Use of a NAM-Enhanced Read-Across and Weight of Evidence Approach to Avoid Chronic Fish Testing for Alkyl Isethionate Surfactants Under EU REACH

Lauren McAnally[1], Jay Dawick[1], Jayne Roberts[2], Steve Gutsell[2], Geoff Hodges[2], Marc Geurts[3]
[1] Innospec Limited, Ellesmere Port, Cheshire, United Kingdom
[2] Safety, Environmental and Regulatory Science (SERS), Unilever, Sharnbrook, United Kingdom,
[3] Nouryon Specialty Chemicals, Deventer, Netherlands

Introduction

A common approach for addressing EU REACH standard information requirement (SIR) is the use of read-across or grouping argumentation where information from one or a number of analogous (or 'source') substance(s) is used to support or predict missing endpoint data for one or more 'target' substances. Methods to support read-across arguments are outlined in the Read Across Assessment Framework (RAAF).

In this specific case study, a NAM-enhanced read-across approach (in line with RAAF scenario 2) was used to fulfil the information requirements for long-term toxicity testing on fish requested in the ECHA Dossier Compliance Check (CCH) for the high-volume alkyl isethionate substances registered under EU REACH.

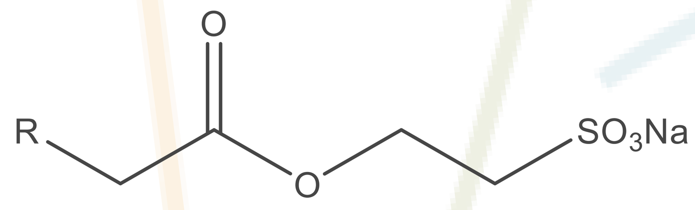
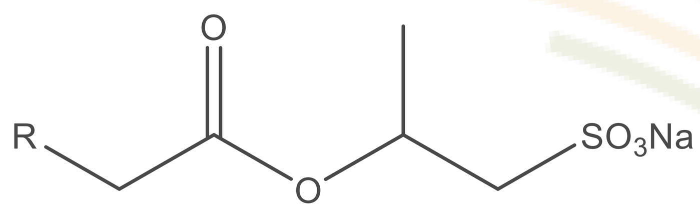
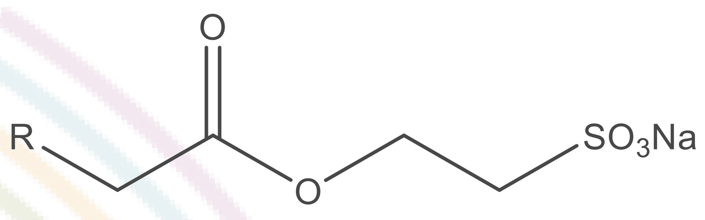
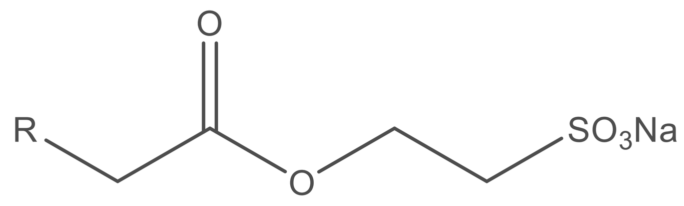
Substance	Sodium Lauryl Isethionate (SLI)	Sodium Lauryl Methyl Isethionate (SLMI)	Sodium Cocoyl Isethionate (SCI-1)	Sodium Cocoyl Isethionate (SCI-2)
Structure	 $R = C_{10}H_{21}$	 $R = C_{10}H_{21}$	 R = C6-C16 predom C10-12 (even numbered)	 R = C6-C16 (even numbererd) + C16 unsaturated

Table 1. Structure of the alkyl isethionate substances registered under REACH.

Trends in toxicity

The read-across approach that was developed focused on the (eco)toxicological similarity of the isethionate substances through the following:

- ✓ Common hydrophilic head group structure
- ✓ Overlapping alkyl chain length distributions
- ✓ Physical-chemical properties
- ✓ Environmental fate properties
- ✓ Mode of Action (MoA).

Additional algal and Daphnia data was generated via new testing to strengthen the read across argument. Trends in (eco)toxicity were then established based on alkyl chain length and residual fatty acid content, which was used to implement a worst-case, protective read-across approach to avoid new chronic fish testing.

	Source SCI-2	Target 1 SCI-1	Target 2 SLMI	Target 3 SLI
Alkyl Chain Length	C8-18 (+C18 unsaturated)	C8-18 (predom 12-14)	C12	C12
Historic Acute Fish	✓ →	✓ [Insufficient - RA from source]	✗ [RA from source]	✗ [RA from source]
RTGill EC50	→	→	→	→
Historic Chronic Fish	✓ →	✗ [RA from source]	✗ [RA from source]	✗ [RA from source]

Table 2. Read-across visual representation

RTGill Cell Line (OECD 249)

To support the read-across approach, new acute *in vitro* RTGill-W1 (OECD 249) testing was commissioned for the whole category of isethionate substances which, when combined with existing/new data on toxicity to algae and daphnia, further established the trends used to address the chronic fish toxicity endpoint for the entire group.

RTGill-W1 LC50 values were obtained for four isethionate substances, SLI, SLMI, SCI-1 and SCI-2. The LC50 values obtained for substances containing longer alkyl chain lengths (SCI-1 and SCI-2l) slightly lower than the C12 mono constituent substances (SLI and SLMI). This indicates comparability of aquatic toxicity between the isethionate substances.

Bioaccumulation

A tiered *in silico* modelling approach was applied to address the bioaccumulation endpoint. All components of the Isethionate substances were shown to have low bioaccumulation potential when considering the key process of biotransformation. Rapid biotransformation is confirmed in fish by *in-vitro* S9 clearance data and is supported by similar data obtained as part of the human hepatocyte stability assay where the substances were shown to rapidly metabolise to sodium isethionate and fatty acid via cleavage of the ester bond. More detailed information on the bioaccumulation approach for isethionate substances was presented in a platform presentation at SETAC 2024[1].

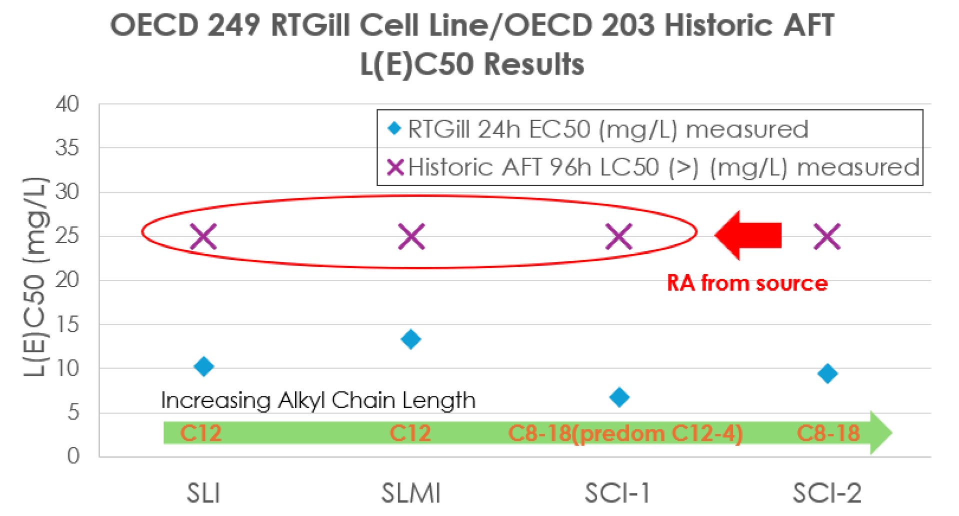


Figure 1. RTGill cell line EC50 and historic Acute Fish Toxicity (AFT) LC50 values of isethionate substances.

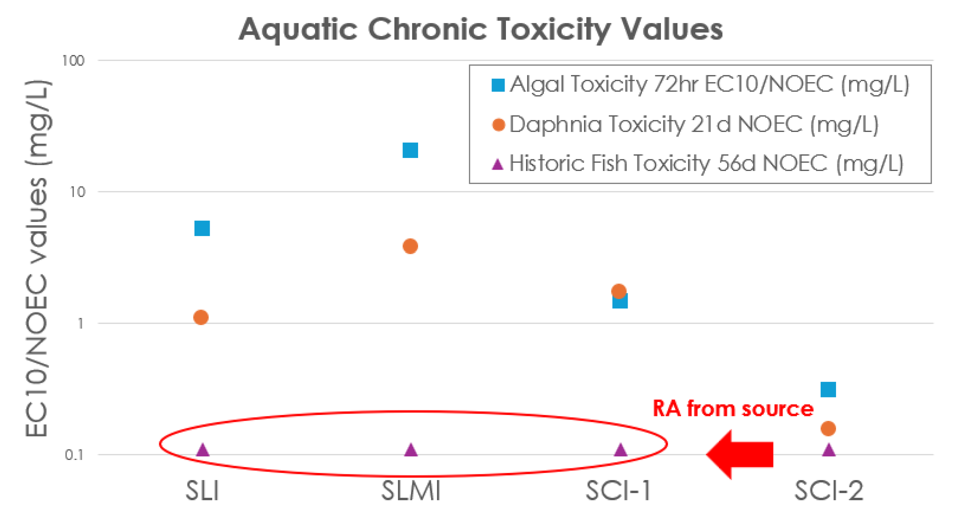


Figure 2. Chronic ecotoxicity data of isethionate substances (Algal OECD 201 EC10 (SCI-2 NOEC), daphnia OECD 211 NOEC and historic fish OECD 210 NOEC values)

Biodegradation

A strong environmental fate-based argument against the need for additional chronic fish testing was also developed which involved making use of data on biodegradation in sewer (OECD 314A), aerobic/anaerobic biodegradation (OECD 301/311), aerobic Sewage Treatment Plant (STP) simulation (OECD 303A) and biodegradation within the effluent-surface water mixing zone (OECD 314D). These data exemplify the rapid and complete biodegradation of the alkyl isethionate surfactants under environmentally relevant conditions and their negligible potential for long term adverse effects in the receiving environment.

Ready Biodegradability (OECD 301)

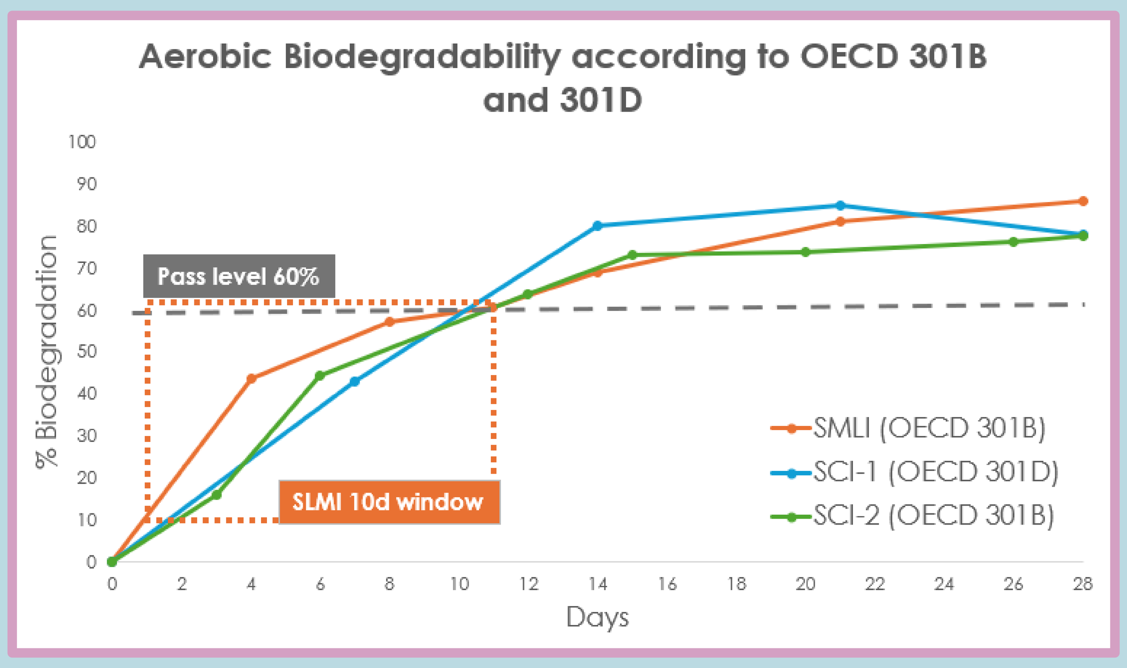


Figure 3. Ready biodegradation results for SLMI and SCI

Anaerobic Biodegradability (OECD 311)

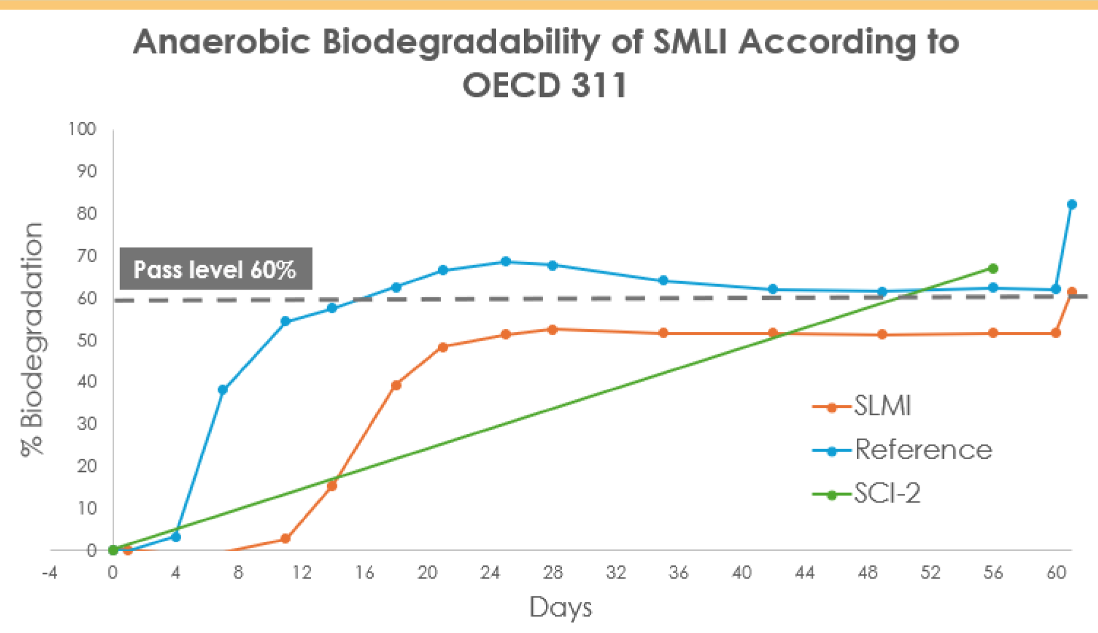


Figure 5. Anaerobic biodegradation results of SLMI.

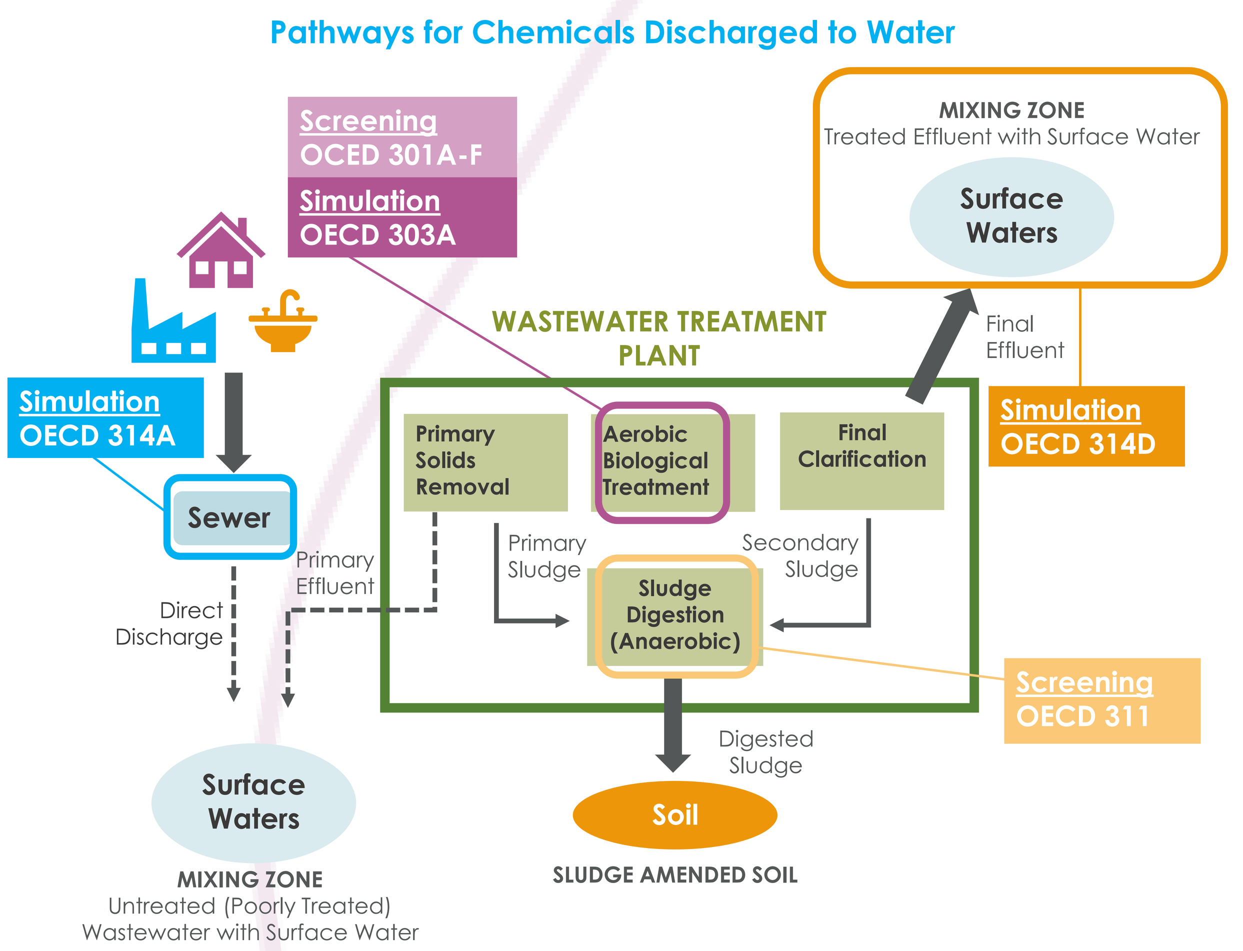


Figure 4. Pathways for chemicals discharged into water. Figure adapted from [2]

Sewer Simulation Biodegradation (OECD 314A)

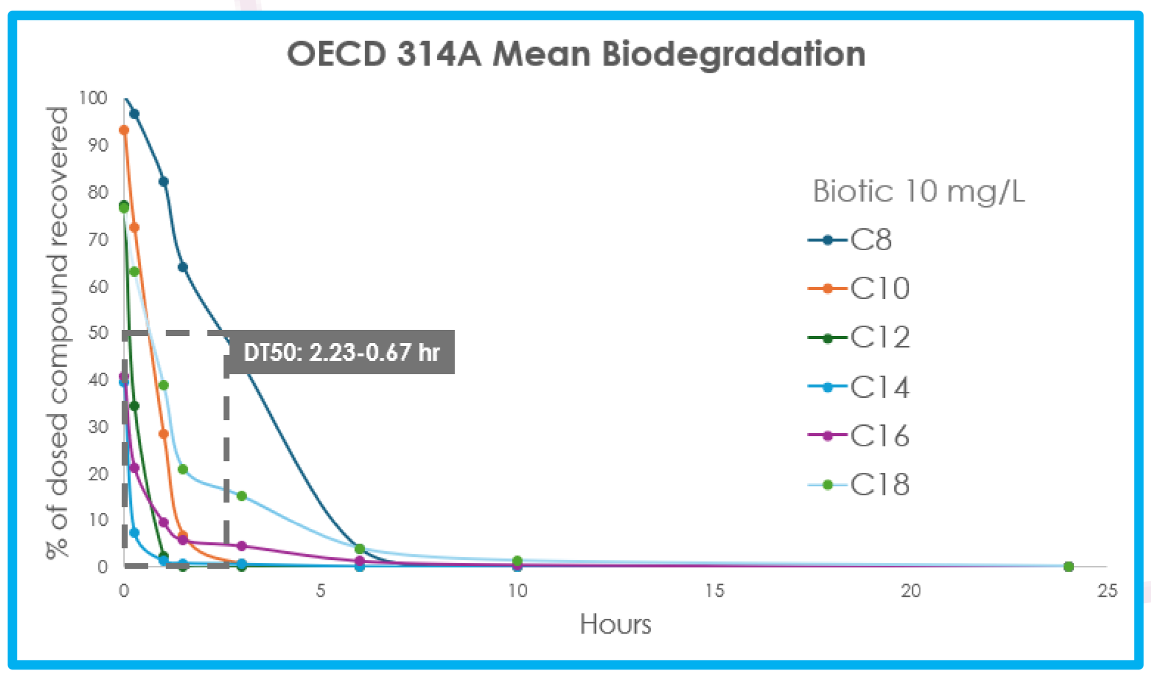


Figure 6. Sewer simulation percentage recovery of results of SCI-2.

STP Simulation Degradation (OECD 303A)

Test Compound	%Parent		
	Degraded	Effluent	Sludge
Sodium [^{14}C] Lauryl Isethionate	**99.99**	0.01	0.00
Sodium [^{14}C] Stearyl Isethionate	**99.61**	0.11	0.28

Table 3. STP simulation degradation results for SLI and SSI

Mixing Zone Degradation (OECD 314D)

Test System	Test Substance	DT50	DT90
Biotic	Sodium [^{14}C] Lauryl Isethionate	0.21h **(12min)**	0.69h (41min)
	Sodium [^{14}C] Stearyl Isethionate	0.36h **(22min)**	1.2h (72min)
Abiotic	Sodium [^{14}C] Lauryl Isethionate	30d	100d
	Sodium [^{14}C] Stearyl Isethionate	23d	76d

Table 4. Mixing zone degradation results for SLI and SSI
DT50 = time taken for concentration of compound to be reduced by 50%
DT90 = time taken for concentration of compound to be reduced by 90%

- ✓ Isethionates are biodegradable under aerobic and anaerobic conditions
- ✓ Rapid biodegradation can be demonstrated under environmentally relevant conditions
- ✓ Negligible potential for long-term adverse effects in receiving environment
- ✓ Extensive primary and ultimate biodegradation clearly demonstrated
- ✓ Trace amounts only remain in effluent and sludge solids

Conclusion

This case study on alkyl isethionate surfactants demonstrates how a combination of NAM-enhanced read across and weight of evidence approaches can be used to avoid chronic fish testing under EU REACH and help registrants uphold the last resort principle.

References

[1] Roberts, J . (2024) Using An In Silico NAMs Approach To Predict Bioaccumulation In Fish: A Case Study For Anionic Surfactants Within A Regulatory Context [PowerPoint presentation] Available at: https://sers.unilever.com/files/using-an-in-silico-nams-approach-to-predict-bioaccumulation-in-fish-a-case-study-for-anionic-surfactants-within-a-regulatory-context-rodbk9.pdf (Accessed 28 April 2025

[2] OECD (2006), *Test No. 311: Anaerobic Biodegradability of Organic Compounds in Digested Sludge: by Measurement of Gas Production*, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, https://doi.org/10.1787/9789264016842-en.