IDEA Meeting of the

Hydroperoxides Task Force

December 3rd, 2015 from 9:30 to 16:30

Vlor (Vlaamse Onderwijsraad)
6, Av des Arts (6th floor)
1210 Brussels (Belgium)

Draft minutes

Participants:

Jean-Marie Aubry (JMA, University of Strasbourg), Hans Bender (HB, Moderator if the IDEA Workshops), Annabelle Besson (AB, IFF), Michael Calandra (MC, Firmenich, by phone), Alain Chaintreau (AC, Firmenich), Elise Corbi (EC, Chanel), André Düsterloh (AD, DSM), Cécile González (CG, IDEA Management Team), Elena Giménez-Arnau (EGA, University of Strasbourg), Hans Leijs (HL, IFF), Andreas Natsch (AN, Givaudan), Ulrika Nilsson (UN, University of Stockholm), Neil Owen (NO, Givaudan), Véronique Rataj (VR, University of Strasbourg), Constance Stiefel (CS, Wala, by phone), Matthias Vey (IDEA Management Team).

1. **Opening of the meeting**

AC opened the meeting at 9h45. A tour de table was done to present the new participants to the group. AC presented the objectives of this meeting (Att. 01), which were:

- Synthetizing the last results,
- Defining the next steps for the development of the analytical method,
- Drawing the conclusions from the TF that will be presented at the next IDEA Annual Review.

IDEA Management Team
Avenue des Arts, 6
1210 Brussels, Belgium
Tel: +32-2 214 20 61
Fax: +32-2 214 20 69

www.ideaproject.info

2. Antitrust statement

The Chairman reminded the participants of the IDEA Hydroperoxide TF of the constraints of the antitrust law. All agreed that there shall be no discussions of agreements or concerted actions that may restrain competition. This prohibition includes the exchange of information concerning individual prices, rates, coverages, 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. Presentation from each participant working on the action plan and discussion of the results:

3.1. Presentation Michael Calandra (Att. 02):

The presentation from MC was focused on the identification of the late eluting compounds that could explain the observed low analytical recoveries. Indeed, at the previous "Round Robin Testing", the IDEA Hydroperoxides TF members observed that the recovery of terpene hydroperoxides were low in most of the methods used for the analysis of essential oils and fragrance oils. Such late eluting compound or adduct would be peroxyhemiacetal (PHA) and are the result of the reaction between terpene hydroperoxides and aldehydes (e.g. octanal, decanal) contained in the essential oil. This reaction is reversible and seems to occur easily at room temperature with many fragrance aldehydes. JMA explained to the group that this reaction has been known for many years but this is the first time it is recognized in fragrances.

The stability of the PHA and the impact of the solvent in its formation remains to be investigated. However, there is an influence of the solvent polarity in the equilibrium. A hydrophobic solvent would drive the reaction toward the formation of PHA. The reaction with the aldehydes seem to happen very quickly and would depend on the type of aldehyde with which the hydroperoxide is reacting to.

3.2. Presentation from Andreas Natsch (Att. 03):

AN reported on the adducts that are formed when orange oil and the Lili matrix are spiked with linalool or limonene hydroperoxides. The results found are very similar to the ones presented by MC. AN pointed out that this intermediate product is also seen in commercial qualities of orange oil, and its level increased as the orange oil is spiked with hydroperoxides. The aldehydes involved in the adduct formation in the orange oil matrix are octanal and decanal, whereas the aldehydes reacting with the hydroperoxide in the Lili matrix are tropional (= heliopropional).

AN also studied the effect of the adduct formation on the recovery by analyzing samples of orange oil spiked with linalool or limonene hydroperoxides before or after the dilution of the orange oil in ethanol. When the hydroperoxides is added to the orange oil prior to its dilution in ethanol, a 50% of quenching is

observed (in particular, 60% for the linalool and 40% for the limonene hydroperoxides). When the hydroperoxide is added after the dilution of the orange oil in ethanol, no quenching and no adducts are observed, meaning that adduct formation mainly occurs in neat oils. In the Lili matrix, the results are similar as the orange oil. The hydroperoxide from linalool is more reactive than the hydroperoxide from limonene. Again when the Lili matrix is spiked before its dilution in ethanol, significant quenching (up to 80% for linalool hydroperoxide) is observed, while quenching is much lower if hydroperoxides are added after the dilution. Nevertheless, as opposed to orange oil, in the case of Lili with very high aldehyde content, some adduct was also observed being formed after dilution, even if the effect was ca. 7-fold reduced as compared to reaction in neat oil.

AN provided a list of open questions:

- Can adduct be isolated by column chromatography?
- Does adduct revert to free hydroperoxide in oils and/or in products? Does the adduct play a role of reservoir?
- What happens to adduct upon TTP reduction?
- Is adduct measured by iodometric titration?
- Can adduct be reverted to hydroperoxide upon mild treatment with base?
- Is there a rapid equilibrium anyway?
- Do we have to care for adduct when measuring HP in consumer products?

AC answered that all attempts to isolate the adduct by UIPLC or column chromatography failed. Indeed, it is likely that the adduct reacts with the active silica of direct phases or is destroyed due to the presence of water in reverse chromatography.

The group agreed that it would be useful to detect the presence of the adduct via iodometric titration in order to better understand its behavior in the essential oil and, further, in the product. MC explained that this measurement might be possible to be performed although it might require the adaptation of the current IFRA method, as the time for incubation needs to be extended.

3.3. Presentation from Alain Chaintreau (Att. 04):

AC presented results on the stability of the pure standards and on the measurements of hydroperoxides by silylation/GC-FID/predicted RRFs in orange oil and Lili matrices.

The linalool and limonene hydroperoxides standards show a good calibration linearity and a satisfactory recovery down to 500ppm. The recovery from the linalool hydroperoxide standard is higher than the recovery from the limonene hydroperoxide standard.

The linalool and limonene hydroperoxides standards are now available in pure state and can be purchased at Greenpharma. Their purity can be rapidly evaluated through silylation/GC-FID/predicted RRFs and in a

more precise way via quantitative ^{1}H-NMR with the use of an internal standard. The results show that the stability is increased when the standards are stored at -80°C, although only a slight decrease in the purity is observed for the standards stored at -18°C, except for limonene-1-hydroperoxides. AC therefore recommended to the group to store when possible the samples and standards at -80°C, in particular for the standards and samples spiked with limonene-1-hydroperoxide, which seems to be the most sensible to temperature variability. This is a possible explanation for the higher reactivity and stronger sensitizing properties that are observed with the limonene-1-hydroperoxide, in comparison to the linalool hydroperoxide.

The presence of the reaction between the hydroperoxides and the aldehydes, and the consequent formation of adducts (i.e. PHA) is very much reduced in highly pure samples. MC added that probably the metal impurities present in the samples, could significantly increase the rate of such reaction. The inclusion of a stabilizer to trap metals as complexes would need more detailed studies to ensure its effectiveness and its regulatory compliance.

The linalool and the limonene hydroperoxides can rapidly be quantified by silylation/GC-FID/predicted RRFs. The level of the aldehydes and the hydroperoxides present in the orange oil and the Lili matrices were measured by silylation/GC-FID/predicted RRFs without the need of a standard. The results show that the adduct is formed in almost the same proportion as the aldehydes are consumed. The group concluded that, as there are no aldehydes in the samples for patch testing, this reaction is therefore unlikely to occur on patch testing studies. EGA pointed out that due to the absence of structural alerts, the PHA is unlikely to be a sensitizing molecule itself. However, as PHA decomposes easily to form the former hydroperoxides and aldehydes, skin sensitization must probably occur. PHA could thus act as a reservoir of sensitizing compounds.

4. Preparation of the IDEA Annual Review 2015

With the support of HB, the group prepared the slides that will be presented at the IDEA Annual Review on December 16th, 2015 in Luxembourg (Att. 05).

5. Outcome of the IDEA Workshop Strategy and next steps

AC presented a short summary of the accomplishments from the IDEA Hydroperoxides TF, which can be summarized in 4 main topics:

- The synthesis of pure standards, available at Greenpharma,
- The purity measurement of standards and samples,
- The improvement of the quantification method,

- The new compound identified (PHA) during the analysis of samples containing linalool or limonene hydroperoxides.

Further work is required regarding the development of the analytical method. The group agreed that the method of silylation/GC-FID/predicted RRFs can be used for non-alcoholic products but that it is not applicable for complex matrices such as the finished cosmetic products. For routine quality control, FID might be the best option as there is no need for a sample or a standard and the standard is acceptable. MC proposed to continue the method development of POV and TTP. The group also agreed that there is a need to better understand the significance of the amount quantified by HLPC and GCMS. Initial work on final consumer products will start on fine fragrances and will be progressively applied to more complex matrices such as deodorants and body lotions.

The actions and next steps agreed by the group are summarized in the following table:

<table>
<tr><th colspan="3">ACTIONS</th></tr>
<tr><th>Variability of TPP-GC method in the same consumer products (fine fragrance at a first stage)</th><th>Application of other methods on the same samples (orange oil and Lili matrices, linalool and limonene hydroperoxide standards.</th><th>Role of the adduct in complex samples</th></tr>
<tr><td>Chanel
DSM
Givaudan
IFF (to confirm)
Firmenich (MC)</td><td>Chanel: MDGC-TOF (to confirm)
U. Stockholm (UN): LC-MS
Givaudan: LC-MS only for Lin-HP
IFF: LC-HRMS
Firmenich: HPLC-CL + POV (MC)
DSM: SFC</td><td>Firmenich: HPLC-CL (MC)
IFF: LC-HRMS, qualitatively
Givaudan: LC-HRMS, qualitatively</td></tr>
<tr><td>AN to prepare a small protocol on the TPP-GC method and share it with the group</td><td>UN: Communicate to the group improvements on the separation method</td><td></td></tr>
<tr><td colspan="3">AN to write a short project description to be circulated within the group and outline the sample requirement from Greenpharma.
TF participants that volunteered in the Round Robin Test to communicate to IFRA their needs on the linalool and limonene standards and spiked samples.
IFRA to organize with Greenpharma the elaboration of a new set of samples/standards to be distributed among the participants.</td></tr>
</table>

Greenpharma will need to send the Standards (spiked and not spiked, in order to know the presence of a significant level of hydroperoxides in the product). In addition, a blank, two spike levels and an additional sample for parallel experiments are needed. AN proposed to foresee 2 samples, in case the results are not available for the first sample.

6. Election of new Chair(wo)man

AC explained to the participants that he was resigning from his position of Chair, due to his retirement by the end of 2015.

The IDEA Management Team will organize a doodle poll for the electronic vote for election of the new Chair and Vice Chair before the end of December.

ACTION: IDEA Management Team to organize an electronic vote to elect the Chair and Vice Chair.

7. Next meeting

The IDEA Management Team will set up a doodle poll to determine the date of the next meeting.

ACTION: The IDEA Management Team to propose dates and organize the next IDEA Hydroperoxides TF meeting.

8. Attachments

Att. 01 IDEA Hydroperoxide Task Force meeting, December 3, 2015
Att. 02 Understanding terpene hydroperoxide chemistry: peroxyhemiacetals (MC and AC)
Att. 03 Hydroperoxides TF meeting: recent results from Givaudan (AN)
Att. 04 Rapid quantification of hydroperoxides without standards (MC and AC)
Att. 05 IDEA Annual Review – Report from the Hydroperoxides TF meeting

Minutes: CG (December 8, 2015)
First review: December 9, 2015 (AC, AN)
Review by the Hydroperoxides TF: December 10, 2015
Final: December 15, 2015