Advancing Regulatory Compliance with NAMs: A Read-Across Case Study for a Mixture to Address Short-Term Repeated Dose Toxicity

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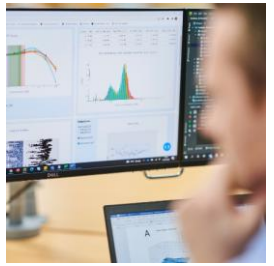
SERS
Safety, Environmental & Regulatory Science

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AIM of the CASE STUDY

Fill the data gap for short-term repeated dose toxicity study (28 days), one species, male and female (oral and dermal exposure) for

FATTY ACIDS, C12-18 AND C18-UNSATD., 2-SULFOETHYL ESTERS, SODIUM SALTS (DEFI)

Following the ECHA Read-Across Assessment Framework (RAAF)

https://echa.europa.eu/documents/10162/17221/raaf_en.pdf/614e5d61-891d-4154-8a47-87efebd1851a

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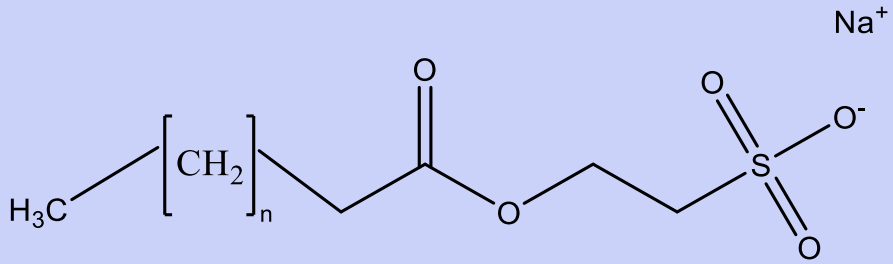
TARGET - IDENTIFICATION & CHARACTERISATION

Chemical Name	**FATTY ACIDS, C12-18 AND C18-UNSATD., 2-SULFOETHYL ESTERS, SODIUM SALTS**
Synonyms	**Direct Esterified Fatty Isethionate (DEFI)**
Type	**Multi-constituent substance**
CAS RN	**85408-62-4**
EC No	**287-024-7**
General structure	 **n = 5, 7, 9, 11, 13, 15**

Constituents	**Boundary Composition of DEFI (%)**
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)	
Impurities	
Sodium Isethionate	
Other	

- Test material identity (TMI) for 2 independent samples
- Full characterisation using battery of analytical methods: LC-MS, MS, NMR, metal analysis
- All constituents present in a concentration at or above 1 % are identified
- The compositional information is completed up to 100%

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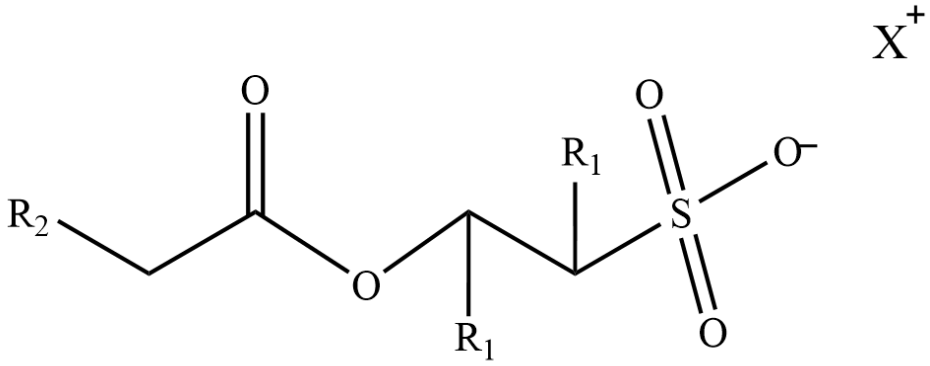
ANALOGUE(S) SELECTION



R_1 = H, CH_3
R_2 = C6 – C16 (even numbered)
X^+ = Na, NH_4

Similarity in the isethionate core, chain type, length, counterion

Availability of high-quality data to fill data gap

Availability of the detailed TMI of the tested substance

FATTY ACIDS, COCO, 2-SULFOETHYL ESTERS, SODIUM SALTS (SCI)

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READ-ACROSS HYPOTHESIS

- Target and source substances are different substances with the same type of effect(s) – this corresponds to **RAAF scenario 2** – analogue approach
- Additionally, **RAAF for multi-constituent substances and UVCBs** has been also considered
- The premise is that both substances consist of the same constituents, with differences in their concentrations, which can impact some physico-chemical properties, however, they both have similar biological behaviour
- This hypothesis will be supported by structural and physico-chemical properties, toxicokinetic data, a battery of NAMs data (both *in silico* and *in vitro*) and existing toxicological studies

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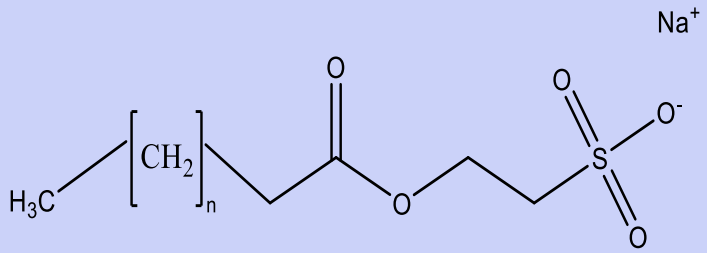
SOURCE - IDENTIFICATION & CHARACTERISATION

AE A.1

Chemical Name	**FATTY ACIDS, COCO, 2-SULFOETHYL ESTERS, SODIUM SALTS**
Synonyms	**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**

Constituents	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	
Other	

- TMI for 5 independent samples
- Full characterisation using battery of analytical methods: LC-MS, MS, NMR, metal analysis
- All constituents present in a concentration at or above 1 % are identified
- The compositional information is completed up to 100%

EXPERIMENTAL DATA FOR SHORT-TERM REPEATED DOSE TOXICITY STUDY

1. OECD 410 (Repeated Dose Dermal Toxicity: 28-day Study in Rodents), 1991

SCI was applied topically at doses of **0, 80, 910 and 2070** mg/kg bw/day to groups of 10 rats/sex. Treatment was under occlusive coverage on 6 hours/day for 28 consecutive days. No clinical signs of toxicity (including local toxicity), no mortality and no effects on any other investigated parameter (body weight, body weight gain, food consumption, water consumption, haematology, clinical chemistry, organ weights, gross pathology, and histopathology) were observed up to and including the highest dose level.

NOAEL = 2070 mg/kg bw/day

2. OECD 407 (Repeated Dose Oral Toxicity: 28-day Study in Rodents), 1995

SCI was administered in the diet to groups of 10 rats/sex at concentrations of **0, 0.1, 0.3, and 1.0%** for 28 days. There were no clinical signs of toxicity attributable to treatment, no mortality, and no adverse effects on any other investigated parameter (body weight, body weight gain, food consumption, water consumption, haematology, clinical chemistry, organ weights, gross pathology, and histopathology) observed up to and including the highest dose level.

NOAEL = 627 mg/kg bw/day for males

NOAEL = 720 mg/kg bw/day for females

AE A.3

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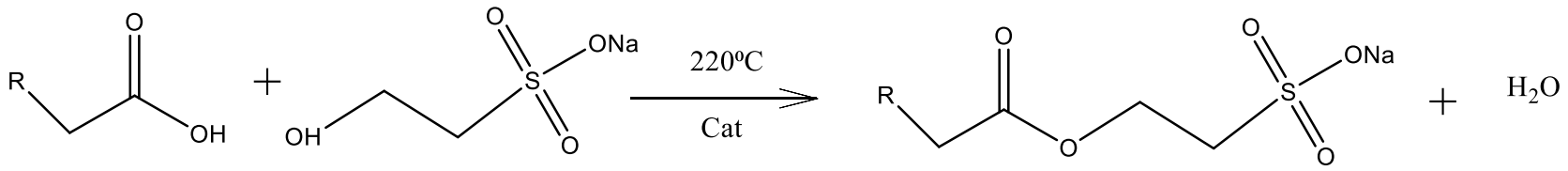
SIMILARITY JUSTIFICATION

- **Manufacturing & exposure**
- **Structural**
- **Physico-chemical**
- **Toxicokinetics**
- **Toxicodynamics**

MANUFACTURING & EXPOSURE

❑ Common route of synthesis: esterification



R = C6 – C16 (even numbered)

❑ Usage

DEFI

- Cosmetic (soap bars) products produced by Unilever

SCI

- Cosmetic products
- Washing, cleaning, and maintenance products (i.e. homecare)

Due to their properties as an anionic surfactants, they can solubilise fats, and form foams and emulsions with fats and oils

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MANUFACTURING & EXPOSURE

❑ **Common route of synthesis: direct esterification**

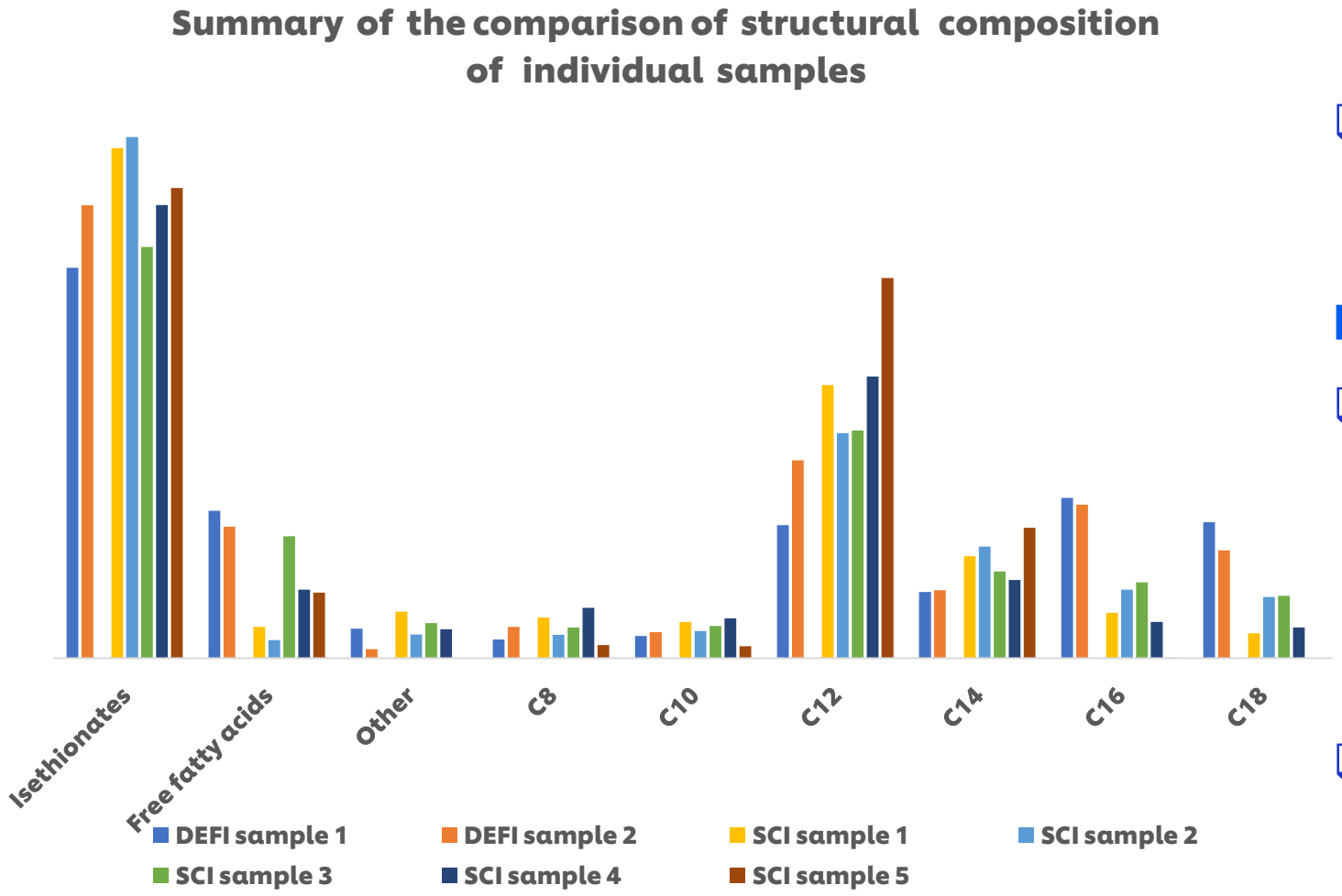
DEFI and SCI have:

- ❑ **The same route of synthesis**
- ❑ **Similar occupational and consumer exposure**

maintenance products (i.e. homecare).

fats and oils

STRUCTURAL SIMILARITY



Similarities in composition

- Both substances contain the same constituents
- Sodium lauroyl isethionate is the most abundant constituent in both substances

Dissimilarities in composition

- The target and the source substances differ in the chain length distributions:
  - DEFI has higher proportion of C16 and C18
  - SCI has a higher proportion of C12 and C14
- DEFI has higher proportion of free fatty acids (except of one SCI sample 3), especially longer alkyl chains of C16 and C18

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AE A.2

STRUCTURAL SIMILARITY

Similarities in composition

- Both substances contain the same

DEFI and SCI have:

- **The same constituents**
- **Different chain length distribution**
- **Different levels of free fatty acids**

3), especially longer alkyl chains of C16 and C18

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PHYSICO-CHEMICAL SIMILARITY

Property	DEFI	SCI
Physical state at 20°C & 101.3kPa	Solid	Solid
Calculated LogP at 20°C	2.78	1.28
Melting Point (°C)	199.85-237.85	225
Boiling Point (°C)	>275	>300
Relative density	1.21 at 22°C	1.11 at 20°C
Vapour Pressure at 25°C (Pa)	$3.5x10^{-6}$	<0.002-0.006
Calculated Water Solubility (g/L)	0.27	3.49
Calculated pKa at 25°C	Isethionate = 1.1 Fatty acids = 4.8	Isethionate = 1.1 Fatty acids = 4.8
Surface tension (mN/m) at 1 g/L	42.5	24

Membrane-water partitioning							
Log Kmw	**Single component STD @~200uM**			**DEFI Sample 2 @ 600uM**			**Mean**
	Replicate Analyses			Replicate Analyses			
C8 Isethionate	-	-	-	3.39	3.43	3.67	**3.50**
C10 Isethionate	3.54	3.70	3.47	3.62	3.63	3.82	**3.63**
C12 Isethionate	4.60	4.58	n/a	4.30	4.39	4.35	**4.42**

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PHYSICO-CHEMICAL SIMILARITY

The structural differences have impact on some physico-chemical properties like logP, WS, VP and surface tension

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TOXICOKINETICS SIMILARITY

AE 2.1 & AE 2.2 & AE 2.4

The tested samples:

- The individual alkyl isethionate constituents (C8-C18)
- DEFI mixture
- For skin absorption, radio-labelled single chain of C12 and C18 and for C12 in the DEFI mixture

The battery of *in vitro* assays to determine ADME:

- Dermal absorption
- Metabolism
  - *In vitro* skin metabolism assay
  - Stability in biological matrices assay
  - Hepatocyte stability (CLint) assay
- Blood to plasma ratio (BP ratio)
- Plasma protein binding (ultrafiltration assay) (PPB)

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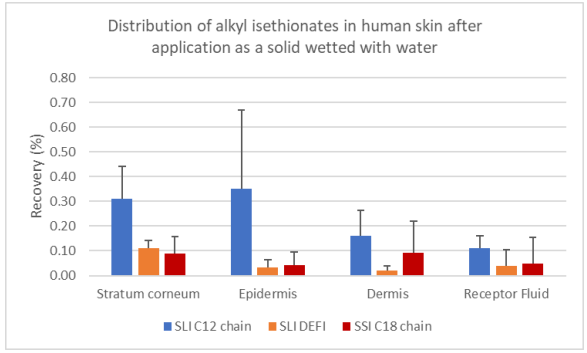
TOXICOKINETICS SIMILARITY

AE 2.1 & AE 2.2 & AE 2.4



	C12 single chain length	C12 in DEFI mixture	C18 single chain length
Dermal Absorption (%)	**1.1**	**0.26**	**0.40**

All five isethionates (C10, C12, C14, C16 and C18) in the artificial mixture (20% each) showed extremely high affinities for plasma proteins

PPB ~99%

Low skin absorption

Remain in the plasma

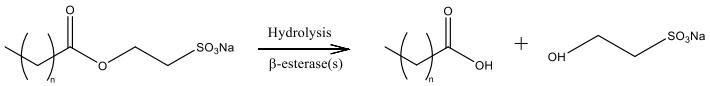
Highly bound to plasma proteins

Rapidly metabolised to the same metabolites

No binding affinity for red blood cells (RBC)

$K_{RBC/Plasma} < 1$

Similar metabolism in skin and hepatocytes:



half-life ($t_{1/2}$) less than 22 min

Chain length does not have significant influence on clearance

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TOXICOKINETICS SIMILARITY

The structural & physico-chemical differences do not impact ADME properties

Based on ADME data for five isethionates

DEFI and SCI have similar toxicokinetics behaviour

Na

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TOXICODYNAMICS SIMILARITY - *in silico* DATA

AE 2.2

AIM: to investigate the impact of structural differences on the systemic bioactivity of the substances

Derek Nexus by Lhasa Limited is a knowledge-based expert system and contains a set of structural alerts for a variety of toxicological endpoints

- 41 endpoints relevant to systemic toxicity have been selected

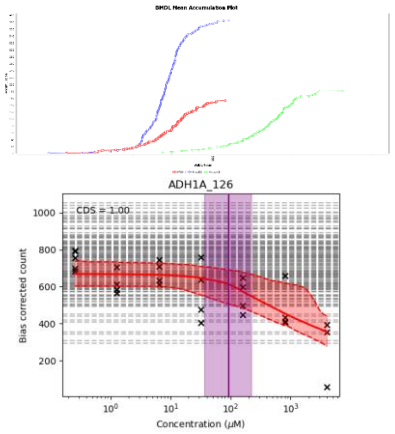
All constituents of DEFI & SCI do not have any alerts for systemic toxicity endpoints

TOXICODYNAMICS SIMILARITY - *in vitro* DATA AE 2.2 & AE 2.3

Non-specific effects

High-Throughput transcriptomics (HTTr)

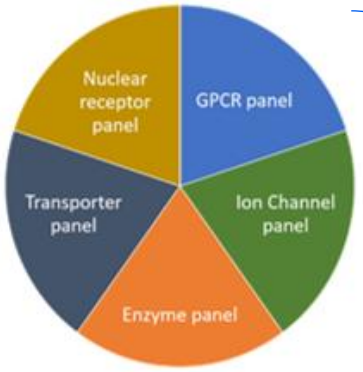
- TempO-seq technology – full gene panel
- 24hr exposure
- 7 concentrations
- Various cell models (e.g. HepG2, MCF7, HepaRG)
- Dose-response analysis using BMDExpress2 and BIFROST model



Reynolds et al. 2020. Comp Tox 16: 100138
Baltazar et al. 2020. Toxicol Sci 176(1): 236–252

Specific effects

In vitro pharmacological profiling



~79 targets

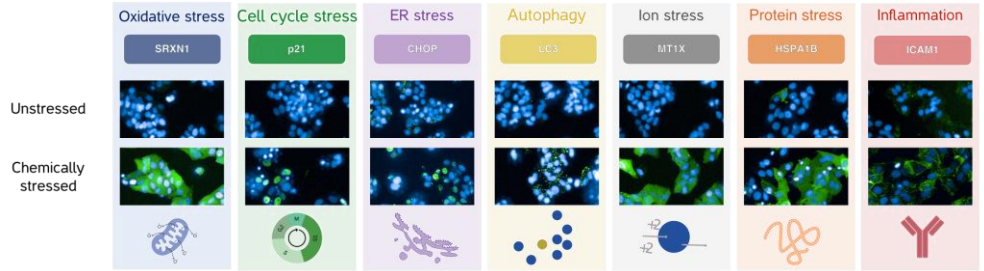

PERSPECTIVES

Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

eurofins | Cerep

Non-specific effects

ToxProfiler



- 7 biomarkers covering 7 cell stress pathways
- HepG2
- 24hr exposure
- 7 concentrations
- Dose-response analysis to derive PoD

Ter Braak et al. 2024. Toxicology 509, 153970

Non-specific effects

Cell stress panel (CSP)

- 36 biomarkers covering 10 cell stress pathways
- HepG2
- 24hr exposure
- 8 concentrations
- Dose-response analysis using BIFROST model

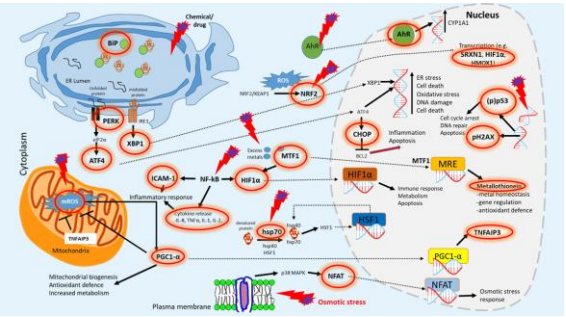


Image kindly provided by Paul Walker (Cyprotex)

Hatherell et al. 2020. Toxicol Sci 176(1): 11-33

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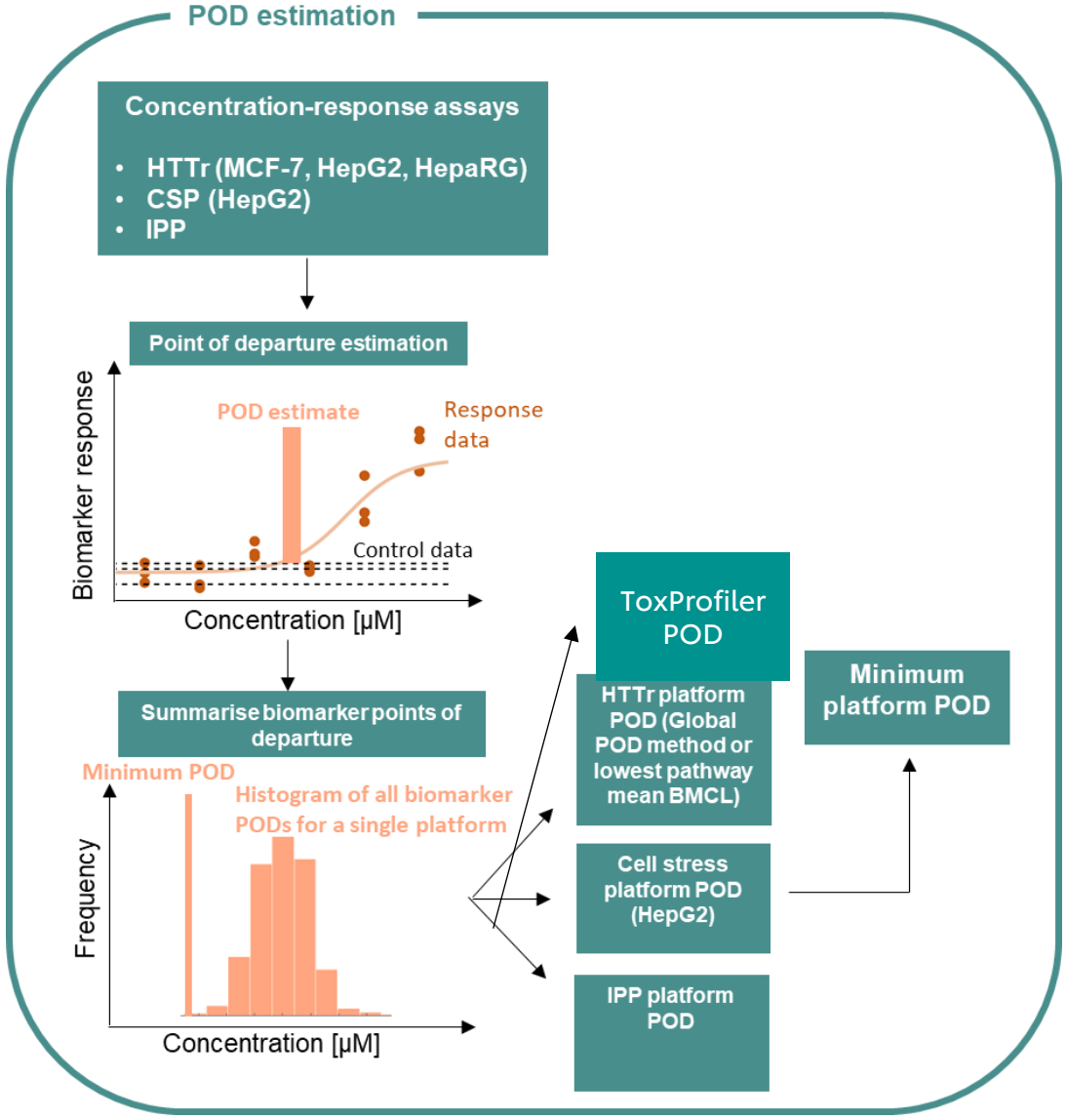
TOXICODYNAMICS SIMILARITY - Point of Departure (POD) ESTIMATION



- DEFI and SCI showed high similarity in the biological activity and potencies
- The lowest PODs for DEFI and SCI were derived from transcriptomics assays

DEFI: POD (µM) = 0.92

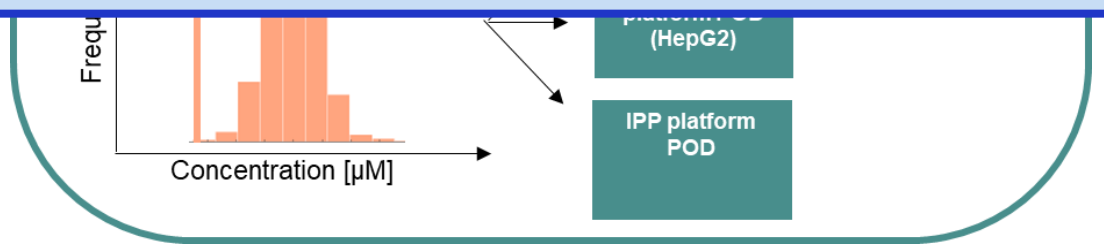
SCI: POD (µM) = 0.86

AE 2.2 & AE 2.3

TOXICODYNAMICS SIMILARITY - *in silico* & *in vitro* DATA

POD estimation

Both, *in silico* & *in vitro* data showed similar biological behaviour for DEFI and SCI



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BRIDGING STUDIES

AE 2.5

ASSAY	DEFI	SCI
Acute Oral Toxicity **OECD TG 401**	Results: LD50 (rat, m/f) 8400 mg/kg bw/day	1.Results: LD50 (rat, m/f) >2000 mg/kg bw/day 2. Results: LD50 (rat, m/f) >5000 mg/kg bw 3.Results: LD50 (rat, m/f) >5000 mg/kg bw
Skin irritation/corrosion (*in vivo*) **OECD TG 404**	Non-guideline study Results: Moderately irritating	Results: Slightly irritating
Eye irritation (*in vivo*) **OECD TG 405**	Non-guideline study Results: Moderately irritating	1. Results: Irritating 2. Results: Irritating 3. Results: Irritating
Genetic toxicity (mutagenicity): Bacterial Reverse Mutation test OECD TG 471	Not mutagenic in absence or presence of metabolic activation	Not mutagenic in absence or presence of metabolic activation
Genetic toxicity ToxTracker ACE	Not genotoxic	Not genotoxic

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BRIDGING STUDIES

Existing toxicological data showed that

DEFI and SCI are non-genotoxic, no acute toxicant & mild skin and eye irritants

Genetic toxicity ToxTracker ACE	Not genotoxic	Not genotoxic

UNCERTAINTY ASSESSMENT

AE A.4

SOURCE OF UNCERTAINTY	
Difference in chain length distributions	Difference in the level of free fatty acids

↓

Differences in some physico-chemical properties such as solubility, log P, surface tension and vapour pressure

Low uncertainty related to the source data to be read from SCI to DEFI

- High quality data (OECD guideline and GLP compliant)
- Detailed substance identity (TMI)

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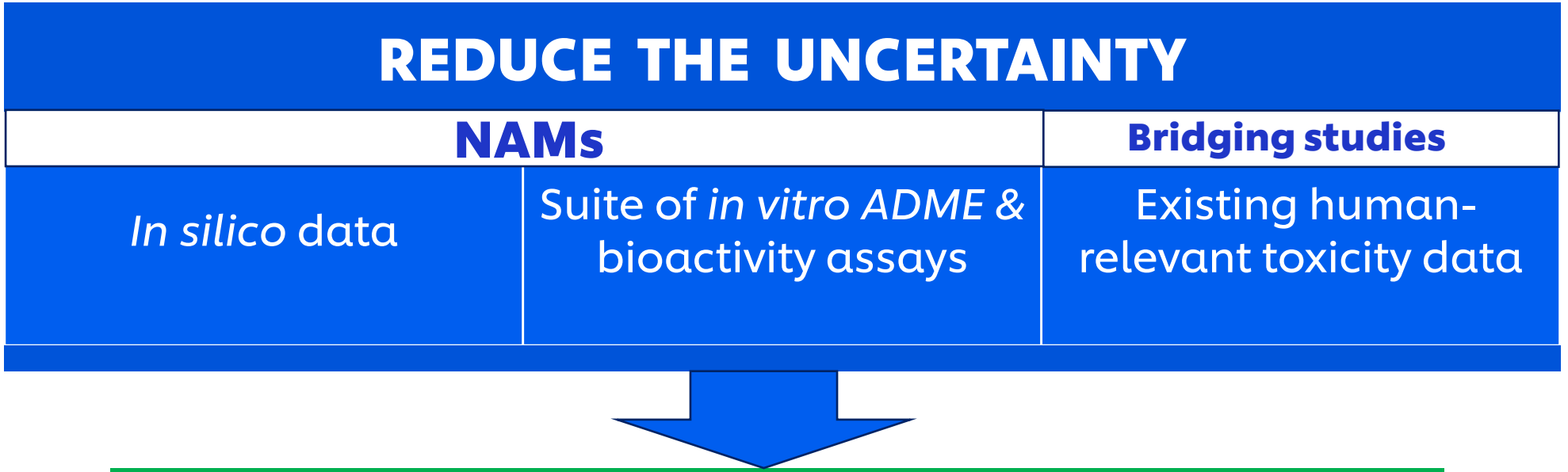
UNCERTAINTY ASSESSMENT

AE A.4



Demonstrate the similar biological (toxicokinetics and toxicodynamics) behaviour of the target (DEFI) and the source (SCI)

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READ-ACROSS SUMMARY

Target and source are different compounds with qualitatively similar properties

SIMILARITY JUSTIFICATION	Target DEFI	Source SCI	Similarities	Dissimilarities
Manufacture and exposure	• Esterification process • Usage in cosmetics	• Esterification process • Usage in cosmetics and home care	• Synthesis route • Occupational and consumer exposure	• Usage in home care
Structural/ compositional	• Contain alkyl isethionates and fatty acids of C8 to C18	• Contain alkyl isethionates and fatty acids of C8 to C18	• The same constituents • C12 isethionate the most abundant component	• Alkyl chain distribution • Level of free fatty acids
Physico-chemical	• Solid, non-volatile substance	• Solid, non-volatile substance	• MP, density, pKa	• Small differences in logP, WS, VP and γ
Toxicokinetics	• Low skin absorption low BP ratio • High PPB • Rapid metabolism to SI and FA	• Low skin absorption low BP ratio • High PPB • Rapid metabolism to SI and FA	• Low skin absorption • Remain in the plasma • Highly bound to plasma proteins • Rapidly metabolised to the same metabolites	NO
Toxicodynamics/ toxicity	• Lowest PoD = 0.92 • *In silico*: lack of alerts • No genotoxic • No acute toxicity • Skin and eye irritant	• Lowest PoD = 0.86 • *In silico*: lack of alerts • No genotoxic • No acute toxicity • Skin and eye irritant	Similar biological behaviour and potencies	NO

READ-ACROSS SUMMARY

Target and source are different compounds with qualitatively similar properties

Provided reliable scientific evidence that the source substance's (SCI) data for the 28-day oral and dermal toxicity studies can be read across to the target substance (DEFI)

/toxicity	• No genotoxic • No acute toxicity • Skin and eye irritant	• No genotoxic • No acute toxicity • Skin and eye irritant		

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CONCLUSIONS

- Demonstrated the regulatory compliant read-across case study for the complex mixtures (both target and source) for higher tier endpoint (short-term repeat dose)
- Strictly followed RAAF Scenario 2 (analogue) and RAAF for mixtures
- Robust similarity justification provided:
  - Manufacturing and exposure
  - Structural
  - Physico-chemical
  - Toxicokinetics
  - Toxicodynamics
- Demonstrated how the implementation of NAMs data (*in vitro* and *in silico*) can result in improved justification and reduced uncertainty
- Read-across justification requires multi-disciplinary team effort

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Mesha Williams
Kathryn Wolton
Adam Wood

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Thank You

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SELECTION of *in vitro* NAMs

- Internal project on developing strategy for NGRA – two phase study published in Pilot study (Middleton et al., 2022) and Extended evaluation(Cable et al., 2025)
- A set of in vitro assays was evaluated to investigate the exposure modelling and bioactivity coverage
- The selected assays have broad biological coverage relevant for both systemic and DART
- Assays with standardisation of study design & experimental protocols have been selected

Middleton AM, et al. Toxicol Sci. 2022, 25;189(1):124-147 https://doi.org/10.1093/toxsci/kfac068

Cable S, et al. Toxicol Sci. 2025, 204(1), 79–95 https://doi.org/10.1093/toxsci/kfae159

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Calculation of POD

- PODs were calculated using internal statistical model – BIFROST – for all biomarkers and genes across all cell lines
- The global POD represents an estimate of the minimum effect concentration across all biomarkers or genes. The method quantifies uncertainty in the POD as a probability distribution for each gene.

Reynolds J. et al. Computational Toxicology, 2020, 16, 100138,

https://doi.org/10.1016/j.comtox.2020.100138