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Indirect Assessment of 4,4′-Diphenylmethane Diisocyanate (MDI) Exposure by Evaluation of Specific Humoral Immune Responses to MDI Conjugated to Human Serum Albumin

Boris D. Lushniak, MD, MPH,[1] Christopher M. Reh, MS,[1]* David I. Bernstein, MD,[2] and Joan S. Gallagher, PhD[2]

Background: *A study of occupational asthma among workers exposed to 4,4′-Diphenylmethane Diisocyanate (MDI).*

Objective: *To demonstrate if serum concentrations of MDI-specific IgG or IgE are sensitive biological markers of disease or of MDI exposure.*

Methods: *The study group consisted of nine MDI-exposed workers and nine nonexposed workers. Air sampling for MDI and polymethylene polyphenyl isocyanate, occupational and medical histories, respiratory physical exams, pre- and postshift spirometry, and self-administered peak expiratory flow rates were performed. Serum specific IgE and IgG antibodies to an MDI-human serum albumin (HSA) conjugate were assayed by the radioallergosorbent test and the enzyme-linked immunosorbent assay, respectively, and compared to nine nonexposed laboratory controls.*

Results: *No definitive cases of occupational asthma were documented. The mean level of MDI-specific IgG was significantly greater among exposed workers compared to nonexposed workers and laboratory controls ($p = 0.04$). Mean levels of TDI and HDI-specific IgG were also increased.*

Conclusion: *This study demonstrates that serum concentrations of MDI-specific IgG appear to be a moderately sensitive biological marker of MDI exposure, but not an indicator of occupational asthma. Workers with IgG antibodies specific for one diisocyanate-HSA conjugate exhibit cross-reactivity to antigens prepared with other diisocyanates.* Am. J. Ind. Med. 33:471–477, 1998. *© 1998 Wiley-Liss, Inc.*†

KEY WORDS: air sampling; biological marker; 4,4′-diphenylmethane diisocyanate (MDI); foaming operations; IgG; isocyanates; occupational asthma

Abbreviations: ACGIH, The American Conference of Governmental Industrial Hygienists; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; FEV, forced expiratory volume in 1 second; FVC, forced vital capacity; HHE, health hazard evaluation; HDI, 1,6-hexamethylene diisocyanate; HPLC, high performance liquid chromatography; HRF, histamine releasing factor; HSA, human serum albumin; MDI, 4,4′-diphenylmethane diisocyanate; MOPIP, 1-(2-methoxyphenyl)piperazine; $\mu g/m^3$, micrograms per cubic meter of sample air; mg/ml, milligrams per milliliter; ml, milliliters; MQC, minimum quantifiable concentrations; mol/l, moles/liter; NDI, 1,5-naphthalene diisocyanate; NIOSH, National Institute for Occupational Safety and Health; OA, occupational asthma; OSHA, Occupational Safety and Health Administration; PEFR, peak expiratory flow rate; PBS, phosphate buffered saline; RAST, radioallergosorbent test; REL, recommended exposure limit; TLV, threshold limit value; TDI, 2,4- and 2,6-toluene diisocyanate.

[1]Hazard Evaluations and Technical Assistance Branch, Division of Surveillance, Hazard Evaluations, and Field Studies, National Institute for Occupational Safety and Health, Cincinnati, OH

[2]Department of Internal Medicine, Division of Immunology, College of Medicine, University of Cincinnati, OH

*Correspondence to: Christopher M. Reh, NIOSH, Alice Hamilton Laboratory, 4676 Columbia Parkway, Mailstop R-11, Cincinnati, OH 45226.

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INTRODUCTION

Asthma is a common illness estimated to affect between 9 and 12 million persons in the United States [Evans et al., 1987]. The worldwide prevalence of asthma has been estimated to be between 5 and 10% [Chan-Yeung and Malo, 1995]. Occupational asthma (OA) was described as early as 1713 by Bernardo Ramazzini. The proportion of newly diagnosed adult asthma cases in the U.S. that are due to occupational exposure has been estimated to be 15%, which is similar to estimates in Japan [Chan-Yeung and Malo, 1995]. A recent analysis of the data presented by Weiss et al. [1992] indicates that the annual cost of OA is in the range of $205 million to $410.5 million (in 1992 dollars) [personal communication, Dr. Lee Petsonk, NIOSH 1996].

OA is defined as a generalized airway obstruction that is usually reversible, and is caused by inhalation of substances found in the workplace. A worker suspected of having OA will present with the acute symptoms of asthma—coughing, wheezing, shortness of breath, tightness in the chest, and nocturnal awakening. After sensitization, any exposure, even to levels below any occupational exposure limit or standard, can produce an asthmatic response which may progress to respiratory distress. This asthmatic reaction may occur minutes after workplace exposure (immediate response), several hours after exposure (late response), or a combination of both immediate and late components after exposure (dual response).

Some of the most widely used asthmagenic compounds are the diisocyanates. The unique feature common to all diisocyanates is the two $-N=C=O$ (isocyanate) functional groups attached to an aromatic or aliphatic parent compound. Generally, the diisocyanates are referred to by their specific abbreviation; e.g., TDI for 2,4- and 2,6-toluene diisocyanate, HDI for 1,6-hexamethylene diisocyanate, MDI for 4,4′-diphenylmethane diisocyanate, NDI for 1,5-naphthalene diisocyanate. Diisocyanate-containing compounds are widely used in many products including surface coatings, waterproofing coatings, paints, polyurethane foams, insulation, adhesives, resins, elastomers, binders, and sealants. Past research has shown that prevalence estimates for diisocyanate-induced asthma in exposed worker populations vary from 5 to 30% [Butcher et al., 1993; Weill, 1979; White et al., 1988; Porter et al., 1975; Adams, 1975; Diem et al., 1982].

Specific IgE and IgG antibodies to TDI, MDI, HDI, and the polyisocyanate forms of MDI and HDI, conjugated to human serum albumin (HSA), have been measured in exposed workers, but are not always detected in sensitized (asthmatic) workers [Zammit-Tabona et al., 1983; Karol et al., 1979a,b; Karol, 1981; Butcher et al., 1980; Game, 1982; Grammar et al., 1988; Broughton et al., 1988; Liss et al., 1988; Tse et al., 1985; Chang and Karol, 1984]. Considerable evidence exists indicating that workers with IgG or IgE antibodies specific for one diisocyanate-HSA conjugate exhibit cross-reactivity to antigens prepared with other diisocyanates, to which there have been no workplace exposures [O'Brien et al., 1979; Baur, 1983; Baur and Fruhman, 1981; Malo et al., 1983]. A recent study of workers with confirmed diisocyanate-induced asthma found that diisocyanate-HSA antigens stimulated the production of histamine releasing factor (HRF) by the peripheral blood mononuclear cells [Herd and Bernstein, 1994]. The presence of IgE or IgG antibodies did not correlate with HRF activity, nor with a diagnosis of OA. These data suggest that specific cellular immune responses could be more important than humoral mechanisms in understanding the pathogenesis of diisocyanate-induced asthma. Thus, the role of specific antibodies in the diisocyanate exposure-response continuum is unclear. The purpose of this study was to determine if there was any relationship between the presence of antibodies to diisocyanates and a worker's exposure or disease status.

The National Institute for Occupational Safety and Health (NIOSH) conducted a health hazard evaluation (HHE) at a facility that manufactured refrigerated tractor trailers (known as reefers) for the trucking industry. The HHE request centered on exposures to diisocyanates in the polyurethane foaming areas of the facility, and on the risk of asthma among exposed workers. A two-component polyurethane foam system was used to insulate the walls, ceiling, floor, and doors of the reefer. Component A of this system consisted of 40–50% MDI and 50–60% of MDI-based polyisocyanate (polymethylene polyphenyl isocyanate); component B contained a mixture of polyether polyols, surfactants, catalysts, and blowing agents. The two components are mixed in the nozzle of the spray gun, and injected into the space inside the reefer's walls. An expanding foam is formed that fills the space and forms a layer of insulation.

MATERIALS AND METHODS

A cross-sectional study design was used to determine if 1) workers exposed to MDI had respiratory symptoms and pulmonary function decrements consistent with OA, and 2) if there was any relationship between the presence of antibodies to MDI-HSA and a worker's exposure or disease status. The protocol for this study was reviewed and approved by the NIOSH Human Subjects Review Board. The study group consisted of nine MDI-exposed workers (eight current foamers and 1 painter who had formerly been a foamer) and nine nonexposed painters. None of the workers had previous known exposures to TDI or HDI. Diisocyanate-containing polyurethane paints were never used at this facility, and the polyurethane foaming and the painting areas were separated by a considerable distance. In addition, most of the painting was performed in a booth, which isolated the painters from exposures related to other operations. Workers in the painting area donned air-purify-

ing, half-face respirators with organic vapor cartridges, and each worker received annual fit-testing and respirator training. None of the foamers were provided with or wore respiratory protection. The study design included three investigatory elements—respiratory, immunologic, and industrial hygiene.

Respiratory Evaluation

The respiratory evaluation consisted of a self-administered questionnaire, pre- and postshift spirometry over the course of one mid-week shift, pulmonary exams, and measurement of peak expiratory flow rates (PEFRs). Occupational and medical histories were obtained using the questionnaire, which included questions on the existence of respiratory symptoms. Workers were defined as being symptomatic of possible OA if they reported at least two of the following workplace-related symptoms: wheezing, chest tightness, and shortness of breath. The spirometry generated forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) data for each worker to determine the presence of a pulmonary function decrement, and if a change in pulmonary function occurred across the work shift. Abnormal pulmonary function was evaluated using standard values adjusted for age, gender, and height [Knudson et al., 1976]. The physical examination was considered abnormal if wheezing was present on auscultation. Finally, the workers were given a brief training session on how to measure PEFRs with a portable Wright peak flow meter, and were provided with a meter and a log for recording their serial PEFRs every three hours (when awake) during a 7-day period.

Immunologic Evaluation

Preparation of conjugates.

Serum samples were collected via peripheral venipuncture to determine if the workers had an immunologic response to MDI, TDI, or HDI. These diisocyanates were conjugated to HSA (1% phosphate-buffered solutions) by rapid stirring at room temperature at a pH of 7.4 [Gallagher et al., 1981]. The reaction was stopped by addition of an equal volume of 2 moles/liter (mol/l) of ammonium carbonate, centrifuged at 3,000*g* for 20 minutes, and dialyzed extensively against 0.1 mol/l of ammonium carbonate. Resulting diisocyanate-protein conjugates were then precipitated with equal volumes of 20% trichloroacetic acid, centrifuged at 3,000*g* for 20 minutes, redissolved in sodium hydroxide, and dialyzed extensively against distilled water. Quantitative estimates of total diisocyanates bound to carrier proteins were determined by a modified Guttman assay [Tse and Pesce, 1979]. MDI-HSA conjugate prepared in this manner exhibited a hapten to protein molar ratio of 3:1.

Specific IgE antibody to diisocyanate-HSA conjugates.

Modifications of the radioallergosorbent test (RAST) were used to measure specific IgE antibodies against the HSA conjugates of MDI, TDI, and HDI [Gallagher et al., 1981; Wide et al., 1967; Maccia et al., 1976]. Five milligrams per milliliter (mg/ml) of conjugated proteins were coupled to methylcellulose disks activated with cyanogen bromide dissolved in acetonitrile. The conjugate-bound disks and the test serum were incubated for 3 hr at room temperature. Subsequently, the disks were washed with phosphate buffered saline (PBS) and incubated with 50,000 counts per minute of ^{125}I-radiolabeled rabbit antihuman IgE antibodies for 16 hr. After a final washing with PBS, the counts per minute of ^{125}I-labeled anti-IgE bound was measured and specific IgE was expressed as percent binding (counts per minute of ^{125}I-labeled anti-IgE bound divided by total counts per minute added to each tube and multiplied by 100). A positive test was defined as percent binding that was three standard deviations greater than the mean binding of control sera from nine individuals not exposed to diisocyanates. The control sera were obtained from a bank of sera from nonexposed individuals, which was collected by a university-operated immunology laboratory (referred to as laboratory controls).

Specific IgG antibody to diisocyanate-HSA conjugates.

IgG antibodies were determined using the enzyme-linked immunosorbent assay (ELISA) [Bernstein et al., 1982; Liss et al., 1984; Voller et al., 1976]. Aliquots of 0.2 milliliters (ml) of diisocyanate-HSA (concentration of 200 micrograms per milliliter) were diluted in 0.1 mol/l of sodium bicarbonate (pH 8.6), and incubated in micro-ELISA plates at 4°C for 14 hr. After washing with PBS, a 1:100 dilution of test serum diluted in 5% bovine serum albumin (BSA) was incubated for 2 hr at room temperature. Preliminary studies were performed with serial serum dilutions of 1:5, 1:10, 1:50, 1:100, 1:500, and 1:1,000. The 1:100 dilution was selected because it was determined to be the optimal serum dilution with high sensitivity for detection of IgG and a negligible degree of nonspecific binding. Goat antihuman IgG alkaline phosphatase conjugates, diluted 1:1,000 in 1% BSA, were then added for 2 hrs at room temperature. Finally, 0.2 ml of 0.006 mol/l of p-nitrophenyl phosphate disodium diluted in glycine buffer (pH 10.4) was added. Enzymatic activity was terminated at 10 minutes with 2 N sodium hydroxide. Optical density was read using a micro-ELISA MR 592 Spectrophotometer, and was considered significant if the absorbance reading was three standard deviations above the mean for control sera from nine

individuals with no prior diisocyanate exposure (laboratory controls).

Industrial Hygiene Evaluation

The industrial hygiene survey consisted of workplace monitoring for both MDI and MDI-based polyisocyanate in the foaming areas. Personal breathing zone air samples were collected using OSHA Method 47, which consists of drawing air through a glass fiber filter coated with 1-(2-pyridyl)-piperazine [Burright, 1983]. The sample device was attached to the collar of each worker's shirt, which is considered to be within the worker's breathing zone. The samples were desorbed in 4.0 millimeters of 10% dimethyl sulfoxide in acetonitrile and analyzed by high performance liquid chromatography (HPLC). The minimum quantifiable concentrations (MQC) for MDI and MDI-based polyisocyanate were 0.3 and 0.1 micrograms per cubic meter of sample air ($\mu g/m^3$), respectively.

Area air sampling was performed using NIOSH Method 5521, which utilizes an impinger containing 15 milliliters of 1-(2-methoxyphenyl)piperazine (MOPIP) in toluene solution [NIOSH, 1989]. After sampling, the impinger solutions were evaporated to dryness in a nitrogen atmosphere, leaving a sample residue which consisted of the MOPIP-diisocyanate urea derivatives. The residue was re-dissolved in 2 ml of 0.5% acetic acid in acetonitrile and analyzed by HPLC. The MQC for both MDI and MDI-based polyisocyanate was 3.7 $\mu g/m^3$.

Statistical Methods

Data were entered and analyzed using the statistical package Epi Info version 6.02 [Centers for Disease Control and Prevention, 1994]. Mean values and standard deviations for specific IgG and IgE antibodies to diisocynate-HSA conjugates are reported for MDI-exposed and nonexposed groups. Kruskal-Wallis nonparametric analysis was used to determine levels of significance between the group means.

RESULTS

The cohort consisted of 18 workers from the foaming and painting areas, divided into exposed and nonexposed groups. The exposed group included nine workers (seven males and two females: their mean age was 32.4 ± 4.4 years; eight were white, one was black; and the mean years on their current job was 5.7 ± 4.9). The nonexposed group included nine workers (all males; mean age of 33.7 ± 7.2 years; eight were white, one was black; and the mean years on their current job was 3.6 years ± 1.0). There was no significant difference in the age or years on current job between the two groups.

Data from the questionnaires revealed that four workers in the exposed group and three workers in the non-exposed group reported respiratory symptoms consistent with possible OA. Results from the pre- and postshift spirometry indicated that two of 18 workers had abnormal preshift FEV_1/FVC ratios (less than 70% of predicted); one of these workers was a smoker. Both individuals were in the exposed group and reported respiratory symptoms on the questionnaire. The remainder of the pre- and postshift spirometry tests were considered normal with no intrashift changes in FEV_1 of greater than 10%. Sixteen of the 18 workers reported PEFRs for at least 1 day. None showed more than a 20% variability over a 24-hr period, and therefore all were considered normal. Finally, the respiratory exams were not remarkable.

The industrial hygiene data showed that exposures to MDI were below the NIOSH recommended exposure limit (REL) and the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) of 50 $\mu g/m^3$ (8-hr, time-weighted average) [NIOSH, 1992]. Personal breathing zone air sampling measured MDI levels ranging from none detected to 9.1 $\mu g/m^3$, with a mean exposure level of 1.5 $\mu g/m^3$. MDI-based polyisocyanate exposures ranged from none detected to 0.6 $\mu g/m^3$, with 12 of 17 concentrations below the MQC. Area air sampling measured levels of MDI below the MQC, and levels of MDI-based polyisocyanate ranging from 16.9 to 72.5 $\mu g/m^3$, with an average of 44.6 $\mu g/m^3$.

Six of nine workers in the exposed group had elevated IgG antibodies specific for the MDI-HSA conjugate (Figure 1). Of this group, two were symptomatic and had a decreased FEV_1/FVC ratio indicative of possible asthma. When comparing the exposed workers to nonexposed workers and non-exposed laboratory controls, mean MDI-HSA IgG antibodies were significantly higher in the exposed workers. The mean optical density for exposed workers was 1.143 ± 0.982 compared to 0.057 ± 0.056 for non-exposed workers and laboratory controls (Kruskal-Wallis nonparametric analysis, $P = 0.04$). IgG antibody level was not associated with years of MDI exposure, or with specific job titles (data not presented here).

The six workers with elevated IgG to MDI-HSA also exhibited antibody responses to the other diisocyanate-HSA antigens. All six had elevated IgG antibodies to TDI-HSA and five had elevated IgG antibodies to HDI-HSA. When comparing the exposed workers to nonexposed workers and nonexposed laboratory controls, mean TDI-HSA and HDI-HSA IgG antibodies were significantly higher in the exposed workers. For TDI-HSA, the mean optical density for exposed workers was 1.065 ± 0.765 compared to 0.209 ± 0.133 for nonexposed workers and laboratory controls ($P = 0.004$). For HDI-HSA, the mean optical density for exposed workers was 0.964 ± 0.918 compared to 0.171 ± 0.069 for nonexposed workers and laboratory controls ($P = 0.02$).

None of the MDI-exposed workers demonstrated increased diisocyanate-specific IgE antibodies when com-

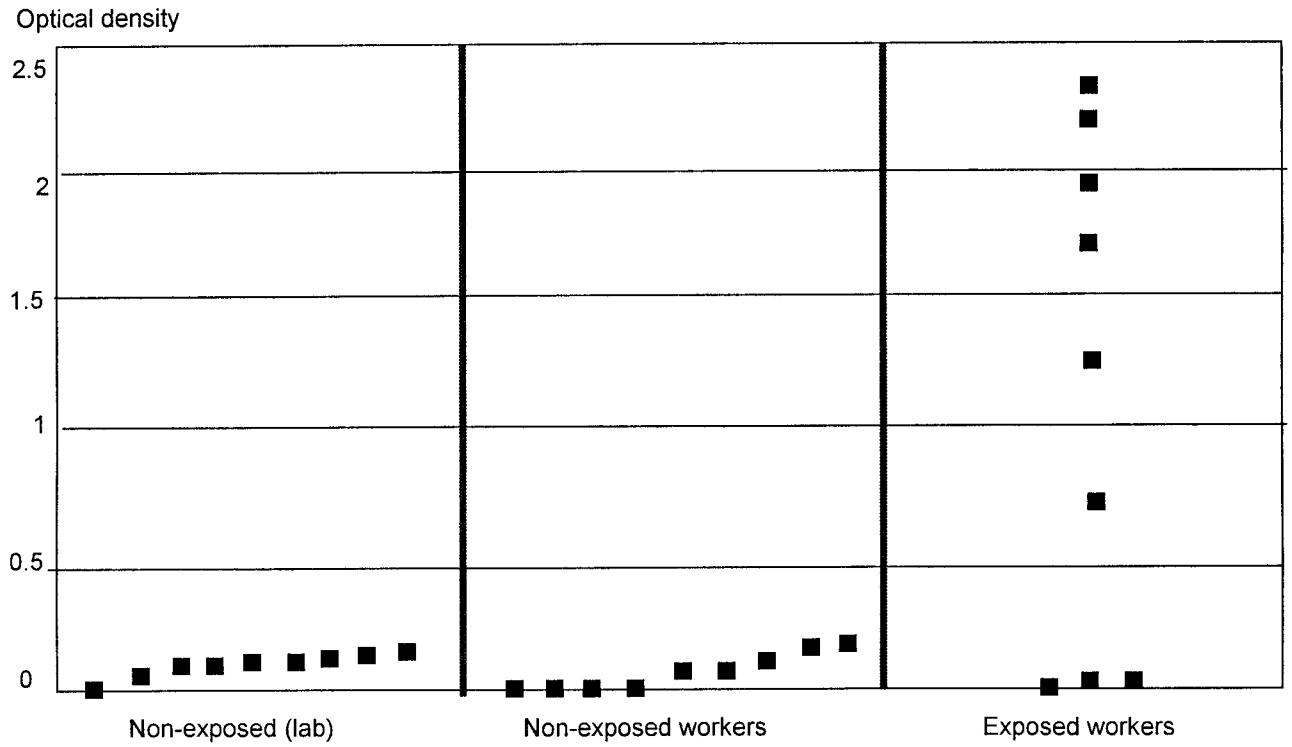

FIGURE 1. MDI-HSA specific IgG antibodies for each participant by exposure group (NIOSH HHE 87-350).

pared to nonexposed workers or to nonexposed laboratory controls. The mean percent binding of IgE to MDI-HSA for exposed workers was 0.911 ± 0.232 compared to 0.933 ± 0.137 for nonexposed workers and laboratory controls ($P = 0.4$). The mean percent binding of IgE to TDI-HSA for exposed workers was 0.922 ± 0.067 compared to 0.933 ± 0.157 for nonexposed workers and laboratory controls ($P = 0.3$). Finally, the mean percent binding of IgE to HDI-HSA for exposed workers was 0.889 ± 0.078 compared to 0.922 ± 0.140 for nonexposed workers and laboratory controls ($P = 0.5$).

Issues related to the use of the IgG antibodies to the MDI-HSA conjugate as a biological marker of MDI exposure in this group of workers are presented in Table I. Our data demonstrate the test to have moderate sensitivity (67%), and excellent specificity (100%). The data also show that the proportion of workers who exhibit the MDI-HSA specific IgG antibody and are MDI exposed (positive predictive value) is 100%; the workers without the antibody expression and no MDI exposure (negative predictive value) is 75%. The false positive rate is 0%, and the false negative rate is 33%.

TABLE I. Comparison of MDI-HSA Specific IgG Antibody Expression in the MDI Exposed and Nonexposed Workers*

	MDI exposure		
	(+)	(−)	Totals
MDI-HSA Specific IgG Antibody Expression (+)	6[a]	0[b]	6
MDI-HSA Specific IgG Antibody Expression (−)	3[c]	9[d]	12
Totals	9	9	18

*Sensitivity, a/a+c = 6/9 = 67%; Specificity, d/b+d = 9/9 = 100%; Positive Predictive Value, a/a+b = 6/6 = 100%; Negative Predictive Value, d/c+d = 9/12 = 75%; False Positive Rate, b/b+d = 0/9 = 0%; False Negative Rate, = c/a+c = 3/9 = 33%.

DISCUSSION

Two days of air monitoring demonstrated that foamers at this plant were exposed to MDI at levels below occupational exposure limits. However, information not available at the time of the NIOSH study leads us to believe that the MDI and MDI-based polyisocyanate exposure concentrations may have been underestimated. OSHA Method 47 has been shown to estimate MDI concentrations more than three times lower than those found using other air sampling and analytical methods [personal communication, Dr. Rosa Key-Schwartz, NIOSH, 1996]. In addition, recent data have shown that sampling time, humidity, temperature, and the presence of a back-up pad in the filter cassette all contribute to the loss of the derivatizing reagent used to impregnate the filter [Meyer and Czarnecki, 1996]. Nonetheless, personal breathing zone and area air sampling data confirmed the workers in the exposed group were actually exposed to MDI.

Due to the above mentioned problems with OSHA Method 47, and the lack of long-term MDI exposure data at this facility, this study could not determine whether MDI exposures below the NIOSH REL and the ACGIH TLV could induce the MDI-HSA specific IgG antibody, or the spirometry abnormality observed in two of the exposed workers.

In this investigation, no workers had demonstrable reversible airway obstruction as assessed by intrashift changes in spirometry (specifically FEV_1) or by serial PEFRs. However, two of the 18 employees may have OA. Both workers were in the MDI-exposed group, and both had an obstructive pulmonary function pattern.

The level of specific IgE antibodies to the MDI-HSA antigen was not associated with the workers' exposure status or with the diagnosis of possible OA. Previous studies have shown that detecting diisocyanate-specific IgE antibodies supports but does not establish a diagnosis of OA. Also, since only 10–30% of workers with diisocyanate-induced asthma have detectable diisocyanate-specific IgE antibodies [Bernstein, 1993], failure to detect specific IgE antibodies does not rule out the diagnosis.

The significance of an IgG antibody response in relation to OA is unclear. Several previous studies have found no association between elevated levels of specific IgG antibodies to the MDI-HSA antigen, and confirmed OA [Zammit-Tabona et al., 1983; Tse et al., 1985; Herd and Bernstein, 1994; Lummus et al., 1996]. In this investigation, three workers with an increased IgG antibody level were symptomatic, three were asymptomatic, and none had symptoms suggestive of hypersensitivity pneumonitis. Considering the findings in this and previous studies, it appears that the humoral immune system may not play a significant mechanistic role in the pathogenesis of diisocyanate-induced OA.

Nonetheless, this investigation suggests the potential use of the diisocyanate-specific IgG antibody as a biological marker of diisocyanate exposure. Our results demonstrate that increased levels of IgG antibodies to the MDI-HSA conjugate were found in six of nine workers in the MDI-exposed group, in none of the nine workers in the nonexposed group, and in none of the nine nonexposed laboratory controls. In addition, mean levels of the specific IgG antibody were significantly higher in the exposed group when compared to the nonexposed groups.

We anticipate that the MDI-HSA specific IgG antibody may be useful in the clinical evaluation of a suspected case of MDI-induced asthma, and in epidemiologic investigations. Considering the information in Table I, the clinician can be confident that any worker exhibiting the antibody will have been exposed to MDI, whereas the absence of the antibody will indicate no MDI exposure in 75% of the cases. However, we must emphasize the MDI-HSA specific IgG antibody can only be used to confirm MDI exposure; other diagnostic tools must be used to determine the presence of an obstructive lung disorder (asthma) and the work-relatedness of this disorder. This biological marker could be used to confirm qualitatively that workers with potential for MDI exposure were actually exposed to MDI. However, inferences related to the quantitative exposure cannot be made using this marker. Also, we do not recommend using the absence of this marker as confirmation that workers are not exposed to MDI. The 33% false negative rate could be a significant source of misclassification.

We must stress that caution needs to be taken when using the IgG antibody as a marker for exposure to a particular diisocyanate. As previously discussed, workers exposed to a given diisocyanate may exhibit an increased reaction to other diisocyanates. Cross-immunologic reactivities between different diisocyanates were found in this study; therefore, a knowledge of the process and the exposures involved was key in the interpretation of the results. In addition, the findings and conclusions of this study were based on a small cohort. Further investigations are needed to validate the relationship between this marker and MDI exposure. We recommend against the generalization of these findings to other diisocyanates. We contend that studies should be performed to investigate the relationship between diisocyanate exposure and the expression of specific IgG antibodies to other diisocyanate-HSA antigens (e.g., TDI-HSA, HDI-HSA, IPDI-HSA, etc.) before any conclusions can be made related to these exposure-response models. In addition, the influence of MDI dermal exposures on the exhibition of the IgG antibody marker should be investigated.

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