Regulatory Toxicology and Pharmacology 89 (2017) 268–278

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Commentary

Azodicarbonamide (ADCA): A reconsideration of classification as a respiratory sensitiser

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ARTICLE INFO

Article history:
Received 18 April 2017
Received in revised form
30 June 2017
Accepted 19 July 2017
Available online 20 July 2017

Keywords:
Occupational asthma
Skin sensitisation
Respiratory allergy

ABSTRACT

Azodicarbonamide (ADCA) is widely used by industry in the manufacture of a variety of products. ADCA has been classified as a respiratory allergen, and the purpose of this article was to consider whether this classification is appropriate based upon the available data. Here both clinical experience and relevant experimental data have been reviewed. Although there have been reports of an association between workplace exposure to ADCA and symptoms of respiratory allergy and occupational asthma, the evidence is less than persuasive, with in many instances a lack of properly controlled and executed diagnostic procedures. In addition, ADCA fails to elicit positive responses in mouse and guinea pig predictive tests for skin sensitisation; a lack of activity that is regarded as being inconsistent with respect to respiratory sensitising potential. Collectively, the data reviewed here do not provide an adequate basis for the classification of ADCA as a respiratory allergen.

1. Introduction

Azodicarbonamide [diazene-1,2-dicarboxamide] (ADCA) (CAS number: 123-77-3) is a fine yellow powder, and is used widely in industry as a blowing and expanding agent in the manufacture of a wide variety of products.

ADCA was initially employed in the 1940s as a foaming agent of rubber articles. Use of ADCA during the 1970s grew in parallel with increased manufacture of plastics and rubber goods, and probably accounted (alone, or in combination with other agents) for approximately 80% of foamed rubber and thermoplastic goods within Europe. As ADCA decomposes during processing the chemical is either undetectable, or deemed low and encapsulated, in finished articles.

ADCA as a base substance is no longer manufactured in the European Union with supplies now imported primarily from Asia; the volume of such imports being estimated to be approximately 20,000 tonnes per annum.

Supply of ADCA is limited exclusively to industrial use, and this is well-established at downstream sites. Many such sites have handled ADCA for years/decades, with the larger volume industries using several thousand tonnes each year. As will be discussed later the volume and long duration of use of ADCA is relevant when considering the question on which this article is focused.

ADCA is currently classified as being a respiratory sensitiser, and the aim here is to explore whether that classification is appropriate. With that objective in mind it is important to consider briefly how such classification is defined.

Under the terms of the Globally Harmonized System (GHS) of Classification, ADCA is currently classified as a respiratory sensitiser (H334), the Hazard Statement being: '*may cause allergy or asthma symptoms or breathing difficulties if inhaled*' (GHS, 2011). It is important in this context to note that the guidance provided by the European Chemicals Agency (ECHA) for the implementation of REACH (ECHA, 2016a,b), states that a respiratory sensitiser is defined '*as an agent that will lead to hypersensitivity of the airways following inhalation exposure of that agent.*' The Guidance also makes the point that: '*Respiratory sensitisation (or hypersensitivity) is a term that is used to describe asthma and other related respiratory conditions (rhinitis, extrinsic allergic alveolitis), irrespective of the mechanism (immunological or non-immunological) by which they are caused.*'

The difficulty that this definition causes is that it serves to embrace both true respiratory allergens which, by definition, induce effects via immunological mechanisms, and non-allergenic asthmagens in which case adverse effects are caused by non-immunological mechanisms (Kimber et al., 2001). WHO (1999)

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http://dx.doi.org/10.1016/j.yrtph.2017.07.018
0273-2300/

considered ADCA an asthmagen.

True chemical respiratory allergy is characterised by immunological priming that results in allergic sensitisation of the respiratory tract. If the sensitised subject is exposed subsequently by inhalation to the same chemical then an accelerated and more aggressive secondary immune response will be provoked that elicits a respiratory reaction recognised clinically as respiratory allergy, and in a workplace setting described as occupational asthma. Reactions may become more severe with repeated exposure as the level of sensitisation increases (Kimber et al., 2011).

The purpose of this article is therefore to review briefly the scope and toxicology of chemical respiratory allergy, and to consider, with respect to ADCA, the approaches available for the identification of chemicals that have the ability to cause sensitisation of the respiratory tract.

2. Chemical respiratory allergy

Chemical respiratory allergy resulting in rhinitis and asthma is an important occupational health issue. It is associated with high levels of morbidity, and has significant financial costs (Kimber and Wilks, 1995; McDonald et al., 2005; Bakerly et al., 2008; Ayres et al., 2011; Kenyon et al., 2012; Feary et al., 2016). In contrast to skin sensitisation and allergic contact dermatitis, where many hundreds of chemicals have been confirmed as causative agents, there are less than 100, and probably no more than 80, chemicals that have been implicated as having the potential to cause allergic sensitisation of the respiratory tract (Kimber et al., 2014a). Among the more common and better characterised classes of chemical respiratory allergens are the diisocyanates, chlorplatinate salts, acid anhydrides, and some reactive dyes (Baur, 2013; Baur and Bakehe, 2014).

Chemical respiratory allergy is associated with a variety of occupational health and toxicological challenges, and these have been examined elsewhere in a number of review articles (Holsapple et al., 2006; Kimber et al., 2007, 2014a; 2014b; Boverhof et al., 2008; Isola et al., 2008; Basketter and Kimber, 2011; Dotson et al., 2015; Cochrane et al., 2015; North et al., 2016).

One issue has been the routes of exposure through which sensitisation of the respiratory tract to chemicals can be acquired. It has commonly been assumed that inhalation exposure is necessary for sensitisation. However, there is evidence from both experimental studies and clinical observations that effective sensitisation of the respiratory tract can result from skin contact with chemical respiratory allergens. That is, in susceptible individuals, topical exposure to a chemical respiratory allergen can result in the class of immune response required for respiratory sensitisation (Rattray et al., 1994; Arts et al., 1998; Kimber and Dearman, 2002; Kimber et al., 2014a). As will be discussed in a later section, the relevance of skin exposure for respiratory sensitisation to chemicals is an important consideration when interpreting data from sensitisation studies in experimental animals.

Perhaps the most significant challenge has been the absence of methods for the identification and characterisation of chemical respiratory allergens. The fact is that despite research that has spanned more than 40 years there are still no validated methods for assessment of the respiratory sensitising potential of chemicals. Indeed, there are not even approaches that are widely recognised within the scientific community, or by regulatory agencies. In large part this is due to continuing uncertainty regarding the immunological mechanisms through which sensitisation is acquired, and in particular about the role of IgE antibody (Kimber et al., 1998, 2014a; 2014b; Kimber and Dearman, 2002).

Nevertheless, some progress has been made, and during the last 3 decades a variety of *in vivo, in vitro* and *in silico* strategies have been explored (Botham et al., 1988; Griffiths-Johnson and Karol, 1991; Sarlo and Clark, 1992; Satoh et al., 1995; Hilton et al., 1996; Dearman and Kimber, 2001; Arts et al., 2008; Pauluhn, 2008; Lalko et al., 2011; Enoch et al., 2012). However, none of these initiatives has yet resulted in a standard approach that can be used with confidence for the identification of respiratory sensitising chemicals.

Although there is no agreement about a mandatory role for IgE antibody in the development of respiratory sensitisation to chemicals, there is a growing consensus about the cellular requirements. Thus, there is now reason to believe that chemical respiratory allergy is closely associated with the elicitation of selective T helper (Th)-2 type immune responses. While much of the evidence for this is based on experimental studies in which chemical respiratory allergens have been found to induce preferential Th2-type T cell responses (Dearman et al., 1995, 1996; 2005; Van Och et al., 2002; Kimber et al., 2011), there are data indicating that a similar Th2 selectivity is found in humans also (Ouyang et al., 2013; Newell et al., 2013; Kimber et al., 2014c). The importance of T lymphocyte responses for the acquisition of respiratory sensitisation to chemical allergens will be returned to later in this article.

It is against this background of the absence of a validated and universally recognised test for the identification of chemical respiratory allergens that the classification of ADCA as a respiratory sensitiser must be considered. There are two main sources of information that can inform decisions about the presence or absence of respiratory sensitising potential: (a) experimental studies in animals and (b) clinical observations and investigations. We will turn first to evidence available from experimental studies.

3. ADCA and respiratory sensitisation: experimental animal studies

In the absence of methods for the identification of chemical respiratory allergens it has been necessary to adopt alternative approaches. One strategy has been to examine whether the behaviour of chemical respiratory allergens in test methods for the evaluation of skin sensitisation potential is informative. For this purpose attention has focussed largely on the local lymph node assay (LLNA) (Kimber and Weisenberger, 1989; Kimber and Basketter, 1992; Kimber et al., 1994, 2002; Basketter et al., 2002), but data from predictive guinea pig tests (primarily the guinea pig maximisation test [GPMT] and the occluded patch test of Buehler) are relevant also (Buehler, 1965; Magnusson and Kligman, 1969).

The LLNA is a fully validated method for the identification of low molecular weight skin sensitising chemicals. The method is predicated on the fact that skin sensitising chemicals will induce lymphocyte (primarily T cell) activation and proliferation in regional lymph nodes draining the site of skin exposure (Kimber et al., 2011). In practice the test is conducted using CBA strain mice. Animals are exposed daily, for 3 consecutive days, to various concentrations of the test chemical, or to the same volume of the relevant vehicle alone. Five days following the initiation of exposure mice are injected with a source of radiolabelled thymidine. Animals are sacrificed 5 hours following injection and the level of lymphocyte proliferation by draining (auricular) lymph node cells is measured as a function of thymidine incorporation using β scintillation counting. Chemicals that at one or more test concentrations elicit a 3-fold or greater increase in proliferation (thymidine incorporation) compared with vehicle controls are classified as skin sensitisers (Kimber et al., 2002).

Many hundreds of chemicals have now been tested in the LLNA (for example, Kern et al., 2010) and during the course of those investigations an important observation has been made about the activity of chemical respiratory allergens in the assay. It has been

found that, in addition to skin sensitisers, all known chemical respiratory allergens that have been tested elicit positive responses in the LLNA (Dearman et al., 2013).

There is also extensive experience with the GPMT and the occluded patch test of Buehler. The principle of these guinea pig tests is the same. In both cases groups of animals are exposed to the test material, and then at some time thereafter they are challenged with a non-irritant concentration of the same chemical. Sensitisation is measured as a function of challenge-induced erythematous reactions in test animals compared with controls (animals that are challenged but without prior induction). The difference between the two assays is that induction in the GPMT is performed using both topical and intradermal exposure (the latter with Freund's Complete Adjuvant to 'maximise' the response), whereas the Buehler assay employs 3 consecutive weekly topical exposures for induction (Buehler, 1965; Magnusson and Kligman, 1969). With only a single exception (piperazine), chemical respiratory allergens also test positive in these guinea pig test methods (Dearman et al., 2013).

Before considering the use of this information as part of the characterisation of respiratory sensitising potential, it is worth exploring the mechanistic basis for this relationship. Although it might appear unexpected that chemical respiratory allergens elicit positive LLNA responses, the data are consistent with what is known of the development of sensitisation of the respiratory tract to chemicals. Thus, as summarised above, respiratory allergens will induce T cell responses and these are required for the acquisition of sensitisation. Furthermore, while the LLNA is based on the evaluation of immune responses induced following skin contact, it is now established that this is a relevant route of exposure for sensitisation of the respiratory tract to chemicals. The conclusion drawn is that there is good reason for chemical respiratory allergens to induce T cell activation and proliferation in regional lymph nodes following skin exposure, and to elicit positive responses in the LLNA.

It must be appreciated, however, that although both contact allergens and respiratory allergens test positive in the LLNA, it is known that they induce different qualities of T cell response. As indicated above, chemical respiratory allergens elicit selective Th2-type responses, whereas contact allergens are associated with preferential Th1 responses (Dearman et al., 1995, 1996; 2005; Van Och et al., 2002; Kimber et al., 2011). However, such differential qualities of T cell response are not registered in the LLNA which simply measures the proliferation of all lymph node cells.

It is assumed that respiratory allergens also elicit positive responses in guinea pig assays for the same reason; that they provoke T cell responses that drive skin reactions following challenge of sensitised animals.

Given the mechanistically legitimate activity of chemical respiratory allergens in the LLNA (in mice and rats), and in guinea pig assays, it has become apparent that this provides a useful tool for examining the respiratory sensitising potential of chemicals. The value is that chemicals that fail to elicit positive responses in the LLNA can be regarded as lacking not only skin sensitising activity, but also the potential to induce sensitisation of the respiratory tract. That is, chemicals that test negative in the LLNA can be eliminated with confidence from further consideration as possible chemical respiratory allergens. In the absence of standard tests for the identification of respiratory sensitising chemicals the above paradigm has proven very useful (Dearman et al., 2013).

In terms of considering whether or not ADCA has respiratory sensitising properties it is appropriate therefore to examine the behaviour of this chemical in skin sensitisation predictive tests, and there are available both LLNA and guinea pig test data.

In a well-conducted standard LLNA, ADCA (purity $\geq$ 99%) was tested at 3 concentrations (10%, 25% and 50%) using propylene glycol as a vehicle. The results were uniformly negative (WIL Research Europe BV, 2014a).

In a GPMT animals (10 test animals and 5 controls) were treated at the induction stage with 10% ADCA (purity $\geq$ 99%) by intradermal injection, and with 50% of the chemical by topical application. Subsequently all animals were challenged topically with 50% of the test chemical. The vehicle was 1% aqueous carboxymethyl cellulose. No skin reactions were observed, and the test was uniformly negative (WIL Research Europe BV, 2014b).

Somewhat earlier a Buehler study had been conducted using 10 test and 10 control guinea pigs. Both induction (weekly topical application for 3 consecutive weeks) and challenge were performed using 50% ADCA in aqueous solution. Again, no skin reactions were observed and the test was uniformly negative (Huntingdon Research Centre, 1988).

Taken together these results indicate that in 3 standard animal tests for skin sensitisation, carried out under the conditions of Good Laboratory Practice, ADCA was entirely negative. On that basis, and according to the documented relationship between chemical respiratory allergens and activity in mouse and guinea pig skin sensitisation test methods, the conclusion reached would be that ADCA lacks the potential to cause sensitisation of the respiratory tract.

However, before confirming that conclusion, it is relevant to ensure the legitimacy of the tests conducted, and the interpretation of the results obtained.

In the standard LLNA summarised above the highest concentration tested was 50%, and the vehicle used (propylene glycol) would be expected to promote skin penetration. Nevertheless, it could conceivably be argued that the result obtained was a false negative due to inadequate transit of the test material across the stratum corneum to gain access to the viable epidermis, possibly as the result of low solubility. However, there is no reason to suppose that this was in fact the case, and precedent indicates that suspensions will elicit positive responses in the LLNA. Moreover, it has recently been reported that there is a '*widespread misconception that the ability to efficiently penetrate the stratum corneum is a key determinant of sensitisation potential and potency*' (Fitzpatrick et al., 2017a; b). With regard to the possibility of poor access of the test chemical across the skin it is also important to consider the results obtained in the GPMT. As summarised above, this assay employs both topical administration and intradermal injection as the induction regimen, with the latter route of exposure effectively bypassing the barrier of the stratum corneum. Collectively, therefore, these data provide reassurance that the negative results obtained with the LLNA (and with the guinea pig tests) are robust and that ADCA does not have the potential to cause sensitisation of the respiratory tract.

Consistent with that conclusion appears to be the fact that ADCA does not have the ability, under physiological conditions, to form sufficiently stable associations with proteins. The reaction of the primary adduct on the N=N double bond with an additional thiol group ultimately leads to oxidation of the SH groups to form disulfides, and reduction of ADCA to the bis-urea. Thus, in biological systems in particular, a rapid oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG) takes place. The reaction mode is also the basis of the use of ADCA as a flour additive for oxidation of the free SH groups in gluten (Tsen, 1963). In *in vivo* studies on the antiviral effect of ADCA, apparently no protein conjugation was observed or detected (Huang et al., 1998; Maynard et al., 1998). The absence or limitation to form stable associations with proteins is relevant in the context of both skin and respiratory sensitisation as low molecular weight chemicals (those less than 500 Da) are not of sufficient size to engage effectively with the

immune system.

In order to provoke an immune response chemicals must be inherently electrophilic, or must be transformed *in vivo* to an electrophilic species (Kimber et al., 2011). In the absence of stable association with protein, chemicals will fail to trigger an immune response and will not cause allergic sensitisation. The inability of ADCA to form sufficiently stable associations with proteins under physiological conditions is consistent with this chemical lacking the potential to cause sensitisation of the respiratory tract.

Finally, although no validated methods are available for the direct assessment of the respiratory sensitising potential of chemicals, there has been one report in the literature of an animal (guinea pig) study aimed at investigating the respiratory sensitising potential of ADCA. Animals were exposed for 6 hours per day, for 5 days per week, for 4 weeks to aerosols of ADCA (99.4% pure; biurea content less than 1%) at concentrations of 51 or 200 mg/m^3. Controls were exposed to filtered air. One week before the exposures started, and 3 days after the last exposure, animals were exposed to ADCA at the same concentrations (or to 200 mg/m^3 in the case of controls) for 10 min (to monitor for specific sensitisation), or with histamine (to measure non-specific airway reactivity). There was no evidence that inhalation exposure to ADCA resulted in either specific sensitisation or non-specific airway reactivity, and the authors concluded that ADCA is not a pulmonary sensitiser. An intradermal skin test was also performed on 50% of the animals one week after the inhalation challenge, and there was no evidence of skin sensitisation either (Gerlach et al., 1989).

In conclusion, therefore, all of the experimental animal data available indicate that ADCA lacks the potential to cause allergic sensitisation of the respiratory tract or respiratory allergy.

4. Clinical observations and investigations

4.1. Workplace studies

In a study by Ferris et al. (1977; Table 1), 11 workers in a grinding company were investigated as several had complained about productive cough, nocturnal cough, shortness of breath, fatigue and leg cramps when grinding ADCA. This company grinded various materials including a herbicide, nylon terpolymer, sulfanilamide, aluminum oxide, amorphous silica and fiber glass. After a 4-day non-working period, in 3–4 workers spirometry was conducted on the first Monday, then on Friday and the following Monday. ADCA concentrations measured were 2.0, 0.7 and 2.1 mg/m^3 on Friday and 1.9 mg/m^3 on Monday. Count median particle diameters were 3.9 μm before and 2.2 μm after grinding with a geometric standard deviation (GSD) of 2. In general the workers showed a decrease in FEV_1 (Forced Expiratory Volume in one second) and FVC (Forced Vital Capacity), the decrease in FEV_1 fell slightly more than FVC, and the decrease on the first Monday was greater than on the following 2 days (FEV_1: −21, −8, and −10%, respectively; FVC: −17, −5 and −6%, respectively). The authors concluded that this preliminary study called attention to the possible pulmonary reactivity of inhaled finely ground ADCA and thus engineering controls are indicated. In addition, they concluded that the results suggested an acute respiratory effect and not a chronic respiratory disease.

In a prevalence study reported by Slovak (1981) undertaken in an ADCA production factory in the UK, 151 workers who had been, or were then currently, engaged in manufacture, servicing and quality control were investigated. They had been diagnosed as having asthma based on a history of repeated episodes of wheezing or chest tightness (± cough) related to exposure to ADCA. In total 28 cases (18.5%) were identified. Workers were placed in 3 groups: (a) diagnosed as having had asthma due to ADCA, (b) all asymptomatic process workers in daily exposure who had been on the plant for more than one year, and (c) a control process worker population without any contact with the product, or with any known respiratory sensitisers. ADCA dust was measured and was between 2 and 5 mg/m^3. Of the 28 cases, more than 50% had developed asthmatic symptoms within 3 months, and 75% had developed it within the first year; 6 (22%) workers had immediate asthmatic reactions, 16 (56%) had late onset asthma, and 6 (22%) had a dual reaction. Additional symptoms reported were cough (39%), rhinitis (29%), conjunctivitis (25%) and rash (4%). Pre- and post-shift spirometry did not reveal any differences among the groups. Identical levels (48%) of atopy were found in groups (a) and (b). Skin prick tests were carried out with common allergens and with ADCA (0.1, 1 and 5% in DMSO); these were negative in groups (a) and (b).

In a study by Ahrenholz et al. (1985; Table 1) workers in a factory for the production of pastes and plastisols, using ADCA as raw material amongst other substances, were asked about their symptoms after some workers experienced nose bleeds, irritation of mucous membranes and skin reactions. The high incidence of reported symptoms (eye and nasal irritation, nocturnal coughing, shortness of breath, wheezing and chest tightness) prompted further investigation. In addition, 7 exposure measurements were made that yielded detectable levels only in 2 cases, with ADCA levels of 2.1 and 3.1 mg/m^3. In the main study, six weeks later, levels reported were 0.15–12 mg/m^3 (n = 3 samples, median 3.8 mg/m^3) in one building, and 0.6–4.8 mg/m^3 (n = 5 samples, median 1.6 mg/m^3) in another building. Indirect exposures (apart from the workplace with ADCA handling) ranged from non-detectable to 0.03 mg/m^3 (n = 11 samples) and from non-detectable to 0.1 mg/m^3 (n = 14 samples). Worker exposures were divided into three categories: direct (i.e. short-term while handling the compound); indirect (full-shift, working in the vicinity of where the compound was used); and unexposed (full-shift with no expected exposures). Arithmetic means (and ranges) for the three groups were 3.6 mg/m^3 (0.59–12 mg/m^3), 0.01 mg/m^3 (from none detected to 0.04 mg/m^3), and 0.001 mg/m^3 (from none detected to trace), respectively. The findings in a group of 30 workers (previously) exposed to ADCA, most of whom were symptomatic (n = 18), were compared with a group of 16 employees without prior exposure. Of the 18 workers reporting asthmatic symptoms, the cause of the symptoms was believed (on the basis of their temporal characteristics) by the investigators to be non-occupational (n = 3), occupational but not ADCA-related (n = 7), or occupational and ADCA-related (n = 8). One of the employees without prior exposure also reported lower respiratory tract symptoms. Ten subjects (56%) of the 18 symptomatic workers and 3 subjects (25%) of 12 without symptoms had a prior history of allergies. The workers wore disposable dust respirators but the degree of protection was questionable since some of the workers had beards. In addition, respirators were also observed lying around the area when they were not in use. The average pre-/post-shift pulmonary function measurements performed on the ADCA-exposed workers were not significantly different from those of non-exposed workers. Although there was some exposure dependence, no individual measurements were reported and the average decrease of FEV_1 and FVC was very low, approximately 2%–3%. The incidence of symptoms reported at this time also did not differ between the two groups. No specific IgE antibody to ADCA-human serum albumin conjugate was detected, and increases in specific IgG were only found at relatively high concentrations (at a dilution of 1:10), whereas values ≥ 3 times the control value of at least a 1:50 dilution were considered medically significant. The authors concluded that the potential for ADCA to produce sensitisation, and whether this is mediated through an immune mechanism, was not clear.

In another study (Ahrenholz and Anderson, 1985; Table 1)

Table 1
ADCA worker studies.

	Ferris et al. (1977)	Slovak (1981)	Ahrenholz et al. (1985)	Ahrenholz and Anderson (1985)	Whitehead et al. (1987)
Company	Grinding	ADCA production	Production of pastes and plastisols using ADCA	Manufacture of plastic parts	Additional information to Ahrenholz and Anderson (1985)
Other chemicals	Herbicide, nylon terpolymer, sulfanilamide, aluminium oxide, amorphous silica, fiber glass				Urazol, biurea, cyamelide, cyanuric acid; the latter may form into cyanic acid
ADCA concentrations	0.7–2.1 mg/m^3	2-5 mg/m^3	0.15–12 mg/m^3 (one building), 0.6–4.8 mg/m^3 (other building)	0-40 μg/m^3	
Particle size	2.2–3.9 μm (CMD)				
Number of workers investigated	11	151	3 groups: (1) direct exposure (n = 30; mean: 3.6 mg/m^3, range 0.59–12 mg/m^3), (2) indirect exposure (mean: 0.01 mg/m^3, range 0–0.04 mg/m^3) (3) unexposed (n = 16; 0 –0.001 mg/m^3)	227/242 employees; divided as (1) never worked in injection molding (n = 57), (2) formerly worked (n = 60), (3) currently working (n = 110). Groups 2 and 3 were divided in (a) significant ADCA exposure (n = 136), and (b) no significant ADCA exposure (n = 34)	
Complaints	Productive cough, nocturnal cough, shortness of breath, fatigue, leg cramps	Repeated episodes of wheezing or chest tightness (± cough)	Nose bleeds, eye and nasal irritation, nocturnal cough, shortness of breath, wheezing, chest tightness and skin reactions	Respiratory problems and occasional skin irritation	
Number of asthma cases/ cases with symptoms	11	28/151 (18.5%)	18/30 (60%)	Respiratory symptoms were reported more often in group (a) when compared to group (b)	
Measurement results (spirometry)	Decrease in FEV1 (8 –21%) and FVC (5 –17%)	No differences in pre- and post-spirometry between the asthma cases and 2 control groups (asymptomatic workers and workers without contact to ADCA)	No significant differences in pre- and post-spirometry between the ADCA exposed and non-exposed workers (decrease in FEV1 and FVC of 2–3%)	Only small decreases in FEV1 (30–141 mL) and FVC (8–120 mL)	
Skin prick test with ADCA		Negative (0.1, 1.0 and 5.0% in DMSO)			
Other		Identical levels of atopy (48%) in symptomatic and asymptomatic workers	No specific IgE to ADCA-HSA conjugate detected; increases in specific IgG only found at low dilution (1:10 not at the usual dilution of 1:50)	No specific IgE to ADCA-HSA conjugate detected; increases in specific IgG only found at low dilution (1:10 not at the usual dilution of 1:50)	Percentage of asthma before working at this plant was 4–8% and was not different between groups 1-3

results were reported for workers in the manufacture of plastic parts for the automobile, computer and other industries who complained of frequent respiratory problems and occasional skin irritation, believed to be a result of exposure to ADCA. Respiratory health variables were studied cross-sectionally in 227 out of 242 employees in the plastics molding facility using ADCA. The group of 227 employees was divided into (a) never worked in injection molding (n = 57), (b) formerly worked in injection molding (n = 60), or (c) currently working in injection molding (n = 110). The highest average values for FEV_1 and FVC were identified in the 60 employees who had previously worked in injection molding (4.16 and 3.37 L, respectively), while the 110 currently employed (4.13 and 3.35 L, respectively) and the 57 workers who were never employed (4.08 and 3.25 L, respectively) showed slightly lower values. A strong association was observed for injection molding workers for eye/nose/throat irritation, cough, and wheezing. The workers were then divided in 2 other groups: those with a significant exposure to ADCA (n = 136), and those with no significant exposure to ADCA (n = 34; this group had been employed before 1 January 1980 which was before the introduction of ADCA, or were employed during the 4–5 months prior to the study when use declined markedly). Respiratory symptoms were reported more often in the 136 exposed workers than in the 34 non-exposed or low level exposed workers. Wheezing, chest tightness, and symptoms of chronic bronchitis were strongly associated with work in injection molding during periods in which ADCA was in use. However, no respiratory symptoms occurred when exposed to the low concentrations in the workplace measured at the time of the investigations. Individual lung function measurements were not documented. Only 17 of the workers exposed during injection molding reported “average” pulmonary function values (pre-/post-shift). Of these 17 workers, seven reported coughing, three reported wheezing, and two reported chest tightness. In pulmonary function tests (at ADCA concentrations determined at the workplace of 0–20, 20–40 and ≥40 μg/m^3, respectively), only small decreases were found in the average values of FEV_1 (−141, −35 and −30 ml,

respectively), and FVC (−120, −8 and −50 mL, respectively). Detailed information on individual measurements, which could show corresponding effects, was absent. However, it was stated that the pulmonary function measurements on these 17 subjects showed no consistent trend indicative of a concentration -response relationship. In addition, 32 personal exposure measurements were made. Thus, in those exposed while working in injection molding, values between 1 and 368 μg/m^3 (average of 36.1 μg/m^3) were found. The highest concentration of 752 μg/m^3 was found at a mixing station where ADCA was mixed with plastic resins and binders. Ten months earlier, air concentrations up to ca. 280 μg/m^3 and personal exposures up to 1800 μg/m^3 were measured. The authors also discussed the possibility of a healthy worker effect. In addition, the concentrations at the time of the second examination were very low since the use of ADCA had been reduced dramatically ca. 4–5 months earlier. Finally, no specific IgE antibody for an ADCA-human serum albumin conjugate could be detected. However, details of how this was established were not given. Specific IgG was only found at a dilution of 1:10, but not with the usual dilution of 1:50. In addition, the differences between the exposed and non-exposed workers were only slight. Even when compared with the laboratory control, there was no clear increase in specific IgG in any of the groups. Almost the same data were reported by Whitehead et al. (1987). Additional information consisted of: the percentage of those with asthma before working at this plant was between 4 and 8% and was not different between the 3 groups. Solid by-products formed included urazol, biurea, cyamelide, and cyanuric acid. The latter may form into cyanic acid. Several of these would be expected to be potentially irritant. These results suggested that the respiratory symptoms observed could have been caused by ADCA itself, reaction products of ADCA during injection molding, or other unidentified agents from this process.

4.2. Incidence data from national registers

In the UK, from 1989 to 1991, a total of 1528 cases of occupational asthma were reported, including 17 cases in which ADCA was cited as the cause; in the subsequent periods 1992–1994 and 1995–1997, the number of reported ADCA cases dropped to 11 out of a total of 2,857, and 2 out of 3002 cases, respectively (McDonald et al., 2000; Table 2). Physicians reported the agent suspected as having been responsible for their patients' asthma. One more asthma case was reported in 2008 (ECHA, 2012). By contrast, out of a total of 1670 occupational asthma cases recorded over the period from 2003 to 2014, no ADCA-associated cases were found (HSE, 2015).

In Finland, ADCA was not mentioned as being among the cause of 2602 recorded occupational asthma cases in the period 1989–1995 (Karjalainen et al., 2000), whereas a later publication by the same group listed 3 ADCA cases in that period (1989–1995); overall, 9 ADCA cases were identified out of a total of 5591 cases for the period 1986–2002 (Piipari and Keskinen, 2005). When chemical induced occupational asthma was suspected, PEF surveillance was used at work and on days off.

Kwon et al. (2015) reported 2 ADCA cases out of 236 occupational asthma cases in Korea in the period 2004–2009. Individual patients were written up as case reports and sent to the KOWAS (Korea Work-Related Asthma Surveillance) program headquarters. Two subtypes of work-related asthma were included: work-exacerbated asthma and new-onset occupational asthma. New-onset occupational asthma was further divided into sensitiser-induced occupational asthma and irritant-induced occupational asthma, including reactive airways dysfunction syndrome (RADS). It is not clear into which subtypes the 2 ADCA cases had been categorized.

It has also been reported that at the Dutch National Center for Occupational Diseases, one ADCA occupational asthma case was recorded in the period 2000–2012; this was an analyst of a pharmaceutical company (no further information; ECHA, 2012).

Several other national lists have been made available (see Table 2); none of these mentioned ADCA as cause of occupational asthma.

4.3. Case reports – respiratory symptoms

In a study by Malo et al. (1985) 2 workers showed a late asthmatic reaction after provocation with ADCA, preceded by an immediate bronchoconstriction in one of the subjects. The subject showing the late reaction also displayed an increase in non-allergic bronchial hyperreactivity; this was not seen in the other subject. The subject showing the late reaction, was atopic and reacted in skin prick tests to ragweed pollen, house dust and house dust mite. The other subject was non-atopic. No skin prick tests were performed with ADCA (due to insolubility). The subjects were aware of the nature of the provocations as ADCA is a bright yellow powder (see Table 3).

Another worker showed a continuous decrease of FEV_1 following provocation with ADCA with a maximum decrease 5 hours after challenge. This subject did not react in skin prick tests to 50 common inhalant allergens. He also tested negative to ADCA in a skin prick test using DMSO as solvent, but a patch test with ADCA in petrolatum showed a positive reaction at 1.0% w/w, but not at 0.1% w/w, 48 and 96 h after application (Kim et al., 2004).

Normand et al. (1989) studied 4 workers in the plastics industry. The first subject underwent a provocation test (no details provided); he did not show an immediate reaction, but reported having an asthma attack 14 h later. The second subject developed a steadily decreasing FEV_1 value reaching a maximum decrease 3–4 h after provocation exposure. The two other subjects did not receive a provocation test; asthmatic reactions were reported when working with ADCA during one fortnight a year. The authors indicated that the possibility that substances other than ADCA could have caused asthmatic reactions could not be excluded, but that the inhalation challenge with ADCA in 2 workers and the association with the use of ADCA in the other 2 subjects pointed to ADCA, or possibly a contaminant, as the causative agent.

Valentino and Comai (1985) reported an ADCA worker complaining about rhinitis during the shift, and cough and dyspnea during the evening and night. A 60-min provocation test with 100 g ADCA on a moving glass plate at 37 °C did not result in changes in FVC or FEV_1 whereas workplace exposure resulted in a continuous decrease of FEV_1 with a maximum decrease of up to 50% 4 h after work exposure.

Due to technical problems, a 48-year old worker in the production of porophore using ADCA was exposed to unusually high concentrations of the chemical. Three weeks later he developed rhinitis and cough. His lung function was, however, normal and the acetylcholine test was negative. This subject was given Terfenadin (H1 anti-histamine) and he quickly recovered; the authors suggested a 'hyperreactivity cough' (Alt and Diller, 1988).

4.4. Case reports – skin effects

Only a few cases of allergic contact dermatitis possibly due to ADCA have been reported. Nava et al. (1983) described a 59-yr old baker, suffering from bakers' allergy, who had worked for 36 years and had eczema on arms and face during the last 20 years. He tested positive to ADCA in a patch test; however, no information was provided on test substance, concentration, and reaction outcome.

Table 2
Incidences of occupational asthma, including ADCA, as recorded in national registers.

Country	Year	Number of occupational asthma cases	Number of ADCA cases	Reference
UK (SWORD)	1989–1991	1528	17	McDonald et al. (2000)
UK (SWORD)	1992–1994	2857	11	McDonald et al. (2000)
UK (SWORD)	1995–1997	3002	2	McDonald et al. (2000)
UK	2006–2015	915	0	HSE (2015)
Finland (FROD)	1989–1995	2602	ADCA not listed	Karjalainen et al. (2000)
Finland (FROD)	1986–2002	5591	9	Piipari and Keskinen (2005)
Korea (KOWAS)	2004–2009	236	2	Kwon et al. (2015)
Dutch National Center for Occupational Diseases	2000–2012	?	1	ECHA (2012)
Germany	2003	835	ADCA not listed	Latza and Baur (2005)
UK West-Midlands (SHIELD)	1990–1997	948	ADCA not listed	Di Stefano et al. (2004)
France (ONAP)	1996–1999	2178	ADCA not listed	Ameille et al. (2003)
Australia (SABRE)	1997–2001	170	ADCA not listed	Elder et al. (2004)
Australia -New South Wales (SABRE)	2001–2008	89	ADCA not listed	Hannaford-Turner et al. (2010)
US (California, Massachusetts, Michigan and New Jersey- SENSOR)	1993–1995	768	ADCA not listed	Jajosky et al. (1999)
US California	1993–1996	444	ADCA not listed	Reinisch et al. (2001)
Canada (Quebec)	1986–1988	214	ADCA not listed	Lagier et al. (1990)
Singapore	1983–1999	90	ADCA not listed	Kor et al. (2001)

A 33-yr old man working in a plastic transforming factory in the UK had eczema of the body, but not on face or arms (Bonsall, 1984). This subject had suffered from occasional eczema of the forearms and legs for the previous 10 years; he did not have any respiratory problems. Following an accidental over-exposure to ADCA he developed dermatitis over various parts of the body, but not on the face, hands or wrists. He was patch tested to ADCA at 0.1, 1.0 and 10.0% w/w in petrolatum. A positive reaction was present at 48 and 72 h, but only at the 1.0% concentration. Ten other people handling ADCA in this factory also complained about skin problems; however, 5 of them available for skin testing, each tested negative.

A 58-yr old textile worker using earplugs developed bilateral otitis externa. A positive reaction to his earplug was seen at 48 and 96 h; 3 months later he was patch tested with 0.1, 1 and 5% ADCA in petrolatum. A positive reaction was seen at 48 and 96 h, with 1.0 and 5.0%. The manufacturer had received only one other notification of an allergic reaction in over a decade of selling millions of earplugs (Yates and Dixon, 1988).

In the study by Kim et al. (2004) a subject tested negative to ADCA in a skin prick test using DMSO as solvent, but a patch test with ADCA in petrolatum showed a positive reaction (erythematous papules with edema) at 1.0% w/w (but not at 0.1% w/w) 48 and 96 h after application.

In the study of Normand et al. (1989), 3 out of 4 subjects working in the plastics industry also showed eczematous dermatitis, two of them when formerly working in a bakery.

One case of development of eczema due to ADCA was reported in an operator in a rubber foam producing factory in the Netherlands; no further information was available (National Center for Occupational Diseases, 2000–2012; ECHA, 2012).

5. Discussion

The appearance of respiratory symptoms in workers in ADCA manufacturing or processing factories may possibly point at occupational asthma. However, this alone is not sufficient for the diagnosis of allergic asthma. As stated previously, the term occupational asthma embraces (a) sensitiser-induced asthma (allergic asthma), (b) irritant-induced asthma (non-allergic, including Reactive Airways Dysfunction Syndrome (RADS)), or (c) work-exacerbated asthma (pre-existing asthma unrelated to work that is made worse by a workplace exposure) (Vandenplas, 2011; Tarlo and Lemiere, 2014). In order to confirm a definite diagnosis of allergic occupational asthma the following criteria must be met: (1) diagnosis of asthma by a physician, (2) an association between the symptoms of asthma and work, (3) workplace exposure to an agent or process previously associated with work-related asthma, and at least two of the following criteria: (4) significant work-related changes in spirometry, (5) significant work-related changes in non-specific airway hyperresponsiveness, and (6) a positive response to an inhalation provocation with the specific agent to which the individual is exposed at work (Klees et al., 1990).

In the available worker studies summarised above, there were reported associations between symptoms of asthma and work. However, pre- and post-shift spirometry did not reveal any significant differences between ADCA-exposed and non-exposed workers (Slovak, 1981; Ahrenholz et al., 1985; Ahrenholz and Anderson, 1985), whereas in the study by Ferris et al. (1977) the decrease in FEV_1 and FVC on the first Monday was greater than on the following 2 exposure days. Non-specific airway hyperresponsiveness and inhalation provocation tests were not conducted.

Additional immunological measurements are of considerable value in confirming a diagnosis of allergic asthma, as even with the 6 criteria mentioned it is difficult to discriminate between occupational asthma of types (a), (b) and (c). In one of the studies described above, skin prick tests with ADCA in workers with and without symptoms were negative (Slovak, 1981). In two other studies, no specific IgE could be detected, and only low level specific IgG antibody was found (Ahrenholz et al., 1985; Ahrenholz and Anderson, 1985). In addition, the differences between exposed and non-exposed workers were only slight. Even when compared with the laboratory control, there was no clear increase in specific IgG antibody in any of the groups (Ahrenholz and Anderson, 1985). The interpretation is that ADCA was not able to provoke a strong IgG antibody response, and was found not to be associated with IgE antibody production. However, a more detailed description of the methodology, in particular on the preparation of the ADCA-human serum albumin conjugate, is missing.

In some instance there may be an element of circular reasoning involved. That is, if one (e.g. the physician and/or the worker) is convinced that a certain chemical is a sensitiser, it could be difficult not to conclude that the chemical is the cause of the occupational asthma, in the absence of any additional investigations.

Table 3
Case reports of ADCA.

	Malo et al. (1985)	Malo et al. (1985)	Kim et al., 2004	Normand et al. (1989)	Normand et al. (1989)	Normand et al. (1989)	Valentino and Comai (1985)
Number of cases	1	1	1	1	1	2	1
Age	35	56	56	50	45	50/54	41
Industry (job)	Plastics	Plastics	ADCA production (quality control)	Plastics (pigment grinder)	Plastics (pigment grinder)	Plastics	Plastics (injection molding)
Co-exposure	?	?	?	>30 substances	>30 substances	?	Polyurethanes
Exposure	3-4 years; 1–2 weeks during 3–4 times/year	4-5 years irregular exposure	10 years	Ca. 1 year?	2 months	2 weeks/year	1 year
Symptoms	Irritation of eyes, cough, dyspnea and wheezing	Shortness of breath, wheezing, coughing, nocturnal asthmatic symptoms and rhinitis	Cough, dyspnea, and wheezing	Shortness of breath	Shortness of breath	Attacks of asthma/ asthmatic bronchitis	Rhinitis, cough and dyspnea
Latency	Few months	Few months	7 years	Ca. 9 months?	No?	10 years?	?
Provocation method	Move powder from 1 tray to another/exposure chamber	Move powder from 1 tray to another/ exposure chamber	Move powder from 1 tray to another/ exposure chamber	No details	No details		(a) Moving glass plate (100 g; 37 °C) (b) workplace provocation
Substance used for provocation	Lactose-ADCA 1:1	Lactose-ADCA 1:1	Lactose-ADCA 1:1 (30 g)	No details	No details		As present in workplace
Analytical check on provocation substance	No	No	No	No	No		No
Provocation duration	15 sec	15 sec	10 min	40 min	40 min		(a) 60 min (b) during work shift
Reaction type after provocation	Late (3 h after exposure, max at 6 h)	Dual (ca. 0–30 min and 3–6 h)	Continuous reaction with max after 5 h	Late (14 h after exposure)	Continuous reaction with max after 3–4 h		(a) No reaction (b) Continuous reaction with max 4 h after end of shift
Max fall of FEV_1	24%	26 and 23%	22%	<7%	22%		(a) – (b) 50%
Non-specific bronchial hyperreactivity after provocation	Yes (PC20H* dropped to 0.28 mg/ml after 5 days)	No (PC20H* of 1.2 mg/ml)	Yes (PC20M* of 0.47 mg/ml)				
Histamine/methacholine challenge (PC20H/M) prior to provocation	2 mg/ml	0.5 mg/ml	0.85 mg/ml (prior to medication) 4.69 mg/ml				
Neg control used	Lactose*	Lactose*	Lactose				
Skin prick test to common inhalation allergens	Pos (ragweed pollen, house dust, house dust mite)	Negative	Negative				
Skin prick test with ADCA	ND (due to solubility issue)	ND (due to solubility issue)	Negative (0.1, 1.0 and 5.0% w/v in DMSO)				
Patch test with ADCA			Positive at 48 and 96 h (1.0% w/w in petrolatum) (0.1% was negative)				
Other	Atopic; previous history of angioedema after eating seafood; infantile eczema	No personal or familial atopic history	No medical history (apart from respiratory symptoms); smoker for 20 years (age: 30–50)	Former smoker and a chronic alcoholic; previously breathlessness and eczematous dermatitis of the face	Non-smoker; eczema of hands and forearms when working previously as a baker	Ex-smoker; eczema of hands and forearms (1st subject)/(2nd subject had chronic eczema and attacks of asthma; former job in bakery)	Non-smoker, 500 ml alcohol/day; no family history of asthma

ND = Not Determined; *As ADCA is a bright yellow powder, the subject was well aware of the nature of the powder he handled; PC20H/PC20M = Provocation concentration resulting in a fall of 20% of $FEV_1$1 (H = histamine, M = methacholine).

It is also relevant to consider that many work-related asthma cases may not be associated with exposure to allergens. For instance when reporting new asthma cases in California, of the classifiable cases of work-related asthma, 65% consisted of new-onset work-related asthma cases. However, of these new-onset cases, only 13.4% were associated with exposure to a previously documented allergen. In contrast, 77.2% were associated with exposure to agents that were not known to be allergens, and 9.3% was based on an irritant mechanism. Moreover, approximately one third (35%) of the classifiable cases were identified as work-aggravated asthma (Reinisch et al., 2001). Individuals with predisposing risk factors for asthma, atopy and a family history of asthma are likely to first develop asthma due to non-work exposures, but then experience work-related exacerbation of pre-existing asthma. Information on atopy or predisposing factors was only (partly) available in the case reports; it was generally absent in the worker studies and for the cases included in the national registers.

Only 3 cases with respiratory reactions associated with ADCA have been fully documented (Malo et al., 1985; Kim et al., 2004). The 3 subjects received a provocation with the negative control lactose or the test substance (1:1 lactose-ADCA). In addition, non-specific bronchial hyperreactivity following provocation was measured using either histamine or methacholine. However, the airway reactions of these 3 subjects were quite different, with one subject showing a late reaction, the second subject showing a dual reaction, and the third subject a continuous decrease in FEV_1 with a maximum after 5 h. The maximum fall in FEV_1 was not large, viz. 22–26%.

The other subjects that have been investigated (Normand et al., 1989; Valentino and Comai, 1985; Ferris et al., 1977) received either a provocation test with ADCA (without adequate details reported), or respiratory function was measured during and after workplace exposure. Reactions in these subjects varied from no reaction, to a continuous decrease in FEV_1 with a maximum 3–4 h after provocation, or a decrease in FEV_1 during the day, or a late reaction (14 h after provocation). Most reactions were 'progressive', that is, starting immediately after provocation, and reaching a maximum after several hours. Although such a reaction can be seen following exposure of sensitised subjects to diisocyanates, it has also been observed following exposure to some irritant substances such as chlorine containing cleaning products, and to hypochlorite solutions (Burge et al., 2012).

With regard to the 3 well documented cases for which ADCA was considered to be the cause, one of the three subjects was atopic, showing positive skin prick results to common respiratory allergens. Another subject did not show a change in non-specific bronchial hyperreactivity following ADCA provocation, and therefore there was no formal proof to conclude that ADCA could induce non-specific hyperreactivity in already sensitised subjects. Moreover, the duration of the provocation in the case reports varied to a significant extent: varying between 15 sec and 60 min, and in one study workers were followed during 3 shifts. In none of the provocations was the source and purity of the ADCA samples checked, and therefore it is not known whether an impurity or impurities may have played a role. Also, during workplace exposure(s) co-exposure to other substances cannot usually be excluded. Thus, considering these case reports collectively, it is not possible to conclude with certainty that ADCA has the potential to induce immunologically mediated respiratory allergy in humans.

With regard to allergic contact dermatitis, there is very little evidence that ADCA is a skin sensitiser. This is despite the fact that there is a widespread and extensive use of this chemical in industrial settings. In none of the case reports published was the source or purity of the ADCA used reported, and therefore the evidence for a causal relationship between exposure to ADCA and the development of skin sensitisation is very weak.

As indicated earlier, in contrast to classification of skin sensitisers (H317; may cause an allergic skin reaction) classification of respiratory sensitisers (H334) according to GHS/CLP is not clear-cut. With regard to respiratory sensitisers: any chemical that induces a specific respiratory hypersensitivity reaction at a low to moderate (cat. 1B) or high (cat. 1A) frequency in humans should be considered a respiratory sensitiser. However, there remain many uncertainties regarding classification as H334. For instance, it is not clear how to define low, moderate and high frequencies. Similarly, it is not clear how the word specific is used in the above definition, and whether it denotes a response to a particular test substance, or a specific immunological reaction (an immunological reaction specific for the substance). However, immunological mechanisms do not have to be demonstrated, and given the very large number of workers that have been exposed to ADCA it is remarkable that there have been only a very small number of cases reported.

The guidance further indicates that appropriate lung function tests or bronchial challenge tests should be conducted according to accepted guidelines in order to provide evidence for respiratory sensitisation. It is relevant to consider, therefore, whether the lung function tests conducted with ADCA were appropriate and performed according to accepted guidelines. In none of the provocation tests was the source and purity of the ADCA samples checked, and it must be appreciated that during workplace exposure co-exposures to other substances can rarely be excluded. For the cases included in the national registers such information is completely absent. In addition, occupational, medical and smoking histories were unknown for cases included in the national registers. The classification guidance further implies that in case of existing bronchial hyperreactivity, symptoms of asthma by irritation do not count. If it can be demonstrated that these substances induce symptoms of asthma by irritation only in people with bronchial hyperreactivity, they should not be considered respiratory sensitisers. For the subjects who underwent non-specific bronchial hyperreactivity tests it cannot be concluded, but neither can it be excluded, that ADCA induced symptoms of asthma by irritation.

Moreover, according to ECHA guidance (ECHA, 2016a,b), based on current knowledge there are no chemical respiratory allergens that do not elicit positive responses in skin sensitisation predictive tests (Kimber et al., 2007), and therefore, any chemical testing negative in a skin sensitisation test such as the LLNA, should NOT be considered a respiratory sensitiser either. In accord with this ADCA should not be considered a respiratory sensitiser based on the negative results in the LLNA, GPMT and Buehler test, supported by a negative repeated inhalation exposure study in guinea pigs. Although the latter study is not a standard test, several known respiratory sensitisers have proven to be positive in comparable inhalation tests either with guinea pigs (Karol, 1983; Sarlo and Clark, 1992; Pauluhn and Mohr, 1994; Pauluhn et al., 2000) or with rats (Pauluhn and Poole, 2011).

6. Concluding comments

In conclusion, the data reviewed here do not support the classification of ADCA as a respiratory allergen. With only a single exception, associations between workplace exposure to ADCA and symptoms of respiratory allergy were reported before 2000, and in only 3 cases were the symptoms and clinical investigations well documented (Malo et al., 1985; Kim et al., 2004).

ADCA is a fine, respirable, dry dust, which following inhalation exposure at sufficiently high concentrations may result in non-specific respiratory reactions. Apart from those described by Malo et al. (1985), the respiratory reactions reported to be associated with ADCA have not been typical of those that normally

characterise respiratory allergy. In contrast, the "progressive" reactions that have been frequently reported with ADCA have started immediately following provocation, reaching a maximum after a few hours. Such reactions have also been observed following exposure to irritating substances such as chlorine (Burge et al., 2012). Moreover, in none of the cases describing an association between ADCA and respiratory reactions was the source and purity of the chemical used in the provocation tests reported. The possibility of co-exposure to respiratory irritants and/or to respiratory allergens cannot, therefore, be formally excluded.

Taken together, the evidence available from clinical investigations is insufficient to conclude that ADCA has the potential to cause allergic sensitisation of the respiratory tract, and on that basis should not be classified as a respiratory allergen.

That conclusion is consistent with the results recorded with ADCA in the LLNA and in guinea pig tests for skin sensitisation. ADCA was uniformly negative in all predictive tests for skin sensitisation. This is a behaviour not observed previously with known chemical respiratory allergen; such chemicals invariably eliciting positive responses (Dearman et al., 2013).

Collectively, the data available from clinical and experimental animal studies indicate that ADCA is not a true respiratory allergen. In addition, the available data do not support classification as a respiratory allergen.

Acknowledgements

The authors would like to acknowledge expertise provided by the industry ADCA Task Force (ACDA TF). The authors were supported by, and were *ad hoc* scientific contributors to, the ADCA TF during the preparation of this article.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2017.07.018.

References

Ahrenholz, S., Anderson, K., 1985. Health Hazard Evaluation Report of Leon Plastics. US Dept of Health and Human Services, Grand Rapids, MI, Cincinnati, OH (Report no. HETA-83-156-16722; PB89-143200).

Ahrenholz, S., Morawatz, J., Liss, G., 1985. Health Hazard Evaluation Report of Armstrong World Plastics. US Dept of Health and Human Services, Lancaster, PA, Cincinnati, OH (Report no. HETA-83-451-1547; PB86-105582).

Alt, E., Diller, W., 1988. "Hyperreactivity cough" in occupational medicine. Zbl. Arbeitsmed. 38, 22–26.

Ameille, J., Pauli, G., Calastreng-Crinquand, A., Vervloet, D., Iwatsubo, Y., Popin, E., Bayeux-Dunglas, M.C., Kopferschmitt-Kubler, M.C., 2003. Reported incidence of occupational asthma in France, 1996-99: the ONAP programme. Occup. Environ. Med. 60, 136–141.

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., 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 sensitizers. Toxicol. Sci. 106, 423–434.

Ayres, J.G., Boyd, R., Cowie, H., Hurley, J.F., 2011. Costs of occupational asthma in the UK. Thorax 66, 128–133.

Bakerly, N.D., Moore, V.C., 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.

Basketter, D.A., Evans, P., Fielder, R.J., Gerberick, G.F., Dearman, R.J., Kimber, I., 2002. Local lymph node assay – validation and use in practice. Fd. Chem. Toxicol. 40, 593–598.

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

Baur, X., 2013. A compendium of causative agents of occupational asthma. J. Occup. Med. Toxicol. 8, 1–8.

Baur, X., Bakehe, P., 2014. Allergens causing occupational asthma: an evidence-based evaluation of the literature. Int. Arch. Occup. Environ. Health 87, 339–363.

Bonsall, J.L., 1984. Allergic contact dermatitis to azodicarbonamide. Contact Dermat. 10, 42.

Botham, P.A., 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.

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

Buehler, E.V., 1965. Delayed contact hypersensitivity in the Guinea pig. Arch. Dermatol. 91, 171–177.

Burge, P.S., Moore, V.C., Robertson, A.S., 2012. Sensitization and irritant-induced occupational asthma with latency are clinically indistinguishable. Occ. Med. 52, 129–133.

Cochrane, S.A., Arts, J.H., Hindle, S., Hollnagel, H.M., Poole, A., Suto, H., Kimber, I., 2015. Thresholds in chemical respiratory sensitisation. Toxicology 333, 179–194.

Dearman, R.J., Basketter, D.A., Kimber, I., 1995. Differential cytokine production following chronic exposure of mice to chemical respiratory and contact allergens. Immunology 86, 545–550.

Dearman, R.J., Basketter, D.A., Kimber, I., 1996. Characterization of chemical allergens as a function of divergent cytokine secretion profiles induced in mice. Toxicol. Appl. Pharmacol. 138, 308–316.

Dearman, R.J., Basketter, D.A., Kimber, I., 2013. Inter-relationships between different classes of chemical allergens. J. Appl. Toxicol. 33, 558–565.

Dearman, R.J., Humphreys, N., Skinner, R.A., Kimber, I., 2005. Allergen-induced cytokine phenotypes in mice: role of $CD4^+$ and $CD8^+$ T cell populations. Clin. Exp. Allergy 35, 498–505.

Dearman, R.J., Kimber, I., 2001. Cytokine fingerprinting and hazard assessment of chemical respiratory allergy. J. Appl. Toxicol. 21, 153–163.

Di Stefano, F., Siriruttanapruk, S., McCoach, J., Di Gioacchino, M., Burge, P.S., 2004. Occupational asthma in a highly industrialized region of UK: report from a local surveillance scheme. Eur. Ann. Allergy Clin. Immunol. 36, 56–62.

Dotson, G.S., Maier, A., Siegel, P.D., Anderson, S.E., Green, B.J., Stefaniak, A.B., Codispoti, C.D., Kimber, I., 2015. Setting occupational exposure limits for chemical allergens – understanding the challenges. J. Occup. Environ. Hyg. 12 (Suppl. 1), S82–S98.

ECHA (European Chemicals Agency), 2012. Annex XV Dossier: Diazene-1,2-dicarboxamide [C,C′-azodi(formamide0) (ADCA). Proposal for Identification of a Substance as a CMR CAT 1A or 1B, PBT, vPvB or a Substance of an Equivalent Level of Concern. http://echa.europa.eu/documents/10162/d9e11c88-481a-47a9-8fff-915b48086ddb.

ECHA (European Chemical Agency), 2016a. Guidance for the Implementation of REACH.

ECHA (European Chemical Agency), 2016b. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7a: Endpoint Specific Guidance. Version 5.0, December 2016. https://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.pdf.

Elder, D., Abramson, M., Fish, D., Johnson, A., McKenzie, D., Sim, M., 2004. Surveillance of Australian workplace Based Respiratory Events (SABRE): notifications for the first 3.5 years and validation of occupational asthma cases. Occup. Med. 54, 395–399.

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.

Feary, J., Pinnock, H., Cullinan, P., 2016. Occupational asthma. Brit. Med. J. 353 http://dx.doi.org/10.1136/bmj.i2658.

Ferris, B.G., Peters, J.M., Burgess, W.A., Cherry, R.B., 1977. Case report – apparent effects of an azodicarbonamide on the lungs. J. Occup. Med. 19, 424–425.

Fitzpatrick, J.M., Roberts, D.W., Patlewicz, G., 2017a. What determines skin sensitization potency: myths, maybes and realities? The 500 molecular weight cut-off: an updated analysis. J. Appl. Toxicol. 37, 105–116.

Fitzpatrick, J.M., Roberts, D.W., Patlewicz, G., 2017b. Is skin penetration the determining factor in skin sensitization potential and potency? Refuting the notion of a logKw threshold for skin sensitization. J. Appl. Toxicol. 37, 117–127.

Gerlach, R.F., Medinsky, M.A., Hobbs, C.H., Bice, D.E., Bechtold, W.E., Cheng, Y.S., Gillett, N.A., Birnbaum, L.S., Mauderly, J.L., 1989. Effect of four-week repeated inhalation exposure to unconjugated azodicarbonamide on specific and non-specific airway sensitivity of the Guinea pig. J. Appl. Toxicol. 9, 145–153.

GHS (Globally Harmonized System of Classification and Labelling of Chemicals). 2011. New York and Geneva: United Nations.

Griffiths-Johnson, D.A., Karol, M.H., 1991. Validation of a non-invasive technique to assess development of airway hyperreactivity in an animal model of immunologic pulmonary hypersensitivity. Toxicology 65, 283–294.

Hannaford-Turner, K., Elder, D., Sim, M.R., Abramson, M.J., Johnson, A.R., Yates, D.H., 2010. Surveillance of Australian workplace Based Respiratory Events (SABRE) in New South Wales. Occup. Med. 60, 376–382.

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.

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: considerations of stability and controls. J. Appl. Toxicol. 16, 165–170.

HSE (Health and Safety Executive), 2015. Occupational Asthma: New Cases of Assessed Disablement by Year, Agent & Percentage Disablement (2006 to Latest Year). http://www.hse.gov.uk/statistics/tables/iidb09.xlsx.

Huang, M., Maynard, A., Turpin, J.A., Graham, L., Janini, G.M., Covell, D.G., Rice, W.G.,

1998. Anti-HIV agents that selectively target retroviral nucleocapsid protein zinc fingers without affecting cellular zinc finger proteins. J. Med. Chem. 41, 1371–1381.
Huntingdon Research Centre, 1988. Delayed Contact Hypersensitivity in the Guinea-Pig with Unifoam AZ SO-NL. Report No: 88708D/OCI 68/SS.
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.
Jajosky, R.A.R., Harrison, R., Reinisch, F., Flattery, J., Chan, J., Tumpowsky, C., Davis, L., Reilly, M.J., Rosenman, K.D., Kalinowski, D., Stanbury, M., Schill, D.P., Wood, J., 1999. Surveillance of work-related asthma in selected U.S. states using surveillance guidelines for state health departments-California, Massachusetts, Michigan, and New Jersey, 1993-1995. MMWR CDC Surveill. Summ. 48, 1–20.
Karjalainen, A., Kurppa, K., Virtanen, S., Keskinen, H., Nordman, H., 2000. Incidence of occupational asthma by occupation and industry in Finland. Am. J. Ind. Med. 37, 451–458.
Karol, M.H., 1983. Concentration-dependent immunologic response to toluene diisocyanate (TDI) following inhalation exposure. Toxicol. Appl. Pharmacol. 68, 229–241.
Kenyon, N.J., Morrissey, B.M., Schivo, M., Albertson, T.E., 2012. Occupational asthma. Clin. Rev. Allergy Immunol. 43, 3–13.
Kern, P.S., Gerberick, G.F., Ryan, C.A., Kimber, I., Aptula, A., Basketter, D.A., 2010. Local lymph node assay data for the evaluation of skin sensitization alternatives: a second compilation. Dermatitis 21, 8–32.
Kim, C.-W., Cho, J.-H., Leem, J.H., Ryu, J.-S., Lee, H.-L., Hong, Y.-C., 2004. Occupational asthma due to azodicarbonamide. Yonsei Med. J. 45, 325–329.
Kimber, I., Agius, R., Basketter, D.A., Corsini, E., Cullinan, P., Dearman, R.J., Gimenez-Arnau, E., Greenwell, L., Hartung, T., Kuper, F., Maestrelli, P., Roggen, E., Rovida, C., 2007. Chemical respiratory allergy. Opportunities for hazard identification and characterization. Altern. Lab. Anim. 35, 243–265.
Kimber, I., Basketter, D.A., 1992. The murine local lymph node assay: a commentary on collaborative trials and new directions. Fd. Chem. Toxicol. 30, 165–169.
Kimber, I., Basketter, D.A., Gerberick, G.F., Ryan, C.A., Dearman, R.J., 2011. Chemical allergy: translating biology into hazard identification and characterization. Toxicol. Sci. 120 (S1), S238–S268.
Kimber, I., Basketter, D.A., Roggeband, R., 2001. Chemical respiratory allergy: classification and labelling. Toxicology 167, 159–162.
Kimber, I., Basketter, D.A., Thyssen, J.P., Dearman, R.J., McFadden, J.P., 2014c. Chemical allergy in humans: fresh perspectives. J. Immunotoxicol. 11, 203–204.
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., Dearman, R.J., Basketter, D.A., Boverhof, D.R., 2014a. Chemical respiratory allergy: reverse engineering an adverse outcome pathway. Toxicology 318, 32–39.
Kimber, I., Dearman, R.J., Basketter, D.A., 2014b. Diisocyanates, occupational asthma and IgE antibody: implications for hazard characterization. J. Appl. Toxicol. 34, 1073–1077.
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 Derm. 47, 315–328.
Kimber, I., Dearman, R.J., Scholes, E.W., Basketter, D.A., 1994. The local lymph node assay: developments and applications. Toxicology 93, 13–31.
Kimber, I., Warbrick, E.V., Dearman, R.J., 1998. Chemical respiratory allergy, IgE and the relevance of predictive test methods: a commentary. Hum. Exp. Toxicol. 17, 537–540.
Kimber, I., Weisenberger, C., 1989. A murine local lymph node assay for the identification of contact allergens: assay development and results of an initial validation study. Arch. Toxicol. 63, 274–282.
Kimber, I., Wilks, M.F., 1995. Chemical respiratory allergy. Toxicological and occupational health issues. Hum. Exp. Toxicol. 14, 735–736.
Klees, J.E., Alexander, M., Rempel, D., Beckett, W., Rubin, R., Barnhart, S., Balmes, J.R., 1990. Evaluation of a proposed NIOSH surveillance. Case definition for occupational asthma. Chest 98, 212S–215S.
Kor, A.C., Lee, H.S., Chee, C.B.E., Wang, Y.T., 2001. Occupational asthma in Singapore. Singap. Med. J. 42, 373–377.
Kwon, S.-C., Song, J., Kim, Y., Calvert, G.M., 2015. Work-related asthma in Korea – findings from the Korea Work-related Asthma Surveillance (KOWAS) program, 2004-2009. Allergy Asthma Immunol. Res. 7, 51–59.
Lagier, F., Cartier, A., Malo, J.L., 1990. Medico-legal statistics on occupational asthma in Quebec between 1986 and 1988. Rev. Mal. Resp. 7, 337–341.
Lalko, J.F., Kimber, I., Dearman, R.J., Gerberick, G.F., Sarlo, K., Api, A.M., 2011. Chemical selectivity measurements: potential for characterization of respiratory chemical allergens. Toxicol. In Vitro 25, 433–445.
Latza, U., Baur, X., 2005. Occupational obstructive airway diseases in Germany: frequency and causes in an international comparison. Am. J. Ind. Med. 48, 144–152.
Magnusson, B., Kligman, A.M., 1969. The identification of contact allergens by animal assay. The Guinea pig maximization test. J. Invest. Dermatol. 52, 268–276.
Malo, J.L., Pineau, L., Cartier, A., 1985. Occupational asthma due to azobisformamide. Clin. Allergy 15, 261–264.
Maynard, A.T., Huang, M., Rice, W.G., Covell, D.G., 1998. Reactivity of the HIV-1 nucleocapsid protein p7 zinc finger domains from the perspective of density-functional theory. Proc. Nat. Acad. Sci. U. S. A. 95, 11578–11583.
McDonald, J.C., Keynes, H.L., Meredith, S.K., 2000. Reported incidence of occupational asthma in the United Kingdom. Occup. Environ. Med. 57, 823–829.
McDonald, J.C., Chen, Y., Zekveld, C., Cherry, N.M., 2005. Incidence by occupation and industry of acute work related respiratory diseases in the UK, 1992-2001. Occup. Environ. Med. 62, 836–842.
Nava, C., Beretta, F., Elena, A., Ghizzi, A., Pattarin, R., Villa, L., 1983. Le dermatiti allergiche da miglioranti e da altri additivi per farine. Med. Lav. 74, 376–379.
Newell, L., Polak, M.E., Perera, J., Owen, C., Boyd, P., Pickard, C., Howarth, P.H., Healy, E., Holloway, J.W., Friedmann, P.S., Ardern-Jones, M.R., 2013. Sensitization via healthy skin programs Th2 responses in individuals with atopic dermatitis. J. Invest. Dermatol. 133, 2372–2380.
Normand, J.-C., Grange, F., Hernandez, C., Ganay, A., Davezies, P., Bergeret, A., Prost, G., 1989. Occupational asthma after exposure to azodicarbonamide: report of four cases. Br. J. Ind. Med. 46, 60–62.
North, C.M., Ezendam, J., Hotchkiss, J.A., Maier, C., Ayoyama, K., Enoch, S., Goetz, A., Graham, C., Kimber, I., Karljalainen, A., Pauluhn, J., Roggen, E.L., Selgrade, M., Chen, C.L., 2016. Developing a framework for assessing chemical respiratory sensitization: a workshop report. Regul. Toxicol. Pharmacol. 80, 295–309.
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., Mohr, U., 1994. Assessment of respiratory hypersensitivity in Guinea-pigs sensitized to diphenyl-methane-4,4-diisocyanate (MDI) and challenged with MDI, acetylcholine, or MDI-albumin conjugate. Toxicol 92, 53–74.
Pauluhn, J., Thiel, A., Emura, M., Mohr, M., 2000. Respiratory sensitization to diphenyl-methane-4,4-diisocyanate (MDI) in Guinea pigs: impact of particle size on induction and elicitation of response. Toxicol. Sci. 56, 105–113.
Pauluhn, J., 2008. Brown Norway rat asthma model of diphenylmethane-4,4′-diisocyanate (MDI): impact of vehicle for topical induction. Regul. Toxicol. Pharmacol. 50, 57–66.
Pauluhn, J., Poole, A., 2011. Brown Norway rat asthma model of diphenylmethane-4,4'-diisocyanate (MDI): determination of the elicitation threshold concentration of after inhalation sensitization. Toxicol 281, 15–24.
Piipari, R., Keskinen, H., 2005. Agents causing occupational asthma in Finland in 1986-2002: cow epithelium bypassed by moulds from moisture-damaged buildings. Clin. Exp. Allergy 35, 1632–1637.
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.
Reinisch, F., Harrison, R.J., Cussler, S., Athanasoulis, M., Balmes, J., Blanc, P., Cone, J., 2001. Physician reports of work-related asthma in California, 1993-1996. Am. J. Ind. Med. 39, 72–83.
Sarlo, K., Clark, E.D., 1992. A tier approach for evaluating the respiratory allergenicity of low molecular weight chemicals. Fundam. Appl. Toxicol. 1992 (18), 107–114.
Satoh, T., Kramarik, J.A., Tollerud, D.J., Karol, M.H., 1995. A murine model for assessing the respiratory hypersensitivity potential of chemical allergens. Toxicol. Lett. 78, 57–66.
Slovak, A.J.M., 1981. Occupational asthma caused by a plastics blowing agente, azodicarbonamide. Thorax 36, 906–909.
Tarlo, S.M., Lemiere, C., 2014. Occupational asthma. N. Engl. J. Med. 370, 640–649.
Tsen, C.C., 1963. The reaction mechanism of azodicarbonamide in dough. Cereal Chem. 40, 638–647.
Vandenplas, O., 2011. Occupational asthma: etiologies and risk factors. Allergy Asthma Immunol. Res. 3, 157–167.
Van Och, F.M.M., Van Loveren, H., De Jong, W.H., Vandebriel, R.J., 2002. Cytokine production induced by low-molecular weight 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.
Valentino, M., Comai, M., 1985. Asma professionale da azodicarbonamide: caso clinico. G. Ital. Med. Lav. 7, 97–99.
Whitehead, L.W., Robins, T.G., Fine, L.J., Hansen, D.J., 1987. Respiratory symptoms associated with the use of azadicarbonamide foaming agent in a plastics injection molding factory. Am. J. Ind. Med. 11, 83–92.
WHO, 1999. Azodicarbonamide. Concise International Chemical Assessment Document 16. World Health Organization, Geneva, Switzerland (including corrigenda published by 12 April 2005).
WIL Research Europe BV, 2014a. Assessment of Contact Hypersensitivity in the Mouse (Local Lymph Node Assay). Project 506582.
WIL Research Europe BV, 2014b. Assessment of Contact Hypersensitivity to ADCA in the Albino Guinea Pig (Maximisation-test). Project 506583.
Yates, V.M., Dixon, J.E., 1988. Contact dermatitis from azodicarbonamide in earplugs. Contact Dermat. 19, 155–156.