IDEA
International Dialogue for the Evaluation of Allergens

IDEA Meeting of the

Hydroperoxides Task Force

March 24th, 2014 from 2:00pm to 6:00pm

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Final Minutes

Participants: Jean-Marie Aubry (Lille university), Hans Bender (IDEA), Anna Börje (Gothenburg university), Hugues Brevard (Robertet), Alain Chaintreau (Firmenich), Elena Gimenez (Strasbourg university), Fred Lebreux (IDEA), Andreas Natsch (Givaudan), Ulrika Nilsson (Stockholm university), Neil Owen (Givaudan), Véronique Rataj (Lille university), David Roberts (Liverpool university), Clémentine Marteau (I.F.F.), Matthias Vey (IDEA).

1. Adoption of the agenda

As a result of the election conducted in March, Alain Chaintreau was appointed chairman of the IDEA Hydroperoxides TF. An informal agreement was taken between the two candidates who applied for the chairman position: Alain Chaintreau will lead the group until completion of the chemical part, then Andreas Natsch will take care of the follow-up application of developed methods to risk assessment.

The chairman opened the meeting at 2:00pm by welcoming the participants and organized a tour de table. The agenda was adopted such as provided. The Chairman's general presentation is attached to the minutes (att.01).

2. Antitrust statement

The Chairman reminded the constraints of the antitrust law to the participants. 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,

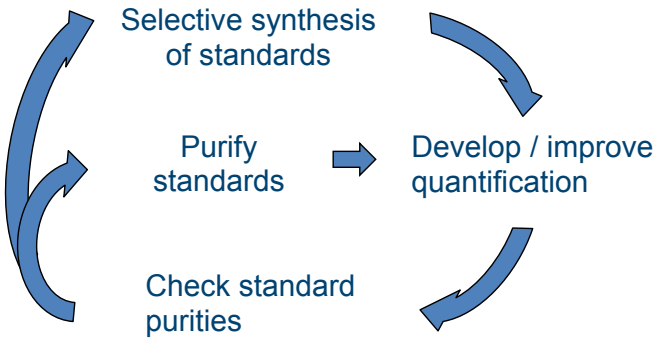
claims settlement practices, or any other competitive aspect of an individual company's operation. Each participant is obligated to speak up immediately for the purpose of preventing any discussion falling outside these bounds.

3. Key objectives of this group: presentation and discussion

The key objectives of this group were summarized as follows:

- Select and ensure the general availability of a suitable range of pure references resulting from abiotic transformation of fragrance ingredients; this step implies the development of procedures to prepare and purify haptens currently not commercially available. Furthermore, and because some products of abiotic transformation may be unstable (i.e. hydroperoxides), the half-life of these chemicals as well as any other parameters related to their conservation should also be investigated.
- Use these references to develop new analytical methods for the detection and the quantification of chemically-defined haptens, resulting from abiotic transformations, in fragranced products. These methods should be sensitive, specific, with target limits of quantification below the estimated induction levels and limits of detection below the estimated elicitation levels.
- Make all relevant haptens resulting from the abiotic transformation of fragrance ingredients readily available for patch-testing. In case of success, these new patch-testing references will be presented to the dermatology community and potentially introduced in patch test baseline series.

The participants agreed that the hydroperoxides issue can only be solved via an iterative approach as pure hydroperoxides are necessary to develop a reliable quantification method and a reliable quantification method is necessary to assess the purity of hydroperoxides.



4. Hydroperoxides of concern

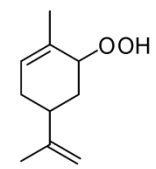
- <u>Terpenes:</u>

  The group agreed that hydroperoxides resulting from limonene, linalool and linalyl acetate oxidation are the most important ones and should be studied in priority. This choice is justified by several elements including the consumer exposure, the oxidation potential, the existing knowledge already collected on these allergens and the great difference of skin sensitization potency with parent materials. Citronellol

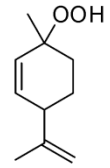
hydroperoxides was added to the list because its synthesis is simpler (one double bond vs two double bonds in geraniol), well controlled and because the skin sensitization potency of citronellol is limited compared to its oxidized form. Other hydroperoxides could be considered in the future once light is shed on the ones outlined above.

- Isomers:

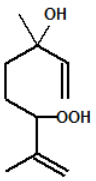
  The group agreed that the research should stay limited to a few isomers: limonene-1-OOH, limonene-2-OOH, linalool-6-OOH and linalool-7-OOH are the major isomers resulting from limonene / linalool autoxidation and seem to have the strongest skin sensitization potency.



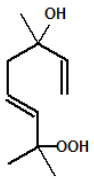
***cis/trans*-Limonene-2-OOH**
EC3 = 0.83%



cis/trans-Limonene-1-OOH
EC3 = 0.33%



Linalool-6-OOH



Linalool-7-OOH

EC3 of 5:3 mixture = 1.6%

5. The synthesis of hydroperoxides

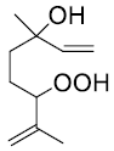
- Background science:

  The chemistry of hydroperoxides has been studied and several reactions can be used to selectively synthesize some of the substances of interest (limonene-1-OOH, limonene-2-OOH, linalool-6-OOH, linalool-7-OOH and citronellol hydroperoxides). One of the most common reactions to produce organic hydroperoxides is the Schenck reaction[1].

  Except for very hydrophobic substrates, the use of microemulsions was regarded as useless and even counterproductive. This complex system should be avoided for the synthesis of terpenes hydroperoxides and replaced, for instance, by methanol.

[1] Singlet oxygen ($^1O_2$), the oxidative specie, is made *in situ* by peroxymolybdate-catalyzed (MoO_4^{2-}) disproportionation of hydrogen peroxide (H_2O_2). In theory, singlet oxygen is only necessary to initiate the reaction, then a radical propagation starts. A careful adjustment of hydrogen peroxide and peroxymolybdate is therefore necessary to optimize reaction's kinetic.

The main shortcomings of all existing methods are the limited yields and the difficulty to purify hydroperoxides.
Elena Gimenez gave a short presentation highlighting the merits of derivatization (via peroxysilane formation) to separate isomers and enhance their purity (att.02). Her group can selectively synthesize limonene-2-OOH from carveol (one step, overall yield of 48%) and linalool-7-OOH from linalool (3 steps, overall yield of 31%).



The group remarked that the form of linalool-6-OOH not reacting with one equivalent of TBDPSCl (reported at the left) was not recovered. The recovery of this material might lead to another source of pure hydroperoxides and therefore should be investigated. Elena Gimenez agreed to check with the trainee who developed this methodology. She mentioned that the student will leave the laboratory in June therefore a rapid confirmation is necessary.

ACTION: Elena Gimenez will check with her student whether or not the unreacted linalool hydroperoxide isomer can be recovered.

Andreas Natsch's group has developed a method to synthesize a mixture 5:3 of linalool-7-OOH and linalool-6-OOH. The isomers of this mixture are very difficult to separate (at least in GC-MS conditions) but the synthesis is efficient and Givaudan could provide this reference sample to the group if needed.

Anna Börje and Ulrika Nilsson developed conditions to efficiently synthesize limonene-1-OOH.

ACTION: The participants will share articles focusing on the synthesis of selected hydroperoxides with the task force.

The question was asked if the isomers separation is really needed as the objective is to understand what happens in raw materials and consumer products (where the hydroperoxides formation is mainly not selective). Furthermore, it was mentioned that cross-reactivity between several (if not all) forms of hydroperoxides cannot be excluded. If this is the case, there would be no reason to separate isomers. The group agreed that this needs to be verified but, at least for the analytical development, isolated isomers need to be available.

The Chairman asked if correlations could be made between bond dissociation energies (BDE) and chemical reactivity, allergenic activity or stability. Regarding allergenic activity there is limited interest because all hydroperoxides have a comparable EC3 (between 0 and 2). Rather than BDE, Jean-Marie Aubry suggested to calculate charge densities.

- <u>Approach to adopt and establishment of criteria to select a contract research laboratory:</u>

The Chairman asked to the participants who should take the lead on this project and summarized the specifications that the ideal synthesis lab should meet:

1. Selective synthesis of isomers (without taking the chirality into account). Alternatively, a preparative isolation from a mixture of isomers could be considered, if the resulting quantities and timeframe comply with items 4 and 6 reported below.
2. Structural confirmation of the compound: To be checked, notably by NMR (^{1}H and ^{13}C).
3. Purity: the highest possible purity of each isomer (>90%)
4. Quantities to be delivered: at least 10 g / isomer to be shared between the laboratories developing the quantification.
5. Stability: it should be checked in the course of the development
6. If the stability of some isomers in pure state is too low, alternatives should be investigated so that the analytical laboratories receive samples at known concentrations.
7. Maintain the availability of most frequent standards during all the project.
8. Timeframe: 6 months
9. Partnership: The synthesis lab should work in partnership with (the) analytical lab(s).

The fragrance industry does not have the resources and expertise to entirely manage it. The academic laboratories around the table explained that this work requires an important manpower and none of the universities have engineers who could work on it. Ideally, this should be a Ph.D. thesis subject but some participants felt that the technical difficulties inherent to this project and the limited opportunities to publish are prone to discourage candidates. The name of a well-known company specialized in the manufacturing of hydroperoxides-containing patch-test devices was mentioned. The group felt that it might be a solution for the access to hydroperoxides mixtures but probably not to pure chemically-defined hydroperoxides.

ACTION: The participants will send to the FL the contact details of laboratories which could take care of the synthesis part of this project.

- <u>Specific questions (hazardous chemicals transporter, stability upon storage):</u>
  The group confirmed that the common belief that hydroperoxides are unstable is mainly wrong. Most of hydroperoxides resulting from terpenes oxidation do not degrade below 80°C. The rule saying that "organic hydroperoxides with more than 10 carbons do not explode and are even pretty stable" is almost always verified. Terpenes are just at the limit but, based on researchers' experience, it can already be considered as stable.

  The Chairman found contact details of a consultant who might take care of hydroperoxides transportation. Although the group did not regard it as necessary, this consultant is able to ship materials at -180°C and -90°C.

Hazard classification needs to be determined to transport materials by airways. However the consultant explained that this can be done by reading-across of other hydroperoxides.

The bibliography indicates that the exchange of hydroperoxides between research laboratories is already something usual and the transportation issue was not regarded as a real problem.

6. The quantification of hydroperoxides

- Background science:

  Several analytical methods (HPLC-MS/MS, HPLC-CL, TMS+GC-MS, PPh_3+GC-MS, LC-MS) have been published and led to relatively accurate results in well-controlled conditions (given hydroperoxides, given matrices, etc.) However, the group agreed that there is no general / ideal method: the existing ones are complementary and still need to be optimized / validated. This optimization / validation work cannot be achieved until pure reference materials become available.

  The group agreed that the ideal method should not require sample pre-treatment or chemical reactions, be specific and sensitive and avoid hydroperoxides degradation during the analysis.

  It was mentioned that the iodometric titration, although non-specific (dosage of all hydroperoxides), can provide useful information on the general oxidation state of mixtures. This method might become very useful in case cross-reactivity between all hydroperoxides is proved.

- Establishment of criteria to select a contract analytical laboratory:

  The Chairman summarized the specifications that should be met by a contract research laboratory in charge of developing the analytical method:

  1. Selectivity towards the hydroperoxides, or convenient means to locate hydroperoxides in a chromatogram.
  2. When the calibration of hydroperoxides has been achieved once, use of recorded (relative) responses to avoid the further use of standards.
  3. Alternatively, if these two criteria cannot be met by a single method, several methods would be developed if each of them meets one of these criteria.
  4. Methods based on a spectrometric detection should comply with the state-of-the-art practices. Notably, the identity of quantified peaks should be checked to avoid analyte confusion and detect coelutions.
  5. Purity and stability of standards: in partnership with the synthesis laboratory, the purity and stability of standards as a function of time will be checked, to determine possible storage conditions and shelf-life.
  6. The proposed method should be submitted to a pre-validation (intermediate precision) by its author.

7. Method delivery one year after the beginning of the synthesis project. The quantification development should start before the availability of pure standards, to support the synthesis project in the purity determination of standards.
8. Partnership: The analytical laboratory should work in partnership with the synthesis laboratory.

The group briefly discussed the financial aspects of this project and it was recommended that the industry applies for EU funds (e.g. Horizon 2020). Elena Gimenez knows the process quite well and is ready to participate in a small group to determine whether or not the hydroperoxides project has the potential to be EU-funded. In case the group decides to opt for this strategy, a rapid preparation is necessary. In general it is a two-step selection process for all the calls. The first step is a pre-proposition (short) and if selected then one month is granted for the second-step which consists in submitting a full proposition. Elena Gimenez recommended that the task force looks at the calls for 2015 and explained that the participation of IFRA (fragrance industry) as co-founder might be important. A consultant would have to do the administrate part of this preparation and this is quite a big project to set up therefore it was agreed to determine first whether or not hydroperoxides are considered a public health priority by the Commission.

ACTION: A small group will be formed to determine whether or not the hydroperoxides project has the potential to be EU-funded.

- <u>Next steps:</u>
  It was regarded as appropriate to first evaluate the existing methods via a kind of ring-test (identical samples would be sent to all participant laboratories for analysis of hydroperoxides content). Orange oil was considered an excellent matrix to quantify limonene-1-OOH and limonene-2-OOH. Shiu oil will be used to quantify linalool-6-OOH and linalool-7-OOH. Robertet agreed to provide both essential oils to the participants. Givaudan agreed to spike Shiu oil with a mixture of linalool-6-OOH and linalool-7-OOH and also to provide the reference material to all participants. The IDEA Management Team still has to find a reliable source of limonene hydroperoxides. Andreas Natsch will investigate internally. At a later stage, and based on first ring-test's results, hydroalcoholic products could be investigated too.

  ACTION: Andreas Natsch to check whether or not Givaudan can provide limonene hydroperoxides. If not, the IDEA Management Team will look for alternative sources.

  ACTION: Hugues Brevard to provide Orange oil and Shiu Oil to the laboratories responsible for spiking and distribution (to be confirmed for Orange oil).

  ACTION: IDEA Management Team to coordinate the ring-test to come.

As a second step, it was suggested that labelled hydroperoxides (^{2}D or ^{13}C) be synthesized to facilitate their analysis. The group agreed that it might be a good idea.

ACTION: As per Jean-Marie Aubry's request, Givaudan, Firmenich and Robertet will check if qualities of limonene without BHT are commercially available. Such qualities would facilitate the research on antioxidants.

FL reminded that hydroperoxides degradation products are always less potent sensitizers than the parent hydroperoxides. Beyond the usual risk management measures that can be derived from better analytical tools, catalysts might then be developed to promote the degradation of hydroperoxides once formed. This "scavenger" would act in tandem with antioxidants and allow the global reduction of hydroperoxide-content in consumer products. The group agreed that this point is worthy of consideration and it might the object of future Hydroperoxides TF meetings along with other topics such as the chemistry of antioxidants.

Preparation, 28/03/2014
F. Lebreux
IDEA Management Team

First Review, 09/04/2014
A. Chaintreau
Chairman of the IDEA Hydroperoxides TF

Final Review, 23/04/2014
IDEA Hydroperoxides TF