, long chain hydrocarbon molecules, which reduce its potential for skin penetration. Furthermore, without provision of measured data, ECHA concludes that your claim that the physicochemical properties suggest a significant rate of absorption through the skin cannot be justified.

ECHA further notes that in the registration dossier, you reported that the Substance contains about [redacted] (from [redacted] to [redacted] of aromatic hydrocarbons, but only limited further data on percentages and identity of PAH were provided, with an estimate of [redacted] PAH of >2-rings (testing strategy document p.9). You have not provided information about the Substance as a whole, or the majority of the chemical constituents, showing that the physicochemical properties suggest there is a significant rate of absorption, and indeed you argue that many constituents are unlikely to cross the dermal barrier.

(2) Conditions listed under the third (3) indent of column 2 are not met:
You did not provide any studies conducted with the Substance which would demonstrate that toxicity is observed in the acute dermal toxicity test at lower doses than in the oral toxicity test.
You did not provide robust study summaries in the registration dossier. Without assessing the analogue approach, in the absence of robust study summaries, reporting on exact dosages and composition of test substances, the relevance of data cannot be assessed and thus cannot be used for supporting a proposed dermal route.

Therefore, ECHA concludes that the dermal route is not an appropriate route of administration for testing.

Notwithstanding the conclusion that the dermal route is an inappropriate route, ECHA has evaluated the choice of most appropriate route. The oral route is the default one because it is assumed to maximise systemic availability (internal dose) of most substances. By contrast, the physicochemical characteristics of numerous components of the Substance indicate that there will be very limited absorption. Further, you argue that a number of the Petroleum Substances are associated with dermal irritation, and this also argues against the dermal route, since dermal irritation will tend to limit the applied dose and systemic availability of the substance, and it is assumed that there will be less topical irritation after oral exposure. ECHA therefore concludes that the oral route is the most appropriate route.

This conclusion is not affected by your comments on the draft decision:

(i) ECHA notes that the proposed study is a standard information requirement pursuant to the Art 10 and Annex IX of the REACH Regulation. The criteria on the appropriate route for testing are defined in the Column 2 of Annex IX, Section 8.6.2. As already outlined in its draft decision, the criteria for selecting a dermal route of administration are not met and therefore maintains its request to modify a proposed test and to conduct an oral study according OECD TG 408 with the Substance.

(ii) As stated above, based on reported uses and exposure scenarios, dermal exposure is a significant route of exposure for workers. ECHA has not stated that the oral route is the most likely route of human exposure, but rather it is the most appropriate route of exposure to examine repeated-dose toxicity.

(iii) ECHA notes that guidance documents from other bodies and for other legislations do not apply to the relevant legal requirements of REACH (e.g. the column 2 provisions of Annex IX, 8.6.2). You have also referred to ECHA's Guidance for repeated-dose toxicity, which states "*to decide on a specific route, it requires first to identify the appropriate routes. If more than one route is appropriate, a decision on the most appropriate route of administration is required.*" (p.450). Further, you have not identified a specific problem with oral-to-dermal extrapolation for this substance for repeated-dose toxicity testing, but generalise that such extrapolation should be avoided. ECHA further notes that you have not identified an error with ECHA's reasoning in the decision, but

rather have a different scientific opinion.

(iv) You have not identified specific problems for route-to-route extrapolation for the registered substance.

(v) You have not addressed the reasoning set out in the decision in relation to the criteria for the appropriateness of the dermal route or for the choice of most appropriate route.

In summary, ECHA considers that you have not provided adequate reasoning to justify that dermal is an appropriate, or the most appropriate route of exposure.

Under Article 40(3)(c) of REACH, you are requested to carry out the additional test with the Substance as indicated above.

3. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method OECD TG 414) in a first species (rat or rabbit), oral route;

A pre-natal developmental toxicity (PNDT) study in one species is a standard information requirement in Annex IX to REACH.

You have provided the following studies for this endpoint in your dossier:

Key study:

i. A dermal pre-natal developmental toxicity study in rats conducted with the Substance, equivalent or similar to OECD TG 414, GLP compliance not specified, 1989.

Supporting studies:

ii. An oral (gavage) pre-natal developmental study in rats, conducted with highly refined white oil (CAS No. 8012-95-1), equivalent or similar to OECD TG 414, GLP compliance not specified, 1987.

iii. An inhalation pre-natal developmental study in rats, conducted with white mineral oil (CAS No. 8042-47-5), equivalent or similar to OECD TG 414, GLP compliance not specified, 1987.

We have assessed this information and identified the following issue(s):

A. In order to be considered compliant and enable assessing if the Substance is a developmental toxicant, the studies have to meet the requirements of OECD TG 414. The key parameter(s) of this test guideline include:

- testing of at least three dose levels and a concurrent control;
- highest dose level should aim to induce some developmental and/or maternal toxicity;
- 20 female animals with implantation sites for each test and control group;
- examination of the foetuses for sex and body weight/external, skeletal and soft tissue alterations (variations and malformations)/number of resorptions and or live foetuses/ measurement of anogenital distance in live rodent foetuses.

The key study you have provided was conducted with two dose levels.
The highest dose level in this study did not induce any developmental and/or maternal toxicity and you have not shown that the aim was to induce toxicity.
Furthermore, this study was conducted with 15 pregnant females for each test group.

In the key study you have provided, you only reported that "*No effects were noted in litter size and weights; number viable (number alive and number dead); sex ratio; postnatal growth (if applicable); postnatal survival (if applicable); grossly visible abnormalities; external, soft tissue and skeletal abnormalities*". The sex and body weight of the foetuses has not been reported. External, skeletal and soft tissue alterations (variations and malformations) / number of resorptions and or dead foetuses have not been recorded and anogenital distance has not been measured in live foetuses.

ECHA further notes that both supporting studies do not meet the requirements of OECD TG 414. In both supporting studies, only one dose level was used. The sex and body weight of the foetuses has not been reported. External, skeletal and soft tissue alterations (variations and malformations) / number of resorptions and or dead foetuses have not been recorded and anogenital distance has not been measured in live foetuses.

Therefore, none of the provided studies fulfil the key parameters for an OECD TG 414.

B. Both supporting studies have been conducted on substances other than the Substance and you did not provide any justification on read-across. In the absence of such a justification, and having no data on the composition of both other substances, these studies cannot be acceptable. ECHA did not assess your read-across approach.

In your comments to the draft decision, you acknowledge the limitations and deficiencies of the provided key study. Consequently, you acknowledge the need of generating new data and propose to conduct new PNDT studies on two species.

Selection of test material: proposed read-across approach

In your comments to the draft decision, ECHA understands that you propose to meet this information requirement by testing a different RAE category member, as addressed above. You do not agree with ECHA's arguments about the need to conduct two PNDT studies in two species on both of the substances as you do not agree with ECHA's conclusions on read-across.

You also highlight that the planned repeated dose and reproductive toxicity testing on both substances in the RAE category would provide the necessary reassurance on the read-across approach.

Your read-across approach is, however, rejected for the reasons provided in the Appendix on general considerations (section (i)). Further, future data cannot be currently taken into account in the context of strengthening your read-across hypothesis.

Route of administration
In your comments to the draft decision, you proposed testing the substance by the dermal route. ECHA concludes that the test proposed should be conducted via oral route of exposure for the same reasons as provided under Appendix C, Section 1 below.

The rat or rabbit is the preferred species under the OECD TG 414. Testing should be performed with the rabbit or rat as a second species, depending on the species tested in the first pre-natal developmental toxicity study.

Under Article 40(3)(c) of REACH, you are requested to carry out the additional test with the Substance as indicated above.

Appendix C: Reasons for the requirement applicable to all the Registrants subject to Annex X of REACH

This decision is based on the examination of the testing proposals you submitted.

1. Pre-natal developmental toxicity study (Annex X, Section 8.7.2., column 2) in a second species

Pre-natal developmental toxicity (PNDT) studies (OECD TG 414) in two species is a standard information requirement under Annex X to REACH.

You have submitted a testing proposal for a PNDT study under the OECD TG 414 to be conducted with Substance 2, (extracts (petroleum), deasphalted vacuum residue solvent, EC No 295-332-8, CAS RN 91995-70-9). As outlined in the Appendix on general considerations, your read-across adaptation is rejected.

You provided your considerations concluding that there were no alternative methods which could be used to adapt the information requirement for which testing is proposed. ECHA has taken these considerations into account.

ECHA notes that your technical dossier does not contain information on a pre-natal developmental toxicity study in a second species (Annex X, Section 8.7.2.). Consequently there is an information gap and it is necessary to provide information for this endpoint.

In your comments to the draft decision, you do not agree with the need to conduct OECD 414 studies in two species on both of the substances. You do not agree with ECHA's conclusions on read-across (see general comments above) within the category to the similar substance (CAS 91995-70-9). ECHA has addressed your comments on the read-across approach in Appendix on general considerations of the draft decision (section (i)).

Route of administration

You proposed testing the substance with the rabbit and by the dermal route. You referred to the criteria listed in the Column 2 of Annex IX, 8.6.2 or REACH and justified the selection of the dermal route of administration by consideration of the following aspects:

- Skin contact in production and/or use is likely, as dermal and inhalation routes are the predominant routes of exposure for workers; and
- Systemic effects have been observed following repeated dermal administration to rats of similar petroleum substances and thus confirming dermal penetration.

You provided references to various scientific publications and reports, and submitted records of several sub-chronic, pre-natal developmental and chronic toxicity studies performed on rats via dermal exposure with other petroleum substances. You concluded that investigation of the potential for systemic toxicity for this type of substances can be achieved using the dermal route of application.

In your comments on the draft decision, you argue that the dermal route is most appropriate for the following reasons:

i. For substances where there is no or minimal expected human exposure by the oral route, you consider that the rationale justifying the oral route as the likely route of human exposure is not clearly documented in the draft decision letter or elsewhere.

ii. You argue that exposure should be performed by the likely route of human exposure (which would obviate the problems of route-to-route extrapolation) and cite ECHA guidance R.7a p.450, plus other guidance documents.
iii. You argue that there are specific difficulties with route-to-route extrapolation, and that the absence of appropriate route-specific data to understand if effects observed orally are relevant to man means there is uncertainty.
iv. You argue that the data provided meet the spirit of the conditions of Annex IX, 8.6.2 (column 2), and that there is evidence that specific components of the Substance are absorbed.

ECHA disagrees for the following reasons.

Firstly, ECHA notes that your considerations based on the criteria outlined in Column 2, Annex IX 8.6.2 address route of administration for a sub-chronic toxicity study but are not applicable for a pre-natal developmental toxicity study, which is addressed under Annexes IX and X, Section 8.7.2.

Secondly, the most appropriate route for reproductive toxicity studies is via oral administration, which is the default route recommended in the test method guideline (OECD TG 414). ECHA notes that for hazard identification, risk assessment and/or classification of a substance for reproductive toxicity, the oral route is the default route of administration. You have not provided any argument that there is particularly high dermal penetration with the Substance, that the dermal route produces a higher relevant internal dose, nor have you argued that dermal application leads to a specific toxicity, or leads to more potent toxicity as compared with oral application. Furthermore, the 'Guidance document on mammalian reproductive toxicity testing and assessment' (OECD No 43, ENV/JM/MONO(2008)16) outlines that "the dermal route of exposure is not recommended for reproductive toxicity studies. The technical difficulties associated with reproductive toxicity testing by administration by the dermal route outweigh the advantages of mirroring the human exposure. Other studies, such as ADME studies should be undertaken to facilitate extrapolation from the oral to dermal route, if this is required".

ECHA concludes that the test proposed should be conducted via oral route of exposure. This conclusion is not affected by your comments on the draft decision:

i. ECHA has not stated that the oral route is the most likely route of human exposure, but rather it is the most appropriate route of administration, having regard to the likely route of human exposure. ECHA acknowledges that dermal exposure to the substance is a significant route of human exposure, but, in line with ECHA's guidance, this is outweighed by the importance of identification of hazard, which is more thoroughly addressed by oral exposure.
ii. The use of the oral route is in line with ECHA's Guidance R.7a which sets out considerations for reproductive toxicity in section R.7.6.2.3.2, including that the selection of the "most appropriate route of administration" focuses on identification of hazards (see the Introduction to this Guidance, R.7a and sub-section "Selection of the appropriate route of administration for toxicity testing", under R.7.2 Human health properties or hazards) and depends on the most appropriate route for identification of the intrinsic properties of the substance for reproductive hazard. You have not provided justification for the dermal route in line with the legal criterion and the Guidance.
iii. You have not identified specific problems for route-to-route extrapolation for the Substance.
iv. Annex IX, 8.6.2 is not relevant for reproductive toxicity testing. In any case, there is also physico-chemical evidence that some constituents will be poorly absorbed by the

dermal route, and a lack of evidence that a sufficient amount of constituents will be absorbed by the dermal route.

Species

The rat or rabbit is the preferred species under the OECD TG 414. Testing should be performed with the rabbit or rat as a second species, depending on the species tested in the first pre-natal developmental toxicity study.

Under Article 40(3)(b) of REACH, you are requested to carry out the proposed test under modified conditions, with the Substance as specified above.

Before performing a pre-natal developmental toxicity study in a second species you should consider the specific adaptation possibilities of Annex X, Section 8.7.2, column 2 and general adaptation possibilities of Annex XI. If the results of the test in the first species (the 1st PNDT study requested under B.3 above or any other new information) enable such adaptation, testing in a second species should be omitted and the registration dossier should be updated containing the corresponding adaptation statement and underlying scientific justification.

Appendix D: Procedural history

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 22 November 2018.

ECHA held a third party consultation for the testing proposals from 28 February 2019 until 15 April 2019. ECHA did not receive information from third parties.

For the purpose of the decision-making, this decision does not take into account any updates of registration dossiers after the date on which you were notified the draft decision according to Article 50(1) of REACH.

The decision making followed the procedure of Articles 50 and 51 of REACH, as described below:

ECHA notified you of the draft decision and invited you to provide comments within 30 days of the notification.

ECHA notes that two different sets of comments were submitted to the draft decision.

Firstly, one registrant submitted general comments agreeing with all observations as outlined in ECHA's draft decision. This registrant also expected the Lead Registrant submitting comments on behalf of all registrants and performing all tests as suggested by ECHA. ECHA acknowledges the member's support to ECHA's draft decision.

Secondly, and in contrast to supportive comments from that registrant, the Lead Registrant submitted extensive comments challenging the requests outlined in ECHA's draft decision. ECHA notes that in their comments, the Lead Registrant did not address his disagreement with the other registrant's supportive comments. Therefore, in it's draft decision, ECHA addressed in details only the comments submitted by the Lead Registrant.

ECHA took into account your comments and did not amend the requests.

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix E: Observations and technical guidance

1. The information requirement under Section 8.7.3. of Annex IX/X to REACH (Extended one-generation reproductive toxicity study, EOGRTS) is not addressed in this decision, because the information from the Sub-chronic toxicity study (90-day), requested in the present this decision, is relevant for the design of the EOGRTS.

2. This testing proposal examination decision does not prevent ECHA from initiating compliance checks at a later stage on the registrations present.

3. Failure to comply with the requests in this decision, or to otherwise fulfil the information requirements with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State(s).

4. Test guidelines, GLP requirements and reporting

   Under Article 13(3) of REACH, all new data generated as a result of this decision needs to be conducted according to the test methods laid down in a European Commission Regulation or according to international test methods recognised by the Commission or ECHA as being appropriate.

   Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses must be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

   Under Article 10 (a) (vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide: 'How to report robust study summaries'[8].

5. Test material

   Selection of the test material(s)

The registrants of the Substance are responsible for agreeing on the composition of the test material to be selected for carrying out the tests required by the present decision. The test material selected must be relevant for all the registrants of the Substance, i.e. it takes into account the variation in compositions reported by all members of the joint submission. The composition of the test material(s) must fall within the boundary composition(s) of the Substance.

While selecting the test material you must take into account the impact of each constituent/impurity on the test results for the endpoint to be assessed. For example, if a constituent/impurity of the Substance is known to have an impact on (eco)toxicity, the selected test material must contain that constituent/impurity. Any constituents that have harmonised classification and labelling according to the CLP Regulation (Regulation (EC) No 1272/2008) must be identified and quantified using the appropriate analytical methods.

In your IUCLID dossier, you specify that the Substance includes batches which have a Mutagenicity Index (MI) ≥0.4. You argue in your read-across justification that the hazardous properties of the substance are determined by ≥3-ring PAH, which have effects on multiple human health endpoints (including mutagenicity). Thus the batches of substance which have

[8] https://echa.europa.eu/practical-guides

MI ≥0.4 may have different and more potent human heath properties than batches of the substance which have an MI <0.4. Testing of a batch of Substance with an MI <0.4 would not provide relevant information for the batches of substance with MI ≥0.4 Accordingly, it is necessary to perform testing on a batch of the substance which has been tested for MI, and which has an MI which is representative of batches of the Substance with an MI ≥0.4. ECHA suggests that the MI should be in the 95th percentile of substances with MI ≥0.4, but you should under any circumstances justify your choice of test material with respect to the MI.

The OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 11 [ENV/MC/CHEM(98)16] requires a careful identification of the test material and description of its characteristics. In addition, the Test Methods Regulation (EU) 440/2008, as amended by Regulation (EU) 2016/266, requires that "*if the test method is used for the testing of a* [...] *UVCB* [...] *sufficient information on its composition should be made available, as far as possible, e.g. by the chemical identity of its constituents, their quantitative occurrence, and relevant properties of the constituents*".

In order to meet this requirement, all the constituents of the test material used for each test must be identified as far as possible. For each constituent the concentration value in the test material must be reported in the Test material section of the endpoint study record.

Technical Reporting of the test material for UVCB substances

The composition of the selected test material must be reported in the respective endpoint study record, under the Test material section. The composition must include all constituents of the test material and their concentration values and other parameters relevant for the property to be tested. In this case, given the intrinsic compositional variability of the test substance, information as specified below has to be provided:

a) Detailed information on the composition of the sample tested: this must include information on the identity and concentration of all known constituents. In reporting, the chemical composition, both individual constituents as well as "major hydrocarbon classes" should be presented;

b) An explanation why the composition of the sample tested represents the composition of the substance subject to the present decision;

c) You should demonstrate based on the detailed analytical composition on the test material and the intrinsic variability of the Substance, that the sample selected for testing does not result in an underestimation of hazard.

Based on the analytical information currently provided by you, ECHA concludes that the sample selected for testing must be analysed for Mutagenicity Index, and must also be analysed for PAC content (Method II in Appendix A. of Gray et al., Regulatory Toxicology and Pharmacology 67 (2013) S4–S9), in addition to the other analytical methods described in your category justification (SIMDIS-GC, TLC-FID, LC). There must be specific justification for the choice of test sample and its representativity for the Substance, particularly with respect to Mutagenicity Index and PAH (PAC) content.

Without such detailed reporting, ECHA may not be able to confirm that the test material is relevant for the Substance and to all the registrants of the Substance.

Technical instructions are available in the manual "How to prepare registration and PPORD dossiers"[9].

6. List of references of the ECHA Guidance and other guidance/ reference documents[10]

QSARs, read-across and grouping
Guidance on information requirements and chemical safety assessment, Chapter R.6 (version 1.0, May 2008), referred to as ECHA Guidance R.6 in this decision.

ECHA Read-across assessment framework (RAAF, March 2017)[11]

Toxicology
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

Environmental toxicology and fate
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), referred to as ECHA Guidance R.7b in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

PBT assessment
Guidance on information requirements and chemical safety assessment, Chapter R.11 (version 3.0, June 2017), referred to as ECHA Guidance R.11 in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.16 (version 3.0, February 2016), referred to as ECHA Guidance R.16 in this decision.

OECD Guidance documents
Guidance Document on aqueous –phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD23.

[9] https://echa.europa.eu/manuals
[10] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[11] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

Appendix F: List of the registrants to which the decision is addressed and the corresponding information requirements applicable to them

Registrant Name	Registration number	(Highest) Data requirements to be fulfilled
[redacted]	[redacted]	[redacted]