Expanding the applicability domain of NAMs for skin sensitization testing: A case study using GARDskin for assessment of metals

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Summary

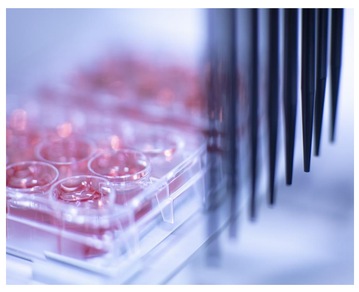
- Limited data are currently available to support the inclusion of metals into the applicability domain of the OECD TG 442 series of assays.
- GARD®skin (OECD TG 442 E) correctly predicted 11/12 metals in this study, including nickel which is a false negative in LLNA.
- GARD®skin has a potential to reduce the need for animal testing for the endpoint of skin sensitization within the metal production and medical device sectors.

Introduction

A suite of New Approach Methods (NAMs) for hazard assessment of skin sensitizers has been adopted into OECD TG 442 to replace traditional animal models. However, further characterization of the applicability domain (AD) of these assays is critical to understand limitations and to facilitate regulatory uptake in other industrial sectors. The potential to use the current OECD TG 442 assays for assessing the skin sensitizing potential of metals has not yet been systematically evaluated. This constitutes a significant challenge for those seeking to replace animal models for risk assessment during production of new metal salts with unknown sensitizing potential, or to evaluate leachables from metal containing medical devices. **The aim of this study is to evaluate the applicability domain of GARD®skin (OECD TG 442) for assessing the skin sensitizing potential of metals.**

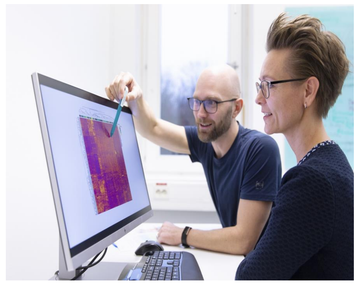
Methods

- A total of 13 metal salts with available reference data from LLNA, GPMT or Human evidence were provided by Johnson Matthey (Table 1).
- Testing was performed in the GARD®skin assay according to the standard protocol as per the OECD TG 442 E (Fig.1), using DPBS, H_20, or cell media (a-Mem) as vehicles.
- Extended gene expression analysis was performed by comparing spearman correlation coefficients for pairwise comparison between log fold changes of genes induced by metals and LMW organic chemicals in the GARD®skin training set.



Step 1
Expose Cells (the SenzaCell™ cell line) to the test sample at determined concentration.

Step 2
Measure the gene expression levels of 196 biomarkers, the genomic biomarker signature.



Step 3
GARD® Data Analysis Application makes a binary prediction based on gene expression analysis.

Figure 1. GARD®skin (OECD TG 442E) in three steps
Testing of the metals was performed according to the GARD®skin protocol described in OECD TG 422E. In short: **(Step 1)** Cells were exposed to test item under 24h. **(Step 2)** Total RNA were isolated from the cells, and the gene expression of the 196 genes in the GARD® Prediction Signature was measured. **(Step 3)** Gene expression data were uploaded into the cloud-based GARD® Data Analysis Application (GDAA) harbouring the data analysis pipeline, including the Support Vector Machine (SVM) based prediction model.

Table 1. Test chemicals

Chemical	CAS nr.	Oxidation state	Reference classification	Reference source
Cisplatin	15663-27-1	II	S	Human
Cobalt chloride	7646-79-9	II	S	WoE
Diammonium hexachloroplatinate	16919-58-7	IV	S	WoE
Nickel (II) sulphate hexahydrate	10101-97-0	II	S	Human
Palladium di(4-oxopent-2-en-2-oate)	14024-61-4	II	S	LLNA
Potassium dichromate	7778-50-9	VI	S	LLNA
Tetraammine palladium (II) hydrogen carbonate	134620-00-1	II	S	GPMT
(trans) Diamminedichloropalladium	14323-43-4	II	NS	LLNA
Hydrogen hexahydroxy platinate	51850-20-5	IV	NS	WoE
JM proprietary Pt salt	Confidential	Confidential	NS	LLNA
Potassium permanganate	7722-64-7	VII	NS	GPMT
Tetraammine platinum (II) hydrogen carbonate	123439-82-7	II	NS	GPMT
Zinc sulphate	7733-02-0	II	NS	LLNA

WoE: Weight of evidence classification based on available data (Human data prioritized if available).
S: Sensitizer, NS: Non-Sensitizer. LLNA: Local Lymph Node Assay. GPMT: Guinea Pig Maximization Test.

Results & Conclusions

GARD®skin classifications are shown in **Fig.2** and performance values are summarized in **Table 2**. Results from the extended gene expression analysis **(Fig.3)** demonstrated an overall high similarity between toxicity pathways activated by metals and organic chemicals, and further support the inclusion of metals into the applicability domain of GARD®skin.

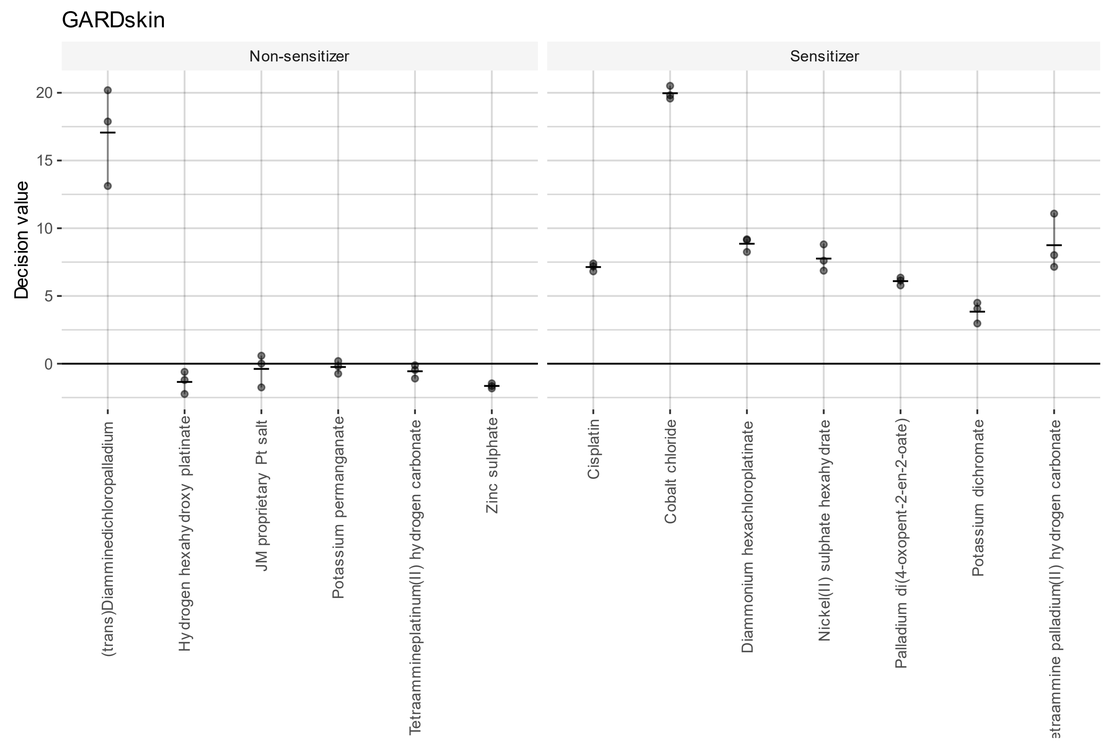


Figure 2. GARD®skin classifications
GARD®skin DVs from replicates (n=3). Mean DV > 0 is classified as skin sensitizer.

Table 2. Performance values

Reference Classifications		NS (6)	S (7)
GARD®skin	NS	5	0
	S	1	7
Accuracy		92.3%	
Sensitivity		100%	
Specificity		83.3%	

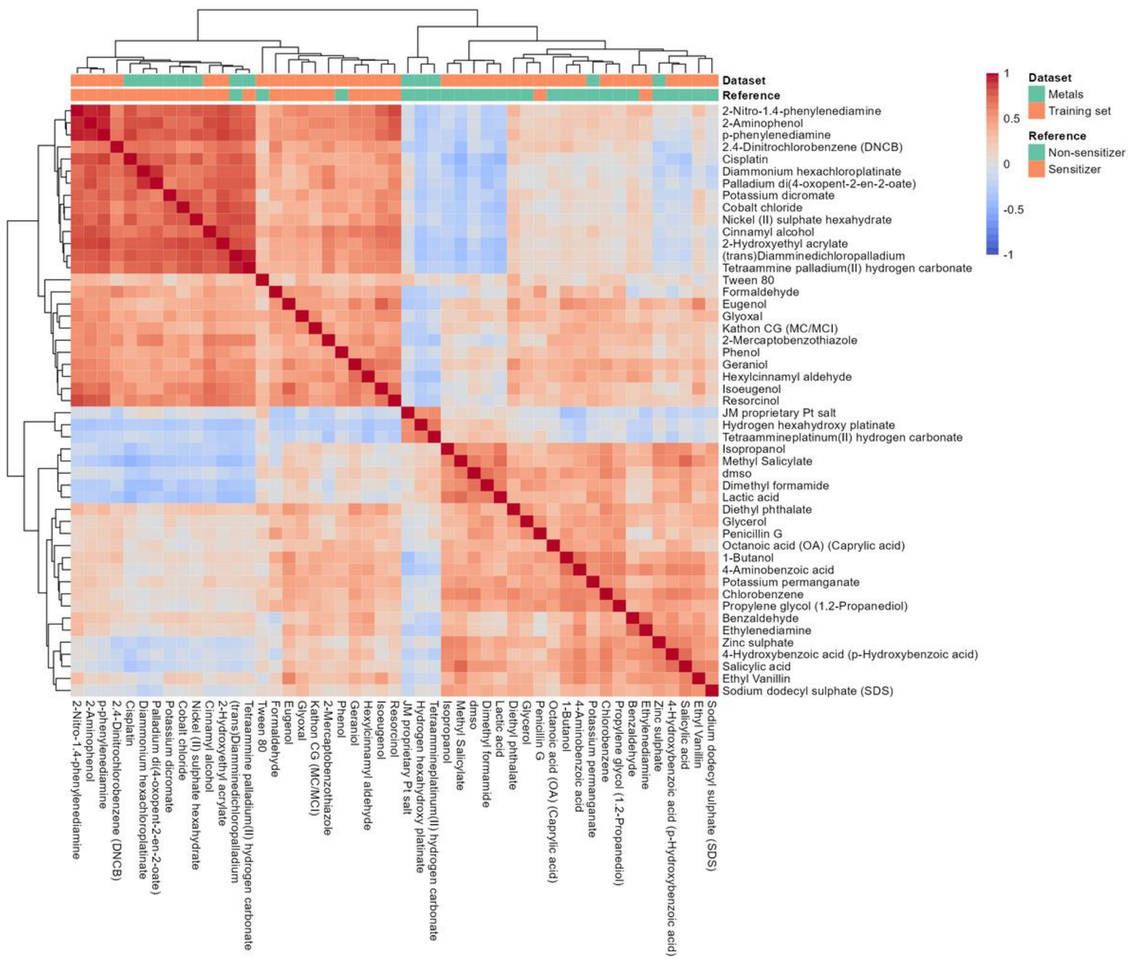


Figure 3. Spearman correlation heatmap
Figure comparing gene expression induced by the metals to historical GARD®skin training data

Conclusion

Results from this study support inclusion of metals into the AD of GARD®skin, which is an important step to ensure scientific/ regulatory confidence to reduce the need for animal testing within the metal production and medical device sector.

Reference

Forreryd A, Gradin R, Larne O, Rajapakse N, Deag E, Johansson H. The GARD®skin assay: Investigation of the applicability domain for metals. ALTEX. 2023;40(3):425-438. doi: 10.14573/altex.2203021. Epub 2022 Nov 3. PMID: 36343115.

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