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Sensitizing potency of chemical respiratory allergens in humans

Can animal data be used to estimate human potency?

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J. van Amsterdam | H. van Loveren | J. Ezendam

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Colophon

J. van Amsterdam
H. van Loveren
J. Ezendam

Contact:
J. Ezendam
RIVM
Janine.Ezendam@rivm.nl

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Summary

Respiratory allergies, such as asthma and rhinitis, induced by chemicals are an important occupational problem. There are currently no predictive animal models that are validated for the identification of these respiratory allergens. Classification and labelling is based on human evidence. According to the Globally Harmonized System (GHS) respiratory sensitizers should be categorized in strong and other sensitizers. It is however unclear if human data can be used to predict the potency of these compounds. To improve the classification and labelling of these compounds, it would be of great value if existing animal data could be used for hazard identification and potency estimation.

A literature survey was conducted to investigate if it is possible to derive potency estimates from published animal studies. If so, a subsequent literature study was performed to find human potency data of the same compounds and assess if these correlate to the potency derived from animal studies. The literature survey focused on respiratory allergies induced by diisocyanates, since these are the most common cause of occupational asthma.

The most important criterion to include animal studies was the availability of dose-response data from diisocyanates. These data were available in the Local Lymph Node Assay (LLNA), the Mouse Ear Swelling Test (MEST) and the respiratory LLNA. From these studies toluene diisocyanate (TDI) and hexamethylene diisocyanate (HDI) were selected since these compounds displayed different potencies in the respiratory LLNA and the MEST; TDI being more potent than HDI. Unfortunately, the literature survey for human studies yielded no appropriate studies that could be used for potency estimates. Important drawbacks were that in most studies the exposure to the compound was not given or that in studies were exposure was assessed no clinically relevant tests were performed.

Although there were no human studies available that could be used to estimate potency of HDI or TDI, the literature survey confirmed that diisocyantes are a leading cause of occupational asthma, despite many preventive measures, including reduction of exposure levels. Additionally, skin exposure and peak exposures appear to be very important contributing factors in the development of occupational asthma.

In conclusion, with the current knowledge it is not possible to derive human potency for chemical respiratory allergens from animal data. At the moment the only way to decide that a chemical is a respiratory allergen are observations in humans. Monitoring adverse respiratory effects in humans is therefore still advocated. Additionally, predictive animal models are still of great importance since these would be of value in hazard identification of newly emerging respiratory allergens.

1   Background

Occupational respiratory allergies are common occupational diseases, with an estimated prevalence of 9-15% of all asthma cases in adults. These respiratory allergies comprise allergic rhinitis and asthma and can cause severe morbidity. In most cases the symptoms worsen with continuing exposure. The only possibility to improve the symptoms is avoidance of exposure (Malo & Chan-Yeung, 2001; Gezondheidsraad, 2008; Dykewicz, 2009).

Around 250 substances have been identified as known causes of occupational asthma. These include high-molecular-weight allergens (greater than 1000 daltons), mostly from biological origin, for example proteins and enzymes. In addition, low-molecular-weight chemicals (less than 1000 dalton) can cause the disease as well and almost 100 chemicals were identified (Van Kampen et al., 2000) (Table 1). Of these low-molecular-weight chemicals, diisocyanates are the most common cause of occupational asthma, followed by acid anhydrides (Bernstein, 2003; Di Stefano et al., 2004; Gezondheidsraad, 2008). The scope of this letter report is on occupational respiratory allergies induced by low-molecular-weight chemicals. Table 1 provides an overview of known respiratory allergens together with occupations in which exposure commonly occurs.

Table 1 Overview of chemical respiratory allergens

Chemical class	Examples	Common occupations
Diisocyanates	Toluene diisocynate Hexamethylene diisocyanate Diphenylmethane diisocyanate	Spray painters, polyurethane industry, manufacturers of plastics, rubber
Acid anhydrides	Phthalic anhydride Trimellitic anhydride	Manufacturers of plastics, epoxy resin
Amines	2-ethanoldiamine Piperazine	Cleaners, photography
Metal and their compounds	Chromium (VI) salts Cobalt sulphate Nickel sulphate Hexachloroplatinate	Electroplaters, welders, hard metal industry
Medicines	Hydralazine Penicillamine	Pharmaceutical industry, pharmacists
Reactive dyes (hair dyes, textile dyes)	Basic blue 99 p-phenylene diamine Azo dyes	Textile workers, hair dressers
Persulphate salts	Ammonium persulphate Sodium persulphate Potassium persulphate	Hair dressers
Other	Glutaraldehyde Colophony Chloramine-T	Health care workers Electronic workers Cleaners

According to the globally harmonized system (GHS) for classification and labeling respiratory allergens are classified with the R42 risk phrase: 'May cause sensitization by inhalation'. Classification and labeling of respiratory allergens is currently done based on human evidence, since there is no predictive animal model that be used for hazard identification. According to GHS, respiratory allergens should be categorized in strong and other sensitizers. Strong respiratory sensitizers, labelled with subcategory 1A, are substances that show a

high frequency of occurrence in humans and/or severity of reaction within an exposed population. Less potent substances are categorized in subcategory 1B, and are substances that show a low to moderate frequency of occurrence in humans and/or severity of reaction within an exposed population.

It is unclear if data from humans can be used to categorize respiratory allergens in the correct potency class, mainly based on absence of clear information on exposure and dose-response relationships. The lack of predictive animal models has hampered classification and labelling for these compounds. Predictive animal models would be valuable tools to identify newly emerging respiratory allergens before they give rise to occupational asthma. Furthermore, in animal models dose-response relationships can readily be assessed, which can be used to quantify the response and assess the potency. Effects of respiratory allergens have been assessed in several different animal models. These models use different routes of sensitization and challenge, including inhalation, dermal and intranasal exposures. Up to now, further development of these tests towards predictive test methods have been hindered by practical difficulties, lack of reproducibility and the need for specific equipment and expertise necessary for inhalation exposures (Arts et al, 2007).

In previous studies we have shown that both respiratory and skin allergens could be detected in a newly developed respiratory LLNA (Arts et al., 2008, De Jong et al., 2009). The potency ranking of the tested allergens was different in this assay compared to the dermal LLNA. It was speculated that the route of exposure is an important factor that drives the potency. However, due to the lack of data on human potency of the tested respiratory allergens it was impossible to conclude which test method was the most accurate.
In this letter report the predictability of the potency from these animal tests for human potency was further investigated. Additional dose-response information from other published animal studies will be included in this evaluation as well. Subsequently, it will be investigated if human studies are available from which human potency can be derived to establish if the animal data correlate to human potency.

2   Approach

To establish that the animal models have good validity and predictability for the human potency of respiratory allergens, a set of allergens must show the same or a similar dose-response relationship both in human and animal studies. A first step in this approach was to select compounds, which have been tested in a dose-response manner in the same experimental animal model. Potency can then be estimated by analyzing the dose-response curves and assessing if respiratory allergens induce different magnitudes of effects. Finally, it should be established whether these results correspond to those in humans.

The prevalence of the specific occupational allergy has been chosen as a selection criterion. Diisocynates have been selected as model compounds, as they, and most notably TDI, present the most common cause of occupational asthma in developing countries, with a prevalence of 3-13% in exposed workers (Park et al., 1992).

✓   Scientific literature has been searched to obtain dose-response data from animal models in which diisocyanates were tested

✓   The information from animal models will be used to select compounds for the literature search on human data. Diisocyanates for which potency estimates were available were selected and scientific literature has been searched to obtain the required human data and to assess whether the difference in potency observed in animal testing is also found in humans exposed to the diisocyanates.

2.1   Relevant animal test models

The local lymph node assay (LLNA) is used to test the potential and potency of low molecular weight (LMW) compounds to induce sensitization via the skin (Kimber & Weisenberger, 1989; Gerberick et al., 2007). The assay relies on lymphocyte proliferation in the draining lymph nodes which is a hallmark of the skin sensitization response during the induction phase. This test allows dose-response assessment, which is used to estimate the potency of a chemical (Van Loveren, 2008). It has been shown that potencies assessed in the LLNA correlate relatively well with thresholds determined in humans (Gerberick et al., 2001; Griem et al., 2003; Schneider & Akkan, 2004; Basketter et al., 2005). The potency in the LLNA is expressed as the EC3 value, which is the interpolated concentration that induces a three-fold increase in proliferation (van Och et al., 2000). Besides skin allergens, respiratory allergens have been tested in the LLNA and are positive in this assay.

In the respiratory LLNA, a modification of the LLNA, mice are exposed by inhalation to the substances on three consecutive days. After a two day rest period, the mice are sacrificed and proliferation in the mandibular lymph nodes is measured. This test allows dose-response assessment and the ED3 as a measure for potency is the dose at which a 3-fold increase in proliferation was obtained. In the respiratory LLNA both respiratory and skin allergens were tested. It was shown that the potency of the different substances differed between the (skin) LLNA and the respiratory LLNA, indicating that the route of exposure has an impact on the potency of chemicals (Arts et al, 2008).

The mouse ear swelling test (MEST) is a method to assess the skin sensitizing potential of chemicals as well. Mice are sensitized by topical application on the abdomen an four days later they receive a challenge on the ears. Ear swelling is measured as a read-out for a delayed-hypersensitivity response. Dose-response measurements focus on the sensitization phase and use a fixed challenge dose. This approach offers the possibility to calculate the dose at which 50% of the mice is sensitized, the SD50 dose (Gad, 1994). In this animal model diisocyanates have been tested as well.

The main adverse respiratory health effects associated with repeated or long-term exposure to occupational allergens are bronchial asthma and the rapid decline in lung function. The various clinically relevant measures to assess occupational allergy are diagnostic tests for hypersensitivity (skin prick and RAST), serum IgG or IgE levels, bronchial hyperresponsiveness and a variety of novel biomarkers. The abovementioned animal models do not assess any of these clinical parameters. These clinically relevant parameters have been assessed in other animal models, predominantly in guinea pigs and mice after inhalation exposure. Only in a limited number of these studies dose-response relationships were assessed after inhalation exposure. In these studies NESILs (no-expected-sensitization-induction-level) for sensitization or elicitation were reported for different compounds (Karol, 1983; Stadler & Karol, 1984; Pauluhn et al., 2000; Pauluhn et al., 2002; Arts et al., 2004; Pauluhn, 2008; Pauluhn & Poole, 2011). However, the experimental design differed considerably between these studies, including differences in species, and the exposure route and number of exposures for sensitization or challenge. Since in none of these studies more than one compound was tested, it was not possible to compare the NOAELs from these studies, because the method chosen for induction of sensitization and/or elicitation has a significant influence on the threshold dose. Therefore, these studies were not included in this report.

2.2 Selection of relevant human studies

In the literature survey for human studies the following aspect (cf. paragraphs 2.2.1 to 2.2.3) have to be considered in the selection of human studies relevant for the research question.

2.2.1 Criteria of studies

To answer the research questions posed, human studies have to be retrieved from literature, which compare the prevalence of occupational asthma of the two selected diisocynates in either the same human study (same cohort) or in different human studies (different cohorts), but with a similar design. For proper comparison it is further required that the exposure levels (dose and time) of the test compounds are the same or comparable. When data are found in different studies, the sample size, population characteristics, and read out parameter and their assay must be the same or at least be comparable.

2.2.2 Genetic and other risk factors

The influence of genetic factors or other lifestyle or environmental factors on the development of occupational respiratory allergy is not well understood either. Therefore, it is unknown if these factors might influence dose and effect relationships.

2.2.3   *Exposure*

Exposure to the respiratory allergen is the most important event in the development of occupational respiratory allergy. However, several other factors might have an effect as well on the development. Important determinants at the exposure site, are exposure conditions, exposure pattern (peak versus chronic), exposure route (inhalation versus skin), and exposure to other substances. However, the current scientific knowledge on the impact of these exposure factors is insufficient to understand which factors will have the largest impact (Gezondheidsraad, 2008).

In the evaluation of the human studies, quantitative information on exposure levels is necessary. Airborne exposure is practically and technically difficult to quantify. Furthermore, the internal dose of the allergens may deviate substantially from that measured using external monitors, especially in subjects using personal protective equipment (Anonymous, 2006; Hogberg et al., 2005), which may give remarkable and unexpected bias of exposure results. Gloves and protective clothing are presumed to protect against isocyanate skin exposure. In addition, the usage of the allergic compound in the workplace varies in time, adding further time variability between exposure and the biomarkers of exposure (Pronk et al., 2006).

In addition to air measurements, biological monitoring for diisocyanates has been available for many years and is based on the analysis of isocyanate derived diamines released by hydrolysis of protein adducts in urine or plasma. For instance, in occupational studies of exposure to TDI during manufacture of polyurethane foams (Kaaria et al., 2001; Maitre et al., 1993) show a good correlation between airborne TDI and toluene diamine (TDA) in urine. Other biomarkers of exposure reflecting exposure to diisocyanates have been proposed, but not yet validated.

Abovementioned issues are taken into account in the evaluation of the human studies.

2.3   **Clinical parameters**

Clinically relevant outcome measures in humans are airway complaints, like shortness of breath, chest tightness, and wheezing. In the diagnosis of occupational asthma the methacholine challenge test is a very suitable test to diagnose airway hyperreactivity (shortness of breath) to the allergen. However, this method is invasive and requires specific skills and equipment. In human studies, assays based on immunological markers for sensitization, such as allergen-specific IgE and IgG are commonly used as diagnostic tests. These methods are easier to implement in human studies and are less invasive.

IgE and IgG have been used in diagnosing workers with isocyanate asthma (Cartier et al., 1989; Ye et al., 2006a; Wisnewski, 2007). However, with respect to diisocyanates, there is doubt about the validity of IgE to identify isocyanate sensitization in humans, since in only 14-20% of the cases IgE antibodies to diisocyanates have been detected (Baur, 1983; Baur 1994; Keskinen et al., 1988; Wisnewski et al., 2006; Mapp et al., 2005). The short half-life of unbound serum IgE of approximately 2 days is particularly important to occupational disease, in which exposure to the causative agent occurs exclusively in the workplace (Infuhr et al., 2005; Patterson et al., 1975). Brief periods (weeks) away from the workplace (i.e. exposure) may result in a decrease in serum IgE levels, to levels undetectable by conventional RAST tests(Tee et al., 1998a). If undetectable, the outcome of the test would provide a false-negative finding and

bias the outcomes. Allergen-specific IgG probably also is a promising marker of occupational allergy, primarily of exposure, in particular when assessed long time (several weeks) after exposure (Wisnewski et al., 2006; Welinder et al., 1988b; Liss et al., 2006). IgG directed at isocyanate-albumin conjugates is almost never detected in the blood of unexposed individuals (Aul et al., 1999; Bernstein et al., 2002; Bernstein et al., 2006; Wisnewski et al., 2004a; Ye et al., 2006b). Among exposed workers, the serum isocyanate-specific IgG titer correlates closely with occupational exposure levels (Wisnewski et al., 2004b).

In the evaluation of the available human literature these issues will be considered. Immunological markers alone are not sufficient for occupational asthma and should be further substantiated with clinical relevant outcomes.

3 Results

3.1 Animal data

It appeared that only very few animal studies are available, which have tested a set of allergens in a dose-response manner in the LLNA, respiratory LLNA or MEST test. The results are summarized in Table 2. In this Table potency is derived from dose-response curves by setting cut-off limits. The potency in these assays is therefore the amount of chemical needed to reach this cut-off. Strong potent respiratory allergens will reach this cut-off at lower doses than weak potent respiratory allergens. For the LLNA and respiratory LLNA a 3-fold induction of proliferation was used as a cut-off and EC3 and ED3 values are shown, respectively. For the MEST assay, the dose that sensitized 50% of the mice was determined, the SD50 value.

Based on these animal data two diisocynates were selected: toluene diisocynate (TDI) and hexamethylene diisocyanate (HDI) to be further evaluated in humans. The selection of the two compounds is based on the largest difference in potency as shown in relevant animal models. Depending on the test used, the potency ratio (TDI/HDI) 1.55 (respiratory LLNA) to 8.1 (MEST).

Table 2 Potency of diisocyanates determined in different animal models

Compound		LLNA EC$_3$	Respiratory LLNA [c] ED$_3$	MEST [d] SD$_{50}$ (in mg/kg bwt)
Toluene diisocynate	TDI	0.11 [a]	28	0.73
Hexamethylene diisocyanate	HDI	-	18	0.09
Diphenylmethane diisocyanate	MDI	0.3 [b]	-	5.3
Dicyclohexylmethane diisocyanate	HMDI	-	-	0.24
Isophorone diisocyanate	IPDI	-	44	-

[a]Van Och et al., 2000; [b]Corsini et al., 2009; [c]Arts et al., 2008a and [d]Thorne et al., 1987

3.2 Evaluation of the available human data

No appropriate human studies, matching the requirements outlined in chapter 2 have been published. No studies have been described where a cohort is exposed to at least two occupational allergens, belonging to the same chemical class. Most studies to occupational allergy or asthma refer to populations exposed to one unique allergen i.e. not two allergens or use a marker (read out value) which is not reliable or validated. An important drawback of most studies is that the exposure to the allergen has not been given. Unfortunately, most of the studies, where exposure has been assessed, do not report clinically relevant measures. Visa versa, in many studies where clinically relevant tests have been performed (in relatively small cohorts), no exposure data are given. Without accurate exposure information, negative alllergen-specific IgE assays may lead to misdiagnosis and false conclusions about pathogenic mechanisms.

3.3 Human data on diisocyanate-induced respiratory allergy

The available literature confirms that isocyanates (di- and poly-) are a leading cause of occupational asthma. For example, vehicle paint sprayers using isocyanate-based paints were reported to be 80 times more likely to get occupational asthma than other workers (HSE, 2009). Respiratory exposures have been reduced through improved protection measures and the use of less-volatile isocyanates. Yet isocyanate asthma continues to occur, frequently in settings with minimal inhalation exposure but opportunity for skin exposure. Based on clinical and epidemiologic studies, there is agreement that isocyanate skin exposure occurs in the workplace and can increase the risk for sensitization and isocyanate asthma. Reducing exposure reduces the risk but even at this level, a risk of respiratory sensitization may remain (ACGIH, 2010). Indeed, allergic contact urticaria and asthma following direct hand contact with MDI glue has been documented based on a positive MDI-IgE, MDI patch test, and MDI inhalation challenge (Valks et al., 2003). This is consistent with case reports about the development of MDI asthma in settings where skin exposure to MDI occurred and where MDI air levels, if measured, were non-detectable or extremely low. Fewer cases of isocyanate asthma have generally been reported in settings with lower respiratory exposures, but cases continue to occur in settings with consistently low reported air levels (Baur, 2003; Tarlo et al., 1997a). Skin exposure (Bernstein et al., 1993b; Lenaerts-Langanke, 1992) or intermittent peak exposures, which could also entail both respiratory and skin exposure (Tarlo et al., 1997b), have been considered important contributing factors in such cases. Typically, such exposures are unpredictable and frequently accidental, making them difficult to investigate and quantify.

Although there are no human studies available to estimate human potency, they do provide some insight on the frequency of occurrence of diisocyanate-induced asthma.

- Petsonk et al. (2000) investigated a group of new workers exposed to MDI in a facility with minimal airborne exposure. They noted new asthma-like respiratory symptoms in 27% of the workers with the highest potential for MDI exposure, which were associated with MDI skin exposure.
- Bernstein et al. (1993a) studied a cohort of 243 workers in a urethane molding plant with low MDI airborne exposures (< 5 ppb). Isocyanate asthma and/or sensitization (MDI-IgE) was diagnosed in several workers, most of whom were reported likely to have had MDI skin exposure.
- The group of Wisnewski (Redlich et al., 2001; Woskie et al., 2004) has characterized workplace isocyanate exposures in more than 200 auto body shop workers with isocyanate-specific immune responses but without documented isocyanate asthma. Individual respiratory and skin exposures were estimated, based on exposure algorithms. HDI-specific IgG, present in 21% of the workers, was strongly associated with inhalation exposure, but skin exposure also contributed (Stowe et al., 2006).
- The same group has also detected MDI-specific IgG in > 30% of about 100 workers in a factory that uses MDI, but with consistently very low air values, but documented MDI skin exposure (Liss et al., 2006). In both work settings, isocyanate skin exposure appeared to contribute to the development of isocyanate-specific IgG, which has been associated with isocyanate exposure (Welinder et al., 1988a; Wisnewski et al., 2004c; Ye et al., 2006c).
- Darcey et al. (Darcey et al., 2002) investigated 38 community residents who complained of symptoms they attributed to exposure to air emissions from nearby manufacturing facilities. Common complaints included headache,

mucosal irritation, and shortness of breath, chest tightness, and wheezing. Airway hyperreactivity was observed in 22% of individuals tested. Six individuals (18.2%) had antibodies to at least 1 of the 3 common industrial diisocyanates.

4 Summary and conclusion

In summary, the available human studies had several limitations:
- Potency of more than one respiratory allergens has not been measured in one or multiple human studies, which makes it impossible to compare compounds
- Diisocyanate allergy is presumably only partly mediated by the IgE pathway giving doubt to the validity of IgE titer as a marker of occupational asthma/allergy
- Exposure levels have not been measured in studies where the clinical outcome was assessed of exposure to two or more occupational allergens
- Exposure may vary in time and in the different workers of the cohort
- Exposure depends on the protection measures taken and compliance of the workers

These limitations make it difficult to adequately estimate the potency of different diisocyanates in humans and to evaluate the capacity of the available dose-response data from animal models to predict potency of respiratory allergens.

At the moment the best indicators, to decide whether a chemical compound is potentially hazardous, are observations in humans. However, despite the non-validated predictability of the available animal models, the data obtained from those models may sustain observations about intrinsic allergenicity of these compounds in humans. In the future, increased knowledge on the risk factors for occupational asthma, such as impact of the skin as a route of exposure and other exposure parameters, might lead to development of predictive animal models in which dose-response measurements are possible. For a correct interpretation of outcomes of these animal models good human data are required. Therefore it is still advocated to monitor adverse effects to potential occupational allergens in humans (incidence, severity of clinical symptoms, exposure levels).

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