Summary of read-across case studies selected by EFSA

In this guide

In this guide

This is a paper for discussion. It does not reflect the views of the Committee and should not be cited.

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.

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.

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.