Integration of New Approach Methods in Read Across. A Case Study with Fatty Acids, C12-18 and C18-unsatd., 2-sulfoethyl Esters, Sodium Salts (DEFI) for Sub-Acute Toxicity

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Background

Toxicological read-across is a vital method in regulatory toxicology, enabling the prediction of toxicological properties for one chemical based on data from similar chemical(s), thereby replacing the need for animal testing. However, the regulatory acceptance of read-across is often limited due to challenges in demonstrating toxicokinetic (TK) and toxicodynamic (TD) similarity[a], which are key aspects highlighted in authoritative guidance on read-across such as the ECHA Read-Across Assessment Framework (RAAF) [b]. Fatty acids, coco, 2-sulfoethyl esters, sodium salts (SCI), a structurally similar substance was selected as a possible source substance and here we present a case study, applying non animal, new approach methodologies (NAMs) to demonstrate chemical and biological similarities for complex multi constituent/UVCB substances.

Aims

The aim of this work was to fill a data gap for short-term (28 day) repeated dose toxicity study (oral) for **Fatty acids, C12-18 and C18-unsatd., 2-sulfoethyl esters, sodium salts** (DEFI) using a read across approach.

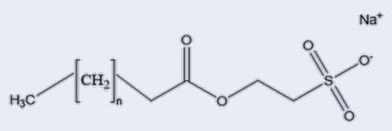
Read Across Hypothesis

The target and source substances contain the same components and differ only in their concentrations. It is therefore hypothesised that despite potential minor differences in physicochemical properties, they are expected to have similar biological properties (RAAF scenario 2, analogue approach).
This hypothesis is supported by structural, physicochemical, toxicokinetic, toxicodynamic, and existing toxicological data (bridging studies).

Target Characterisation

Target substance, DEFI, is multi-constituent product of an esterification reaction of fatty acids with sodium isethionate under catalytic conditions. The chemical identification of DEFI is shown in **Table 1**.
Two independent DEFI samples were analytically characterised (LC-MS, MS, and NMR).

Table 1. Identification of Target Substance DEFI

Chemical Name	FATTY ACIDS, C12-18 AND C18-UNSATD., 2-SULFOETHYL ESTERS, SODIUM SALTS
Synonym	DEFI
Type	Multi-constituent substance
CAS RN	85408-62-4
EC No	287-024-7
General Structure	 n = 5, 7, 9, 11, 13, 15

Analogue Selection

When searching for an appropriate analogue to read across from there were some key structural and data quality requirements to consider as demonstrated by **Figure 1.**

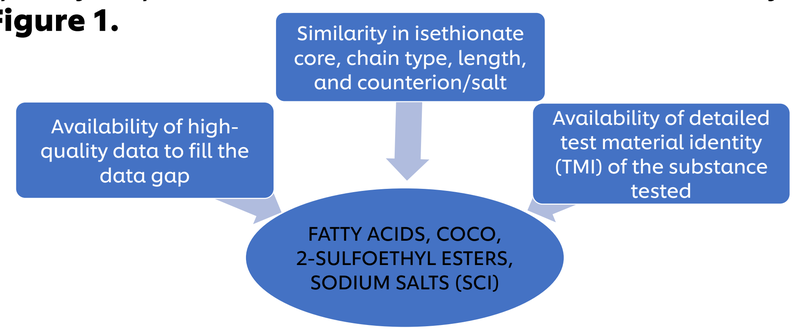
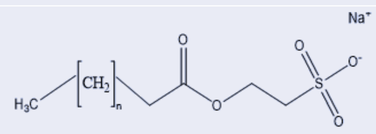


Figure 1. Key Requirements Considered When Searching for an Appropriate Source Analogue

Table 2. Identification of Source Substance SCI

Chemical Name	FATTY ACIDS, COCO, 2-SULFOETHYL ESTERS, SODIUM SALTS
Synonym	SCI
Type	Unknown or variable composition, complex reaction products or biological materials (UVCB)
CAS RN	61789-32-0
EC No	263-052-5
General structure	 n = 3, 5, 7, 9, 11, 13, 15

Fatty acids, coco, 2-sulfoethyl esters, sodium salts (SCI) was selected as the most appropriate analogue, possessing high structural similarity to DEFI as shown in **Table 3** and **Figure 2.**

Table 3. A Comparison of the Boundary Compositions of DEFI and SCI

Components	Boundary Composition of DEFI (%)	Boundary Composition of SCI (%)
Sodium caproyl isethionate (C6)		
Sodium capryloyl isethionate (C8)		
Sodium caproyl isethionate (C10)		
Sodium lauroyl isethionate (C12)		
Sodium myristoyl isethionate (C14)		
Sodium palmitoyl isethionate (C16)		
Sodium stearoyl isethionate (C18)		
Caprylic acid (C8)		
Capric acid (C10)		
Lauric acid (C12)		
Myristic acid (C14)		
Palmitic acid (C16)		
Stearic acid (C18)		
Sodium Isethionate		
Others		

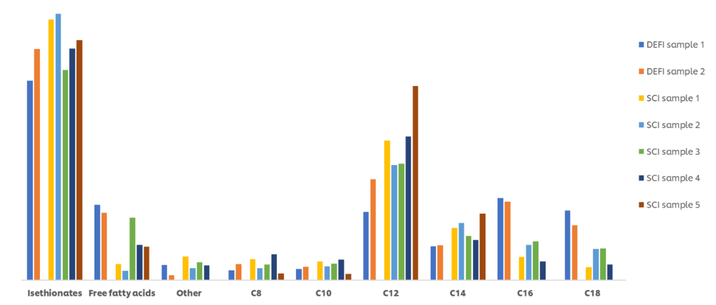


Figure 2. Structural Composition Comparison of Characterised DEFI and SCI Samples

Similarity Justification

Chemical and biological comparisons were made between both substances in order to strengthen the read across justification. This included establishing the physicochemical, toxicokinetic, and toxicodynamic similarities and differences.

Physicochemical Properties

Table 4. The Physicochemical Properties of DEFI and SCI

Property	DEFI	SCI
Physical state	Solid	Solid
LogP	2.78	1.28
MP (°C)	199.85-237.85	225
BP (°C)	>275	>300
Density	1.21 at 22°C	1.11 at 20°C
VP (Pa)	$3.5x10^{-6}$	<0.002-0.006
WS (g/L)	0.27	3.49
pKa	Isethionate = 1.1 Fatty acids = 4.8	Isethionate = 1.1 Fatty acids = 4.8
Surface tension (mN/m)	42.5	24

Both substances are non-volatile solids, with similarities in melting point, density, and pKa. Slight differences occur for vapour pressure, logP, surface tension, and water solubility.

Toxicokinetics

Both substances have low skin penetration, high binding affinity to plasma protein, no affinity for binding to red blood cells and are rapidly metabolised (in the skin and liver) in the same way.

Bridging studies (taking worst case):
- Acute oral toxicity of both substances is >2000 mg/kg bw
- Both substances were mildly irritating to both eye and skin.
- Both are negative in the Ames test
- Both were found to be non-genotoxic in the ToxTracker assay

Toxicodynamics

In silico profiling was performed for both substances for physicochemical and systemic-related endpoints. This produced identical output for both substances with no toxicological concerns.

A broad suite of human relevant *In vitro* bioactivity assays were performed on DEFI and SCI, these NAMs poses a large biological coverage of molecular and cellular events relating to systemic toxicity and have been used in previous NGRA case studies and workflows[c].

- **High Throughput Transcriptomics (HTTr)** – non-targeted gene expression effects
- **ToxProfiler** - captures cellular stress response pathways
- **Cell Stress Panel** - captures cellular stress biomarkers over multiple assays
- ***In vitro* Pharmacological Profiling** – targeted biological receptor effects
- **Chemically Activated Luciferase Expression, CALUX** – functionally relevant receptor follow up

Both substances behave similarly with the most sensitive assay being a point of departure derived from HTTr data (Bifrost model analysis) resulting in values of 0.92 and 0.86 for DEFI and SCI, respectively.

Uncertainty Assessment

Sources of uncertainty:
- Differing chain length distributions and level of free fatty acids.
- Some differences in physicochemical properties
- Low uncertainty regarding experimental source data

Uncertainty was reduced by comparing both substances using NAMs

Conclusions

Source substance, SCI, is suitable for use in reading across to target substance, DEFI for this data gap – short term repeat dose toxicity. Both substances express similarities in their structure, use, synthesis, some physicochemical properties, toxicokinetics, and toxicodynamics. The findings of this work support and advance the use of read across as a scientifically valid approach to meet regulatory requirements strengthened by the integration of NAMs.

References: [a]Ball et al., 2014, 212-221. [b]ECHA, 2017, ISBN 978-92-9495-758-0. [c]Cable et al., 2024, kfae159.

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