National Institute of Environmental Health Sciences
Division of Translational Toxicology

The Skin Allergy Risk Assessment-Integrated Chemical Environment (SARA-ICE) Model for UN GHS classification – an evaluation and application in case studies

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Introduction

In chemico and *in vitro* OECD test guideline methods are available for use in skin sensitization assessment. No single method can currently be used to determine skin sensitization but can be used as part of a defined approach (DA). DAs allow new approach methods (NAMs) to be used in combination via a fixed data interpretation procedure. Currently the DAs accepted for regulatory use only provide information for skin sensitisation hazard and potency classification and are not suitable for point of departure (PoD) determination for use in quantitative risk assessment.

A collaboration between Unilever and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) has developed the Skin Allergy Risk Assessment-Integrated Chemical Environment (SARA-ICE) Model, a defined approach (DA) developed upon principles of the Unilever SARA Model (Reynolds *et al*., 2019, Reynolds *et al*., 2022). The SARA-ICE Model is designed to provide a weight-of-evidence (WoE) PoD and United Nations Globally Harmonized System for Classification and Labelling of Chemicals (GHS) classification prediction for use in skin sensitisation assessments.

The SARA-ICE core dataset utilises data within the publicly available Integrated Chemical Environment (ICE) database in addition to the published Unilever SARA database and Cosmetics Europe database. The model is constructed within the Bayesian statistical framework and allows for determination of a human relevant PoD termed the ED_{01}, defined as the dose with a 1% chance of inducing sensitisation following a human predictive patch test (HPPT) exposure. The PoD can be inferred using any combination of HPPT (human repeat insult patch test or human maximisation test), *in vivo* local lymph node assay (LLNA), and new approach methods (NAM [*in chemico* direct peptide reactivity assay (DPRA) and kinetic DPRA and *in vitro* KeratinoSens™, h-CLAT, or U-SENS™]) data. For a chemical of interest, the model returns the probability of each GHS classification conditional on the distribution of the ED_{01}.

Here we show some initial outputs of the SARA-ICE Model evaluation and its application for GHS classification of methylisothiazolinone (MIT) as a case study. Isothiazolinones (ITs) are widely used as antimicrobial preservatives in cosmetics and are known to have skin sensitising potential. This SARA-ICE analysis builds upon the work conducted by Strickland et al., 2022, where Shiseido Artificial Neural Networks (ANN) non-animal defined approaches (DA) for skin sensitization were evaluated for PoD estimates for use in quantitative risk assessment for ITs.

SARA-ICE Training Dataset

The SARA-ICE DA uses a core database of 434 chemicals with study results from 871 HPPTs, 535 LLNAs, 653 DPRAs, 361 kDPRAs, 1,030 KeratinoSens™, 483 h-CLATs and 388 U-Sens™. The number of studies per chemical is distributed heterogeneously, with a minimum of two studies for any single chemical.

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ED_{01} for Chemicals in SARA-ICE Database

The SARA-ICE Model can be used to obtain sensitiser potency estimates and UN GHS classifications from:

- NAM data only (DPRA, kDPRA, h-CLAT, KeratinoSens™, U-Sens™)
- *in vivo* data only (HPPT and/or LLNA)
- combinations of both for a weight-of-evidence estimate

SARA-ICE explicitly quantifies the uncertainty in both the continuous metric of sensitiser potency and discrete GHS classification.

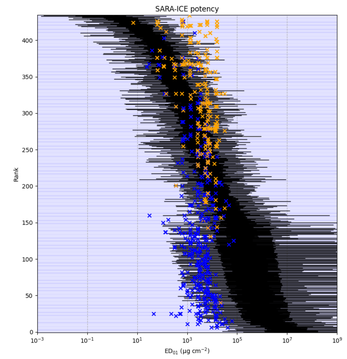


Figure 1. Estimates of ED_{01} for chemicals in SARA-ICE database. Blue x – the HPPT induction dose following which no individual was sensitised Orange x – the HPPT induction dose following which at least one subject was sensitised. ED_{01} estimates vary in precision. Precision in estimates a function of data availability. Standard deviation of estimates ranges from 0.3 – 1.8 units on the log10 scale

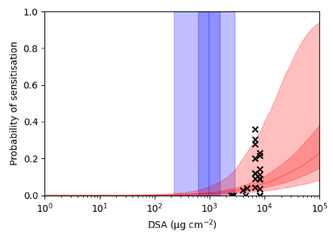


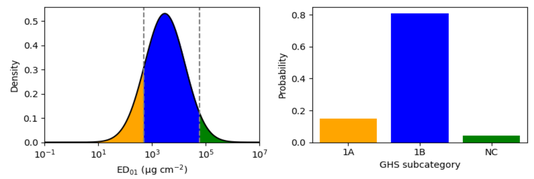
Figure 2. Red: Estimate of the average sensitisation rate to cinnamic alcohol given HPPT data only. Bands indicate uncertainty in estimates after accounting for inter and intra study variability (median, 50% and 95% intervals). Blue: ED_{01} estimates - dermally applied dose resulting in a 1% sensitisation rate (median, 50% and 95% intervals). '**x**' shows probability of sensitisation given ED_{01} estimates from a single HPPT study.

GHS Classification Probabilities

Continuous probability distribution of ED_{01} approximated into discrete probability distribution for GHS subcategories 1A, 1B and NC.

- Uses threshold of 500 μg/cm^2 for 1A/1B boundary (UN, 2021)
- Uses threshold of 60,000 μg/cm^2 for 1B/NC boundary (maximum dermal dose in a standard HPPT)
- Probability mass of each GHS subcategory equal to area under curve between thresholds of ED_{01} distribution

Figure 3. (a) Example estimate of ED_{01} distribution with overlay of GHS subcategories 1A, 1B and NC defined thresholds, (b) probability of each GHS subcategory from ED_{01} distribution



Distribution across GHS classes does not by itself result in a GHS classification. A decision model needs to be defined in order to obtain distinct SARA-ICE classifications. The proposed decision model requires two confidence thresholds to be defined, one for binary classification, one for subcategory classification conditional on binary class "1" being chosen. For example:

Binary classification threshold, θ_{bin}
Prior probability of binary class 1 is 0.67.
p(NC) for single NAM inputs <0.8
Therefore, set $\boldsymbol{\theta_{bin}=0.8}$

Subcategory classification threshold, θ_{sub}
Prior probability of 1A and 1B, given binary class 1, is 0.50.
Therefore, set $\boldsymbol{\theta_{sub}=0.55}$

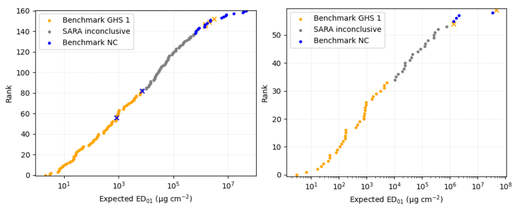


Figure 4. OECD LLNA (left) and human (right) benchmark binary classifications (OECD, 2021) based upon θ_{bin}=0.8. Grey – inconclusive classification. Yellow – sensitiser (GHS 1A/1B). Blue – non-sensitiser (GHS NC). Yellow points to the right of the grey are incorrect classifications, blue points to the left of the grey are incorrect classifications.

Methylisothiazolinone (MIT) Input Data

The skin sensitization potential of MIT was evaluated using both NAM data (*in chemico* DPRA and kDPRA, *in vitro* KeratinoSens™, h-CLAT, and U-Sens™. and *in vivo* data (LLNA and HRIPT).

NAM

- 1 DPRA study with 97.9% depletion of the cysteine peptide and 0% depletion of lysine peptide (Hoffmann et al., 2022).
- 1 kDPRA study with a log Kmax of -0.25 M-1s-1 (Natsch & Gerberick, 2022).
- 1 KeratinoSens™ study with an EC1.5 of 11.78 μM and an IC50 of 138.98 μM (Hoffmann et al., 2022).
- 1 h-CLAT study with a CD86 EC150 of 9.23 μg ml-1, a CD54 EC200 of 7.89 μg ml-1 and an IC50 of 24.7 μg ml-1 (Hoffmann et al., 2022).
- 1 U-Sens™ study with a CD86 EC150 of 9 μg ml-1 (Hoffmann et al., 2022).

In vivo

- 3 LLNA EC3s at 0.4%, 1.9% and 2.2% (Hoffmann et al., 2022).
- 6 HRIPTs with the following results (Giménez-Arnau, A. M. (2016):

Table 1. SARA-ICE Input HRIPT Data for MIT (Giménez-Arnau, A. M., 2016)

Induction dose (μg cm^{-2})	Number tested	Number sensitised
5	97	0
10	100	0
15	98	0
20	116	1
25	210	1
30	75	0

Methylisothiazolinone (MIT) Results

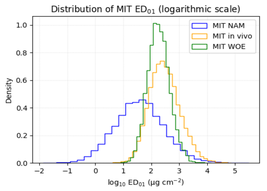


Figure 5. Distribution of ED_{01} for MIT given;
Blue – NAM data only
Orange – *in vivo* data only
Green – NAM + *in vivo* data (WoE)

Figure 6. Probability of each GHS subcategory from ED_{01} distribution given;
Blue – NAM data only
Orange – *in vivo* data only
Green – NAM + *in vivo* data (WoE)

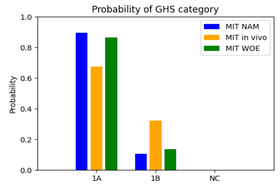


Table 2. SARA-ICE estimated ED_{01} and GHS sub-category call with probabilities of each class, dependent on input data of either NAM data only, *in vivo* data only or NAM and *in vivo* data

	ED_{01} percentiles (μg cm^{-2})					GHS categories			
	2.5th	25th	50th	75th	97.5th	Subcategory call	Prob. 1A	Prob. 1B	Prob. NC
NAM	0.75	9.7	37	140	2,400	1A	0.90	0.10	~0
In vivo	32	130	280	670	4,300	1A	0.68	0.32	~0
NAM + in vivo	33	100	180	330	1,200	1A	0.87	0.13	~0

Discussion

The SARA-ICE Model is a probabilistic method which is able to integrate multiple skin sensitisation data inputs in various combinations and will support GHS classification of skin sensitisers, in addition to providing a human-relevant point of departure, with quantified uncertainty, for quantitative risk assessment. Currently, SARA-ICE is undergoing evaluation via the OECD Defined Approach Skin Sensitisation (DASS) Expert Group for potential inclusion in Guideline 497: Defined Approaches on Skin Sensitisation. Ultimately, the SARA-ICE Model will be publicly available in the NICEATM Integrated Chemical Environment.

Binary classification performance of the SARA-ICE Model using NAM inputs only against LLNA and human benchmarks results in an inconclusive rate of around 33% for benchmark class 1 and 40% for the NC benchmark. Sensitivity, specificity and balanced accuracy for conclusive predictions was 95%, 89% and 92%, respectively versus LLNA benchmarks, and sensitivity, specificity and balanced accuracy for conclusive predictions was 94%, 100% and 97%, respectively for human benchmarks.

The SARA-ICE Model estimates with high probability that MIT is a sensitiser and most likely to be in the 1A category, with the most confident prediction of 1A resulting from use of NAM data only (0.90). The Scientific Committee on Consumer Safety (SCCS), identified a NESIL of 15μg/cm^2. In comparison, the SARA-ICE Model estimates a median ED01 of between 37-260μg/cm^2 for estimates based upon NAM data and *in vivo* data, respectively. The 2.5th of the ED01 was estimated as between 0.75-33μg/cm^2 based upon NAM data and NAM + *in vivo* data, respectively. These estimates are comparable to the DSA metric of 210μg/cm^2 transformed from the ANN D_hC_KS estimated EC3 of 0.83%.

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