

# Read-Across Assessment Framework (RAAF)

Considerations on multi-constituent substances and UVCBs



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## Read-Across Assessment Framework (RAAF) - considerations on multi-constituent substances and UVCBs

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## 1. Overview

The focus of the Read-Across Assessment Framework (RAAF) published in May 2015 is on mono-constituent substances.

Although the assessment framework may also be applied in an analogous manner to multi-constituent substances and 'substances of unknown or variable composition, complex reaction products or biological materials (UVCBs); ECHA considered that additional issues need to be addressed when read-across cases involving such substances have to be assessed.

The first step towards a future extension of the RAAF is to identify such additional issues emerging in read-across cases involving multi-constituent substances and/or UVCBs.

This document describes the additional key issues for the ECHA experts assessing such cases. Such considerations may also be relevant for registrants in the justification of read-across cases. However, due to the case-specific nature of read-across cases for multi-constituent substances and/or UVCBs, the document does not provide considerations on how to deal with the identified issues when adaptations using read-across are developed.

The starting point of the analysis presented in this document is the substance definition in the REACH Regulation: a registered substance under REACH may comprise a set of chemical structures corresponding to different constituents, impurities and/or additives, i.e. not only one relevant chemical structure is present. If the registered substance itself is tested, it does not matter whether the substance is a mono-constituent substance, multi-constituent substance or a UVCB. The obtained test results reflect the inherent properties of all constituents. However, detailed knowledge on the test material is needed to be able to conclude that the tested material is representative of the registered substance.

If a prediction is attempted for a multi-constituent or a UVCB target substance based on the test results obtained with individual constituents or based on the test results obtained with other multi-constituent substances or UVCBs, then the precise nature of the composition of source and target substances matters greatly.

To illustrate this complexity, model cases were constructed and analysed. Generic conclusions were derived from the analysis of the model cases. The model cases focus on analogue one-to-one read-across approaches, however, the identified issues are also considered to apply to category approaches.

In comparison with rather pure mono-constituent substances, multi-constituent substances and UVCBs involve more than one and up to many relevant chemical structures<sup>1</sup>. Consequently, read-across approaches for such substances require additional justifications and assessments to account for the increasing complexity of the composition of the substance.

All the chemical structures involved need to be considered; grouping of substances based on structural similarity must take account of all constituents (including any impurity and additive), and the predictions within proposed groups must likewise consider the impact of all constituents. Hence, detailed information on the composition of source substances and the test material used in the conducted source studies is needed to establish their relation to the target substance in terms of grouping and predictions.

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<sup>1</sup> The RAAF acknowledges that mono-constituent substances may have up to 20 % impurities, which need to be assessed with regard to their impact on the prediction.

The variations in concentrations of constituents, which are characteristic for many multi-constituent substances and UVCBs, also add complexity to predictions involving multi-constituent substances or UVCBs.

The combined exposure to several constituents and the possible associated influences on each other's toxicity was found to be a relevant consideration. Uncertainty about the consequences of such influences makes the interpretation of the test results difficult when used for predictions. Similarly, the outcome of combined chemical exposure when only data on individual constituents exists was an important consideration.

The hypothesis on why a prediction based on read-across is possible may involve complex mechanistic explanations since all the involved chemical structures are addressed. Such explanations need sufficient supporting information (e.g. information on toxicokinetics/ metabolism and/or mechanism of toxicity for the individual constituents).

'Bridging studies' are comparable studies on the source and target substance, and these bridging studies allow side-by-side comparison of the substances for a particular property (e.g. properties as determined in a 90-day study). Bridging studies may demonstrate that two multi-constituent substances or UVCBs have similar properties for a particular endpoint, and thus play a key role in a read-across justification. In the absence of such an empirical demonstration, read-across may be difficult to justify for complex compositions.

The analysis described in this document confirmed the complexity of read-across approaches for multi-constituent substances and UVCBs. Therefore, it was deemed premature to cover such substance types by a full extension of the RAAF. More work is needed to develop further the RAAF based on the findings described in this document.

## 2. Introduction

'Read-across and grouping', or 'read-across', is one of the most commonly used alternative approaches for data gap filling in registrations submitted under the REACH Regulation. It entails the use of relevant information from analogous substances (the 'source' information) to predict properties for the 'target' substance(s) under consideration.

The conditions under which read-across can be used to adapt the standard testing regime are listed in Annex XI, Section 1.5 to the REACH Regulation. Prediction of a property based on read-across has to be reliable, can be used for risk assessment and/or classification and labelling, and complies in general with the provisions in REACH for the substance under consideration.

The Read-Across Assessment Framework ([RAAF](#)) was developed by ECHA as an internal tool for assessing predictions, based on read-across, of the human health, fate and environment properties of substances in the context of the REACH Regulation. It also was made publicly available to help improve the use of read-across by experts developing read-across cases aimed at fulfilling the requirements of the REACH Regulation.

The RAAF provides a framework to evaluate consistently the scientific aspects of a proposed read-across case, resulting in an output that is suitable for subsequent regulatory consideration of the read-across case. In this context, different read-across approaches are described in the form of 'scenarios'.

The scenarios thereby categorise the type of read-across approach used to allow a systematic assessment of the crucial scientific aspects. Each 'scenario' has different 'assessment elements', which address different scientific considerations deemed crucial to judge the validity and the reliability of the read-across. A read-across case is appraised against each of the respective assessment elements. The appraisal is then used to inform decision-making.

The focus of the RAAF is mono-constituent substances. However, the framework of the RAAF may also be applied in an analogous manner to multi-constituent substances and UVCBs.

The importance of considering multi-constituent substances and UVCBs in read-across approaches may be illustrated by the share of such substances in REACH registrations.

By 25 January 2017, ECHA had received 50 748 registrations (including NONS updates), of which many are joint registrations. On the substance level, ECHA has identified 11 998 registered substances. Further analysis of the substance types, as declared by the registrants<sup>2</sup> shows that about 69 % of these are mono-constituent substances, 10 % are multi-constituent substances and 21 % are UVCBs,

ECHA has not quantified the number of read-across cases involving multi-constituent substances and/or UVCBs. However, the total number of read-across cases identified in the registration dossiers (see the [Second Report under Article 117\(2\) of the REACH Regulation](#)), combined with the percentage of registered multi-constituent substances and UVCBs, indicate that read-across approaches involving such substances are likely to be numerically substantial.

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<sup>2</sup> registrants may not always have allocated the substances into the substance types correctly

### 3. Purpose and scope of the document

The purpose of the analysis presented in this document is to identify the additional key issues arising when assessing read-across cases involving multi-constituent substances and/or UVCBs.

Although many aspects are applicable to environmental (and fate) properties as well, the emphasis is on human health. A full extension of the RAAF to cover multi-constituent substances and UVCBs for all current RAAF elements was deemed premature. It was perceived that the complexity involved in building and assessing read-across cases involving multi-constituent substances and UVCBs needs to be better understood.

Experts should consider the key issues identified when assessing read-across approaches involving multi-constituent substances and UVCBs. Due to the case-specific nature of read-across cases for multi-constituent substances and UVCBs, the document does not provide considerations on how to deal with the identified issues when adaptations based on read-across are developed.

It is not a purpose of this document to address the way the RAAF and the additional considerations described here are implemented in ECHA's processes nor to describe how the shortcomings identified in the scientific assessment are evaluated in the course of dossier evaluation under REACH.

## 4. Problem description

In this chapter, some relevant general aspects are highlighted. The RAAF explains the legal basis, basic concepts and terminology for grouping of substances and read-across under REACH.

These concepts apply to read-across approaches involving multi-constituent substances and UVCBs as well. The RAAF chapters on “What is grouping of substances?” and “What is read-across?” are in particular important for understanding the issues described in the current document. The appendix to this document provides a listing of terms and definitions.

The basis for grouping and read-across under REACH is the structural similarity between substances; therefore, the substance composition has a major impact for all read-across approaches.

### 4.1 SUBSTANCE CONCEPT UNDER REACH

The substance concept in REACH is defined in the REACH Regulation and further clarified in the [Guidance for identification and naming substances under REACH and CLP](#).

In Article 3(1) of the REACH Regulation, the definition of a substance for the purpose of this regulation is provided:

*“Substance: means a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition;”*

As a direct consequence of Article 3(1), the definition of “substance” goes beyond a pure chemical compound defined by a single molecular structure.

Substances registered under REACH may be:

- “Well-defined substances”: substances with a defined qualitative and quantitative composition that can be sufficiently identified based on the identification parameters in Annex VI, Section 2 to the REACH Regulation. Variability of composition for well-defined substances is specified by upper and lower limit of the concentration range(s) of the main constituent(s).
  - Mono-constituent substances defined by their quantitative composition, in which one main constituent is present to at least 80 % (w/w). Mono-constituent substances usually have impurities (up to 20 % w/w), i.e. constituents present in a substance as produced which are not intentionally added.
  - Multi-constituent substances consisting of more than one main constituent present at concentrations  $\rightarrow$  10 % and  $<$  80 % (w/w). Multi-constituent substances usually also have impurities (i.e. constituents which are not the main constituents).
- “UVCBs” are substances that cannot be sufficiently identified by the identification parameters in Annex VI, Section 2 to the REACH Regulation. UVCBs cannot be sufficiently identified by their chemical composition, because the number of constituents is relatively large and/or the composition is, to a significant part, unknown and/or the variability of composition is relatively large or poorly predictable<sup>3</sup>.

<sup>3</sup> However, it is acknowledged that there are also UVCBs which are quite well characterised in terms of their composition.

- These definitions clarify that a registered substance under REACH may comprise a set of chemical structures corresponding to different constituents, impurities and/or additives.

## 4.2 THE INFLUENCE OF THE COMPOSITION COMPLEXITY ON TESTING AND PREDICTIONS

There are two fundamental ways to address the information requirements for any substance registered under REACH. Either the substance is tested as such or adaptations are used.

If the substance itself is tested and is representative for the registered substance, it does not matter whether the substance is a mono-constituent substance, multi-constituent substance or a UVCB<sup>4</sup>.

If a prediction is attempted for a multi-constituent or a UVCB target substance based on the test results obtained with individual constituents or based on the test result obtained with other multi-constituent substances or UVCBs, then the precise nature of the composition of source and target substances matters greatly. This is further explained in the next sections.

## 4.3 CONSEQUENCES OF COMBINED EXPOSURE TO TWO OR MORE CONSTITUENTS

For the consideration of read-across cases involving multi-constituent substances and/or UVCBs, the impact of combined exposures deserves particular scrutiny. It seemed therefore appropriate to first clarify the terms normally used to analyse mixtures and how they have been adapted in this document.

A full discussion of the available literature was not conducted nor was it deemed necessary for the purpose of this document. This document relies on concepts developed for the assessment of combined exposures to multiple chemicals by international bodies (WHO/IPCS 2011)<sup>5</sup> and EU bodies (SCHER, SCENHIR, SCCS 2012<sup>6</sup>; EFSA 2013<sup>7</sup>; Kortenkamp et al. 2009<sup>8</sup>; Danish Veterinary and Food

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4 In dossier evaluation decisions registrants are reminded that the sample of substance used for the new test(s) must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirements for the range of substance compositions manufactured or imported by the joint registrants. It is the responsibility of all joint registrants who manufacture or import the same substance, to agree on the appropriate composition of the test material and to document the necessary information on their substance composition.

5 Meek M.E. *et al.* (2011) Risk assessment of combined exposure to multiple chemicals. A WHO/IPCS framework. *Reg Toxicol Pharmacol* 60, S1-S14.

6 SCHER, SCENIHR, SCCS (2012) Toxicity and assessment of chemical mixtures. European Union, Directorate-General for Health and Consumers.

7 EFSA (2013). International framework dealing with human risk assessment of combined exposure to multiple chemicals. *EFSA Journal* 11(7):3313

8 Kortenkamp A. *et al.* (2009). State of the art report on mixture toxicity. Final report for study contract 070307/2007/485103/ETU/D.1. Contractor: The School of Pharmacy, University of London (ULSOP)

Administration, 2003<sup>9</sup>; IGHRC, 2008<sup>10</sup>).

Due to the substance concept under REACH, the terms developed for other purposes have to be slightly adapted to be useful for the analysis of read-across approaches involving REACH substances.

### Combined exposure to chemicals (mixtures)

The aspects of “mixture toxicology” have received quite some attention in the past decades. There are many research publications on various aspects of mixtures as reviewed by the EU Scientific Committees SCHER, SCENHIR and SCCS (2012)<sup>4</sup>.

“Mixture toxicology” might mean the investigation of combined effects of pure chemicals or the investigation of real world complex mixtures such as tobacco smoke. Mixtures, as defined in the REACH and CLP regulations, are intentional mixtures of substances obtained by blending of two or more substances without a chemical reaction. Such mixtures are not to be confused with the substances to be registered under REACH (see 3.1).

The WHO/IPCS framework (2011)<sup>3</sup> avoids the term “mixture” due to its ambiguous meaning in different contexts. Therefore, the term mixture is also not used in this document. The WHO/IPCS framework preferred the term “combined exposure to multiple chemicals”. In the context of read-across approaches for REACH substances, this translates to “combined exposure to multiple constituents (originating from one substance)”.

Combined exposure to multiple chemicals (constituents) may lead to adverse health effects that were not anticipated from knowledge of the properties of individual chemicals. The phenomenon therefore has general relevance for exposure to chemicals, including human populations, and some examples are given below.

- N-hexane causes neuropathy in humans and animals through the formation of 2,5-hexanedione. In combination with methyl ethyl ketone, n-hexane neurotoxicity is increased, thought to be by influence on the formation/excretion of 2,5-hexanedione. This appears to be responsible for an outbreak of occupational neuropathy in workers.<sup>7</sup>
- Carbontetrachloride and ethanol are both hepatotoxic, but together they produce much more liver injury than the sum of the individual effects on liver at a given dose would suggest<sup>11</sup>
- Furafullyline is a methylxanthine derivative that was introduced in the hope of being a long-acting replacement for theophylline in the treatment of asthma. It was observed that the substance is a potent inhibitor of the N3-demethylation of caffeine by highly selective and potent inhibition of P4501A2 in humans<sup>12</sup>. Upon combined exposure of volunteers to furafullyline and caffeine, a rapid accumulation of caffeine was observed leading to a 10-fold increase in the caffeine serum level. This led to the onset of adverse side effects (e.g. heartburn and mental confusion).

9 Danish Veterinary and Food Administration (2003). Combined actions and interactions of chemicals in mixtures. Fodevare Rapport 2003:12

10 IGHRC (2008). Chemical mixtures a framework for assessing risks to human health (CR14) Institute of Environment and Health, Cranfield University, UK

11 Casarett and Doull's Toxicology, Curtis D. Klaassen (ed.) 2007, p. 17

12 E. Tarrus et al. (1987). Accumulation of caffeine in healthy volunteers treated with furafullyline. Br. J. clin. Pharmac. 23, 9-18.

- Melamine (2,4,6-triamino-1,3,5-triazine) and cyanuric acid (1,3,5-triazine-2,4,6-triol) are structural analogues. Individually, both substances are regarded as not very toxic.
- When both compounds occurred combined in pet food, outbreaks of acute renal failure in cats and dogs were observed. Illegal adulteration of food and feed with melamine has resulted in illness and deaths of human infants. These effects are attributed to the formation of melamine-cyanuric acid co-crystals in nephrons. In 2010, EFSA<sup>13</sup> concluded that "...the currently available information does not allow identification of a factor by which the toxicity is increased by co-exposure. Therefore the TDI for melamine is not applicable if there is significant concomitant exposure to cyanuric acid, [...]".

### No interaction/interactions

When there is combined exposure to multiple constituents, there are two possible outcomes, which are discussed below:

- that the constituents when dosed in combined exposure have exactly the same magnitude and type of effects as when dosed individually – this is "no interaction"; or
- that the constituents when dosed in combined exposure have a different magnitude and/or type of effects as when dosed individually – this is "interaction".

*"No interaction"* assumes that constituents do not influence each other's toxicity, i.e. they do not interact with each other at the biological target site or with regard to toxicokinetic aspects; two principle situations are possible:

- Similar action: constituents act through the same mode of action and/or at the same target cell or organ or tissue, and may differ only by their potency. The effects can be estimated directly from the sum of the doses/concentrations. In principle, doses or concentrations of the individual constituents are added after being multiplied by a scaling factor that accounts for differences in the potency of the individual constituents (dose/concentration addition).<sup>14</sup>
- Dissimilar action: constituents act independently from each other, usually through different modes of action that do not influence each other, or at different target cells, tissues or organs. The effects can be estimated directly from the probability of responses to the individual constituents (response addition) or the sum of biological responses (effects addition).<sup>15</sup>

13 EFSA (2010). Scientific Opinion on Melamine in Food and Feed. EFSA Journal 8(4):1573.

14 The EU Scientific Committees point out that dose/concentration additivity is assumed over the entire dose range, including doses/concentrations below the NOAELs/NOAECs of the individual constituents. This means, that effects can be expected if the summed dose/concentration is high enough to exceed the threshold of toxicity for the combined exposure, even when the dose/concentration level of each individual constituent is below its own individual NOAEL/NOAEC (SCHER, SCENHIR, SCCS 2011)

15 If an individual constituent is below its individual NOAEL/NOAEC (and these values represent true zero-effects levels) it will not add to the joint effects caused by the combined exposure. The EU scientific committees point out that experimentally determined NOAELs/NOAECs do not necessarily represent zero-effect levels. In such cases, it can be expected that exposures to such levels still contribute to the joint effects of the combined exposure to the constituents.

“*Interaction*” assumes that constituents influence each other’s toxicity<sup>16</sup>, i.e. they interact with each other at the biological target site, then the combined effects of two or more constituents may be stronger (synergism, potentiation) or weaker (antagonism, inhibition, masking) than expected on the basis of dose or effects addition. Consequently, the two principle situations are:

- Synergism, potentiation: synergism occurs when the effect resulting from the combined exposure to constituents is stronger than estimated for additivity on the basis of the toxicities of the constituents. Potentiation occurs when a constituent that itself does not have a toxic effect on a biological system increases the effect of a second constituent on that system (EFSA 2013)<sup>5</sup>.
- Antagonism, inhibition, and/or masking: antagonism occurs when the effect of the combined exposure to multiple constituents is weaker than estimated for additivity on the basis of the toxicities of the individual constituents. Inhibition occurs when a constituent that does not have a toxic effect on a biological system decreases the apparent effect of a second constituent on that system. Masking occurs when constituents produce opposite or functionally competing effects on the same biological system and diminish the effects of each other, or one overrides the effects of the other (EFSA 2013)<sup>5</sup>.

Interactions may occur at a toxicokinetic and/or toxicodynamic level.

- Toxicokinetic interactions, i.e. constituents influence aspects of
  - their absorption (e.g. surface active chemicals can enhance the absorption of other chemicals through the skin);
  - their distribution (e.g. a more lipophilic compound may compete with the binding sites of blood proteins and replace the less lipophilic compound, thereby increasing the concentration of unbound compound with associated toxic effects);
  - their metabolism (enzyme inhibition/induction resulting in reduction or increase in toxicity depending on whether the parent compound or a metabolite is responsible for the toxicity);
  - their excretion (influence on active processes).
- Toxicodynamic interactions, i.e. interactions between the biological responses resulting from the exposure to individual constituents, for instance resulting from similar targets (ligand-receptor interactions).

#### 4.4 PREDICTIONS BASED ON READ-ACROSS FROM SOURCE STUDIES

Depending on the nature of the source data used for predictions for a multi-constituent substance or a UVCB, two principle approaches are possible for the prediction.

Constituent-based approach: the source data come from test results obtained with the individual constituents of the target substance. If a prediction is attempted for a multi-constituent substance or

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<sup>16</sup> In principle, there might be interaction of chemicals on the biological level and on the chemical level (chemical-chemical interactions with subsequent formation of different chemical structures and different toxicity profiles compared to the original chemicals). Whereas in other regulatory areas dealing with combined exposure, chemical interactions also need to be considered (IGHRC, 2008). This appears to be not needed for the circumstances considered here, since the substance identity of the manufactured or imported substance is described and confirmed with analytical methods and no interactions on the chemical level between constituents are expected. Therefore, only interactions occurring in the biological test system (toxicokinetic or toxicodynamic interactions) are further considered here.

a UVCB target substance based on the properties of individual constituents, then the composition and concentrations of the constituents in the target substance must be accounted for in the justification for the prediction. The test results of the individual constituents alone will have no information on possible interactions of these constituents when combined exposure to these constituents occurs.

Substance-based approach: the source data come from test results obtained with the source substance (a multi-constituent substance or a UVCB) as such, and the result of the test is used to predict the properties for a target substance. All possible toxicokinetic and toxicodynamic interactions among the source substance's constituents are inherently reflected in the test result. The test result does not discriminate the specific contribution of the constituents in the type of effect(s) observed or on their individual potency or on their possible interaction on the toxicokinetic and/or toxicodynamic level. Therefore, if a prediction is attempted for a mono-constituent or multi-constituent substance or UVCB based on test results obtained with a source substance containing more than one main constituent, the composition and concentrations of the constituents of the target and source substances must be taken into account in the justification for the prediction. The impact of possible influences of the constituents on each other's toxicity needs to be considered to assess the predictions for the target substance containing only one of the source substance's constituents, or containing partly the same and partly different constituents, or containing structurally similar constituents, or the same constituents but at different concentrations.

Predictions based on category approaches may use the constituent-based approach and/or the substance-based approach. In comparison with an analogue one-to-one read-across approach, the principle issues described above remain similar. If the structural variations of category members are covered by sufficient data points for the properties under consideration, the confidence in a particular prediction may potentially be strengthened.

## 5. Analysis of model cases

### 5.1 BACKGROUND AND SCOPE FOR THE ANALYSIS

When discussing scientific issues related to read-across approaches between substances with several constituents, a large number of theoretical cases can be envisioned.

To identify the key scientific issues that arise when analysing read-across approaches involving multi-constituent substances and/or UVCBs, a number of model cases were constructed and systematically examined. The model cases were constructed without describing concrete (chemical) structural features and comprehensive details. Specific structural features were deliberately avoided to avoid case-specific considerations stemming from the actual chemical structure dominating the analysis.

It is recognised that the model cases do not fully reflect the complex reality as more complex variations are encountered in real world read-across approaches. Moreover, the model cases are not meant to exhaustively describe all possible hypothetical situations. However, focusing the discussions around the model cases was found to be useful to illustrate the key scientific considerations stemming from complex composition(s) of source and/or target substances.

The model cases were systematically varied with increasing complexity of the involved compositions for source and/or target substances. For each model case, the key scientific issues of the proposed read-across were identified and discussed building on the fundamental principles laid down in the RAAF. In addition, the experience acquired from the evaluation of real read-across cases was taken into account.

Under the assumption that the issues identified are similar for analogue and category read across approaches, the analysis was limited to one-to-one analogue approaches. To limit the scope of the analysis, it was assumed that the properties to be predicted are systemic effects after repeated administration. Such predictions have two aspects: type of effects and strength of effects. To simplify the cases further, impurities were not considered, i.e. they were assumed to be lower than 0.1 % for mono- and multi-constituent substances and of no toxicological relevance. Similarly, for model cases involving UVCBs, it was assumed that the constituents/groups of constituents above 0.1 % and of toxicological relevance were known.

It is worth noting that the consequences for classification and labelling were not analysed. The CLP Regulation<sup>17</sup> has defined the provisions to be followed for “mixtures”, which are applicable for REACH substances containing several constituents as well: *“Where the mixture itself has not been tested ..., but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data shall be used in accordance with the bridging rules set out in section 1.1.3.”* When data are available for all or some ingredients of the mixture, the concentration limits provided in the CLP Regulation under the individual hazardous properties apply.

### 5.2 ILLUSTRATION OF THE APPROACH

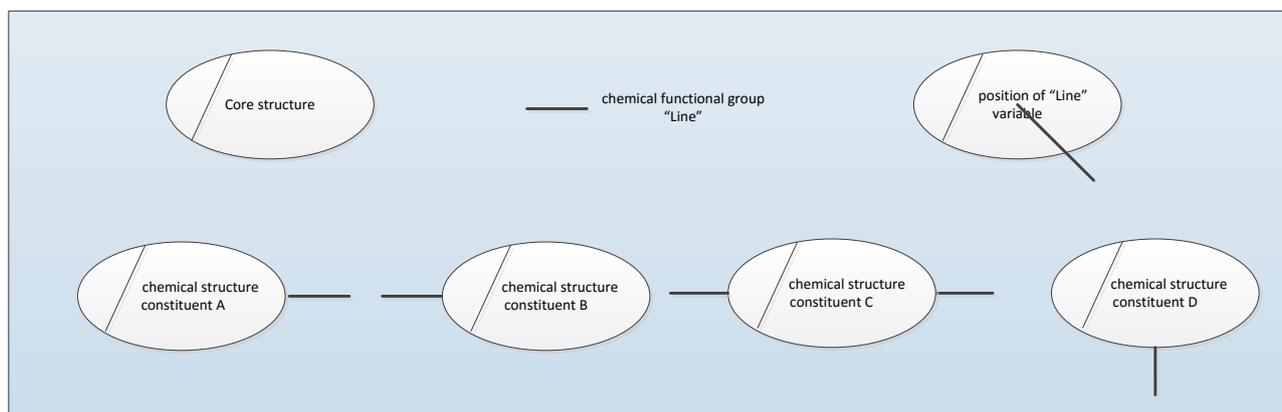
An ellipse and a triangle are used to illustrate two different chemical core structures. In addition, a “Line” and “T-Line” are used to illustrate two different functional groups, which may be attached to different positions

<sup>17</sup> Regulation (EC) No 1272/2008, OJ L 353 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures - amending and repealing Directive 67/548/EEC and 1999/45/EC, and Regulation (EC) No 1907/2006.

of the core structure.

The resulting constructs are representations of structurally similar constituents characterised by different core structures and different numbers and positions of the functional groups. In the model cases, the core structures (ellipse or triangle) are combined with the functional groups (“Line” or “T-Line”) to illustrate the different constituents present in the source and target substances.

**“Pool Ellipse” of structurally related constituents**



**“Pool Triangle” of structurally related constituents**

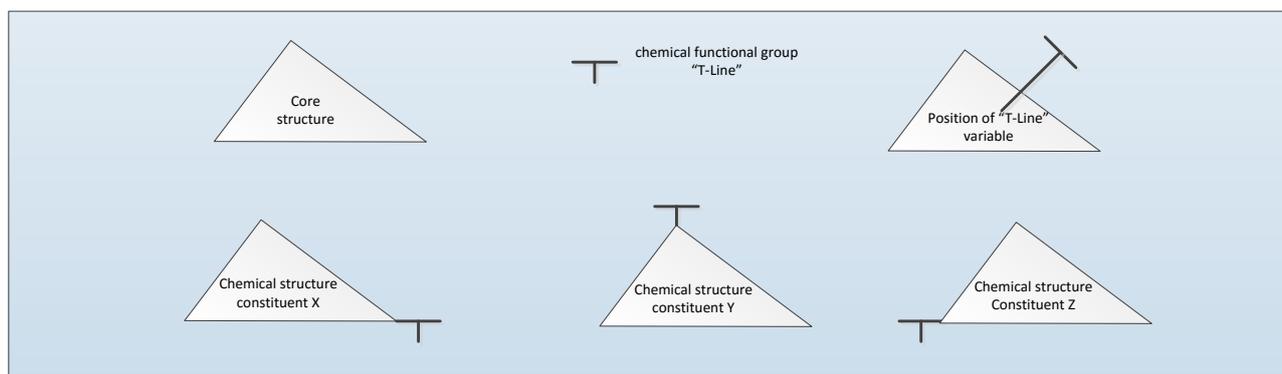


Figure 1. The image shows two “pools” of substances. An ellipse and a triangle are used to illustrate two different chemical core structures. The ellipse has an additional line-bridge to make it non-symmetrical and the triangle is neither equilateral nor right angled. In addition, the “Line” and the “T-Line” represent two different functional groups. The “Line” and the “T-Line” may be attached to different positions of the core structure. It is also possible that the exact position of the functional group is not defined. The “Line” and “T-Line” are meant to possess different chemical reactivity and may also be subject to metabolic conversion. The different chemical structures of the ellipse or the triangle are grouped in “pools” of structurally similar substances. 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.

Substances with the same core structure (ellipse or triangle) are considered structurally similar. These substances can be grouped into a “pool” of structurally similar substances irrespective of the position and number of attached functional groups<sup>18</sup>.

Each individual pool consists of structurally similar substances, but the two different pools are not similar to each other. For examples of chemical structures, which may be considered as pools, see the appendix. ECHA has invented this terminology and is not aware that the rather neutral expression “pool” is used elsewhere to describe groups of structurally similar constituents, which may be distributed over several substances.

Figures 1.1 and 1.2 illustrate how the graphic elements are used to describe the prediction for a mono-constituent substance. Figure 1.1 describes a typical read-across case, where RAAF Scenarios 1 or 2 would apply (depending on the mechanistic explanation). Figure 1.2 illustrates a scenario where read-across is not possible, since there is no structural similarity between the two substances.

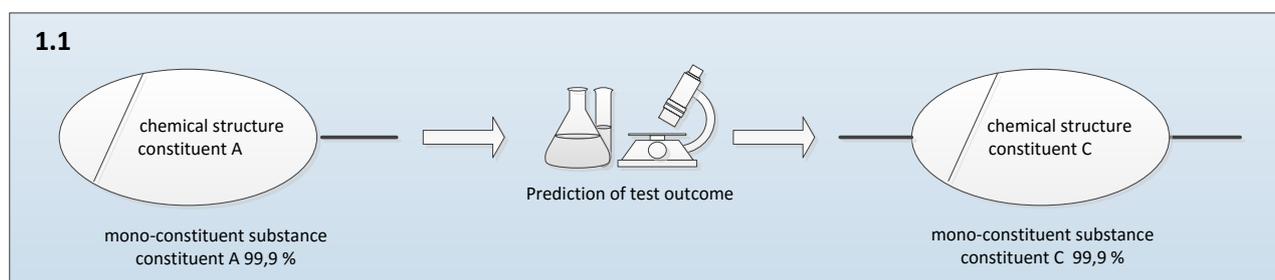


Figure 1.1 illustrates a situation where structural similarity exists between the main constituent A of the mono-constituent source substance and the main constituent C of the mono-constituent target substance. Data from guideline studies conducted with constituent A are available for read-across to constituent C.

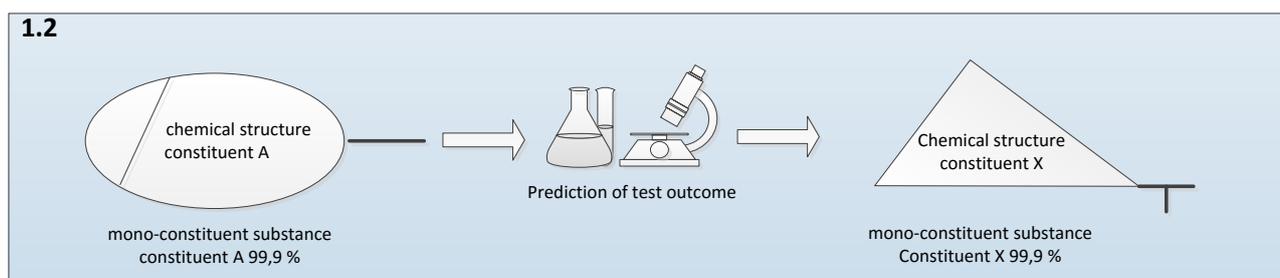


Figure 1.2 illustrates a situation where structural similarity does not exist between the main constituent A of the mono-constituent source substance and the main constituent X of the mono-constituent target substance. Data from guideline studies conducted with constituent A are available for read-across to constituent X. However, there is no structural similarity as a basis for a prediction, since the core structure as well as the functional groups are different.

<sup>18</sup> The term “pool” is used to avoid confusion with the term “group” which is used in Annex XI, Section 1.5. to describe groups of substances, whereas here “pools” (or groups) of constituents are in focus.

### 5.3 CONSIDERATION OF MODEL CASES

It is emphasised that the model cases are hypothetical but reflect principle situations encountered in real cases. They start from the premise that the only data available comes from tests conducted with the source substances. This data is proposed to be read across to the target substance. The critical issues derived by analysing the model cases, are considered as applicable to real situations.

It is noted that it is possible to envisage supporting information that would address the key issues identified in the analysis of the model cases, thereby strengthening the justification for the prediction. However, it is not considered here which specific data would address e.g. the uncertainties related to the impact of the combined exposure or variations in constituent concentrations, nor is the evaluation or acceptability of such supporting information considered.

#### 5.3.1 Prediction from two mono-constituent substances to a multi-constituent substance

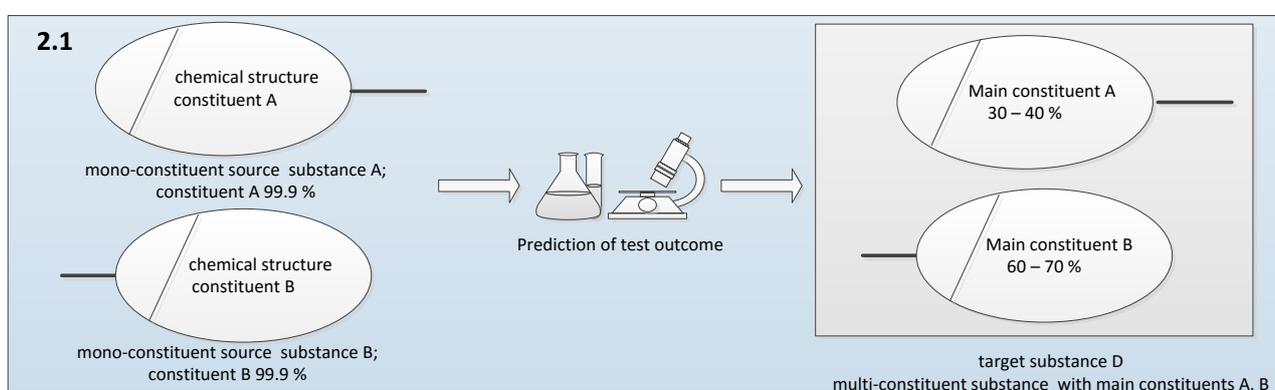


Figure 2.1 illustrates a “constituent-based approach” (see 3.3.3). Data from guideline studies conducted with mono-constituent substance A and with mono-constituent substance B are available for read-across to target substance D. Substance D is a multi-constituent substance with constituent A at 30 – 40 % and constituent B at 60 – 70 %.

Figure 2.1 shows a model case where the constituents of the multi-constituent substance D are identical to the mono-constituent substances A and B. Furthermore, both constituents A and B belong to the ellipse-pool, so structural similarity as a basis for the grouping may be assumed. In comparison to a read-across approach between mono-constituent substances, e.g. from mono-constituent substance A to mono-constituent substance B, the assessment of the case requires additional considerations.

#### Critical issues

Without further information, it is not clear which of the situations described below apply (see 3.3.2 and glossary for explanations of terms):

- a) There is no interaction for the constituents A and B when combined exposure occurs; the types of effects observed for A and B will predict the type of toxicity to be expected for D. For quantitative predictions the following situations are possible:
  - If there is dissimilar action, the test results for A and B may be regarded as a worst-case scenario in comparison with D since they have been tested as a pure substance (higher doses when compared to doses which would be achieved in testing D).
  - If there is similar action, the effects observed for A and B are to be treated as additive when quantitative predictions for D are made. The effects for D may be estimated directly from the

sum of the doses/concentrations from the test results obtained with A and B.<sup>19</sup>

It is noted that information on the critical effect on the biological target for both constituents and the associated NOAELs is necessary to determine how to make the prediction.

- b) If there are toxicokinetic and/or toxicodynamic interactions between A and B, changes in the type of effect and/or in the strengths of effects are likely to be observed (if substance D would be tested) when compared to the effects observed with A and B alone.

In conclusion, the prediction for substance D needs to take into account which impact the combined exposure to constituents A and B could have on the type and the strengths of effects. There is a need for additional information<sup>20</sup> that establishes which of the situations above are applicable and what the consequences for the prediction are.

### 5.3.2 Prediction from a multi-constituent substance to a mono-constituent substance

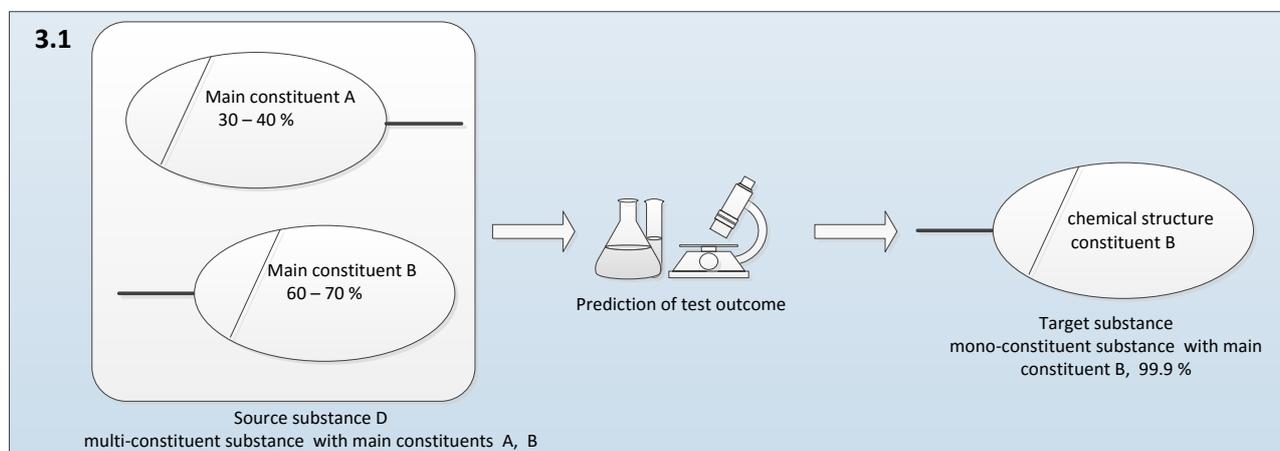


Figure 3.1 illustrates a situation where test results obtained with multi-constituent substance D containing the two main constituents A and B are used to predict the test results for a mono-constituent substance, which contains the constituent B of substance D as the only main constituent. Data from guideline studies conducted with source substance D are available for read-across to substance B.

In this model case, constituent B is the same in both substances. Furthermore, both constituents A and B belong to the ellipse-pool, structural similarity as a basis for the grouping may be assumed.

The test result of substance D reflects the effects caused by the combined exposure to both constituents A and B. All possible toxicokinetic and toxicodynamic interactions are inherently reflected in the test result. The test result does not discriminate the specific contribution of constituents A and B in the type of effect

<sup>19</sup> IGHRC (2008) expressed that the “general held view is that dose addition should be assumed for groups of chemicals that produce the same toxic effect in the same target organ via the same mechanism” And further: “Where chemicals affect the same target organ and there is uncertainty about the mechanism of action it is more precautionary to assume that the effects of co-exposure to these chemicals will be additive rather than independent”

<sup>20</sup> As stated in the introduction to 5.3, it is noted that it is possible to envisage supporting information that would address the key issues identified in the analysis of the model cases, thereby strengthening the justification for the prediction. However, which specific data would address e.g. the uncertainties related to the impact of the combined exposure or variations in constituent concentrations is not considered here, and nor is the evaluation or acceptability of such supporting information.

observed or on their individual potency or on their possible interaction on the toxicokinetic or toxicodynamic level.

This is a model case of a “substance-based” approach (see 3.3.3), since the source data for the prediction come from testing a whole substance with several constituents. This approach needs additional consideration in comparison with predictions based on read-across between mono-constituent substances.

### Critical issues

Without further information, it is not clear which of the following situations apply:

- a) The effects observed in the source study are caused by constituent B (or its conversion products) alone, constituent A is proposed to cause no adverse effects in this study type and there is no interaction between A and B (= no interaction, dissimilar action).
- b) The effects observed are caused by the action of both constituents A and B (or their conversion products) in substance D, but there is no interaction (= no interaction, similar or dissimilar action).
- c) The effects observed are caused by the action of both constituents A and B and there is an interaction between constituents A and B (= interaction possibly leading to different type of effects and/or change in the strength of effects).
- d) No effects are observed for D. The study maybe not be sufficient for hazard identification of B alone since the limit dose or the dose causing toxicity may not have been reached for B, or A may antagonise/inhibit/mask the toxicity of B (interaction).

There is a need for additional information<sup>19</sup> establishing which of the situations above are applicable. Each situation has a different outcome for the prediction in terms of the type of effect and/or strength of effect.

If there is an absence of information on the influence of A and B on each other's toxicity (concern here would be that A antagonises/inhibits or masks the toxicity of B), then the test results obtained with substance D do not allow a prediction of constituent B alone.

Since constituents A and B are structurally similar, it may be claimed that they have the same type of effect on the basis of this structural similarity, although only information for one of the constituents (A or B) is available.

As explained in the RAAF, structural similarity alone between the two constituents A and B is not sufficient to claim similar properties, but supporting evidence needs to be available<sup>21</sup>.

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<sup>21</sup> This would be the most simple example for what may be called a “nested” read-across, i.e. there is a read-across approach for constituents of the source substance which “nests” within the read-across approach for the source and target substance. A “nested” case for the two constituents A and B within substance D needs to be assessed in the same way as a read-across approach applied for mono-constituents according to the principles of the RAAF.

### 5.3.3 Prediction from a multi-constituent substance to a multi-constituent substance (constituents are members of the same pool)

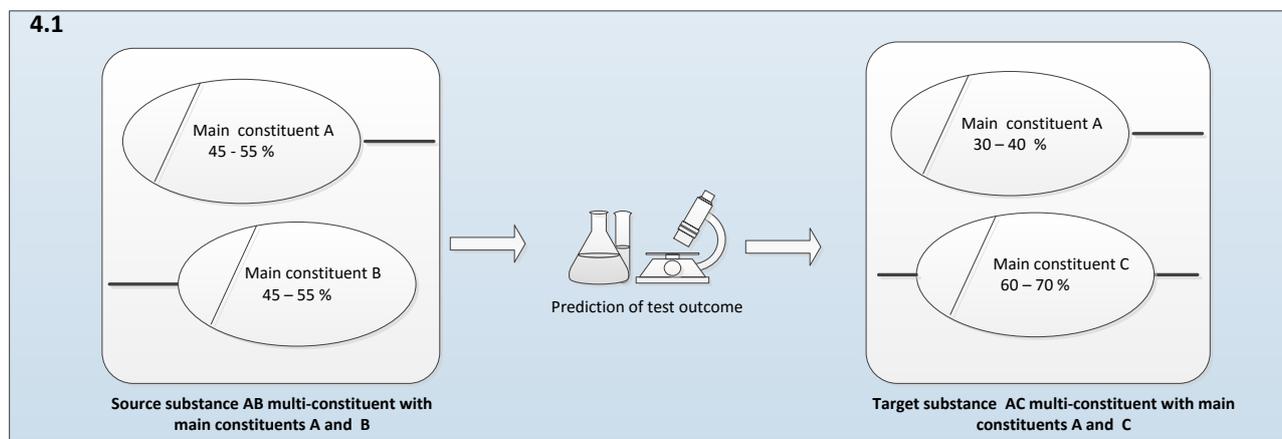


Figure 4.1 illustrates a situation where a test result obtained with multi-constituent substance AB containing the constituents A and B at 45 – 55 % each is used to predict the outcome of such a test for another multi-constituent substance also containing the constituent A (at 30 – 40 %) and additionally constituent C (at 60 – 70 %). Data from guideline studies conducted with substance AB are available for read-across to substance AC.

In this model case, constituent A is the same in both substances. Furthermore, all constituents belong to the ellipse-pool, so structural similarity of the constituents as a basis for the grouping may be assumed.

The test result of substance AB reflects the effects caused by the combined exposure to both constituents A and B. All possible toxicokinetic and toxicodynamic interactions are inherently reflected in the test result. The test result does not discriminate the specific contribution of constituents A and B on the type of effect observed or on their individual potency or on their possible interaction on the toxicokinetic or toxicodynamic level.

If substance AC would be tested, a similar description would apply. Test results would reflect the effects caused by the combined exposure to both constituents A and C. All possible toxicokinetic and toxicodynamic interactions would be inherently reflected in the test result. The test result would not discriminate the specific contribution of constituents A and C on the type of effect observed or on their individual potency or on their possible interaction on the toxicokinetic or toxicodynamic level.

This is a model case of a “substance-based” approach (see 3.3.3), since the source data for the prediction come from testing a whole substance with several constituents. In this model case, the target substance also has several constituents. This approach needs additional considerations in comparison with predictions based on read-across between mono-constituent substances.

#### Critical issues

Lacking information on whether constituents A or B or both cause the observed toxicity in the test with substance AB prevents a scientifically-sound prediction for substance AC. Without further information, it is not possible to establish which of the following situations apply:

- a) The effects observed in the source study are caused by constituent B (or its conversion products) of substance AB and constituent A is proposed to cause no adverse effects in this study type and there is no interaction between A and B or A and C (= no interaction, dissimilar action).

- b) The effects observed in the source study are caused by constituent A (or its conversion products) and constituent B is proposed to cause no adverse effects in this study type and there is no interaction between A and B (= no interaction, dissimilar action).
- c) The effects observed in the source study are due to both constituents A and B (or their conversion products), in substance AB, but there is no interaction (no interaction dissimilar or similar action).
- d) The effects observed in the source study are due to both, constituents A and B (or their conversion products) and there is an interaction between constituents A and B (= interaction possibly leading to different types of effect and/or change in strength of effects).
- e) If no effects are observed for the test conducted with AB, the conclusion that such outcome can be predicted for AC is not supported on the test result with AB alone.

There is a need for additional information<sup>19</sup> that establishes which of the situations above are applicable. Each situation has a different outcome for the prediction in terms of type of effect and/or strength of effect. If there is an absence of information on the influence of A and B on each other's toxicity (interaction or no interaction), then the test results obtained with substance AB do not allow a prediction of substance AC, in particular since it is also not known whether A and C interact with each other.

The concentration of constituent A in substance AB is 45 - 55 % whereas in substance AC it is 30 to 40 %. The possible concentration difference of up to 25 % needs to be addressed. The possible concentration variation of C in substance AC may also be relevant for the prediction.

#### 5.3.4 Prediction from a multi-constituent substance to a multi-constituent substance (constituents are members of two different pools)

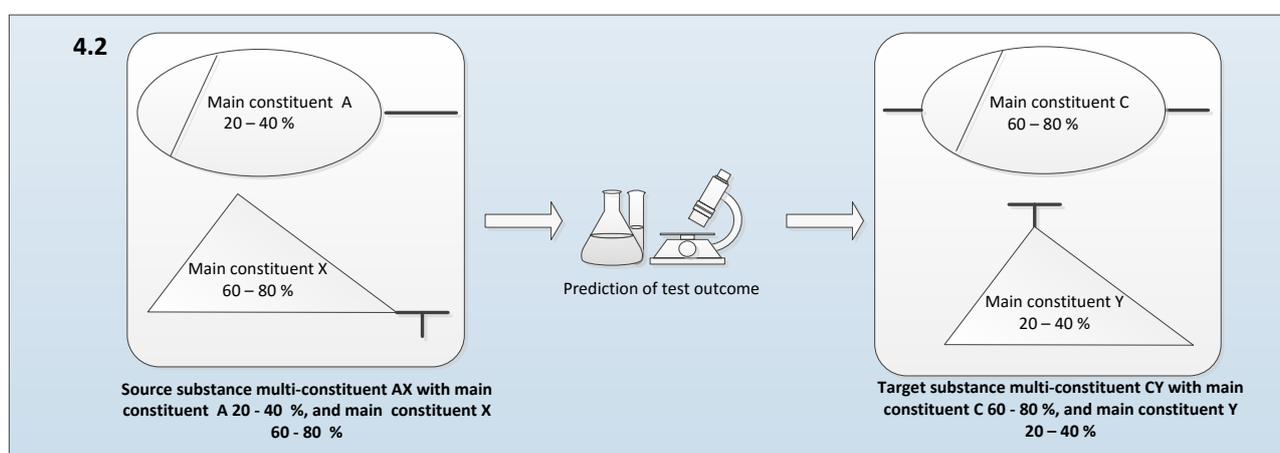


Figure 4.2 illustrates a situation where a test result obtained with a multi-constituent substance containing the main constituents A at 20 - 40 % and X at 60 - 80 % is used to predict the outcome of such test for another multi-constituent substance containing the main constituent C at 60 - 80 % and the main constituent Y at 20 - 40 %. Data from Guideline studies conducted with substance AX are available for read-across to substance CY.

In this model case, there are two different pools of core structures involved, the ellipse and the triangle. The triangles have the functional group "T-Line", the ellipse the functional group "Line". The ellipse pool and the triangle pool have structural similarity within the pool, but between the pools, there is no structural similarity. Structural similarity of the constituents within the defined pools as a basis for the grouping may be assumed.

The test result of substance AX reflects the effects caused by the combined exposure to both constituents A and X. All possible toxicokinetic and toxicodynamic interactions are inherently reflected in the test result. The test result does not discriminate the specific contribution of constituents A and X on the type of effect observed or on their individual potency or on their possible interaction on the toxicokinetic or toxicodynamic level.

If substance CY would be tested, a similar description would apply. Test results would reflect the effects caused by the combined exposure to both constituents C and Y. All possible toxicokinetic and toxicodynamic interactions would be inherently reflected in the test result. The test result would not discriminate the specific contribution of constituents C and Y on the type of effect observed or on their individual potency or on their possible interaction on the toxicokinetic or toxicodynamic level.

This is a model case of a “substance-based” approach (see 3.3.3), since the source data for the prediction come from testing a whole substance with several constituents. In this model case, the target substance also has several constituents. This approach needs additional considerations in comparison with predictions based on read-across between mono-constituent substances.

### Critical issues

Lacking information on whether A or X or both cause the observed toxicity in the test with substance AX prevents a scientifically-sound prediction for substance CY. Constituent C in the target substance is structurally similar to constituent A in the source substance. Constituent Y in the target substance is structurally similar to constituent X in the source substance.

In analogy to 4.1 (a – e) there is a need for additional information<sup>19</sup> that establishes which of the situations are applicable. Each situation has a different outcome for the prediction in terms of type of effect and/or strength of effect. If there is an absence of information on the influence of A and X on each other’s toxicity (interaction or no interaction), then the test results obtained with substance AX do not allow for a prediction of substance CY, in particular since it is also not known whether C and Y interact with each other.

In contrast to the previous model case 4.1, in this model case 4.2 there are two pools of core structures involved (ellipse and triangle) with different functional groups (line and t-line) and therefore two mechanistic explanations are needed why a prediction is possible. One mechanistic explanation, which addresses constituent A versus constituent C and one which addresses X versus constituent Y. Such explanations require sufficient knowledge of the toxicodynamic and toxicokinetic properties to justify the prediction of the properties of C and Y from A and X, respectively. The RAAF needs to be applied to assess the presented explanations.

In addition, the possible interactions arising from combined exposure, and the consequent impact on the prediction, need to be assessed for the pair A and X and the pair C and Y.

The third issue to be addressed is the difference in proportions. Whereas a member of the pool ellipse is present in 20 – 40 % in substance AX, another member of the pool ellipse is present in substance CY in 60 – 80 %. For X and Y, it is the other way around. The impact of this variation in proportion on the attempted predictions needs to be analysed to come to credible conclusions on the prediction.

In this model case, a new issue becomes evident compared to the previous cases. There are two pools with different core structures and different functional groups involved: besides the expected toxicodynamic differences, the constituents of these different pools may well have different toxicokinetic characteristics. This may lead to a situation that due to differences in uptake, distribution, metabolism and/or excretion, the systemically available concentration-relations for members of the two pools may considerably differ from the original concentrations-relations of the constituents in the substances. Different metabolic pathways may further complicate the picture of the internal exposure.

Moreover, with repeated administration and differences in lipophilicity associated with differing bioaccumulation potential, the concentrations-relations may change over time for the individual constituents. The assessment of the explanations therefore needs to take into account such possibilities and need to clarify whether they are affecting source and target substances in the same way and what their impact on the prediction is.

### 5.3.5 Prediction from a complex multi-constituent substance to complex multi-constituent substance

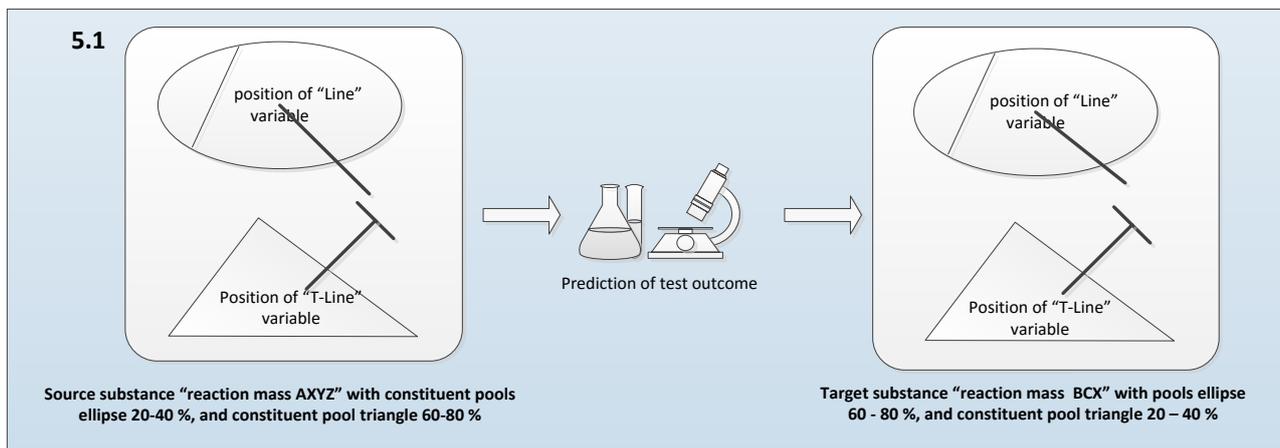


Figure 5.1 illustrates a situation with a multi-constituent substance containing several constituents from the pool ellipse at 20 - 40 % and several constituents of the pool triangle at 60 - 80 %. A test result obtained with this substance is used to predict the outcome of such a test for another multi-constituent substance containing several constituents from the pool ellipse at 60 - 80 % and several constituents from the pool triangle at 20 - 40 %. Data from Guideline studies conducted with substance XYZ are available for read-across to substance BCX.

Table 1: Compositions of substances XYZ and BCX. For structures of A to Z see Figure 1

	SUBSTANCE "REACTION MASS XYZ" [TYPICAL % (RANGE OF %)]	SUBSTANCE "REACTION MASS BCX" [TYPICAL % (RANGE OF %)]
Constituent A (ellipse)	13 % (6 - 20 %)	3 % (< 8 %)
Constituent B (ellipse)	5 % (2 - 12 %)	45 % (30 - 64 %)
Constituent C (ellipse)	9 % (2 - 12 %)	10 % (6 - 24 %)
Constituent D (ellipse)	4 % (< 5 %)	6 % (< 10 %)
Constituent X (triangle)	49 % (36 - 64 %)	26 % (16 - 36 %)
Constituent Y (triangle)	10 % (6 - 16 %)	5 % (2 - 8 %)
Constituent Z (triangle)	10 % (6 - 16 %)	5 % (2 - 8 %)

The triangles have the functional group “T-Line”, the ellipse the functional group “Line”. The ellipse pool and the triangle pool have structural similarity within the pool, but not between each other. The constituent concentrations are listed in the table above. A test result obtained with “reaction mass XYZ” containing the typical constituent concentrations provided in the table is used to predict the outcome of such a test for “reaction mass BCX”.

The test result of substance ‘reaction mass XYZ’ reflects the effects caused by the combined exposure to all constituents A, B, C, D, X, Y and Z. All possible toxicokinetic and toxicodynamic interactions are inherently reflected in the test result. The test result does not discriminate the specific contribution of the constituents on the type of effect observed or on their individual potency or on their possible interaction on the toxicokinetic or toxicodynamic level.

If substance ‘reaction mass BCX’ would be tested, a similar description would apply. Test results would reflect the effects caused by the combined exposure to all constituents A, B, C, D, X, Y and Z. All possible toxicokinetic and toxicodynamic interactions would be inherently reflected in the test result. The test result would not discriminate the specific contribution of the constituents on the type of effect observed or on their individual potency or on their possible interaction on the toxicokinetic or toxicodynamic level.

This is a model case of a “substance-based” approach (see 3.3.3), since the source data for the prediction come from testing a whole substance with several constituents. In this model case, the target substance also has several constituents. This approach needs additional considerations in comparison with predictions based on read-across between mono-constituent substances.

### Critical issues

In contrast to the model cases discussed so far, the grouping justification needs to get more attention. Although the core structures and functional groups within the ellipse and triangle pools, respectively, are the same, variability in the differences in concentrations between the members of the pools and between the pools are quite high. The assessment of the additional explanations is therefore needed which describe why the substances are regarded as structurally similar and can be grouped on the basis of the constituents’ structures and concentration variations.

Lacking information on whether one or more of the ellipse-type constituents, or one or more of the triangle-type constituents, or both types in combined action cause the observed effects in the test with substance XYZ prevents a scientifically-sound prediction for substance BCX.

The individual ellipse-type constituents are present in the source and target substance, although in different concentrations. Similarly, the individual triangle-type constituents are present in the source and target substance, also in different concentrations. Without further information it is not possible to establish which of the following situations apply:

- a) The effects observed in the source study are caused by ellipse-type constituents (or their conversion products) of substance XYZ and it is proposed that constituents X, Y and Z do not cause adverse effects in this study type (also not in the target substance) and there is no interaction between triangle-type and ellipse-type constituents (= no interaction, dissimilar action in relation to the pools).

The prediction transforms to a read-across from A, B, C and D in the source substance to the same constituents in the target substance. However, in this model case, several individual ellipse-type constituents are present in the source substance. Therefore, the question of which one causes the observed effects profile becomes relevant.

The possible interaction of the ellipse-type constituents and how these may impact the prediction also becomes relevant. Moreover, A is the major ellipse-type constituent in XYZ but still only present at a typical concentration of 12 %. In BCX, B is present at a typical concentration of 45 %. What this variation in ellipse-type constituent concentrations means in terms of predictions has to be addressed. Another aspect is that the test results with XYZ reflect a range of ellipse-type constituents between 20 – 40 %, whereas in BCX they reflect 60 – 80 %.

- b) The effects observed in the source study are caused by triangle-type constituents (or their conversion products) of substance XYZ and it is proposed that constituents A, B, C, and D do not cause adverse effects in this study type and there is no interaction between triangle-type and ellipse-type constituents (= no interaction, dissimilar action in relation to the pools).

The prediction transforms to a read-across from X, Y and Z in the source substance to the same constituents in the target substance. However, in this model case several individual triangle-type constituents are present in the source substance. Therefore, the question of which one causes the observed effects profile becomes relevant.

The possible interaction of the triangle-type constituents and how these may impact the prediction also becomes relevant. X is the major triangle-type constituent at a typical concentration of 49 % in XYZ and also the major triangle-type in BCX but at a typical concentration with a lower value (26 %). In this situation, the read-across approach from XYZ to BCX needs to address the potential interactions between the triangle-type constituents as well as the concentration differences.

- c) The effects observed in the source study are caused by constituents of both pools or their conversion products (similar or dissimilar action) and/or there is an interaction between constituents of these pools and/or among each other (= interaction possibly leading to different type of effects and/or change in strengths of effects).

It is evident that mechanistic explanations need to address the different chemical structures present, the concentration variations between the pools in the source and target substance and the possible interactions.

In model case 5.1, the constituents are defined in terms of chemical structure and concentrations and read-across justifications are possible on this basis. If reduced to the principle issue, a prediction is made from the triangle pool (with “minor” constituents from the ellipse pool) to the ellipse pool (with “minor” constituents from the triangle pool); if data on all individual pool members is not available, then this requires a “nested read-across”<sup>19</sup> between the pool members before the read-across between the substances. In addition, possible interactions need to be covered. In a way, this is a complex version of example 4.2.

Due to the further increase in composition complexity in model case 5.1 (now several pool members present instead of only one in model case 4.2), the associated explanations must also become more complex. The systemic exposure profile of parent compounds and their conversion products will probably not be clear. The identification of chemical structures causative for observed effects will therefore also not be clear. Without considerable further information<sup>19</sup> on individual constituents and/or bridging studies, the confidence in any predictions will be low.

## 5.3.6 Prediction from a UVCB to a UVCB

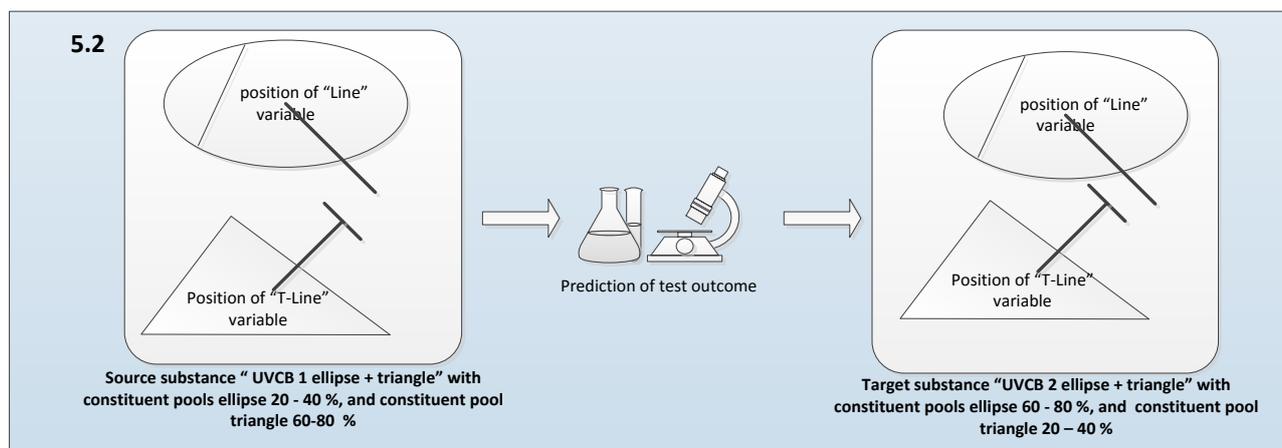


Figure 5.2 illustrates a situation where test results obtained with a UVCB containing several constituents from the pool ellipse and several constituents of the pool triangle is used to predict the outcome of such a test for another UVCB containing several constituents from the pool ellipse and several constituents from the pool triangle. Data from Guideline studies conducted with substance are available for read-across to substance UVCB2.

Table 2: Compositions of substances UVCB1 and UVCB2. For structures of A to Z see figure 1

	SUBSTANCE "UVCB 1 ELLIPSE+TRIANGLE" (RANGE OF %)	SUBSTANCE "UVCB 2 ELLIPSE+TRIANGLE" (RANGE OF %)
Constituent A (ellipse)	concentration variable, A+B = 20 - 40 %	Not present
Constituent B (ellipse)	concentration variable, A+B = 20 - 40 %	Not present
Constituent C (ellipse)	Not present	concentration variable, C + D = 60 - 80 %
Constituent D (ellipse)	Not present	concentration variable C + D = 60 - 80 %
Constituent X (triangle)	49 % (36 - 64 %)	20 % (10 - 30 %)
Constituent Y (triangle)	< 10 %	< 10 %
Constituent Z (triangle)	< 10 %	< 10 %

Figure 5.2 illustrates a situation where two different pools of core structures are involved, the ellipse and the triangle pool. The triangles have the functional group "T-Line", the ellipse the functional group "Line". The ellipse pool and the triangle pool have structural similarity within the pool, but not between the pools. Each

of these pools consist of varying concentrations of several constituents, distinguished by the positions of the functional groups. For the constituents of the pool ellipse, it is not known at which concentrations the individual members are present.

Substance UVCB1 has a total concentration of A+B of 20 – 40 % whereas for substance UVCB2 the total concentration C+D is 60 – 80 %. For the constituents of the pool triangle, the concentrations of the individual constituents are known. The resulting compositional information is listed above in the table.

### Critical issues

Although the core structures and functional groups within the pools ellipse and triangle, respectively, are the same, variability in the differences in concentrations between the members of the pools and between the pools are high. In addition, some constituents are only present in one substance but not the other. The consequences on the grouping justification need to be assessed. The assessment of the additional explanations is therefore needed which describe why the substances are regarded as structurally similar and can be grouped on the basis of the constituents' structures and concentration variations.

In terms of a proposed prediction, the constituents of the pool ellipse are known, but the concentrations of the individual constituents of the pool are not known; only the total concentration value of A+B or C+D is known.

Furthermore, A+B are not present in UVCB2 at all, whereas B+C are not present in UVCB1. With regard to A+B and C+D the prediction relies on "nested read-across". It needs to be assessed if A, B, C and D are indeed belonging to the same pool and whether and why they have a similar pattern or a predictable pattern for the property under consideration. Without further information<sup>19</sup>, it is not possible to establish which of the principle following situations apply:

- a) The effects observed in the source study are caused by ellipse-type constituents (i.e. (A or B) or (A and B) or their conversion products in the source substance). Furthermore, it is proposed that constituents X, Y and Z do not cause adverse effects in this study type (also not in the target substance) and there is no interaction between triangle-type and ellipse type constituents (= no interaction, dissimilar action in relation to the pools).

The prediction transforms to a read-across from A and B in the source substance to C and D in the target substance. However, in this model case, several individual ellipse-type constituents are present in the substances. Therefore, the question of which one causes the observed effects profile becomes relevant.

The possible interaction of the ellipse-type constituents and how these may impact the prediction becomes relevant. Moreover, the concentrations of A + B in UVCB1 and C + D in UVCB2 are only known as a sum. Furthermore, A + B only have a concentration sum of 20 – 40 % in UVCB1, but C + D have a concentration sum of 60 – 80 % in UVCB2. It has to be addressed what this variation in ellipse-type constituent concentrations means in terms of predictions.

- b) The effects observed in the source study are caused by triangle-type constituents (i.e. X or Y or Z or their combination or their conversion products in the source substance). Furthermore, it is proposed that constituents A and B do not cause adverse effects in this study type and there is no interaction between triangle-type and ellipse-type constituents (= no interaction, dissimilar action in relation to the pools).

Under the assumption that C and D also do not cause adverse effects in this study type, the prediction transforms to a read-across from X, Y and Z in the source substance to the same

constituents in the target substance. However, in this model case, several individual triangle-type constituents are present in the source substance. Therefore, the question of which one causes the observed effects profile becomes relevant.

The possible interaction of the triangle-type constituents and how these may impact the prediction also becomes relevant. X is the major triangle-type constituent at a typical concentration of 49 % in UVCB1 and also the major triangle-type in UVCB2 but at a typical concentration with a lower value (20 %). In this situation, the read-across approach from UVCB1 to UVCB2 needs to address the potential interactions between the triangle-type constituents as well as the concentration differences.

- c) The effects observed in the source study are caused by constituents or conversion products of both pools (similar or dissimilar action) and/or there is an interaction between constituents of these pools and/or among each other (= interaction possibly leading to different type of effects and/or change in strengths of effects).

It is evident that mechanistic explanations need to address the different chemical structures present, the concentration variations between the pools in source and target and the possible interactions.

Due to the further increase in composition complexity in model case 5.2 (now the concentrations of some pool members are not known, both pool ellipse members are different in the substances), the associated explanations must also become more complex.

The systemic exposure profile of parent compounds and their conversion products will probably not be clear. The identification of chemical structures causative for observed effects will therefore also not be clear. Without considerable further information<sup>19</sup> on individual constituents and/or bridging studies, the confidence in any predictions will be low.

## 6. Concluding observations

The conclusions were derived on the basis of the analysed model cases and integrate experience from evaluating real read-across cases. They are grouped into general observations, observations for structural similarity, and observations for predictions.

Although in this paper the focus was on the analysis of model cases constructed for an analogue read-across approach, the observations are applicable for categories as well.

Provided that there are good quality data, categories have the advantage of several data points for the properties under consideration and thereby potentially provide more confidence in a particular prediction.

### 6.1 GENERAL OBSERVATIONS

The general principles laid down in Annex XI, Section 1.5 to the REACH Regulation and discussed in the RAAF for mono-constituent substances are also applicable to read-across approaches involving multi-constituent substances and UVCBs.

Detailed compositional information on the source substance (composition and concentrations of the constituents) and the test material used in the conducted source studies is fundamental to establish the relation to the target substance in terms of grouping and predictions. For the assessment of such cases, the detailed information on the composition of the source substances forms the basis for the evaluation of the proposed prediction.

In comparison with (rather pure) mono-constituent substances<sup>1</sup>, multi-constituent substances and UVCBs involve more than one (sometimes many) relevant chemical structures.

Consequently, read-across approaches for such substances require additional justifications and assessments to account for the increasing complexity of the composition of the substances and its impacts on the predictions.

Additional issues that are important for predictions with multi-constituent substances and UVCBs include:

- Predicting the outcome of combined constituent exposure when
  - only data on individual constituents is available;
  - only results obtained with other combined constituent exposures are available.
- Predicting the outcome when there is variation in the concentration of constituents.

### 6.2 OBSERVATIONS FOR THE STRUCTURAL SIMILARITY OF SUBSTANCES

The prerequisite for predictions based on read-across under REACH is the grouping of substances on the basis of structural similarity (of the constituents of the substances).

In this step of a read-across approach, the structural similarities between the group members are established.

The REACH Regulation does not mention any quantitative measure of structural similarity, but lists aspects on which the similarities may be based (common functional groups, common precursors and/or common

breakdown products, constant pattern in the changing of potency of the properties).

The assessment of proposals for grouping based on the structural similarities of constituents is not conducted in isolation but in the context of the properties of the involved substances and in the context of the purpose, i.e. the prediction. In the observations described here, the structural similarity considerations are separated from the considerations on predictions to better illustrate the issues encountered.

For typical mono-constituent substances, grouping on the basis of structural similarity appears to be straightforward, since only one main constituent per substance needs to be considered (exceptions: impurity considerations). On this basis, the criteria for the group membership can be clearly defined.

For multi-constituent substances the grouping on the basis of structural similarity is more complex. In contrast to mono-constituent substances, structural similarity for more than one constituent per substance needs to be considered. Several principal situations may occur:

- It is claimed that all constituents in the source and target substances are structurally similar (e.g. all are saturated alcohols with the same carbon number, i.e. are members of one “pool” of structurally similar substances);
- It is claimed that structural similarity is based on two or more “pools” of structurally similar constituents for which the pool proportions are similar in source and target substances (e.g. pool I: saturated alcohols with increasing carbon number about 30 – 40 %, pool II: monofunctional amines with increasing carbon number about 60 – 70 %);
- It is claimed that structural similarity is based on two or more “pools” of structurally similar constituents, although the proportions of the pools of constituents are different in source and target substances. Such differences clearly require extensive explanations and justified criteria for group membership (e.g. composition of a substance called “more I than II”: 60 % of constituents belonging to pool I and 35 % belonging to pool II; composition of another substance called “more II than I”: 30 % of constituents belonging to pool I and 65 % belonging to pool II);
- It is claimed that there is structural similarity although there is only one pool of structurally similar constituents present in source and target substances and the other constituents are not structurally similar. Such grouping proposals also clearly require extensive explanations and justified criteria for group membership.

For all the situations above (and many more which are a combination of these), it needs to be assessed whether there is a basis for structural similarity. Also, the proportions of the individual pool members in the source and target substances need to be considered and the variations of the constituents’ concentrations also need attention.

For UVCBs, grouping on the basis of structural similarity may become even more complex, e.g. due to the presence of more constituents in the substances, potentially higher variations in the concentrations of the constituents and sometimes unknown constituents. Such grouping proposals also clearly require extensive explanations and justified criteria for group membership.

### 6.3 OBSERVATIONS FOR PREDICTIONS

The RAAF focuses on mechanistic explanations on why and how predictions are possible within a group. The fundamental types of mechanistic explanations are explained in the scenarios of the RAAF. Such

explanations are of course also required, if multi-constituent substances and /or UVCBs are involved in a prediction based on read-across approaches.

For multi-constituent substances and UVCBs, how the mechanistic explanation(s) cover the constituents involved needs to be assessed. This means that several mechanistic explanations may have to be assessed which simultaneously address the variety of structures present in the substances and consequently also more than one RAAF scenario may be needed to assess the case.

The assessment elements of the scenarios in the RAAF can also be applied to multi-constituent substances and UVCBs for each proposed mechanistic explanation, but additional issues are proposed to be assessed by ECHA experts. These are explained below.

Predictions may rely on a constituent-based approach (source data come from the individual constituents) or a substance approach (source data come from tests with the complete source substance). Regardless of the type of source data, consideration is needed on how the following issues were addressed and taken into account in the prediction model:

- The impact of the combined exposure to two or more constituents on the prediction. This means that the explanations need to outline whether and how the constituents influence or do not influence each other's toxicity. Such influences may be of toxicodynamic and/or toxicokinetic nature and may impact predictions for the type of effects and/or strength of effects.
- Variations in the concentrations of the structurally similar constituents (or pool of constituents) and the impact of these variations on the predicted type and the strength of effects. The variations in proportion of constituents may influence the assumed dose response of the substance. Consequently, the quantitative nature (i.e. magnitude of the effects) of the predicted effect is a further issue that has to be assessed, taking account of the precise proportion of constituents in the source substance, in relation to the precise proportion of constituents in the target substance.

The two issues explained above are hurdles for a robust read-across approach involving multi-constituent substances and/or UVCBs. It is noted that the type of supporting information presented to overcome these hurdles may vary somewhat between the approaches:

- For the constituent-based approach, the additional information on the toxicodynamic and the toxicokinetic characteristics of the individual constituents needs to be assessed. The assessment of the read-across approach needs to evaluate how the type of effects predicted has been derived on the basis of the information from the individual constituents, and in particular how possible interactions are taken into account and how the variations of the constituents' concentrations are addressed. The prediction model used for quantitative predictions needs scrutiny as well.
- For the substance-based approach, the toxicodynamic and toxicokinetic characteristics of individual components may not be known specifically from the test conducted with the source substance that is proposed to be used for the prediction. They are already reflected in the test results obtained with the test material representing the source substance. The test results obtained with a test material containing several constituents do not provide information on the individual contribution of the constituents to the observed toxicity or their possible interactions. The assessment of the read-across approach needs to evaluate what further information is presented by bridging studies and/or mechanistic explanations to explain why and how the results from the source substance are used to predict the properties of the target substance taking into account also possible interaction between constituents in the target substance.

Bridging studies are comparable studies on the source and target substance, and these bridging studies allow side-by-side comparison of the substances for a particular property (e.g. properties as determined in a 90-day study). Bridging studies may enable the demonstration that two multi-constituent substances or UVCBs have similar properties for a particular endpoint, and thus play a key role in a read-across justification. In the absence of such an empirical demonstration, read across may be difficult to justify for complex compositions.

ECHA has assessed and also accepted predictions based on read-across adaptations involving substances with complex compositions. Prerequisites are scientifically credible explanations and sufficient supporting information (mechanistic data as well as bridging studies).

## 7. Summary

This document describes the additional key issues proposed to be considered by ECHA experts when predictions based on read-across cases involving multi-constituent substances and/or UVCBs are used to adapt standard information requirements.

All chemical structures involved need to be considered; grouping of substances on the basis of structural similarity must take account of all constituents, and the predictions within proposed groups must likewise consider the impact of all constituents.

The analysis described in this document confirmed the complexity of read-across approaches for multi-constituent substances and UVCBs.

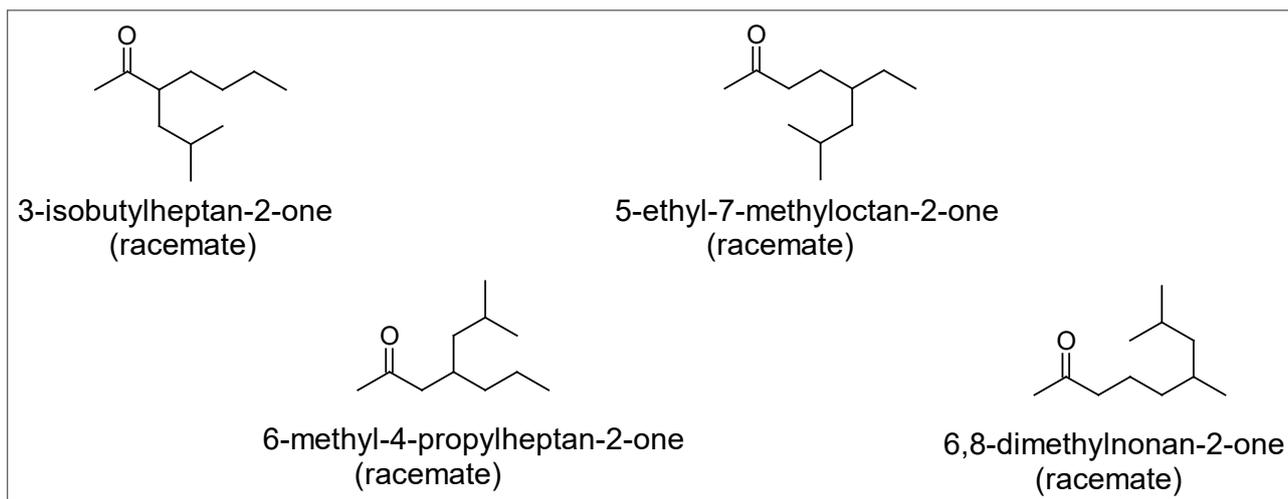
More work is needed to further develop the RAAF based on the findings described in this document.

## Appendix

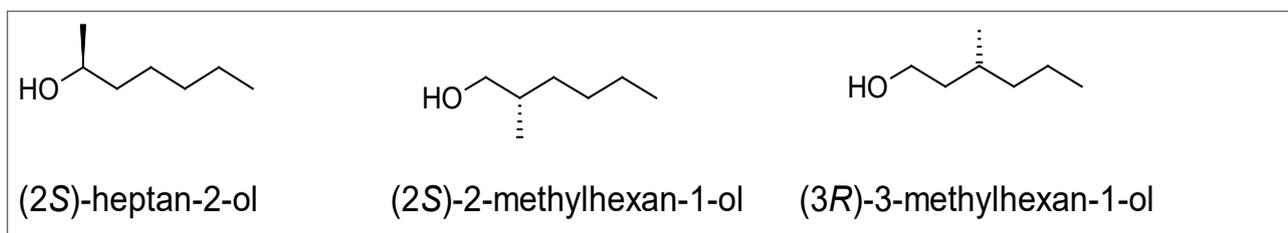
### POOLS

**Illustrative examples** for groups of substances, which may be regarded as “pools” of structurally similar substances/constituents (without analysis on similar properties)

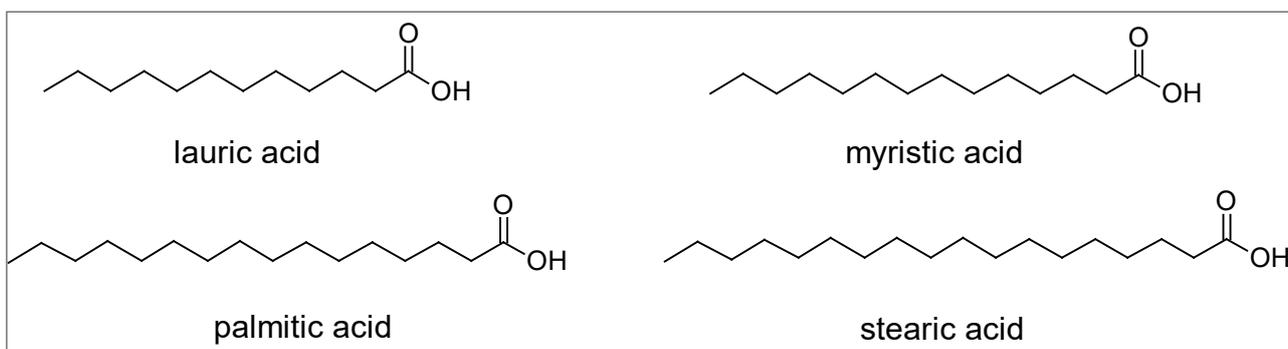
#### POOL A



#### POOL B



#### POOL C



## TERMS AND DEFINITIONS

TERM	DEFINITION	SOURCE
Additive	A substance that has been intentionally added to stabilise a substance.	ECHA guidance <sup>22</sup>
Analogue approach	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 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.	RAAF
Antagonism	Occurs when the effect of the mixture is less than estimated for additivity on the basis of the toxicities of the individual constituents.	EFSA5
Applicability domain	The set of inclusion/exclusion rules that identify the ranges of values within which a reliable prediction can be made for category members.	RAAF
(Bio)transformation	A series of chemical changes in a compound as a result of enzymatic or other activity in a living organism.	RAAF
Bridging study	Studies on the source and target substance which allow side-by-side comparison of the toxicity profile between the substances for the property under consideration. Example: OECD 407 studies are available for both, source and target substances. The results are supporting the prediction for a 90-day study (OECD 408) which is based on read-across from a study conducted with the source substance.	This document
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).	RAAF
Category definition	Includes a category hypothesis, description of the applicability domain of the category and details on the identity and purity/impurity profiles of the category members.	RAAF

<sup>22</sup> Guidance for identification and naming of substances under REACH and CLP. December 2016. Version 2.0

TERM	DEFINITION	SOURCE
Category hypothesis	Explanation as to why property(ies) of category members may be predicted from reference substances within the category. This explanation must be based on a relationship between structural similarity and the predicted property(ies).	RAAF
Category justification	Reasoning and associated supporting evidence that are provided to verify the scientific validity and robustness of the category hypothesis.	RAAF
Component	Substance intentionally added to form a mixture.	ECHA guidance <sup>22</sup>
Constituent	Any single species present in a substance that can be characterised by its unique chemical identity.	
	ECHA Guidance <sup>22</sup>	
Data matrix	A table that summarises all available study results of the source and target substances per REACH information requirement/ endpoint and including planned studies. The data should be arranged to reflect the regular pattern identified and used in the prediction. The IUCLID dossier should contain (robust) study summaries of each study referred to in the data matrix to allow an independent assessment of the data.	RAAF
Dissimilar action	<p>Constituents act independently from each other, usually through different modes of action that do not influence each other, or at different target cells, tissues or organs.</p> <p>The effects can be estimated directly from the probability of responses to the individual constituents (response addition) or the sum of biological responses (effects addition).</p> <p>If an individual constituent is below its individual NOEL/NOAEC (and these values represent true zero-effects levels), it will not add to the joint effects caused by the combined exposure.</p> <p>Synonym: independent action.</p>	SCHER, SCENHIR, SCCS <sup>4</sup>
Group	Under REACH, substances that are structurally similar with physicochemical, toxicological, ecotoxicological and/or environmental fate properties that are likely to be similar or to follow a regular pattern may be considered as a group of substances. Within a group of substances, a data gap might be filled by read-across, as described below.	RAAF

TERM	DEFINITION	SOURCE
Impurity	<p>An unintended constituent present in a substance, as produced. It may originate from the starting materials or be the result of secondary or incomplete reactions during the production process. While impurities are present in the final substance, they were not intentionally added.</p> <p>All constituents (except additives) which are not the main constituent(s) in the mono-constituent substance or a multi-constituent substance are considered to be impurities.</p> <p>For UVCBs, the concept of impurities is not applicable.</p>	ECHA guidance <sup>22</sup>
Inhibition	Inhibition occurs when a constituent that does not have a toxic effect on a biological system decreases the apparent effect of a second constituent on that system.	EFSA <sup>5</sup>
Interaction	The combined effect of two or more chemicals as stronger (synergistic, potentiating, supra-additive) or weaker (antagonistic, inhibitive, subadditive, infra-additive) than would be expected on the basis of dose/concentration addition or response addition.	SCHER, SCENHIR, SCCS <sup>4</sup>
Main constituent	A constituent, not being an additive or impurity, in a substance that makes up a significant part of that substance and is therefore used in substance naming and detailed substance identification.	ECHA guidance <sup>22</sup>
Masking	Occurs when constituents produce opposite or functionally competing effects on the same biological system and diminish the effects of each other, or one overrides the effect of the other.	EFSA <sup>5</sup>
Mixtures	Intentional mixtures of substances obtained by blending two or more substances without a chemical reaction and are consequently not to be considered as multi-constituent substances.	ECHA guidance <sup>22</sup>
Mono-constituent substance:	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 (i.e. unintended constituents present in a substance as produced which are not intentionally added).	ECHA guidance <sup>22</sup>
Multi-constituent substance	A substance consisting of several main constituents present at concentrations generally $\geq 10\%$ and $< 80\%$ (w/w) (a multi-constituent substance is the result of a manufacturing process. Multi-constituent substances usually also have impurities, i.e. unintended constituents present in a substance as produced which are not intentionally added).	ECHA guidance <sup>22</sup>
Potentiation	Occurs when a constituent that itself does not have a toxic effect on a biological system increases the effect of a second constituent on that system.	EFSA <sup>5</sup>

TERM	DEFINITION	SOURCE
Prediction	In the context of read-across, the property of target substance(s) is estimated from the property of source substance(s). The prediction may be made by means of read-across or by observation of a regular pattern.	RAAF
Prediction of absence of effect(s)	The situation where no effects have been observed in a source study and this result, i.e. absence of effect(s), is read-across to a target substance. This situation is also often referred to as "negative read-across".	RAAF
Property	Refers to inherent characteristics of a substance which can be studied in a defined experimental study type. These characteristics may relate to physico-chemical or toxicological aspects. The properties of a substance can be determined from the results of experimental studies.	RAAF
REACH	Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals	RAAF
Read-across	Under REACH, a technique for predicting endpoint information for one substance (target substance), by using data from the same endpoint from (an)other substance(s), (source substance(s)). Consequently, the read-across approach has to be considered as property specific.	RAAF
Read-across approach	Can be either an analogue or category approach, composed of elements addressing the structural similarity, a read-across hypothesis, a read-across justification and the prediction of property(ies) of the target substance(s).	RAAF
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.	RAAF
Read-across justification	The reasoning and associated supporting evidence provided to verify the scientific validity and robustness of the read-across hypothesis.	RAAF
Regular pattern	Refers to the observation of regular behaviour in a property among the category members. This can consist of no observed differences in a property across the category or in a regular change in that property across the category.	RAAF
Similar action	Constituents act through the same mode of action and/or at the same target cell or organ, and may differ only by their potency. The effects can be estimated directly from the sum of the doses/concentrations. In principle, doses or concentrations of the individual constituents are added after being multiplied by a scaling factor that accounts for differences in the potency of the individual constituents.  Synonym: dose/concentration addition, similar joint action.	SCHER, SCENHIR, SCCS <sup>4</sup>

TERM	DEFINITION	SOURCE
Supporting evidence	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.	RAAF
Synergism	Occurs when the effect resulting from the combined exposure to constituents is greater than estimated for additivity on the basis of the toxicities of the constituents.	EFSA <sup>5</sup>
Test material/test substance	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.	RAAF
Toxicodynamic interactions	Interactions between the biological responses resulting from the exposure to individual constituents, for instance, resulting from similar targets (ligand-receptor interactions).	SCHER, SCENHIR, SCCS <sup>4</sup>
Toxicokinetic interactions	Constituents modify the absorptions of other constituents, the active transport mechanism of other constituents (uptake, clearance), or the metabolism of other constituents.	SCHER, SCENHIR, SCCS <sup>4</sup>
UVCB	<p>Substances of unknown or variable composition, complex reaction products or biological materials. UVCBs cannot be sufficiently identified by their chemical composition, because:</p> <ul style="list-style-type: none"> <li>- the number of constituents is relatively large; and/or</li> <li>- the composition is, to a significant part, unknown; and/or</li> <li>- the variability of composition is relatively large or poorly predictable.</li> </ul> <p>For a UVCB substance, all known constituents and all constituents present at concentrations <math>\geq 10\%</math> should be specified by at least an English-language IUPAC name and preferably a CAS number; the typical concentrations and concentrations ranges of the known constituents should be given as well.</p>	ECHA guidance <sup>22</sup>
Well-defined substances	Substances with defined qualitative and quantitative composition that can be sufficiently identified based on the identification parameters of REACH Annex VI section 2.	ECHA guidance <sup>22</sup>
Worst-case approach	The strength of effect(s) in the target substance is actually expected to be lower than the strength of effect(s) observed for the source substance; hence, using the value obtained from the source substance, the prediction constitutes a worst-case approach that will not lead to an underestimation of the effects that would be observed in a study with the target substance if it were to be conducted.	RAAF

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