National Institute of Environmental Health Sciences
Division of Translational Toxicology

Application of Skin Allergy Risk Assessment-Integrated Chemical Environment Defined Approach (SARA-ICE DA) to Assess Skin Sensitization Potency of Isothiazolinone Compounds

Abstract 3506
Poster P742

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Introduction

- In chemico and in vitro OECD test guideline methods are available for use in skin sensitization assessment. None of these methods can currently be used individually to determine skin sensitization potential but can be used as part of a defined approach (DA).
  - DAs allow non-animal new approach methodologies (NAMs) to be used in combination via a fixed data interpretation procedure. DAs currently accepted for regulatory use only provide information for skin sensitization hazard and potency classification and are not suitable for point of departure (PoD) determination 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 developed upon principles of the Unilever SARA Model (Reynolds et al. 2019, Reynolds et al. 2022). 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.
- SARA-ICE utilizes 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 ED01, defined as the dose with a 1% chance of inducing sensitization following a human predictive patch test (HPPT) exposure. The PoD can be calculated using any combination of HPPT in vivo local lymph node assay (LLNA), and NAM data. NAMs used include the in chemico direct peptide reactivity assay (DPRA) and 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 ED01.
- Here we apply the SARA-ICE Model to assess the skin sensitization potency of six isothiazolinones (Table 1) as a case study. Isothiazolinones are widely used as antimicrobial preservatives/biocides and are known to have skin sensitizing potential. This SARA-ICE analysis builds upon previous work (Strickland et al. 2022), where Shiseido Artificial Neural Networks (ANN) non-animal DAs for skin sensitization were evaluated for PoD estimates for use in quantitative risk assessment for isothiazolinones.

Figure 1. SARA-ICE Model

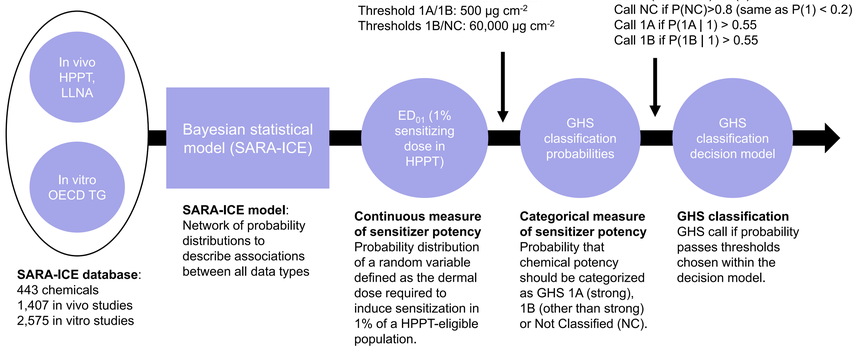


Table 1. Isothiazolinones Considered in This Study

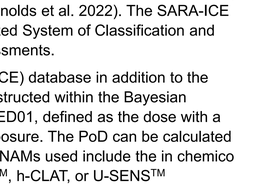
Abbreviation	Chemical Name	CASRN	Molecular Weight	Chemical Structure
MIT	2-Methyl-4-isothiazolin-3-one	2682-20-4	115.16	
CMIT/MIT	Mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one	55965-84-9	141.36 (Average MW assuming CMIT: MIT ratio of 0.761: 0.239)	
BIT	1,2-Benzisothiazolin-3-one	2634-33-5	151.18	
OIT	2-n-Octyl-4-isothiazolin-3-one	26530-20-1	213.34	
DCOIT	4,5-Dichloro-2-octyl-3(2H)-isothiazolone	64359-81-5	282.23	
BBIT	1,2-Benzisothiazolin-3-one, 2-butyl	4299-07-4	207.29	

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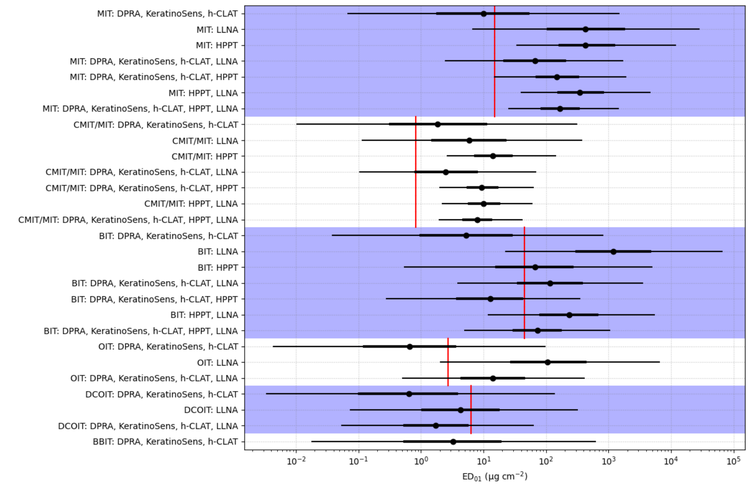
Table 2. SARA-ICE Input Data for Isothiazolinones

Study Type	Chemical						Source
	MIT	**CMIT/MIT**	**BIT**	**OIT**	**DCOIT**	**BBIT**	
DPRA	Cysteine depletion: 100% Lysine depletion: 0%	Cysteine depletion: 100% Lysine depletion: 10.6%	Cysteine depletion: 100% Lysine depletion: 0%	Cysteine depletion: 100% Lysine depletion: 1.3%	Cysteine depletion: 100% Lysine depletion: 11.6%	Cysteine depletion: 100% Lysine depletion: 0%	NICEATM IT report, Appendix A, Table 2
KeratinoSens™	$EC_{1.5}$: 9.54 µM IC_{50}: 108 µM	$EC_{1.5}$: 3.41 µM IC_{50}: 19.9 µM	$EC_{1.5}$: 3.14 µM IC_{50}: 57.8 µM	$EC_{1.5}$: 2.19 µM IC_{50}: 12.7 µM	$EC_{1.5}$: 1.32 µM IC_{50}: 4.65 µM	$EC_{1.5}$: 3.84 µM IC_{50}: 53.0 µM	NICEATM IT report, Appendix A, Table 5
h-CLAT	CD54 EC_{200}: 11.8 µg ml^{-1} CD86 EC_{150}: 11.8 µg ml^{-1} CV_{75}: 24.6 µg ml^{-1}	CD54 EC_{200}: 2.63 µg ml^{-1} CD86 EC_{150}: 2.81 µg ml^{-1} CV_{75}: 3.04 µg ml^{-1}	CD54 EC_{200}: 7.83 µg ml^{-1} CD86 EC_{150}: 7.84 µg ml^{-1} CV_{75}: 13.1 µg ml^{-1}	CD54 EC_{200}: 0.95 µg ml^{-1} CD86 EC_{150}: 7.26 µg ml^{-1} CV_{75}: 8.8 µg ml^{-1}	CD54 EC_{200}: 0.92 µg ml^{-1} CD86 EC_{150}: >1.08[1] µg ml^{-1} CV_{75}: 0.9 µg ml^{-1}	CD54 EC_{200}: 3.01 µg ml^{-1} CD86 EC_{150}: 3.15 µg ml^{-1} CV_{75}: 3.3 µg ml^{-1}	NICEATM IT report, Appendix A, Tables 7 & 8
LLNA	EC_3: 0.4% to > 4.5% (4 studies)	EC_3: 0.0049% to 0.048% (9 studies)	EC_3: 1.5% to 32.4% (7 studies)	EC_3: 0.2% to 0.66% (4 studies)	EC_3: 0.0041% to 0.011% (2 studies)		NICEATM IT report, Appendix C
HPPT	DSA: 10 µg/cm^2 to 30 ug/cm^2 N_{tested}: 75 to 210 $N_{sensitizer}$: 0 to 1 (6 studies)	DSA: 0.83 µg/cm^2 to 79 ug/cm^2 N_{tested}: 45 to 602 $N_{sensitizer}$: 0 to 7 (13 studies)	DSA: 45 µg/cm^2 to 91 ug/cm^2 N_{tested}: 54 to 58 $N_{sensitizer}$: 0 to 5 (2 studies)				Strickland et al., 2023; Herzler et al., 2024

$EC_{1.5}$, effective concentration producing a 1.5-fold response; IC_{50}, concentration producing 50% inhibition; $EC_{150/200}$, Effective concentration producing a 150% or 200% increase; EC_3, effective concentration producing a 3-fold response (stimulation index); DSA, dose per skin area

Results: SARA-ICE Estimates for Isothiazolinones

Figure 2: SARA-ICE ED01 Estimates for Six Isothiazolinones based on Combinations of In Vitro and In Vivo Data



ED01 estimates represented as centered 95% credible intervals (thin line), 50% credible intervals (thick line) and median (bullet). Red lines are plotted at the reference "no expected sensitization induction level" (NESIL) values listed in Table 3 (below).

Table 3: SARA-ICE ED01 and GHS Subcategory Probability Estimates (benchmark reference values included for context)

Chemical	Input Data Combination	SARA-ICE Mean ED01 (µg cm^{-2})	SARA-ICE Probability GHS Subcategory			SARA-ICE GHS Call	Reference Values	
			1A	1B	NC		NESIL (µg cm^{-2})	GHS Subcategory
MIT	DPRA, KeratinoSens™, h-CLAT	9.9	0.94	0.06	0.00	1A	15[1]	1A[2]
	LLNA	4.3e+02	0.53	0.46	0.01	Inconclusive		
	HPPT	4.8e+02	0.54	0.45	0.00	Inconclusive		
	DPRA, KeratinoSens™, h-CLAT, LLNA	66	0.89	0.11	0.00	1A		
	DPRA, KeratinoSens™, h-CLAT, HPPT	1.5e+02	0.84	0.16	0.00	1A		
	HPPT, LLNA	3.7e+02	0.61	0.39	0.00	1A		
	DPRA, KeratinoSens™, h-CLAT, HPPT, LLNA	1.7e+02	0.86	0.14	0.00	1A		
CMIT/MIT	DPRA, KeratinoSens™, h-CLAT	1.9	0.98	0.02	0.00	1A	0.83[3]	IA[2]
	LLNA	6	0.98	0.02	0.00	1A		
	HPPT	15	1.00	0.00	0.00	1A		
	DPRA, KeratinoSens™, h-CLAT, LLNA	2.6	1.00	0.00	0.00	1A		
	DPRA, KeratinoSens™, h-CLAT, HPPT	9.8	1.00	0.00	0.00	1A		
	HPPT, LLNA	10	1.00	0.00	0.00	1A		
	DPRA, KeratinoSens™, h-CLAT, HPPT, LLNA	8.1	1.00	0.00	0.00	1A		
BIT	DPRA, KeratinoSens™, h-CLAT	5.3	0.96	0.04	0.00	1A	45[4]	1[2]
	LLNA	1.2e+03	0.33	0.64	0.03	1B		
	HPPT	63	0.83	0.16	0.00	1A		
	DPRA, KeratinoSens™, h-CLAT, LLNA	1.2e+02	0.81	0.19	0.00	1A		
	DPRA, KeratinoSens™, h-CLAT, HPPT	12	0.98	0.02	0.00	1A		
	HPPT, LLNA	2.4e+02	0.69	0.31	0.00	1A		
	DPRA, KeratinoSens™, h-CLAT, HPPT, LLNA	73	0.93	0.07	0.00	1A		
OIT	DPRA,KeratinoSens™, h-CLAT	0.66	0.99	0.01	0.00	1A	2.7[5]	1A[2]
	LLNA	1.1e+02	0.78	0.22	0.00	1A		
	DPRA, KeratinoSens™, h-CLAT, LLNA	14	0.98	0.02	0.00	1A		
DCOIT	DPRA, KeratinoSens™, h-CLAT	0.64	0.99	0.01	0.00	1A	6.3[5]	1A[2]
	LLNA	4.4	0.98	0.02	0.00	1A		
	DPRA, KeratinoSens™, h-CLAT, LLNA	1.7	1.00	0.00	0.00	1A		
BBIT	DPRA, KeratinoSens™, h-CLAT	3.2	0.97	0.03	0.00	1A	N/A	1[2]

References for benchmark reference values: 1) ECHA database; 2) SCCS 2016; 3) Burnett et al. 2021; 4) Novick et al. 2013; 5) Ladics 2020.

Discussion

- SARA-ICE DA is a probabilistic model that integrates multiple skin sensitization data inputs in various combinations.
- SARA-ICE DA supports GHS classification of skin sensitizers and provides a human-relevant point of departure, with uncertainty, for quantitative risk assessment.
- SARA-ICE DA was applied to six isothiazolinones as case studies to explore DA performance when applied to a data-rich family of compounds with known sensitization potential.
- Of the six isothiazolinones evaluated here, all are currently classified as either GHS category 1 or 1A for skin sensitization (note: BIT and BBIT are classified as category 1). SARA-ICE DA estimated that all compounds are likely to be GHS category 1A, except for MIT when LLNA or HPPT data are the only input values and BIT when LLNA data are the only input values.
- Benchmark NESIL values have been derived for all isothiazolinone case studies except BIT (Table 3). The values for the NESILs vary from 0.83 to 45 µg/cm^2.
- The correlation of NESILs and SARA-ICE DA ED01 varies with each type of input (Figure 3, Table 3), with most of the estimates from only NAMs-based data being more conservative than the NESILs. In this case, CMIT/MIT was the only chemical with an ED01 estimate (median) greater than the NESIL (1.8 µg/cm^2 vs. 0.83 µg/cm^2).
- For the purposes of deriving safe-exposure levels, SARA-ICE predictions using only NAMs-based data would provide a more protective PoD for risk assessment than the NESIL.

Next Steps

- 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 (OECD 2021).
- Ultimately, the SARA-ICE Model will be publicly available as a containerized version available in GitHub and eventually housed on the NICEATM ICE platform (https://ice.ntp.niehs.nih.gov).

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