# In vitro assays for assessment of the skin sensitization hazard and potency of isobornyl acrylate

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## 1. Introduction

The acrylate monomer isobornyl acrylate (IBOA) was named Allergen of the Year in 2020 by the American Contact Dermatitis Society due to the increased number of patients that were sensitized to IBOA found in glucose sensors and pumps. IBOA is also present in other medical devices in plastics, coatings, sealants, glues, adhesives, and inks. As a result, it is important to find alternatives to current animal methods for assessing the skin sensitization potential of IBOA in medical devices to avoid the risk of sensitizing more individuals to this chemical.

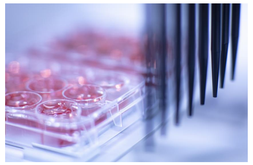
Here, we explore the ability of the GARDskin Medical Device assay to detect the skin sensitizing potential of IBOA in a mixture of chemicals extracted from a soft silicone material (NuSil MED-2000) in both saline and olive oil extracts. The skin sensitizing potency of IBOA was also investigated using the novel GARDskin Dose-Response assay.

## 2. Methods

The GARDskin assay (OECD TG 442E) is an *in vitro* method that was developed for hazard identification of skin sensitizers. In the GARDskin assay a chemical or mixture is classified as a skin sensitizer if the decision value (DV) is positive and as a non-sensitizer if the DV is negative. [1]. The procedure for conducting the GARDskin assay is illustrated in **Figure 1**. For assessment of skin sensitizers in medical device extracts according to ISO 10993-12, the GARDskin protocol was adapted to create the GARDskin Medical Device assay.

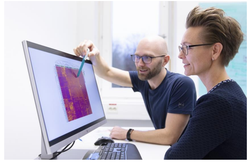
The GARDskin Medical Device assay was used for assessment of the skin sensitizing hazard of simulated medical device extracts spiked with IBOA, in line with the proposed validation strategy described in ISO/DTS 11796 prepared by ISO/TC194/WG8. In short, extracts were prepared of a soft silicon material (NuSil MED-2000, Medtronic) in the extraction vehicles saline and olive oil. The silicon material extracts were then spiked with IBOA (CAS 5888-33-5) before testing with the GARDskin Medical Device assay.

For assessment of the skin sensitizing potency of IBOA, a modification of the standard GARDskin protocol was used, in which the test chemical is evaluated in a titrated range of concentrations to investigate the dose-response relationship between GARDskin classifications and test chemical concentration. The readout of the GARDskin Dose-Response assay is a $cDV_0$ value corresponding to the lowest concentration required to retrieve a classification as a skin sensitizer in GARDskin. This concentration correlates significantly with LLNA EC3 and human NOEL values and linear regression models have been established to exploit these relationships for potency predictions [2].



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

Step 2
Measure the gene expression levels of 200 biomarkers, the genomic biomarker signature.



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

**Figure 1.** The GARD®skin assay in three steps

## 3. Results & Discussion

The silicon material extracted in both saline and olive oil were classified as a non-sensitizer in both extracts with a negative DV in the GARDskin Medical Device assay and hence could be used as simulated medical device extracts in this study.

When testing IBOA as a pure chemical 90% viability of the cells was observed in the GARD input finder step at 45.5 µM. However, when testing the silicon material extracts spiked with IBOA there were no observed cytotoxicity in the GARD input finder step for the same in well-concentration. This is probably because the IBOA was not fully dissolved in the silicon extracts but may also indicate that IBOA was not bioavailable for the cells in the test system.

The saline extract of the silicon material spiked with IBOA was correctly classified as a skin sensitizer showing that the IBOA was bioavailable for the cells in the saline extract. The olive oil extract of the silicon material spiked with IBOA was however classified as non-sensitizer which indicates that IBOA was not bioavailable for the cells in the assay in the olive oil extract, results shown in **Figure 2**.

The chemical IBOA was also tested in a dose-response manner using the GARDskin Dose-Response assay, which provided an observed $cDV_0$ value of 5.91 µM as shown in **Figure 3**. The LLNA EC3 value was predicted to 0.848% and the NOEL value was predicted to 230 µg/cm$^2$, as shown in **Table 1**, which is in agreement with results of human skin sensitizing potency reported in the literature and information in IBOA's ECHA registration dossier.

The results from this study provide evidence that the GARDskin Medical Device assay is sensitive enough to detect low concentrations of device-related skin sensitizers in a mixture of extracted chemicals. The sensitivity of the GARDskin test system was confirmed by the results from the GARDskin Dose-Response assay where the $cDV_0$ value of IBOA is lower or in line with the IBOA concentration typically found in extracts from medical devices like insulin pumps that are known to cause allergy in diabetic patients [3].

Hence, the GARDskin Medical Device assay has the potential to replace *in vivo* tests for risk assessment of skin sensitizers in medical devices and can be also be used as an important tool in combination with GARDskin Dose-Response assay during development and production of acrylate materials to avoid residues of IBOA, thus reducing the risk of allergic reactions in patients.

**Table 1.** Summary of the quantitative potency results from the GARDskin Dose-Response assay for the tested acrylate monomer IBOA

| Test Item | Observed $cDV_0$ (µM) | Predicted EC3 (%) | NOEL (µg/cm$^2$) | Predicted Human potency |
|---|---|---|---|---|
| IBOA | 5.91 (4.31, 8.64) | 0.848 (0.39, 1.84) [I] | 230 (65.7, 803) | 2 (0.305) [II] |

I) 95 % confidence interval for $cDV_0$ and EC3 estimate

II) Predicted probability for the Human Potency Classification

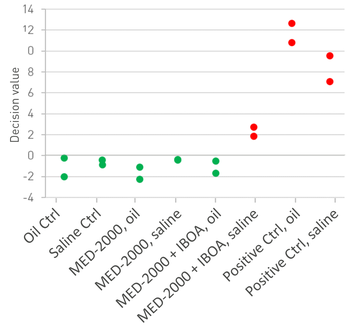



**Figure 2.** DV provided in the GARDskin Medical Device assay for the extraction vehicles olive oil and saline, the silicon material (MED-2000) extracts in oil and saline, the silicon material extracts spike with IBOA, and the positive control (PPD) in oil and saline.
Green / DV < 0 = non-sensitizer
Red / DV ≥ 0 = skin sensitizer

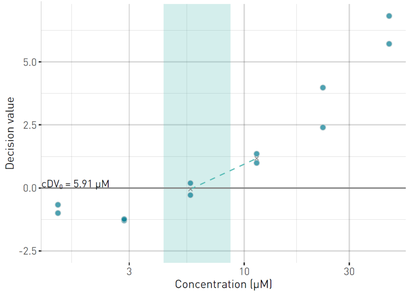



**Figure 3.** The dose-response curve and the corresponding $cDV_0$ values for IBOA provided in the GARDskin Dose-Response assay.

References:
1. Johansson et al 2019, Toxicological Sciences, 170 (2), p 374–38
2. Gradin et al 2021, Nature Scientific Reports 11, 18904
3. Herman A et al 2017 Contact Dermatitis 77 (6) p 367-373

## 5. Conclusions

The GARD®skin Medical Device assay can be used

- with the extraction vehicles saline and oil as described in ISO 10993.
- to detect low concentrations of skin sensitizers in a mixture and have the potential to replace *in vivo* tests for risk assessment of skin sensitizers in medical devices.
- as risk assessment tool in combination with the GARD®skin Dose-Reponse assay during development of medical devices containing acrylates.

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