

**Subject:** LLNA evaluation

**Date:** Wednesday, October 24, 2007 3:38 AM

**From:** Ann-Therese Karlberg

Dear Dr Allen,

Since my group is one of the groups in academia that performs the Local lymph node assay most frequently (one a week for many years) as part of our research program I received your mail from a Danish college.

The thing that I want to comment on is the lack of thorough chemical considerations in the choice of the substances used for testing. The substances chosen for testing should be pure, with conclusive structures and no mixtures in different ways. I will give you two examples among the substances discussed in the lists: 1. Abietic acid is considered a moderate sensitizer. In our investigations of abietic acid we found it extremely easily oxidized when exposed to oxygen in air. Abietic acid itself is not an allergen but is activated by air exposure on normal storage and handling so that allergenic oxidation products are formed in a complex mixture. The most prominent allergens identified are the hydroperoxides which as such also are unstable. In fact it is not possible to keep abietic acid pure and non-oxidized unless it is stored under argon. This makes abietic acid an unsuitable compound for evaluation of LLNA since the activity can vary depending on storage conditions of the substance. 2. Citral consists of the two stereoisomers geranial and neral which are both moderate allergens according to LLNA in our hands. Whether the results obtained in the tests with citral are due to reactions to geranial or neral or both have never been discussed. What can be said is that the dose estimated is not conclusive. Since both geranial and neral are available on the market there is no need to test them in a mixture and get non-conclusive results.

Furthermore, I think it is important that substances with an allergenic activity based on different types of reactive sites should be included to eliminate that only certain types of reactive chemicals are tested. If there are things that you want to discuss more in detail I would be happy to discuss with you.

Best regards,

Ann-Therese Karlberg  
Professor  
Dermatochemistry and Skin Allergy  
Department of Chemistry  
Göteborg University  
SE-412 96 Gothenburg  
Sweden