#275

EFFECT OF A MODULATOR ON THE SKIN SENSITIZATION POTENCY OF COSMETIC FRAGRANCE FORMULATIONS

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INTRODUCTION & AIM

Perfume long lasting is an important concern widely addressed in fragrance research and innovation. There is a need for new technologies to prolong the perception and intensity of fragrances over time. The intended function of fragrance modulators is to slow down the evaporation rate of some perfume raw materials, with a potential impact on the toxicological profile of the final formulation and in particular the risk of skin sensitization. This hypothesis has been investigated using the GARDskin Dose-Response assay.
The aim was to evaluate complex mixtures such as fragrance samples and to obtain continuous potency predictions, crucial point for comparing the modulators effects on formulations skin sensitizing potency. Furthermore, in the context of Integrated Approaches to Testing and Assessment, the intention was to address a spectrum as broad as possible of the skin sensitization Adverse Outcome Pathways, which was made possible by the use of the GARDskin method.

MATERIAL & METHOD

The potential of three different modulators to reduce the skin sensitization potential of fragrance formulations containing different concentrations of fragrance oils were investigated using the GARDskin Dose-Response assay.

The GARDskin Dose-Response assay is based on the validated protocols of GARDskin (OECD TG 442E) and incorporates dose-response measurements to derive continuous potency predictions. The readout of the assay corresponds to the lowest concentration required to exceed the binary classification threshold in GARDskin (DV≥ 0), referred to as the cDV_0 value. This concentration is inversely correlated to skin sensitizing potency and can be used to predict a corresponding LLNA EC3/human NESIL value or be directly interpreted on its own to quantify differences in skin sensitizing potency between investigated samples.

In this study, a total of 12 fragrance formulations were evaluated. Dose-response curves were supplemented with 95% confidence intervals (CIs), and non-overlapping CIs for cDV_0 values were used to determine statistically significant differences in potency between investigated samples. The analysis was split in 2 sample waives using 2 different fragrance formula chassis (see table 1 for details) :

- ✓ Formula 1 (MP22001 to MP22006) allowed to compare the effect of 3 different modulators on the sensitizing potential of a first fragrance formulation. All samples were compared to the control sample without added modulator (MP22001).
- ✓ Formula 2 (MP22007 to MP22012) analyzed the impact of modulator 1 on the sensitizing potential of a second type of formulation, designed with different fragrance levels. Each fragrance level had its own negative control.

Table 1 : Tested items

Test Item	Formulation Chassis	Modulator	Modulator Level	Fragrance Oil Level
MP22001	1	-	-	++
MP22002	1	1	+++	++
MP22003	1	1	++	++
MP22004	1	1	+	++
MP22005	1	2	+++	++
MP22006	1	3	+	++

Test Item	Formulation Chassis	Modulator	Modulator Level	Fragrance Oil Level
MP22007	2	1	-	++
MP22008	2	1	+++	++
MP22009	2	1	+++	+++
MP22010	2	1	-	+++
MP22011	2	1	+++	+
MP22012	2	1	-	+

RESULTS & ANALYSIS

The table 2 below gathers the different cDV_0 values and 95% CIs calculated for the 12 analyzed samples.

Table 2 : Estimated cDV_0 concentrations

Test Item	cDV_0 (ppm)
MP22001	184 [165 – 205][1]
MP22002	256 [216 – 314][1]
MP22003	423 [283 – 647][1]
MP22004	208 [96.3 – 329][1]
MP22005	185 [137 – 242][1]
MP22006	189 [172 – 209][1]

Test Item	cDV_0 (ppm)
MP22007	209 [129 – 308][1]
MP22008	614 [335 – 1150][1]
MP22009	254 [212 – 300][1]
MP22010	289 [171 – 487][1]
MP22011	685 [378 – 1170][1]
MP22012	363 [273 – 466][1]

1- 95% confidence intervals for respective endpoints

The Figures 1 to 6 represent dose response curves illustrating the Decision Values (y-axis) at different concentrations (x-axis) for the different studied samples. The shaded area represents a 95% CIs of the cDV_0 calculations.

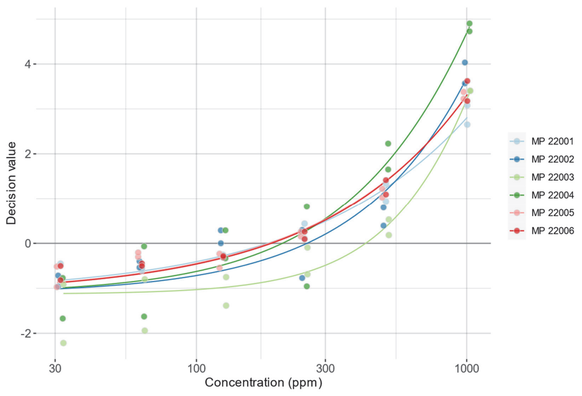

Figure 1 : MP22001 to MP22006

When comparing the cDV_0 values of test samples MP22002, MP22003 and MP22004, only the first two are significantly different from the control MP22001 showing they are relatively weaker sensitizers.
cDV_0 values of samples MP22005 and MP22006 are equivalent to that of the control MP22001.

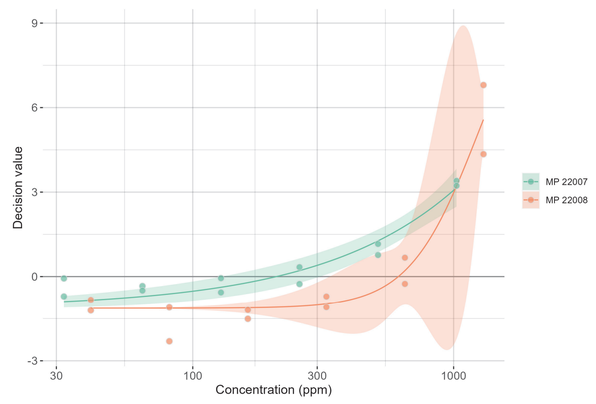

Figure 2 : MP22007 and MP22008

The cDV_0 value of MP22008 is significantly higher than which of its control MP22007, indicating its relatively weaker potential to induce sensitization. The results obtained for these samples are correlated with those observed for MP22001 and MP22002 sharing the same modulator and fragrance oil levels.

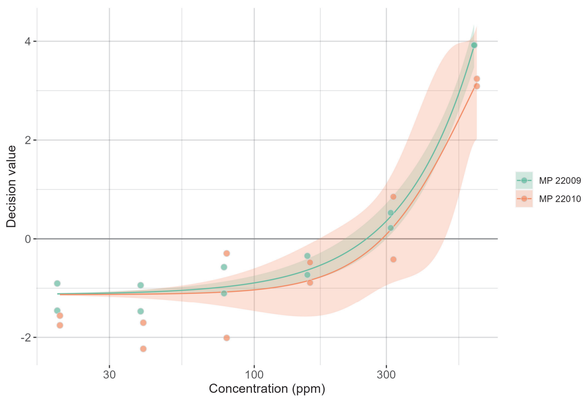

Figure 3 : MP22009 and MP22010

cDV_0 values of test samples MP22009 and MP22010 are not statistically different.

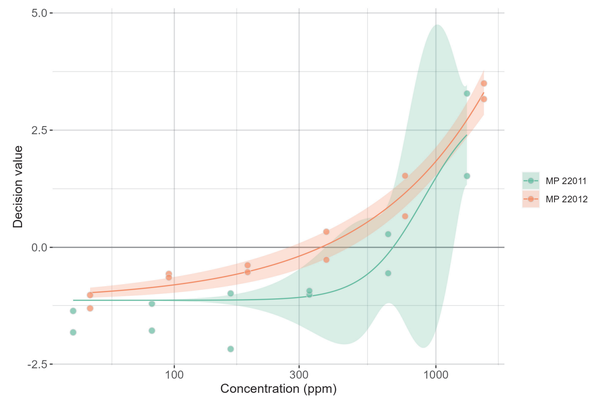

Figure 4 : MP22011 and MP22012

There is a difference in cDV_0 values of samples MP22011 and MP221012 where the test item appears to be relatively weaker sensitizer compared to its control, although not statistically significant due to the large CIs. Increasing the number of assayed concentrations close to the classification threshold may help to improve the resolution and to detect smaller differences in potency between test and control samples.

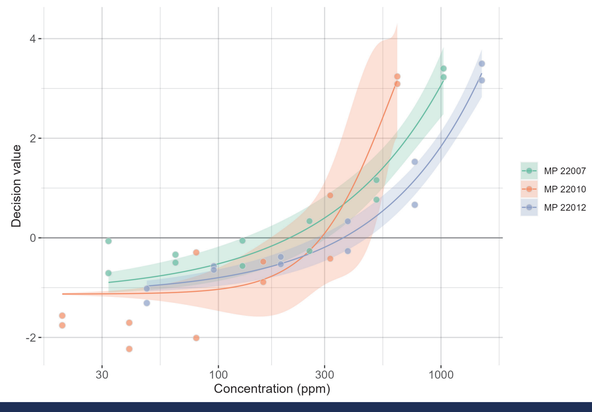

Figure 5 : MP22007, MP22010 and MP22012

No significant differences in cDV_0 values are observed within the group of control samples.

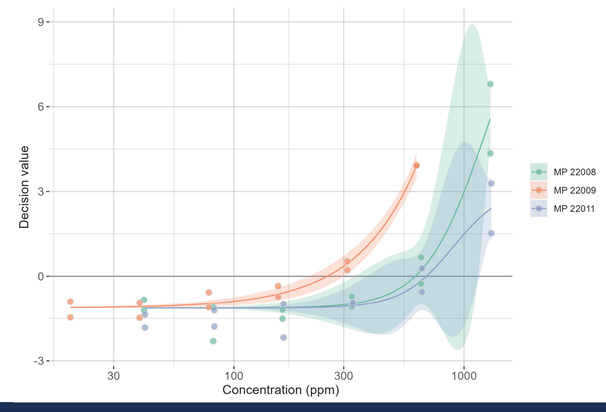

Figure 6 : MP22008, MP22009 and MP22011

Comparing the group of test samples, the cDV_0 value of MP22009 is significantly different from the ones of MP22008 and MP22011 indicating that MP22009 is a relatively more potent skin sensitizer than other test samples within the group.

CONCLUSION

The GARDSkin Dose Response assay, thanks to its ability to obtain continuous potency predictions, in our case on complex mixtures, allowed to identify a reduction of the skin sensitization potency following the spike of a modulator into fragrance formulations.

Analyzing different fragrance modulators at varying doses, it can be concluded that modulator 1 allowed a reduction of the sensitizing potential of Formula 1 at its two highest tested doses. The observed effect was not dose dependent. At its highest concentration, the same effect was observed in Formula 2 for the two samples that contain the same amount of fragrance oil than Formula 1. The effect of modulator 1 was observed on the two fragrance designs studied.
In contrast, modulators 2 and 3 were not efficient in impacting the sensitizing effect of Formula 1 at the tested concentrations. Only modulator 1 was of interest regarding sensitizing potency reduction in a fragrance formula.

The addition of the modulator 1 on the 2 lowest concentrations of perfume oil in Formula 2 generated, as previously, a similar reduction of the sensitization, contrary to the highest concentration for which no effect was observed. By varying the proportion of fragrance oil in the Formula 2, we were able to demonstrate the existence of a threshold from which the effect of modulator 1 on the sensitizing potential of this formula was no longer detected.

In conclusion, modulator 1 appeared to be of interest showing an ability to reduce the sensitizing potential of the tested fragrance formulations. It could be interesting to evaluate its impact on other cosmetic or fragrance formulas containing different fragrance oils and perfume raw materials. Another next step should be to define the lowest concentration of modulator 1 allowing a significant reduction of the skin sensitizing275 potential of perfume formulas.