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TOX/2025/42

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT)

Summary of the European Food Safety Authority's Scientific Opinion on the Guidance on the use of Read-across for Chemical Safety Assessment in Food and Feed

Introduction

1. The European Food Safety Authority (EFSA) Scientific Committee (SC) have recently published a Scientific Opinion on the guidance on the use of read-across for chemical safety assessment in food and feed (EFSA SC, 2025). The document briefly reviews existing frameworks on read-across from organisations such as the European Chemicals Agency (ECHA) and the Organisation for Economic Cooperation and Development (OECD). The guidance goes on to describe a structured workflow to standardise and justify the read-across approach as a non-animal testing method for filling data gaps in chemical safety assessments, along with a discussion on the applicability domain and characterisation of the boundaries for read-across. The opinion also includes a series of appendices on read-across processes (Appendix A), information on available *in vitro* methods for toxicological characterisation of chemical substances (Appendix B), an uncertainty assessment template (Appendix C), case study examples (Appendix D) and a glossary of relevant terms and definitions (Appendix E).

2. This paper provides a brief overview of the EFSA Scientific Opinion. Its purpose is to support discussion around the guidance developed by EFSA. Members are invited to review the Scientific Opinion and share any comments or feedback

they may have, with a view to determining whether they would be content to use the guidance.

Background

3. Read-across is a method used in chemical risk assessment for screening, classification, prioritisation and hazard assessment of data-poor target substances based on toxicological data from one or more data-rich source substances that are structurally and mechanistically similar. It is one of the most common alternatives to animal testing.

4. The read-across prediction can be applied through two ways of chemical groupings, known as the analogue and category approaches. An analogue approach compares the properties of a target substance with a limited number of closely related source substances, whereas a category approach is based on the premise that structural similarity among several source substances can predict the target substance's properties.

5. Read-across involves a number of steps (i.e. problem formulation, data gap analysis, source substance identification and evaluation, data gap filling and uncertainty assessment), each of which may carry a certain level of uncertainty. Therefore, it needs to be carried out in as transparent, standardised and unbiased a manner as possible to make the overall conclusions scientifically justified and reliable.

6. A number of read-across frameworks have been proposed, such as the OECD’s Guidance on Grouping of Chemicals (OECD, 2014), and ECHA's Read-Across Assessment Framework (ECHA, 2017). Moreover, EFSA has previously considered the use of read-across in specific risk assessments, including those for smoke flavourings, feed additives, and pesticide active substances, and has drawn on related guidance documents that refer to its application (EFSA FAF Panel, 2021; EFSA SC, 2019 and ECHA/EFSA, 2023). However, these documents mention read-across only as a supporting line of evidence, without offering detailed guidance on its

implementation. Therefore, EFSA argues that a generic and flexible framework, together with a standardised workflow that provide a scientific basis for the use of read-across for applicants and risk assessors is needed.

7. The present EFSA guidance outlines a stepwise approach for applying read-across to fill data gaps in the chemical safety assessments of individual substances in food and feed. The guidance also explains how to integrate different types of New Approach Methodologies (NAMs) data at relevant steps to support the read-across. In addition, it provides guidance on performing a thorough analysis of the uncertainties pertaining to each step of the read-across and assessing the overall uncertainty, along with a discussion on the applicability domain of read-across.

Stepwise read-across guidance

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.

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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.

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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.

Applicability domain of read-across

34. The applicability domain of a method refers to the chemical, biological, or functional space where its predictions or measurements are considered reliable. For read-across, EFSA highlights that defining this domain means identifying suitable similar substances.

35. A clearly defined applicability domain is especially important for category-based read-across, which relies on patterns across multiple substances within a category. This allows the same prediction to apply to several target substances, provided they meet the category criteria. In contrast, analogue-based read-across is more limited, as its outcome applies only to the specific target substance unless another substance is very closely related.

36. After identifying similar substances, filters are often applied to refine the selection. These filters help define the boundaries of the read-across and should be explicitly stated. The domain is more clearly established when data interpolation is used.

37. EFSA advises using multiple source substances to strengthen read-across, as this increases the number of matching features and expands the applicability domain. If only one source is used, it must be highly similar to the target, with minimal differences that could affect the outcome.

38. Read-across is always endpoint-specific. Therefore, the applicability domain depends on the availability and density of chemical and biological data for the specific toxicological endpoint.

Conclusions

39. The concept of read-across is based on the principle that structurally or mechanistically similar molecules tend to exhibit similar properties. This methodology involves identifying data-rich source substances that closely resemble a data-poor target substance and using their toxicological data to estimate the potential toxicity of the target.

40. EFSA’s guidance provides a structured framework for applying read-across to assess the toxicological hazard of a chemical substance, supporting safety evaluations within the food and feed chain. However, EFSA emphasises that read-across is not a substitute for a full risk assessment. Instead, it serves as a supporting line of evidence within hazard assessment, which itself is a key component of risk assessment. Like other structure-activity relationship-based approaches, read-across alone is generally insufficient for regulatory conclusions—particularly when asserting the absence of toxicity for a specific endpoint. Such evidence is more robust when integrated into a WoE analysis alongside other data sources.

41. The guidance outlines the key steps in conducting a read-across: problem formulation, target substance characterisation, source substance identification and

evaluation, data gap filling, and uncertainty analysis. Each step may introduce varying degrees of uncertainty, which collectively determine the overall uncertainty level—low, moderate, or high. Whether this level is acceptable depends on the context of the risk assessment. Therefore, the risk assessor must define a tolerable level of uncertainty during the initial problem formulation. The guidance also addresses strategies to manage and minimise uncertainty throughout the process.

42. While expert judgement is necessary at certain stages, EFSA stresses that read-across must be conducted transparently, using standardised and unbiased procedures. Scientific justification should be clearly provided to support the overall conclusions.

Questions for the Committee

43. Do Members have any comments on:

a) The structured workflow to standardise and justify the read-across approach.

b) Any other comments on this scientific opinion.

c) Would they be content to use this guidance?

Secretariat

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Abbreviations

(Q)SAR	(Quantitative) Structure–Activity Relationship
ADME	Absorption, Distribution, Metabolism and Excretion
AOP	Adverse Outcome Pathway
CI	Mitochondrial Complex I
CIII	Mitochondrial Complex III
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
DART	Developmental and Reproductive Toxicity
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
FAF	Food Additives and Flavourings
GLP	Good Laboratory Practice
IATA	Integrated Approaches to Testing and Assessment
KE	Key Event
LogP	Partition Coefficient Logarithm
MHA	2-Methylhexanoic Acid
MoA	Mechanism of Action
NAMs	New Approach Methodologies
N-NA	N-Nitrosamine
OECD	Organisation for Economic Cooperation and Development
SC	Scientific Committee
WoE	Weight of Evidence

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Annex A to TOX/2025/42

Summary of read-across case studies selected by EFSA

1. EFSA have drawn a total of six examples from historical assessments that informed the development of their guidance on the use of read-across for chemical safety assessment in food and feed. EFSA notes that these case studies should not be viewed or used as models for applying the guidance; instead, future practice should follow the methodology described in the Scientific Opinion. A summary of these examples is provided below.

2. The first three cases are directly related to EFSA activity (e.g. outsourced projects that specifically addressed the applicability and performance of read-across, by exploring different strategies using rich data from pesticide active substances and their metabolites), while the subsequent three cases were developed within the OECD integrated approaches to testing and assessment (IATA) project.

Case study 1: In vitro genotoxicity of pesticide metabolites

3. Although comprehensive toxicological dossiers are typically developed for pesticide active substances, data on the toxicological properties of their metabolites are often limited or absent. To address this, EFSA has recommended using (quantitative) structure–activity relationships ((Q)SARs) and read-across methods to assess the genotoxic potential of all metabolites as an initial step in defining residue levels for risk assessment (Benigni *et al.*, 2020).

4. Two read-across strategies were proposed and evaluated for their ability to predict *in vitro* Ames mutagenicity and chromosomal aberrations:

- Approach 1: Assessed similarity between a metabolite and its parent pesticide using three parameters: molecular weight, partition coefficient (logP), and structural similarity (dice/atom-centered).

- Approach 2: Applied a decision theory framework (Dempster–Shafer theory) to integrate multiple evidence sources supporting a MoA hypothesis. This included evaluating biological similarity between parent substances and metabolites based on pesticidal MoA, coded via substructural motifs. Chemical structure, physicochemical properties, and metabolic reactivity were also considered. Metabolic similarity was quantified by comparing shared potential metabolic reaction sites.

5. Both strategies showed strong predictive performance for Ames mutagenicity. However, predictions for chromosomal aberrations were less reliable, likely due to limited data quality and the small size of the chromosomal aberration dataset.

Case study 2: Carcinogenicity of N-nitrosamines

6. EFSA recently applied read-across to address data gaps in evaluating the carcinogenic risk of N-nitrosamines (N-NAs) found in food (EFSA CONTAM Panel, 2023). N-NAs are typically metabolised via α-hydroxylation, forming diazonium ions that can create DNA adducts—leading to mutations and potentially initiating carcinogenesis. The factors influencing this reactivity are well understood.

7. Analogue identification and evaluation were guided by mechanistic and structural insights. Available data on mutagenicity, metabolism, and DNA adduct formation supported the assessment. Dice similarity calculations also helped confirm suitable source substances.

8. Using read-across and trend analysis, EFSA successfully predicted the carcinogenic activity and potency of 18 N-NAs lacking direct data.

Case study 3: Repeated dose toxicity of pesticides

9. Irwan *et al.* (2024) developed a modular read-across assessment framework that integrates chemical and mechanistic data along with observed metabolites.

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10. To evaluate the framework, a data-rich class of pesticides was used. The modular approach was applied to identify source substances related to repeat-dose toxicity—specifically liver toxicity, developmental toxicity, and general systemic toxicity. The added value of incorporating *in vivo* absorption, distribution, metabolism and excretion (ADME) data was also explored. Modules were tested in three different combinations:

- Combining chemical and mechanistic similarity proved most effective, especially when the target substance's MoA was known. However, demonstrating mechanistic similarity depends heavily on data availability, and low data density can serve as an indicator of uncertainty.

- Initiating source substance identification using NAM data alone was overly broad, generating an unmanageable number of candidates.

- Incorporating common metabolites efficiently narrowed down the pool of source substances to the most relevant ones. However, some potentially relevant substances were missed due to data gaps.

Case study 4: Parkinsonian hazard liability of deguelin

11. Deguelin, a naturally occurring rotenoid from Fabaceae plants, has been shown to induce Parkinson-like symptoms in rats. Its potential to cause similar effects in humans remains uncertain, making it the target substance in this IATA case study (OECD, 2020a).

12. Epidemiological data suggest a statistically significant association between occupational exposure to rotenone (another rotenoid) and increased Parkinson's disease incidence. Rotenone was therefore selected as the source substance for the read-across approach. An established adverse outcome pathway (AOP) for rotenone indicates that inhibition of mitochondrial complex I (CI) in nigrostriatal neurons leads to parkinsonian motor deficits. This read-across analysis hypothesised that deguelin elicits similar biological interactions and activates key events (KEs) within the AOP

as rotenone, albeit with differing potency. The testing strategy included both *in silico* and *in vitro* assays.

13. *In silico* results showed high structural similarity and shared pharmacophores between rotenone and deguelin. Both exhibited comparable metabolism and toxicokinetics *in vitro* and *in vivo*, and they both inhibit CI and trigger mitochondrial dysfunction. Overall, the read-across confirmed that deguelin shares rotenone's mode of action but with reduced potency.

Case study 5: Potential neurotoxicity of azoxystrobin and other strobilurins

14. Synthetic strobilurins are fungicides that act by binding to the quinol oxidation site of cytochrome b in mitochondrial complex III (CIII). *In vitro* studies have indicated potential neurotoxicity via a CIII-mediated mechanism. This study aimed to assess the potential CIII-mediated neurotoxicity of azoxystrobin using NAMs through a read-across approach (OECD, 2020b).

15. Source substances included other strobilurin fungicides with comparable chemical structures, pesticidal MoA, toxicophores, neurotoxic potential, and toxicokinetic profiles to azoxystrobin. Regulatory *in vivo* data for both source and target substances were reviewed, focusing on ADME, neurotoxicity, and target organ toxicity. These data showed no evidence of neurotoxicity in either neurotoxicity studies or repeat-dose toxicity studies for the source substances. The scientific hypothesis was: can the absence of neurotoxic potential via CIII inhibition be predicted using toxicodynamic and toxicokinetic NAM data?

16. Analysis of the read-across data did not indicate a higher neurotoxic potential for azoxystrobin compared to the source compounds. Given that the source compounds do not exhibit neurotoxicity *in vivo*, it was concluded that azoxystrobin is also not a neurotoxicant.

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Case study 6: Developmental toxicity of methyl hexanoic acid

17. 2-Methylhexanoic acid (MHA) lacks data from developmental and reproductive toxicity (DART) studies. To explore the potential for read-across, seven structurally related aliphatic carboxylic acids with available *in vivo* DART data were identified (OECD, 2020c).

18. Among these analogues, some demonstrated clear developmental toxicity, while others did not. Recognising that structural similarity alone does not reliably predict developmental toxicity, MHA and the selected source substances were assessed using a battery of *in vitro* assays relevant to developmental toxicity. These results were integrated with toxicokinetic modelling to estimate effective cellular concentrations and corresponding *in vivo* exposure levels.

19. The NAM-based assessment correctly identified four source substances as developmental toxicants and two as non-toxicants. Based on the observed NAM similarity, it was concluded that MHA may not be entirely devoid of developmental toxicity potential.

Lessons learnt from case studies

20. These case studies illustrate diverse approaches to Steps 3 and 4 of the read-across framework.

21. Case studies 1 and 2 primarily applied cheminformatics methods, leveraging the fact that their general MoAs are known. In contrast, case studies 4 to 6 addressed more specific MoAs and therefore focused on AOP communality between target and source substances. This communality was supported by various NAMs. Additionally, the OECD IATA case studies included a standardised uncertainty table. Case study 3 explored multiple combinations of chemical similarity, biological NAMs, and physiologically based kinetic modelling to support the read-across.

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22. EFSA concluded that the selection of case studies demonstrates how read-across strategies can be tailored to address specific scientific questions. The importance of systematically analysing uncertainty was emphasised.

Secretariat

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