

Specific IgE to isocyanates: A useful diagnostic role in occupational asthma

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Background: Isocyanates are the most frequent cause of occupational asthma in industrialized countries.

Objective: We sought to investigate the utility of specific IgE measurement in the diagnosis of isocyanate-induced asthma.

Methods: Fifty-eight of 101 patients referred for investigation were diagnosed as having isocyanate-induced occupational asthma by means of history, serial peak flow records, and bronchial provocation tests. Specific IgE antibodies to toluene diisocyanate:human serum albumin (HSA), diphenylmethane diisocyanate:HSA, and hexamethylene diisocyanate:HSA were measured in all patients by Phadebas RAST.

Results: Twenty patients had a RAST ratio of 2 or greater to at least one isocyanate. Thirteen (28%) of the 46 patients with a positive provocation test response had a RAST ratio of 2 or greater, and nine (20%) had a RAST ratio of 3 or greater. Raising the RAST cut-off from 2 or greater to 3 or greater reduced its sensitivity but increased the specificity of the test to 100%. RAST measurement was most likely to be positive within 30 days of exposure. Serial measurements suggested that the half-life of the IgE antibodies was approximately 6 months. Evidence of cross-reactivity between isocyanate RAST responses was found in eight subjects.

Conclusion: Specific IgE to isocyanates is a more specific than sensitive index of occupational asthma. With a RAST score of 3 or greater, it is wholly specific and therefore diagnostic of isocyanate-induced asthma. The sensitivity of specific IgE measurement is highest when blood is taken less than 30 days from last exposure, which is consistent with the observed half-life. (*J Allergy Clin Immunol* 1998;101:709-15.)

Key words: Isocyanates, IgE, occupational asthma, bronchial provocation test

Isocyanates are currently the most frequent cause of occupational asthma (OA) in the industrialized world.^{1,2} They are used extensively in the manufacture of polyurethane polymers in flexible and rigid foams, elastomers, adhesives, paints, surface coatings, and foundry cores.^{3,4} Isocyanates cause a spectrum of respiratory diseases, the most frequent being OA. OA occurs in 5% to 10% of exposed workers.⁵ Allergic alveolitis and

Abbreviations used

BPT:	Bronchial provocation test
HDI:	Hexamethylene diisocyanate
HSA:	Human serum albumin
MDI:	Diphenylmethane diisocyanate
NDI:	Naphthalene diisocyanate
OA:	Occupational asthma
TDI:	Toluene diisocyanate

isolated systemic symptoms also occur, but they are uncommon.⁶ Several mechanisms, allergic and nonallergic, have been suggested for isocyanate-induced asthma. The latter include reflex and pharmacologic responses, destructive histopathologic changes, and β -adrenergic blockade.⁷ These do not, however, explain the characteristic allergic features of a latent interval, the development of IgE sensitization in a minority of those exposed, or the evidence of activated T-lymphocytes in the bronchial mucosa in isocyanate-induced asthma.⁸

Specific inhalation tests in the laboratory are still considered the gold standard in the diagnosis of occupational asthma.^{3,9} However, they are expensive, require prolonged monitoring of patients, and can only be carried out in specialized centers. It is therefore worth exploring the utility of the measurement of specific IgE antibody levels to isocyanates in the diagnosis of OA.

METHODS

We studied all persons exposed to isocyanates at work who were referred to Royal Brompton Hospital for investigation of occupational asthma between 1974 and 1996 from whom blood was available for measurement of specific IgE antibodies, and in whom a firm diagnosis (isocyanate-induced OA present or absent) was reached. One hundred one patients ranging in age from 18 to 62 years (93% men), fulfilled these criteria. Diagnoses were made on the basis of clinical history, and in all but 15 patients this was supported by serial peak flow records (90 patients), bronchial provocation tests (BPTs) (70 patients), or both. Diagnoses were made without knowledge of the patient's specific IgE level.

Peak flow records

Patients performed serial measurements of peak flow every 2 hours from waking to sleeping for a period of at least 4 weeks, including a period of 1 week away from work. Records were plotted and examined visually.

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TABLE I. Specific and total IgE levels and results of BPTs in all patients with specific IgE to an isocyanate

Patient no.	TDI		MDI		HDI		Total IgE (kU/L)	OA
	BPT	RAST ratio	BPT	RAST ratio	BPT	RAST ratio		
1	Dual	1.2	Dual	2.6	ND	6.6	ND	+
2	ND	0.9	Early	1.1	ND	2.1	366	+
3	ND	2.2	ND	2.6	ND	1.7	788	—
4	ND	2.0	Late	6.2	ND	2.6	500	+
5	ND	17.3	Dual	40.7	ND	5.3	28	+
6	ND	62.2	Early	72.5	ND	41.0	324	+
7	ND	2.5	ND	0.8	ND	2.2	61	—
8	ND	22.0	Early	42.0	ND	17.7	62	+
9	ND	1.8	ND	2.4	Neg	2.5	210	—
10	ND	6.7	ND	1.3	ND	3.0	317	+
11	Neg	2.3	ND	1.5	ND	1.5	489	—
12	ND	1.7	ND	2.2	ND	1.8	84	+
13	ND	13.2	Late	14.7	ND	15.2	11,939	+
14	ND	3.0	ND	6.1	ND	2.8	20	+
15	Early	1.0	ND	5.0	ND	0.7	24	+
16	Dual	2.2	Dual	1.4	Dual	1.2	773	+
17	Early	1.5	ND	4.8	ND	3.5	555	+
18*	Neg	1.6	Neg	8.1	ND	2.1	39	+
19	Neg	1.4	Late	1	ND	2.6	13	+
20	ND	2.1	ND	1.8	Late	2.2	ND	+

Positive provocation test responses in the presence of specific IgE are shown in bold type.

ND, Not done; Neg, negative.

*Late-phase reaction on BPT to NDI

BPTs

Single-blind inhalation tests were done with one or more diisocyanates: toluene (TDI), diphenylmethane (MDI), and hexamethylene (HDI). Two patients were tested with naphthalene diisocyanate (NDI), and one was tested with 4 cyclohexyl isocyanate. BPTs were standardized throughout the study and have been described before.¹⁰ Briefly, TDI challenges were performed by asking the patient to brush paint TDI/varnish on a cardboard surface, with the varnish base alone used as a negative control test. For MDI challenges, crystals were heated on a sandbath, with sugar crystals used as a control test. For HDI challenges, spray painting was carried out with HDI-cured paint, using paint without hardener as a placebo. Responses were measured by FEV₁ hourly for 12 hours and by nonspecific bronchial hyperreactivity at 24 hours after control and test exposures. Airway reactivity to histamine was measured as described by O'Brien et al.,¹¹ and a change of reactivity of at least one doubling dose from the pretest value was considered significant. No patient had increased airway reactivity who did not have either an immediate or late reaction. Continuous measurement of atmospheric concentrations of TDI, MDI, and HDI ensured that isocyanate exposures remained within the appropriate maximum exposure limit. A repeatable 15% or greater fall in FEV₁ or increase in bronchial hyperreactivity after isocyanate challenge as compared with control exposure was considered a positive response.

Skin prick tests

Skin prick tests were done with commercial extracts (Bencard, Brentford, U.K.) of B2 grass pollens (4100), *Dermatophagoides pteronyssinus* (2800), cat fur (3204), dog (3205), *Aspergillus fumigatus* (2000), *Alternaria alternata* (1100), *Cladosporium herbarum* (1300), and house dust (4701ED; Miles, Bridgend,

U.K.). Atopy in this study was defined as the presence of a positive test response (wheal ≥ 3 mm in diameter) to one or more of these extracts.

IgE measurements

Specific IgE antibodies to TDI:human serum albumin (HSA), MDI:HSA, HDI:HSA, and HSA were measured in all subjects by Phadebas RAST (Pharmacia and Upjohn Ltd., Uppsala, Sweden) with ALLERGO-DISCS R (MIAB, Uppsala, Sweden). Tests were made in duplicate and were repeated if the coefficient of variation was greater than 30%. To ensure standardization of the assays, a 5-point standard curve (0.35 to 52.5 PRU/ml, Pharmacia and Upjohn Ltd.) was included. Sera were stored at -20° C. Repeat assays on a proportion of sera showed no deterioration in specific IgE levels over time ($p = 0.81$).

For each patient, the ratio of the isotope percentage binding of the hapten disc to that of the HSA disc was calculated as follows:

$$\text{RAST ratio} = \frac{\% \text{ isotope binding of isocyanate: HSA disc}}{\% \text{ isotope binding of HSA disc}}$$

Initially a ratio of 2 or higher was taken as positive. A cut-off ratio of 3 or greater was also examined, which was the level recommended by Baur⁶ and Wass and Belin.¹² In six subjects RAST measurements were determined on serial blood withdrawals, allowing estimation of the half-life of IgE antibodies to isocyanates.

Total IgE levels were determined in 18 of 20 patients with specific IgE to isocyanates and compared with 51 study subjects who did not have specific IgE.

TABLE II. Presence of specific IgE antibodies in patients with OA caused by isocyanates and patients with OA caused by isocyanates confirmed by a positive BPT response

RAST ratio	OA ⁺	OA ⁻	BPT ⁺	BPT ⁻	Sensitivity (%)	Specificity (%)
All patients						
≥2	16/58	4/43	—	—	28	91
≥3	11/58	1/43	—	—	19	98
Patients undergoing BPTs						
≥2	—	—	13/46	2/24	28	92
≥3	—	—	9/46	0/24	20	100

Diagnostic indices

The sensitivity of the test was calculated as the proportion of patients with asthma induced by isocyanates in whom specific IgE antibodies were detected, and its specificity was calculated as the proportion of patients who did not have asthma induced by isocyanates in whom specific antibodies were not detected. In a test that is wholly specific, there are no false-positive results (i.e., a positive test result is diagnostic of the disease). The statistical significance of differences between diagnostic indices was assessed by treating them as independent proportions.

Fifty-eight patients were considered by these criteria to have OA caused by isocyanates, 46 with positive BPT responses and seven with serial peak flow records supporting the history. In 15 patients the diagnosis was reached on the basis of a clinical history alone (five with OA and 10 without OA). Those with OA were a little older (mean age, 42.4 years) than those without OA (mean age, 39.1 years). There were 58 positive provocation test responses, which included 80% of those challenged with TDI, 65% of those challenged with MDI, and 42% of those challenged with HDI. Eight subjects had positive BPT responses to more than one isocyanate, which included the isocyanate to which they were exposed. The two patients challenged with NDI and the one challenged with 4 cyclohexyl isocyanate also had positive reactions. Of the 46 subjects with positive BPT responses, seven had isolated immediate reactions, 11 had isolated late reactions, and 28 had dual reactions to at least one isocyanate. In the 87 subjects in whom atopic status was recorded, 52% were atopic (49% of those with OA [$n = 51$] and 56% of those without OA).

RESULTS

IgE measurements

Twenty of the 101 study patients had a RAST ratio of 2 or greater to TDI:HSA, MDI:HSA, HDI:HSA, or a combination thereof; eleven had a ratio of 3 or greater. These results are shown in Table I, with corresponding BPT results.

Relationship of OA and RAST results

Sixteen (28%) patients with RAST ratios of 2 or greater were in the OA group, and four (9%) were not. When the RAST ratio cut-off was raised to 3 or greater, 11 patients (19%) remained in the OA group, and one remained in the group without OA. Raising the RAST ratio cut-off from 2 to 3 decreased the sensitivity of the

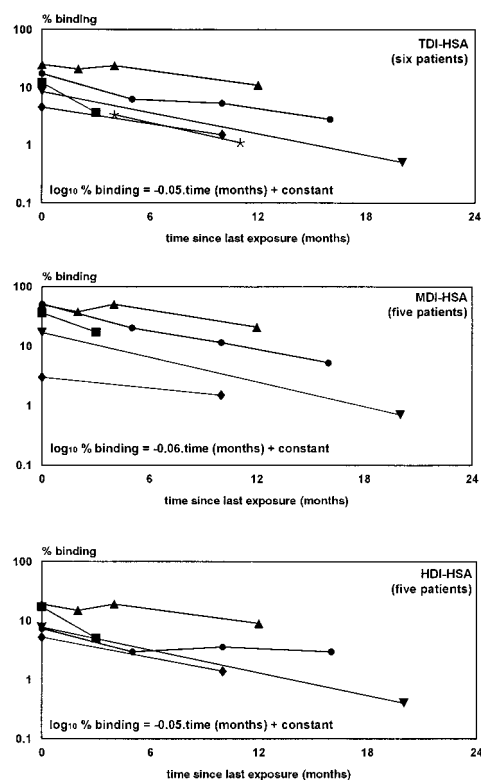


FIG. 1. Serial measurements of IgE antibodies to isocyanate:HSA conjugates.

test (28% to 19%) but raised its specificity (91% to 98%) (Table II), although this difference did not reach statistical significance ($p > 0.05$).

Relationship of BPT and RAST results

Specific IgE results were further examined in patients whose diagnosis had been confirmed by BPT (Table II). Thirteen (28%) of the 46 patients with a positive BPT response had specific IgE (RAST ratio ≥ 2) compared with two (9%) who had a negative challenge test response. Only nine (20%) of those with a positive challenge test response had specific IgE with a ratio of 3 or greater, and none with a negative provocation test response had such a result (specificity = 100%). Eight (23%) of the 35 subjects who had an immediate BPT response had specific IgE (RAST ratio ≥ 2), and five (45%) of the 11 subjects who had only an isolated late response also had a positive RAST result. Of the 15 subjects who had specific IgE and who had positive BPT responses, eight (53%) had immediate reactions, and eight had late reactions, including three with dual responses.

Measurement of specific IgE on serial blood withdrawals

The decline in specific IgE antibodies in six patients' sera over up to 2 years is shown in Fig. 1. The half-life of IgE antibodies, calculated from the time of stopping

TABLE III. Specificity and sensitivity for specific IgE by time interval between last exposure date and date of blood withdrawal

Time from last exposure to blood withdrawal (days)	n	RAST ≥ 2		RAST ≥ 3	
		Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
≤ 0	32	41	100	32	100
$>0-\leq 30$	29	38	81	15	94
>30	24	14	90	2	100
>360	12	17	83	—	—
All	85	33	89	22	97

TABLE IV. Patients with specific IgE to HSA

Patient no.	TDI:HSA		HSA (%b)
	RAST (%b)	RAST ratio	
1	5.5	1.1	5.0
2	5.1	1.0	5.1
3	2.2	1.8	1.2
4	45.0	13.2	3.4
5	6.1	1.8	3.4
6	2.4	1.0	2.4
7	3.9	1.5	2.6

%b, Percentage isotope binding.

exposure, was 5.8 months (95% confidence interval: 4.6 to 7.7) for TDI, 5.4 months (95% CI: 4.3 to 7.1) for MDI, and 6.7 months (95% CI: 4.6 to 12.0) for HDI.

Time interval between date of blood withdrawal and exposure

The time interval between the date of blood withdrawal for specific IgE and the date of last exposure to isocyanates was examined. Results at four different time intervals are shown in Table III. When blood was taken during exposure, the sensitivity was 41% at RAST ratios of 2 or greater (32% at RAST ratio ≥ 3) but fell markedly to 14% (2%) when blood was taken more than 30 days after exposure had ceased. The specificity of the test was 100% when the patient was still exposed.

Cross-reactivity among isocyanates

Evidence of cross-reactivity among TDI, MDI, and HDI came from both airway and IgE responses, although agreement was not found between the tests. Fifteen study subjects (one exposed to MDI, one exposed to NDI, and the remainder exposed to TDI) were challenged with more than one isocyanate. Eight had positive BPT responses to more than one isocyanate, with four of these having positive responses to all three. However, no analysis of cross-reactivity shown by airway responses could be made because multiple challenges were not systemically performed.

In the 20 subjects who had RAST ratios of 2 or greater, eight had IgE to two isocyanates, and six had

IgE to all three isocyanates. In 13 of these subjects, all but one had positive responses to the isocyanate to which they were exposed. The fourteenth patient (no. 18) had specific IgE to MDI and HDI despite having a negative BPT response to MDI but a positive BPT response to NDI.

Specific IgE antibodies to HSA

Seven subjects (7%) in the study had isotope binding greater than 1% to HSA. Table IV shows IgE binding and RAST scores to TDI:HSA in the four patients with the highest binding to HSA (nos. 1 to 4), with results from a further three subjects not included in this study (nos. 5 to 7). Only subject 4 had a positive RAST ratio to TDI:HSA, whereas all seven subjects would be considered to have a positive RAST ratio if the isotope binding to HSA alone was not taken into account. Results were similar with MDI:HSA and HDI:HSA.

Total IgE and atopy

The median total IgE in patients with specific IgE was 264 kU/L compared with 49 kU/L in those who did not have specific IgE. This difference was not explained by atopy, which was present in 53% and 51% of patients, respectively.

DISCUSSION

Our results indicate that measurement of specific IgE to isocyanates is a specific but relatively insensitive test for asthma induced by isocyanates. Its sensitivity is greatest within 1 month of exposure and declines rapidly with increasing time from exposure, consistent with our observed half-life in serum of specific IgE to isocyanates. A RAST ratio of 3 increased the specificity of specific IgE antibody from 92% to 100% when the gold standard used was a positive response to an inhalation test. Specific IgE to isocyanates was, however, a less sensitive test of isocyanate-induced asthma, with an overall sensitivity for a RAST ratio of 3 or greater in this study of 20% for patients with a diagnosis determined by BPTs. This low figure in part reflects the short half-life in serum of specific IgE to isocyanates, with sensitivity falling from 41% at RAST ratios of 2 or greater (32% at RAST ratios ≥ 3) for sera taken during exposure to 14% (2%) for sera taken more than 30 days from last

TABLE V. Diagnostic value of specific IgE antibodies to isocyanate:HSA conjugates in seven studies with more than 50 subjects each

Subjects (n)	Work-related symptoms		Sensitivity (%)	Specificity (%)	Criterion for IgE+	Reference
	+	–				
232	7/174	0/58	4	100	ratio/HSA ≥ 3	12
1780	148/1095	1/685	14	100	≥ 0.35 kU/L	4
BPTs						
	+	–				
56	5/26	0/30	19	100	ratio/HSA ≥ 2	13
56	4/26	1/30	15	97	cpm $> c(\bar{x} + 3SD)$	13
78	11/28	0/50	39	100	NBR $> c(\bar{x} + 4SD)$	14
81	7/35	0/46	20	100	ratio/HSA ≥ 2	15
62	8/29	1/33	28	97	OD $> c(2\bar{x})$	16
63	1/34	1/29	3	97	cpm $> c(\bar{x} + 2SD)$	17

NBR, Net bound radioactivity; OD, optical density; c, control group IgE.

exposure. The interesting finding that the half-life of the IgE antibodies was approximately 6 months needs to be confirmed in larger populations.

Other studies have reported specific IgE antibody to isocyanates in 3% to 39% of patients with isocyanate-induced asthma (Table V).³ Our results fall within this range: 20% with RAST ratios of 3 or greater and 28% with RAST ratios of 2 or greater. Several factors may contribute to the variation in prevalence of specific IgE in different studies. In addition to the timing of the test and different methods and criteria for a positive test response, the prevalence of a positive test response varies with the criteria used for case identification. Wass and Belin¹² found only 4% with specific IgE in patients with “suspected isocyanate-induced respiratory symptoms,” whereas Pezzini et al.¹⁴ found specific IgE in 39% of 28 workers with isocyanate-induced asthma diagnosed by inhalation testing.

Specific IgE has also been found more frequently in patients with asthma induced by MDI and HDI than by TDI.^{14–16} In our study 59% of patients with positive BPT responses were found to have specific IgE to MDI, 86% had specific IgE to HDI, but only 33% had specific IgE to TDI. Karol et al.¹⁷ suggested that specific IgE might be associated with the provocation of an early (rather than a late) asthmatic response in inhalation testing. Keskinen et al.¹⁵ found all seven patients in her series with specific IgE had an immediate asthmatic response, and none of those with late responses had specific IgE. In our series only 53% of patients with specific IgE had an immediate response, similar to the 66% reported by Pezzini et al.,¹⁴ and eight (53%) had late asthmatic responses provoked by inhalation testing.

There is a substantial, although variable degree of cross-reactivity between IgE antibodies induced by different isocyanates. New antigenic determinants result from the interaction of human respiratory proteins and isocyanates.^{12, 18, 19} Specific IgE antibodies predomi-

nantly recognize these new determinants, which may be very similar in different isocyanate compounds and could explain the cross-reactivity found between them.²⁰ In vitro cross-reactivity between isocyanates has been demonstrated in several studies^{19, 21, 22} by RAST and ELISA inhibition. Because of the different specificities of the antibodies, cross-reactivity between different isocyanate conjugates differs from patient to patient. Baur²¹ estimated that approximately 60% of his patients' sera showed variable cross-reactivity. In our study 14 of the 20 (70%) subjects who had a RAST score of 2 or greater showed cross-reactivity between TDI:HSA, MDI:HSA, and HDI:HSA. Bronchial cross-reactivity among isocyanate compounds has been reported,¹¹ but in this study agreement with specific IgE was not found. One reason for this discrepancy may be that BPTs were done with the chemical alone, whereas specific IgE was done with the conjugate. There is not the same homogeneity of results for BPTs as for measurements of specific IgE, in which all 101 patients were tested for three isocyanates. Of the 70 patients who underwent a BPT, only 16 were tested with more than one isocyanate, but of these, eight subjects did demonstrate cross-reactivity. On the other hand, in the patients who fail to show cross-reactivity, the importance of the use of the appropriate isocyanate for testing has been demonstrated.¹¹ In this study two subjects (nos. 18 and 19) had positive BPT responses to one type of isocyanate but not to others. Similarly, two subjects (nos. 10 and 15) had specific IgE antibodies to certain isocyanates only.

Lenkei and Ghetie²³ postulated that anti-HSA antibodies have a physiologic role in the removal of aged albumin molecules. Cartier et al.¹⁶ have suggested that isocyanates may alter HSA in a similar manner to this senescence process, increasing the level of anti-HSA antibodies. It is therefore important that the IgE response to HSA is taken into account when raised levels of specific isocyanate IgE are detected. By using RAST

ratio analysis, only one of the seven subjects with raised IgE to HSA in Table IV was found to have a truly positive isocyanate:HSA RAST result.

In the light of clinical evidence that the majority of cases of isocyanate-induced asthma are the manifestation of a specific hypersensitivity response, the low prevalence of specific IgE to isocyanates in patients with isocyanate-induced asthma is possibly surprising. A number of authors have suggested that this implies the operation of nonimmunologic pathways in the development of the disease. Although such pathways may operate in the clinical manifestations of established disease, the pattern of development of isocyanate-induced asthma (an initial symptom-free period of exposure and subsequent provocation of asthma by the specific agent in less than toxic concentrations of which the affected individual was previously tolerant) suggests, in most cases, a hypersensitivity response. Recent studies have also identified the presence of activated T_{H2} cells within the airways of patients with isocyanate-induced asthma,⁸ and Bignon et al.²⁴ have described specific molecular mosaics in HLA Class 2 molecules that confer protection and susceptibility to sensitization in exposed workers, suggesting the participation of a specific immunologic response.

Isocyanates are highly reactive molecules, which react readily with OH and NH_2 groups on protein molecules. It is likely that isocyanates bind to several different proteins in the airways in addition to HSA, any of which may be immunogenic. Tests for specific IgE that make use of isocyanate-HSA conjugates are likely to identify only those patients in which binding to HSA has stimulated IgE production, not those in which IgE may have been stimulated by other conjugates. In keeping with this, Baur et al.⁴ found that only patients with specific IgE to isocyanate-HSA conjugates had asthmatic responses provoked by inhalation of these conjugates. Current understanding of the central role of specific T_{H2} lymphocyte responses in the development of the eosinophilic bronchitis characteristic of asthma suggests that IgE may not be an essential mediator in the development of asthma. Therefore specific IgE may not be found in some patients with isocyanate-induced asthma. Alternatively, it may only be produced locally.²⁵

In summary, we have found that measurement of specific IgE to isocyanates is a specific test for isocyanate-induced asthma, and misleading results may occur if specific IgE to HSA is not taken into account. By using a RAST ratio of 3 or greater, the presence of specific IgE is 100% specific (i.e., there are no false-positive results) and therefore diagnostic of isocyanate-induced asthma. Because specific IgE is present only in a minority of exposed workers, a negative test response does not exclude the diagnosis. However, sensitivity falls markedly with time from last exposure, and to maximize sensitivity, serum for testing should be taken during or within 1 month of exposure. Our results indicate that tests for specific IgE have a valuable role to play in the diagnosis, and possibly in

the surveillance, of exposed workers with isocyanate-induced asthma.

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