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Adaptation of the Next-Generation Risk Assessment Framework for Skin Sensitization for the use of Natural Complex Substances

Y. Gao[1], P. Kern[2], J. Ebmeyer[3], N. Alépée[4], H. Mizumachi[5], S. Suzuki[5], J. Kühnl[3], K. Brandmair[3], S. Oeda[6], Y. Hatakeyama[6], T. Otake[6], M. Hirota[6], M. Aleksic[7], F. Boislève[8], K. Mewes[9], Y. Arezki-Faivre[10], I. Lee[11], H. Raabe[12], J. Andersson[13], A. Forreryd[13], E. van Vliet[14], A. Cuciureanu[15], D. Allen[15]

1 Procter & Gamble Corporation, Beijing, China
2 Procter & Gamble Corporation, Brussels, Belgium
3 Beiersdorf AG, Hamburg, Germany
4 L'Oréal R&I, Aulnay-sous-Bois, France
5 Kao Corporation, Tochigi, Japan
6 Shiseido Global Innovation Center, Takashima, Japan
7 Unilever, Bedford, United Kingdom
8 Chanel, Neuilly, France
9 Henkel, Düsseldorf, Germany
10 Shiseido Group EMEA, Ormes, France
11 The Research Institute for Fragrance Materials, Mahwah, United States of America
12 Institute for In Vitro Sciences, Inc., Gaithersburg, United States of America
13 SenzaGen AB, Lund, Sweden
14 Innovitox B.V. Consulting & Services, Houten, The Netherlands
15 International Collaboration on Cosmetics Safety, Mount Royal, United States of America

Introduction

- Natural complex substances (NCS) are widely used in cosmetics, but safety assessments remain difficult due to variable compositions and complex multi-constituent mixtures.
- Traditional *in vivo* assays and New Approach Methodologies (NAMs) were designed for single chemicals, which limits their direct applicability to complex matrices.
- Building on a concept proposed by Gao et al. (2024) and outlined in Figure 1, the NCS working group is evaluating a Next Generation Risk Assessment (NGRA) framework that integrates exposure waiving, fit-for-purose NAMs, and Weight-of-Evidence (WoE) across three tiers.

Tier 0: exposure-based waiving

- Potential skin sensitizers in NCS are evaluated across chemical classes like sesquiterpenoids, phenolics, flavonoids, and quinones, with potency and concentration ranges (Table 1).
- Sensitizing species cluster in specific botanical families, notably *Anacardiaceae*, *Asteraceae*/*Compositae*, *Primulaceae*, *Liliaceae*/*Amaryllidaceae*, *Rutaceae*, and *Apiaceae,* with individual sensitizers typically present at less than 1% dry weight.
- Based on Gao et al. (2026), the framework establishes a provisional exposure-based waiving threshold of **10 µg/cm²** (AEL) for complex mixtures, which is adjustable based on concentration ratios (e.g., 1 g of preparation made from how many grams of dried plant material) (Figure 1).

Tier 1: weight-of-evidence assessment using available data

- This tier is triggered when Tier 0 thresholds are exceeded, utilizing human topical use history, allergy case reports, clinical data, NAM data, historical animal data, and composition (Figure 1).
- Case studies in Table 2 show that:
  - **Poison ivy** has strong evidence of having sensitization hazard and its potency is likely moderate when considering dried plant at its natural composition. This is estimated from human epidemiology, historical *in vivo* data and urushiols.
  - **German chamomile** has some sensitizing evidence based on mixed data and its potency is likely to be weak considering guinea pig studies and sensitizers concentrations in plant.
  - **Aloe vera** shows consistent non-sensitizing clinical and NAM evidence.
- The approach provides a holistic interpretation while acknowledging botanical variability and interpretation challenges.

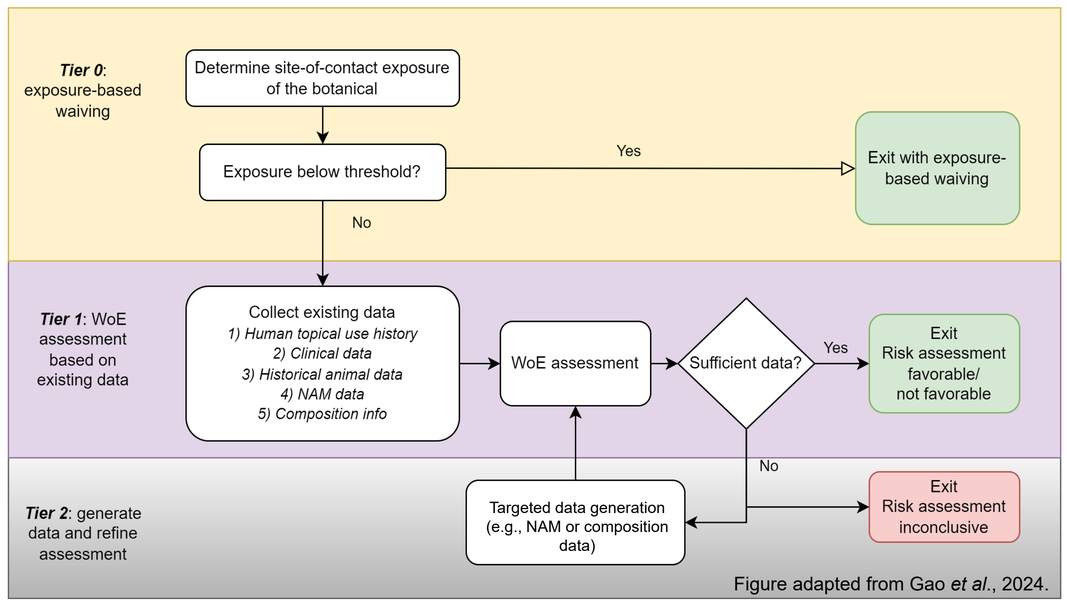

Figure adapted from Gao *et al.*, 2024.

Table 1: Potency and concentration of representative sensitizing phytochemicals

Substance group	Substance	CAS RN	Potency	Point of departure (µg/cm²)	Concentration range in plants (%)
Sesquiterpenoids and Sesquiterpene lactones	Lactucin	1891-29-8	Weak	1000	0.00687-0.2
	Lactucopicrin	65725-11-3	Weak	1000	0.00096-0.39
	Parthenolide	20554-84-1	Weak (read across)	1000	0.047-0.676
	Parthenin	508-59-8	Weak (read across)	1000	0.02-0.3
	Frullanolide	27579-97-1	Mod-strong	10	0.0013-0.172
	Eremofrullanolide	62870-69-3	Strong (read across)	10	Limited data
	alantolactone	546-43-0	Strong	10	0.381-3.18
	Tulipalin A	547-65-9	Moderate	100	0.05-5
	Tulipalin B	38965-80-9	Strong	10	0.05-1.6
Phenolic compounds	Urushiol	Group of chemicals	Strong	10	0.08-2.8
	Anacardic acid	16611-84-0	Strong	10	0.62-12.89
	Cardanol	79353-39-2	Non-sensitizer		
	Ginkgolic acid	22910-60-7	Strong	10	1.26 - 4.72
Quinones	Primin	15121-94-5	Strong	10	0.0525-0.2655
Coumarins	Herniarin (7-Methoxycoumarin)	531-59-9	Weak	1000	0.067
	Umbelliferone (7-hydroxycoumarin)	93-35-6	Weak	1000	0.0075-0.04
	Hymecromone (4-methylumbelliferone)	90-33-5	Weak	1000	Limited data
Disulfides	Allicin (Diallyl thiosulfinate)	539-86-6	Non-sensitizer		
	Diallyl disulfide	2179-57-9	Moderate	100	0.00423-0.04
	Allylisothiocyanate	57-06-7	Strong	10	0.0008-0.354
Flavonoids	Apigenin	520-36-5	Weak	1000	0-13.7
	Luteolin	491-70-3	Weak	1000	0-2.87
	Kaempferol	520-18-3	Weak	1000	0-0.0832
	Quercetin	117-39-5	Very weak	10000	0-0.2338
	Rutin	153-18-4	Very weak	10000	
	Isorhamnetin	480-19-3	Weak	1000	0.0057-0.081
Miscellaneous	Falcarinol (Panaxynol)	21852-80-2	Strong	10	0.001-0.212
	Evernic acid	537-09-7	Weak	1000	Limited data
	Atranorin	479-20-9	Moderate	100	0.0023-1.275
	Usnic acid	125-46-2	Weak	1000	0.508 - 2.64
	EGCG (Epigallocathechin Gallate)	989-51-5	Moderate	100	0.546-5.4

Table 2: WoE assessment case studies

Species	Common name	Plant part	Human topical use history	Human clinical data		Compositional data, known sensitizers	In vivo data	NAM data		Hazard	Potency
Toxicodendron radicans	Poison ivy	Leaf/stem/fruit	Long history of exposure, well-known sensitizing	Positive diagnostic patch tests		Urushiols	Positive guinea pig and mouse studies	No data found*		Strong evidence - Sensitizing	Moderate
Matricaria chamomilla	German chamomile	Flower	Long history of exposure, occasional reports of ACD	Positive diagnostic patch tests	Negative HMT and HRIPTs	Sesquiterpene lactones, coumarins	Weakly sensitizing in guinea pigs	Positive in KeratinoSens™	Negative in DPRA and B-PPRA	Some evidence - Sensitizing	Weak
Aloe barbadensis	Aloe vera	Leaf inner gel	Long history of exposure, limited case reports of ACD	No sensitization in photoallergy studies and clinical trial		No known sensitizers	No data found	Negative in DPRA and B-PPRA		Strong evidence – Not sensitizing	NA

*Poison ivy does not have published NAM data, however the urushiols showed reactivity in the DPRA.

Tier 2: targeted generation of NAM data

- This tier is applied when existing data is insufficient, utilizing NAMs that cover AOP key events KE1–KE3 (Figure 1 & Table 4).
- 6 NCS, including German chamomile, Feverfew, Centella asiatica, Rosemary, Green tea, and Aloe vera were evaluated alongside targeted characterization of their main components (Table 3).
- Testing demonstrated the technical feasibility of detecting low-level sensitizers (1–2%) in mixtures, yielding results consistent with overall composition and WoE (Table 4).

Table 3: NCS samples tested

NCS sample tested	Plant part	Reason of choice	Characterization of main component
Green tea dried extract	Leaf	High concentrations of potential sensitizers. Likely to be positive *in vitro*. Low prevalence in human	Total polyphenols > 55%, Catechin content 30-45%, EGCG content 15-25%
Aloe vera spray dried juice	Inner leaf gel	No known sensitizers. Consistent evidence of non-sensitizing to human	NA (no known sensitizers)
German chamomile dried extract	Flower	Low concentrations of sensitizers. The mixture is likely to have weak sensitizing potency. The NCS has long history of huma topical use and associated with some skin sensitization case reports	Apigenin min. 1.2%
Feverfew dried extract	Aerial part		Parthenolide min. 0.10%
Centella dried extract	Aerial part		≥ 5.00% total triterpenoid derivates expressed as asiaticoside
Rosemary dried extract	Leaf		Total hydroxycinnamic derivatives, expressed as rosmarinic acid content: 5-7%

Table 4: NAMs conducted for each NCS sample

KE in AOP	NAMs	Preliminary findings
KE1	DPRA	Results consistent with sample composition and other WoE. Reactivity profiling provided mechanistic insights into adduct formation for green tea, feverfew, and rosemary extracts.
	ADRA	
	kDPRA	
	Reactivity profiling	
KE2	KeratinoSens™	Both negative or positive results for weak samples.
	EpiSensA	Consistent with sample composition and other WoE, except for green tea.
KE3	H-CLAT	Positive results, especially for CD54 may be driven by lipopolysaccharide (LPS) present in samples
	U-SENS™	Negative or borderline positive results for weak samples. Green tea induced cytotoxicity
	GARD® skin Dose-Response	Predicted NESIL values in line with NCS sample composition and other WoE
	In general	NAMs technically feasible and able to detect low levels of sensitizers at 1-2% in mixtures

Conclusion & Next steps

- This work establishes an early, tiered NGRA approach for the skin sensitization assessment of NCS.
- Case studies demonstrate that a structured WoE approach can successfully integrate heterogeneous data sources to reach transparent and defensible conclusions.
- Tier 2 testing proves that NAMs spanning KE1–KE3 are technically feasible for NCS, successfully detecting low levels of sensitizers (1–2%) in mixtures with results generally consistent with composition and other lines of evidence.
- The provisional 10 µg/cm² threshold is likely not protective for potent or high-concentration constituents such as urushiols in poison ivy.
- Priorities for future work include refining the Tier 0 threshold as further compositional and potency data are analyzed, developing clearer guidance for integrating component-level data and interpreting conflicting evidence, and continuing to characterize NAM performance and applicability domains for complex matrices.

References

- Gao Y, *et al.*, A botanical reference set illustrating a weight of evidence approach for skin sensitization risk assessment. Food Chem Toxicol. 2024; 184: 114413.
- Gao Y, *et al.*, Development of a skin sensitization Site-of-Contact Exposure Threshold (SoCET) for botanicals and natural substances. Food Chem Toxicol. 2026; 207: 115846.