

## Letter to the Editor

# Reactivity assays, substances, samples and *in vitro* alternatives: why should we care?

Dear Sir,

The link between ability of low molecular weight chemicals to act as skin sensitizers and their capacity to react covalently with proteins or peptides has long been recognised (1–3). Over the last three decades, quantitative correlations between sensitizing potency and reactivity parameters, based on kinetics with model nucleophiles or calculated by physical organic chemistry, have been observed for many groups of chemicals. In some cases the correlation is based on reactivity alone (e.g. 4), while in other cases a hydrophobicity parameter is also involved (e.g. 5).

Recently, much activity has been directed at the development of reactivity assays for screening of chemicals for skin sensitization (reviewed in 6). These assays typically use synthetic peptides with the chemical in large excess. It is proposed that a significant level of peptide depletion is taken as an indication of the chemical's ability to bind covalently to protein and hence its potential to be a sensitizer. However, the suitability of such an assay depends on the purpose for which the information is required. There are various reasons for wanting to predict skin sensitization potential, or the lack of it:

- (1) For regulatory classification and/or labelling purposes.
- (2) For human safety assessment associated with product development.
- (3) For quality control or other purposes.

The science of organic chemistry is very well developed, and for many compounds the ability or the inability to react covalently with proteins or peptides is obvious enough to make testing seem completely unnecessary to an organic chemist. However, organic chemistry expertise is not always available, and even when it is, there are cases where reactivity or lack of it cannot be predicted with confidence—but all of that can mask an important problem. One of the fathers of modern contact dermatitis, the late Jan Wahlberg, would always demand to know the purity of a substance under assessment for the simple reason that impurities have often been shown to play an important part in skin sensitization. This issue remains as important

in the 21st century as when Jan was asking us this question in the 1980s and 1990s.

The potential of reactive impurities to impact the outcome of reactivity assays depends on the level of the impurities and on the ratio of the test chemical to the peptide nucleophile. This is illustrated by the following examples:

One per cent of a reactive impurity would be enough to give 100% peptide depletion, with a 100:1 ratio of test material to peptide, or 10% depletion with a 10:1 ratio.

Twenty per cent depletion of peptide could result from 0.2% of a reactive impurity with a 100:1 ratio of test material to peptide, or from 2% impurity with a 10:1 ratio.

A theoretical estimation of reactivity by a competent organic chemist will inform, in our view, as least as well as a peptide reactivity assay in relation to a substance of defined molecular structure. To date, much of the development of the various reactivity assays has deployed laboratory grade compounds which are usually of high purity. However, consideration of a material such as limonene is instructive. Theoretical analysis or assays of high purity material will indicate, quite rightly, an absence of skin sensitization hazard, but the relative ease by which oxidation occurs is well known to lead to the production of allergenic impurities (7, 8). However, with a number of industrial chemicals, the situation may often not be clear cut, with the nature and levels of impurities depending on detailed aspects of how the chemical has been manufactured and stored (9). What impact does this kind of consideration have as we progress to non-animal assays?

In what follows, it is assumed that the chemistry is not known or predictable in advance. A key decision when considering doing a reactivity assay is whether it is the pure substance that is of interest or whether it is a particular sample of the substance, for example a raw material for use in a formulation. If it is the *sample* you're interested in and you want to determine if it has the capacity to sensitize, even if the sensitization may be due to an impurity, then you should do a reactivity assay with an excess of the sample—so even if the main compound is

unreactive, the reactive impurity will show itself by a significant depletion of the nucleophile. In contrast, if it is the *pure substance* that is of interest, you should use an excess of nucleophile, so that any reactive minor impurities that happen to be in your particular sample don't cause confusion.

The first situation is important when you want to check a batch of material delivered to the factory or made in your chemicals plant (quality control). It is also potentially very relevant for assessment of multicomponent natural products, for example if one wanted to assess a range of aromatherapy oils for sensitization potential. The second situation is important when you're deciding whether to go ahead with a novel raw material in a new product. You want to assess if X itself is likely to be a sensitizer, and if not and you go ahead with it, any issues with sensitizing impurities can be dealt with at a later stage in the development programme by further reactivity studies which will assist in defining and implementing appropriate manufacturing specifications.

So, why should we really care about all of this? One simple answer is that we want to achieve the optimum balance between ensuring consumer safety and exploiting the benefits that chemical innovation can bring. However, the analysis above is really an example of a more general problem: that is the confusion between regulatory classification and labelling on the one hand and the more important business of ensuring human health by adequate safety assessment on the other. If we validate *in vitro* assays only in the context of the first point, we may unwittingly miss out on all of the others, including human safety.

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