# Behaviour of chemical respiratory allergens in novel predictive methods for skin sensitisation

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A B S T R A C T

Asthma resulting from sensitisation of the respiratory tract to chemicals is an important occupational health issue, presenting many toxicological challenges. Most importantly there are no recognised predictive methods for respiratory allergens. Nevertheless, it has been found that all known chemical respiratory allergens elicit positive responses in assays for skin sensitising chemicals. Thus, chemicals failing to induce a positive response in skin sensitisation assays such as the local lymph node assay (LLNA) lack not only skin sensitising activity, but also the potential to cause respiratory sensitisation. However, it is unclear whether it will be possible to regard chemicals that are negative in in vitro skin sensitisation tests also as lacking respiratory sensitising activity. To address this, the behaviour of chemical respiratory allergens in the LLNA and in recently validated non-animal tests for skin sensitisation have been examined. Most chemical respiratory allergens are positive in one or more newly validated non-animal test methods, although the situation varies between individual assays. The use of an integrated testing strategy could provide a basis for recognition of most respiratory sensitising chemicals. However, a more complete picture of the performance characteristics of such tests is required before specific recommendations can be made.

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## 1. Introduction and background

Chemical respiratory allergy, associated with asthma and rhinitis, is an important occupational health issue (Kimber and Wilks, 1995; Bakerly et al., 2008; Kenyon et al., 2012; Feary et al., 2016). Probably no more than 80 low molecular weight chemicals have been confirmed as having the potential to cause allergic sensitisation of the respiratory tract, among some of the better characterised being diisocyanates, acid anhydrides, chloroplatinate salts, and certain reactive dyes (Baur, 2013; Baur and Bakehe, 2014).

From a toxicological perspective, chemical respiratory allergy remains problematic, and the challenges have been highlighted in a number of review articles (Holsapple et al., 2006; Kimber et al., 2007, 2014a; b; Boverhof et al., 2008; Isola et al., 2008; Cochrane et al., 2015; North et al., 2016). One of the key issues is that, despite a keen interest in this area for more than 3 decades, there are still no validated, or even widely accepted, methods available for the identification and characterization of chemical respiratory allergens. That does not imply a complete lack of progress, however. In fact, a variety of strategies (based on the use of mice, rats and guinea pigs models, in vitro approaches, and on the identification of structural alerts) have been considered (Botham et al., 1988; Griffiths-Johnson and Karol., 1991; Satoh et al., 1995; Hilton et al., 1996; Dearman and Kimber, 2001; Arts et al., 2008; Pauluhn, 2008; Lalko et al., 2011; Enoch et al., 2012), but none of these has gained traction within the scientific community as a preferred method, and none has been viewed as being of sufficient merit to warrant progression to a formal validation process.

To a large extent the failure to identify an agreed method for the identification of chemical respiratory allergens is due to uncertainty about the mechanisms through which sensitisation of the respiratory tract to chemicals is acquired. In particular, there is a continuing debate about whether, and to what extent, IgE antibody is implicated. This issue has been discussed at some length in the literature (Kimber and Dearman, 2002; Kimber et al., 2014a; b), and will be addressed again later.

Against this background it has been necessary to adopt

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alternative measures to help ascertain the presence or absence of respiratory sensitisation hazards. One approach has been to explore whether the behaviour of chemicals in predictive animal tests for skin sensitisers is able to inform consideration of respiratory sensitisation potential. In this context, it is the mouse local lymph node assay (LLNA) (Kimber and Basketter, 1992; Basketter et al., 2002; Kimber et al., 1994, 2002) that has been the main focus of attention. However, results derived from guinea pig predictive tests — the guinea pig maximisation test (GPMT) (Magnusson and Kligman, 1969) and the occluded patch test (Buehler, 1965) - are relevant also.

The LLNA is a fully validated method for the identification of skin sensitising chemicals. The assay is based on an understanding that the acquisition of skin sensitisation requires, and will be associated with, the activation and proliferation of allergen-responsive T lymphocytes. Such T cell responses take place in regional lymph nodes draining the sites of exposure to a chemical allergen, and it is upon these events that the LLNA is based (Kimber et al., 2011). Subsequently, it has become apparent that, in addition to skin sensitisers, chemicals that are known to cause sensitisation of the respiratory tract elicit positive responses in the LLNA (Dearman et al., 2013). Moreover, it has been found, where data are available, that (with a single possible exception [piperazine]) chemical respiratory allergens also test positive in the GPMT (Dearman et al., 2013).

At face value it might be considered that these data are unexpected. That is, chemical respiratory allergens are known to favour sensitisation of the respiratory tract, and are most commonly associated clinically with occupational respiratory disease. Nevertheless, there is good reason to suppose that, at least in animal models of skin sensitisation, chemical respiratory allergens will elicit positive responses. To explore why this is the case it is necessary to review briefly some basic features of immune responses to chemical allergens.

Although with many chemical respiratory allergens there are reports of cases of allergic contact dermatitis (ACD), there is a clear preference for sensitisation of the respiratory tract (Kimber et al., 2011), and in some instances, such as with phthalic anhydride, chemical respiratory allergens appear to have no potential to cause skin sensitisation (Basketter and Kimber, 2016).

It is now believed that the basis for the selectivity of chemical allergens to elicit preferentially either skin sensitisation associated with ACD, or sensitisation of the respiratory tract associated with occupational asthma, is governed by the quality of immune response elicited. That is, contact allergens have been shown preferentially to induce the development of selective Th1-type T cell responses, whereas chemical respiratory allergens favour the induction of preferential Th2-type responses (reviewed Kimber et al., 2011). It must be appreciated that this is something of an over-simplification, insofar as other factors and other functional subpopulations of T lymphocytes also play important roles. However, the fact remains that such differential selectivity exists and is a pivotal factor in the elicitation by contact and respiratory allergens of different qualities of immune response, and different forms of allergic disease. Although much of the evidence for the induction by chemical allergens of distinct T cell responses derive from studies in rodents (Dearman et al., 1995, 1996; 2005; Van Och et al., 2002; Kimber et al., 2011), there is evidence that the same pattern and relationships pertain in humans (Ouyang et al., 2013; Newell et al., 2013; Kimber et al., 2014c).

Chemical respiratory allergens therefore favour the development of selective Th2-type immune responses. As such responses, in turn, promote IgE antibody production, it appears reasonable to speculate that IgE responses play a pivotal role in sensitisation of the respiratory tract to chemical allergens, and the elicitation of respiratory allergic reactions. Certainly, IgE antibody is known to play a pivotal role in allergic sensitisation of the respiratory tract to proteins. However, the role played by IgE antibody in chemical respiratory allergy remains uncertain. The main cause of that uncertainty is that in a significant number of cases of chemical respiratory allergy (and in particular respiratory allergy associated with diisocyanates) it has not been possible routinely to identify allergen-specific IgE antibody in the plasma of symptomatic subjects (Cullinan, 1998; Kimber et al., 1998, 2010; Liu and Wisnewski, 2003).

It has been argued elsewhere (Kimber and Dearman, 2002; Kimber et al., 2011, 2014a), that there is good reason to suppose that there is a much closer relationship between IgE antibody and chemical respiratory allergy than is currently appreciated. Nevertheless, uncertainty resulting from the difficulty of detecting specific antibody in some patients with occupational asthma has prevented the acceptance of methods based on measuring IgE antibody responses. Despite a continuing debate about the relevance of IgE antibody, there is developing more of a consensus that Th2 cell responses play an important, and probably mandatory, role in sensitisation of the respiratory tract to chemical allergens (reviewed in Kimber et al., 2014b).

The important point that emerges from this situation is that, irrespective of whether the important effector mechanism is Th2 cells or IgE antibody (the production of which is dependent upon Th2 cells), effective sensitisation of the respiratory tract will require the activation, proliferation and differentiation of allergen-responsive T lymphocytes. This being the case, it therefore should come as no surprise that chemical respiratory allergens will test positive in the LLNA where a positive response is characterised by the activation and proliferation of lymphocytes, including T cells, in draining lymph nodes. That is, that the ability to stimulate T cell responses is a common feature of contact and respiratory allergens. Where these classes of allergens differ is not in their ability to induce T cell activation, but the differentiation pathway that will be adopted in driving the development of selective Th1-type, or selective Th2-type, immune responses. It is known that chemical respiratory allergens also test positive in the GPMT due to the elicitation of a T cell response (Dearman et al., 2013).

In conclusion, therefore, there is a sound mechanistic basis for the elicitation by chemical respiratory allergens of positive responses in the LLNA (and other animal models of skin sensitisation). The clear implication is that the LLNA is not able to discriminate between contact and respiratory allergens. However, the benefit of this lack of discrimination is that a chemical that fails to test positive in the LLNA can be safely regarded as lacking not only skin sensitising properties, but also the ability to cause allergic sensitisation of the respiratory tract and occupational asthma. This paradigm has proven very useful in providing reassurance that chemicals that test negative in the LLNA can be eliminated from consideration as potential respiratory allergens.

However, times are changing, and there are currently three validated alternative test methods for skin sensitisation hazard identification that do not require the use of animals (OECD, 2015a; b; 2016). In addition there are several other *in vitro* skin sensitisation tests that are in various stages of development, and some of these are nearing potential validation (Reisinger et al., 2015; Ezendam et al., 2016).

These issues were discussed at a recent Workshop convened by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). This workshop titled 'Chemical Respiratory Allergy: Clinical Information and How to use it and Improve it' took place in Madrid on October 27th and 28th 2016. It was agreed that although the innovations resulting in the availability of novel non-animal test methods are to be welcomed and applauded, it is necessary