

Poor correlation between stated and found concentrations of diphenylmethane-4,4'-diisocyanate (4,4'-MDI) in petrolatum patch-test preparations

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Diphenylmethane diisocyanate (MDI) is widely used in its polymeric form in the manufacturing of polyurethane products. Previous reports on MDI-related contact allergy have shown a pattern, where patients seem to react to their own MDI-based work material but not to commercial patch-test preparations, which contain 4,4'-MDI. Therefore, we performed chemical analyses of 14 commercial test preparations of 4,4'-MDI obtained from 8 European and 4 American dermatology departments as well as 2 preparations from 2 major European suppliers of patch-test allergens. A new method for monitoring 4,4'-MDI in petrolatum preparations was developed and the determination of 4,4'-MDI as the MDI-dibutylamine derivative using liquid chromatography-mass spectrometry was performed. None of the preparations obtained from the dermatology departments contained more than 12% of the concentration stated on the label. In most cases, 4,4'-MDI content was only a few percentages or less of the concentration stated. 7 of the 14 preparations were analysed before the expiry date. Yet, only 1 of them, a preparation directly obtained from the supplier, came close to the concentration stated on the label. Thus, using these preparations, patients will be tested with a lower concentration than intended, leading to possible false-negative reactions.

Key words: allergic contact dermatitis; CAS 101-68-8; CAS 101-77-9; chemical analysis; diaminodiphenylmethane; diphenylmethane-4,4'-diisocyanate; false-negative reactions; MDA; MDI; occupational; polyurethane. © Blackwell Munksgaard, 2004.

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Isocyanates are highly reactive chemicals that are widely used in the plastics industry, mainly because of their ability to form polyurethane upon addition reaction with multifunctional alcohols (polyols). Depending on their molecular structure, isocyanates can be divided into 2 groups, aromatic and aliphatic. The aromatic isocyanates toluene diisocyanate (TDI) and diphenylmethane diisocyanate (MDI) account for more than 95% of global isocyanate production. Aliphatic isocyanates are generally more expensive to produce but are used when the performance of aromatic diisocyanates is inadequate, mainly due to their tendency to undergo oxidative discolouration upon exposure to light and moisture (1). At the end of the 1970s, TDI and MDI were being produced in roughly equal quantities in the USA but, since then, the growth rate in MDI has exceeded that of TDI and, by 1990, 20% more MDI than TDI was produced in the USA (2).

The most well-known health risk of isocyanate exposure is the adverse effect on the respiratory tract, including both airway irritation and immunologically mediated outcomes, which include for example hypersensitive pneumonitis and diisocyanate-induced asthma (2). These symptoms can arise even at low air concentrations of isocyanate. Less risk of problems with the respiratory tract is associated with MDI-related products compared to TDI, because MDI has a lower vapour pressure. However, it is difficult to monitor the exposure of possible allergens by routine measurements, because technical qualities used in the industry contain a complex mixture of 25–80% monomeric 4,4'-MDI as well as oligomers containing 3–6 rings and other minor isomers such as 2,4'-MDI and 2,2'-MDI (3, 4). These mixtures are referred to as polymeric MDI, and the exact composition varies with the manufacturer (4).

Compared to respiratory symptoms, delayed hypersensitivity and allergic contact dermatitis from isocyanates are less frequently reported (5). Recently, Goossens et al. (3) reported 13 patients with positive patch-test reactions to isocyanate-based products and, where 6 patients reacted to products based on MDI, 1 patient reacted to a product based on TDI, and 1 to a product based on 1,6-hexamethylene diisocyanate. In the remaining 5 patients, the precise nature of the isocyanate product could not be obtained. 1 of these 5 patients reacted to 4,4'-MDI which is used in commercial test preparations for diagnosing allergic contact dermatitis from MDI. In no other case described above did a patient react to any commercial preparation of isocyanate. However, Goossens et al. (3) argue that diphenylmethane-4,4'-diamine (MDA) in the isocyanates series seems to be a good marker for isocyanate sensitivity, because 10 of the 13 patients with a contact allergy to isocyanate-based products also reacted to MDA. A similar pattern of reactivity supporting Goossens et al. (3) was reported by Frick et al. (6) when investigating 5 workers at a company manufacturing boards coated with a lacquer based on MDI. 3 of the workers were tested with the lacquer, and contact allergy was established with concurrent reactions to MDA. Only 1 of the 3 showed a simultaneous reaction to the commercial 4,4'-MDI test preparation. Of 2 individuals not tested with the lacquer, 1 reacted to both 4,4'-MDI and MDA, while the other did not react to any isocyanate-related substance but instead to a soap used at the work place. In 1999, Tait and Delaney (7) made a similar observation when investigating a fitter exposed to MDI when cleaning a filter contaminated with an MDI-based product. The fitter reacted with a strong allergic reaction to MDA and a weak allergic reaction to the MDI-based product but was negative to the commercial 4,4'-MDI test preparation.

The above-mentioned studies, where patients seemed to react more frequently upon patch testing with work materials containing MDI than on patch testing with the commercial 4,4'-MDI test preparations, caused us to doubt the quality of these test preparations. Therefore, we performed chemical analyses on test preparations of 4,4'-MDI used at 12 dermatology departments as well as from 2 major European suppliers of patch-test allergens.

Materials and Methods

Chemicals

Toluene, isooctane and methylene chloride were obtained from Lab-Scan (Dublin, Ireland), metha-

nol and ethanol from Kemetyl (Stockholm, Sweden), di-*n*-butylamine (DBA) from Aldrich Chemie (Steinheim, Germany), $^2\text{H}_{18}$ -labelled di-*n*-butylamine (DBA- d_{18}) from CDN Isotopes (Quebec, Canada), caffeine from Sigma Chemical (St. Louis, MO, USA). 4,4'-MDI was obtained from supplier 1 and supplier 2. MDI-DBA and MDI-DBA- d_{18} were prepared as previously described (8).

Patch-test preparations

To acquire a general view of the status of the petrolatum (pet.) patch-test preparations containing 4,4'-MDI used around the world, we contacted departments that would give a geographical distribution of samples and got a positive reply from 8 European and 4 American departments. We also directly obtained 4,4'-MDI test preparations from the 2 major European suppliers of patch-test allergens. Table 1 summarizes which supplier was used by the different departments.

Apparatus

Liquid chromatography mass spectrometry (LC-MS)

Analyses of 4,4'-MDI-containing preparations were performed using a mass spectrometer (Platform LCZ; Micromass, Altrincham, Cheshire, UK) in the electrospray mode monitoring positive ions. The cone voltage was 40 V and a capillary voltage of 3.5 kV. The temperature of the ion source was 110°C, and the desolvation temperature was 200°C. Quantifications were made by selected ion recording monitoring the molecular ions $[\text{MH}]^+$. Quantitative measurements were made by calculating the response ratio between the analyte and the deuterium-labelled internal standard.

The MS instrument was connected to Shimadzu LC10 ADvp LC pumps (Shimadzu, Kyoto, Japan). 20- μl loop injections containing a 4- μl sample plug surrounded by 16 μl of water were made with a CTC-Pal LC autosampler (CTC, Zwingen, Switzerland). 4,4'-MDI-DBA and 4,4'-MDI-DBA- d_{18} derivatives were analysed using linear gradient elution for 10 min with a mobile phase composition of water/acetonitrile/formic acid from 45/55/0.05 to 0/100/0.05 (v/v/v) with a flow rate of 100 $\mu\text{l}/\text{min}$. The LC column used was an Ace[®] C18, 50 $\times$ 1.0 mm with 3- μm particles from Advanced Chromatography Technologies (Aberdeen, Scotland).

Work-up procedure

About 100 mg of the pet. preparation was accurately weighed into a test tube. 4,4'-MDI in the pet. preparation was reacted with DBA by adding

Table 1. Stated and found concentrations of 4,4'-diphenylmethane diisocyanate in the petrolatum preparations and the departments providing preparations as well as expiry date and coefficient of variation when analysed in July 2003^a

	Origin	Supplier	Stated concentration (% w/w)	Found concentration ^b (% w/w)	Ratio between stated/found	C.V. ^c (%)	Expiry date
Department 1	Europe	Supplier 2	1	0.12	8.3	4.9	2003–11
Department 2	USA	Supplier 1	2	0.095	21.1	5.8	2003–03
Department 3	USA	Supplier 1	2	0.082	24.4	24	1997–01
Department 4	Europe	Supplier 2	1	0.036	27.8	36	2003–10
Department 5	Europe	Supplier 2	1	0.026	38.5	33	2003–12
Department 6	USA	Supplier 1	2	0.031	64.5	6.8	1997–09
Department 7	Europe	Supplier 2	1	0.015	66.7	20	2003–10
Department 8	Europe	Supplier 1	2	0.024	83.3	30	Not declared
Department 9	Europe	Supplier 1	2	0.010	200	6.0	1998–05
Department 10	Europe	Supplier 2	1	0.001	1000	25	2003–12
Department 11	Europe	Unknown	0.1	<0.0005 ^d	>200		Not declared
Department 12	USA	Supplier 1	2	<0.0005 ^d	>4000		Not declared
Supplier 1		Supplier 1	2	1.3	1.5	11	2005–02
Supplier 2		Supplier 2	1	0.19	5.3	11	2003–09

^aPreparations from department 4 were analysed in September 2003.

^bMean value of the triplicate samples.

^cCoefficient of variation (C.V.) for the triplicate samples.

^dLess than quantification limit for samples treated according to the work-up procedure and quantified in the calibrated range.

5.0 ml of 0.010 M DBA in methylene chloride (Fig. 1) and the sample was treated in an ultrasonic bath for 30 min. Thereafter, 5.0 ml of toluene was added followed by treatment in the ultrasonic bath for another 20 min. The sample was diluted 10× in toluene. Samples from clinics 3 and 5 were not diluted, because they contained a much lower concentration than the other preparations. 1.0 ml was transferred into a new test tube and 100 µl of 4,4'-MDI-DBA-d₁₈ (10 µg/ml) serving as internal standard (IS) was added. The sample was evaporated to dryness in a vacuum centrifuge and extraction was performed by adding 5.0 ml of isooctane and 5.0 ml of methanol/water (9/1; v/v) and by shaking vigorously for 10 min. The isooctane phase was discarded, and the methanolic phase was washed with 5.0 ml of isooctane whereafter it was evaporated to dryness. The residue was dissolved in 5.0 ml of methanol for LC-MS analysis. 3 samples of each pet. preparation of 4,4'-MDI were analysed.

A calibration series was made by spiking aliquots of 1.0 ml of toluene with 4,4'-MDI-DBA to 7 concentrations in the range of 0–3.0 µg/ml of 4,4'-MDI. The sample work-up procedure was

then performed, and analysis resulted in a linear calibration curve. For the samples from clinic 3 and 5, a calibration series was made in the same way as described above but where 4,4'-MDI-DBA was pipetted into test tubes containing 1.0 ml of methylene chloride instead of toluene.

Extraction recovery

Aliquots of 1 ml of toluene containing about 100 µg of dissolved pet. were transferred to 9 test tubes, and 2.5 µg of 4,4'-MDI-DBA was added into each test tube. Sample sets of 3 were extracted 1, 2 or 3× using the extraction system described in the work-up procedure. Thereafter IS was added, and they were washed according to the work-up procedure. The remaining methanolic phases were evaporated to dryness, and the residues were dissolved in 5.0 ml of methanol and analysed using LC-MS.

Reference solutions

Reference solutions containing 4,4'-MDI-DBA and 4,4'-MDI-DBA-d₁₈, which were used for the quantification of samples, were characterized using liquid chromatography followed by chemiluminescent nitrogen detection. The procedure has previously been described by Karlsson et al. (8).

Purity of 4,4'-MDI

To investigate the purity triplicate samples of 4,4'-MDI from suppliers 1 and 2 were accurately weighed in test tubes. 5.0 ml of 0.010 M DBA in

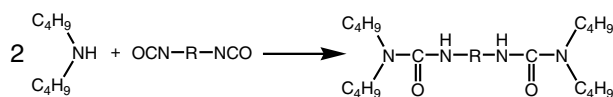


Fig. 1. General reaction describing the conversion of reactive diisocyanate into a stable urea derivative by reacting the diisocyanate with the secondary amine, dibutylamine (DBA). DBA is added in surplus to ensure that all diisocyanate is reacted.

methylene chloride was added to each test tube, and the tubes were placed in an ultrasonic bath for 30 min. Thereafter, each sample was diluted 1000× in toluene and 1.0 ml was separated from each tube and spiked with 100 µl IS. The samples were evaporated to dryness in a vacuum centrifuge, and the residues were dissolved in 5.0 ml of methanol and analysed. In addition, 4,4'-MDI from supplier 1 was ground and 3 samples were weighed and treated in the same manner as the other samples (Table 2).

Repeatability

To determine the repeatability of the method, the sample with the highest amount of 4,4'-MDI, namely the preparation directly obtained from supplier 1 (Table 1), was used. Aliquots of 100 µl of the stock solution, where the pet. preparation had been diluted in 5.0 ml of 0.010 M DBA in methylene chloride and thereafter 5.0 ml of toluene, were transferred to 10 test tubes and diluted in 900 µl of toluene, treated according to the work-up procedure and analysed.

Results

The amounts of 4,4'-MDI in each of the preparations, as well as the coefficient of variation of the triplicate samples, are summarized in Table 1. The preparations used at the clinics contained a few percentages or less of the concentration stated on the labels, while the preparations obtained directly from the suppliers were somewhat higher. The extraction recovery for the work-up procedure described was 83% and it was shown that for a work-up procedure with 2 more extractions with methanol/water (9/1; v/v), the recovery increased to 87%. On average, the purity of 4,4'-MDI was 87% (w/w) for supplier 1, 94% for supplier 2 and 88% for the ground 4,4'-MDI from supplier 1 (Table 2). The coefficient of variation for the 10 samples analysed in order to determine the repeatability for 4,4'-MDI determination of pet. patch-test preparations was 2.9%.

Discussion

It is well known that isocyanates are highly reactive and their stability in patch-test preparations has been discussed previously. Estlander et al. (9) tested 1 patient with a patch-test preparation of 1.5% 4,4'-MDI in pet. manufactured 5.5 months prior to the testing, and 1 patient with a preparation manufactured 15.5 months earlier, to see whether the preparations could elicit positive patch-test reactions. Both patients reacted positively to these preparations, and the authors concluded that 4,4'-MDI preparations could be used for over a year. However, no chemical analyses were done.

There are several methods described in the literature on the monitoring of MDI in air (8, 10). However, to our knowledge, there are no methods published that are suitable for analysis of MDI in patch-test preparations. The method developed in this study is based upon a method used for monitoring of airborne isocyanates (8), which was modified to involve an extraction procedure for isocyanates from pet. and a suitable solvent. An investigation of the recovery of the extraction showed that the extraction procedure had a yield of 84%, which was only marginally increased to 87% by doing repeated extractions. However, all losses due to the extraction procedure are compensated for by the use of IS. The pet. patch-test preparations were dissolved in methylene chloride containing DBA. This solvent was chosen on the basis of its capacity to dissolve 4,4'-MDI. Chemical analysis of 4,4'-MDI test preparations using this method was performed on patch-test preparations obtained from 2 suppliers, as well as preparations used at 12 dermatology departments from USA and Europe. 4 of the preparations had passed their expiry date, 3 were not labelled with an expiry date and 5 had a valid expiry date. Department 1 had the highest amount of 4,4'-MDI, corresponding to a concentration of 0.12%, even though it was not the freshest sample. The samples from departments 10 and 5, which were from the same supplier as the preparation from department 1, were fresher but contained only 0.001% and 0.026%, respectively. Most preparations contained only a few

Table 2. Purity of 4,4'-diphenylmethane diisocyanate when dissolved in methylene chloride

	Supplier 1 [Purity (% w/w)]	Supplier 1, ground [Purity (% w/w)]	Supplier 2 [Purity (% w/w)]
Sample 1	87	94	88
Sample 2	88	82	95
Sample 3	86	89	99
Mean value	87	88	94
C.V. ^a (%)	1.2	6.8	5.9

^aCoefficient of variation (C.V.) for the triplicate samples.

percentages or less of the concentration stated on the label. Only 1 preparation directly obtained from the supplier 1 came close to the declared concentration, as it contained 1.3% 4,4'-MDI. Isaksson et al. (11) stated that, according to personal communications with the manufacturers, the prerequisite of the content of the preparations from the TRUE TestTM (Pharmacia AS, Hillerød, Denmark) and patch-test allergens from Hermal (Hermal, Reinbeck, Germany) is $\pm 20\%$ of the concentration given on the label. None of the preparations investigated, not even the preparations obtained directly from the suppliers, fulfilled these requirements. This could be due to the fact that isocyanates are highly reactive and polymerize in the syringes. Furthermore, the results suggest that this happens rather rapidly, because not even the fresher preparations contained the required amount 4,4'-MDI.

If these preparations are used, patients will be tested with a much lower concentration of 4,4'-MDI than intended, leading to a high risk of false-negative reactions and thereby under-diagnosis of allergic contact dermatitis from 4,4'-MDI. These findings suggest that patch testing with fresh preparations of the patients' own work material is desirable when aiming at diagnosing contact allergy from MDI. However, as stated previously (5, 6), if clinics lack the possibility/knowledge to test with the patients' own work material, positive reactions to MDA should be taken into account because this might be an important marker for isocyanate sensitivity.

The results also show that the purity of 4,4'-MDI in the raw material provided by the suppliers varied somewhat between the samples (Table 2). As mentioned, this could among other things depend on the fact that the raw material had already polymerized to some extent. When investigating the behaviour of polymeric MDI in water, Yakabe et al. (12) reported that solid and insoluble polyurea is formed, which encrusts the isocyanate and gradually spreads through it. 4,4'-MDI might react with water in the air during storage so that a polymerized crust is formed on the surface of the MDI flake. Aromatic diisocyanates are also able to react with the moisture that is always present in organic solvents and thus give rise to a number of products, including for example polyureas (13). Nevertheless, because the purity of the raw material was rather high, this alone could not account for the low concentrations found in relatively fresh preparations. As we were told that supplier 1 ground their 4,4'-MDI before dispersing it in pet., we also made a comparison with ground 4,4'-MDI on account of purity. There were no major differences between

the samples, which indicate that the losses occur at a later stage. As summarized in Table 1, there is a slight variation between the triple samples. However, because the repeatability trials showed that the coefficient of variation between 10 samples from the same stock solution was less than 3%, the variations between the triple samples ought to be due to factors other than methodological deficiencies. One explanation might be that the test preparations themselves are not homogeneous with regard to both the dispersion of MDI in pet. and differences in the degree of possible polymerization in different parts of the syringe.

Conclusion

An analytical method for monitoring of 4,4'-MDI in pet. test preparations shows that the concentrations in commercial test preparations are so low that patch testing with the same cannot be considered reliable. Therefore, when aiming at diagnosing contact allergy from MDI-related substances, it seems desirable to test with the patient's own fresh work material.

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References

1. Ulrich H. *Chemistry and technology of isocyanates*. Chichester/New York/Brisbane/Toronto/Singapore: Wiley, 2001: 315.
2. Klees J E, Ott M G. Diisocyanates in polyurethane plastics application. *Occup Med* 1999; 14: 759–776.
3. Goossens A, Detienne T, Bruze M. Occupational allergic contact dermatitis caused by isocyanates. *Contact Dermatitis* 2002; 47: 304–308.
4. Anonymous. TA: Concise International Chemical Assessment Document (CICAD) PG:25 p YR:2001 IP: VI:27.
5. Estlander T, Kanerva L, Jolanki R, et al. Polyurethane resins. In: Kanerva L, Elsner P, Wahlberg J E, Maibach H I (eds). Berlin: Springer, 2000: 597–601.
6. Frick M, Isaksson M, Björkner B, Hindsén M, Pontén A, Bruze M. Occupational allergic contact dermatitis in a company manufacturing boards coated with isocyanate lacquer. *Contact Dermatitis* 2003; 48: 255–260.
7. Tait C P, Delaney T A. Reactions causing reactions: allergic contact dermatitis to an isocyanate metabolite but not to the parent compound. *Australas J Dermatol* 1999; 40: 116–117.
8. Karlsson D, Dahlin J, Skarping G, Dalene M. Determination of isocyanates, aminoisocyanates and amines in air formed during the thermal degradation of polyurethane. *J Environ Monit* 2002; 4: 216–222.

9. Estlander T, Keskinen H, Jolanki R, Kanerva L. Occupational dermatitis from exposure to polyurethane chemicals. *Contact Dermatitis* 1992; 27: 161–165.
10. Tinnerberg H, Spanne M, Dalene M, Skarping G. Determination of complex mixtures of airborne isocyanates and amines – Part 1. Liquid chromatography with ultraviolet detection of monomeric and polymeric isocyanates as their dibutylamine derivatives. *Analyst* 1998; 121: 1095–1099.
11. Isaksson M, Gruvberger B, Persson L, Bruze M. Stability of corticosteroids patch-test preparations. *Contact Dermatitis* 2000; 42: 144–148.
12. Yakabe Y, Henderson K M, Thompson W C, Pemberton D, Tury B, Bailey R E. Fate of methylenediphenyl diisocyanate and toluene diisocyanate in aquatic environment. *Environ Sci Technol* 1999; 33: 2579–2583.
13. Seel K, Walber U, Herbold B, Kopp R. Chemical behaviour of seven aromatic diisocyanates (toluenediisocyanates and diphenylmethanediisocyanates) under in vitro conditions in relationship to their results in the Salmonella/microsome test. *Mutat Res* 1999; 438: 109–123.

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