

## Formation of Skin Sensitizers from Fragrance Terpenes via Oxidative Activation Routes – Chemical Analysis, Structure Elucidation and Experimental Sensitization Studies

LINA HAGVALL

*Department of Dermatology and Venereology, Sahlgrenska University Hospital, Gothenburg, Sweden. E-mail: lina.hagvall@vregion.se*

Lina Hagvall defended her Ph. D. thesis in Gothenburg, Sweden, on January 30, 2009. Her opponent was Dr. Andreas Luch, Head of the Department of Product Safety and Scientific Services, Federal Institute for Risk Assessment, Berlin, Germany. The thesis was supervised by Professor Ann-Therese Karlberg and Dr. Anna Börje, Dermatochemistry and Skin Allergy, Department of Chemistry, Faculty of Science, University of Gothenburg, Gothenburg. The thesis can be found at: <http://hdl.handle.net/2077/18951>. Lina Hagvall is now working as a chemist/occupational hygienist at the Department of Dermatology and Venereology, Sahlgrenska University Hospital, Gothenburg, Sweden.

The work presented in this thesis comprises the identification and analysis of compounds susceptible to oxidative activation and the formation of strong contact allergens. These studies formed part of a research program whose overall goal was to enhance understanding of how skin sensitizers are formed by autoxidation following exposure to air or by cutaneous metabolism from compounds with no or low sensitizing capacity.

Exposure to fragrances is difficult to avoid in daily life. Many fragrances are skin sensitizers and testing for traditional markers of contact allergy has shown them to be the second commonest cause of contact allergy, after nickel. As the immunological memory formed during the development of contact allergy persists throughout life, and symptomatic treatment of allergic contact dermatitis is the only option currently available option, preventive work is pivotal. Many fragrance compounds are converted to strong sensitizers as a result of exposure to air, and understanding the mechanisms behind the oxidative activation of non-sensitizers to strong contact allergens is an important part of the preventive work

Activation via autoxidation upon air exposure of the terpenes limonene and linalool, ubiquitously used as fragrance compounds, has previously been studied. It was found that strongly sensitizing oxidation products were formed following their exposure to air. Among these, hydroperoxides are the main sensitizers. Clinical studies in dermatitis patients have shown autoxidized limonene and linalool to be two of the most common contact allergens. Cutaneous metabolism, an alternative pathway of oxidative activation, has not been extensively investigated in the context of contact allergy, although it is known that certain compounds are activated in this way.



Lina Hagvall (*left*) and opponent Dr Andreas Luch, Head of the Department of Product Safety and Scientific Services, Federal Institute for Risk Assessment, Berlin, Germany.

The aim of this thesis was to investigate the effects of autoxidation and/or metabolism on the sensitizing capacities of the fragrance terpenes geraniol, geranial and linalyl acetate, and an essential oil, lavender oil, by identification of their main oxidation products and/or metabolites and subsequent characterization of their sensitizing capacity.

Geraniol is an important fragrance terpene, originally extracted from rose petals and used for fragrance allergy screening of consecutive dermatitis patients. Geranial is its corresponding aldehyde. Citral, consisting of equal amounts of geranial and neral, is present in high concentrations in lemon and lime leaf oils. It is also included in the test panel for fragrance allergy screening of dermatitis patients. In the thesis, it was shown that both geraniol and geranial are susceptible to autoxidation,

forming oxidation products with higher sensitizing capacity than the non-oxidized compounds.

The autoxidation products of geraniol are formed via two pathways. The first corresponds to the autoxidation of the previously studied linalool, as a hydroperoxide similar to those identified in air-exposed linalool could be identified. In the second pathway, oxidation of the allylic alcohol to geranial was observed. The autoxidation of geraniol greatly influenced its sensitizing capacity. Both pathways are important for increasing sensitizing capacity, as both geranial and the hydroperoxide formed are believed to be major contributors to sensitizing capacity.

The autoxidation of geranial generated high concentrations of an epoxide but yielded no hydroperoxides similar to those previously shown to be produced by the autoxidation of geraniol. The epoxide was shown to be a strong sensitizer, with a sensitizing capacity similar to those of previously studied hydroperoxides. Autoxidation of geranial thus increased its sensitizing capacity, although the pattern of identified oxidation products was different from those of previously studied terpenes.

The metabolic activation of geraniol and geranial was studied using a cocktail of recombinant human cytochrome P450 enzymes that have previously been shown to be expressed in human skin. Both geraniol and geranial were found to be metabolized by these enzymes to compounds with increased sensitizing capacities. Geranial was shown to be the main metabolite of geraniol, and geranial epoxide the main metabolite of geranial. As these compounds were also formed via autoxidation, the two activation routes were linked in a novel way. This indicates that geranial may play an important role in the sensitization to geraniol and that cross-reactions between geraniol and citral, the commercially used mixture of geranial and neral, are possible.

Lavender scent is one of the most commonly used fragrances in the world. Lavender oil is produced in high quantities from lavender due to its high yield and the even higher demand for it. It consists mainly of the terpenes linalool and linalyl acetate. However, synthetic lavender scent produced using synthesized linalool and/or linalyl acetate is most often used in commercial products. It has been shown that linalool of synthetic origin is susceptible to autoxidation, forming hydroperoxides, which are common causes of contact allergy in dermatitis patients.

The autoxidation of lavender oil was studied in order to investigate if the essential oil protects against autoxidation. The results were compared to those from studies of the autoxidation of synthetic linalyl acetate and linalool. Linalyl acetate was found to autoxidize in a similar manner to linalool, with hydroperoxides its main sensitizing oxidation products. These hydroperoxides had a similar sensitizing capacity to the linalool hydroperoxides.

In studying the autoxidation of lavender oil, it was demonstrated that the autoxidation of linalool and linalyl acetate proceeded in the same way in lavender oil as in pure preparations of these compounds. The same oxidation products were formed and the most important sensitizers were the hydroperoxides of linalool and linalyl acetate. Thus, it was concluded that the risk of sensitization by hydroperoxides formed from linalool and linalyl acetate in lavender oil is equal to that of hydroperoxides formed from synthetic compounds.

The results of the present work increase our understanding of the activation of fragrance terpenes, which themselves have a weak allergenic effect. Oxidation products other than hydroperoxides, such as aldehydes and epoxides, were shown to be major sensitizers formed by both autoxidation and metabolic activation. It is likely that other fragrance compounds are susceptible to conversion to strong sensitizers via both autoxidation and metabolism.

Which test materials are relevant for use in the diagnosis of contact allergy to fragrances? The choice of test materials can lead to a false negative diagnosis if the relevant hapten is not included. Contact allergy to air-exposed linalool and limonene is common in dermatitis patients, whereas reactions to the pure materials are very rare. Using too low a test concentration can also result in a false negative diagnosis, particularly for compounds activated via cutaneous metabolism to metabolites with increased sensitizing capacities. In such cases, the concentration of metabolite formed in the skin will be too low to elicit an allergic reaction. It is probable that many cases of contact allergy to oxidation products or metabolites of fragrance materials are not diagnosed as the relevant haptens are still not commercially available for testing.

The results presented in this thesis provide a scientific basis for decisions on possible preventive measures available for producers of fragranced products, and for political decisions regarding regulation of the use of fragrances in consumer products to reduce the risk of contact sensitization to fragrance compounds.