Occupational asthma and extrinsic alveolitis due to isocyanates: current status and perspectives

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Isocyanates are used for the large scale production of polyurethane polymers, which have an almost endless variety of applications in the manufacture of flexible and rigid foams, elastomers, adhesives, and surface coatings.¹ Acute or chronic exposure to high concentrations of isocyanates can result in respiratory health hazards through a direct irritant effect.^{2,3} Isocyanates are of special interest, however, because, in some exposed workers, they can cause occupational asthma or extrinsic alveolitis through an apparently sensitising mechanism.^{2,3} Because of their wide industrial use, isocyanates are the principal cause of occupational asthma which is now the most common respiratory disease linked to the working environment.^{4,5} This review focuses on recently studied aspects of occupational asthma and extrinsic alveolitis related to exposure to isocyanates.

Isocyanates

CHEMISTRY

The isocyanates are a class of low molecular weight chemicals that contain highly reactive isocyanate (NCO) groups. The NCO groups are attached to an organic radical that can be aromatic, aliphatic, or cycloaliphatic.¹ They have a high affinity for compounds containing reactive hydrogen atoms bound to oxygen, nitrogen, or sulphur. They react with hydroxylic (OH) groups to form a urethane (fig 1). This is the basic reaction in the production of high molecular weight polymers referred to as polyurethanes. Isocyanates also combine with primary and secondary amines, water, ureas, and urethanes. On the other hand, the elementary molecules of isocyanates, called monomers, are capable of reacting with themselves to form dimers, trimers, and other oligomers. The self polymerisa-

tion of isocyanates occurs spontaneously and can be accelerated by heating.

INDUSTRIAL USES

The production of polyurethane polymers requires at least two (diisocyanates) or more (polyisocyanates) NCO groups in the molecule. Figure 2 shows the structure of the principal monomers of diisocyanates. Toluene diisocyanate (TDI) is an aromatic diisocyanate that is liquid and highly volatile at ambient temperature. It exists in two isomeric forms, the 2,4- and the 2,6- isomers usually mixed in a ratio of 80:20. The aromatic diphenylmethane diisocyanate (MDI) was introduced in the late 1960s because it has a lower vapour pressure than TDI. The monomer of MDI is a crystalline solid but MDI is usually available commercially in liquid mixtures containing the monomer and various oligomers of MDI. About 95% of all polyurethanes are based on TDI and MDI. The world production of isocyanates was estimated in 1986 to be 650 000 tonnes of TDI and 850 000 tonnes of MDI.⁶ The third most commercially important diisocyanate is the aliphatic hexamethylene diisocyanate (HDI) which, like TDI, is highly volatile at room temperature. There are other diisocyanates available including naphthylene diisocyanate (NDI), isophorone diisocyanate (IPDI) and dicyclohexylmethane diisocyanate or hydrogenated MDI (HMDI) but their use is restricted to a few specialised applications.

The molecular structures of polyurethanes vary from rigid cross linked polymers to linear, highly extensible elastomers. The properties of the final product can be tailored by the proper selection of the isocyanates, polyols, and additives such as catalysts, blowing agents, foam stabilisers, cross linking

Figure 1 Addition reaction between an isocyanate (NCO) and a hydroxyl (OH) group to give a urethane linkage. This reaction is the basic mechanism for the production of high molecular weight polymers called polyurethanes.

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Monomers of isocyanates

Aromatic radical

Toluene diisocyanate (TDI)

Diphenylmethane diisocyanate (MDI)

1,5-naphthylene diisocyanate (NDI)

Aliphatic radical

Hexamethylene diisocyanate (HDI)

Cycloaliphatic radical

Isophorone diisocyanate (IPDI)

4, 4'- dicyclohexylmethane diisocyanate (hydrogenated MDI, HMDI)

Figure 2 Structures of the monomers of diisocyanates used in industry. They are classified according to the nature of their organic component (aromatic, aliphatic, or cycloaliphatic).

Prepolymers of isocyanates

Derived from toluene diisocyanate (TDI)

Derived from hexamethylene diisocyanate (HDI)

Trimer of HDI (isocyanurate ring)

Biuret structure of HDI

Derived from diphenylmethane diisocyanate (MDI)

Polymethylene polyphenyl diisocyanate (PPI)

Figure 3 Structures of some of the principal prepolymers of diisocyanates used in industry. The prepolymers of HDI include the biuret and the trimer (isocyanurate ring) structures of HDI. The most common prepolymer of MDI is an oligomer often referred to as polymethylene polyphenyl diisocyanate. Note that the prepolymers of TDI and HDI have three functional NCO groups.

and chain extending compounds. The main application of TDI is in the manufacture of flexible foams for cushioning in the furniture and automobile industries. MDI has progressively replaced TDI and is widely used in the production of rigid foams, thermal and sound insulation products, packaging materials, binding agents, elastomers, and coatings. HDI and IDPI are almost exclusively used in the formulation of coatings and paints because the presence of the aliphatic radical provides excellent light and weather resistance in the polyurethane end product.

New types of isocyanates, commonly referred to as prepolymers, have been introduced to reduce respiratory hazards due to inhalation of monomer vapours. These result either from the reaction of a polyhydroxyl compound with water, or an excess of diisocyanate molecules, or from the self combination of diisocyanate monomers.¹ Figure 3 shows the ideal structure of the principal prepolymers of isocyanates. Commercial prepolymers are most often a complex mixture of various compounds with different molecular weights but, as a rule, the prepolymers are less volatile than their parent monomer. Over the past two decades, isocyanate prepolymers have gained increasing importance, particularly as moulded foams and surface coatings, because they offer several processing advantages. Although products based on prepolymers usually contain only trace amounts of the volatile monomer (less than 1%), it is important to note that they still contain considerable amounts of reactive isocyanate groups on the non-volatile prepolymer.⁷

SOURCES AND MODE OF EXPOSURE

Exposure to isocyanates may result from the inhalation of vapours generated by TDI and HDI at ambient temperature and by MDI when heated.⁸ A considerable source of exposure to non-volatile isocyanates (MDI and prepolymers) comes from the aerosols generated during spraying processes.^{8,9} Less often, exposure results from the thermal decomposition of polyurethanes—for example, during welding on surfaces coated with a polyurethane.^{8,10}

Methods have been developed for analysis of isocyanates in working environments.⁷ High performance liquid chromatography (HPLC) is now considered as the reference method but is discontinuous and time consuming. The paper tape monitors are based on a colorimetric method. They are less accurate than chromatography and have not been validated for the assessment of prepolymers. The tape monitors are, however, currently the only way to perform continuous monitoring of isocyanate concentrations.

To prevent the irritant effects of isocyanates, the threshold limit values (TLVs) have been estab-

lished at 5 ppb for a 10 hour workday time weighted average (TWA) and at 20 ppb as a ceiling value.^{7,11,12} Some countries have also established similar permissible exposure limits for the prepolymers of isocyanates.^{7,9} At present it is not known whether the observance of these exposure limits is capable of preventing or decreasing the occurrence of respiratory hypersensitivity among exposed workers. Furthermore, hypersensitive workers can develop clinical manifestations at exposures well below these recommended limits.

Asthma

Occupational asthma is variable airflow limitation causally related to exposure to non-irritant levels of isocyanates.¹³ In 1951, Fuchs and Valade described asthma like symptoms in seven workers exposed to TDI.¹⁴ They suggested for the first time that contact with diisocyanates might lead to the development of hypersensitivity rather than direct toxicity. This initial observation as well as subsequent reports from Europe and North America showed that the bronchial response in some workers fulfilled the clinical criteria for hypersensitivity;¹⁵ only a minority of workers equally exposed to isocyanates develop asthma after a variable symptom-free latency period, as opposed to what has been described under the heading of reactive airways dysfunction syndrome (RADS).¹⁶ Furthermore, the symptoms recur on each exposure even to concentrations at which most subjects do not develop respiratory symptoms.

NATURAL HISTORY AND EPIDEMIOLOGY

Little is known about the natural history and the factors predisposing to occupational asthma. In a recent collaborative study comparing subjects with occupational asthma due to low molecular weight chemicals (isocyanates, $n = 107$ and plicatic acid contained in western red cedar, $n = 433$) and various high molecular weight agents ($n = 121$), we found that the latency period was shorter for low molecular compared with high molecular weight agents.¹⁷ Forty per cent of workers with occupational asthma due to isocyanates and plicatic acid developed symptoms within the first year of exposure compared with 20% of those with occupational asthma caused by high molecular weight agents. After five years of exposure, 60% of subjects exposed to isocyanates and high molecular weight agents had symptoms compared with 80% of those exposed to western red cedar. The mean latency period between the start of work exposure and the onset of symptoms was 7.03 years for isocyanates, 3.63 years for plicatic acid, and 7.34 years for high molecular weight agents. A continuous mode of exposure increased the rate of developing symp-

Table 1 Prevalence of occupational asthma among various populations of workers exposed to isocyanates according to the criteria used for establishing the diagnosis

Isocyanate	Population	No of workers	Estimated prevalence of occupational asthma No (%)	Diagnostic criteria*	Positive inhalation challenges†	Reference No
TDI	TDI production	166	29 (17)	Questionnaire	9/13	32
TDI	Automotive industry	192	57 (30)	Questionnaire	3/6	33
TDI, MDI	Various industries	195	55 (28)	Questionnaire or spirometry	12/17	34
MDI	Foundry	78	12 (15)	Questionnaire and NSBH	7/11	21
MDI, PPI	Paint shops	51	7 (14)	Questionnaire or spirometry and NSBH	6/6	29
MDI	Foundry	26	7 (27)	Questionnaire	ND	35
HDI	Paint shops	150	1 (0.6)	Questionnaire	ND	36

*The criteria used for diagnosing occupational asthma included a questionnaire (history of work related symptoms) with or without spirometry (presence of airflow limitation) and/or assessment of non-specific bronchial hyperreactivity (NSBH); †number of positive inhalation challenges/number of tested workers; PPI is polymethylene polyphenyl diisocyanate; ND: not done.

toms compared with intermittent exposure,¹⁷ but the prevalence of isocyanates does not seem to correlate closely with the cumulative level of exposure at work. Nevertheless, there is some evidence that the development of occupational asthma is associated with acute, often accidental, exposures to high concentrations of isocyanates (spills).^{16, 18} Atopy, smoking habits, and even pre-existing asthma or airway hyperresponsiveness have not been shown to be significant risk factors for occupational asthma.

The most commonly used isocyanates, including TDI,¹⁹ MDI,^{20, 21} HDI,^{22, 23} IPDI,²⁴ and NDI,²⁵ cause asthma in various occupational and even non-occupational²⁶ settings. Occupational asthma has generally been ascribed to the monomers of these diisocyanates. A role for the prepolymers in the development of occupational asthma has been suspected but not firmly shown.²⁷⁻²⁹ We recently described two subjects in whom specific inhalation challenges elicited asthmatic reactions after exposure to a prepolymer of TDI present in a wood varnish but who were not affected after exposure to the monomer of TDI.³⁰ We conducted a prospective study among 20 workers referred for possible occupational asthma caused by spray paints containing both an HDI monomer and HDI prepolymers.³¹ The workers underwent inhalation challenges with the monomer and prepolymers of HDI separately. Among the 10 subjects who had positive inhalation challenges, four developed an asthmatic reaction only after exposure to the prepolymer and not after exposure to the monomer. These observations suggest that prepolymers of isocyanates themselves can cause occupational asthma.

Isocyanates are the principal cause of occupational asthma accounting for about 25% of all cases identified in industrialised countries.^{4, 5} Information on the prevalence of isocyanate induced occupational asthma in exposed workers is scarce and

restricted to a few cross sectional studies (table 1).^{21, 29, 32-36} Cross sectional surveys are likely to underestimate the actual prevalence of occupational asthma as workers with work related respiratory symptoms tend to leave their jobs. Furthermore, the prevalence of isocyanate asthma may be influenced by the type of industry and the criteria used for diagnosis (questionnaires, assessment of non-specific bronchial responsiveness, inhalation challenges). According to the results of studies with objective diagnostic tests, a prevalence of about 10% seems to be a reasonable approximation.^{21, 29}

PATHOGENESIS

A number of mechanisms have been proposed for isocyanate induced asthma. A classic IgE mediated mechanism has been suggested,^{37, 38} but the presence of a diisocyanate specific IgE has been shown in only a few workers with symptoms.³⁹ More recently, assessment of specific IgG antibodies has been found to give better results than IgE.⁴⁰ Reflex and pharmacological mechanisms have also been proposed to explain isocyanate induced asthma and isocyanate induced airway hyperresponsiveness, but these mechanisms cannot explain the development of sensitisation to isocyanates in only a certain number of exposed subjects.⁴¹ Isocyanates inhibit the stimulation of cyclic adenosine monophosphate (cAMP) in human lymphocytes by isoproterenol and postaglandin E1, whereas at some concentrations isocyanates act as cAMP stimulants. In susceptible subjects, specific lymphocyte blastogenic activity and lymphokine leucocyte inhibitory factor responses to toluene diisocyanate (TDI) have been found.⁴² There are recent data to suggest an immune response after exposure to TDI. In fact an increased number of circulating CD8+ lymphocytes has been shown 48 hours after inhalation challenge with TDI.⁴³

Airway inflammation is a constant finding in isocyanate induced asthma primarily in dual and late

asthmatic reactions, which are more common than early reactions after inhalation challenges with isocyanates. The mechanisms of the late asthmatic response are still unknown. It has been proposed that isocyanates cause late asthmatic reactions and increases in non-specific airway responsiveness by causing an acute inflammatory process in the airways. The airway response to isocyanates does not show an asthmatic response after exposure to TDI.⁴⁴ Airway responsiveness to methacholine, moreover, increases only in subjects who develop a dual or late asthmatic reaction.⁴⁵ Late asthmatic reactions are associated with an influx of neutrophils followed by an influx of eosinophils in the airways, and with an increase in albumin in lavage supernatant liquid, suggesting the development of an acute inflammatory reaction in the airways and the development of microvascular leakage and oedema formation.^{46,47} Inflammatory mediators may be released by epithelial cells, alveolar macrophages, or others. During late asthmatic reactions induced by TDI, an increase in leukotriene B₄ has been found.⁴⁸ As leukotriene B₄ is also released by neutrophils, it is not known whether LTB₄ is the cause or the result of the influx of neutrophils in the airways. Whether other chemotactic factors such as interleukin 8 and 5 (IL-8, IL-5) are involved in this model remains to be clarified.

Steroids block the late asthmatic reaction, the associated increase in non-specific airway responsiveness, the influx of neutrophils and eosinophils, and the extravasation of albumin, suggesting that airway inflammation plays an important part in late asthmatic reactions induced by exposure to TDI.^{47,49-51}

Airway inflammation was seen at necropsy of a car painter with occupational asthma induced by TDI who died at work.⁵² The bronchi were constricted by the spasm of a thickened smooth muscle, and there was a massive infiltration of inflammatory cells, particularly eosinophils. The oedematous airways were plugged with mucus, exudate, and inflammatory cells. This is an extremely severe stage of the disease, however, and it is not clear to what extent these postmortem findings reflect the pathology of occupational asthma in real life, particularly in the intervals between attacks.

The nature and degree of mucosal inflammation of the airways in TDI induced asthma in the intervals between attacks has recently been clarified by performing bronchial biopsies in patients with occupational asthma induced by TDI and in control subjects.⁵³ Subjects with TDI induced asthma exhibit an increased number of inflammatory cells in the airway mucosa compared with control subjects. Eosinophils were increased in the epithelium,

in the more superficial layer of the submucosa, and in the total submucosa, whereas CD45 positive cells were increased in the epithelium and in the more superficial layer of the submucosa, and mast cells were increased only in the epithelium (fig 4). Both eosinophils and mast cells seemed to be degranulated. A thicker subepithelial reticular layer was present in patients with TDI asthma, where deposition of collagen III has been shown by immunohistochemistry. Seen through electron microscopy, the intercellular spaces between basal cells were wider in the asthmatic subjects, contributing to the development of epithelial fragility. The pathological features of TDI induced asthma are similar to those described for non-occupational asthma. We recently investigated whether cessation of exposure to the sensitising agent is able to reverse pathological findings in TDI induced asthma.⁵⁴ In subjects sensitised to TDI, bronchial biopsies were performed at diagnosis and six months after cessation of exposure to TDI. The thickness of the reticular basement membrane was significantly reduced compared with that at diagnosis, and it decreased to values similar to those of control biopsies (fig 5). The inflammatory cell infiltrate persisted in most of the asthmatic subjects, and

Figure 4 Numbers of inflammatory cells in the bronchial epithelium of controls and of subjects with TDI asthma. From reference 53 with permission.

degranulation of eosinophils and mast cells was still present. At the end of the study, airway responsiveness to methacholine, or sensitivity to TDI, or both, also persisted in most of the asthmatic subjects, despite the cessation of exposure and the disappearance of asthmatic symptoms. These results in patients with occupational asthma induced by TDI suggest that non-exposure for six months is able to reverse the thickening of the reticular membrane in the bronchial mucosa, but the inflammatory cell infiltrate, the specific sensitivity to TDI, and the non-specific airway hyperresponsiveness may persist. Whether a longer period of cessation of exposure is required to reverse these morphological and functional changes still remains to be investigated.

More recently, an *in vitro* model to study the mechanism of action of isocyanates has been established.⁵⁵⁻⁵⁸ Exposure to TDI contracts the bronchial smooth muscle in guinea pigs through the activation, at least in part, of capsaicin sensitive primary afferents. It is likely that the contraction is produced indirectly by the generation of a prostanoind that activates capsaicin sensitive primary afferents via a ruthenium red sensitive mechanism.⁵⁶

The products of reaction between TDI and water, such as toluenediamine (2, 4- TDA), also contract the bronchial smooth muscle through the same mechanism. Whether this mechanism plays a part in the development of the adverse effects on the respiratory tract seen after exposure to TDI, and whether it contributes to the development of airway inflammation in subjects sensitised to isocyanates remains to be elucidated.

In conclusion, all of these studies suggest that airway inflammation is significant in the late asthmatic reaction induced by exposure to TDI. The pathology of TDI induced asthma is similar to that of non-occupational asthma. The mechanism of accumulation of inflammatory cells in the airways and the role of an immunological mechanism in the pathogenesis of isocyanate induced asthma remain to be established.

DIAGNOSIS

Several procedures can help the physician to establish the diagnosis of occupational asthma. It is

essential to take a careful clinical history but this proved to be an unsatisfactory diagnostic tool compared with objective testing.⁵⁹ A typical history of occupational asthma (occurrence or worsening of chest symptoms at work or after work and improvement on days off work) had a low positive predictive value (46%) although the negative predictive value was better (83%). The low predictive value of a suggestive history probably results from the fact that asthma can be exacerbated at work for several reasons other than hypersensitivity to isocyanates—that is, cold weather, exercise, or exposure to non-specific irritant substances. Also subjects may tend to exaggerate their symptoms at the prospect of compensation.

The many efforts made to show immunological sensitisation to isocyanates have so far led to disappointing results.^{35 40 60-64} The presence of IgE antibodies to isocyanates has been documented in 20% to 39% of subjects with proved occupational asthma but has also been found in unaffected subjects (table 2).^{21 37 40 61 63} An increased concentration of specific IgE antibodies was more often associated with asthmatic reactions of the immediate type in some studies^{61 63} but not in others.^{40 61 63} More recently, assessment of IgG antibodies to isocyanates has been found to be more sensitive than assessment of IgE but also less specific.⁴⁰ Specific IgE and IgG antibodies are more often detected in subjects with occupational asthma due to HDI and MDI than in those with occupational asthma due to TDI.^{40 61 63} Welinder *et al* suggested that the concentrations of specific IgG to HDI could be more closely related to the amount of exposure than to the presence of respiratory symptoms.⁶² These results probably reflect the difficulties in preparing suitable isocyanate antigens for antibody assessment. Isocyanates act as haptens and need to be conjugated to an autologous carrier protein to induce an immunological response.⁶⁰ So far, most immunological studies have been performed using human serum albumin (HSA) although the precise nature of the respiratory tract component to which isocyanates bind *in vivo* remains unknown. Isocyanates are highly reactive chemicals that can combine with OH, NH₂, and SH groups on human proteins leading to variable degrees of intra and

Table 2 Diagnostic value of specific IgE and IgG antibodies to isocyanates compared with the results of inhalation challenges

Isocyanate	Presence of specific IgE antibodies		Presence of specific IgG antibodies		Reference No
	Positive inhalation challenge (sensitivity)	Negative inhalation challenge (specificity)	Positive inhalation challenge (sensitivity)	Negative inhalation challenge (specificity)	
	No (%)	No (%)	No (%)	No (%)	
TDI	5/26 (19)	0/30 (100)	—	—	37
MDI	1/7 (14)	1/4 (75)	2/7 (28)	8/32 (75)	21
TDI, MDI	11/28 (39)	0/51 (100)	—	—	61
TDI, HDI, MDI	7/35 (20)	0/46 (100)	—	—	63
HDI, MDI	9/29 (31)	1/33 (97)	21/29 (72)	8/32 (75)	40

interprotein cross linking.^{64 65} Thus the interaction of isocyanates with human proteins induces modifications of their structure that can result in the development of new antigenic determinants. Some studies on animal models and in humans indicate that antibodies are directed to these new antigenic determinants rather than to the isocyanate itself.^{60 66 67} This could account for the observed immunological cross reactivities between different types of isocyanates.⁶⁵⁻⁶⁸ This could also explain why the use of monofunctional isocyanates, such as *p*-tolyl monoisocyanate,^{37 69} is not more valuable than difunctional isocyanates in the preparation of antigen substrates. Finally, assessing antibodies directed to the prepolymers of isocyanates rather than to the monomers should be further assessed.^{30 31 62 36 70}

Non-specific bronchial hyperresponsiveness to histamine or methacholine may be absent in seven to 45% of subjects with occupational asthma at the time of the diagnosis^{40 71} especially when the subject has not been exposed to isocyanates for a long period. Indeed, non-specific bronchial responsiveness can increase or even develop progressively as the subject is exposed to isocyanates⁷²⁻⁷⁴ and decrease or disappear after the last exposure.⁷² There are only two documented case reports in which the subjects did not exhibit non-specific bronchial hyperresponsiveness either before or after asthmatic reactions to MDI.^{75 76} The bronchial reactions to isocyanates could, however, have been due to a non-specific effect as the concentrations during the tests were in the irritant range (≥ 30 ppb in both cases).

A firm diagnosis of occupational asthma requires objective demonstration that exposure to isocyanates results in an asthmatic reaction.⁷⁷ Several procedures have been proposed to confirm this causal relation. Assessment of forced expiratory volume in one second (FEV₁) before and after a workshift is clearly an inadequate method.⁷⁸ Prolonged recording of peak expiratory flow rates (PEFs) during periods at work and away from work has been suggested as a valuable means of establishing the diagnosis of occupational asthma.⁷⁹ Although simple and inexpensive, measurements of PEF are effort dependent, require substantial motivation and honesty, and may not be feasible in workers who have left their jobs.^{80 81} Recording of PEFs does not make it possible to distinguish between bronchial response due to isocyanates and that due to non-specific agents present in the workplace. The monitoring of PEFs is less sensitive and less specific than inhalation challenges even when it is combined with serial assessments of non-specific bronchial responsiveness.^{81 82}

Specific inhalation tests in the laboratory should still be considered as the reference standard for

establishing the diagnosis of occupational asthma.⁶ Inhalation challenges should be performed in specialised centres by trained personnel and under close medical supervision.⁷⁷ The generation of isocyanates in test chambers can be achieved in several different ways. In the original procedure described by Pepys and coworkers in the 1970s,^{10 19 23} the subjects were challenged by brushing or spraying the commercial isocyanate compound to which they were exposed at work. More simply, vapours of the highly volatile TDI and HDI can be generated by evaporation of the corresponding pure monomers at room temperature.^{6 83-86} Products containing primarily HDI prepolymers of HDI and only trace amounts of the monomer can be used provided that they are nebulised.⁸⁶ Exposure to MDI, which is not volatile at room temperature, can be achieved by heating it to $> 80^{\circ}\text{C}$ ^{21 23 87} or spraying the product.²⁹ Concentrations of isocyanate are influenced considerably by room temperature and humidity, absorption on surfaces other than stainless steel and teflon, opening of the door during the tests, and the subject's own breathing⁸⁸ and should therefore be continuously assessed with a tape monitor. Although challenge rooms may be adapted,⁸³ it is our experience that they do not allow for a fine regulation of concentrations of isocyanate because of their large volume. The concentrations can therefore exceed the recommended ceiling and result in unduly severe asthmatic reactions or in non-specific irritant responses. We have recently developed a closed circuit system for the generation of isocyanates in gaseous form.⁸⁹ Briefly, vapours of isocyanates are generated by passing a controlled flow of air on to the surface of pure monomers maintained at constant temperature, mixed with a second airstream that could be regulated for flow, humidity, and temperature, and then sent to a 0.25m³ cylindrical reservoir coated with teflon. The subject inhales gaseous isocyanates through an orofacial mask supplied with a unidirectional valve and isocyanate vapours are removed to the outside of the laboratory. The pressure in the reservoir is maintained at a constant concentration despite the subject's breathing via a system of solenoid valves connected to an exhaust pump. The results obtained in 20 subjects showed that the concentrations of isocyanates were significantly more stable with the closed circuit method than with the challenge room method. The closed circuit method was also more effective for maintaining the concentration of isocyanates below the recommended limit of 20 ppb. Since we found selective asthmatic reactions to prepolymers of isocyanates^{30 31} the closed circuit methodology is currently being developed to allow for the generation of stable and preselected concentrations of isocyanate prepolymers because they are not volatile and cannot be generated in a

Figure 5 Bronchial mucosal biopsy from a patient with TDI asthma. Upper panel, at diagnosis when a thickened reticular basement membrane and an inflammatory cell infiltrate is apparent. Lower panel, six months later after cessation of exposure to TDI when the basement membrane thickness is reduced, but the inflammatory cell infiltrate is still present. Magnification $\times 600$; stain: haematoxylin-eosin; light microscopy. From reference 54 with permission.

gaseous form.

Baseline FEV₁ must be > 21 or 60% of the predicted value before the inhalation challenge is initiated. A control test should be carried out to ensure that the patient's FEV₁ is stable—that is, fluctuations of FEV₁ are less than 10% over an eight hour period. During the control day, the subject should be exposed to a control substance—namely, one with an irritant but no known sensitising effect, to distinguish between specific and non-specific bronchial responses. For isocyanate challenges, the control substance could be the polyol components (for example, paint diluents) that are mixed with the isocyanates to produce the desired final polyurethane.^{29 40 86} A standardised schedule of exposure to isocyanates has not yet been adopted. In most centres, subjects are exposed to isocyanates for a fixed period (15–30 minutes)^{6 23 85 90–92} and sometimes the concentration is increased from one test to the next.^{6 71} It is our experience that some subjects require more prolonged exposure to exhib-

it an asthmatic reaction so that we gradually increase the duration of exposure on separate days according to the protocol presented in fig 6. There is only limited information on the factors that can affect the bronchial response to isocyanates in sensitised subjects. Burge *et al*⁷¹ found that the concentration of TDI required to induce a reaction in sensitised subjects was inversely correlated to the baseline of histamine reactivity, although histamine reactivity accounted for only 30% of the variation in threshold doses of TDI. With our closed circuit method,⁸⁹ we recently assessed the dose response relation in two subjects with asthma due to TDI by challenging them with different concentrations of TDI and different durations of exposure while the level of non-specific reactivity remained constant throughout the tests. We found that the dose of isocyanate (concentration times duration of exposure) required to induce an asthmatic reaction, although variable from one subject to the other, remained constant for each subject. These data suggest that increasing the duration of exposure for a given concentration is equivalent to increasing the concentration for a given duration of exposure, and is probably less hazardous.

After challenge exposure to isocyanates, FEV₁ should be monitored at 10 to 15 minute intervals for the first hour, 30 minute intervals for the second hour, then hourly for at least eight hours to detect late reactions.⁷⁷ A significant bronchial response should be considered as a sustained fall in FEV₁ of more than 20% from the prechallenge value in the absence of such changes on a control day. Several patterns of bronchial response after specific inhalation challenges have been described, including immediate or early, early late, late, and dual reactions.¹⁰ Atypical patterns of reaction have also been noted after exposure to isocyanates.^{21 29} In a recent study,⁸⁶ we found that these atypical reactions occurred more often in subjects with isocyanate induced occupational asthma (9/23) than in subjects with occupational asthma caused by high molecular weight agents and plicatic acid (6/46). The reactions were mainly of the progressive type, starting within minutes after the end of exposure and reaching a maximum five to six hours later. Less common patterns were referred to as square waved (similar to dual reactions but without recovery between the two components of the response) and prolonged immediate (similar to immediate but with functional recovery taking up to eight hours). In some subjects, inhalation challenges with isocyanates can induce recurrent nocturnal asthma for several days.^{90 91} Asthmatic cross reactivity between different isocyanates has been documented.^{23 28 92} Innocenti *et al*⁹³ found that nearly 50% of subjects with asthma induced by TDI also exhibited asthmatic reactions to MDI, which they were never

Figure 6 Schematic representation of the protocol used for inhalation challenges with isocyanates in our laboratory.

exposed to at work. The assessment of non-specific bronchial responsiveness before and after a specific inhalation test can provide further information as it has been shown that late and dual but not immediate reactions are associated with a significant increase in non-specific bronchial responsiveness.⁴⁴ In a retrospective study that included a large number of subjects, however,⁹⁴ we found that 41 of 101 (41%) subjects with late reactions but also 11 of 63 (17%) of subjects with immediate reactions had a significant increase in non-specific bronchial responsiveness.

Inhalation challenges may be falsely negative because the subject has been away from work for a long period and lost his or her sensitivity to isocyanates.⁶ The subject may have been exposed to the wrong agent. We have already mentioned that some subjects develop asthma only after exposure to prepolymers of isocyanate and not after exposure to the parent monomers.³⁰⁻³¹ False positive reactions could occur after exposure to irritant concentrations of isocyanates, particularly in subjects with pronounced non-specific bronchial responsiveness. Indeed, the temporal pattern of immediate reactions due to hypersensitivity to isocyanates is similar to what has been described for reactions induced by non-specific agents.⁹⁵ For obvious ethical reasons, the degree of exposure to isocyanates that could result in irritant reactions has never been established for humans. Nevertheless, some studies have shown that concentrations of TDI below 20 ppb do not induce bronchial reactions in non-exposed asthmatic and non-asthmatic subjects.⁴⁴⁻⁹⁰ The generation of low and stable concentrations of isocyanates as provided by the closed circuit methodology described should increase the diagnostic reliability of inhalation challenges.

Some physicians are reluctant to perform inhalation tests because they can induce severe asthmatic reactions. Inhalation challenges with isocyanates can be a safe method provided that the mentioned precautions are taken. The data obtained using our closed-circuit system indicate that it induces less severe reactions especially in the case of the immediate component.⁸⁹ This suggests that improving the methodology of inhalation challenges to ensure more accurate control of exposure to isocyanates

will further increase the safety of the tests. The use of specific inhalation tests has also been restricted because they are expensive and time consuming. In this respect, it should be kept in mind that a diagnosis of occupational asthma implies considerable medical, financial, and social consequences. We believe that the costs of inhalation tests are largely counterbalanced by those related to the wrong diagnoses that inevitably result when inhalation challenges are not used.

OUTCOME AND MANAGEMENT

There is now accumulating evidence that removal of the workers with occupational asthma from exposure to isocyanates does not lead to a complete recovery even though their condition often improves. Several follow up studies have shown that more than 50% of affected workers still have symptoms of asthma and non-specific bronchial hyperresponsiveness after cessation of exposure even after several years (table 3).⁹⁶⁻⁹⁹ Specific bronchial reactivity to isocyanates may also persist for a long time after exposure.¹⁸⁻⁹⁹⁻¹⁰⁰ Little is known about the factors that can predict the remission or persistence of asthma after exposure to isocyanates ends. Studies on occupational asthma due to various agents have shown that the persistence of asthma after cessation of work was associated with a longer duration of symptoms before diagnosis and with a more severe asthma as defined by the degree of airway obstruction and non-specific bronchial hyperresponsiveness at the time of diagnosis.¹⁰¹⁻¹⁰² These findings outline the importance of early diagnosis. The mechanisms by which specific and non-specific airway reactivity persist in most affected subjects after removal from exposure to the offending agent are still unknown. Studies with bronchoalveolar lavage and bronchial biopsies showed that airway inflammation persisted while the thickening of basement membrane reversed in subjects with persistent bronchial hyperresponsiveness after removal from exposure.⁵⁴⁻¹⁰³

Affected subjects who remain exposed to even very low concentrations of isocyanates at work develop chronic asthma and progressive worsening of lung function and non-specific bronchial responsiveness.⁹⁷⁻¹⁰⁴ This is consistent with our finding

Table 3 Outcome of isocyanate induced asthma: persistence of symptoms and non-specific bronchial hyper-responsiveness after removal from work exposure

No of subjects	Duration of follow up* (y)	Persistence of asthma symptoms (%)	Persistence of non-specific bronchial hyper-responsiveness(%)†	Reference No
12	1-3	66	7/12 (58)	96
50	> 4	82	12/19 (63)	97
20	0.5-4	50	9/12 (75)	98
22	1	77	17/22 (77)	99

*Mean or extreme values depending upon available data; †number of subjects with persistent bronchial hyper-responsiveness / number of subjects where bronchial responsiveness was reassessed during follow up.

that low concentrations of isocyanates can still induce asthmatic reactions provided that the duration of exposure is long enough (unpublished data). There is so far no evidence that respiratory protective devices are efficient in preventing chronic asthma and impairment of lung function. Furthermore, exposure to isocyanates at the workplace in sensitised subjects may lead to life threatening asthma attacks.⁵² Subjects with occupational asthma should therefore be definitively removed from exposure to isocyanates and benefit from adapted compensation and rehabilitation programmes.¹⁰⁵ The guidelines used to determine permanent disability should accurately reflect the severity of asthma and include the degree of airway obstruction, the level of non-specific bronchial hyperresponsiveness, and the need for anti-asthma medication.

The effectiveness of preventive measures on the frequency of occupational asthma has so far been assessed only rarely.¹⁰⁶ Pre-employment screening programmes aimed at identifying susceptible workers—for example, those with atopy, non-specific bronchial hyperresponsiveness, or smoking habits—are inappropriately discriminatory as none of these features has been shown to be a significant risk factor. Thus preventive measures should be primarily aimed at reducing the level of exposure to isocyanates. Changes in production processes should be considered whenever possible. The replacement of the highly volatile TDI and HDI by MDI and prepolymers that are less volatile has not been successful, as the last can also cause asthma.

Extrinsic alveolitis

Isocyanates have been found to cause a pattern of reaction characterised by features of parenchymal lung involvement (restrictive breathing defect, impairment of diffusion capacity for carbon monoxide, and parenchymal infiltrates on chest radio-

graph) associated with systemic symptoms (general malaise, chills, fever and increased blood leucocyte count)^{28 107-119} These features are similar to those described in extrinsic alveolitis due to various organic substances.¹²⁰

NATURAL HISTORY AND EPIDEMIOLOGY

By contrast with asthma, extrinsic alveolitis has only been described in isolated case reports (table 4).^{28 107-119} Using specific inhalation challenges, we demonstrated extrinsic alveolitis in 8/167 (4.7%) workers exposed to a resin containing MDI in a wood chipboard manufacturing plant.¹²¹ The subjects we investigated were all those who filled a claim at the local Workers' Compensation Board after the introduction of MDI to the plant as a thorough medical survey of the whole workforce could not be conducted. The 4.7% figure is therefore probably an underestimate of the actual prevalence of extrinsic alveolitis in the plant. Although we have no definite explanation for this high prevalence, the possibility that extrinsic alveolitis might have been overlooked in previous cross sectional surveys should be considered. Indeed, in our study, systemic symptoms were so severe that affected subjects had to leave the plant shortly after the onset of symptoms. No single predisposing factor could be identified but exposure to high concentrations of MDI due to inadequate safety controls during the first weeks after the use of the MDI resin began at the plant should be suspected as the essential factor. Furthermore, it is interesting to note that only one of our eight subjects was working permanently near the source of the MDI, suggesting that even intermittent exposure can lead to extrinsic alveolitis. Our study indicates that under some undefined circumstances, extrinsic alveolitis due to isocyanates could be a more common occurrence than is generally thought.

Table 4 Hypersensitivity pneumonitis caused by isocyanates: main features of the subjects reported in the literature

Isocyanate	No	Inhalation challenge	Specific IgE	Specific IgG	Precipitating antibodies	Bronchoalveolar lavage	Reference No
TDI	3	ND	+	ND	ND	ND	107
HDI	1	ND	ND	ND	ND	ND	107
TDI	1	+	ND	—	—	ND	108
MDI	1	ND	+	+	+	ND	109
MDI	1	+	ND	+	ND	ND	110
HDI	1	+	—	+	ND	ND	111
MDI	1	+	—	+	ND	ND	112
HDI	1	+	—	ND	—	ND	113
MDI(?)	1	+	ND	ND	ND	40%Ly, $T_H/T_S < 1$	114
MDI	1	+	ND	+	ND	67%Ly, anti-MDI IgG	115
MDI	1	ND	+	+	—	ND	116
HDI	1	ND	—	+	ND	ND	117
TDI	1	ND	ND	+	ND	74%Ly, $T_H/T_S < 1$	118
						anti-TDI IgG and IgA	
HDI, TDI	1	NR	+	+	ND	ND	119
MDI	8	+	8/8	8/8	ND	24%Ly, 17%Ne, $T_H/T_S < 1$	121
						37%Ly, 32%Ne	
						anti-MDI IgG and IgE	

ND = Not done; Ly = lymphocytes; Ne = neutrophils; T_H = T Helper lymphocytes (CD4); T_S = T suppressor lymphocytes (CD8).

PATHOGENESIS

The mechanisms leading to the extrinsic alveolitis type of reaction caused by isocyanates remain largely unknown. Hypersensitivity to isocyanates rather than a direct toxic effect is strongly supported by the fact that symptoms occur after a latency period and colleagues working in the same environment fail to show any adverse effect. As for hypersensitivity due to organic agents,¹²¹ there are several lines of evidence supporting involvement of both type III IgG-dependent and type IV cellular immunity. Specific IgG antibodies to isocyanates have been detected in the serum of affected subjects,^{28 109 110 115-118 122} although they may also be present in exposed workers with no symptoms.⁶¹ Specific antibodies have also been found in bronchoalveolar lavage fluid of two subjects^{115 118} but the role of these antibodies in the development of extrinsic alveolitis has not so far been established. An excess of alveolar T lymphocytes, predominantly of T suppressor lymphocytes, has been shown to occur in five subjects in which bronchoalveolar lavage was performed,^{114-116 121} suggesting that cell mediated immunity may also play an important part. Conjugates of TDI with human serum albumin were found to be capable of inducing an in vitro proliferation of bronchoalveolar lavage lymphocytes from one affected subject.¹¹⁸ As occurs for extrinsic alveolitis due to organic agents,¹²³ an increase in alveolar neutrophils may precede the lymphocytic alveolitis.

DIAGNOSIS

The subjects with extrinsic alveolitis induced by isocyanates typically experience a general malaise—a “flu-like syndrome” including headaches, nausea, muscle aches, chills, and fever—which occurs late in the workshift or even after it. Most subjects fail to show interstitial infiltrates and a restrictive breathing pattern. These features may also be absent in bird fancier's or farmer's lung disease.¹²⁰ The presence of IgG antibodies directed at the incriminated isocyanate should not be regarded as being pathognomonic of isocyanate induced extrinsic alveolitis. In our study, high concentrations of specific IgG to MDI were found in only three of the eight subjects. Thus the diagnosis of isocyanate induced extrinsic alveolitis should not be based solely on the clinical history, chest radiograph, and baseline function tests but on the objective demonstration that exposure to non-irritant concentrations of isocyanates causes alveolar and systemic reactions. A rise in body temperature above 37.2°C, an increase in circulating neutrophils of 2500/mm³ or more, and a fall in FVC $\geq$ 15% are the most sensitive and specific indices of a positive alveolar reaction.¹²⁴ Some degree of airways obstruction can be seen in association with the

alveolar response.^{28 122} The monitoring of body temperature and assessments of white blood cell count should be included as a routine procedure for inhalation challenges with isocyanates.

OUTCOME AND MANAGEMENT

Little is known about the prognosis of subjects with isocyanate induced extrinsic alveolitis. There has only been one case of life threatening respiratory failure due to a haemorrhagic pneumonitis in a subject exposed to MDI who had specific IgG antibodies.¹²² Some affected subjects show a significant functional impairment at the time of diagnosis¹⁰⁷ but there is no information on the long term prognosis for these subjects. Most subjects, however, have no permanent disability after ceasing work. There is one report of a subject with extrinsic alveolitis who remained without symptoms despite continuous exposure to TDI;¹¹⁸ however, the last assertion has been seriously questioned.¹²⁵ Complete removal from exposure at work should therefore be recommended for all subjects.

Conclusion

Because of their high chemical reactivity and widespread industrial use, isocyanates are now the principal cause of occupational asthma in industrialised countries. Every isocyanate, regardless of its molecular form or volatility, should be considered as potentially hazardous. The mechanisms leading to isocyanate induced occupational asthma are still a matter of intense research. An IgE or IgG mediated mechanism has so far not been convincingly shown but should not be categorically excluded because immunological studies are limited by difficulties in preparing adequate antigen substrates. There is also evidence that inflammation and morphological changes of the bronchial mucosa and direct neurogenic mechanisms could have an important role. The frequency of occupational asthma among workers exposed to isocyanates is still uncertain because there have been few population based surveys and all of them were cross sectional. Careful longitudinal studies of exposed populations are required to determine the actual frequency of the disease as well as to investigate its natural history and identify potential risk factors. It is important to confirm the diagnosis through objective tests and to recommend complete removal from exposure to isocyanates. Methods that allow for a more accurate control of the level of exposure to isocyanates during inhalation challenges should be further developed. Such methods will increase the safety of the tests and will contribute to a more satisfactory standardisation of inhalation challenges performed for diagnostic and research purposes. The study of isocyanate induced asthma can further our under-

standing of non-occupational asthma from the pathophysiological and epidemiological points of view. Isocyanate induced asthma is a good model because subjects can be examined before exposure, to the causative agent, when they develop asthma, and after they have been removed from exposure, which is not feasible for non-occupational asthma.

Isocyanates have also been reported to cause alveolar and systemic reactions consistent with extrinsic alveolitis. The pathogenesis of these reactions is far from being elucidated. Current data suggest that both IgG mediated and cellular immunological mechanisms are involved. Although extrinsic alveolitis has only been described in anecdotal case reports, the possibility that such reactions have been overlooked in cross sectional surveys of exposed workers should be considered.

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- Schauerte K, Dahm M, Diller W, Uhlig K. Sonderdruck aus Oertel Polyurethane Handbook. Raw materials. München: Carl Hanser Verlag, 1985:116.
- Musk AW, Peters JM, Wegman DH. Isocyanates and respiratory disease: current status. *Am J Ind Med* 1988;13:331-49.
- Davies RJ. Respiratory hypersensitivity to diisocyanates. *Clin Immunol Allergy* 1984;4:103-24.
- Lagier F, Cartier A, Malo JL. Medico-legal statistics on occupational asthma in Quebec between 1986 and 1988. *Rev Mal Respir* 1990;7:337-41.
- Meredith SK, Taylor VM, McDonald JC. Occupational respiratory disease in the United Kingdom 1989: a report to the British Thoracic Society and the Society of Occupational Medicine by the SWORD project group. *Br J Ind Med* 1991;48:292-8.
- Banks DE, Sastre J, Butcher BT, et al. Role of inhalation challenge testing in the diagnosis of isocyanate-induced asthma. *Chest* 1989;95:414-23.
- Silk SH, Hardy HL. Control limits for diisocyanates. *Ann Occup Hyg* 1983;27:333-9.
- Hardy HL, Devine JM. Use of organic isocyanates in industry. Some industrial hygiene aspects. *Ann Occup Hyg* 1979;22:421-7.
- Törnling G, Alevanderson R, Hendenstiema G, Plato N. Decreased lung function and exposure to diisocyanates (HDI and HDI-BT) on re-examination 6 years after initial study. *Am J Ind Med* 1990;17:299-310.
- Pepys J, Hutchcroft BJ. Bronchial provocation tests in etiologic diagnosis and analysis of asthma. *Am Rev Respir Dis* 1975;112:829-59.
- American Conference of Governmental Industrial Hygienists. *TLVs. Threshold limit values and biological exposure indices for 1986-1987*. ACGIH, Cincinnati: ACGIH, 1987.
- US Department of Health, Education and Welfare. Occupational exposure to diisocyanates. NIOSH criteria document for a recommended standard. Washington DC: US Department of Health, Education and Welfare, ACGIH, 1987.
- Newman Taylor A. Occupational asthma. *Thorax* 1980;35:241-5.
- Fuchs S, Valade P. Etude clinique et expérimentale sur quelques cas d'intoxication par le Desmodur T (diisocyanate de toluylène 1-2-4 et 1-2-6). *Archives des maladies professionnelles de médecine du travail et de sécurité sociale* 1951;12:191-6.
- Brugsch HG, Elkins HB. Toluene di-isocyanate (TDI) toxicity. *N Engl J Med* 1963;268:353-7.
- Brooks S M. The evaluation of occupational airways disease in the laboratory and workplace. *J Allergy Clin Immunol* 1982;70:56-66.
- Malo JL, Ghezzi H, D'Aquino C, L'Archevêque J, Chan-Yeung M. Natural history of occupational asthma: relevance of type of agent and other factors in the rate of development of symptoms in affected subjects. *J Allergy Clin Immunol* 1993 (in press).
- Moller DR, McKay RT, Bernstein IL, Brooks SM. Persistent airways disease caused by toluene diisocyanate. *Am Rev Respir Dis* 1986;134:175-6.
- Pepys J, Pickering CAC, Terry DJ. Asthma due to inhaled chemical agents—tolylene di-isocyanate. *Clin Allergy* 1972;2:225-36.
- Tanser AR, Bourke MP, Blandford AG. Isocyanate asthma: respiratory symptoms caused by diphenyl-methane diisocyanate. *Thorax* 1973;28:596-600.
- Zammit-Tabona M, Sherkin M, Kijek K, Chan H, Chan-Yeung M. Asthma caused by diphenylmethane diisocyanate in foundry workers. Clinical, bronchial provocation, and immunologic studies. *Am Rev Respir Dis* 1983;128:226-30.
- Cockcroft DW, Mink JT. Isocyanate-induced asthma in an automobile spray painter. *Can Med Assoc J* 1979;121:602-4.
- O'Brien IM, Harries MG, Burge PS, Pepys J. Toluene di-isocyanate-induced asthma. I. Reactions to TDI, MDI, HDI and histamine. *Clin Allergy* 1979;9:1-6.
- Clarke CW, Aldons PM. Isophorone diisocyanate induced respiratory disease (IPDI). *Aust NZ J Med* 1981;11:290-2.
- Harries MG, Burge PS, Samson M, Newman Taylor AJ, Pepys J. Isocyanate asthma: respiratory symptoms due to 1,5-naphthylene di-isocyanate. *Thorax* 1979;34:762-6.
- Peters JM, Murphy RLH. Hazards to health: Do it yourself polyurethane foam. *Am Rev Respir Dis* 1971;104:432-3.
- Belin L, Hjortsberg U, Wass U. Life-threatening pulmonary reaction to car paint containing a prepolymerized isocyanate. *Scand J Work Environ Health* 1981;7:310-2.
- Malo JL, Ouimet G, Cartier A, Levitz D, Zeiss R. Combined alveolitis and asthma due to hexamethylene diisocyanate (HDI), with demonstration of crossed respiratory and immunologic reactivities to diphenylmethane diisocyanate (MDI). *J Allergy Clin Immunol* 1983;72:413-9.
- Séguin P, Allard A, Cartier A, Malo JL. Prevalence of occupational asthma in spray painters exposed to several types of isocyanates, including polymethylene polyphenylisocyanates. *J Occup Med* 1987;29:340-4.
- Vandenplas O, Malo JL, Cartier A. Occupational asthma caused by a prepolymer but not the monomer of toluene diisocyanate (TDI). *J Allergy Clin Immunol* 1991;87:A645.
- Vandenplas O, Cartier A, Lesage J, et al. Prepolymers of hexamethylene diisocyanate (HDI) as a cause of occupational asthma. *J Allergy Clin Immunol* 1993 (in press).
- Butcher BT, Jones RN, O'Neil CE, et al. Longitudinal study of workers employed in the manufacture of toluene-diisocyanate. *Am Rev Respir Dis* 1977;116:411-21.
- White WG, Sudgen E, Morris MJ, Zapata E. Isocyanate-induced asthma in a car factory. *Lancet* 1980;i:756-60.
- Baur X, Fruhmman G. Specific IgE antibodies in patients with isocyanate asthma. *Chest* 1981;80:73-6S.
- Liss GM, Bernstein DI, Moller DR, Gallagher JS, Stephenson RL, Bernstein IL. Pulmonary and immunologic evaluation of foundry workers exposed to methylene diphenyldiisocyanate (MDI). *J Allergy Clin Immunol* 1988;82:55-61.
- Grammer LC, Eggum P, Silverstein M, Shaughnessy MA, Liotta JL, Patterson R. Prospective immunologic and clinical study of a population exposed to hexamethylene diisocyanate. *J Allergy Clin Immunol* 1988;82:627-33.
- Butcher BT, O'Neil CE, Reed MA, Salvaggio JE. Radioallergosorbent testing of toluene diisocyanate-reactive individuals using p-tolyl isocyanate antigen. *J Allergy Clin Immunol* 1980;66:213-6.
- Karol MH, Alarie Y. Antigens which detect IgE antibodies in workers sensitive to toluene diisocyanate. *Clin Allergy* 1980;10:101-9.
- Patterson R, Hargreave FE, Grammer LC, Harris KE, Dolovich J. Toluene diisocyanate respiratory reactions 1. Reassessment of the problem. *Int Arch Allergy Appl Immunol*

- 1987;84:93-100.
- 40 Cartier A, Grammer L, Malo JL, *et al.* Specific serum antibodies against isocyanates: association with occupational asthma. *J Allergy Clin Immunol* 1989;84:507-14.
- 41 Davies RJ, Butcher BT, O'Neil CE, Salvaggio JE. The in vitro effect of toluene diisocyanate on lymphocyte cyclic adenosine monophosphate production by isoproterenol, prostaglandin, and histamine. A possible mode of action. *J Allergy Clin Immunol* 1977;60:223-9.
- 42 Gallagher JS, Tse CST, Brooks SM, Bernstein IL. Diverse profiles of immunoreactivity in toluene diisocyanate (TDI) asthma. *J Occup Med* 1981;23:610-6.
- 43 Finotto S, Fabbri LM, Rado V, Mapp CE, Maestrelli P. Increase in numbers of CD8 positive lymphocytes and eosinophils in peripheral blood of subjects with diisocyanate. *Br J Ind Med* 1991;48:116-21.
- 44 Mapp CE, Di Giacomo GR, Omini C, Broseghini C, Fabbri LM. Late, but not early, asthmatic reactions induced by toluene-diisocyanate are associated with increased airway responsiveness to methacholine. *Eur J Respir Dis* 1986;69:276-84.
- 45 Mapp CE, Polato R, Maestrelli P, Hendrick DJ, Fabbri LM. Time course of the increase in airway responsiveness associated with late asthmatic reactions to toluene diisocyanate in sensitized subjects. *J Allergy Clin Immunol* 1985;75:568-72.
- 46 Fabbri LM, Boschetto P, Zocca E, *et al.* Bronchoalveolar neutrophilia during late asthmatic reactions induced by toluene diisocyanate. *Am Rev Respir Dis* 1987;136:36-42.
- 47 Boschetto P, Fabbri LM, Zocca E, *et al.* Prednisone inhibits late asthmatic reactions and airway inflammation induced by toluene diisocyanate in sensitized subjects. *J Allergy Clin Immunol* 1987;80:261-7.
- 48 Zocca E, Fabbri LM, Boschetto P, *et al.* Leukotriene B4 and late asthmatic reactions induced by toluene diisocyanate. *J Appl Physiol* 1990;68:1576-80.
- 49 Fabbri LM, Chiesura-Corona P, Vecchio L, Dal, *et al.* Prednisone inhibits late asthmatic reactions and the associated increase in airway responsiveness induced by toluene-diisocyanate in sensitized subjects. *Am Rev Respir Dis* 1985;132:1010-4.
- 50 DeMarzo N, Fabbri LM, Crescioli S, Plebani M, Testi R, Mapp CE. Dose-dependent inhibitory effect of inhaled beclomethasone on late asthmatic reactions and increased airway responsiveness to methacholine induced by toluene diisocyanate in sensitized subjects. *Pulmonary Pharmacology* 1988;1:15-20.
- 51 Mapp C, Boschetto P, Dal Vecchio L, *et al.* Protective effect of antiasthma drugs on late asthmatic reactions and increased airway responsiveness induced by toluene diisocyanate in sensitized subjects. *Am Rev Respir Dis* 1987;136:1403-7.
- 52 Fabbri LM, Danieli D, Crescioli S, *et al.* Fatal asthma in a subject sensitized to toluene diisocyanate. *Am Rev Respir Dis* 1988;137:1494-8.
- 53 Saetta M, Stefano A, Di, Maestrelli P, *et al.* Airway mucosal inflammation in occupational asthma induced by toluene diisocyanate. *Am Rev Respir Dis* 1992;145:160-8.
- 54 Saetta M, Maestrelli P, DiStefano A, *et al.* Effect of cessation of exposure to toluene diisocyanate (TDI) on bronchial mucosa of subjects with TDI induced asthma. *Am Rev Respir Dis* 1992;145:169-74.
- 55 Mapp CE, Chitano P, Fabbri LM, *et al.* Evidence that toluene diisocyanate activates the efferent function of capsaicin-sensitive primary afferents. *Eur J Pharmacol* 1990;180:113-8.
- 56 Mapp CE, Boniotti A, Graf PD, Chitano P, Fabbri LM, Nadel JA. Bronchial smooth muscle responses evoked by toluene diisocyanate are inhibited by ruthenium red and by indomethacin. *Eur J Pharmacol* 1991;200:73-6.
- 57 Mapp CE, Graf PD, Boniotti A, Nadel JA. Toluene diisocyanate contracts guinea pig bronchial smooth muscle by activating capsaicin-sensitive sensory nerves. *J Pharmacol Exp Ther* 1991;256:1082-5.
- 58 Mapp CE, Chitano P, Fabbri LM, Patacchini R, Maggi CA. Pharmacological modulation of the contractile response to toluene diisocyanate in the rat isolated urinary bladder. *Br J Pharmacol* 1990;100:886-8.
- 59 Malo JL, Ghezdo H, L'Archevêque J, Lagier F, Perrin B, Cartier A. Is the clinical history a satisfactory means of diagnosing occupational asthma? *Am Rev Respir Dis* 1991;143:528-32.
- 60 Bernstein IL. Isocyanate-induced pulmonary diseases: a current perspective. *J Allergy Clin Immunol* 1982;70:24-31.
- 61 Pezzini A, Riviera A, Paggiaro P, *et al.* Specific IgE antibodies in twenty-eight workers with diisocyanate-induced bronchial asthma. *Clin Allergy* 1984;14:453-61.
- 62 Welinder H, Nielsen J, Bensryd I, Skeruing S. IgG antibodies against polyisocyanates in car painters. *Clin Allergy* 1988;18:85-93.
- 63 Keskinen H, Tupasela O, Tiikkainen U, Nordman H. Experiences of specific IgE in asthma due to diisocyanates. *Clin Allergy* 1988;18:597-604.
- 64 Tse CST, Pesce AJ. Chemical characterization of isocyanate-protein conjugates. *Toxicol App Pharmacol* 1979;51:39-46.
- 65 Kochman S, Lefebvre S, Bernard J, *et al.* Toluene diisocyanate-induced conformational changes of serum albumin: a study on repeated inhalations in guinea-pigs. *Toxicol Lett* 1990;50:165-71.
- 66 Wass U, Belin L. Immunologic specificity of isocyanate-induced IgE antibodies in serum from 10 sensitized workers. *J Allergy Clin Immunol* 1989;83:126-35.
- 67 Grammer LC, Harris KE, Malo JL, Cartier A, Patterson R. The use of an immunoassay index for antibodies against isocyanate human protein conjugates and application to human isocyanate disease. *J Allergy Clin Immunol* 1990;86:94-8.
- 68 Baur X. Immunologic cross-reactivity between different albumin-bound isocyanates. *J Allergy Clin Immunol* 1983;71:197-205.
- 69 Karol MH, Ioset HH, Alarie YC. Toluyl-specific IgE antibodies in workers with hypersensitivity to toluene diisocyanate. *Am Ind Hyg Assoc J* 1978;39:454-8.
- 70 Karol MH, Hauth BA. Use of hexyl isocyanate antigen to detect antibodies to hexamethylene diisocyanate (HDI) in sensitized guinea pigs and in a sensitized worker. *Fundam Appl Toxicol* 1982;2:108-13.
- 71 Burge PS. Non-specific bronchial hyper-reactivity in workers exposed to toluene di-isocyanate, diphenyl methane di-isocyanate and colophony. *European Journal of Respiratory Diseases* 1982;63(suppl 123):91-6.
- 72 Butcher BT, O'Neil CE, Reed MA, Salvaggio JE, Weill H. Development and loss of toluene diisocyanate reactivity: immunologic, pharmacologic, and provocative challenge studies. *J Allergy Clin Immunol* 1982;70:231-5.
- 73 Hargreave FE, Ramsdale EH, Pugsley SO. Occupational asthma without bronchial hyperresponsiveness. *Am Rev Respir Dis* 1984;130:513-5.
- 74 Mapp CE, Dal Vecchio L, Boschetto P, Marzo N, De, Fabbri LM. Toluene diisocyanate-induced asthma without airway hyperresponsiveness. *Eur J Respir Dis* 1986;68:89-95.
- 75 Smith AB, Brooks SM, Blanchard J, Bernstein IL, Gallagher J. Absence of airway hyperreactivity to methacholine in a worker sensitized to toluene diisocyanate. *J Occup Med* 1980;22:327-31.
- 76 Banks DE, Jr Barkman HW, Butcher BT, *et al.* Absence of hyperresponsiveness to methacholine in a worker with methylene diphenyl diisocyanate (MDI)-induced asthma. *Chest* 1986;89:389-93.
- 77 Cartier A, Bernstein IL, Burge PS, *et al.* Guidelines for bronchoprovocation on the investigation of occupational asthma. Report of the subcommittee on bronchoprovocation for occupational asthma. *J Allergy Clin Immunol* 1989;84:823-9.
- 78 Burge PS. Single and serial measurements of lung function in the diagnosis of occupational asthma. *European Journal of Respiratory Diseases* 1982;63:47-59.
- 79 Burge PS, O'Brien IM, Harries MG. Peak flow rate records in the diagnosis of occupational asthma due to isocyanates. *Thorax* 1979;34:317-23.
- 80 Venables KM, Dally MB, Burge PS, Pickering CAC, Newman Taylor AJ. Occupational asthma in a steel coating plant. *Br J Ind Med* 1985;42:517-24.
- 81 Perrin B, Lagier F, L'Archevêque J, *et al.* Validity of investigating occupational asthma with serial monitoring of peak expiratory flow rates and bronchial responsiveness as compared to specific inhalation challenges. *J Allergy Clin Immunol* 1991;87:200 (abstract).
- 82 Côté J, Kennedy S, Chan-Yeung M. Sensitivity and specificity of PC 20 and peak expiratory flow rate in cedar asthma. *J Allergy Clin Immunol* 1990;85:592-8.
- 83 Butcher BT. Inhalation challenge testing with toluene diisocyanate. *J Allergy Clin Immunol* 1979;64:655-7.
- 84 Gerblach AA, Horowitz J, Chester EH, Schwartz HJ, Fleming GM. A proposed standardized method for bronchoprovocation tests in toluene diisocyanate-induced asthma. *J*

- Allergy Clin Immunol* 1979;64:658-61.
- 85 Mapp CE, Boschetto P, Dal Vecchio L, Maestrelli P, Fabbri LM. Occupational asthma due to isocyanates. *Eur Respir J* 1988;1:273-9.
 - 86 Perrin B, Cartier A, Ghezzi H, et al. Reassessment of the temporal patterns of bronchial obstruction after exposure to occupational sensitizing agents. *J Allergy Clin Immunol* 1991;87:630-9.
 - 87 Huynh CK, Doc T Vu, Savolainen H. Controlled dynamic generation of standard 1,6 hexamethylene diisocyanate aerosols. *Ann Occup Hyg* 1989;33:573-81.
 - 88 Hammad YY, Rando RJ, Abdel-Kader H. Considerations in the design and use of human inhalation challenge delivery systems. *Folia Allergologica Immunologica* 1985;32:37-44.
 - 89 Vandenplas O, Malo JL, Cartier A, Perrault G, Cloutier Y. Closed-circuit methodology for inhalation challenges with isocyanates. *Am Rev Respir Dis* 1992;145:582-7.
 - 90 Chester EH, Martinez-Catnichi FL, Schwartz HJ, et al. Patterns of airway reactivity to asthma produced by exposure to toluene diisocyanate. *Chest* 1979;75:229-31.
 - 91 Siracusa A, Curradi F, Abbritti G. Recurrent nocturnal asthma due to tolylene di-isocyanate: a case report. *Clin Allergy* 1978;8:195-201.
 - 92 Paggiaro PL, Innocenti A, Bacci E, Rossi O, Talini D. Specific bronchial reactivity to toluene diisocyanate: relationship with baseline clinical findings. *Thorax* 1986;41:279-82.
 - 93 Innocenti A, Cirila AM, Pisati G, Mariano A. Cross-reaction between aromatic isocyanates (TDI and MDI): a specific bronchial provocation test study. *Clin Allergy* 1988;18:323-9.
 - 94 Malo JL, Ghezzi H, L'Archevêque J, Cartier A. Late asthmatic reactions to occupational sensitizing agents: frequency of changes in nonspecific bronchial responsiveness and of response to inhaled beta-2 adrenergic agent. *J Allergy Clin Immunol* 1990;85:834-42.
 - 95 Cartier A, Malo JL, Bégin P, Sestier M, Martin RR. Time-course of the bronchoconstriction induced by inhaled histamine and methacholine. *J Appl Physiol* 1983;54:821-6.
 - 96 Paggiaro PL, Loi AM, Rossi O, et al. Follow-up study of patients with respiratory disease due to toluene diisocyanate (TDI). *Clin Allergy* 1984;14:463-9.
 - 97 Lozewicz S, Assoufi BK, Hawkins R, Newman-Taylor AJ. Outcome of asthma induced by isocyanates. *Br J Dis Chest* 1987;81:14-27.
 - 98 Rosenberg N, Garnier R, Rousselin X, Mertz R, Gervais P. Clinical and socio-professional fate of isocyanate-induced asthma. *Clin Allergy* 1987;17:55-61.
 - 99 Mapp CE, Corona PC, Marzo N de, Fabbri L. Persistent asthma due to isocyanates. A follow-up study of subjects with occupational asthma due to toluene diisocyanate. *Am Rev Respir Dis* 1988;137:1326-9.
 - 100 Moller DR, Brooks SM, McKay RT, Cassidy K, Kopp S, Bernstein IL. Chronic asthma due to toluene diisocyanate. *Chest* 1986;90:494-9.
 - 101 Chan-Yeung M, MacLean L, Paggiaro PL. Follow-up study of 232 patients with occupational asthma caused by western red cedar (*Thuja plicata*). *J Allergy Clin Immunol* 1987;79:792-6.
 - 102 Hudson P, Cartier A, Pineau L, et al. Follow-up of occupational asthma caused by crab and various agents. *J Allergy Clin Immunol* 1985;76:682-7.
 - 103 Paggiaro P, Bacci E, Dente FL, et al. Bronchoalveolar lavage and morphology of the airways after cessation of exposure in asthmatic subjects sensitized to toluene diisocyanate. *Chest* 1990;98:536-42.
 - 104 Banks DE, Rando RJ, Barkman HW. Persistence of toluene diisocyanate-induced asthma despite negligible workplace exposures. *Chest* 1990;97:121-5.
 - 105 Malo JL. Compensation for occupational asthma in Quebec. *Chest* 1990;98:236S-9S.
 - 106 Wang JD, Huang PH, Lin JM, Su SY, Wu MC. Occupational asthma due to toluene diisocyanate among velcro-like tape manufacturers. *Am J Ind Med* 1988;14:73-8.
 - 107 Charles J, Bernstein A, Jones B, et al. Hypersensitivity pneumonitis after exposure to isocyanates. *Thorax* 1976;31:127-36.
 - 108 Fink JN, Schlueter DP. Bathruber's lung: an unusual response to toluene diisocyanate. *Am Rev Respir Dis* 1978;118:955-9.
 - 109 Zeiss CR, Kanellakes TM, Bellone JD, Levitz D, Pruzansky JJ, Patterson R. Immunoglobulin E-mediated asthma and hypersensitivity pneumonitis with precipitating anti-hapten antibodies due to diphenylmethane diisocyanate (MDI) exposure. *J Allergy Clin Immunol* 1980;65:346-52.
 - 110 Malo JL, Zeiss CR. Occupational hypersensitivity pneumonitis after exposure to diphenylmethane diisocyanate. *Am Rev Respir Dis* 1982;125:113-6.
 - 111 Malo JL, Ouimet G, Cartier A, Levitz D, Zeiss R. Combined alveolitis and asthma due to hexamethylene diisocyanate (HDI), with demonstration of crossed respiratory and immunologic reactivities to diphenylmethane diisocyanate (MDI). *J Allergy Clin Immunol* 1983;72:413-9.
 - 112 Baur X, Dewair M, Rommelt H. Acute airway obstruction followed by hypersensitivity pneumonitis in an isocyanate (MDI) worker. *J Occup Med* 1984;26:285-7.
 - 113 Nielsen J, Sangø C, Winroth G, Hallberg T, Skerfving S. Systemic reactions associated with polyisocyanate exposure. *Scand J Work Environ Health* 1985;11:51-4.
 - 114 Vergnon JM, Wiesendanger T, Pages J, Cordier G, Momex JF, Brune J. Pneumopathie d'hypersensibilité aux isocyanates. Intérêts et dangers du test de provocation réaliste. *Revue des Maladies Respiratoires* 1985;2:83-90.
 - 115 Bascom R, Kennedy TP, Levitz D, Zeiss CR. Specific bronchoalveolar lavage IgG antibody in hypersensitivity pneumonitis from diphenylmethane diisocyanate. *Am Rev Respir Dis* 1985;131:463-5.
 - 116 Walker CL, Grammer LC, Shaughnessy MA, Duffy M, Stoltzfus VD, Patterson R. Diphenylmethane diisocyanate hypersensitivity pneumonitis: a serologic evaluation. *J Occup Med* 1989;31:315-9.
 - 117 Seldén AI, Belin L, Wass U. Isocyanate exposure and hypersensitivity pneumonitis—report of a probable case and prevalence of specific immunoglobulin G antibodies among exposed individuals. *Scand J Work Environ Health* 1989;15:234-7.
 - 118 Yoshizawa Y, Ohtsuka M, Noguchi K, Uchida Y, Suko M, Hasegawa S. Hypersensitivity pneumonitis induced by toluene diisocyanate: sequelae of continuous exposure. *Ann Intern Med* 1989;110:31-4.
 - 119 Patterson R, Nugent KM, Harris KE, Eberle ME. Immunologic hemorrhagic pneumonia caused by isocyanates. *Am Rev Respir Dis* 1990;141:226-30.
 - 120 Salvaggio JE. Hypersensitivity pneumonitis. *J Allergy Clin Immunol* 1987;79:558-71.
 - 121 Vandenplas O, Lévesque J, Cartier A, Grammer LC, Malo JL. Hypersensitivity pneumonitis among workers exposed to diphenylmethane diisocyanate. *Am Rev Respir Dis* 1993 (in press).
 - 122 Patterson R, Nugent KM, Harris KE, Eberle ME. Immunologic hemorrhagic pneumonia caused by isocyanates. *Am Rev Respir Dis* 1990;141:226-30.
 - 123 Fournier E, Tonnel AB, Gosset P, Wallaert B, Ameisen JC, Voisin C. Early neutrophil alveolitis after antigen inhalation in hypersensitivity pneumonitis. *Chest* 1985;88:563-6.
 - 124 Hendrick DJ, Marshall R, Faux JA, Krall JM. Positive "alveolar" responses to antigen inhalation provocation tests: their validity and recognition. *Thorax* 1980;35:415-27.
 - 125 Grammer LC. Hypersensitivity pneumonitis and toluene diisocyanate (letter). *Ann Intern Med* 1989;111:690-1.

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