Validating Read-Across Analogues Accounting for Metabolic Similarity. Application to Environmental Fate Endpoints

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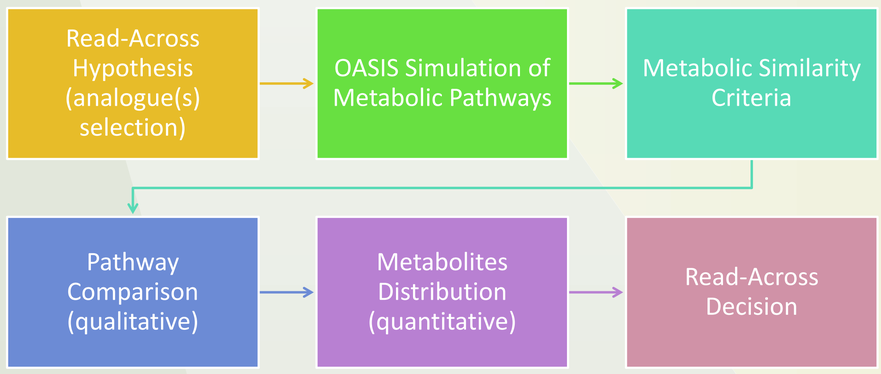
INTRODUCTION

Read-across is one of the most frequently used non-animal approaches for filling toxicological data gaps. Traditionally, analogue selection has been based on physicochemical, structural, and mechanistic similarity, while metabolic similarity has often been overlooked. This can lead to inconsistencies, as structurally similar substances may exhibit conflicting properties due to differences in their metabolic fate. There is now broad recognition that considering the metabolic fates of the target substance and proposed source analogue(s) is critical for accepting or rejecting a read-across hypothesis.

The present work demonstrates the value of metabolic similarity, particularly in cases where structural similarity alone is insufficient to assess the suitability of a source analogue for data gap filling [1].

WORKFLOW



SIMULATION OF METABOLISM IN OASIS

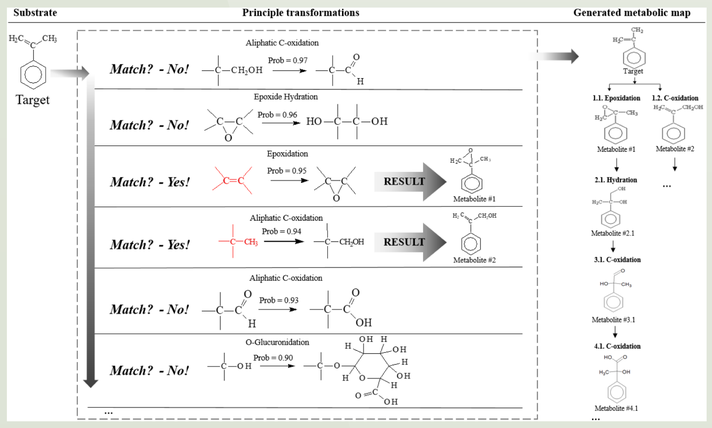
Unique Advantages of OASIS Metabolic Simulators:

Enzyme-Specific Modeling

Enzyme specificity enables environment- and tissue-specific customization

Objective Pathway Propagation

Quantitative feasibility assessment confines propagation to biologically plausible pathways

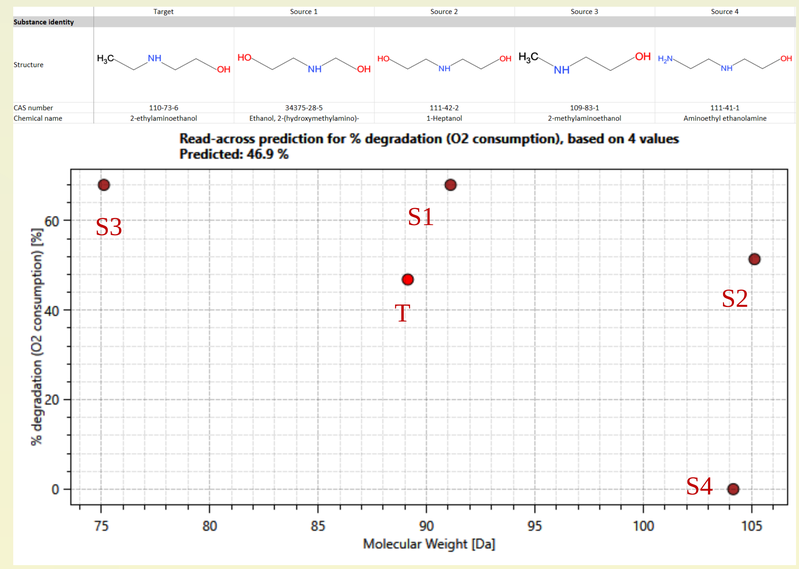


CASE STUDY. ANALOGUES SELECTION IN QSAR TOOLBOX

Target: CAS# 110-73-6

Endpoint: Biodegradability

Read-across: The target is predicted as ***Not Readily Degradable*** based on four structurally similar analogues (having same Organic Functional Groups according to OECD QSAR Toolbox 4.8)



Questions

? Is the structural similarity sufficient to support biodegradation of two parent chemicals?

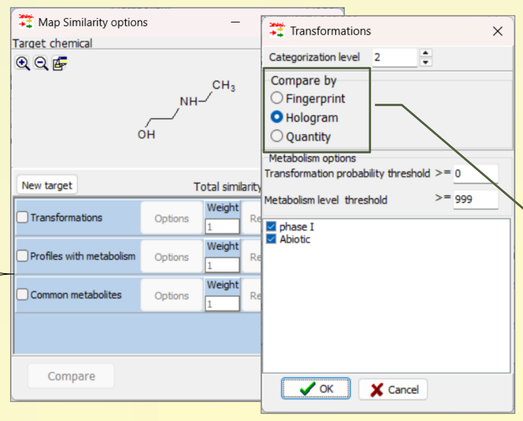
? How to justify conflicting biodegradability data among structurally similar analogues?

CASE STUDY. METABOLIC SIMILARITY CRITERIA AND OPTIONS IN OASIS

Criteria

Commonality with respect to:

- molecular transformations
- structural/mechanistic alerts (using QSAR Toolbox profilers)
- simulated metabolites/degradants

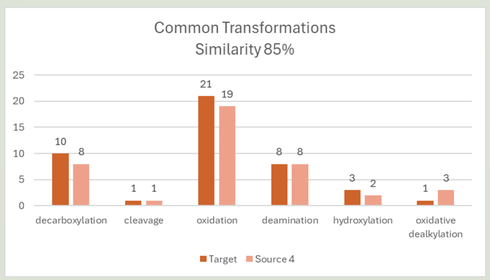


Options

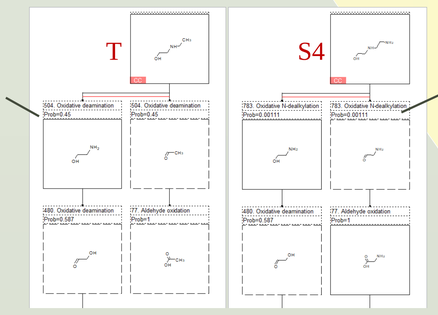
- *Fingerprint*: is the transformation applied (yes/no)
- *Hologram*: what is the frequency of application
- *Quantity*: what is the quantity of each metabolite/degradant

CASE STUDY. COMPARISON OF EACH TARGET–ANALOGUE PAIR BASED ON COMMON MOLECULAR TRANSFORMATIONS

RA analogues	Similarity estimate
Target (T)- Source 1 (S1)	65%
Target (T) - Source 2 (S2)	45%
Target (T) - Source 3 (S3)	73%
Target (T) - Source 4 (S4)	85%



45%



0.1%

The couple of *Target* and *Source 4* demonstrates high metabolic similarity based on common metabolic transformations (at all levels).

HOWEVER:

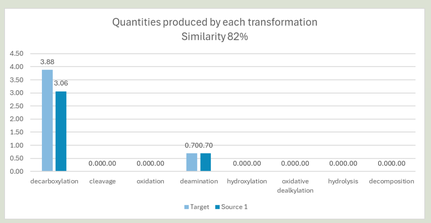
Target: Deamination occurs at first metabolic level (45% feasibility)

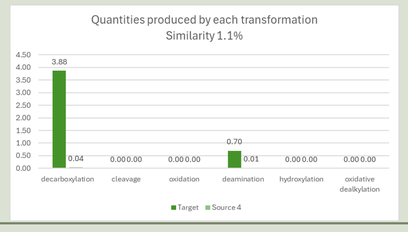
Source 4: N-Dealkylation occurs at first metabolic level (0.1% feasibility)

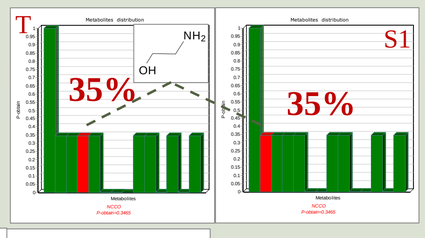
Conclusion: Source 4 is metabolically similar to the Target, but dissimilar with respect to expected BOD.

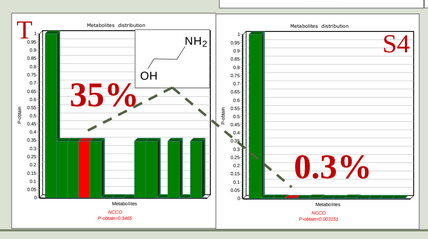
CASE STUDY. COMPARISON OF EACH TARGET–ANALOGUE PAIR BASED ON METABOLITE FORMATION PROBABILITY

RA analogues	Similarity estimate
Target (T) - Source 1 (S1)	82%
Target (T) - Source 2 (S2)	88%
Target (T) - Source 3 (S3)	77%
Target (T) - Source 4 (S4)	1.1%









The couple of *Target* and *Source 1* demonstrates the highest similarity based on metabolites distribution (35% vs. 35% to form the same product).

The couple of *Target* and *Source 4* demonstrates the lowest similarity based on metabolites distribution (35% vs. 0.3% to form the same product).

Conclusion: The couple T-S4 shows conflicting biodegradation behavior, while the couple T-S1 biodegrades in a similar way.

STRUCTURAL SIMILARITY ≠ METABOLIC SIMILARITY → READ-ACROSS DECISION

RA analogues	Structural Similarity (based on TB)	Metabolic Similarity (based on OASIS)	Suitable for Read-Across
Source 1	High	High	Yes
Source 2	High	Moderate	No
Source 3	High	High	Yes
Source 4	High	Low	No

✓ **Keep Source Analogues 1 and 3**

✗ **Eliminate Source Analogues 2 and 4**

CONCLUSIONS

1. Structural similarity alone is insufficient for reliable read-across predictions in biodegradation assessment.
2. Metabolic similarity provides an objective basis for analogue validation.
3. Differences in transformations feasibility and kinetics can significantly alter environmental fate interpretation.
4. Incorporating metabolic similarity enhances robustness, transparency, and regulatory credibility of non-animal approaches.

REF: [1] Kuseva et al. *Computational Toxicology*. **2021**, 19, 100173.