Summary of the EFSA Scientific Opinion on the Guidance on the use of Read-across for Chemical Safety Assessment in Food and Feed

Stepwise read-across guidance

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8. Figure 2 in EFSA's opinion illustrates the read-across workflow, while Table 2 summarises each step.

9. EFSA recommends organising the data collected for both the target and the source substances in a matrix. This data matrix should be structured in a

tabular format and information should be arranged in a suitable order to facilitate the integration of the supporting evidence into the read-across process (examples are given in Appendix A).

Step 1: Problem formulation

10. A read-across assessment generally begins when the target substance lacks data—or has unreliable data—for the endpoint of interest. According to EFSA's guidance, the problem formulation step sets the purpose and expectations of the assessment and outlines the available approaches to achieve the objective. These considerations must be framed within the relevant regulatory context and the specific endpoints being addressed.

11. The problem formulation also involves defining the level of uncertainty that can be tolerated for the read-across to be considered acceptable, in light of its contribution to the overall weight of evidence (WoE). It includes identifying data gaps for the endpoint in question and evaluating whether there is sufficient justification for the read-across. This step will also inform potential future strategies to reduce uncertainty (see *Step 6: uncertainty assessment*).

Step 2: Target substance characterisation

12. EFSA's opinion indicates that this step should unambiguously identify the target substance, as well as consider the hazard information and the data gaps that need to be addressed. The characterisation of the target substance forms the basis for the read-across hypothesis and informs the selection of an initial set of source substances.

13. At this stage, all available information should be collected, including physicochemical properties, metabolic transformation, toxicokinetic and toxicodynamic aspects, as well as *in vivo*, *in vitro* and *in silico* predictions, and any structural alerts for the target substance. While EFSA does not prescribe specific requirements or limitations for these data, the guidance emphasises that the information should be aligned with the needs identified during problem formulation.

14. EFSA also recommends consulting multiple data sources and, where appropriate, gather evidence through a systematic review. This process should adhere to general principles for the adequacy of data.

Step 3: Source substance identification

15. EFSA refers to this step as the process of searching for candidate source substances that are similar to the target substance. The overarching similarity rationale should be stated and justified within the read-across and will dictate how the search for a source substance will be conducted.

16. Structural and chemical similarity provides the starting point and is at the basis of all the other phenomena. It can be measured in various ways, offering different numerical results. However, the guidance states that additional relevant data must also be carefully considered, such as:

- Physicochemical characteristics, e.g. structural alerts, stability and chemical reactivity or conformation in space that might impact active-site binding.
- Endpoint under consideration and the mechanism of action (MoA) of the target substance, if known.
- Toxicokinetic profile.
- Metabolic profile, e.g. generation of common metabolites between target and source substance or when the target substance is a metabolite of the source substance, and thus, similar biological properties may be expected.
- Breakdown products not associated with metabolism, e.g. generation of common breakdown products or when the source substance is a breakdown product of the target substance. The latter assumes that the toxicity data on the breakdown product would be expected to be representative of the toxicity expected from the parent substance.
- Manufacturing process.

17. The identification of a source substance can follow a supervised and or an unsupervised search method.

18. In a supervised approach, similar source substances with the same MoA as the target substance are filtered. EFSA’s guidance recommends proposing a conceptual scheme that outlines the hierarchical sequence of events leading to the observed effect in the target substance. This scheme should then be compared to determine whether the same sequence applies to the source substance.

19. In the absence of information on the MoA underlying the adverse effect, an unsupervised approach should be applied. This involves using all similarity metrics described in paragraph 16. However, EFSA notes that this method carries greater uncertainty regarding the relevance of the specific features used to

establish similarity. Therefore, multiple similarity metrics should be applied to strengthen the justification. The unsupervised approach may also be employed to predict non-specific toxicity or the absence of toxicity, though it typically requires extensive supporting evidence.

Step 4: Source substance evaluation

20. EFSA notes that Step 4 is the formal process to identify and justify those analogues that are most similar to the target substance in terms of the metrics described in paragraph 16. Data and other information relevant to the endpoint being read across should be prioritised.

21. This step can be performed manually by an expert or by using an *in silico* system (examples can be found in Appendix A, Table A.1). Expert evaluation should consider the reliability and relevance of the data associated with the selected source substances and whether the data were obtained by systematic techniques. *In silico* and *in vitro* methods can also be used in this step to generate new supporting information, confirm the suitability of selected source substances, further characterise potency trends across analogues, or to support the case for excluding certain source substances.

22. Differences between the target and the source substances could result in changing the read-across hypothesis based on the assembled data. Thus, Step 4 might require several iterations, and/or refinement of the read-across strategy.

Step 5: Data gap filling

23. EFSA’s guidance recommends implementing a strategy for filling data gaps in the data matrix to support read-across and enable prediction of the target substance's endpoint(s) of interest.

24. Different strategies can be applied, either data-driven (e.g. similarity weighted averages, closest neighbour based on similarity) or expert-driven, although EFSA considers that the preferable option is a data-driven approach with the least contribution of expert judgement, since the latter could introduce additional uncertainty factors.

25. Finalising the data matrix and deciding on a data gap filling strategy will indicate whether the data available on the analogues are sufficient to support a conclusion based on the read-across results, or whether at this point additional

data need to be retrieved/generated before continuing.

Step 6: Uncertainty assessment

26. EFSA explains that the primary purpose of this step is to determine whether the read-across is scientifically robust and fit for purpose, or if further data or refinement are needed to reduce uncertainty to a tolerable level. The process consists in characterising the level of uncertainty at each step of the read-across process and assess whether it remains within tolerable limits, defined during problem formulation. The process for the assessment of uncertainty in a read-across is summarised in Figure 3 of the Scientific Opinion.

27. The uncertainty assessment can be conducted either qualitatively (i.e. through narrative descriptions) or quantitatively (i.e. using probabilistic or semi-quantitative methods). It is expected that the uncertainties will be documented in the read-across report using an appropriate template (an example is provided in Appendix C, based on the semi-quantitative method proposed by Pestana *et al.* (2021)).

28. Each step of the read-across workflow carries inherent uncertainties and these should be characterised. EFSA recommends that the assessor characterises the uncertainties at each step as they progress through the assessment. The primary sources of uncertainty associated with these steps are summarised in Table 4 of the guidance. Briefly, in Step 1, it is key to capture an acceptable level of uncertainty, whereas in Step 2 different levels of uncertainty are tolerated based on the specific regulatory context. In Step 3, the impact of the choice of structural representation used to conduct the searches should be considered, and whether this can be systematically evaluated. Three uncertainty sources should be considered in Step 4, i.e. the strength of the similarity rationale and the quality of supporting data, integration of multiple lines of evidence and inclusion of transparent and scientifically justified expert judgement. In Step 5, greater uncertainty in the overall assessment may arise when the data matrix is sparse, either due to few endpoints being filled or because the target substance lacks most endpoint data. In contrast, uncertainty is reduced when the relevant data gaps for both source and target substances have already been addressed.

29. The overall characterisation of uncertainty, which considers all aspects of uncertainty and requires expert judgement to reach a final evaluation, should be performed with reference to EFSA's Guidance on Uncertainty (EFSA SC, 2018). If all steps and criteria indicate low uncertainty, the overall impact may be

considered low. In such a case, a narrative account of any residual uncertainties should be sufficient to justify the validity of the read-across. Conversely, if appropriate procedures have not been followed at one or more steps, the overall uncertainty may range from moderate to high.

30. When uncertainty is too high for a read-across to be deemed fit for purpose, it should be further evaluated—either through more detailed uncertainty analysis (e.g. semi-quantitative evaluation, quantitative statistical analysis) or by incorporating additional lines of evidence (NAMs). If uncertainty remains high despite these improvements the read-across approach may not be feasible, and experimental testing of the target substance may be necessary.

31. It is worth noting that the uncertainty discussed in EFSA’s guidance pertains to hazard assessment. In contrast, risk assessment may tolerate moderate or high levels of uncertainty, depending on other lines of evidence and/or the application of additional uncertainty factors.

32. EFSA highlights the importance of applying standardised procedures throughout the read-across workflow to minimise uncertainty and improve regulatory acceptance. When each step is conducted using recognised methods—such as OECD testing guidelines or studies performed under Good Laboratory Practice (GLP)—the overall uncertainty is considered low. In contrast, reliance on non-guideline studies, non-GLP data, or NAMs without sufficient documentation can increase uncertainty.

33. Overall, ensuring unambiguous identification and detailed characterisation of both target and source substances is critical. Using transparent, reproducible, and scientifically justified procedures throughout the read-across process helps maintain robustness and regulatory confidence, especially when uncertainty needs to be kept within acceptable limits.