National Institute of Environmental Health Sciences
Division of Translational Toxicology

SARA-ICE: A Self-contained Model for Predicting a Human-relevant Point-of-departure for Skin Sensitization

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Background

- Under the OECD Test Guidelines (TG) Programme, Defined Approaches (DAs) allow non-animal new approach methodologies (NAMs) to be used in combination via fixed data interpretation procedures for endpoints such as skin sensitization.
- DAs currently accepted for regulatory use under TG 497 only provide information for skin sensitization hazard and potency classification and are not yet suitable for determining a point of departure (PoD) for use in quantitative risk assessment.
- Unilever and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) have developed the **Skin Allergy Risk Assessment-Integrated Chemical Environment (SARA-ICE) Model**, a DA based on the Unilever SARA Model [1,2]. The SARA-ICE Model provides a weight-of-evidence (WoE) PoD and United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) classification prediction for use in skin sensitization assessments [3].
- SARA-ICE uses data from the publicly available Integrated Chemical Environment (ICE; https://ice.ntp.niehs.nih.gov/) database and the Unilever SARA and Cosmetics Europe databases. The model uses a Bayesian statistical framework to define the ED01, a human-relevant PoD representing the dose with a 1% chance of inducing sensitization in a human predictive patch test (HPPT) population. The ED01 is calculated using any combination of HPPT, local lymph node assay (LLNA), and/or NAM data. NAMs include the in chemico direct peptide reactivity assay (DPRA) or kinetic DPRA and the in vitro KeratinoSens™, h-CLAT, or U-SENS™ assays. For a chemical of interest, the model returns the probability of each GHS classification conditional on the distribution of the ED_{01}.
- A free, publicly available, software container with user-friendly graphic user interface (GUI) has been developed for SARA-ICE.
- Here we present a case study using the SARA-ICE Model to assess the skin sensitization potency of six isothiazolinones (Table 1). Isothiazolinones are widely used as antimicrobial preservatives/biocides and are known to have skin sensitizing potential (GHS category 1 or 1A for the isothiazolinones assessed in this study [4]). This study builds upon previous work [5] that applied non-animal DAs for skin sensitization to generate quantitative risk assessments for isothiazolinones.

Figure 1. SARA-ICE Workflow

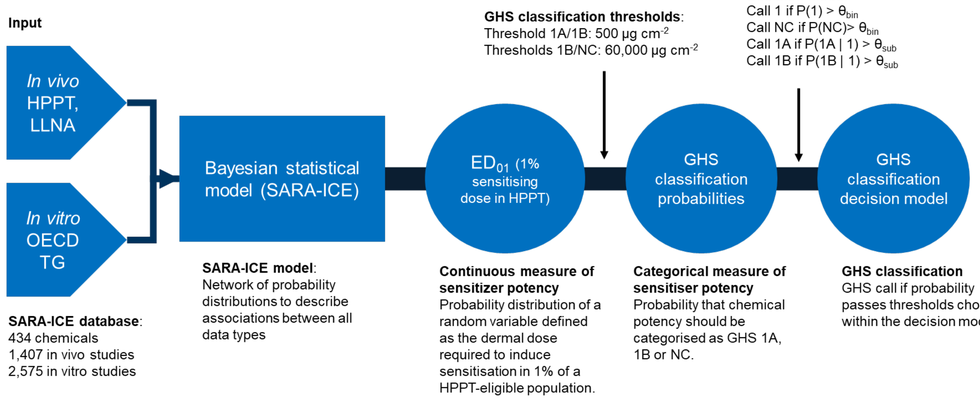
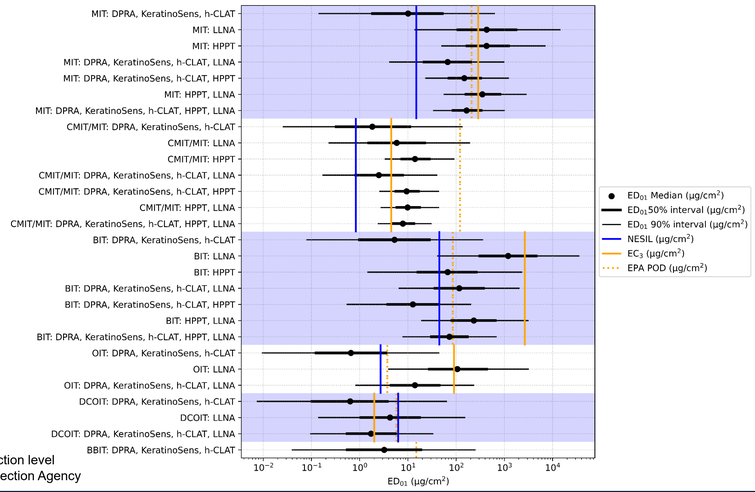


Figure 2. ED_{01} Estimates for Isothiazolinones

- ED_{01} estimates for each isothiazolinone, given different permutations of input data, are represented as centered 90% credible intervals (thin line), 50% credible intervals (thick line) and median (bullet).
- Blue vertical lines indicate the reference NESIL*.
- Dashed yellow lines indicate the US EPA** PoD.
- Solid yellow lines indicate the reference LLNA EC_3.



*NESIL = No expected skin sensitization induction level
**US EPA = United States Environmental Protection Agency

Table 1. Isothiazolinones Considered in this Study

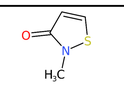
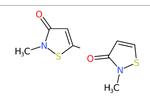
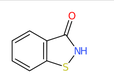
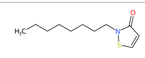
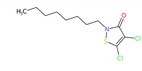
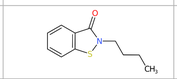
Chemical Name (Abbreviation; CAS RN)	MW	Chemical Structure	Input data: DPRA [6] (% depletion)	Input data: KeratinoSens [6]	Input data: h-CLAT [6]	Input data: LLNA [6]	Input data: HPPT [7,8]	Reference Data: NESIL (µg cm-2)	Reference Data: GHS [4]	Reference Data: EPA POD [9] (µg cm-2)	Reference Data: LLNA EC_3 [10] (µg cm-2)
MIT (2-Methyl-4-isothiazolin-3-one; 2682-20-4	115.16		Cys: 100% Lys: 0%	$EC_{1.5}$: 9.54 µM IC_{50}: 108 µM	CD54 EC_{200}: 11.6 µg/ml CD86 EC_{150}: 11.8 µg/ml CV_{75}: 24.6 µg/ml	EC_3: 0.4% to >4.5% (4 studies)	DSA: 10 µg cm-2 to 30 µg cm-2 Ntested: 75 to 210 Nsensitised: 0 to 1 (6 studies)	15 [11]	1A	210	288
CMIT/MIT (Mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one; 55965-84-9)	141.36 [12]		Cys: 100% Lys:: 10.6%	$EC_{1.5}$: 3.41 µM IC_{50}: 19.9 µM	CD54 EC_{200}: 2.63 µg/ml CD86 EC_{150}: 2.81 µg/ml CV_{75}: 3.04 µg/ml	EC_3: 0.0049% to 0.048% (9 studies)	DSA: 0.83 µg cm-2 to 79 µg cm-2 Ntested: 45 to 602 Nsensitised: 0 to 7 (13 studies)	0.83 [12]	1A	120	4.5
BIT (1,2-Benzisothiazolin-3-one; 2634-33-5)	151.18		Cys: 100% Lys: 0%	$EC_{1.5}$: 3.14 µM IC_{50}: 57.8 µM	CD54 EC_{200}: 7.63 µg/ml CD86 EC_{150}: 7.84 µg/ml CV_{75}: 13.1 µg/ml	EC_3: 1.5% to 32.4% (7 studies)	DSA: 45 µg cm-2 to 91 µg cm-2 Ntested: 54 to 58 Nsensitised: 0 to 5 (2 studies)	45 [13]	1	85	2642
OIT (2-n-Octyl-4-isothiazolin-3-one; 26530-20-1)	213.34		Cys: 100% Lys: 1.3%	$EC_{1.5}$: 2.19 µM IC_{50}: 12.7 µM	CD54 EC_{200}: 0.95 µg/ml CD86 EC_{150}: 7.26 µg/ml CV_{75}: 8.8 µg/ml	EC_3: 0.2% to 0.66% (4 studies)		2.7 [14]	1A	3.75	90.25
DCOIT (4,5-Dichloro-2-octyl-3(2H)-isothiazolone; 64359-81-5)	282.23		Cys: 100% Lys: 11.6%	$EC_{1.5}$: 1.32 µM IC_{50}: 4.65 µM	CD54 EC_{200}: 0.92 µg/ml CD86 EC_{150}: >1.081 µg/ml CV_{75}: 0.9 µg/ml	EC_3: 0.0041% to 0.011% (2 studies)		6.3 [14]	1A	5.8	2
BBIT (1,2-Benzisothiazolin-3-one, 2-butyl; 4299-07-4)	207.29		Cys: 100% Lys: 0%	$EC_{1.5}$: 3.84 µM IC_{50}: 53.0 µM	CD54 EC_{200}: 3.01 µg/ml CD86 EC_{150}: 3.15 µg/ml CV_{75}: 3.3 µg/ml			N/A	1	15	N/A

Table 2. Estimated ED_{01} and GHS Classification for Isothiazolines

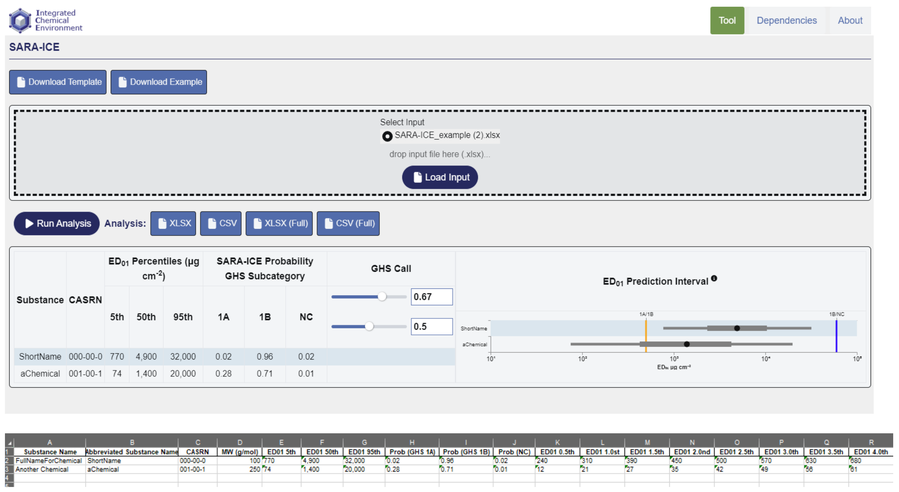
Chemical	Input Data	ED_{01} percentiles (µg cm-2) 5th	50th	95th	SPUR (50th / 5th)	Pr(GHS 1A)	Pr(GHS 1B)	Pr(NC)	Classification GHS binary	Classification GHS subcategory
MIT	DPRA, KeratinoSens, h-CLAT	0.14	10	6.1e+02	70	0.94	0.06	0.00	1	1A
MIT	LLNA	14	4.3e+02	1.4e+04	31	0.53	0.46	0.01	1	Inconclusive
MIT	HPPT	51	4.3e+02	7e+03	8.4	0.54	0.45	0.00	1	Inconclusive
MIT	DPRA, KeratinoSens, h-CLAT, LLNA	4.2	67	9.7e+02	16	0.89	0.11	0.00	1	1A
MIT	DPRA, KeratinoSens, h-CLAT, HPPT	24	1.5e+02	1.2e+03	6.3	0.84	0.16	0.00	1	1A
MIT	HPPT, LLNA	57	3.5e+02	2.8e+03	6.1	0.61	0.39	0.00	1	Inconclusive
MIT	DPRA, KeratinoSens, h-CLAT, HPPT, LLNA	34	1.6e+02	9.9e+02	4.8	0.86	0.14	0.00	1	1A
CMIT/MIT	DPRA, KeratinoSens, h-CLAT	0.026	1.8	1.3e+02	70	0.98	0.02	0.00	1	1A
CMIT/MIT	LLNA	0.23	5.9	1.9e+02	25	0.98	0.02	0.00	1	1A
CMIT/MIT	HPPT	3.4	14	90	4.1	1.00	0.00	0.00	1	1A
CMIT/MIT	DPRA, KeratinoSens, h-CLAT, LLNA	0.18	2.5	39	14	1.00	0.00	0.00	1	1A
CMIT/MIT	DPRA, KeratinoSens, h-CLAT, HPPT	2.6	9.4	43	3.6	1.00	0.00	0.00	1	1A
CMIT/MIT	HPPT, LLNA	2.8	9.9	43	3.5	1.00	0.00	0.00	1	1A
CMIT/MIT	DPRA, KeratinoSens, h-CLAT, HPPT, LLNA	2.4	7.9	30	3.2	1.00	0.00	0.00	1	1A
BIT	DPRA, KeratinoSens, h-CLAT	0.081	5.3	3.5e+02	65	0.96	0.04	0.00	1	1A
BIT	LLNA	42	1.2e+03	3.5e+04	29	0.33	0.64	0.03	1	Inconclusive
BIT	HPPT	1.5	67	2.3e+03	45	0.83	0.16	0.00	1	1A
BIT	DPRA, KeratinoSens, h-CLAT, LLNA	6.7	1.2e+02	2e+03	17	0.81	0.19	0.00	1	1A
BIT	DPRA, KeratinoSens, h-CLAT, HPPT	0.55	13	2e+02	23	0.98	0.02	0.00	1	1A
BIT	HPPT, LLNA	19	2.3e+02	3.1e+03	12	0.69	0.31	0.00	1	1A
BIT	DPRA, KeratinoSens, h-CLAT, HPPT, LLNA	7.9	73	6.7e+02	9.2	0.93	0.07	0.00	1	1A
OIT	DPRA, KeratinoSens, h-CLAT	0.0096	0.66	43	68	0.99	0.01	0.00	1	1A
OIT	LLNA	4	1.1e+02	3.1e+03	26	0.78	0.22	0.00	1	1A
OIT	DPRA, KeratinoSens, h-CLAT, LLNA	0.84	14	2.3e+02	17	0.98	0.02	0.00	1	1A
DCOIT	DPRA, KeratinoSens, h-CLAT	0.0076	0.64	63	84	0.99	0.01	0.00	1	1A
DCOIT	LLNA	0.14	4.3	1.5e+02	30	0.98	0.02	0.00	1	1A
DCOIT	DPRA, KeratinoSens, h-CLAT, LLNA	0.097	1.7	33	18	1.00	0.00	0.00	1	1A
BBIT	DPRA, KeratinoSens, h-CLAT	0.04	3.2	2.5e+02	81	0.97	0.03	0.00	1	1A

SPUR = SARA-ICE Prediction Uncertainty Ratio, Pr = Probability, NC = Not classified

Discussion

- The SARA-ICE DA classified all compounds as likely to be GHS Category 1A in instances where a conclusive classification was possible. For MIT and BIT, SARA-ICE was unable to assign a GHS subcategory for instances where LLNA or HPPT data were the only input values for the DA.
- For the purposes of deriving safe exposure levels, SARA-ICE predictions using only NAM data provided a more protective PoD for risk assessment than the NESILs (referenced in Table 1), except for CMIT/MIT.
- A measure of uncertainty (the SARA-ICE prediction uncertainty ratio, SPUR) provides the width of the interval between the 5th and 50th percentiles. Generally, the greater the number of data inputs, the narrower the SPUR estimates and the more certainty in the ED_{01} estimate.
- A GUI allowing easy access to the SARA-ICE model has been developed (Figure 3). It provides estimates of ED_{01} at the 5th, 50th, and 95th percentiles and the ability to predict GHS subcategories based on varying confidence thresholds. Outputs also include the ED_{01} estimate for the 0.5th to 99.5th percentiles in a downloadable format.
- The SARA-ICE model provides a human relevant PoD for use in risk assessment. The model is currently under evaluation for inclusion in TG 497.

Figure 3. SARA-ICE Software Container GUI



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