Results of Ring Testing for the Selection of Reference Substances for Improving Chemical Persistence Assessment in Higher Tier OECD 309 Simulation Test

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Introduction

- The OECD 309 test guideline (TG) is the primary method under REACH for assessing **aerobic biodegradation in surface waters**, especially for soluble compounds, with increasing global use and regulatory importance.
- Degradation **kinetics can be highly variable** due to variability in test parameters and inoculum characteristics, including microbial diversity, surface water composition, temperature, and storage conditions.
- Existing reference compounds (**aniline and sodium benzoate**) may degrade even in suboptimal conditions, potentially overestimating the microbial viability.
- Issues with the current OECD 309 TG highlight the **need for new reference substances** that:
  - Better represent test system performance
  - Require the presence of diverse microbial communities for significant mineralisation
  - Provide benchmarks for persistent substances
- The **Cefic-LRI ECO55 project** aims to address OECD 309 issues, such as by exploring potential new reference substances.

AIM: To conduct an inter-laboratory ring trial to evaluate new reference substances for OECD 309, aiming to improve test consistency and reliability in regulatory persistence assessments.

Methodology

32 candidates
Candidate reference substances were identified via a literature review

8 shortlisted
Based on relevant properties and availability of radiolabelled forms

2 selected
Modified **OECD 301 D** studies led to the selection of **caffeine and 2,4-D**, alongside the existing reference **aniline**

OECD 309 ring trial
9 European labs conducted OECD 309 studies under various conditions (12°C and 20°C, 1 and 10 µg/L, 2x water sampling locations))

Ring Trial Results

Caffeine

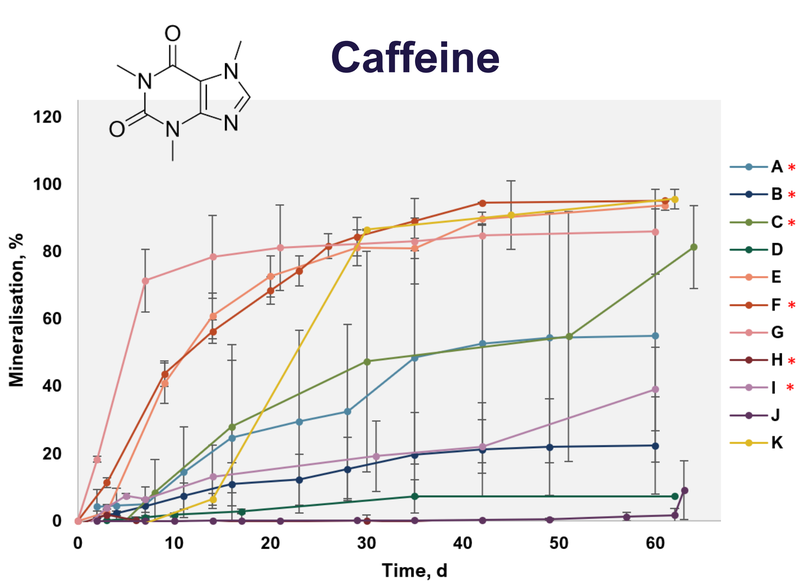

Figure 1. Mineralisation of caffeine across 11 studies by 9 labs. * = tests conducted at 20°C.

Significant variability in the remaining applied radioactivity (AR), which appeared influenced by temperature (not conclusive).

Half-lives ranged from 5.83 to 181 days (mean = 30.5 days).

2,4-D

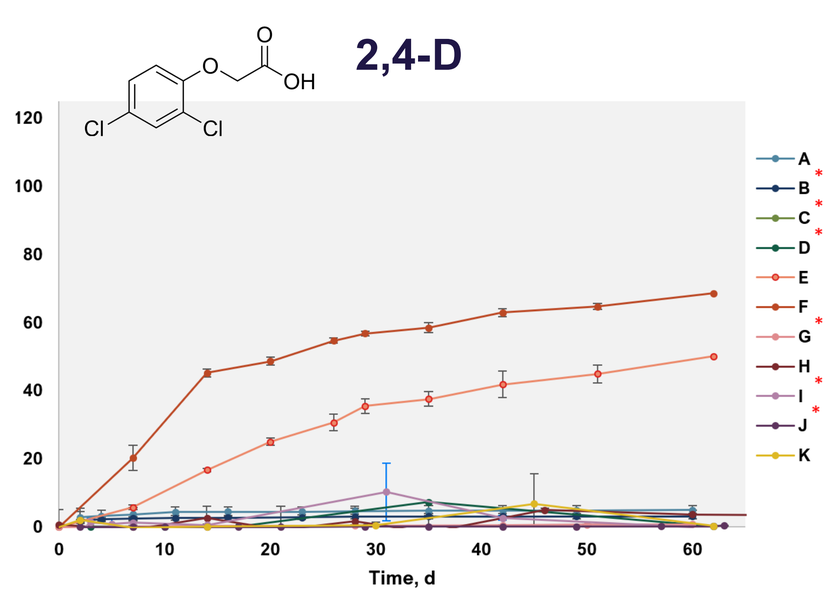

Figure 2. Mineralisation of 2,4-D across 11 studies by 9 labs. * = tests conducted at 20°C.

Little mineralisation and overall less variability, with significant mineralisation only observed in 2 studies from a single laboratory, suggesting that other factors may have led to its mineralisation.

Half-lives ranged from 20.5 to 5849 days (mean = 485 days).

Aniline

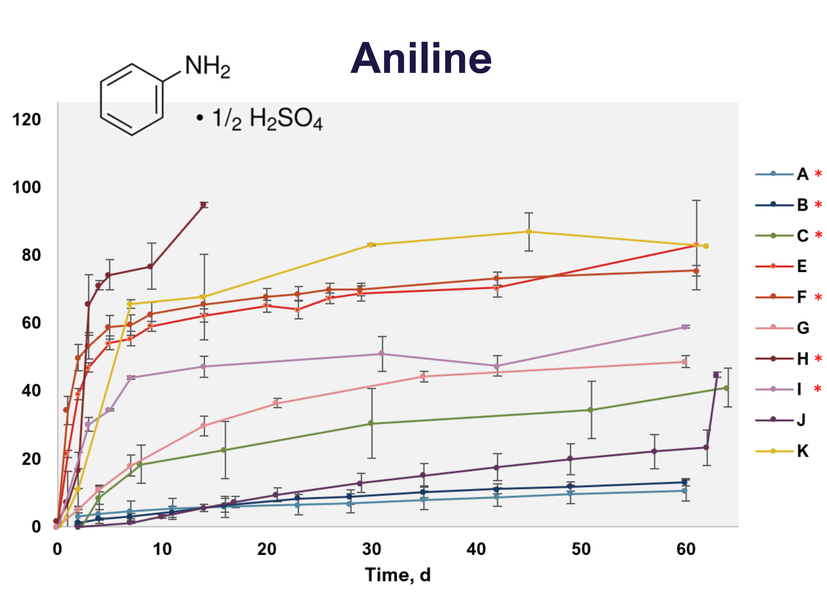

Figure 3. Mineralisation of aniline across 10 studies by 8 labs. * = tests conducted at 20°C.

Aniline mineralisation (AR and half-lives) showed wide variation far exceeding the TG's 14-day mineralisation target.

The other existing reference substance, sodium benzoate, failed to meet validity criteria in one of the two studies.

CONCLUSIONS

- **Large amount of heterogeneity** within the collated ring test data from across the participating labs for both caffeine and 2,4-D.
- It was **not possible to establish validity criteria for either candidate** reference substance under the OECD 309 TG.
- Further work is required to assess and characterise other factors influencing test outcome, such as system type (open vs. closed), total organic carbon, and environmental parameters such as water collection temperature, weather conditions (e.g. rain events), and phosphorus levels.

Table 1. Summary of mineralisation results across the nine participant laboratories

	Aniline	Sodium benzoate	Caffeine	2,4-D
Number of datasets	10	2	11	11
Number of laboratories	8	2	9	9
Mean mineralisation, %	55.3 ± 29.1	83.0 ± 14.5	52.8 ± 41.2	12.1 ± 25.5
Median mineralisation, %	53.8	83.0	60.3	0.6
Max. mineralisation, %	94.8	93.2	95.6	68.6
Min. mineralisation, %	10.7	72.8	-1.2	-7.5
Range, %	84.1	20.5	96.8	76.1