May 2022

Advice on using read-across for UVCB substances

Obligations arising from Commission Regulation 2021/979, amending REACH annexes

1. Introduction

On 17 June 2021, the European Commission implemented the following changes to REACH Annex XI, Section 1.5, which have applied since 8 January 2022[1]. This guidance addresses the text that has changed in the amendment.

In particular, the basis for establishing structural similarity specifically for substances of unknown or variable composition or biological origin (UVCBs) is part of the amended legal text and described in Section 2.

The conditions to be fulfilled by a grouping and read-across adaptation and the supporting documentation that needs to be provided are relevant for all substances and not specific to UVCBs. They have been clarified in the amended legal text and are explained in Sections 3 and 4, respectively.

2. Structural similarity in grouping and read-across

REACH Annex XI, Section 1.5 specifies two conditions that must be fulfilled whenever a read-across adaptation is used:

1) 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; and
2) relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group by interpolation to other substances in the group.

If either of these two conditions is not met, the read-across adaptation fails. Structural similarity is, therefore, a key concept. General guidance is provided for structural similarity and related concepts in Section 2.1, before addressing structural similarity for UVCBs in Sections 2.2 and 2.3.

2.1. Basis of structural similarities in constituents

Establishing structural similarity between substances is a prerequisite for applying grouping and read-across[2]. When structural similarity of substances is not established, it is not possible to adapt an information requirement through grouping and read-across. This applies to all substances regardless of whether they are mono- or multi-constituent, or UVCB substances. The prediction of the properties of substances within a group must be based on the structural similarity that has been identified: without structural similarity,

[1] Amendment link OJ L 216 18.06.2021, p. 121, ELI: http://data.europa.eu/eli/reg/2021/979/oj

[2] See the decision of the Board of Appeal in A-006-2012, paragraph 66, and in A-016-2019. "*Furthermore, Section 1.5. of Annex XI allows for an adaptation if it is established that (i) the substances in a group or category are structurally similar, (ii) the properties of the substances are likely to be similar or follow a regular pattern, and (iii) the similarity of properties or their regular pattern is the result of structural similarity…*".

it is not possible to establish a prediction of properties.

The aim of establishing structural similarity is to set up the basis for predicting properties of substances within a grouping.

Structural similarity is established by comparing the chemical structures of constituents between substances. This comparison must provide a clear characterisation of both the similarities and differences between the chemical structures of the constituents between substances. This comparison and the basis of structural similarity must be clearly described.

In this document, constituent means *discrete chemical structure*, which is separable from its stereo-, regio- and constitutional isomers[3]. For read-across between mono-constituent substances, structural similarity is a simpler concept: since one constituent is the *main* constituent at 80 % or more of the composition, the structural similarity of mono-constituent substances is usually established based on this main constituent. Where relevant, it is also necessary to consider the structural similarity of other constituents (i.e. additives and/or impurities). Less usually, it may be possible to establish structural similarity based on a particular constituent which has potent hazardous properties (e.g. CMR category 1, PBT or vPvB) and which determines the hazardous properties of the substance by itself ("worst-case").

Structural similarities may be based on any of the following:

1) a common functional group;
2) the common precursors and/or the likelihood of common breakdown products through physical and biological processes; and
3) molecular similarity estimations.

For further explanations and details, see the existing guidance on establishing structural similarity in *ECHA guidance R.6*[4] and the read-across assessment framework (RAAF[5, 6]).

It is not always necessary to fully characterise all constituents in a substance, i.e. to resolve all stereo-, regio- and constitutional isomers. However, a justification must be provided – substantiated by scientific evidence – to show why full characterisation of constituents is not necessary for the relevant information requirements. This needs to show that a specific and limited degree of structural variation (e.g. structural isomerism in an alkyl group) does not likely contribute to variation in the properties to be read-across for the information requirements concerned.

> As one example, grouping of substances in relation to skin irritation may be based on the structural similarity of constituents due to a common functional group which determines that the substances are strong acids (pH<2.0); the strong acidity of

[3] Note that for the purposes of substance identity (see *Guidance for identification and naming of substances under REACH and CLP*), mono- and multi-constituent substances may be composed of main constituents, impurities and additives, whereas UVCBs are composed of constituents and additives. In this document, constituent is used for mono- or multi-constituent and UVCB substances to refer to any single species present in a substance that can be characterised by its unique chemical identity.

[4] *Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of chemicals*, May 2008 Link

[5] Read-Across Assessment Framework (RAAF), May 2017 Link

[6] "Read-Across Assessment Framework (RAAF) Considerations on multi-constituent substances and UVCBs" Link

the substance is responsible for the biological property (i.e. corrosion). With appropriate justification, it may not be necessary to characterise e.g. structural isomerism in alkyl side-chains for the purpose of establishing structural similarity between substances for this information requirement.

Establishing structural similarity may be specific to an information requirement when giving a basis for predicting properties of substances within a group.

However, for predicting other properties for the same substances where there is no clear link between the functional group and the biological property, it would be necessary to characterise structural isomerism in alkyl sidechains for the purpose of establishing structural similarity between substances.

2.1.1. Structural similarity and prediction of properties of a substance

Under REACH, a grouping and read-across adaptation must be based on structural similarity between the source and target substances. Consequently, the generation of information on the chemical structure of constituents is essential to demonstrate structural similarity. However, structural similarity alone is not sufficient to justify the possibility to predict properties of the target substance by read-across.

A read-across hypothesis needs to be provided. This hypothesis establishes why a prediction for a toxicological, ecotoxicological or environmental fate property is possible. It must be based on recognition of the structural aspects the chemical structures have in common, and the differences between the structures of the source and target substances.

The read-across hypothesis is also dependent upon the information generated on the chemical structure of constituents. For further details (including whether the test material used represents the source substance as described in the read-across hypothesis, e.g. in terms of constituents, purity and impurities), see *ECHA guidance R.6*[4] and the read-across assessment framework (RAAF[5,6]).

2.1.2. Substance identity and relationship to read-across

Under REACH, all registered substances must be identified and named. In line with Article 10, a registration requires the substance identity to be recorded using the parameters specified in Section 2 of Annex VI to REACH[7]. The requirements for substance identification and naming for registration purposes are separate from the information required to establish structural similarity for the purposes of grouping and read-across according to REACH Annex XI, Section 1.5.

As a consequence, the provision of valid and sufficient information on naming and identification of a substance (i.e. satisfying the requirements of Annex VI, Section 2) does not automatically mean that the information on composition satisfies the requirements of Annex XI, Section 1.5 to establish structural similarity for grouping and read-across.

The requirements for identifying the substance may be different (higher) for the purpose of establishing structural similarity as a basis for grouping and read-across (Annex XI, Section 1.5), than for the purpose of unambiguously identifying the substance (Annex VI, Section 2).

There are distinct requirements for information on composition for substance identity

[7] *Guidance for identification and naming of substances under REACH and CLP* (Version 2.1), May 2017 Link

compared with for grouping and read-across because the purposes of these two provisions are different, i.e. unambiguously identifying a substance versus predicting the properties of a substance from data on other substances, respectively.

However, the information provided for Annex VI, Section 2 may have direct relevance for the ability to use an adaptation according to Annex XI, Section 1.5. If the legal requirements from Section 2 of Annex VI are not fulfilled, then it would normally follow that the identity of the substance is not sufficiently characterised for substance identification, nor for the purpose of making a comparison of structural similarity in the context of an adaptation according to grouping and read-across.

The information submitted within the context of substance identification and naming must be consistent with the information submitted on the composition of the substance in the context of grouping and read-across. When, in accordance with Article 11(1), two or more compositions of the substance within the same dossier are associated with different hazardous properties, the relevant compositions of the substance must be reflected in the compositional information for grouping and read-across.

2.2. Establishing the exhaustive structural similarity of UVCB substances

UVCB substances contain multiple constituents, and so there is not a single main constituent that can be compared to establish structural similarity for UVCB substances.

The structural similarity of a UVCB to another substance is established on the basis of similarities in the structures of constituents between the substances, together with the concentration of these constituents and variability in the concentration of these constituents.

The requirement to establish structural similarity of a UVCB on this basis applies to all UVCBs in a grouping and read-across adaptation, whether they are the source or target substance.

For read-across between UVCBs, there may be two different types of structural similarities. One type of similarity is that the constituents (or their transformation products) of the two UVCBs are identical, but that there are differences in the concentrations of constituents or variability of the concentrations of the constituents between the two substances. Another type of similarity is that the constituents (or their transformation products) of the two UVCBs are not the same, but that all the constituents (or their transformation products) of the first UVCB are structurally related to the corresponding constituents of the second UVCB. In this latter scenario, variation in concentration of constituents would represent an additional source of variation between the substances.

Both types of structural similarity between UVCB substances may be present in a single read-across case. It is necessary to address whether, or to what extent, the similarity arises from *identical* as opposed to *structurally-related* constituents, and whether, or to what extent, the similarity arises from variation in concentration and variability of constituents.

If structural similarity is not demonstrated, then it is not possible to use grouping and read-across as an adaptation.

2.2.1. Identification of constituents and their concentrations

The identification of constituents provides structural information about them, and enables a comparison of constituents between substances. As noted above, constituent means discrete chemical structure, separable from its stereo-, regio- and constitutional isomers, and so the default is that constituents will be defined according to this definition.

Since structural similarity is established by comparing the chemical structures of

constituents between substances, this comparison must provide a clear characterisation of both the similarities and differences between the chemical structures of the constituents between substances. A characterisation of the full composition is also necessary to characterise the differences between constituents (details below).

Sufficient spectral data is required to confirm the structure of a constituent. Several spectroscopic methods can be suitable, for example ultraviolet and visible absorption spectroscopy (UV/Vis), infrared spectroscopy (IR), nuclear magnetic resonance spectroscopy (NMR) and mass spectroscopy (MS).

For inorganic substances, the use of X-ray diffraction (XRD) or X-ray fluorescence (XRF) or atomic absorption spectroscopy (AAS) may be more suitable. The constituents present in a UVCB substance must be tabulated, together with methods used for assignment of identity.

In principle, the name of the constituents should be given in English according to the IUPAC nomenclature rules, but for some constituents, such as enzymes, other conventions are appropriate. Other internationally accepted designations can be given in addition. The constituent is identified by the chemical name and other identifiers (including the molecular and structural formula).

However, it is possible to justify why full characterisation of constituents is not necessary if you are able to show that a specific and limited degree of structural variation (e.g. in an iso-pentyl group in a structure) does not contribute to variation in the properties, which are to be read-across for an information requirement. In general, when you justify that full characterisation of constituents is not necessary, this must be specific and limited; the type and amount of structural variation must be clearly delimited. The aim of establishing structural similarity is to set up the basis for predicting properties of substances within a group. Therefore, the variation of constituents in an incompletely characterised structure (e.g. an iso-pentyl group) should not be unduly large. Otherwise, it is not possible to establish similarities and differences in structures of constituents that would enable the properties of the substances within the group to be predicted.

> For example, characterisation of constituents as 'aromatic compounds' or 'alkanes' would in principle not be acceptable, as the amount of structural variation within such generic descriptors is so large. Therefore, differences in the composition of substances covered by these generic descriptors would not enable structural similarity and differences to be established.

The concentration of constituents must be determined. This aims to quantify the differences or similarities between substances regarding the concentration of constituents. The mere identification of a constituent as being a common constituent in two UVCB substances is not sufficient to demonstrate similarity, as this does not provide information on the concentration of the constituent, and this would not inform on the potential differences between the two substances. Given that constituent concentration can vary significantly (e.g. over orders of magnitude) between UVCBs, the concentration of specific constituents is an essential aspect of comparison to establish structural similarity.

Compositional information must be completed up to 100 % - the total should include the identified constituents and the amount of unknown constituents. Where more than one analytical method is used, the strategy for determining composition must be justified. A description of the analytical methods and/or the appropriate bibliographic references for the identification and quantification of constituents need to be given. This information should be sufficient to allow the methods to be reproduced.

For identifying constituents, chromatographic methods are generally preferred, but other methods may exceptionally be employed to quantify constituents if there is suitable justification. The use of common analytical methods is strongly recommended to ensure

comparability of compositional information between different registrations. For each constituent, the concentration should be tabulated, together with information about variation in concentration (see Section 2.2.2). The concentration should be given in an appropriate metric (e.g. w/w for solids and liquids, v/v for gases).

For all UVCB substances covered by a grouping and read-across adaptation, all constituents present in a concentration at or above 1 % must be identified. Section 1.5 of Annex XI to REACH requires structural similarity to be established on the basis of the structures of constituents, i.e. that constituents in a UVCB are identified. The aim of establishing structural similarity is to set up the basis for predicting properties of substances within a group. It can be reasonably anticipated that constituents present at 1 % (w/w) or greater affect the hazardous properties of a UVCB substance, and that constituents that are known to be hazardous can affect the hazardous properties of a substance at lower concentrations.

Accordingly, it is necessary to identify constituents present at concentrations ≥1 %, and at lower concentrations where there is a concern for the hazardous properties of specific constituents. Specific reasons for identifying constituents when present at <1 % include:

- Where constituents are known or suspected to be of very high concern (meeting the criteria of REACH Article 57), if constituents have hazardous properties such as carcinogenic, mutagenic or reprotoxic (CMR)[8] properties, the following lower concentration thresholds must be applied:
    - 0.1 % for constituents classified as carcinogenic or mutagenic (category 1); and
    - 0.3 % for substances that are toxic to reproduction or development (category 1).
- Where read-across is relevant for persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) properties, the identification of constituents must be consistent with the need for PBT/vPvB assessment to characterise constituents present at ≥0.1 % (as described in *Guidance R.11, R.11.4.1*[9]) unless it is possible to show that the constituents are not relevant for PBT/vPvB properties. Further, the total amount of unidentified constituents relevant for PBT/vPvB properties must not exceed 10 % (w/w) and the total amount of any single unidentified constituent relevant for PBT/vPvB properties must not exceed 1 tonne per year.

The aim of establishing structural similarity is to set up the basis for predicting properties of substances within a group. Therefore, it is necessary to identify the constituents present in a UVCB. Consistent with that aim, it is not possible to establish structural similarity when a substantial proportion of the constituents of a substance (i.e. >20 % w/w) have not been identified and quantified[10]. As such, identified constituents above the thresholds given above must account for a minimum of 80 % of the mass of a UVCB substance.

Under Annex VI, registrants must individually report the identity and concentration of the constituents of the substance they specifically manufacture or import, in their IUCLID dossier. However, to establish structural similarity of UVCBs for grouping and read-across,

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[8] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

[9] *Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment* (Version 3.0), June 2017 Link

[10] This is analogous to the threshold of 80 %, which is used to identify mono-constituent substances ("A mono-constituent substance is a substance in which one constituent is present at a concentration of at least 80 % (w/w) and which contains up to 20 % (w/w) of impurities."). See *Guidance for identification and naming of substances under REACH and CLP* in footnote 7.

the read-across justification must provide the information specified in this section (2.2.1) on the identity and concentration of constituents of the substance as specifically registered by all the registrants.

The read-across justification may refer to information on identity and concentration of constituents provided in the IUCLID dossier to satisfy the requirements of Annex VI, as long as this information provides the information specified in this section (2.2.1) and is representative of the substance as specifically registered by all registrants. The read-across justification must also justify that the hypothesis applies to the substance as specifically registered by all these registrants.

2.2.2. Variability in concentration of constituents

By definition, UVCB substances may exhibit variability in their composition. However, the variability in concentration of constituents in a UVCB substance must enable a comparison to be made in the similarities and differences in concentration of specific constituents between the UVCB substance (whether source or target) and the other substances with which it is grouped.

When constituents of a UVCB substance are known or suspected to pose a concern, e.g. through classification as CMR, STOT-RE, PBT, vPvB, or the suspicion that they have these properties, quantifying the variability in concentration gains particular importance. This is because these constituents may determine the hazardous properties of the substance. As such, the similarities and differences in concentrations of these constituents in the UVCB substance, compared to other substances within the group, are particularly relevant for establishing structural similarity.

It is an obligation to determine the variability in the concentration of constituents in UVCB substances used in grouping and read-across. To measure variability in the concentration of constituents, the concentration of constituents in multiple independent samples of the substance must be determined. The independent samples must be representative of the substance as produced by all registrants of the substance. The resulting information must be used to determine the variability in concentration of the constituents. The dataset must be sufficient to derive the nature of the distribution of concentrations. It must provide statistical measures of the variability with reasonable certainty.

Determining the concentration of constituents in too few samples results in very high uncertainty due to the high contribution of individual samples, with the consequence that the estimations of variability in concentration are highly uncertain. Therefore, the concentration of constituents in at least five independent samples of the substance must be measured. The independent measurements must be from different production batches of the substance as produced by all the registrants. These measurements must take account of the aspects of production of the substance that lead to variation of the composition of the substance as jointly submitted. These aspects of production could be, for instance, the geographic origin of the starting material, the time at which the starting material is sourced, etc. The selection of the samples must be documented to justify the representativeness of variability.

Where there is reason to believe that there is the potential for uncharacterised variability in concentration of constituents (e.g. a large number of registrants, variations in starting material or process of production), additional measurements of the concentration of constituents should be undertaken in independent samples to characterise the variability of concentration of constituents within the substance as jointly registered. To compare the concentration of constituents from different samples, the same analytical methodology must be used to measure the concentration of constituents in the samples of the substance.

This approach will give an indication of the type and scale of variability in the concentration of constituents in the UVCB substance. Not all constituents will be present (or detectable)

in each batch of a UVCB, so the variability in some constituents may not be statistically quantifiable or may have very large uncertainty limits.

However, when constituents in a UVCB substance are known or suspected to pose a concern, further batches of the substance should be measured for these particular constituents to ensure that the variability in their concentrations in the substance can be robustly characterised.

There must be adequate and reliable documentation of the basis for determining variability in concentrations of constituents to ensure that the information provided is reliable, reproducible and serves as a basis for the read-across justification.

For each constituent identified and quantified as set out in Section 2.2.1, the concentration of the constituent in at least five determinations must be provided. The dossier must also contain the corresponding characterisation of variability in concentration of the constituent, such as the nature of distribution and the mean and standard deviation of measurements, or the range of concentrations with justification if a statistical analysis is not possible or has high uncertainty.

The basis for characterising the variability must be explained at a level sufficient to allow the characterisation of variability to be reproduced. Under Annex VI, registrants must report the concentration range of constituents of the substance they specifically manufacture or import in Section 1.2 of their IUCLID dossier.

The read-across justification must provide the information specified in this section (2.2) to establish structural similarity of UVCBs for grouping and read-across, on the identity and concentration of constituents and their variability – as specifically registered by all the registrants.

2.3. Derogation to establishment of exhaustive structural similarity of UVCB substances

There may be cases where determining the exhaustive structural similarity of a UVCB substance is not technically possible or impractical (Section 2.3.1). If it is not technically possible or impractical to identify all individual constituents of a UVCB substance, the comparison of the chemical structures of constituents is not possible and the read-across adaptation can thereby not be justified. Even in these situations, the structural similarity would need to be sufficiently demonstrated by other means (Section 2.3.2).

2.3.1. Identification of all constituents in a UVCB is technically not possible or impractical

Section 2.2.1 sets out requirements for identifying constituents in a UVCB and determining the concentration of the constituents, and Section 2.2.2 sets out requirements for the determination of variability in concentration of constituents.

If those requirements are not met for a UVCB substance, it is not possible to establish structural similarity based on similarity in the structures of constituents. If it can be demonstrated that these requirements were not met because it was not technically possible or was impractical[11], it may be possible to demonstrate structural similarity by other means (see Section 2.3.2).

In this case, there must be a comprehensive justification with reliable supporting evidence

[11] The phrase 'not technically possible' to identify the individual constituents in a UVCB means that it is impossible, whereas 'impractical' means that it is possible but disproportionate to do so.

to show why it is not technically possible or impractical to do so.

A justification that the identification of constituents is 'not technically possible' should address the absence of knowledge about the constituents and the absence of published methods for identifying the constituents. However, it should also address why the identification of the constituents is not technically possible. It is not sufficient to state that there is not a published method for analytical separation of a particular constituent for identification to be considered as 'not technically possible'.

> An example where it would be 'not technically possible' to identify all individual constituents in a UVCB substance could be the following: A UVCB substance contains >20 % constituents which cannot be resolved by chromatographic means (i.e. there is no known method for resolving such constituents) or otherwise be identified. It is not technically possible to meet the criteria of Section 2.1 for identifying 80 % of constituents.

Concerning the demonstration that it is 'impractical' to identify the individual constituents in a UVCB, Article 13(1) states that "*information shall be generated whenever possible by means other than vertebrate animal tests*". In addition, Annex XI, Section 1.5 requires that "*Structural similarity for UVCB substances shall be established on the basis of similarities in the structures of the constituents, together with the concentration of these constituents and variability in the concentration of these constituents*".

As such, the standard for the impracticality of identifying constituents requires that extensive efforts are made to apply existing knowledge of analytical separations to resolve and identify constituents in line with the requirements of Section 2.2.1 and 2.2.2; the application of existing methodology to quantify known constituents present at ≥1 % (and hazardous constituents present at a lower levels - see Section 2.2.1) will normally be mandatory.

Where constituents present at ≥1 % (w/w) account for >80 % of the mass of the substance, and the resolution of such constituents is not impossible, there will need to be exceptional justifications to explain why it is impractical to identify and quantify constituents. It will also be necessary to provide a detailed justification as to why the identification and quantification of unknown constituents in a UVCB is impractical.

> An example where it would be 'impractical' to identify all individual constituents in a UVCB substance could be the following: a UVCB substance may contain many thousands of constituents, many of which are present at <0.1 % and are not relevant for hazard assessment concerning CMR or PBT/vPvB. The sum of identified constituents, present at >1 %, accounts for less than 80 % of the substance. Under these circumstances, it is not required to identify the constituents present at <0.1 %, and the sum of identified constituents is less than 80 %. It may be technically possible to identify constituents present at <0.1 %, but it is impractical to identify hundreds of constituents present at a low concentration, and thereby meet the criteria of Section 2.2.1 for identifying 80 % of constituents.

The scope of what is not technically possible or is impractical depends on detailed considerations arising from the nature of the substance. In this context, the evolution of methodologies for analysing constituents is critical. It is, therefore, inappropriate to provide detailed guidance in this document on analytical methods which may become rapidly outdated. Consequently, registrants must inform themselves on a regular basis about the capabilities of existing analytical methods and the development of new analytical methods.

2.3.2. Condition: demonstrating structural similarity by other means for a UVCB

When a registrant justifies that the identification of all individual constituents is not technically possible or impractical, the structural similarity must be demonstrated by other means to enable a quantitative and qualitative comparison of the actual composition between substances.

As noted in Section 2.2, the structural similarity between the source and target substances is a prerequisite to any read-across adaptation. The read-across hypothesis establishes that a prediction of property is possible. This must be based on a recognition of the structural aspects that the chemical structures have in common and on the differences between the structures of the source and target substances. The quantitative and qualitative comparison of the actual compositions of the substances must, therefore, set up the basis for predicting properties of substances within a group.

There may be quantitative differences in the composition of constituents between two UVCBs (i.e. that the same constituents are present but in different concentrations). There may also be qualitative differences in the composition of constituents between two UVCBs (i.e. that constituents present in one UVCB are not present in the other UVCB, or that there is variability of constituent concentrations in one but not the other substance).

There are different considerations for characterising quantitative versus qualitative differences in composition, and these must both be addressed if present. The actual composition refers to all the constituents that compose a substance. Therefore, the comparison must be informative for all the constituents of the substances. A quantitative comparison should enable the concentrations of all constituents in common between the UVCB substances to be compared. A qualitative comparison should enable the constituents which do not have common structures to be compared and should also inform on the nature of the difference in structure between the constituents present in the UVCB substances.

If it can be demonstrated that the identification of all individual constituents is not technically possible or impractical, the structural similarity must be demonstrated by other means. Therefore, the registrant must provide a justification explaining why the other means enable a quantitative and qualitative comparison of the actual composition between substances.

The demonstration of structural similarity by other means is dependent on substance-specific considerations, and the evolution of spectroscopic and chromatographic methods continuously changes the capabilities of the relevant methods – registrants need to be aware of such details.

> An example of demonstrating structural similarity by other means could be "fingerprinting" of constituents and their concentrations in compositions using chromatographic methods to provide an overview (fingerprint) of the constituents, particularly where there are common constituents. Suitable methods could include 2D-gas-chromatography, linked to an appropriate detection method capable of quantitative detection of analytes and structural characterisation, e.g. tandem mass spectrometry. Key issues in evaluating the acceptability of the fingerprinting method will be:
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> - the provision of information on a sufficient proportion of constituents in a substance (i.e. covering >95 % of the constituents of a substance);
> - the provision of information on constituents of the substance which are known or suspected to be of very high concern (meeting the criteria of REACH Article 57) as set out in Section 2.2.1;
> - sufficient resolution of constituents so that there is confidence that variation in constituents is not obscured by low resolution in the chromatography;

- the demonstration that there is quantitation of the analytes;
- characterisation of the variability in constituents for each substance; and
- information on the structural nature of constituents which are not in common between the substances being compared.

The comparison of substances would also need to provide a quantitative and qualitative comparison of the actual composition between substances.

Another example of other means of demonstrating structural similarity can involve pre-existing information from starting materials and the (same) manufacturing process. Given that there is a detailed definition of starting materials, and definition of the manufacturing process (including identity and ratio of reactants, the reaction mechanisms), it may be possible to conclude on the reactions that are possible and consequently the identity of the constituents of the UVCB that is produced, without identifying all constituents of the UVCB. This may be particularly relevant when the two UVCBs to be compared are a) the starting material and b) the product resulting from the starting material after the production process. There must be a rationale explaining why it is possible to undertake a quantitative and qualitative comparison of the actual composition between substances, together with the comparison of all constituents between substances. The key issues in evaluating the acceptability of the fingerprinting method are also applicable in this example.

When neither the identification of constituents nor other means to justify similarity between UVCB substances is possible, then grouping and read-across cannot be used.

3. Studies that shall normally be performed for a particular information requirement

REACH Annex XI, Section 1.5 requires the grouping and read-across adaptation to fulfil all of the following conditions:

- be adequate for the purpose of classification and labelling and/or risk assessment;
- have adequate and reliable coverage of the key parameters addressed in the corresponding study that must normally be performed for a particular information requirement; and
- cover an exposure duration comparable to or longer than the corresponding study that shall normally be performed for a particular information requirement if exposure duration is a relevant parameter.

The REACH amendment[1] introduced an additional condition in Section 1.5 of Annex XI:

- "the corresponding study that shall normally be performed for a particular information requirement".

The "corresponding study" refers to the applicable test guideline/method for a specific information requirement set under Annexes VI to X to the REACH Regulation. *Guidance R.7a* describes the test guidelines/methods that are appropriate for each information requirement[12]. The Column 1 information requirement may be adapted by specific provisions laid down in Column 2. Article 13(3) provides "*where tests on substances are required to generate information on intrinsic properties of substances, they shall be conducted in accordance with the test methods laid down in a Commission Regulation or in accordance with other international test methods recognised by the Commission or the Agency as being appropriate.*" As a consequence, the corresponding study must be a test method described in Commission Regulation (EC) No. 440/2008, or an OECD test

[12] *Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance* (Version 6.0), July 2017 Link

guideline.

> As an example, if a study on a source substance is used to fulfil the information requirement of Annex IX, 8.6.2 for a sub-chronic toxicity study (90-day), the reference for assessing the coverage of the key parameters/exposure duration from these source studies is the set of parameters listed in the test guidelines for a 90-day study, i.e. OECD test guidelines 408 for oral, 411 for dermal and 413 for inhalation route, and the corresponding EU test methods B.26 for oral, B.28 for dermal and B.29 for inhalation route.

Note that the choice of route (and test guideline) may be constrained by the information requirement.

> For example, Annex IX, 8.6.2 specifies that the study must be by the *"most appropriate route of administration, having regard to the likely route of human exposure"*. A study on a source substance that has been performed via a route other than the most appropriate route for the target substance cannot be considered as "the corresponding study that shall normally be performed for a particular information requirement".
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> Consequently, if the information requirement is for Annex IX, 8.6.2, sub-chronic toxicity study (90-day), and the most appropriate route is the oral route, then the corresponding study that shall normally be performed would be according to OECD test guideline 408 or test method B.26. The source studies performed on the source substances must have adequate and reliable coverage of the key parameters of OECD TG 408 or test method B.26. Similarly, for this information requirement, the studies performed on the source substances must have an exposure duration of 90 days or more.
>
> As an example for the information requirement of Annex VII, 9.1.1, short-term toxicity testing on invertebrates (preferred species *Daphnia*), the corresponding study that shall normally be performed would be according to EU test method C.2 or OECD TG 202. The source studies performed on the source substances must have adequate and reliable coverage of the key parameters of EU test method C.2 or OECD TG 202.

Finally, note that not all the information requirements set out in Annex VII to X of REACH are covered by a test method described in Commission Regulation (EC) No. 440/2008 or an OECD test guideline.

4. Adequate and reliable documentation

REACH Annex XI, Section 1.5 states that adequate and reliable documentation must be provided for the applied method, i.e. the grouping and read-across. The content of this requirement was further clarified. Indeed, *ECHA Guidance R.6.2*[4] provides further information, including reporting formats in tables R.6.2.6.1 for analogue approaches and R.6.2.6.2 for category approaches.

The documentation of the applied method must include the elements listed below. However, depending on the specific justification of the grouping and read-across adaptation, it may also be necessary to submit other adequate and reliable documentation to justify the adaptation (e.g. the category justification).

For further details, the read-across assessment framework (RAAF[5]) and *ECHA Guidance R.6* provide a framework to assess whether all the important scientific aspects of the grouping and read-across adaptation have been considered, and to conclude on the robustness of the prediction of the properties.

4.1. A robust study summary for each source study used in the adaptation

A robust study summary (RSS) means a detailed summary of the objectives, methods, results and conclusions of a full study report providing sufficient information to make an independent assessment of the study minimising the need to consult the full study report. For guidance on how to prepare and report RSS, see *ECHA practical guide 3*[13].

An RSS must be available for each source study on a source substance.

'Source' and 'target' substances in read-across are defined in ECHA's RAAF[5]. A 'source study' is performed on a source substance and is used to provide the hazard information for the target substance for the relevant information requirement (i.e. is being "*read* from the source substance *across* to the target substance"). Depending on the read-across case, there may be multiple source studies per information requirement. Source studies are distinguished from supporting information, which provides e.g. information to support the read-across hypothesis, or information on composition of substances, etc.

> As an example, consider a read-across for the 90-day information requirement, based on a hypothesis of hydrolysis to a common product for the target (T) and another substance – the source (X). The source study would be the 90-day study on substance X, which is read-across to show the hazardous properties of the target substance in a 90-day study. Supporting information could include studies on hydrolysis of source and target to a common product, composition of source and target, and/or studies on other information requirements than the 90-day (e.g. a 28-day study) for source and target. Such supporting information would provide the evidence to support the mechanistic basis of the hypothesis, and to compare and demonstrate similarity of effects in support of the hypothesis.

4.2. An explanation why the properties of the registered substance may be predicted from other substances in the group

An explanation why properties of the target substance may be predicted from source substances must include:

a) A read-across hypothesis – the *ECHA Guidance R.6.2*[4] establishes that a read-across hypothesis explains why the read-across can be performed, i.e. why a specific property of a target substance may be predicted from one or more source substance. According to the ECHA RAAF, "*this hypothesis must be based on a relationship between structural similarity and the predicted property(ies) and needs to be supported by read-across justification*". The read-across hypothesis will typically correspond to the two scenarios identified in the RAAF, i.e.
   - '(Bio)transformation to common compound(s)'; or
   - 'Different compounds have the same type of effect(s)',

   and the accompanying justification will provide sufficient information so that the hypothesis can be independently assessed. The hypothesis will include consideration of any difference in potency between source and target substance, and how this impacts the prediction of properties of the target substance.

b) A justification, i.e. reasoning to verify the scientific validity and robustness of the read-across hypothesis, based on the provided supporting evidence. ECHA Guidance R.6 considers that documentation of an analogue or category approach is an integral part of the assessment report, and R.6.2.6.1 and R.6.2.6.2 indicates the need that the experimental data verify that the read-across is justified.

[13] *How to report robust study summaries Practical Guide 3* (Version 2.0), November 2012 Link

4.3. Supporting information to scientifically justify such explanation for prediction of properties

Information to support the read-across hypothesis is supporting information (different from source study, as defined under Section 4.1). This supporting information is used (as described in Section 4.2) to verify the scientific validity and robustness of the read-across hypothesis. A particular piece of information may function as a source study for one information requirement but may also serve as supporting information to support the read-across hypothesis.

The justification for a read-across hypothesis provided in the dossier is the documentation that will be assessed by ECHA. ECHA only evaluates the documentation provided in the dossier. It does not undertake extra analysis or research to further develop a justification that would be insufficient. ECHA does not complement the supporting documentation that would be incomplete. Consequently, failure to provide supporting information for a key aspect of the read-across hypothesis will result in this key aspect not being demonstrated to be scientifically valid. This would result in a failure of the grouping and read-across adaptation.

The information that is needed to support a read-across hypothesis for a specific case of grouping and read-across is dependent upon the hypothesis and the substances concerned. ECHA's RAAF identifies the issues that are analysed when evaluating a read-across adaptation, and these should be addressed by supporting information when appropriate.

Supporting information includes a variety of types of information. Adequate identification of all source substances, together with compositional information (see Section 2.2) is mandatory. However, the nature of the remaining supporting information is dependent on the hypothesis and the substances concerned. Supporting information may correspond to REACH information requirements from Annexes VI-XI or may be other information. It may consist of studies performed by a test method or test guideline, alternative methods (including *in vitro* and *in silico* methods) or other analysis. The support that this information provides for the read-across hypothesis must be explained as detailed under Section 4.2b).

Where a study is supposed to support a critical scientific aspect of the read-across hypothesis (i.e. corresponding to the scientific issue addressed by an assessment element under the RAAF framework[14]), ECHA must be able to independently assess this study. This supporting information must be submitted with a detailed summary of the objectives, methods, results and conclusions of each study so as to enable an independent scientific assessment of these studies (see *How to report robust study summaries*[15]). ECHA must be able to verify that supporting evidence substantiates the scientific validity and robustness of the read-across hypothesis (as set out in Section 4.2b).

The detail that is required from a supporting study is determined by the support that the study provides to the read-across hypothesis. Accordingly, where multiple studies establish the same issue, it is not necessary to provide a detailed summary enabling an independent scientific assessment for all the studies, but it is necessary to provide a detailed summary enabling an independent scientific assessment of the key study(ies).

Likewise, where an issue is peripheral to, or unnecessary for, the support of the read-across hypothesis, it is not necessary to provide a detailed summary enabling an independent scientific assessment for all the studies. Where a detailed summary of a study enabling an independent scientific assessment is not required, the study should

[14] Read-Across Assessment Framework (RAAF), May 2017 Link

[15] *How to report robust study summaries Practical Guide 3* (Version 2.0), November 2012 Link

nevertheless be reported by providing a summary of the objectives, methods, results and conclusions of the study providing sufficient information to make an assessment of the relevance of the study.

5. Glossary

Abbreviation/ Term	Explanation/ Definition
Analogue approach	The term analogue approach is used when read-across is employed between a few, very structurally similar substances for which it is not possible to establish a trend or a regular pattern. As a result of the structural similarity, a given (toxicological or other) property of one substance (the source) is used to predict the same property for another substance (the target), for which this property is not available but is needed to fulfil a REACH information requirement. The outcome of a study conducted with the source substance is read-across for all investigated parameters to the target substance. A worst-case approach may also be used.
(Bio)transformation	A series of chemical changes in a compound as a result of enzymatic or other activity in a living organism. The term "transformation" used for environmental endpoints refers to abiotic and biotic degradation.
Category approach	Used when read-across is employed between several substances that have structural similarity. These substances are grouped together on the basis of defined structural similarity and differences between the substances. As a result of the structural similarity, one or more toxicological properties are proposed to be similar or to follow a regular pattern. The predictions are made within the group for the target substance(s) based on the observed regular pattern. Alternatively, the prediction is based on a read-across from a category member in a conservative manner (worst case).
CMR	Carcinogenic, mutagenic or reprotoxic.
Constituent	Discrete chemical structure, which is separable from its stereo-, regio- and constitutional isomers
Mono-constituent substance	A mono-constituent substance is a substance, defined by its quantitative composition, in which one main constituent is present to at least 80 % (w/w).
PBT	Persistent, bioaccumulative and toxic.
REACH	Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals.
Read-across hypothesis	Hypothesis on the basis of which property(ies) of target substance(s) may be predicted from source substance(s). This hypothesis must be based on a relationship between structural similarity and the predicted property(ies) and needs to be supported by read-across justification.
Read-across justification	The reasoning and associated supporting evidence that are provided to verify the scientific validity and robustness of the read-across hypothesis.
Robust study summary	A detailed summary of the objectives, methods, results and conclusions of a full study report providing sufficient information to make an independent assessment of the study minimising the need to consult the full study report (REACH Article 3 (28)).
Source substance	Read-across is regarded as a technique for predicting endpoint information for one substance, by using data from the same endpoint from (an)other substance(s), i.e. the source substance(s)
Supporting information	Any scientific evidence provided to support the read-across hypothesis. Such supporting evidence may be, for example, information on the toxicokinetic properties of the substances, information from valid (Q)SARs, in vitro or in vivo experimental data addressing specific aspects of the read-across hypothesis.

Target substance	read-across is regarded as a technique for predicting endpoint information for one substance (i.e. the target substance), by using data from the same endpoint from the source substance(s).
Test material	The substance actually tested in the source study(ies). The identity and composition (including impurities) of this test substance should be representative of the source substance described in the read-across hypothesis.
Transformation	A series of chemical changes in a compound as a result of biotic or abiotic degradation.
UVCB	Substances of unknown or variable composition, complex reaction products or biological materials.
vPvB	Very persistent and very bioaccumulative.