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Supplementary Information for

Predicting Skin Irritation, Corrosion, and Sensitization Potential of Nanomaterials Using OECD-Validated New Approach Methodologies (NAMs)

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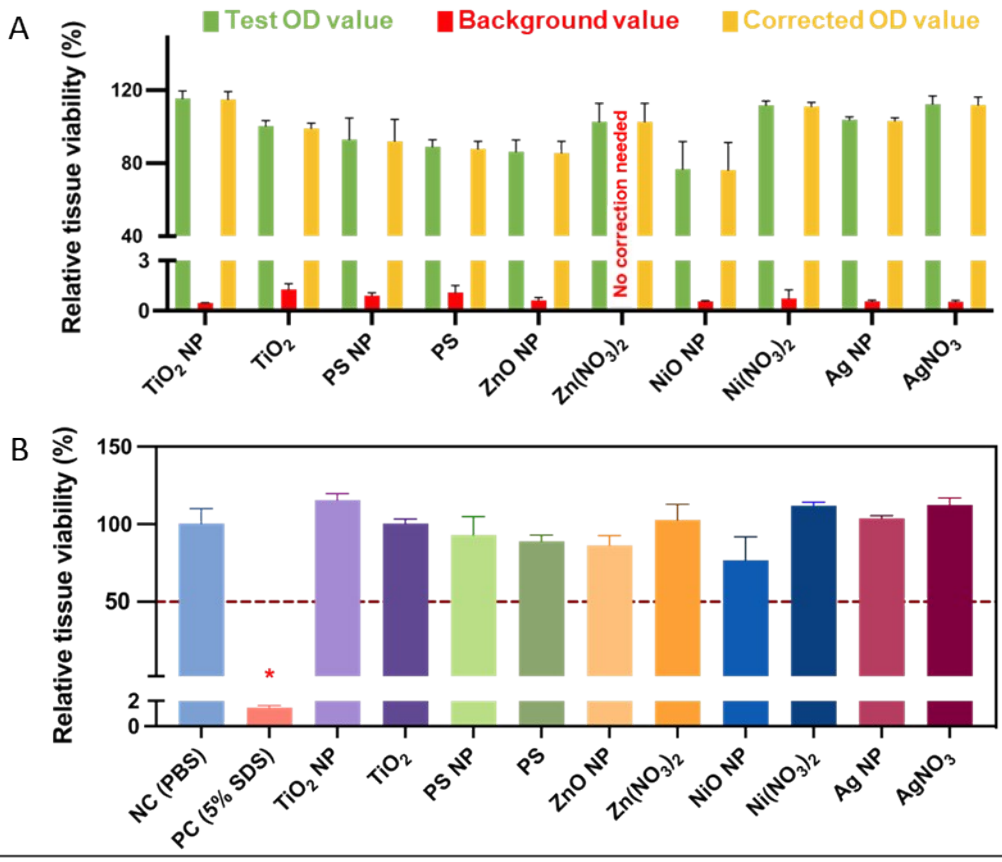

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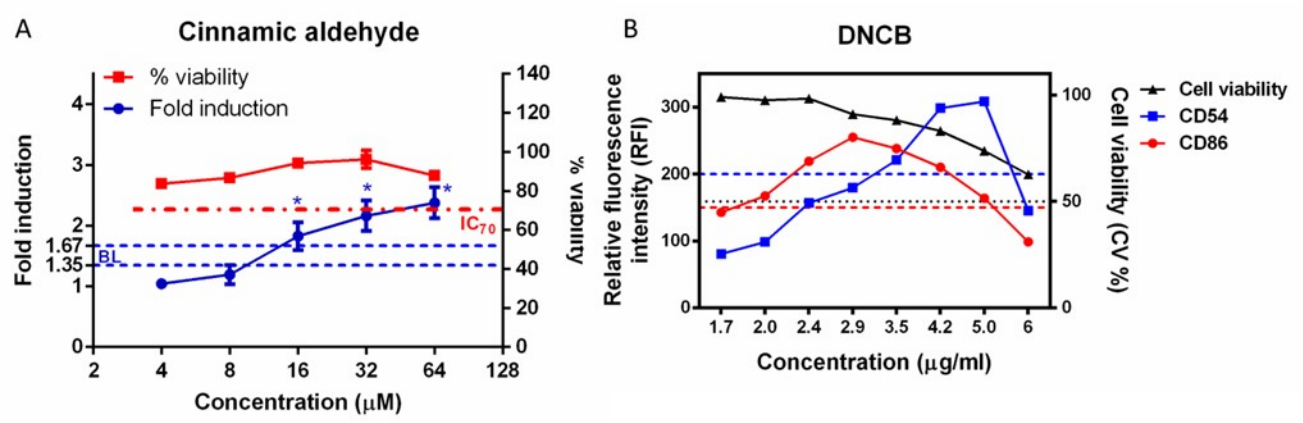
EPiTRI-SIT results (High concentration: 1000 µg/mL)						
Nanomaterials (NMs)			Corresponding materials (bulks or metal ions)			
Test substance	Relative tissue viability (%)	UN GHS Classification	Test substance	Relative tissue viability (%)	UN GHS Classification	
1000 µg/mL TiO_2 NPs	115.8 ± 4.1	No category	1000 µg/mL TiO_2	100.4 ± 2.9	No category	bulks
1000 µg/mL PS NPs	93.0 ± 11.8	No category	1000 µg/mL PS	89.0 ± 3.9	No category	bulks
1000 µg/mL ZnO NPs	87.0 ± 6.5	No category	82.3 µM $Zn(NO_3)_2{\cdot}6H_2O$*	102.7 ±10.2	No category	released metal ions
1000 µg/mL NiO NPs	76.8 ± 15.1	No category	54.2 µM $Ni(NO_3)_2{\cdot}6H_2O$*	112.4 ± 2.3	No category	released metal ions
1000 µg/mL Ag NPs	104.2 ± 1.6	No category	0.528 µM $AgNO_3$*	112.8 ± 4.4	No category	released metal ions
PBS (NC)	100.0 ± 8.8	No category	5% SDS (PC)	1.5 ± 0.2	Skin irritant	

Figure S1. (A) Relative cell viability results from the functional check prior to skin irritation/corrosion tests using the 3D RHE model. Green bars represent the measured OD value, red bars represent the background OD value of each test substance, and yellow bars represent the corrected OD value (green − red). (B) Quantification of relative tissue viability after 30-minute exposure to 1 mg/mL suspensions of the five nanoparticles (NPs) and their corresponding bulk materials or released ions (measured by ICP-MS). (C) Classification of test substances according to OECD TG 439 (bottom-up approach). Mean ± S.D ($n \geq 3$); * p-value ≤ 0.05 compared to control.



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Positive control substances	KeratinoSens™ assay results			
Cinnamic aldehyde	I_{max}	$EC_{1.5}$ (μM)	IC_{70} (μM)	Prediction
	2.49	8.72	> 64	Positive
DNCB	h-CLAT assay results			
	CD 86 RFI	CD 54 RFI	CV 50 (μg/ml)	Prediction
	> 150%	> 200%	> 6	Positive

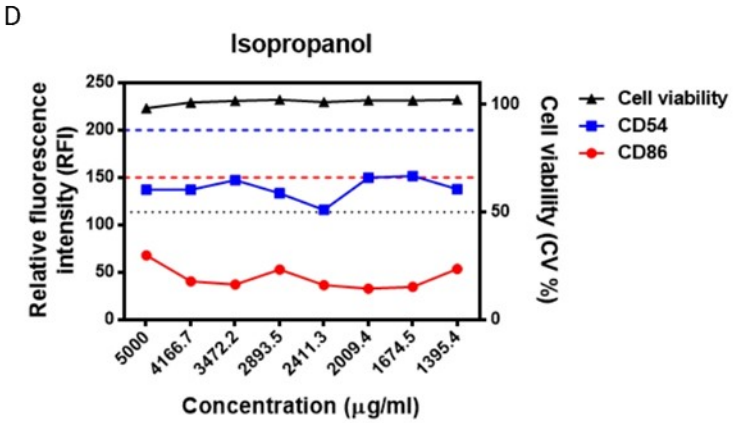

Figure S2. KeratinoSens and h-CLAT results of positive control substances. (A) Dose response curve of luciferase activity induction and cell viability changes of positive control cinnamic aldehyde. (B) Dose response curve of CD86 and CD54 fluorescence induction and cell viability changes of positive control DNCB. (C) Summary results of KeratinoSens™ and h-CLAT assays for positive control substances. (D) Dose response curve of CD86 and CD54 fluorescence induction and cell viability changes of negative control isopropanol.

Supplementary Table 1. Summary of testing challenges and the corresponding technical modifications in this study.

Testing challenges	Modifications relating to OECD TG
DPRA assay	
• Inorganic particles and poorly soluble metal oxides fall outside the applicability domain of TG 442C. • These materials may not act through classical electrophilic haptenation mechanisms (KE1). • Not expected to form stable covalent adducts with model peptides under DPRA conditions.	Not used in this study.
KeratinoSens assay	
Cell viability interference of NPs in the KeratinoSens assay	Calibration for background interference by measuring background absorbance values of NPs
h-CLAT assay	
Fluorescence interference of NPs in h-CLAT assay	Applied DiOC18 cell tracer dye in cell staining to separate nanoparticle and cell in flow cytometry

Supplementary Table 2. Summary of available *in vivo* and *in vitro* skin sensitization data for the NPs used in this study.

Nanoparticles	Skin sensitization results			Ref
	Skin sensitization assay		Prediction	
PS NPs	***In vivo***	LLNA: SI < 2	Negative	[1]
TiO_2 NPs	***In vivo***	LLNA: SI < 2	Negative	[1]
		LLNA: BrdU-FCM assay: SI < 2.7	Negative	[2]
		LLNA: BrdU-FCM assay: SI < 1	Negative	[3]
	In vitro	KeratinoSens: I_{max} = 1.3, IC_{50}: >2000 μM	Negative	[2, 4]
		U-SENS: CD86 EC_{150}: 183.1 μg/mL	Positive	[5]
ZnO NPs	***In vivo***	LLNA: BrdU-FCM assay: SI < 2	Negative	[3]
		LLNA: BrdU-FCM assay: SI < 2.06	Negative	[6]
	In vitro	KeratinoSens: Imax =1.42, IC_{50}: 162.64 μM	Negative (borderline)	[4]
		h-CLAT: CD86 RFI = 217~360 %, CD54 RFI > 3000%	Positive	[7]
ZnO Z-Cote HP1 NPs	***In vitro***	KeratinoSens: Imax =5.17	Positive	[8]
		h-CLAT: CD86 RFI = 163%, CD54 RFI = 2585%	Positive	
NiO NPs	***In vivo***	LLNA: BrdU-FCM assay: SI < 2.7	Negative	[2]
		LLNA: BrdU-FCM assay: SI < 2	Negative	[3]
	In vitro	KeratinoSens: I_{max} =1.05, IC_{50}: >2000 μM	Negative	[2, 4]
Ag NPs	***In vivo***	OECD TG 406: one animal (1/20) showed patchy erythema	Positive	[9]
	In vitro	h-CLAT: CD86 RFI = 156~260 %, CD54 RFI > 234 %	Positive	[7]

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