Assessing Skin Sensitizing Hazard of Drug Products and Active Pharmaceutical Ingredients using GARD®: New Approach Methodology in Occupational Health and Safety

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- **Occupational Health and Safety**
- **Pharmaceutical Manufacturing**
- ***In Vitro* Skin Sensitization Testing**

New Approach Methodology

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1. Introduction

Skin sensitizers are chemicals capable of inducing hypersensitivity reactions, such as Allergic Contact Dermatitis (ACD). Identifying and characterizing these chemicals' skin sensitizing potential is essential for limiting hazardous exposure. Significant efforts have been made in the scientific community to develop New Approach Methodologies (NAMs) to replace animal testing for skin sensitization. Internationally recognized test guidelines as well as integrated strategies have been developed, combining *in vitro, in chemico, and in silico* approaches for predicting skin sensitization potential. While widely accepted in cosmetics and personal care, NAMs' application in the pharmaceutical sector for product development and Occupational Health and Safety (OHS) is less prominent.

The aim of this study is to demonstrate how NAMs, including the *in vitro* GARD® assay, combined with *in silico* data, can be used by the pharmaceutical industry to assess the skin sensitization potential of drug products and Active Pharmaceutical Ingredients (APIs) to take protective measures and improve occupational safety in production environments.

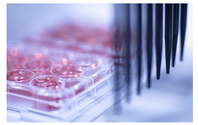
In this study, the drug product is an oral medicine used to treat mental health problems, and the two APIs are compounds that affects how dopamine works in the brain, helping with symptoms like hallucinations or delusions, and helps boost certain chemicals in the brain to improve mood and reduce feelings of depression, respectively. Following reports of allergic reactions in production and quality control, the drug product and two APIs A and B were assessed.

2. Methods

The drug product contained approximately 0.5% of API A and 9.4% of API B. In silico predictions using Nexus Derek software identified all compounds as potential sensitizers. The *in vitro* assay GARD®skin Dose-Response was conducted to confirm these predictions.

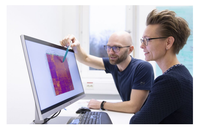
The GARD®skin method (OECD 442E) is an *in vitro* assay for assessment of chemical skin sensitizers. The method provides binary hazard identification of skin sensitizers by evaluation of transcriptional patterns of an endpoint-specific genomic biomarker signature, comprising 196 genes, referred to as the GARD®skin Genomic Prediction Signature (GPS), in the SenzaCell® cell line. Final classifications are provided by a machine-learning prediction algorithm in the form of Decision Values (DV), the sign of which is evaluated by the prediction model. Any test chemical with a positive mean DV is classified as a skin sensitizer. Conversely, any test chemical with a negative mean DV is classified as non-skin sensitizer. (Figure 1)

GARD®skin Dose-Response is an adaptation of the conventional GARD®skin method, in which test chemicals are evaluated by the GARD®skin prediction algorithm in an extended range of concentrations, to investigate the dose-response relationship between DVs and test chemical concentration. It provides a quantitative estimation of sensitizing potency, referred to as cDV_0, which corresponds to the lowest required dose able to generate a positive mean DV. The readouts can predict correlating LLNA EC3 values, which are traditionally used to measure the skin sensitizing potency of chemicals. Furthermore, it can predict human skin sensitizing potency NOEL and GHS/CLP classification (1A or 1B), all with high statistical significance. (Figure 2)



Step 1.
Expose cells (the SenzaCell™ cell line) to the extracts at determined concentration.

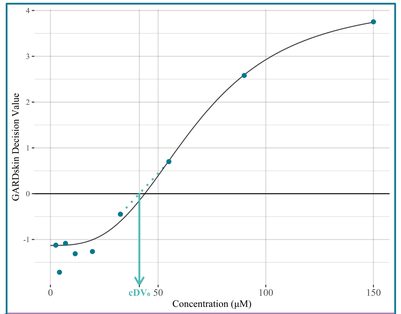
Step 2.
Measure the gene expression levels of 196 biomarkers, the genomic biomarker signature.



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

The readout of GARD®skin is a Decision Value (DV):
- DV ≥ 0 : Sensitizer
- DV < 0 : Non-sensitizer

Figure 1. GARD®skin in three steps. See OECD TG 442E for the full protocol.



	GARD	LLNA
Response value	DV	SI
Binary Threshold	DV=0	SI=3
Readout	cDV_0 (µM)	EC3 (%)

GARD®skin Dose-Response allowing for establishment of the cDV_0 value, which is derived analogously to the LLNA EC3 value.

Figure 2. The experiment setup of GARD®skin Dose-Response.

3. Results

All test items were identified as sensitizers by GARD®skin Dose-Response with the following results:

- Drug product: cDV_0 = 12.8 µg/ml. Predictions: LLNA EC3 = 7.63%, NOEL = 3180 µg/cm², GHS/CLP 1B.
- API A: cDV_0 = 1.51 µg/ml. Predictions: LLNA EC3 = 1.08%, NOEL = 254 µg/cm², GHS/CLP 1A.
- API B: cDV_0 = 1.72 µg/ml. Predictions: LLNA EC3 = 1.19%, NOEL = 315 µg/cm², GHS/CLP 1A.

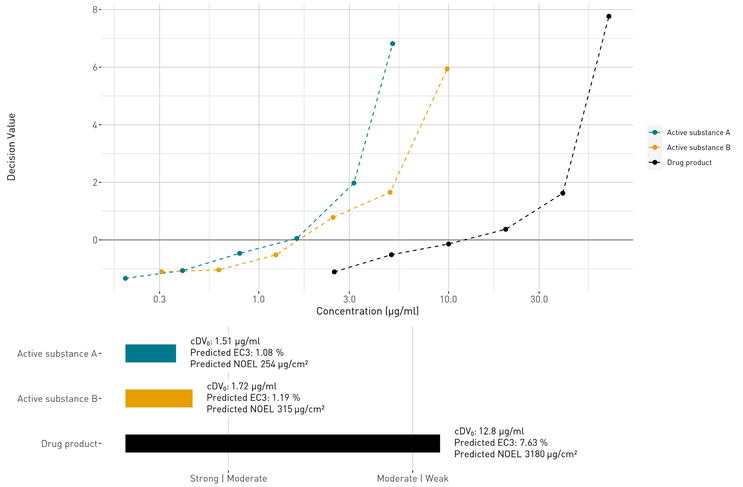


Figure 3. Results from GARD®skin Dose-Response demonstrated a clear dose-dependent increase in DVs for all three test items, confirming their classification as skin sensitizers, with varying potencies. Both APIs were classified as strong sensitizers, while the drug product was classified as a weak sensitizer.

4. Discussion and Conclusions

This study highlights a case where the GARD®skin Dose-Response assay, combined with *in silico* data, was used to assess the skin sensitization potential of a drug product and its APIs in an OHS context.

Both APIs were classified as strong sensitizers, while the drug product was classified as a weak sensitizer, reflecting the dilution effect of excipients. Based on the test outcomes, recommendations were made to improve occupational safety in handling these sensitizers:

- Avoiding dust inhalation and skin/eye contact.
- Minimizing prolonged and/or repeated exposure.
- Using appropriate personal protective equipment (PPE).
- Removing contaminated clothing and washing it before reuse.
- Ensuring thorough hand washing after handling, during breaks, and at the end of each shift.
- Handling substances in closed systems with proper ventilation.

In conclusion, the findings highlight NAMs like GARD®skin Dose-Response as useful tools for enhancing OHS and safety protocols in pharmaceutical manufacturing.

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