IDEA
International Dialogue for the Evaluation of Allergens

Meeting of the
IDEA Hydroperoxides Task Force

December 12th, 2016 – 12h30 to 5h30 pm

IFRA Offices
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Final Minutes

Participants:
Hans Bender (HB, IDEA Management Team), Michael Calandra (MC, Firmenich), Elise Corbi (EC, Chanel), John Elliot (EJ, University of Alberta, by phone), Elena Gimenez-Arnau (EGA, University of Strasbourg), Cécile Gonzalez (IDEA Management Team), Ann-Therese Karlberg (ATK, University of Gothenburg), Hans Leijs (HL, IFF), Andreas Natsch (AN, Givaudan), Ulrika Nilsson (UN, University of Stockholm), Neil Owen (NO, Givaudan), Matthias Vey (MV, IDEA Management Team, by phone)

1) Welcome to the participants.

AN opened the meeting at 13h10 and welcomed the participants. A short tour de table was organized. The agenda was adopted as such.

2) Antitrust statement:

The participants were reminded the constraints of the antitrust law. All agreed that there should be no discussions of agreements or concerted actions that may restrain competition. This prohibition includes the exchange of information concerning individual prices, rates, coverage, market practices, claims settlement practices, or any other competitive aspect of an individual company's operation. Each participant understood the obligation to speak up immediately for the purpose of preventing any discussion falling outside these bounds.

3) Review of data generated on analysis in complex bases.

The paper "Facing the challenge: developing and validating methods to detect and quantify skin sensitizing hydroperoxides in consumer products" from the IDEA Hydroperoxides TF submitted but considered not innovative enough for the journal. AN proposed other publications to be considered by the group to resubmit the paper. There was agreement that the paper should be submitted to Journal of Analytical Toxicology.

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Note added after meeting: Journal of Analytical Toxicology did also not consider the paper in the scope of the journal, it was now submitted to Flavor and Fragrance journal.

4) Review of methods for analysis in complex bases.

The methods for hydroperoxides quantification in complex bases have been:
- LC-MS, as proposed by Firmenich (AC).
- GC-MS: different parameters have been tested.
- Extrelut extraction method adapted from allergen detection work, as proposed by Givaudan (AN).

AN presented the results from Givaudan on the Extrelut extraction method using MTBE (Att. 01) for the lotion, the anti-ageing cream and the non-antiperspirant deodorants (one of the deodorant has all-natural ingredients, which initially contained a low level of Lim and Lin). The results show 80-95%recovery.
The results from Chanel were also presented (Att. 01). In this case, the recovery rate for the hydrophilic cream was lower than for the hydrophobic cream and the deodorant base. The question on whether this is due to the stability of the Lim and Lin remains so far unknown, as hydroperoxides could decay into the alcohols in the bases, which cannot been seen by the analysis performed. The Extrelut method allows to do the reduction in all the products tested. The GC-MS coupled to reduction and Extrelut extraction method is sensitive enough to quantify in complex bases at 50ppm. Moreover, stability of hydroperoxides stored in a fridge does not appear to be an issue for the next ring test.

IFF will try the GC-MS method in the coming weeks. Firmenich has also done some related experiments but not with the same creams.

For LC-MS, MC has tried the extraction method #1 applied to cream #2, which is based on temperature variations. After warming, the sample is cold filtered and shows a yield recovery of 90%. Samples are run in LC-MS at room temperature.
ACTION: MC to briefly explain the steps of the methods.

ATK raised her concern about running a ring test before solving the issue of low recovery for hydrophilic matrixes and the stability of the hydroperoxides. AN pointed out that we cannot avoid to have products with unstable hydroperoxides on the market. It is unlikely that the difference observed in the results here is due to the presence or absence of antioxidant. In addition, the presence or absence of antioxidant seem not to have influence on the reduction.

ATK and EGA proposed that other hydroperoxides could be present in products such those derived from Citronellol and Geraniol. It may be worthy to consider whether they interfere with the analytical protocol used? AN proposed, that he could run these two hydroperoxides through the PPH3 reduction / GC-MS method and check (a) for coelution; (b) include the MS and retention time info into the method package so

that test labs can check their presence. Importantly, NO mentioned (and this was agreed by the group) that the target of the whole exercise are oxidized Limonene and Linalool, and that we should not change scope. However, having this reference data can only help.

HL emphasized that for each ring test the team should publish the results, and take the lead so the information and the objective of the analysis is not misinterpreted. Moreover, the publication should cover the limitations such as the effect of aluminium in deodorants.

5) Plan ring trial in complex bases:

a) products to be tested and test set-up

The IDEA Hydroperoxides TF agreed that similar products tested in previous ring tests should be selected for the next ring test. MC suggested to take the products that have successfully tested previously within this group.

ATK and EGA suggested checking if the samples have Citronellol and Geraniol as if the retention times for Citronellol and Geraniol can interfere with the ones containing also Limonene and Linalool. This can be done as citronellol andd geraniol are "easy" standards.

AN proposed to take an antiaging cream and an body lotion from the market place. Deodorants are simpler in composition than the creams and lotions, so AN recommended not to waste efforts on them. For the publication, the brand will not be revealed.

ACTION: IDEA Management Team to buy the antiaging cream and the body lotion and send them to Greenpharma.

b) methods

The participants agreed that the same methods should be applied for the ring test.

c) participation and timeline

AN asked whether the CRO should be included already at this stage, as there are already items that can be shared with them. MV pointed out that if we want to measure products in the market, to avoid any type of criticism, an external lab should be involved. In addition, the sooner they are involved, the better would be for the development and credibility of the project.

The selection of the CRO should be done before the ring test starts. As a first approach, Eurofins will be contacted. If the lab does not have expertise in fragrance materials analysis, the samples will be sent in two stages so they can perform a learning process in advance to the ring test. HL mentioned that there are CROs that are not keen in sharing the basis (procedure and methods) for obtaining the results.

ACTION: IDEA Management Team to look for an external lab that would like to participate for the GC-MS and/or other methods (e.g. Eurofins or other).

In addition, IFF, Firmenich, Givaudan and Chanel confirmed their participation.

ACTION: IDEA Management Team to ask Wala if they would also like to participate.
ACTION: JE to tell IDEA Management Team his willingness to participate to the ring test.

The timeline will depend on when we set up the collaboration of the CRO.
ACTION: AN to share the action plan with Greenpharma.

6) Review and discuss conclusions for the IDEA Workshop on pre- and pro- haptens on December 13th.
The group reviewed the slides that will be presented during the IDEA Workshop on pre- and pro- haptens (Att. 02). The changes proposed have directly been included in the attached version of the presentation.

7) Review and discuss what products to test and by whom to get to better exposure assessment - proposal to be presented to the participants of the IDEA Workshop on pre- and pro- haptens.
AN proposed an approach to test market samples, aged products and patient's samples by GC-MS after reduction (Att. 01). In this proposal the level of 50ppm has been selected as reporting limit. This level is only based on the analytical feasibility. NO pointed out that the fact that option 2 involved an LC-MS could step back CRO as it implies to have GC-MS and LC-MS in the same facilities. LC-MS should be performed as a cross-confirmation and is considered as optional (directly detecting the hydroperoxide). The participants reviewed and the agreed proposal is outlined in Att. 01.

MV mentioned that at the ESCD interest was raised on the follow up of the products of concern in the clinics. A protocol could jointly be developed with dermatologists. Indeed, there are a few dermatologists that are willing to make analyzed the content on hydroperoxides of the product that is causing the allergy to the patients. The insight from the clinics will also indicate the team which products to test and how many of them. MC pointed out that such activity will lead us to detect certain classes of products as more likely to have sensitizing properties.

AN listed the variables that could influence the products to test in a market overview/samples from consumers and customers:

- Production batches and replicates
- Body regions where the product is applied (link to cumulative exposure) – such feedback would allow to define the product types such as lotion, face or hand cream, deodorant, etc.
- Number of brands in a given product.
- Geographic spread or number of countries. The cities selected should have clinical data.
- Aged samples whether freshly bought. In the case of aged samples, the information regarding the history of the product should be known. The group suggested to ask a market search company and search for fresh batches in shops where old ones are also available.

ATK mentioned that already in the 90s, the positive reaction in Seville to Limonene was much higher than in other regions. The reactions were correctly measured. Recently, a similar result has been found in the Spanish multicenter study coordinated from Barcelona (Att. 03). It is supposed that the exposure in Seville is much higher than in other regions of the world and it is likely due to local products and the exposure to the sun. This would mean that higher levels of allergens could be available. In this case, analytical measurements should be able to help to elucidate this and other similar cases. There was agreement to select London, Leuven, Gothenburg and Seville. If no difference is found between in the cities, then the local products suspected to cause allergy among the population should be carefully studied. In any case, the samples selected for the study should be representative of the market.
EGA mentioned that the Spanish contact allergy group is meeting end of September 2017.

The question of which samples should be included in the proposal will be asked at the 4th IDEA Workshop on pre- and pro- haptens (December 13th) to the entire group.

In the meantime, MC and HL will repeat the analysis on LC and TOF methods.

8) Attachments:

Att. 01 HP analytical TF meeting_December 12, 2016
Att. 02 Presentation for the IDEA WS on pre- and pro-haptens – Update of the HP TF.
Att. 03 Deza et al 2017 - Contact Dermatitis