Toxicology 333 (2015) 179–194

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Review

Thresholds in chemical respiratory sensitisation

Stella A. Cochrane [a,*], Josje H.E. Arts [b], Colin Ehnes [c], Stuart Hindle [d], Heli M. Hollnagel [d], Alan Poole [e], Hidenori Suto [f], Ian Kimber [g]

[a] Unilever Safety and Environmental Assurance Centre, Colworth Science Park, Sharnbrook, Bedfordshire, Mk44 1LQ, UK
[b] AkzoNobel NV, Arnhem, the Netherlands
[c] BASF SE, GUP/PB - Z470, 67056 Ludwigshafen, Germany
[d] Dow Europe GmbH, Bachtobelstrasse 3, CH-8810 Horgen, Switzerland
[e] ECETOC, Avenue Van Nieuwenhuyse 2, Box 8, B-1160 Bruxelles, Belgium
[f] Sumitomo Chemical Co. Ltd. Environmental Health Science Laboratory, 3-1-98 Kasugade-Naka, Konohana-Ku, Osaka 554-8558, Japan
[g] University of Manchester, Faculty of Life Sciences, Michael Smith Building, Oxford Road, Manchester M13 9PT, UK

ARTICLE INFO

Article history:
Received 6 March 2015
Received in revised form 16 April 2015
Accepted 16 April 2015
Available online 8 May 2015

Keywords:
Thresholds
Chemical respiratory allergy
Respiratory sensitisation
Occupational asthma
Hazard characterisation
Sensitising potency

ABSTRACT

There is a continuing interest in determining whether it is possible to identify thresholds for chemical allergy. Here allergic sensitisation of the respiratory tract by chemicals is considered in this context. This is an important occupational health problem, being associated with rhinitis and asthma, and in addition provides toxicologists and risk assessors with a number of challenges.

In common with all forms of allergic disease chemical respiratory allergy develops in two phases. In the first (induction) phase exposure to a chemical allergen (by an appropriate route of exposure) causes immunological priming and sensitisation of the respiratory tract. The second (elicitation) phase is triggered if a sensitised subject is exposed subsequently to the same chemical allergen *via* inhalation. A secondary immune response will be provoked in the respiratory tract resulting in inflammation and the signs and symptoms of a respiratory hypersensitivity reaction. In this article attention has focused on the identification of threshold values during the acquisition of sensitisation.

Current mechanistic understanding of allergy is such that it can be assumed that the development of sensitisation (and also the elicitation of an allergic reaction) is a threshold phenomenon; there will be levels of exposure below which sensitisation will not be acquired. That is, all immune responses, including allergic sensitisation, have threshold requirement for the availability of antigen/allergen, below which a response will fail to develop. The issue addressed here is whether there are methods available or clinical/epidemiological data that permit the identification of such thresholds. This document reviews briefly relevant human studies of occupational asthma, and experimental models that have been developed (or are being developed) for the identification and characterisation of chemical respiratory allergens.

The main conclusion drawn is that although there is evidence that the acquisition of sensitisation to chemical respiratory allergens is a dose-related phenomenon, and that thresholds exist, it is frequently difficult to define accurate numerical values for threshold exposure levels. Nevertheless, based on occupational exposure data it may sometimes be possible to derive levels of exposure in the workplace, which are safe.

An additional observation is the lack currently of suitable experimental methods for both routine hazard characterisation and the measurement of thresholds, and that such methods are still some way off. Given the current trajectory of toxicology, and the move towards the use of non-animal *in vitro* and/or *in silico*) methods, there is a need to consider the development of alternative approaches for the identification and characterisation of respiratory sensitisation hazards, and for risk assessment.

* Corresponding author. Tel.: +44 1234264928.
E-mail address: stella.a.cochrane@unilever.com (S.A. Cochrane).

http://dx.doi.org/10.1016/j.tox.2015.04.010
0300-483X/

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1. Definitions

Allergy: The adverse health effects that might result from the stimulation of an adaptive immune response.

Allergic sensitisation: A state of heightened sensitivity/responsiveness to a specific allergen resulting from previous exposure and immunological priming.

Allergic contact dermatitis/contact allergy: An allergic disease of the skin that is elicited following topical exposure to a chemical allergen to which skin sensitisation has previously been induced.

Allergic sensitisation of the respiratory tract: A state of heightened sensitivity/responsiveness of the respiratory tract to a specific allergen resulting from prior exposure and immunological priming.

Chemical respiratory allergy: An immune mediated hypersensitivity reaction to an exogenous low molecular weight chemical resulting in symptoms such as asthma and rhinitis.

Asthma: A chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, in particular mast cells, eosinophils, T lymphocytes, neutrophils and epithelial cells. In susceptible individuals this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and cough, particularly at night and in the early morning. These episodes are usually associated with widespread but variable airway obstruction that is often reversible, either spontaneously or with treatment. The inflammation also causes an associated increase in the existing bronchial hyperresponsiveness to a variety of stimuli, either irritant or allergenic (NHLBI, 1991; Apter, 2008).

Allergic rhinitis: Inflammation of the mucus membrane of the nose caused by an allergic response.

2. Introduction

A wide variety of natural and man-made materials can cause allergic sensitisation of the skin or respiratory tract in susceptible individuals.

It has been proposed that respiratory sensitisers could be identified as substances of very high concern (SVHC) in the European regulatory context, since it is not routinely possible to identify a threshold for the adverse effects they cause (ECHA, 2012). The primary aim of this document is to review and discuss scientific evidence that chemical respiratory allergy can be regarded as a thresholded effect.

Commonly encountered examples of respiratory sensitisers are proteins (pollen, dust mite excreta and animal dander etc.). In this case the mechanisms resulting in allergic sensitisation are relatively well understood. In susceptible subjects exposure to the inducing allergen provokes an IgE antibody response. Such antibody distributes systemically and associates *via* specific membrane receptors with mast cells, including mast cells in the respiratory tract. At this point sensitisation has been acquired. Subsequent inhalation exposure of the sensitised subject to the inducing allergen will trigger an allergic reaction. The antigen

binds with, and cross-links, IgE antibody bound to the mast cell surface. This causes cellular activation and the release by mast cells of both pre-formed and newly-synthesised inflammatory mediators (including vasoactive amines and leukotrienes). These mediators work in concert to trigger an inflammatory reaction that in turn precipitates the symptoms of respiratory allergy (Kimber and Basketter, 2014).

In addition, it is known that certain low molecular weight (LMW) chemicals are able to cause sensitisation of the respiratory tract resulting in respiratory allergy. Here the cell and molecular mechanisms resulting in the acquisition of sensitisation are not so well established.

In this report chemical respiratory allergy is defined as an immune-mediated hypersensitivity reaction to an exogenous LMW chemical resulting in symptoms such as asthma and rhinitis.

Asthma and asthma-like symptoms can be caused by a variety of stressors and conditions. However, this document will focus solely on LMW chemicals causing respiratory effects *via* an immunological mechanism (allergy). This article will not consider materials that may induce or exacerbate asthma or asthma-like reactions in subjects via non-immunologic mechanisms, such as airway paraesthesia (Siddiqui et al., 2008; Kimber and Dearman, 2002).

Another immune-mediated respiratory hypersensitivity that may develop in response to LMW chemicals is chemical-induced hypersensitivity pneumonitis, also known as extrinsic allergic alveolitis (McSharry et al., 2002). However, due to the relative rarity of this disorder this will not be considered here.

Given the focus of this document, it is important to clarify that the term asthmagen as used in this paper encompasses materials that may be allergens and/or irritants and makes no clear distinction between these. An asthmagen has been defined by the Health and Safety Executive (HSE; United Kingdom) as any material that causes asthma but it does not discriminate between causation *de novo* and the exacerbation of a pre-existing asthmatic condition (HSE, 2001).

There are chemicals and a limited number of chemical classes that have been implicated as occupational respiratory allergens, and some of these are listed in Table 1. In each case these chemicals have been shown to cause occupational asthma based on established clinical criteria (as determined by the US Association of Occupational and Environmental Clinics, and in some cases epidemiological data (Beckett 1994; Friedman-Jimenez et al., 2000; Quint et al., 2008; Tarlo et al., 2008).

It is also important to be aware of the terminology associated with chemical respiratory allergy, and for that purpose the definitions provided in the preface to this report should be consulted.

In theory at least, any type of immune mediated injury may occur in the lung as a result of exposure to a chemical allergen, however, those that cause rhinitis and asthma *via* immunoglobulin E (IgE)- and T helper cell type 2 (Th2)-mediated responses are of particular concern because of the degree of sensitivity induced (Holsapple et al., 2006). As indicated previously, respiratory hypersensitivity (respiratory allergy) can be induced by both high and low molecular weight allergens. High molecular weight (HMW) allergens are mainly proteins, whereas LMW allergens are reactive chemicals (or chemicals that can be converted readily to reactive species). Reactivity is an important feature of chemical allergens because they must associate with proteins to induce a complex of a size required to elicit an immune response (Holsapple et al., 2006).

All forms of allergy develop in two phases: induction (also known as sensitisation) and elicitation. Briefly, induction/sensitisation is a change in immunological status after initial exposure to an allergen (immunological priming) that is commonly free of any clinical symptoms. In contrast, elicitation occurs following subsequent exposure of the sensitised subject to the same (inducing) allergen (and often at a lower concentration than that required for induction). This secondary exposure results in an accelerated and more aggressive secondary immune response that causes inflammation and clinical symptoms of allergy—in the case of chemical respiratory allergy, symptoms of rhinitis and/or asthma.

The cellular and molecular events that are associated with the induction of skin sensitisation and the elicitation of allergic contact dermatitis are, to some extent, well-defined. This is true also of the development of respiratory allergy to proteins. However, this is not the case for sensitisation of the respiratory tract by chemicals. Thus, there remains uncertainty, and some considerable debate, about the important effector mechanisms that result in the acquisition of sensitisation of the respiratory tract. In particular, the role(s) played by IgE antibody in this process remains contentious (Kimber et al., 2014a,b). Moreover, although it is sometimes assumed that sensitisation of the respiratory tract to chemical allergens will necessarily be induced following inhalation exposure, it is now increasingly appreciated that the skin may also represent a relevant route through which respiratory sensitisation can be achieved (see later).

In summary, we have here reviewed the available data to consider the relevant endpoints that can be used to describe chemical respiratory allergy and to inform our understanding of threshold effects. The aim has been to determine whether chemical respiratory allergy should be regarded as a threshold, or as a non-threshold, toxicity, and to recommend appropriate methods for deriving safe concentrations of chemical respiratory allergens.

3. Mechanisms of chemical respiratory allergy

3.1. Background

Chemical respiratory allergy is an immune mediated hypersensitivity response to an exogenous LMW chemical resulting in symptom such as asthma and rhinitis. Responses to HMW substances such as proteins are not considered here, although there exists some overlap with respect to the relevant mechanisms.

As described in the introduction to this report, the term chemical respiratory allergy refers to the overall disease state. In common with other forms of allergy this develops in two stages.

Table 1
Selected chemical respiratory allergens and examples of use and potential occupational exposures.

Class	Use/occupation
Diisocyanates (toluene diisocyanate; diphenylmethane diisocyanate; hexamethylene diisocyanate)	Polyurethane industries, foundries, automobile spray painting
Anhydrides (phthalic anhydride; trimellitic anhydride; tetrachlorophthalic anhydride)	Epoxy resins, adhesives, floor polishes
Metals (platinum; chromium, cobalt; tungsten carbide)	Platinum refinery, metal plating, tanning, hard metal industry
Amines (piperazine; ethylenediamine)	Resins, solvents

The first or induction stage describes the acquisition of sensitisation, and in this case sensitisation of the respiratory tract. This stage is characterised by immunological priming to the inducing allergen. The second or elicitation stage develops when a sensitised subject is exposed to the same allergen for a second or subsequent time. Here the relevant exposure is *via* the respiratory tract. The sensitised subject is equipped to mount an accelerated and more aggressive secondary immune response that causes inflammation in the respiratory tract and the symptoms of clinical respiratory allergy, including rhinitis and asthma (Kimber et al., 2011). It should be noted that although clinical allergic reactions are elicited following inhalation exposure of the sensitised subject, it is believed now that the acquisition of sensitisation of the respiratory tract may be achieved through skin contact as well as inhalation exposure. See Section 4.

3.2. Mechanisms through which sensitisation of the respiratory tract is acquired

Chemicals are too small to induce an adaptive immune response. To acquire immunogenic potential they must form stable associations with protein (hapten–protein conjugates). Mechanistic chemistry studies have revealed that chemical respiratory allergens can be assigned to one of six electrophilic mechanistic domains, with harder (stronger) electrophilic mechanisms such as acylation being more prevalent than softer (weaker) mechanisms, with the hypothesis being that the harder nucleophile lysine is the favoured biological nucleophile for sensitisation of the respiratory tract (Enoch et al., 2012). Details of the mechanisms involved are, however, still poorly understood, though the possible importance of lysine reactivity in sensitisation of the respiratory tract by chemical allergens is supported by some *in chemico* and *in vitro* studies (Hopkins et al., 2005; Lalko et al., 2011, 2012, 2013b).

The induction of an adaptive immune response requires the recognition, internalisation, processing and transport of antigen by dendritic cells (DC; professional antigen-presenting cells). Antigen-bearing DC move from the site of exposure to regional lymph nodes. These cells localise within the paracortex of lymph nodes and it is here that antigen is presented to responsive T lymphocytes. Specific ligation of the antigen receptor expressed by responsive T lymphocytes triggers activation, cellular division and a selective expansion of reactive cells. This is the central event in the initiation of adaptive immune responses. The process of immune activation is orchestrated by DC and their cytokine and chemokine products, and by factors released by other cell types, including epithelial cells. As discussed in greater detail in the next section, there is a growing appreciation that the acquisition of sensitisation of the respiratory tract can be effected by skin contact with the inducing chemical allergen, as well as by inhalation exposure, and this has been reflected increasingly in experimental systems (Adenuga et al., 2012; Arts et al., 1998, 2006; Arts and Kuper, 2003; Boverhof et al., 2008; Farraj et al., 2006, 2007; Holsapple et al., 2006; Kimber and Dearman, 2002; Plitnick et al., 2002; Selgrade et al., 2006).

Irrespective of the route of exposure through which sensitisation is acquired, there is a growing body of evidence that chemical respiratory allergens are associated, in experimental animals at least, with characteristic cytokine secretion patterns. Although experience in some experimental systems has been varied, it appears that, probably irrespective of the route of exposure, chemical respiratory allergens induce selective Th2-type immune responses, characterised by the preferential expression of type 2 cytokines (including interleukins 4, 5 and 13). It is argued also that skin and respiratory chemical allergens induce distinct cytokine secretion patterns (Th1- and Th2-type, respectively), and that this may provide a basis for distinguishing between these allergens in the context of hazard characterisation (Kimber and Dearman, 1997a; Dearman and Kimber, 2001; Dearman et al., 2002; Plitnick et al., 2002; Selgrade et al., 2006; De Jong et al., 2009; Kimber et al., 2011; Adenuga et al., 2012). Although evidence for the differential immunological selectivity of contact and respiratory chemical allergens has been based largely on studies in experimental animals, there is emerging evidence that the same, or at least similar, patterns are seen in humans (Toebak et al., 2006; Kimber et al., 2013; Newell et al., 2013; Ouyang et al., 2013).

There remain many unanswered questions regarding the cellular and molecular mechanisms through which chemicals cause sensitisation of the respiratory tract. Although some progress has been made in trying to define, in broad terms at least, the sequence of events leading from exposure to sensitisation (Kimber et al., 2014b), there are several uncertainties. For instance, it is intriguing that chemical respiratory allergens may favour interaction with lysine residues for creation of hapten–protein conjugates, but it is unclear to what extent such amino acid selectivity impacts on the induction by these chemicals of selective Th2-type immune responses. Perhaps the greatest uncertainty is the role of IgE antibody in sensitisation of the respiratory tract to chemicals. Although the majority, if not all, chemical respiratory allergens have been associated with the production in sensitised subjects of IgE antibody, it is the case that in many instances – and in particular with the diisocyanates – patients with chemical respiratory allergy and occupational asthma lack detectable IgE antibody (Kimber and Dearman, 2002; Isola et al., 2008; Kimber et al., 2014a). For reasons that are outside the scope of this paper, it may be the case that the correlation between IgE antibody production and chemical respiratory allergy is underestimated. It could even be the case that IgE antibody plays a mandatory and universal role in sensitisation of the respiratory tract to chemicals, but this is unproven (Kimber and Dearman, 2002; Jones et al., 2003; Kimber et al., 2014a). Nevertheless, it must be acknowledged that there may exist IgE antibody-independent mechanisms through which sensitisation of the respiratory tract to chemicals may be acquired. Even if there is a continuing debate about the relationship of IgE antibody with chemical respiratory allergy, there is (as described above) reason to believe that the Th2-selective immune responses are a common feature of sensitisation (Kimber et al., 2014b). Work is underway to map out chemical respiratory sensitisation as an adverse outcome pathway (AOP) and it hoped such activities will enable the current mechanistic pathways to considered in greater totality and more depth and drive efforts to fill identified knowledge gaps.

3.3. Elicitation of allergic reactions

Elicitation occurs upon subsequent re-exposure of a sensitised subject to the inducing allergen. A secondary immune response will be elicited, resulting in inflammation and the symptoms of respiratory allergic disease. There is commonly a latency period between initial exposure(s) and the onset of symptoms, and this can be of the order of many months or years.

In those instances where the reactions are mediated by IgE antibody the sequence of events is as follows. Inhalation exposure of the sensitised subject to the relevant chemical allergen will result in cross-linking of IgE antibody bound to plasma membrane receptors on mast cells in the respiratory tract. This causes mast cell activation and degranulation with the release of newly-synthesised and pre-formed inflammatory mediators that act in concert to provoke inflammatory reactions. If, as has been speculated, chemical respiratory allergy can also be effected by processes other than those requiring IgE antibody then the mechanisms involved remain obscure. However, in such instances

it will almost certainly be the case that Th2 cells play a prominent role.

3.4. Risk factors

It is clear that there exist inter-individual differences in susceptibility to chemical respiratory allergy. Not all of those exposed to chemicals associated with occupational asthma will acquire sensitisation of the respiratory tract. It appears that there are important genetic, epigenetic and environmental factors that together determine the likelihood that sensitisation will be induced (Miller and Ho, 2008). It is known, for example, that atopy (a predisposition to mount IgE antibody responses), gender and pre-existing non-specific bronchial hypersensitivity are not associated with the development of chemical respiratory allergy. However, cigarette smoking (Venables et al., 1985; Nielsen et al., 2005) and polymorphisms in anti-oxidant enzyme systems and of the major histocompatibility complex (MHC) Class II do show associations.

There is evidence for an association between susceptibility to sensitisation to chloroplatinate salts and the major histocompatibility complex allele HLA-DR3 (Newman Taylor et al., 1999). There has also been reported an association between specific HLA alleles and respiratory sensitisation to acid anhydrides (Jones et al., 2004). In addition, there has been found associations between anti-oxidant genes and HLA alleles with diisocyanate-induced occupational asthma (Yucesoy et al., 2012, 2014). The influence of genetic polymorphisms on susceptibility to developing chemical respiratory allergy has implications for the development and interpretation of relevant exposure thresholds.

4. Routes of exposure

It is clear that in a sensitised subject the elicitation of a respiratory reaction will require inhalation exposure to a sufficient quantity of the inducing chemical allergen (Arts et al., 2006).

It has often been assumed that inhalation exposure will also be necessary for the effective acquisition of sensitisation of the respiratory tract to chemicals. Although inhalation exposure to relevant chemicals no doubt can and does result in development of respiratory sensitisation, there is no reason to believe that this is the only route of exposure that will be effective. In fact, there is a growing appreciation that topical exposure to an appropriate quantity of a chemical respiratory allergen may result in sensitisation of the respiratory tract (summarised in Kimber and Dearman, 2002; Kimber et al., 2014b; Redlich and Herrich, 2008 Redlich, 2010).

From an immunological perspective the ability of skin exposure to result in sensitisation of the respiratory tract comes as no surprise. It must be appreciated that, by definition, the development of allergic sensitization requires that a specific immune response is induced, and that immune responses are generally designed to provide specific host resistance. There is, therefore, good reason to believe that chemical respiratory allergens will be able to provoke through skin contact the quality of immune response required for acquisition of sensitisation of the respiratory tract.

Studies in experimental animals support this. Thus, intradermal or topical exposure of guinea pigs to known chemical respiratory allergens has been shown to result in effective sensitisation of the respiratory tract (Botham et al., 1989; Rattray et al., 1994). Of particular relevance is the fact that in one series of comparative studies in guinea pigs it was found that either topical or intradermal exposure of animals to diphenylmethane diisocyanate (MDI) was far more effective at inducing sensitisation of the respiratory tract, in terms of the number of animals sensitised, (as measured by the elicitation of pulmonary reactions following subsequent inhalation challenge of animals with atmospheres of MDI) than was inhalation exposure (Rattray et al., 1994). This observation is consistent with the view that inhalation exposure may, in some circumstances at least, induce immunological tolerance rather than priming or sensitisation (Kimber and Dearman, 2002). Moreover, it is possible that in an occupational context, encounter with chemicals at skin surfaces, particularly resulting from accidents and spillages, may facilitate exposure locally to high concentrations of chemical that will favour elicitation of an immune response. In this context it is likely that inhalation exposure will be less likely to result in high local concentrations of the chemical within the respiratory tract (Arts et al., 2008). Moreover, in the case of inhalation exposure it may be that the duration of exposure and concentration are important factors determining the likelihood that immunological priming will occur (Karol et al., 1986).

The question remains whether observations that derive from animal studies translate into an understanding of the development of respiratory sensitisation of humans resulting from workplace exposure. Although it is difficult to be certain, there are indications that in an occupational setting skin contact with chemical respiratory allergens may result in sensitisation of the respiratory tract, particularly in those circumstances where there is acute dermal exposure to high concentrations of chemicals resulting from accidental spillages or splashes. It is possible also that sensitisation of the respiratory tract is acquired from a combination of both skin and inhalation exposure (Karol, 1986; Nemery and Lenaerts, 1993; Kimber, 1996; Tarlo and Malo, 2006; Bello et al., 2007; Redlich and Herrick, 2008; Redlich, 2010).

Irrespective of whether sensitisation is acquired *via* dermal contact or inhalation exposure, current mechanistic understanding of allergic sensitisation is such that it can be assumed there will be threshold effects for immunological priming, in the skin or respiratory tract, respectively. This means that for both the skin and respiratory tract there will be levels of exposure below which sensitisation will fail to develop. The issue however remains regarding how readily such thresholds, particularly for a human population can be defined, and based upon current immunological knowledge and available methodologies this will be very challenging (please refer to Section 6.1 for further information on thresholds and definitions thereof). The standard approach in an experimental animal system would be to define the minimal concentration of the chemical allergen required for effective sensitisation of the respiratory tract, as judged by subsequent elicitation of pulmonary reactions by inhalation challenge. The other approach is to measure, in the tissue through which sensitisation is induced, immune activation, and the elicitation of immune responses, following exposure to the chemical allergen. Although this could be accomplished more easily in the skin (through examination of lymph nodes draining the site of topical exposure), there is no reason why it could not be accomplished also by examination, following inhalation exposure, of lymph nodes in the respiratory tract.

The analysis above addresses *inter alia* the routes of exposure that are relevant for acquisition of sensitisation of the respiratory tract to chemicals, and how an appreciation of the relevant routes can inform animal models that could be used for measurement of threshold values. Against that background it is important to appreciate that a move away from the use of animal models in toxicology is gaining momentum, and that there is a need, therefore, to consider the development and application of alternative approaches that do not rely on the use of experimental animals. It may prove that an investment in the development of an adverse outcome pathway (AOP) for chemical respiratory allergy, which is underway, possibly combined with an improved

appreciation of the available clinical data, may pave the way for design of appropriate *in silico* or *in vitro* methods.

5. Proposed approaches for hazard identification and characterisation

Before considering the strategies that may be available for assessment of the induction of chemical respiratory allergy in the context of dose–response relationships and thresholds, it is relevant to list the various approaches that have been proposed for the purposes of hazard identification and characterisation. The list of methods and approaches provided is not, and is not intended to be, comprehensive or exhaustive, and for a more detailed consideration of the models that have been – or are being – developed there are available numerous relevant review articles (Holsapple et al., 2006; Kimber et al., 2007; Boverhof et al., 2008; Vandebriel et al., 2011; Rovida et al., 2013).

It is important to reiterate here that none of the methods or approaches recorded below has been validated or widely adopted by the toxicology community. Associated with this is the fact that, in practice, respiratory sensitising hazards may be identified on the basis of occupational health effects.

5.1. Quantitative structure activity relationships (QSAR)

There is a long history of interest in the development of (Q)SAR for skin sensitisation. More recently there has been a growing commitment to examining the chemical characteristics of respiratory allergens. Progress is being made, particularly with respect to electrophilic reaction chemistry (Seed et al., 2008; Seed and Agius, 2010; Enoch et al., 2009, 2010, 2012; Dik et al., 2014). However, despite some achievements the models available currently are not validated for the purposes of hazard identification, and are not suited to consideration of dose–response relationships during the acquisition of sensitisation, or the definition of threshold values. Their application initially at least will be in the context of hazard identification.

5.2. In vitro *test systems*

The development of *in vitro* models for the identification of chemical respiratory allergens is still in its infancy. Nevertheless, there is a variety of cell/tissue culture methods that have been proposed (Kimber et al., 2007; Boverhof et al., 2008). One development is based on the Direct Peptide Reactivity Assay that was described initially as a method for the identification of contact allergens in which the interaction of chemicals with model peptides is measured (Gerberick et al., 2004). Using such methods it has been found that under some circumstances contact allergens and chemical respiratory allergens display differential amino acid preferences during the formation of associations with protein; the former favouring cysteine and the latter lysine (Lalko et al., 2011, 2012, 2013a,b). These observations are consistent with proposals deriving from SAR analyses (Enoch et al., 2010). As is the case with QSAR approaches, the *in vitro* methods available currently are not (yet) suitable for determination of thresholds.

5.3. The local lymph node assay

The mouse local lymph node assay was developed initially as a method for the identification of chemicals that have the potential to cause skin sensitisation. More recently the assay has found application also for assessment of sensitising potency for the purposes of risk assessment (Kimber et al., 2001, 2002). Although the assay was not designed to identify chemical respiratory allergens, experience to date has shown that all such allergens tested in the LLNA yield positive results (Dearman et al., 2013). It is for that reason the LLNA is included here. The activity of respiratory sensitising chemicals in the assay indicates the LLNA could, in principle, be used for the assessment of the potency of, and induction thresholds for, chemical respiratory allergens. There is a modification of the assay in which exposure is via inhalation, rather than by skin contact, and that approach could be used in a similar way (Arts et al., 2008; De Jong et al., 2009).

It is important to emphasise that a positive response in the LLNA does not signal that a chemical has the potential to induce allergic sensitisation of the respiratory tract. There is a sound mechanistic basis to explain the ability of both contact allergens and chemical respiratory allergens to elicit positive responses in the assay. In both cases the acquisition of sensitisation (skin sensitisation and respiratory sensitisation) requires that an adaptive immune response is induced. Although the characteristics of immune responses elicited by contact allergens and respiratory allergens differ qualitatively, they are both associated with the activation and proliferation of T lymphocytes in lymph nodes draining the site of exposure. For this reason chemical respiratory allergens (and contact allergens) elicit positive responses in the LLNA when administered topically. It is after that initial activation of responsive T lymphocytes that the immune responses induced by contact allergens and chemical respiratory allergens begin to diverge in a qualitative sense. The important point to emphasise is that a positive response in the LLNA does not imply that a chemical will cause respiratory sensitisation.

5.4. Cytokine fingerprinting

This approach, developed originally in mice, is predicated on the observation that chemical respiratory allergens result in the development of a selective Th2-type immune response characterised by the increased expression of type 2 cytokines such as IL-4, IL-5 and IL-13. In contrast, under the same conditions, skin sensitising chemicals elicit Th1-selective immune responses. These observations raised the possibility that it might be possible to identify chemical respiratory allergens, and to distinguish these from contact allergens, on the basis of cytokine expression profiles during the evolution of immune responses to chemicals. The method has met with some success, although experience has been somewhat variable between laboratories (Dearman et al., 1998b, 2002; Manetz et al., 2001; Dearman and Kimber, 2001; Van Och et al., 2002; Plitnick et al., 2002; Selgrade et al., 2006; De Jong et al., 2009; Adenuga et al., 2012).

5.5. Antibody production

In many instances the development of sensitisation of the respiratory tract to chemicals will be associated with antibody (and in particular with IgE antibody) production. On that basis approaches to hazard characterisation based on such responses have been considered using experimental animals. There are significant challenges associated with the accurate measurement of hapten-specific antibodies, and as IgE antibody is produced only in very small amounts this serves to compound further the technical difficulties. It is probably for these reasons that experience with this approach has been rather limited (Karol, 1983; Botham et al., 1989; Sarlo and Clark, 1992; Blaikie et al., 1995; Zhang et al., 1998; Warbrick et al., 2002a; Pauluhn et al., 2002b).

5.6. Changes in total serum IgE; the mouse IgE test

An approach to hazard characterisation that sought to avoid the need to measure specific antibody responses is the mouse IgE test.

In this approach chemical respiratory allergens are identified as a function of their ability to induce increases in the total serum concentration of IgE immunoglobulin, rather than the stimulation of allergen-specific antibody responses. Adoption of the assay has been limited due to the fact that there is variability between strains of mice, and between laboratories, with respect to chemical driven changes in the serum levels of IgE (Dearman et al., 1992, 1998a; Hilton et al., 1995, 1996). A variant of this approach based on measuring the same responses in rats has also been described (Arts et al., 1997, 1998,b; Warbrick et al., 2002a,b).

5.7. Challenge-induced elicitation reactions in sensitised animals

For this purpose either rats or guinea pigs have been used. The approach taken is to expose animals (*via* an appropriate route of exposure) to the test chemical. Subsequently animals are challenged, *via* inhalation, with the same chemical and the extent of sensitisation measured as a function of induced changes in respiratory rate and/or other pulmonary functions. This method requires that non-irritating concentrations of the test chemical are used for challenge and it is necessary to confirm this by inclusion of non-sensitised controls. This approach has been studied in some detail, but as will be discussed later, is subject to a number of disadvantages (Karol et al., 1981; Botham et al., 1988; Sarlo and Clark., 1992; Rattray et al., 1994; Arts et al., 1998; Pauluhn, 2003; Pauluhn and Eben, 1991; Pauluhn et al., 2000, 2002a).

6. Thresholds and endpoints

6.1. Background

This section explores whether there are available approaches for the derivation of threshold values for the development of sensitisation of the respiratory tract to chemicals. The use of both experimental systems (including putative predictive test systems), and information from human studies or clinical experience are considered.

Kroes et al. (2000) states that in toxicology 'a threshold is defined as a dose at, or below which, a response is not seen in an experimental setting'. It is however recognised that establishing absolute threshold values can be challenging and indeed, in some cases limitations such as subject numbers may make defining a threshold in absolute terms impossible. Additional means used to establish toxicological thresholds include mechanistic information, with the threshold principle based upon the assumption that at or below a threshold, homeostasis is maintained. With respect to chemical respiratory allergy thresholds could apply to both of the aforementioned phases (sensitisation and elicitation), and there is much evidence that thresholds of elicitation can be defined for IgE-mediated allergies (Ballmer-Weber et al., 2015; Taylor et al., 2014), however for the purposes of this document the focus is upon thresholds of sensitisation. Where applicable data on both individual thresholds (exposures that fail to induce sensitisation in individual subjects) and population thresholds (exposures that fail to induce sensitisation in defined groups, or a proportion of a defined group, of subjects, e.g. a worker population), are considered in this review, whilst being mindful of the impact of parameters such as sample sizes.

6.2. Experimental data

This task is made difficult because currently there are no validated or widely accepted methods for the identification and characterisation of chemicals that are able to induce sensitisation of the respiratory tract. The problem is compounded by the fact that, as previously discussed, there remains some uncertainty about the immunobiological mechanisms that result in the development of respiratory sensitisation to chemicals. In particular, the possible role of IgE antibody in the acquisition of sensitisation remains the subject of debate.

To put into context the challenge alluded to above, it is of value to compare briefly the hurdles in determining thresholds for chemical respiratory allergy with the situation that pertains currently to assessment of thresholds for skin sensitisation (Basketter and Kimber, 2011). While the LLNA was developed for hazard characterisation of skin sensitisation, it has provided a useful tool for determination of relative potency of contact allergens. The LLNA measures in mice the potential for skin sensitisation as a function of lymphocyte proliferative responses induced in lymph nodes draining the site of topical exposure to the test chemical (Kimber et al., 2001). Such proliferative responses are required for the initiation of skin sensitisation, but importantly they also correlate quantitatively with the extent to which sensitisation is acquired (equivalent to skin sensitisation potency). For this reason it has been possible to use dose–response analyses in the LLNA as a basis for measuring the relative skin sensitising potency of contact allergens, and for determination of threshold values (Basketter et al., 2000; Kimber et al., 2001; Griem et al., 2003; Schneider and Akkan, 2004). Lack of certainty about relevant biomarkers of respiratory sensitisation by chemicals, linked with the absence of biological correlates of sensitising potency, makes the determination of thresholds in chemical respiratory allergy more difficult to assess (Basketter and Kimber, 2011).

Against this background the various approaches that have been investigated as experimental systems for investigation of the development of chemical respiratory allergy, and that might, in principle, be relevant for the identification of thresholds are considered. These approaches differ in their level of development and in their potential utility in the context of hazard identification and characterisation. Notwithstanding that, the question to be addressed is whether the endpoints utilised in such methods are of value in determining thresholds of sensitisation and definition of no observable effect levels (NOELs). To this end this review has focused primarily on approaches where attempts have been made to evaluate dose–response relationships during the acquisition of sensitisation of the respiratory tract to chemicals, including:

- lymphocyte proliferative responses.
- induced changes in cytokine expression.
- induced IgE antibody production or changes in the total serum concentration of IgE.
- elicitation of respiratory reactions in previously sensitised animals (or humans).

We have not considered here either *in silico* or *in vitro* approaches that are not yet at a stage of development where they could be used for assessment of dose–response relationship or identification of threshold values.

6.2.1. Lymphocyte proliferative responses

As indicated above, the LLNA provides a basis for the identification and characterisation of skin sensitising chemicals (Kimber et al., 2001). However, it has become clear that all known chemical respiratory allergens tested to date also induce positive responses in the LLNA (Dearman et al., 2013). The reason for this is that chemical respiratory allergens are, by definition, able to provoke adaptive immune responses, and such responses will necessarily be associated with lymphocyte proliferation in lymph nodes draining the site of exposure. Furthermore, as discussed above, it is now believed that sensitisation of the respiratory tract by chemicals can be acquired following skin exposure to the allergen. It is therefore appropriate to consider the route of

exposure used in the LLNA as being legitimate for the assessment of chemical respiratory allergens. As a consequence, a case can be made that assessment of relative potency in the LLNA, and determination of NOELs based on lymphocyte proliferative responses in the assay, provide one avenue for definition of thresholds of chemical respiratory allergens (Dearman et al., 2013).

It is important in this context to emphasise again that the LLNA does not provide a mechanism for the identification of chemical respiratory allergens. Thus, the elicitation of lymphocyte proliferative responses in draining lymph nodes does not necessarily signal that a chemical will cause allergic sensitisation of the respiratory tract. What is being suggested here is that one route for defining dose–response relationships for respiratory sensitisation, and for deriving thresholds, might be to examine LLNA responses with chemicals that have already been identified as being respiratory allergens.

A slightly different approach has been the use of a modified version of the LLNA that employs inhalation, rather than topical, exposure. The general approach taken has been to expose mice *via* inhalation, daily for 3 consecutive days, and subsequently to measure lymphocyte proliferation in draining (mandibular) lymph nodes. A number of chemical respiratory allergens has been examined using this method, and in many instances a dose–response relationship could be measured (Arts et al., 2008; De Jong et al., 2009). Some versions of this approach combine measurement of lymph node activation with cytokine production (De Jong et al., 2009; Johnson et al., 2011).

6.2.2. Cytokine production

There has been considerable interest in exploring the possibility that the induced or altered expression of specific cytokines might provide a basis for the identification of chemical respiratory allergens, and for distinguishing these from contact allergens; an approach commonly described as cytokine fingerprinting (Dearman and Kimber, 1999). In most cases the experience has been that exposure (usually repeated exposure) of mice to chemical respiratory allergens is associated with a selective Th2-type cytokine profile reflected by elevated expression of IL-4, IL-5 and IL-13. In such methods it has proven possible to monitor changes in cytokine expression at the levels of both mRNA and protein. It is apparent, however, that this approach yields somewhat variable results in different laboratories and that the cytokine profiles recorded are influenced by the strain of mouse used as well as a number of other factors, including possible the chemical class (Dearman et al., 1998a, 2002; Manetz al., 2001; Dearman and Kimber, 2001; Van Och et al., 2002; Plitnick et al., 2002; Selgrade et al., 2006; De Jong et al., 2009; Adenuga et al., 2012).

Although cytokine fingerprinting usually reflects the fact that sensitisation of the respiratory tract by chemicals is characterised by the selective induction of Th2-type responses, and despite the fact that – in some laboratories at least – the method has been used successfully for hazard identification, the approach is not necessarily well suited to definition of NOELs and thresholds. The detection of changes in cytokine expression is a function of the sensitivity of the detection method employed and this makes difficult the identifications of robust thresholds.

6.2.3. Serum immunoglobulin levels and IgE antibody

One clearly legitimate approach to the identification of chemical respiratory allergens is measurement of induced serological responses. However, it is widely appreciated that, in both humans and animals, the accurate evaluation of antibody responses against chemical allergens in the form of hapten–protein conjugates can be technically demanding.

For that reason one approach that has been explored is measurement of induced changes in the total serum concentration of IgE immunoglobulin (rather than the production of hapten-specific IgE antibodies) in animals (mice and rats) exposed to test chemicals. This strategy is based on two assumptions. The first is that even if there may not be a mandatory requirement for IgE antibody in the acquisition of chemical respiratory allergy, it might still provide an accurate biomarker of exposure (Kimber et al., 2014b). The second assumption is that chemical respiratory allergens will have the potential not only to provoke specific IgE antibody production, but will, in addition, cause an increase in total production of IgE immunoglobulin. Some investigations in mice proved very promising, and it was found that topical exposure of animals to chemical respiratory allergens, but not to contact allergens, induced a significant increase in the total serum concentration of IgE (Dearman et al., 1992; Hilton et al., 1995, 1996).

In principle this approach, the mouse IgE Test, would seem to provide an excellent basis for examination of immunological dose–responses to chemical respiratory allergens, and for derivation of NOELs, particularly as increases in the total serum concentration of IgE can be measured accurately relative to IgE levels in serum drawn from concurrent control (vehicle-treated) mice. A practical difficulty is, however, that on the basis of international inter-laboratory trials it was found that the assay might not 'travel' well. Moreover, increases in IgE appeared to be affected by the strain of mouse used, and by other factors (Dearman et al., 1998a). The conclusion drawn presently is that while the mouse IgE test provides a potentially attractive method for characterisation of chemical respiratory allergens there are hurdles to routine implementation.

However, it might be that other similar approaches can be considered also. For instance, there have been investigations of induced changes in total serum IgE in rats. For this purpose the strain of choice has been the Brown Norway rat (that like BALB/c strain mice has an atopic/Th2-like phenotype). An advantage, in principle at least, of using the rat for such studies is that it is possible to take serial bleeds and therefore measure longtitudinally the changes in the serum level of IgE. Some success has been reported, but there has not been a systematic evaluation of sensitivity and selectivity (Arts et al., 1997, 1998,b; Warbrick et al., 2002a,b).

The alternative strategy is to monitor the induction in exposed animals of specific (hapten-specific) antibodies. As discussed above, this is more challenging technically, but there are nevertheless several studies where it has been possible to demonstrate the induction of apparently hapten-specific IgG and/or IgE antibody following exposure (inhalation, intradermal or topical) of either guinea pigs (Karol, 1983; Botham et al., 1989; Sarlo and Clark., 1992; Blaikie et al., 1995; Zhang et al., 1998; Pauluhn et al., 2002b) or rats (Warbrick et al., 2002a) to a variety of chemical respiratory allergens. While, in theory, the provocation of specific antibody responses, and in particular specific IgE antibody responses, is an attractive and relevant endpoint for determination of thresholds, the technical difficulties associated with the accurate measurement of such responses would probably preclude routine use. One example where antibody responses have been used to measure dose–response relationships is shown in Table 2.

Although, measurement of antibody responses, or of induced changes in total serum levels of IgE, appears – in principle at least – to be a feasible strategy for the assessment of sensitisation thresholds, it must be borne in mind that these approaches, and other strategies considered here, require the use of experimental animals. This might be viewed as a significant limitation to these methods. A reliance on such approaches is inconsistent with the current trajectory of toxicity testing wherein the ambition is to

Table 2
Examples of NOELs determined using approaches assessing different endpoints in animal models.

Species	Endpoint measured: antibody production				
	Chemical allergen(s) and concentrations tested	Exposure methodology	Dose–response?	NOEL	Reference
Guinea pig	Induction TMA; 0.003, 0.01, 0.03, 0.1, 0.3% MDI; 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 1% PA; 0.03, 0.1, 0.3% Elicitation TMA; 7.8–16.8 mg/m^3, MDI; 18–42 mg/m^3 PA; 11–29 mg/m^3 or 9–48 mg/m^3	Induction Single intradermal injection Elicitation Single inhalation challenge with chemical	TMA; yes MDI; yes PA; yes	TMA; not determined MDI; 0.001% PA; not determined	Blaikie et al., 1995

Species	Endpoint measured: elicitation of respiratory reactions in previously sensitised animals				
	Chemical allergen(s) and concentrations tested	Exposure methodology	Dose–response?	NOEL	Reference
Rat	Induction TMA; 1, 5, 25% Elicitation TMA; 25 mg/m^3	Induction Repeated(3) topical application Elicitation Single inhalation challenge with chemical	Yes	1%	Pauluhn et al., 2003

TMA: trimellitic anhydride, MDI: diphenylmethane diisocyanate, PA: phthalic anhydride.

reduce or replace the use of animals in safety assessment. It is hoped that the use of such alternative approaches may improve the relevance of experimental models for humans.

6.2.4. Elicitation of respiratory reactions in previously sensitised animals

A survey of experimental inhalation challenge models is beyond the scope of this article, but excellent reviews are available elsewhere (for example, Pauluhn and Mohr, 2005). In principle, this approach can be used to investigate dose–response relationships with regard to the acquisition of allergic sensitization of the respiratory tract. The approach is to expose animals to various concentrations of the test material. Subsequently animals would be challenged – by inhalation exposure – to a concentration of the same material chosen not to elicit non-specific irritant reactions. In this approach the effectiveness of sensitisation is measured as a function of the elicitation of specific respiratory reactions in challenged animals. A variety of models have been described, using guinea pigs or rats, in which the initial induction of sensitisation is achieved *via* inhalation, intradermal or topical exposure (Karol et al., 1981; Botham et al., 1988; Sarlo and Clark, 1992; Rattray et al., 1994; Pauluhn, 2003; Pauluhn and Eben, 1991; Arts et al., 1998; Pauluhn et al., 2000, 2002a). One example where dose–response relationships for sensitisation have been measured as a function of challenge-induced respiratory reactions is shown in Table 2.

Using this broad approach some of the studies cited above have reported the successful demonstration of dose–response relationships during the induction phase (acquisition) of sensitisation, and the definition of NOELs. However, it must be recognised that investigations relying on inhalation challenge of previously sensitised guinea pigs or rats are very costly to perform and technically demanding requiring special inhalation expertise. This approach also has the same general drawbacks as other animal methods.

6.3. Data from human studies/clinical experience

The objective here is to consider the clinical data available from experience with a number of chemical respiratory allergens, or classes of chemical respiratory allergens (chloroplatinate salts, glutaraldehyde, acid anhydrides and diisocyanates). Before discussing these examples it is relevant to consider briefly the induction in humans of sensitisation of the respiratory tract to protein allergens. It is important to appreciate that in this instance, and in contrast to chemical respiratory allergy, sensitisation is probably most commonly acquired *via* inhalation exposure, although there is increasing evidence that protein allergens can induce IgE-mediated sensitisation *via* skin contact and this may be relevant for respiratory allergy (Lack, 2012; Kimber et al., 2014c).

As explored below, available exposure–response data on the development of sensitisation of the respiratory tract to protein aeroallergens, which also involves the production of allergen-specific IgE antibodies, provides some evidence that this is a threshold immunological mechanism (as is the elicitation by proteins of respiratory allergic reactions in previously sensitised subjects). Although it is acknowledged that no specific thresholds have been defined for occupational respiratory allergy to proteins, there is clear evidence of exposure–response relationships for IgE-mediated allergic sensitisation to such materials. It is also clear that even without an absolute defined threshold, nor a complete understanding of the dose–response relationship and the various factors that can impact upon it, operational thresholds can be established to avoid induction of sensitisation or elicitation of an allergic response. Thus, by setting occupational exposure limits (OELs) and controlling exposures, occupational sensitisation of the respiratory tract to protein allergens can be minimised and symptoms avoided in the workforce (Nielsen et al., 2012). An illustrative example is provided by experience in detergent manufacturing. When enzymes were first used in detergent manufacturing, exposures were in the order of hundreds of nanograms of protein per cubic metre and this resulted in high rates of sensitisation, and symptoms of asthma and rhinitis in the workforce. Subsequent introduction of occupational exposure controls has, however, led to elimination of symptoms and very low rates of sensitisation. An OEL for Subtilisin proteases of 60 ng/m^3 has been set by a number of regulatory agencies, which has been reduced further within the detergent industry to take account of potential adjuvant effects associated with co-exposure to surfactants. Thus, OELs for enzymes in detergent manufacturing are typically in the range of 5–15 ng/m^3. Through use of these limits sensitisation has been minimised and symptoms avoided in workforces handling a range of enzymes (Basketter et al., 2010, 2012; Peters et al., 2001; Sarlo, 2003). It can be concluded, therefore, that in the case of human respiratory sensitisation to proteins there is evidence for thresholds even if they may not currently be specified numerically.

Against this background the issue of thresholds in the development of respiratory sensitisation to chemicals in humans

is considered by reference to (in no particular order of importance): chloroplatinate salts, glutaraldehyde, acid anhydrides and diisocyanates is considered.

6.3.1. Chloroplatinate salts

Insoluble forms of platinum are relatively inert, but the soluble salts formed during the refinement process tend to exhibit the potential for irritation, corrosion and sensitisation (European Commission (SCOEL), 2001). The chloroplatinates of particular interest with respect to sensitisation of the respiratory tract are 6 halogenoplatinates (hexachloroplatinic acid, ammonium tetrachloroplatinate, ammonium hexachloroplatinate, potassium tetrachloroplatinate, potassium hexachloroplatinate and sodium hexachloroplatinate). The current OEL for platinum salts is set at 2 $\mu g/m^3$. However, this is not a health-based level, but rather what is technically achievable and within measurable limits in the workplace (Bullock, 2010).

Studies of relative potency indicate that the most important occupational respiratory allergens are the hexachloroplatinic acid and the tetra- and hexachloroplatinate salts (Cristaudo et al., 2005).

The phenotype and timing of allergic responses to chloroplatinate salts are consistent with an IgE antibody-mediated sensitisation. Typically initial exposure is followed by a latency period (1 month to several years) before a subsequent inhalation exposure results in the development of respiratory hypersensitivity and allergic asthma (Merget et al., 2000; Rauf-Heimsoth et al., 2000). While the weight of evidence suggests that chloroplatinate allergy is associated with IgE antibody induced sensitisation, the fact that skin prick tests apparently fail to identify all symptomatic workers (Calverley et al., 1995), suggest that other mechanisms cannot be excluded. However, it should be noted that skin prick tests are often of insufficient sensitivity to detect IgE antibody-mediated sensitisation to chemical and drug allergens. As previously described (Section 3.4) smoking is a known risk factor for the development of chemical respiratory allergy, however the strength of association varies with allergen and for chloroplatinate salts smoking is reported to be a particularly prominent risk factor (Nielsen et al., 2005).

Also relevant for the assessment of chloroplatinate allergy is consideration of speciation; there being evidence that neutral complexes such as tetraamine platinum dichloride are non-sensitising in the workplace (Linnett and Hughes, 1999; Steinfort et al., 2008). Hexachloroplatinic acid and both tetra- and hexachloroplatinate salts have the structural characteristics required to induce sensitisation (charged with reactive ligand systems), whereas non-halogenated or neutral complexes of platinum fail to induce sensitisation. Platinum allergy appears to be induced by a group of charged compounds with reactive ligand systems, the most potent being hexachloroplatinic acid and the chlorinated salts ammonium hexachloroplatinate, potassium tetrachloroplatinate, potassium hexachloroplatinate and sodium tetrachloroplatinate. Non-halogenated and neutral complexes have been shown to be nonallergenic.

A number of studies describing workplace exposure and associated health effects in catalyst production and recycling factory workers (Bolm-Audorff et al., 1992; Linnett and Hughes, 1999; Merget et al., 2000) have reported direct correlations between inhalation exposure and blood, serum and urine levels of platinum. Merget et al. (2000) performed a 5 year prospective cohort study following workers exposed to either high, consistently low or intermittently low levels of platinum. While a correlation was found between blood levels of platinum and airborne exposure, no cases of respiratory sensitisation were observed in the low exposure group leading to the conclusion that the median recorded value 10 ng/m^3 could be considered as a safe level of exposure. Not unexpectedly, airborne levels display wide variation (10–100 fold), depending on task and sampling methodology and although the majority of reported levels are below current OELs there are instances, for instance during operational malfunctions, where much higher values are recorded (Merget et al., 2000).

Some evidence for the existence of thresholds for sensitisation of the respiratory tract to chloroplatinate salts derives from occupational studies. However, given uncertainties about the timing of sensitisation and the time between sensitisation and the appearance of symptoms, it is difficult to define numerically what such thresholds are. Using the experience obtained from human studies including those of Linnett and Hughes (1999) and Merget et al. (2000) several agencies including the Health Council of the Netherlands (DECOS), 2008) and the US Environmental Protection Agency (Environmental Protection Agency (EPA), 2009) have derived health-based OELs. Although the development of OELs recognises the existence of thresholds for sensitisation of the respiratory tract to chloroplatinate salts it is uncertain how relevant the numerical values are with respect to this specific endpoint threshold.

6.3.2. Glutaraldehyde

Glutaraldehyde is used widely in the healthcare industry as a cold sterilant of medical and surgical instruments, as a fixative for biological tissues in pathology, and as a component of developing solutions for X-ray films. Over the past 30 years evidence has accumulated of occupational asthma associated with the use of glutaraldehyde, including the involvement of IgE antibody, and in particular in endoscopy, radiography and pathology suites (Kimber and Dearman, 1997b; Di Stefano et al., 1999).

The prevalence of respiratory illness in healthcare professionals is much higher than that of the general population (Liss et al., 2011; Bakerly et al., 2008), and exposure to multiple chemicals for prolonged periods of time is almost certainly a contributory factor (Saito et al., 2015; Wiszniewska and Walusiak-Skorupa, 2014). There are well-documented reports of occupational asthma attributable to glutaraldehyde, but the prevalence is low (Vandenplas et al., 2011).

There have been several investigations of the relationship between workplace exposure to glutaraldehyde and occupational asthma. Water et al. (2003) and Cohen and Patton (2006) reported that workers exposed to glutaraldehyde at concentrations ranging from 0.4 to 1.98 mg/m^3 had associated respiratory effects. Two confirmed cases of occupational asthma attributable to glutaraldehyde were reported with exposures ranging from below the limit of detection to 3.32 mg/m^3 (Sutton et al., 2007). However, in another study reported by Teta et al. (1995), where exposure levels ranged from 0.04 to 1.36 mg/m^3 of glutaraldehyde, no cases of occupational asthma were identified.

Several studies (Gannon et al., 1995; Pisaniello et al., 1997; Di Stefano et al., 1999; Vyas et al., 2000) indicate that occupational asthma to glutaraldehyde is not associated with exposure levels at or near the current OEL of 0.2 mg/m^3, even when exposure is sustained for prolonged periods. Evidence suggesting that brief exposures to much higher levels of glutaraldehyde are sufficient to induce allergic sensitization is consistent with the probability that peak exposures to chemicals may drive sensitisation (Arts et al., 2006; Vyas et al., 2000).

6.3.3. Acid anhydrides

It is well established that a variety of acid anhydrides are associated with allergic sensitisation of the respiratory tract and occupational asthma. Among those implicated are the following: phthalic anhydride (PA), tetrachlorophthalic anhydride (TCPA), trimellitic anhydride (TMA), hexahydrophthalic anhydride (HHPA),

methyl hexahydrophthalic anhydride (MHHPA), methyltetrahydrophthalic anhydride (MTHPA), and maleic anhydride (MA) (Kimber and Dearman, 1997b).

Dose–response relationships in the development of sensitisation to acid anhydrides have been described, but it has not been possible to define clear thresholds (Welinder et al., 2001; Nielsen et al., 2001; Drexler et al., 2000; Rosqvist et al., 2003). In a prospective study reported by Welinder et al. (2001), serum IgE antibody was found in 13% of workers exposed to several acid anhydrides (HHPA, MHPA, and MTPHA) with a mean combined exposure level of 15.4 $\mu g/m^3$ (range <1–189 $\mu g/m^3$).

In a cross-sectional study reported by Nielsen et al. (2001), specific IgE antibody was found in 13, 26 and 21% of workers exposed to HHPA at concentrations of less than 10 $\mu g/m^3$, between 10 and 50 $\mu g/m^3$, and greater than 50 $\mu g/m^3$ HHPA, respectively. With MHHPA specific IgE levels were found in 15, 26 and 17% of workers at the low, medium and high levels of exposure, respectively (Nielsen et al., 2001).

Other studies such as that reported by Drexler et al. (2000) found an association between the acquisition of sensitisation and increasing levels of exposure to MTHPA, while Rosqvist et al. (2003) reported that cases with specific IgE antibody, consistent with the acquisition of sensitization, increased with increasing exposures to MTHPA.

Evidence for dose-related sensitisation has also been found by Bernstein et al. (1983) who reported that the number of workers with detectable IgE antibody levels and respiratory symptoms of sensitisation decreased when exposure levels to TMA were reduced from 0.82–2.1 mg/m^3 to 0.01–0.03 mg/m^3. Yokota et al. (1999) compared levels of specific IgE antibody and symptoms of respiratory sensitisation in two industrial sites where workers were exposed to MTHPA at concentrations of between 25 and 64 $\mu g/m^3$ or between 4.9 and 5.5 $\mu g/m^3$. Approximately 65% of the workers at both sites showed specific IgE antibody although allergic symptoms were more common in the plant with higher MTHPA exposure levels (Yokota et al., 1999).

The Health Council of the Netherlands (2010) concluded that the lowest exposure levels to acid anhydrides at which work-related IgE-dependent sensitisation was induced varied from less than 1 $\mu g/m^3$ (TMA, HHPA and MHHPA) and 15 $\mu g/m^3$ (MTHPA), to exposure levels of hundreds of $\mu g/m^3$ (PA and TCPA).

6.3.4. Diisocyanates

Among the diisocyanates that have been shown to cause chemical respiratory allergy and occupational asthma are: toluene diisocyanate (TDI), diphenylmethane diisocyanate (MDI) and hexamethylene diisocyanate (HDI) (Kimber and Dearman, 1997b).

In a prospective study by Karol (1981) the occurrence of TDI specific IgE antibody was examined in one group of 96 workers exposed for 6 months to TDI at concentrations of 20 ppb or less, and in a second group of 20 workers that had experienced acute overexposures within an observation period of 3 years. No IgE antibody or clinical symptoms were found in the workers exposed to 20 ppb or less TDI, whereas IgE antibody that correlated with respiratory symptoms was identified in 4 workers that had experienced acute overexposure. Other workers in this latter group also displayed respiratory symptoms, but in absence of detectable IgE antibody.

Other prospective studies differentiate between low and high exposure atmospheres, but in those cases assessments are based exclusively on clinical parameters (lung function, clinical history), in the absence of verification of sensitization by provocation tests.

Studies based exclusively on clinical parameters such as lung function and clinical history have found evidence of altered lung function in researchers exposed to TDI above 20 ppb (Bruckner et al., 1986), and in spray painters exposed to TDI at concentrations of between 44 and 112 ppb with no effects at 15 ppb (Huang et al., 1991). These results are consistent with those of many other investigations conducted using comparatively large populations of workers over long periods. Bugler et al. (1991) studied a population of 1462 workers employed in polyurethane foam production facility for 6 years and identified 31 subjects with self- reported respiratory symptoms. The majority of these workers were, however, involved in jobs with peak exposures at above 20 ppb. This finding is similar to those reported by Weill et al. (1981) and Ott et al. (2000) who found that cases of occupational asthma were associated with acute over-exposure, or with job profiles in which there was routine short-term exposure to concentrations of diisocyanates of greater than 20 ppb.

In a cross-sectional study of 243 employees in a polyurethane processing facility who were regularly exposed to MDI at a 8 h time weighted average exposure not exceeding 5 ppb three subjects diagnosed with occupational asthma appeared to have acquired sensitisation as the result of intermittent high exposures during non-routine work activities (Bernstein et al., 1993). Analysis of worker populations exposed to controlled atmospheres of MDI below 5 ppb (Gee and Morgan, 1985; Musk et al., 1982; Pham et al., 1988), 10 ppb TDI (Musk et al., 1982), or to 3 ppb HDI (Hathaway et al., 1999; Cassidy et al., 2010) did not identify any increase in the incidence of work-related respiratory symptoms. In contrast, exposure of workers to concentrations of MDI of 5 ppb or more was associated with a more frequent occurrence of work-related asthma (Tarlo et al., 1997).

Overall the human evidence of occupational allergy to diisocyanates suggests there is a threshold for the acquisition of sensitisation. While it is difficult to be precise, the available data suggest that the threshold concentrations for the development of sensitization from inhalation exposure to atmospheres of diisocyanates are in the range of between 5 and 20 ppb.

7. Discussion

There is no doubt that, in common with all other forms of allergy, and indeed all types of adaptive immune response, there are threshold exposure requirements for antigen. That is there is, in all circumstances, a requirement for a minimum level of antigen exposure below which an immune or allergic response will not be provoked. Clearly that threshold requirement will vary substantially based on the responsiveness/susceptibility of the individual (inherited or acquired), the immunogenicity of the antigen itself, the conditions of exposure (amount, duration, frequency and route), and other factors. However, although there is a clear consensus that the elicitation of an immune or allergic response is a threshold phenomenon, identifying what that threshold is can be technically demanding. In this context it is presently the case that there is no sure way of identifying antigen or allergen thresholds other than using *in vivo* methodology, either examining the induction of immune responses in humans, or through the use of appropriate experimental animal systems.

Even in humans or in animal models the identification of thresholds demands that the end-point or event used is known to be a relevant biomarker. Sometimes this is relatively straightforward. Thus, for instance, in the case of attempting to define the overall immunogenicity of a protein it would be legitimate to define the threshold level of exposure as being the lowest amount required to stimulate the induction of a measurable IgE antibody response.

In the case of chemical respiratory allergy the identification of thresholds is particularly difficult because there is no general consensus on the mechanisms through which sensitisation is acquired, or the relevance of putative biomarkers.

It is perhaps useful to summarise briefly the state of the science, based upon the experimental approaches that are currently available.

7.1. QSAR and in vitro methods

At present there are no QSAR or *in vitro* models available that will provide a reliable basis for assessment of dose responses, the identification of thresholds, or the derivation of NOEL values. In time they may evolve to provide relevant information, but that is some way off. To accelerate relevant developments in these areas will require continued investment.

7.2. Local lymph node assay and lymphocyte proliferation in draining lymph nodes

Both the standard LLNA, and the respiratory modification of the method, can be considered. Both provide an assessment of dose–response relationships based on proliferation in lymph nodes draining the site of exposure to chemical. In theory, this approach provides a viable strategy for the identification of thresholds because if the level of exposure to a chemical respiratory allergen is insufficient to drive the induction of a T lymphocyte response (measured here by allergen-induced T lymphocyte proliferation) then an allergic response will not develop and – by definition – the acquisition of sensitisation demands that such a response is induced. This is an attractive and feasible approach. However, this approach does require the use of animals It is necessary to emphasise again that if such an approach is considered then it would be of value only in those circumstances where a chemical had already been identified as a respiratory allergen; the LLNA does not provide a means of hazard identification for respiratory sensitisers.

7.3. Antibody production

This is an approach that could be used, although there is no general consensus that antibody (either IgG or IgE antibody) is required for the development of sensitisation to chemical respiratory allergens. However, it is clear that there are substantial technical difficulties in measuring reliably antibody responses to chemical haptens. Moreover, IgE antibody, without doubt the most relevant class of antibody in the context of chemical respiratory allergy, is produced only in very small amounts and can be very difficult to detect.

7.4. Total serum IgE concentration

In terms of hazard identification and characterisation this is an attractive approach, and one that could, in principle, be used for analyses of dose–response relationships, and determination of threshold levels. However, the method is predicated on the ability of chemicals to provoke changes in the total serum concentration of IgE, and there is no consensus that this is a relevant biomarker of exposure to chemical respiratory allergens. In addition, there is concern that the assay may be somewhat variable in performance.

7.5. Cytokine fingerprinting

This strategy is based on an appreciation that chemical respiratory allergens commonly induce in rodents (and probably in humans also) a selective Th2-type immune response that is associated with the increased expression of type 2 cytokines such as IL-4, IL-5 and IL-13. Although experience with this approach is somewhat varied, there is a case to be made that this is an appropriate method for hazard identification. Notwithstanding the potential value of the method in that context, cytokine fingerprinting is probably not a viable approach to determination of thresholds. The assay is rather complicated to perform, and furthermore does not have a single discrete endpoint. In addition, the level of detection of cytokines that is achievable is dependent upon the sensitivity of the analytical method employed.

7.6. Challenge induced respiratory reactions

The general method that would probably be most widely endorsed by the wider scientific community is measurement of challenge-induced respiratory allergic reactions in previously sensitised animals (guinea pigs or rats). The approach in this case would be to identify the threshold level of chemical allergen required for effective sensitisation such that the treated animal would mount a discernible respiratory reaction following subsequent inhalation challenge with the same chemical. Although this strategy is attractive, insofar as it is based on the measurement of the elicitation of respiratory allergic reactions, there are several important drawbacks. Included among these are: the need for relatively large numbers of animals, the requirement for expensive inhalation facilities and the expertise necessary for the generation of atmospheres and their administration, and for the measurement of respiratory responses. Moreover, the measurement of respiratory responses is not necessarily a very sensitive endpoint, and one that can be confounded by irritant reactions.

Against this current landscape the conclusion drawn is that there is no method suitable for the routine assessment of threshold values for the induction of sensitisation of the respiratory tract by chemicals.

Perhaps the most relevant source of information derives from studies of the development of occupational asthma in humans. In most cases the data available from such investigations clearly point to the existence of threshold levels of exposure below which allergic sensitisation will fail to develop, and based on such observations it is possible to derive OELs. If improved diagnostic tests for respiratory sensitisation to chemical allergens can be developed, and linked with prospective monitoring of workforces with respect to both the development of sensitisation and occupational exposure levels, it may prove possible to identify more accurately threshold levels.

Although the primary focus of this review has been on the identification of threshold values in chemical respiratory sensitisation, there are a number of related issues relevant to chemical respiratory allergy that it is appropriate to highlight for future consideration. These are as follows:

- There is a need for greater clarity about what it is that different models and experimental approaches are trying to achieve (hazard identification, hazard characterisation, assessment of potency and/or identification of threshold values).
- Based on the evidence reviewed here it is clear that currently the only feasible approach to assessment of potency and prioritisation of chemicals is based on consideration of inherent sensitising activity (however measured). There is a need for further research and refinement here.
- Given the current state of the science there are important questions to be asked about how best the workforce can be protected. Given that chemical respiratory allergy is a threshold phenomenon it is important that this is reflected in methods for deriving safe levels.
- Whilst undertaking this review, some authors identified the potential for inappropriate use of data from repeat dose oral toxicity studies to be used to try to derive no effect levels for chemical respiratory sensitisation. It is important that key

stakeholders involved in defining thresholds ensure robust data and approaches are used and relevant expertise applied.

8. Conclusions

- There remains uncertainty about the cellular and molecular mechanisms through which sensitisation of the respiratory tract by chemicals is achieved.
- A number of approaches to the identification and characterisation of chemical respiratory allergens have been proposed.
- However, none of these has been validated or is widely accepted.
- It is the case, therefore, that the safety assessment of chemicals with respect to chemical respiratory allergy remains a significant challenge.
- There is no doubt that, in common with all other forms of allergy, chemical respiratory allergy is a threshold phenomenon (at the level of both sensitisation and elicitation).
- Despite this, there are no approaches available currently that have been proven to provide a method for identifying thresholds for sensitisation of the respiratory tract by chemicals.
- The above position results from the fact that – due to uncertainty about mode of action – there are no accepted biomarkers that would provide reliable endpoints for assessment of threshold values.
- The current experimental approaches that appear, in principle, to be best suited to identification of thresholds values are: evaluation of specific antibody responses, measurement of induced changes in the total serum concentration of IgE immunoglobulin, and assessment of challenge-induced respiratory reactions in previously sensitised animals.
- However, all of the above methods are associated with significant technical challenges and/or substantial cost implications and are presently not suitable for routine measurement of threshold values.
- The other important consideration is the fact that all of the methods cited above as having some potential to provide information of relevance to assessment of thresholds require the use of animals. It must be appreciated that the trajectory of toxicology is now moving towards the development of alternative (non-animal) strategies for safety assessment, and indeed for some purposes the use of experimental animals is no longer permitted. There is, therefore, an urgent imperative to develop approaches to hazard identification and characterisation, and for the determination of threshold values, that obviate the need for animals.
- Against that background, the most relevant source of information regarding thresholds for the development of sensitisation of the respiratory tract to chemicals are human data. Studies of chemical respiratory allergy and occupational asthma in the workplace, although not systematic in nature, do collectively indicate that the acquisition of sensitisation is exposure concentration-related, and that thresholds below which sensitisation will fail to develop can be identified.

References

Adenuga, D., Woolhiser, M.R., Gollapudi, B.B., Boverhof, D.R., 2012. Differential gene expression responses distinguish contact and respiratory sensitisers and non-sensitising irritants in the local lymph node assay. Toxicol. Sci. 126, 413–425.

Apter, A.J., 2008. Advances in the care of adults with asthma and allergy. J. Allergy Clin. Immunol. 121, 839–844.

Arts, J.H.E., Kuper, C.F., 2003. Approaches to induce and elicit respiratory allergy: impact of route and intensity of exposure. Toxicol. Lett. 140–141, 213–222.

Arts, J.H.E., Droge, S.C.M., Spanhaak, S., Bloksma, N., Pennicks, A.H., Kuper, C.F., 1997. Local lymph node activation and IgE responses in Brown Norway and Wistar rats after dermal application of sensitizing and non-sensitizing chemicals. Toxicology 117, 229–237.

Arts, J.H.E., Kuper, C.F., Spoor, S.M., Bloksma, N., 1998. Airway morphology and function of rats following dermal sensitization and respiratory challenge with low molecular weight chemicals. Toxicol. Appl. Pharmacol. 152, 66–76.

Arts, J.H.E., Mommers, C., de Heer, C., 2006. Dose-response relationships and threshold levels in skin and respiratory allergy. Crit. Rev. Toxicol. 36, 219–251.

Arts, J.H.E., de Jong, W.H., van Triel, J.J., Schijf, M.A., de Klerk, A., van Loveren, H., Kuper, C.F., 2008. The respiratory local lymph node assay as a tool to study respiratory sensitisers. Toxicol. Sci. 106, 423–434.

Bakerly, N.D., Moore, V.C., Vellore, A.D., Jaakkola, M.S., Robertson, A.S., Burge, P.S., 2008. Fifteen-year trends in occupational asthma: data from the Shield surveillance scheme. Occup. Med. 58, 169–174.

Ballmer-Weber, B.K., Fernandez-Rivas, M., Beyer, K., Defernez, M., Sperrin, M., Mackie, A.R., Salt, L.J., Hourihane, J.O., Asero, R., Belohlavkova, S., Kowalski, M., de Blay, F., Papadopoulos, N.G., Clausen, M., Knulst, A.C., Roberts, G., Popov, T., Sprikkelman, A.B., Dubakiene, R., Vieths, S., van Ree, R., Crevel, R., Mills, E.N., 2015. How much is too much? Threshold dose distributions for 5 food allergens. J. Allergy Clin. Immunol. 135, 964–971.

Basketter, D.A., Blaikie, L., Dearman, R.J., Kimber, I., Ryan, C.A., Gerberick, G.F., Harvey, P., Evans, P., White, I.R., Rycroft, R.J.G., 2000. Use of the local lymph node assay for estimation of relative contact allergenic potency. Contact Dermatitis 42, 244–348.

Basketter, D.A., Kimber, I., 2011. Assessing the potency of respiratory allergens: uncertainties and challenges. Regul. Toxicol. Pharmacol. 61, 365–372.

Basketter, D.A., Broekhuizen, C., Fieldsend, M., Kirkwood, S., Mascarenhas, R., Maurer, K., Pedersen, C., Rodriguez, C., Schiff, H.-E., 2010. Defining occupational consumer exposure limits for enzyme protein respiratory allergens under REACH. Toxicology 268, 165–170.

Basketter, D.A., Berg, N., Kruszewski, F.H., Sarlo, K., Concoby, B., 2012. Relevance of sensitization to occupational allergy and asthma in the detergent industry. J. Immunotoxicol. 9, 314–319.

Beckett, W.S., 1994. The epidemiology of occupational asthma. Eur. Respir. J. 7, 161–164.

Bello, D., Herrick, C.A., Smith, T.J., Woskie, S.R., Streicher, R.P., Cullen, M.R., Liu, Y., Redlich, C.A., 2007. Skin exposure to isocyanates: reasons for concern. Environ. Health Perspect. 115, 328–335.

Bernstein, D.I., Roach, D.E., McGrath, K.G., Larsen, R.S., Zeiss, C.R., Patterson, R., 1983. The relationship of airborne trimellitic anhydride concentrations to trimellitic anhydride-induced symptoms and immune responses. J. Allergy Clin. Immunol. 72, 709–713.

Bernstein, D.I., Korbee, L., Stauder, T., Bernstein, J.A., Scinto, J., Herd, Z.L., Berstein, I.L., 1993. Clinical aspects of allergic disease: the low prevalence of occupational asthma and antibody-dependent sensitization to diphenylmethane diisocyanate in a plant engineered for minimal exposure to diisocyanates. J. Allergy Clin. Immunol. 92, 387–396.

Blaikie, L., Morrow, T., Wilson, A.P., Hext, P., Hartop, P.J., Rattray, N.J., Woodcock, D., Botham, P.A., 1995. A two-centre study for the evaluation of an animal model for the assessment of the potential of small molecular weight chemicals to cause respiratory allergy. Toxicology 96, 37–50.

Bolm-Audorff, U., Bienfait, H.G., Buckhard, J., Bury, A.H., Merget, R., Pressel, G., Schultze-Weringhaus, G., 1992. Prevalence of respiratory allergy in a platinum refinery. Int. Arch. Occup. Environ. Health 64, 257–260.

Botham, P., Hext, P.M., Rattray, N.J., Walsh, S.T., Woodcock, D.R., 1988. Sensitisation of guinea pigs by inhalation exposure to low molecular weight chemicals. Toxicol. Lett. 41, 159–173.

Botham, P.A., Rattray, N.J., Woodcock, D.R., Walsh, S.T., Hext, P.M., 1989. The induction of respiratory allergy in guinea pigs following intradermal injection of trimellitic anhydride: a comparison of the response to 2,4-dinitrochlorobenzene. Toxicol. Lett. 47, 25–39.

Boverhof, D.R., Billington, R., Gollapudi, B.B., Hotchkiss, J.A., Krieger, S.M., Poole, A., Wiescinski, C.M., Woolhiser, M.R., 2008. Respiratory sensitisation and allergy: current research approaches and needs. Toxicol. Appl. Pharmacol. 226, 1–13.

Bruckner, H.C., Avery, S.B., Stetson, D.M., Dodson, V.M., Ronayne, J.J., 1986. Clinical and immunologic appraisal of workers exposed to diisocyanates. Arch. Environ. Health 16, 619–625.

Bugler, J., Clark, R.L., Hill, I.D., McDermott, M. 1991. The acute and long-term respiratoryeffects of aromatic diisocyanates. A five year longtitudinal study of polyurethane foam workers. International Isocyanate Institute Report 10848, Manchester, UK.

Bullock, J., 2010. Chloroplatinate Toxicity: Use and Misunderstanding of Merget. International Precious Metal Institute.

Calverley, A.E., Rees, D., Dowdeswell, R.J., Linnett, P.J., Kielkowski, D., 1995. Platinum salt sensitivity in refinery workers: incidence and effects of smoking and exposure. Occup. Environ. Med. 52, 661–666.

Cassidy, L.D., Molenaar, D.M., Hattaway, J.A., Feeley, T.M., Cummings, B.J., Simpson, P., Li, S.-H., 2010. Trends in pulmonary functionand prevalence of asthma in hexamethylene diisocyanate workers during a 19 year period. J. Occup. Environ. Med. 52, 988–994.

Cohen, N.L., Patton, C.M., 2006. Worker safety and glutaraldehyde in the gastrointenstinal laboratory environment. Gastroenterol. Nurs. 29, 100–104.

Cristaudo, A., Sera, F., Severino, V., De Rocco, M., Di Lella, E., Picardo, M., 2005. Occupational hypersensitivity to metal salts, including platinum, in the secondary industry. Allergy 60, 159–164.

De Jong, W.H., Arts, J.H.E., de Klerk, A., Schijf, M.A., Ezendam, J., Kuper, C.F., van Loveren, H., 2009. Contact and respiratory sensitizers can be identified by cytokine profiles following inhalation exposure. Toxicology 261, 103–111.
Dearman, R.J., Kimber, I., 1999. Cytokine fingerprinting: characterization of chemical allergens. Methods 19, 56–63.
Dearman, R.J., Kimber, I., 2001. Cytokine fingerprinting and hazard assessment of chemical respiratory allergy. J. Appl. Toxicol. 21, 153–163.
Dearman, R.J., Basketter, D.A., Kimber, I., 1992. Variable effects of chemical allergens on serum IgE concentration in mice. Preliminary evaluation of a novel approach to the identification of respiratory sensitizers. J. Appl. Toxicol. 12, 317–323.
Dearman, R.J., Basketter, D.A., Blaikie, L., Clark, E.D., Hilton, J., House, R.V., Ladics, G. S., Loveless, S.E., Mattis, C., Sailstad, D.M., et al., 1998a. The mouse IgE test: interlaboratory evaluation and comparison of BALB/c and C57BL/6 strain mice. Toxicol. Meth. 8, 69–85.
Dearman, R.J., Basketter, D.A., Kimber, I., 1998b. Selective induction of type 2 cytokines following topical exposure of mice to platinum salts. Food Chem. Toxicol. 36, 199–207.
Dearman, R.J., Filby, A., Humphreys, I.R., Kimber, I., 2002. Interleukins 5 and 13 characterize immune responses to respiratory sensitizing acid anhydrides. J. Appl. Toxicol. 22, 317–325.
Dearman, R.J., Basketter, D.A., Kimber, I., 2013. Inter-relationships between different classes of chemical allergens. J. Appl. Toxicol. 33, 558–565.
Di Stefano, F., Siriruttanapruk, S., McCoach, J., Burge, P.S., 1999. Glutaraldehyde: an occupational hazard in the hospital setting. Allergy 54, 1105–1109.
Dik, S., Ezendam, J., Cunningham, A.R., Carrasquer, C.A., Van Loveren, H., Rorije, E., 2014. Evaluation of in silico models for the identification of respiratory sensitizers. Toxicol. Sci. 142, 385–394.
Drexler, H., Jonsson, B.A., Goen, T., Nielsen, J., Lakemeyer, M., Welinder, H., 2000. Wxposure assessment and sensitisation in workers exposed to organic anhydrides. Int. Arch. Occup. Environ. Health 73, 228–234.
ECHA (European Chemical Agency), 2012. Document: CA/60/2012, Helsinki.
Enoch, S.J., Roberts, D.W., Cronin, M.T.D., 2009. Electrophilic reaction chemistry of low molecular weight respiratory sensitizers. Chem. Res. Toxicol. 22, 1447–1453.
Enoch, S.J., Roberts, D.W., Cronin, M.T.D., 2010. Mechanistic category formation for prediction of respiratory sensitization. Chem. Res. Toxiol. 23, 1547–1555.
Enoch, S.J., Seed, M.J., Roberts, D.W., Cronin, M.T., Stocks, S.J., Agius, R.M., 2012. Development of mechanism-based structural alerts for respiratory sensitization hazard identification. Chem. Res. Toxicol. 25, 2490–2498.
Environmental Protection Agency (EPA), 2009. Draft toxicological review of halogenated platinum salts and platinum compounds. In support of the summary information in the Integrated Risk Information System (IRIS).
European Commission (SCOEL), 2001. Recommendations from the Scientific Committee on Occupational Exposure Limits for platinum and platinum compounds. SCOEL/SUM/150.
Farraj, A.K., Harkema, J.R., Kaminski, N.E., 2006. Topical application versus intranasal instillation: a qualitative comparison of the effect of the route of sensitization on trimellitic anhydride-induced allergic rhinitis in A/J mice. Toxicol. Sci. 92, 321–328.
Farraj, A.K., Boykin, E., Haykal-Coates, N., Gavett, S.H., Doerfler, D., Selgrade, M.K., 2007. Th2 cytokines in skin draining lymph nodes and IgE do not predict airway hypersensitivity to intranasal isocyanate exposure in mice. Toxicol. Sci. 100, 99–108.
Friedman-Jimenez, G., Beckett, W.S., Szeinuk, J., Petsonk, E.L., 2000. Clinical evaluation management, and prevention of work-related asthma. Am. J. Ind. Med. 37, 121–141.
Gannon, P.F., Bright, P., Campbell, M., O'Hickey, S.P., Burge, P.S., 1995. Occupational asthma due to glutaraldehyde and formaldehyde in endoscopy and X ray departments. Thorax 50, 156–159.
Gee, J., Morgan, W.K.C., 1985. A 10-year follow-up studyof a group of workers exposed to isocyanates. J. Occup. Med. 27, 15.
Gerberick, G.F., Vassallo, J.D., Bailey, R.E., Chaney, J.G., Morrall, S.W., Lepoittevin, J.P., 2004. Development of a peptide reactivity assay for screening contact allergens. Toxicol. Sci. 81, 332343.
Griem, P., Goebel, C., Scheffler, R., 2003. Proposal for a risk assessment methodology for skin sensitisation based on sensitisation potency data. Regul. Toxicol. Pharmacol. 38, 269–290.
HSE, 2001. Asthmagen? Critical Assessments of the Evidence for Agents Implicated in Occupational Asthma. Health and Safety Executive.
Hathaway, J.A., DeWilde, A., Shepperly, D.C., Nguyen, L.T., Johnson, J.E., 1999. Evaluation of pulmonary function in workers exposed to hexamethylene diisocyanate. J. Occup. Environ. Med. 41, 378–383.
Health Council of the Netherlands (DECOS), 2008. Platinum and platinum compounds. Health based recommended occupational exposure limit.
Health Council of the Netherlands, 2010. Cyclic Acid Anhydrides- Health-based Recommended Occupational Exposure Limit. The Hague: Health Council of the Netherlands publication no: 2010/020SH, ISBN: 978-90-5549-782-9.
Hilton, J., Dearman, R.J., Basketter, D.A., Kimber, I., 1995. Identification of chemical respiratory allergens: dose–response relationships in the mouse IgE test. Toxicol. Methods 5, 51–60.
Hilton, J., Dearman, R.J., Boylett, M.S., Fielding, I., Basketter, D.A., Kimber, I., 1996. The mouse IgE test for the identification of potential chemical respiratory allergens. J. Appl. Toxicol. 16, 165–170.
Holsapple, M.P., Jones, D., Kawabata, T.T., Kimber, I., Sarlo, K., Selgrade, M.K., Shah, J., Woolhiser, M.R., 2006. Assessing the potential to induce respiratory hypersensitivity. Toxicol. Sci. 91, 4–13.
Hopkins, J.E., Naisbitt, D.J., Kitteringham, N.R., Dearman, R.J., Kimber, I., Park, B.K., 2005. Selective haptenation of cellular and extracellular protein by chemical allergens: association with cytokine polarization. Chem. Res. Toxicol. 18, 375–381.
Huang, J., Wang, X., Chen, B., Ueda, M., Aoyama, K., Matsushita, T., 1991. Immunological effects of toluene diisocyanate exposure on painters. Arch. Environ. Contam. Toxicol. 21, 607–611.
Isola, D., Kimber, I., Sarlo, K., Lalko, J., Sipes, I.G., 2008. Chemical respiratory allergy and occupational asthma: what are the key areas of uncertainty? J. Appl. Toxicol. 28, 249–253.
Johnson, V.J., Reynolds, J.S., Wang, W., Fluharty, K., Yucesoy, B., 2011. Inhalation of ortho-phthalaldehyde vapour causes respiratory sensitization in mice. J. Allergy (Cairo) 2011, 751052.
Jones, M., Cullinan, P., Durham, S., Newman Taylor, A., 2003. Cellular aspects of occupational asthma: immunological studies in isocyanate exposed subjects. Research Reports Series. HSE Books.
Jones, M.G., Nielsen, J., Welch, J., Harrs, J., Welinder, H., Bensryd, I., Skerfving, S., Welsh, K., Venables, K.M., Newman Taylor, A., 2004. Association of HLA-DQ5 and HLA-DR1 with sensitization to organic acid anhydrides. Clin. Exp. Allergy 34, 812–816.
Karol, M.H., Hauth, B.A., Riley, E.J., Magreni, C.M., 1981. Dermal contact with toluene diisocyanate (TDI) produces respiratory tract hypersensitivity in guinea pigs. Toxicol. Appl. Pharmacol. 58, 221–230.
Karol, M.H., 1981. Survey ofindustrial workersfor antibodies to toluene diisocyanate. J. Occup. Med. 23, 741–747.
Karol, M.H., 1983. Concentration-dependent immunologic response to toluene diisocyanate (TDI) following inhalation exposure. Toxicol. Appl. Pharmacol. 68, 229–241.
Karol, M.H., 1986. Respiratory effects of inhaled isocyanates. CRC Crit. Rev. Toxicol. 16, 349–379.
Kimber, I., Basketter, D.A., 2014. Categorisation of protein respiratory allergens: the case of Subtilisin. Regul. Toxicol. Pharmacol. 68, 488–492.
Kimber, I., Dearman, R.J., 1997a. Cell and molecular biology of chemical allergy. Clin. Rev. Allergy Immunol. 15, 145–168.
Kimber, I., Dearman, R.J., 1997b. Chemical respiratory hypersensitivity: an introduction. In: Kimber, I., Dearman, R.J. (Eds.), Toxicology of Chemical Respiratory Hypersensitivity. Taylor & Francis, London, pp. 1–6.
Kimber, I., Dearman, R.J., 2002. Chemical respiratory allergy: role of IgE antibody and relevance of route of exposure. Toxicology 181–182, 311–315.
Kimber, I., Basketter, D.A., Berthold, K., Butler, M., Garrigue, J.L., Lea, L.J., Newsome, C., Roggeband, R., Steiling, W., Stropp, G., Waterman, S., Wiemann, C., 2001. Skin sensitisation testing in potency and risk assessment. Toxicol. Sci. 59, 198–208.
Kimber, I., Dearman, R.J., Basketter, D.A., Ryan, C.A., Gerberick, G.F., 2002. The local lymph node assay: past present and future. Contact Dermatitis 47, 315–328.
Kimber, I., Agius, R., Basketter, D.A., Corsini, E., Cullinan, P., Dearman, R.J., Gimenez-Arnua, E., Greenwell, L., Hartung, T., Kuper, F., Maestrelli, P., Roggen, E., Rovida, C., 2007. Chemical respiratory allergy: opportunities for hazard identification and characterisation. The Report and Recommendations of ECVAM Workshop 60, 35. ATLA, pp. 243–265.
Kimber, I., Basketter, D.A., Gerberick, G.F., Ryan, C.A., Dearman, R.J., 2011. Chemical allergy: translating biology into hazard characterization. Toxicol. Sci. 120 (Suppl. 1), S238–S268.
Kimber, I., Basketter, D.A., Thyssen, J.P., Dearman, R.J., McFadden, J.P., 2013. Chemical allergy in humans: fresh perspectives. J. Immunotoxicol. 11, 203–204.
Kimber, I., Dearman, R.J., Basketter, D.A., 2014a. Diisocyanates, occupational asthma and IgE antibody: implications for hazard identification. J. Appl. Toxicol. 34, 1071–1077.
Kimber, I., Dearman, R.J., Basketter, D.A., Boverhof, D.R., 2014b. Chemical respiratory allergy: reverse engineering an adverse outcome pathway. Toxicology Epub ahead of print.
Kimber, I., Griffiths, C.E.M., Basketter, D.A., McFadden, J.P., Dearman, R.J., 2014c. Epicutaneous exposure to proteins and skin immune function. Eur. J. Dermatol. 24, 10–14.
Kimber, I., 1996. Chemical respiratory allergy: exposure and dose-response relationships. In: Vos, J.G., Younes, M., Smith, E. (Eds.), Allergic Hypersensitivities Induced by Chemicals. Recommendations for Prevention. CRC Press, Boca Raton, pp. 139–147.
Kroes, R., Galli, C., Munro, I., Schilter, B., Tran, L., Walker, R., Würtzen, G., 2000. Threshold of toxicological concern for chemical substances present in the diet: a practical tool for assessing the need for toxicity testing. Food Chem. Toxicol. 38, 255–312.
Lack, G., 2012. Update on risk factors for food allergy. J. Allergy Clin. Immunol. 129, 1187–1197.
Lalko, J.F., Kimber, I., Dearman, R.J., Gerberick, G.F., Sarlo, K., Api, A.M., 2011. Chemical reactivity measurements: potential for characterization of respiratory chemical allergens. Toxicol. In Vitro 25, 433–445.
Lalko, J.F., Kimber, I., Gerberick, G.F., Foertsch, L.M., Api, A.M., Dearman, R.J., 2012. The direct peptide reactivity assay: selectivity of chemical respiratory allergens. Toxicol. Sci. 129, 421–431.
Lalko, J.F., Dearman, R.J., Gerberick, G.F., Troutman, J.A., Api, A.M., Kimber, I., 2013a. Recativity of chemical respiratory allergens in the peroxidase peptide reactivity assay. Toxicol. In Vitro 27, 651–661.
Lalko, J.F., Kimber, I., Dearman, R.J., Api, A.M., Gerberick, G.F., 2013b. The selective peptide reactivity of chemical respiratory allergens under competitive and non-competitive conditions. J. Immunotoxicol. 10, 292–301.

Linnett, P.J., Hughes, E.G., 1999. Twenty years of medical surveillance on exposure to allergenic and non-allergenic platinum compounds: the importance of chemical speciation. Occup. Environ. Med. 56, 191–196.
Liss, G.M., Buyantseva, L., Luce, C.E., Ribeiro, M., Manno, M., Tarlo, S.M., 2011. Work-related asthma in healthcare in Ontario. Am. J. Ind. Med. 54, 278–284.
Manetz, T.C., Pettit, D.A., Meade, B.J., 2001. The determination of draining lymph node cell cytokine mRNA levels in BALB/cmice following dermal sodium lauryl sulphate dinitrofluorobenzene, and toluene diisocyanate exposure. Toxicol. Appl. Pharmacol. 171, 174–183.
McSharry, C., Anderson, K., Bourke, S.J., Boyd, G., 2002. Takes your breath away –the immunology of allergic alveolitis. Clin. Exp. Immunol. 128, 3–9.
Merget, R., Kulzer, R., Dierkes-Globisch, A., Breitstadt, R., Gebler, A., Kniffka, A., Artelt, S., Koenig, H.-P., Alt, F., Vormberg, R., Baur, X., Schultze-Werninghaus, G., 2000. Exposure-effect relationship of platinum salt allergy in a catalyst production plant: conclusions from a 5-year prospective cohort study. J. Allergy Clin. Immunol. 105, 364–370.
Miller, R.L., Ho, S.M., 2008. Environmental epigenetics and asthma: current concepts and call for studies. Am. J. Crit. Care Med. 177, 567–573.
Musk, A.W., Peters, J.M.L., Di Berardinis, L., Murphy, R.L.H., 1982. Absence of respiratory effects in subjects exposed to low concentrations of TDI an MDI. J. Occup. Med. 24, 746–750.
NHLBI, 1991. Guidelines for the diagnosis and management of asthma. national heart, lung and blood institute. National asthma education program. Expert panel report. J. Allergy Clin. Immunol. 88, 425–534.
Nemery, B., Lenaerts, L., 1993. Exposure to methylene diphenyl diisocyanate in coal mines. Lancet 341, 318.
Newell, L., Polak, M.E., Perera, J., Owen, C., Boyd, P., Pickard, C., Howarth, P.H., Healy, E., Holloway, J.W., Friedmann, P.S., Arden-Jones, M.R., 2013. Sensitization via healthy skin programs Th2 responses in individuals with atopic dermatitis. J. Invest. Dermatol. 133, 2372–2380.
Newman Taylor, A.J., Cullinan, P., Lympany, P.A., Harris, J.M., Dowdeswell, R.J., Du Bois, R., 1999. Interaction of HLA phenotype and exposure intensity in sensitization to complex platinum salts. Am. J. Respir. Crit. Care Med. 160, 435–438.
Nielsen, J., Welinder, H., Jonnson, B., Axmon, A., Rylander, L., Skerfving, S., 2001. Exposure to hexahydrophthalic and methylhexahydrophthalic anhydrides—dose response for sensitization and airway effects. Scand. J. Work Environ. Health 27, 327–334.
Nielsen, G.D., Olsen, O., Larsen, S.T., Løvik, M., Poulsen, L.K., Glue, C., Brandorff, N.P., Nielsen, P.J., 2005. IgE-mediated sensitisation, rhinitis and asthma from occupational exposures. Smoking as a model for airborne adjuvants? Toxicology 216, 87–105.
Nielsen, G.D., Larsen, S.T., Hansen, J.S., Poulsen, L.K., 2012. Experiences from occupational exposure limits set on aerosols containing allergenic proteins. Ann. Occup. Hyg. 56, 888–900.
Ott, M.G., Klees, J.E., Poche, S.L., 2000. Respiratory health surveillance in a toluene diisocyanate production until 1967–1997—clinical observations and lung function analyses. Occup. Environ. Med. 57, 43–52.
Ouyang, B., Bernstein, D.I., Lummus, Z.L., Ying, J., Boulet, L.P., Cartier, A., Gautrin, D., Ho, S.M., 2013. Interferon-γ promoter is hypermethylated in blood DNA from workers with confirmed diisocyanate asthma. Toxicol. Sci. 133, 218–224.
Pauluhn, J., Eben, A., 1991. Validation of a non-invasive techniqueto assess immediate or delayed onset respiratory hypersensitivity in guinea pigs. J. Appl. Toxicol. 11, 423–431.
Pauluhn, J., Mohr, U., 2005. Experimental approaches to evaluate respiratory allergy in animal models. Exp. Toxicol. Pathol. 56, 203–234.
Pauluhn, J., Thiel, A., Emura, M., Mohr, U., 2000. Respiratory sensitization to diphenyl-methan-4,4′-diisocyanate (MDI) in guinea pigs: impact of particle size on induction and elicitation of response. Toxicol. Sci. 56, 105–113.
Pauluhn, J., Eidmann, P., Freyberger, A., Wasinska-Kempka, G., Vohr, H.W., 2002a. Respiratory hypersensitivity to trimellitic anhydride in Brown Norway rats: a comparison of endpoints. J. Appl. Toxicol. 22, 89–97.
Pauluhn, J., Eidmann, P., Mohr, U., 2002b. Respiratory hypersensitivity in guinea pigs sensitized to 1,6-hexamethylene diisocyanate (HDI): comparison of results obtained with the monomer and homopolymers of HDI. Toxicology 171, 147–160.
Pauluhn, J., 2003. Respiratory hypersensitivity to trimellitic anhydride in Brown Norway rats: analysis of dose-response following topical induction and time course following repeated inhalation challenge. Toxicology 194, 1–17.
Peters, G., Johnson, G.Q., Golembiewski, A., 2001. Safe use of detergent enzymes in the workplace. Appl. Occup. Environ. Hyg. 16, 389–396.
Pham, Q.T., Teculescu, D., Meyer-Bisch, C.J., Mur, M., 1988. Effects of chronic exposure to diisocyanates. Bull. Eur. Physiopath. Resp. 23, 56–60.
Pisaniello, D.L., Gun, R.T., Tkaczuk, M.N., Nitshcke, M., Crea, J., 1997. Glutaraldehyde exposures and symptoms among endoscopy nurses in South Australia. Appl. Occup. Environ. Hyg. 12, 171–177.
Plitnick, L.M., Loveless, S.E., Ladics, G.S., Holsapple, M.P., Selgrade, M.J., Sailstad, D. M., Smialowicz, R.J., 2002. Cytokine profiling for chemical sensitizers: application of the ribonuclease protection assay and effect of dose. Toxicol. Appl. Pharmacol. 185, 228–230.
Quint, J., Beckett, W.S., Campleman, S.L., Sutton, P., Prudhomme, J., Flattery, J., Harrison, R., Cowan, B., Kreutzer, R., 2008. Primary prevention of occupational asthma: identifying and controlling exposures to asthma-causing agents. Am. J. Ind. Med. 51, 477–491.
Rattray, N.J., Botham, P.A., Hext, P.M., Woodcock, D.R., Fielding, I., Dearman, R.J., Kimber, I., 1994. Induction of respiratory hypersensitivity to diphenylmethane-4,4′-diisocyanate (MDI) in guinea pigs. Influence of route of exposure. Toxicology 88, 15–30.
Rauf-Heimsoth, M., Merget, R., Rihs, H.P., Fohring, M., Liebers, V., Gellert, B., Schultze-Werninghaus, W., Baur, X., 2000. T-cell repertoire expression in workers with occupational asthma due to platinum salt. Eur. Respir. J. 16, 871–878.
Redlich, C.A., Herrick, C.A., 2008. Lung/skin connections in occupational lung disease. Curr. Opin. Allergy Clin. Immunol. 8, 115–119.
Redlich, C.A., 2010. Skin exposure and asthma: is there a connection. Proc. Am. Thorac. Soc. 7, 134–137.
Rosqvist, S., Nielsen, J., Welinder, H., Rylander, L., Lindh, C.H., Jonsson, B.A., 2003. Exposure-response relationships for hexahydrophthalic and methylhexahydrophthalic anhydrides with total plasma protein adducts as biomarkers. Scand. J. Work Environ. Health 29, 297–303.
Rovida, C., Martin, S.F., Vivier, M., Weltzien, H.U., Roggen, E., 2013. Advanced tests for skin and respiratory sensitization assessment. ALTEX 30, 231–252.
Saito, R., Abbas Virji, M., Henneberger, P.K., Humann, M.J., LeBouf, R.F., Stanton, M.L., Liang, X., Stefaniak, A.B., 2015. Characterization of cleaning and disinfecting tasks and product use among hospital occupations. Am. J. Ind. Med. 58, 101–111.
Sarlo, K., Clark, E.D., 1992. A tierapproach for evaluating the respiratory allergenicity of low molecular weight chemicals. Fundam. Appl. Toxicol. 18, 107–114.
Sarlo, K., 2003. Control of occupational asthma and allergy in the detergent industry. Ann. Allergy Asthma Immunol. 90, 32–34.
Schneider, K., Akkan, Z., 2004. Quantitative relationship between the local lymph node assay and human skin sensitisation assays. Regul. Toxicol. Pharmacol. 39, 245–255.
Seed, M., Agius, R., 2010. Further validation of computer-based prediction of chemical asthma hazard. Occup. Med. 60, 115–120.
Seed, M.J., Cullinan, P., Agius, R.M., 2008. Methods for the prediction of low-molecular-weight occupational respiratory sensitizers. Curr. Opin. Allergy Clin. Immunol. 8, 103–109.
Selgrade, M.K., Boykin, E.H., Haykal-Coates, N., Wollhiser, M.R., Wiesconski, C., Andrews, D.L., Farraj, A.K., Doerfler, D.L., Gavett, S.H., 2006. Inconsistencies between cytokine profiles antibody responses, and respiratory hyperresponsiveness following dermal exposure to diisocyanates. Toxicol. Sci. 94, 108–117.
Siddiqui, S., Morris, J., Avery, N., Wyand, S., Rood, D., Silbart, L.K., 2008. Pulmonary eosinophilia correlates with allergen deposition to the lower respiratory tract in a mouse model of asthma. Clin. Exp. Allergy 38, 1381–1390.
Steinfort, D.P., Pilmore, J., Brenton, S., Hart, D.H.L., 2008. Absence of platinum salt sensitivity in autocatalyst workers exposed to tetraamine platinum dichloride. Occup. Med. 58, 215–218.
Sutton, P.M., Quint, J., Prudhomme, J., Flattey, J., Materna, B., Harrison, R., 2007. Glutaraldehyde exposure among workers making bioprosthetic heart valves. J. Occup. Eviron. Hyg. 4, 311–320.
Tarlo, S., Malo, J.L., 2006. An ATS/ERS report: 100 key questions and needs in occupational asthma. Eur. Resp. J. 27, 607–614.
Tarlo, S.M., Liss, G.M., Dias, C., Banks, D.E., 1997. Assessment of the relationship between isocyanate exposure and occupational asthma. Am. J. Ind. Med. 32, 517–521.
Tarlo, S.M., Balmes, J., Balkissoon, R., Beach, J., Beckett, W., Bernstein, D., Blanc, P.D., Brooks, S.M., Cowl, C.T., Daroowalla, F., Harber, P., Lemiere, C., Liss, G.M., Pacheco, K.A., Redlich, C.A., Rowe, B., Heitzer, J., 2008. Diagnosis and management of work-related asthma: American College of Chest Physicians Consensus Statement. Chest 134 (Suppl. 3), 1S–41S.
Taylor, S.L., Baumert, J.L., Kruizinga, A.G., Remington, B.C., Crevel, R.W.R., Brooke-Taylor, S., Allen, K.J., Houben, G., 2014. Establishment of Reference Doses for residues of allergenic foods: report of the VITAL Expert Panel. Food Chem. Toxicol. 63, 9–17.
Teta, J.M., Avashia, B.H., Cawley, T.J., Yamin, A.T., 1995. Absence of sensitization and cancer increases among glutaraldehyde workers. Toxic Subst. Mech. 14, 293–305.
Toebak, M., Moed, H., von Blomberg, M.B., Bruynzeel, D.P., Gibbs, S., Scheper, R.J., Rustemeyer, T., 2006. Intrinsic characteristics of contact and respiratory allergens influence production of polarizing cytokines by dendritic cells. Contact Dermatitis 55, 238–245.
Van Och, F.M.M., van Loveren, H., de Jong, W.H., Vandebriel, R., 2002. Cytokine production inducedby low molecular weiht chemicals as a function of the stimulation index in a modified local lymph node assay: an approach to discriminate contact sensitizers from respiratory sensitizers. Toxicol. Appl. Pharmacol. 184, 46–56.
Vandebriel, R., CallantCransveld, C., Crommelin, D., Diamant, Z., Glazenburg, B., Joos, G., Kuper, F., Natsch, A., Nijkamp, F., Noteborn, H., Pieters, R., Roberts, D., Roggen, E., Rorije, E., Seed, M., Sewald, K., van den Heuvel, R., van Engelen, J., Verstraelen, S., van Loveren, H., 2011. Respiratory sensitization: advances in assessing the risk of respiratory inflammation and irritation. Toxicol. In Vitro 25, 1251–1258.
Vandenplas, O., Lantin, A.-C., Alpaos, V.D., Larbanois, A., Hoet, P., Vandeweerdt, M., Thimpont, J., Speybroeck, N., 2011. Time trends in occupational asthma in Belgium. Resp. Med. 105, 1364–1372.
Venables, K.M., Topping, M.D., Howe, W., Luczynska, C.M., Hawkins, R., Taylor, A.J., 1985. Interaction of smoking and atopy in producing specific IgE antibody against a hapten protein conjugate. Br. Med. J. 290, 201–204.
Vyas, A., Pickering, S.A.C., Oldham, L.A., Francis, H.C., Fletcher, A.M., Merrett, T., Niven, R.M., 2000. Survey of symptoms respiratory function, and immunology and their relation to glutaraldehyde and other occupational exposures among endoscopy nursing staff. Occup. Environ. Med. 57, 752–759.

Warbrick, E.V., Dearman, R.J., Kimber, I., 2002a. IgG and IgE anibody responses following exposure of Brown Norway rats to trimellitic anhydride: comparison of inhalation and topical exposure. Toxicology 172, 157–168.
Warbrick, E.V., Dearman, R.J., Kimber, I., 2002b. Induced changes in total serum IgE concentration in the Brown Norway rat: potential for identification of chemical respiratory allergens. J. Appl. Toxicol. 22, 1–11.
Water, A., Beach, J., Abramson, M., 2003. Symptoms and lung function in healthcare personnel exposed to glutaraldehyde. Am. J. Ind. Med. 43, 196–203.
Weill, H., Butcher, B., Dharmarajan, V., Glindmeyer, H., Jones, R., Carr, J., Neil, C.O., Salvaggio, J., 1981. Respiratory and Immunologic Evaluation of Isocyanate Exposure in a New Manufacturing Plant. U.S. Department of Health and Human Services. National Institute for Occupational Safety and Health (NIOSH) Publication No: 81–125.
Welinder, H., Nielsen, J., Rylander, L., Stahlbom, B., 2001. A prospective study of the relationship between exposure and specific antibodies in workers exposed to organic acid anhydrides. Allergy 56, 506–511.
Wiszniewska, M., Walusiak-Skorupa, J., 2014. Occupational allergy: respiratory hazard in healthcare workers. Curr. Opin. Allergy Clin. Immunol. 14, 113–118.
Yokota, K., Johyama, Y., Yamaguchi, K., Takeshita, T., Morimoto, K., 1999. Exposure-response relationships in rhinitis and conjunctivitis caused by methyltetrahydrophthalic anhydride. Int. Arch. Occup. Environ. Health 72, 14–18.
Yucesoy, B., Johnson, V.J., Lummus, Z.L., Kissling, G.E., Fluharty, K., Gautrin, D., Malo, J.L., Cartier, A., Boulet, L.P., Sastre, J., Quirce, S., Tarlo, S.M., Cruz, M.J., Munoz, X., Luster, M.I., Bernstein, D.I., 2012. Genetic variants in antioxidant genes are associated with diisocyanate-induced asthma. Toxicol. Sci. 129, 166–173.
Yucesoy, B., Johnson, V.J., Lummus, Z.L., Kashon, M.L., Rao, M., Bannerman-Thomson, H., Frye, B., Wang, W., Gautrin, D., Cartier, A., Boulet, L.P., Sastre, J., Quirce, S., Tarlo, S.M., Germolec, D.R., Luster, M.I., Bernstein, D.I., 2014. Genetic variants in the major histocompatibility complex class I and class II genes are associated with diisocyanate-induced asthma. J. Occup. Environ. Med. 56, 382–387.
Zhang, X.D., Lotvall, J., Arakawa, H., Welinder, H., Skerfving, S., 1998. Relationship between IgG1 levels and airway responses in guinea pigs actively and passively sensitized to hexahydrophthalic anhydride. Allergy 53, 20–27.