IDEA
International Dialogue for the Evaluation of Allergens

Rapporteur's Progress Report on the IDEA Workshop on

Characterization of Fragrance Allergens

August 27-29th 2013

Dolce La Hulpe Brussels
Chaussée de Bruxelles, 135
B-1310 La Hulpe, Belgium

1. Background information regarding the International Dialogue for the Evaluation of Allergens (IDEA):

Fragrance Allergy is a topic of high interest for the fragrance industry, its customers and the Authorities as expressed through the 2012 SCCS Opinion on Fragrance Allergens. The fragrance industry is determined to address this issue and provide solutions supported by a broad, multi-stakeholder approach.

To fulfil this objective, a work plan (att.01) was developed in the course of 2012 and submitted to DG Sanco Risk Assessment Unit for scrutiny. All comments and suggestions were taken into consideration and the final document, having received the Commission's support, is a clear roadmap intended to deliver positive outcomes for the consumers, the Authorities and the industry. This work plan has now moved into its execution phase and the International Dialogue for the Evaluation of Allergens (IDEA) represents its transposition into concrete actions and investments. Through the organization of experts' workshops and the planning of scientific studies, IDEA aims at providing an agreed and transparent framework for assessing fragrance sensitizers in a prospective way and, ultimately, to find optimal solutions to the issue of fragrance induced skin allergies.

Some fragrance ingredients have the potential to cause skin sensitization. However, such fragrance allergens may be described in various ways and the need to define them via a collaborative approach between stakeholders was clearly identified. A clear understanding of what is a skin sensitizer, what are the tools to identify it and how to combine these tools adequately remains a prerequisite to characterize a fragrance allergen. The aim of this workshop was to characterize fragrance allergens, which specifically are contact allergens, to define their relevance and to discuss possible ways to improve their identification and the diagnostic of related contact allergies.

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. Rapporteur's progress report

a. Introduction

Allergic Contact Dermatitis (ACD) to fragrance results from a complex combination of factors including (but not limited to) the exposure pattern, the allergen potency, the genetic predisposition and the age. ACD is a disease involving the immune system and producing delayed-type reactions. ACD arises as the result of two essential stages: an induction phase, which primes and sensitizes the immune system for an allergic response, and an elicitation phase, in which this response is triggered. It is now generally accepted by the Dermatology community that both the sensitization and the elicitation phases are triggered by a threshold mechanism, the dose triggering the induction generally being higher than the threshold triggering an elicitation reaction. For this reason, safe use levels preventing induction and/or elicitation can be derived for each allergen via an appropriate risk assessment methodology.

ACD should not be confused with Contact Allergy (CA), an asymptomatic state, which simply means that the immune system has been exposed to an allergen and can recognize it, with subsequent ACD after sufficient exposure. Furthermore ACD has to be distinguished from irritant contact dermatitis (ICD), which is a non-specific reaction of the immune system in response to a chemical or a physical irritant. There is no specific immune memory in ICD, which is transient after an acute contact, although it may become chronic on iterative exposure. In contrast to ICD, ACD represents a serious safety concern for consumers. Therefore the workshop only focused on this second, and less frequent, type of contact dermatitis.

Metals, fragrances and preservatives are the most important contact allergens but other ingredient types (e.g. hair dyes, antioxidants) are also frequently reported as skin sensitizers. In the context of this workshop, only the skin sensitization endpoint was taken into consideration for the characterization of fragrance allergens.

The prevalence of CA and ACD to fragrance allergens in the general population is around, respectively, 6% and 2% and about 15% in all tested patients having an ACD. Apart from a possible genetic predisposition of populations the body region where products are applied also has its importance since the use pattern of products and the genetic predisposition of populations seem to have a clear impact on the prevalence (e.g. Italy versus Sweden).

Physical examination and patient history undertaken by an experienced dermatologist are the initial diagnostic steps to suspect (fragrance) allergens are responsible for a skin reaction. Patch-testing is the only available tool for the identification of contact allergens. The skin sensitization potential is usually examined by hazard assessment tools (animal studies like LLNA, *in vitro* and *in silico* studies). Patch-testing cannot usually provide quantitative information on the allergen potency unless the levels of exposure to sensitizing agents are individually known. However, the degree of individual sensitization can be estimated from the patch test reaction's morphology (shape, size, edge effect, etc.). Beyond the negative, IR (irritant), or ? (doubtful), +, +, ++ or +++ reactions reflect the intensity of sensitization corresponding to the inter-individually varying elicitation thresholds in serial dilution patch testing.

Patch testing for the diagnosis of fragrance allergy usually starts with the application of fragrance mixes (FMI and FMII), which are standardized mixtures of the most common fragrance allergens. In case of a positive reaction to these fragrance mixes their ingredients are tested whenever possible to allow precise identification of the individual

allergen(s). In cases of suspected fragrance/cosmetic allergy, if the patient does not react to the fragrance mixes neither to its ingredients or additional commercial fragrance allergens, the practitioner has to investigate the consumer product that triggered the skin condition. The dermatologist has several options including the patch-testing of ingredients as far as labeled on-pack (by virtue of the EU cosmetic / detergent regulations), with the notable exception of fragrances beyond those 26 presently required to be labeled) and the request to the product manufacturer for information and provision of test materials regarding those fragrances not available as commercial allergens.

Patch-testing can be used for other purposes than the diagnosis of ACD and notably to define the prevalence of CA to specific fragrance allergens (epidemiologic studies) and to ensure that a fragrance allergen does not sensitize humans when used at a specific concentration in the HRIPT (Human Repeated Insult Patch Test). Repeated Open Application Test or Use Test, are another form of human testing that helps to determine a person's elicitation threshold for a fragrance ingredient in real-life conditions and then to evaluate consumer tolerance.

Several parameters need to be carefully tuned in order to get meaningful patch-testing results. For instance, the allergen concentration should not be too low (risk of a doubtful or false-negative reaction) or too high (risk of a false-positive reaction and/or sensitizing the patient). Doubtful reactions should be avoided.

The aim of this workshop was to characterize fragrance allergens, which specifically are contact allergens and to discuss possible ways to improve the tools used for the diagnosis of CA and the clinical relevance of reactions established with these tools. Furthermore, this workshop was intended to discuss the existing diagnostic process in view to enhance industry's responsiveness each time a consumer product is responsible for adverse skin reactions. Finally, this workshop was designed to optimize knowledge sharing on general concepts linked to fragrance allergens between dermatology practitioners and to pave the way forward for a better and easier identification and characterization of fragrance allergens.

b. Definition

All concepts related to the identification of fragrance allergens are tightly linked to the definition of 'contact allergen'. For this reason, the development of a commonly-agreed definition of 'contact allergen' was regarded as a prerequisite for further discussions. Definitions of contact allergen were given by several presentations including the below operational definition used by the SCCS in its recent Opinion on fragrance allergens in cosmetic products:

> *A fragrance substance, or a natural mixture of substances (extract), which (in some cases after chemical modification or bio-activation),*
> - *based on several published reports of sufficient quality, has caused contact sensitization in patients, or*
> - *according to a historical human max. test / HRIPT is a sensitizer, or*
> - *has been identified as contact allergen in guideline animal methods or*
> - *can be categorized as likely allergen if limited human or experimental data is combined with structure activity considerations*

The SCCS used this definition to propose a two-part approach for the identification and characterization of fragrance allergens: a first 'fishnet' aimed at identifying all contact allergens about which consumers/dermatologists should be informed. Based on this list of allergens, a second 'fishnet' was used to identify contact allergens of concern within the subset of 'established allergens in humans' following the SCCS criteria.

This methodology was regarded as valid but, regarding 'fishnet 1', some workshop participants highlighted four potential points of refinement:

- Extensive guidance under the CLP Regulation has been developed meanwhile regarding the interpretation of human data. This guidance, which includes some differences with the SCCS approach, was not available at the time the SCCS did its work and could serve as a reference.
- Have a critical evaluation of the quality of the studies, comparable to the Klimisch criteria for toxicological studies. Relate the clinical data to exposure.
- Complement animal data with human data as much as possible.

Regarding 'fishnet 2', it was suggested that subcategories (such as 'alert', 'concern' and 'high concern') based on defined levels of concern be used to refine the existing classification of contact allergens. Potential criteria for the categorization into these levels of concern might be the relative frequencies from consecutive clinical testing (rather than absolute cases), the clinical data on the proven cases of ACD and the consumer exposure. Finally, blinded ROAT studies with scented products mimicking actual use could be an option to confirm the assignment of contact allergens to above subcategories.
The criteria outlined above could be integrated into an appropriate risk assessment/management system. Additionally, the workshop participants agreed that current evidences on the prevalence of ACD to fragrance ingredients in the general population should be reviewed. In light of conclusions established during this workshop, it was suggested that product category-related exposure information be combined with similarly stratified clinical data. In this regard, it was recommended that definition of product categories be developed, taking into consideration Annex I of the Cosmetics Regulation, CLP, and the IFRA categories.

The workshop participants agreed to set a more general definition of 'contact allergen' and the following proposal was developed during the workshop:

> *A contact allergen is a substance that is capable of inducing delayed type sensitization in humans, which may manifest as allergic contact dermatitis.*
> *The elicitation of allergic contact dermatitis requires sufficient exposure, and is subject to significant inter-individual variability.*

c. Tools to identify contact allergens

Specific emphasis has been laid on the different methodologies to identify a sensitizing fragrance ingredient with the general agreement that, in principle, valid human data should override animal data.
Several tools already exist for the identification and the characterization of contact allergens:

c.1. Predictive Tests (Animal studies)

The Buehler Guinea Pig Test and the Local Lymph Node Assay (EU B42, OECD 429) are regarded as reliable test methods for the identification of contact allergens. Except the Buehler test the other Guinea Pig tests are not considered to provide reliable indication for potency. These predictive animal studies are mainly used for the hazard assessment of fragrance allergens but animal welfare considerations imply the replacement of these tools by *in vitro* / *in silico* testing.

c.2. Tests in Humans

As of today, patch-testing is the gold standard to diagnose ACD and with other skin tests (e.g. ROAT) to carry out tolerance studies in the general population. ACD is a disease with many faces that need to be recognized by dermatologists. It can be difficult to distinguish from other skin problems. As a human in vivo test, special attention should be paid to the analysis of patch-test results to reduce the risk of false negatives and false positives. The great majority of human data is derived from diagnostic tests in patients. Other human studies can present some ethical issues.

The HRIPT (Human Repeated Insult Patch Test) is nowadays used as a confirmatory test in the safety evaluation of skin sensitizers, employing exposure doses/area considered non-inducing, usually consisting of two phases. In the Induction phase (Phase I) the allergen under investigation is applied to the skin a few times (typically 9 x 24-hour or 48-hour exposures) during the course of a 3-week period and to each of 100 to 200 volunteers. This is followed by a 2-week rest period after which the skin is exposed to the allergen again on both the induction site and a naïve site using a 24-hour to 48-hour patch-test (Phase II or Elicitation Phase). A response in Phase II is usually allergic in nature and skin reactions are scored over the subsequent few days.

The repeated open application test (ROAT) or use test is, as its name states, an open exposure test that intends to mimic real-life exposure situations. This dose-response test intends to evaluate the tolerance of the sensitized individual or sub-population to a given allergen and gives qualitative or even quantitative information on the elicitation threshold. A finished product (or a well-defined vehicle containing the allergen under investigation) is applied once or twice per day for a 1-3 week period in sensitized individuals. Several test sites may be challenged at the same time using different test concentrations. Application of the test agent or allergen containing solution is either performed by rubbing the sample at the test site or by using a micropipette followed by spread. The marked challenge site is inspected according to a pre-determined reading schedule (e.g. at day 2, 3, 4, 7, 14 and 21). A positive response usually develops after 2-4 days of application, but for weaker allergens or to lower allergen dose/area it can develop later. Dose-elicitation data can be derived from a group of thus tested sensitized persons in terms of a (fitted) dose-response curve of the cumulative response to doses/area cumulated over time, until first appearance of a positive result.

Large inter-individual variations in the concentration triggering the elicitation reaction is observed. These variations generally reflect the strength of the sensitization determined by varying induction doses/area but might be caused by recent exposures to the investigated allergen. However, there are many other reasons for false positives and

negatives. These include methodological problems as well as insufficient scoring of the test results. The methodological problems include insufficient concentration for elicitation, difficulties to obtain the relevant test samples at appropriate concentrations (a too low concentration of the sensitizing agent may result in a negative patch-test) and inappropriate performance of the test.

Although the human patch test has been formalized by commonly agreed protocols, the individual dermatologist's experience still determines the reliability of the diagnosis to an important extent. For this reason, patch-tests should only be performed by sufficiently trained dermatologists. This expertise in running patch-tests was regarded as particularly crucial in case of slight effects due, for instance, to the weak potency of allergens in relation to their patch test concentration and patients' specific sensitivity. In these situations, expertise plays an important role in the ability to correctly diagnosing patch test reactions and contributes to avoid false positive and negative diagnostic results.

Furthermore, the workshop participants identified other potential targets for improvement and further research, respectively:

- Use of appropriate concentrations and vehicles where these have not yet been firmly established
- Test with patients' own products whenever possible
- Better availability of appropriate test samples for breakdown patch testing.
- More structured and efficient communication between the dermatologists and the products manufacturers (in particular when the culprit cannot be spontaneously identified)
- More dose-response tests (ROAT's) in volunteers with documented contact allergy to a fragrance substance
- Consideration of impact of the vehicle, impurities, additives, etc.
- Standardized documentation and timely reporting of test results including clinical history

Considering that the regulatory agencies and the industry need to collect all available information for respectively regulatory and safety evaluation purposes, there was agreement that any relevant information should be made publicly available, especially in the peer reviewed scientific literature. This is exemplified by part of the sources that were evaluated by the SCCS for the development of its scientific Opinion on fragrance allergens:

- Manual search of the journal "Contact Dermatitis"
- Medline search of CAS numbers identified in reviews and clinical studies already retrieved
- Manual search of all RIFM reviews published in "Food Chem. Tox." (last 20 years)
- "Top 100" substances in terms of volume used and "Top 101-200" substances if R43 (as supplied by IFRA)
- Animal test data (GPMT, LLNA, Buehler test) requested from IFRA, eventually LLNA reports' summaries.

c.3. In vitro / in silico studies

Accelerated by the legal ban of animal testing, the development of *in vitro* and *in silico* alternatives aims at the identification of fragrance allergens but also for the determination of their potency (non-sensitizing, very weak, weak, moderate or strong sensitizers). The existing assays (DPRA, KeratinoSens, h-CLAT, MUSST, TBD, etc.) are

complementary and need to be jointly used according to a sound integrated testing strategy to deliver meaningful conclusions.

However, in spite of the numerous technological progresses made this last decade on in-vitro testing, the determination of allergens potency still remains to be developed (including above-mentioned integrated strategy) and validated. More efforts should be done to improve the existing tools or to create new tools able to deliver quantitative estimates.

d. Tools to monitor contact allergens

Since premarketing methods to characterize contact allergens are not always perfect, the post-marketing surveillance of CA and relevant sensitizers and refine the risk assessment methodologies / risk management measures where necessary. These monitoring tools include:

- Appropriate diagnosis in patients by dermatologists, which is seen as the basis for objective evaluation, and collection, analysis and publication of this clinical data
- Epidemiological studies
- Consumer reports collected by companies, which can be helpful but need to be verified with regard to ACD and the actual sensitizer
- Cosmetovigilance results collected by networks of dermatologists (e.g. REVIDAL in France)

The optimal approach to be followed by dermatologists to identify the contact allergen that has caused a particular case of ACD is adequate documentation of history and skin effects, patch testing with commercial test series and possibly culprit consumer product. Additional breakdown testing of product ingredients is sometimes required, especially if patch testing with commercial allergens does not yield an unequivocal explanation of ACD to a cosmetic product. If the suspected culprit allergen is not part of the commercial test series, further liaising with the product manufacturer is required. The results of this research should be reported to the product's manufacturer (for voluntary risk management measures) and to a "suitable public body" collecting the data in view of further analysis and publication.

The European legislation (e.g. Cosmetic Regulation) foresees that undesirable effects resulting from the use of consumer products should be reported to the competent authorities and Member States shall ensure that the information is made accessible to the relevant specialists.
For instance, the French surveillance network REVIDAL/GERDA promotes intense cooperation between experts and publishes their case collections. In Germany the IDOC (Information and Documentation Centre) for contact allergies provides a broad collection of physicians' reports and publications. One of IDOC's main missions is to liaise with the industry and to provide assistance to dermatologists for the access to chemically-defined allergens. Once the diagnosis completed, test results are communicated to IDOC which, after evaluation, can forward them to the industry. This flexible system allows early detection of hitherto non-considered cosmetic allergens.
However, and similarly to the French network, the sharing of available information maintained by IDOC can be hampered by confidentiality issues. Inconsistent medical quality of the IDOC data can also be a problem.

These surveillance networks are useful but their efficiency depends partly on industry's responsiveness. The workshop participants identified several ways to improve the current diagnostic process in view to enhance the responsiveness of the industry. First, it was remarked that trade associations like IFRA could play a key role on a worldwide basis in the identification of consumer product companies for the dermatologists to contact. Secondly, there is a need for a standardized method on how to supply appropriate samples to dermatologists. This method would formalize samples dilution and vehicles.
Furthermore, a formal mechanism should be developed to ensure an exchange of information between the dermatologists and the industry once the patch-test on provided samples is conducted beyond the case-by-case approach as in IDOC. Additionally, a formal mecchanism would be needed to review the clinical data collected on fragrance allergens with the aim of revising risk assessment and risk management of these ingredients and of activating the pro-active surveillance wherever it is regarded as necessary.

e. Potential improvements of diagnostic patch-testing

As of today, the only established methodology to diagnose CA is patch-testing. Its optimal use requires education and training. Efforts should be made to improve the education of dermatologists beyond academia and to certify their competence in the procedure.

The development of supplementing and/or alternative diagnostic tools is an interesting area of research. At the present time, basic science methodologies (e.g. immunological assays) to study the pathophysiology of ACD already exist but still require evaluation of diagnostic usefulness and eventual translation into clinical practice.
It was agreed that the development of a "blue/red indicator test" allowing a microanalysis of the doubtful or weak positive patch tests could be useful to the dermatology community. This tool should be able to quickly determine whether or not the observed reaction is a true contact sensitization. However, the usefulness and value of such a technique that is not yet on the horizon for regular use would have to be confirmed by scientific clinical trials conducted in selected expert patch-testing centers.

The concept of clinical relevance was intensely debated during the workshop. It was agreed that the assessment of clinical relevance of a positive patch-test is based on clinical evaluation of patient's history, the exposure to that allergen and its temporal relation with ACD and on the characteristics of the allergen and the ACD. The predictive value of a positive patch-test depends on sensitivity and specificity as well as the prevalence of ACD in the tested population. Often, identification of the culprit allergen is complicated by patients reacting to more than one allergen in subsequent patch tests.
ROAT in individual cases will help to assess the clinical relevance in those patients in whom it cannot be determined otherwise. An eczematous ROAT response will distinguish the irritant or allergic nature of the reaction if appropriate controls are included.

f. Recommendations

Based on the discussions and conclusions of the three breakout groups, the workshop participants proposed to further explore the following points:

- While the patch-test is a proven diagnostic instrument, further research should aim at reducing uncertainties (false positives and negatives).
- Improve categorization of contact allergens (high and low concern) for C&L, risk assessment and risk management.
- Improve data collection (e.g. by specific centers) in view to collect, scrutinize and publish all useful clinical data.
- Improve the communication between dermatologists and industry, the main recommendation being to establish a standardized procedure for providing samples to the dermatologists and to communicate results of the clinical investigations to industry.
- Better understand the difference of prevalence of skin sensitization, which may be caused by specific genetic predispositions and consumer habits (e.g. north vs. south of Europe or Europe vs. USA).
- Provide further justification for the notion that many low dose exposures are more potent than a single high dose.
- Better understanding of how best to establish the link between CA and ACD in terms of dose elicitation data.
- Provide *in vitro* methodologies for risk assessment purposes and, notably, for the quantitative determination of allergens potency.
- Consider the development of a new diagnostic tool that might help further improve diagnosis of CA for the benefit of the patient
- Define the concept of 'levels of concerns'.
- Develop definition of product categories (taking into consideration Annex I of the Cosmetics Regulation, CLP, and the IFRA categories).
- Understanding geographical differences in prevalence – for instance, US dermatologists at the workshop noted that positive patch tests to HICC/HMPCC were quite rarely observed in the US. A proposal that this is due to differences in exposure was subsequently confirmed to be an unlikely explanation.

Prof. Helmut Greim
Rapporteur of the workshop

APPENDIX 1: Additional reading

- "A proposed relevance scoring system for positive allergic patch test reactions: practical implications and limitations", J.-M. Lachapelle, *Contact Dermatitis*, **1997**, 36, 39-43.

- "Operational definition of a causative contact allergen – A study with six fragrance allergens", J.J. Hostynek and H.I. Maibach, *Exogenous Dermatology*, **2003**, 2, 279-285.

- "The relationship between exposure dose and response in induction and elicitation of contact hypersensitivity in humans", P.S. Friedmann, *British Journal of Dermatology*, **2007**, 157, 1093-1102.

- "*Risk factors associated with sensitization to hydroxyisohexyl 3-cyclohexene carboxaldehyde*", W. Uter, J. Geier, A. Schnuch and O. Gefeller, *Contact Dermatitis*, **2013**, *69*, 72-77.

- "*Categorization of fragrance contact allergens for prioritization of preventive measures: clinical and experimental data and consideration of structure–activity relationships*", W. Uter, J.-D. Johansen, A. Börje, A.-T. Karlberg, C. Lidén, S. Rastogi, D. Roberts and I. R. White, *Contact Dermatitis*, **2013**.

- "*The critical review of methodologies and approaches to assess the inherent skin sensitization potential (skin allergies) of chemicals*" (Part I, II & III), J. P. Thyssen, E. Giménez-Arnau, J.-P. Lepoittevin, T. Menné, A. Boman and A. Schnuch, *Contact Dermatitis*, **2012**, *66*, 11-70.